EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF VITAMIN A

The attached review of vitamin A is a revised version of the paper presented to the Expert Group on Vitamins and Minerals at the meeting on 2 February 2000. New information on vitamin A and bone mineral was included and the review reconsidered at the meeting in March 2002. Some minor amendments have now been made.

The following annexes are also included with this paper:

Annex 1. Tables and figures referred to throughout the review
Annex 2. Intakes of retinol, β-carotene, total carotene and total vitamin A (retinol equivalents) from food and supplements
Annex 3. Summary table of selected nutrition related information and existing guidance.

Expert Group on Vitamins and Minerals Secretariat
April 2002

Vitamin A

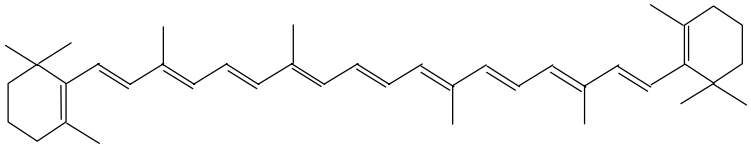
Introduction

1. In this paper, we have reviewed the requirement, function and toxicity of vitamin A in food and nutritional supplements. The preformed vitamin A compounds, retinol and retinyl esters are, therefore, of primary relevance. The safety of the synthetic and therapeutic forms of vitamin A will not be considered here. The proform vitamin A compound, β-carotene, has been reviewed separately. The teratogenicity of retinol has been previously reviewed by the COT. A post-1990 update is provided here.

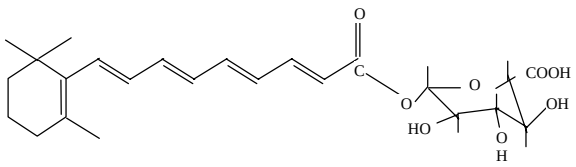
Chemistry and nomenclature

2. Vitamin A is loosely used as a generic term for a group of largely fat-soluble compounds that includes the parent compound, all-*trans*-retinol, its aldehyde and acid forms, retinal and retinoic acid, the active form in vision 11-*cis*-retinal, the major storage and oxidatively more stable form retinyl palmitate, and the water soluble metabolite retinoyl β-glucuronide. These are collectively known as retinoids. The vitamin A family also includes 50 pro-vitamin A carotenoids, a major one being β-carotene. Therapeutically useful analogues include the naturally occurring 13-*cis*-retinoic acid (also known as isotretinoin), and the synthetic aromatic retinoids etretin and etretinate). Structures are shown in Figure 1, below.

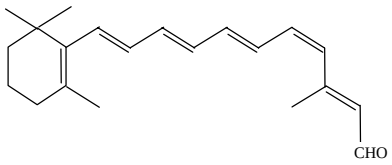
Figure 1: Structures of the major retinoids and β-carotene (adpated from Oldon, 1996)



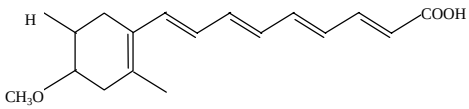
All-trans β-carotene



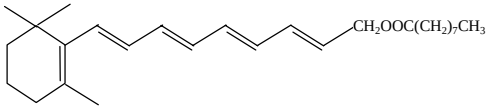
All-*trans* retinoyl β-glucuronide



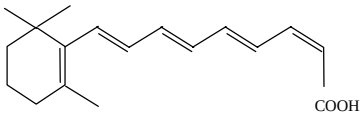
11-*cis* retinal



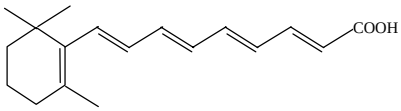
The trimethyl methoxyphenol analog of all-*trans* retinoic acid (etretin, acitretin)



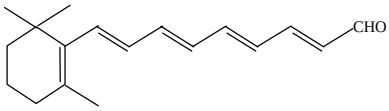
All-*trans* retinyl palmitate



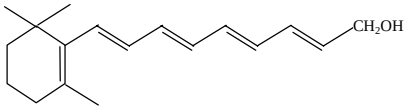
13-*cis* retinoic acid



All-*trans* retinoic acid



All-*trans*-retinal



All-*trans*-retinol

3. The parent compound, all-*trans*-retinol (CAS No. 68-26-8; 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol; $C_{20}H_{30}O$; mol. wt. 286.46; m. pt. 62-64^{0}C; b. pt. 137-138^{0}C) is a long-chain primary alcohol. In the pure state, it exists as pale yellow crystals, soluble in most organic solvents and fats. Structurally, retinol consists of a hydrophobic head made up of a β-ionone ring, a conjugated isoprenoid side chain which is subject to isomerisation in the presence of light, and a polar terminal group which can be enzymatically or chemically modified to form esters or the aldehyde, or be oxidised to the acid.

4. Not all carotenoids have provitamin A activity. However, most carotenoids, including those with provitamin A activity, can serve as singlet oxygen quenchers and as antioxidants. Retinol itself does not possess singlet oxygen quenching properties.

5. Vitamin A is sensitive to ionising radiation. However, retinol and β-carotene are stable throughout most cooking procedures and are resistant to acid and alkali. Industrial food processes, such as pasteurisation, sterilisation, or dehydration, generally result in only small losses (Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

Occurrence in food, food supplements and licensed medicines

Foods

6. Common dietary sources of preformed vitamin A include liver, various dairy products and oily fish. There is a statutory requirement to fortify margarine with vitamin A and some other foods are also fortified with this vitamin. Common sources of provitamin A carotenoids include dark green leafy vegetables, corn, tomatoes, oranges. In the UK, between 66% and 75% of the retinol equivalent (RE) in ingested food is derived from preformed vitamin A.

7. Preformed vitamin A is also available as single- and multi-vitamin and/or mineral supplements, usually in the form of retinol, retinyl palmitate or retinyl acetate.

Licensed Medicinal Products for Oral Use

8. Medicinal products containing vitamin A may be sold without a doctor's prescription providing the recommended dose is no greater that 2250 μg retinol equivalents per day.

9. Thirty-five medicinal products containing vitamin A (in addition to other nutrients) are authorised for supply on general sale, i.e. in supermarkets and other retail outlets. Their licensed uses include the prevention and treatment of nutrient deficiency, use in pregnancy (only at 800 μg per day or less) and lactation, use in children and the elderly, and as a supplement to restricted diets.

10. A further 24 products containing vitamin A may only be sold in pharmacies. The indications for those products include use post-operatively, in catabolic states, in chronic disease and in malabsorption, in addition to the indications outlined in paragraph 9.

11. Due to the concern about the teratogenic potential of vitamin A the Committee on Safety of Medicines recommended in 1990 that products should be labelled with one of the following warnings:

    i) Where the maximum daily dose is greater than the Recommended Daily Allowance (RDA) (currently 800 g RE) the label should state:

    "Do not take vitamin A supplements if you are pregnant or likely to become pregnant except on the advice of a doctor or ante-natal clinic".

    ii) Where the maximum daily dose is the RDA, or lower, the label should state:

    "Do not exceed the stated dose".

Intake/Exposure

Equivalents

12. Following the recommendations of the Food Agricultural Organisation (FAO) and World Health Organisation (WHO), estimations of vitamin A requirement and food content take into account the different activities and variable absorption of vitamin A and provitamin A, and are expressed in terms of "retinol equivalent" (RE) where 1 μg RE = 1 μg retinol = 1.78 μg retinyl palmitate = 6 μg β-carotene = 12 μg other carotenoids with provitamin A activity = 3.33 IU vitamin A activity from retinol. It is important to note, however, that there is much debate about the accuracy of the factor of 6 used for β-carotene. The bioavailability of β-carotene is influenced by a number of factors, including food processing, parasitic infections, individual variation and the presence of fat and fibre, and there is contention over whether any significant amount of β-carotene is absorbed from dark green leafy vegetables (Scott and Rodriquez-Amaya, 2000). Certain authors are now advocating abandoning the current system of retinol equivalents and presenting food composition data with only retinol and the carotenoids as amount per 100 g (Scott and Rodriquez-Amaya, 2000).

Food

13. Mean intakes of adults in the UK population are in excess of the Reference Nutrient Intake (RNI) values (see Annex 2). However, the mean appears to be artificially high, as it is skewed due to high vitamin A intakes in regular consumers of liver and liver products. The median intake in adults has been estimated to be 520 μg RE/day; 97.5th percentile intake was estimated to be 6050 μg RE/day. Post-mortem liver analysis show that most of the UK population has substantial vitamin A reserves (DH, 1991).

Dietary Supplements

UK

14. Vitamin A (as retinol or retinyl ester) is widely available in a wide range of multi-vitamin and multi-vitamin and mineral supplements, frequently with combinations of other anti-oxidant vitamins. The majority contain 800 μg RE (the EU labelling RDA) but higher doses are available.

US

15. In the US, dietary supplements are used extensively and in most cases supplemental intake is 5,000 IU (1,500 μg RE) or more than twice the Reference Daily Intake or RNI of preformed vitamin A. However, vitamin A (retinol, retinyl ester) supplements ≥10,000 IU (3,000 μg RE) are available in US health food stores (Olson, 1996 and references therein) and regional surveys have shown that in some individuals, vitamin A intake from supplements may be far higher (Hathcock *et al*., 1990 and references therein).

Therapeutic forms

16. Tretinoin (all-trans-retinoic derivative of retinoic acid, marketed as Retin A), isotretinoin (13-cis-retinoic acid, naturally occurring, marketed as Roaccutane), and the synthetic analogue acitretin (Neotigason) are available only on prescription and are often prescribed as dermal or oral treatments for skin disorders (Monga, 1997 and references therein).

Recommended amounts

17. Recommended intakes are shown in Table 1. Following FAO/WHO (1988), the US National Research Council (NRC) (1989) and UK COMA (DH, 1991) have adopted the "adequate body pool size" approach to establishing requirements, set at 20 μg (RE) retinol (or equivalent in esterified form)/g wet weight of liver. Recommended intakes for infants have been based upon the amount of vitamin A provided in breast milk. The reference nutrient intake in the UK is 700 μg RE/day for males and 600 μg RE/d for females, with an increment of 100 μg/d in pregnancy and 350 μg/d in lactation (DH, 1991). The Food and Nutrition Board of the Institute of Medicine recently set dietary reference values for North Americans (Trumbo *et al.*, 2001). The recommended dietary allowance (the intake sufficient to meet the needs of 98% of healthy individuals) for vitamin A was 900 μg RE/d for adult men and 700 μg RE/d for adult women with an increment of 70 μg in pregnancy and 600 μg in lactation.

Table 1. Recommended dietary intakes of vitamin A in retinol equivalents (adpated from Olson, 1996):

Group	**RDI (FAO/WHO)**		**RDA (USA)**	**DRV (UK)**		
	Basal	**Safe**		**Lower reference nutrient intake**	**Estimated average requirement**	**Reference nutrient intake**
Infants:						
0-0.5 yrs	180	350	375	150	250	350
0.5-1 yrs	180	350	375	150	250	350
Children:						
1-2 yrs	200	400	400	200	300	400
2-6 yrs	200	400	500	200	300	400
6-10 yrs	250	400	700	250	350	500
Males:						
10-12 yrs	300	500	1000	250	400	600
12+ yrs	300	600	1000	300	500	700
Females:						
10+ yrs	270	500	800	250	400	600
Pregnancy	+100	+100	0			+100
Lactation:						
0-6 mths	+180	+350	+500			+350
>6 mths	+180	+350	+400			+350

Assessment of status

18. Absorbed vitamin A excess to requirement is stored within the liver in an esterified form. Determination of hepatic concentration is therefore one objective determination of vitamin A status, but cannot be readily determined in living individuals. Due to homeostatic control, plasma retinol concentration is tightly maintained around a level of 40-80 μg/100 ml and, as such, is an insensitive indicator of status except in cases of extreme depletion, when levels fall below 20 μg/100 ml, and other signs of deficiency are already evident. However, hypervitaminosis A may be characterised by elevated levels of plasma retinyl esters, which normally contribute to <5% of blood vitamin A. Serum retinol is used in epidemiological surveys as a measure of vitamin A status. Stephensen and Gildengorin (2000) have examined the effect of the acute phase response (measured by elevated C-reactive protein, CRP) on classification of marginal vitamin A deficiency in the NHANES-III data set. Mean serum retinol was significantly lower in people with serum CRP above 10mg/l and the percentage of people classified with marginal deficiency was significantly greater in those with elevated CRP. Infection, arthritis and smoking were all associated with elevated CRP. Other methods used to determine vitamin A status in humans include dietary assessment, clinical evaluation, retinal function tests and assessment of retinol-binding proteins (Basu and Dickerson, 1996; Olson, 1996; Miller *et al.*, 1998). The deuterated-retinol-dilution technique can provide a quantitative estimate of total body stores of vitamin A and has been shown to respond to changes in body stores after supplementation in populations with low vitamin A status (Haskell *et al.*, 1999).

Bioavailability

Lipid intake and bioavailability of vitamin A from food

19. In healthy people, who ingest >10 g fat per day, >80% of dietary vitamin A is absorbed. The extent of absorption is only mildly reduced when vitamin A is increased. In contrast, the extent of absorption of dietary carotenoid (usually approximately 40%) declines as the amounts of carotenoid in the diet increases. Diets low in fat, or individuals with obstruction of the bile duct or suffering from fat malabsorption, may have problems with vitamin A absorption. Plasma concentrations of vitamin A are generally depressed in conditions associated with fat malabsorption, such as cystic fibrosis, obstructive jaundice and sprue (Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

Protein nutrition and bioavailability of hepatic stores

20. Protein nutritional status is an important factor in determining the bioavailability of hepatic stores of vitamin A. Children suffering from protein-energy malnutrition typically have low plasma retinol and retinol binding protein (RBP). This seems to be a reflection of impaired hepatic release of vitamin A due to defective RBP synthesis, since vitamin A status can be improved by administration of protein without the need for increased dietary vitamin A. RBP is rich in aromatic and essential amino acids, has a short half-life (4 hours), a small

body pool and is therefore sensitive to inadequate protein intake (Basu and Dickerson, 1996 and references therein).

Bioavailability of supplements

21. Commercially available forms are mainly retinyl-acetate and retinyl-palmitate. The form of ester *per se* does not affect bioavailability. However, the physical form of the preparation influences the extent of absorption. Higher plasma values and lower faecal losses are observed when vitamin A is given as an aqueous dispersion or emulsion, rather than in oil or as an oil solution in soft gelatin capsules. With comparable doses, symptoms of toxicity appear significantly earlier following ingestion of aqueous dispersions or emulsions than of oil solutions (Bauernfeind, 1980). Singh and Das (1998) have shown that liposome-encapsulated retinol and retinol palmitate may be less toxic *in vivo*, since this form was less lytic to red blood cells *in vitro*.

Absorption and metabolism of liver-derived retinol versus retinol from supplements

22. Buss *et al.* (1994) have shown that the absorption and metabolic handling of vitamin A differ significantly following ingestion of vitamin supplements as compared to the consumption of cooked liver. Plasma C*max* (maximum concentration) and AUC (area under the curve) for all-*trans*-retinoic acid (a principal oxidative metabolite of retinol, and putative teratogen) were 10 - 20 times lower following ingestion of liver, compared to similar doses of vitamin A as supplements. In contrast, plasma concentrations of 13-*cis*-retinoic acid and other metabolites showed larger increases above baseline and smaller differences between liver and supplements.

23. It was suggested that, following administration of retinol in liver, there was a slow absorption and gradual increase in the gastrointestinal mucosa. The resulting low intracellular concentrations of free retinol would bind to cellular retinol binding protein (CRBP), undergo re-esterification and be cleared via the lymphatics, or undergo oxidation to all-*trans*-retinoic acid. The all-*trans*-retinoic acid would then be bound by cellular retinoic acid-binding protein (CRABP) and/or undergo further metabolism before being cleared by the hepatic portal system. In contrast, following administration of retinol in the form of supplements, there was likely to be a more rapid increase in retinol within the intestinal mucosa. Binding to CRBP and/or re-esterification of retinol may become temporarily saturated, resulting in a concomitant increase in oxidation to all-*trans*-retinoic acid. CRABP binding may then also become saturated, allowing increased levels of all-*trans*-retinoic acid to enter the hepatic portal vein and establish elevated levels in the circulation (Buss *et al.*, 1994). Preliminary results, reported by Wiegrand *et al.* (1998), confirmed the findings of Buss *et al*. All plasma retinoid levels were much lower than expected when a liver meal, containing 30,000 μg RE vitamin A, was taken in addition to supplements. This was particularly true for retinoic acid.

24. Together, these findings suggest that the food matrix has a profound influence on the absorption and metabolism of retinol and its esters. Differences in systemic exposure to all-*trans*-retinoic acid may be important for assessing the relative risk

of high doses of vitamin A from liver *vs* supplements regarding potential for teratogenic effects.

Interactions

Alcohol and hepatic storage

25. Chronic alcoholic liver damage results in a decreased capacity for the liver to store vitamin A. However, alcohol may also directly deplete hepatic vitamin A by altering vitamin A metabolism through induction of aldehyde and alcohol dehydrogenases and cytochrome P450 enzymes involved in vitamin A catabolism (Leo and Lieber, 1999).

Alcohol potentiation of vitamin A hepatotoxicity

26. Both alcohol and vitamin A are hepatotoxic, given in sufficient doses. Animal studies have been inconsistent in demonstrating whether the combined effects of alcohol and vitamin A are additive or synergistic, using standard criteria of hepatotoxicity (Hathcock *et al.*, 1990 and references therein). Leo *et al.* (1982) showed altered hepatocyte morphology and mitochondrial function in ethanol-treated rats supplemented with high levels of vitamin A. These effects appear to be additive. Ethanol alone caused increased proliferation of the endoplasmic reticulum (ER), steatosis and moderate alterations in mitochondria. Vitamin A alone also caused proliferation, a lesser level of steatosis and slight enlargement of mitochondria. Combined treatment resulted in more severe fat accumulation and giant mitochondria with filamentous para-crystalline inclusions. Similar inclusions have been observed following biopsy of alcoholic patients being treated with 3,000 µg RE vitamin A/day for sexual dysfunction (Worner *et al.*, 1988) and other patients with hypervitaminosis A (Minuk *et al.*, 1988).

Alcohol, alcohol dehydrogenase and fetal alcohol syndrome

27. Alcohol consumption may lead to fetal abnormalities, not by potentiating vitamin A toxicity, but by interfering with its metabolism. Animal studies have shown that combined administration of ethanol and vitamin A caused a significant increase in incidences of cleft palate, mis-shaped zygomatic arch, moderately enlarged renal pelvis and other abnormalities (Whitby *et al.*, 1994). It has been suggested that interference with vitamin A metabolism, following maternal alcohol ingestion, results in impaired alcohol dehydrogenase activity and reduced retinoic acid synthesis, resulting in functional vitamin A deficiency in the embryo (Deltour *et al*., 1996; DeJonge and Zachman, 1995; Duester, 1994).

Protein

28. In protein malnourished individuals, synthesis of RBP is impaired (see paragraph 21). Consequently, in these individuals, hypervitaminosis A may occur at lower doses than might otherwise be expected (Hathcock *et al.*, 1990).

Zinc

29. Animal studies have shown that zinc deficiency may be accompanied by decreases in plasma retinol and hepatic RBP and repletion of zinc restores vitamin A levels to within the normal range. It has been postulated that zinc plays a regulatory role in protein biosynthesis and fast turnover proteins, such as RBP, may be particularly sensitive to zinc deficiency. A consequential defect in RBP synthesis would culminate in an impaired release of vitamin A from hepatic stores. Zinc status may also influence a critical step in retinol metabolism. The conversion of retinol to retinal occurs ubiquitously, and is a well-characterised step in the visual cycle. The reaction, catalysed by a zinc metallo-enzyme, alcohol (retinol) dehydrogenase (ADH), is decreased in zinc-deficient animals. Zinc deficiency has also been shown to adversely affect absorption of vitamin A in rats, possibly through zinc-induced changes in lipid metabolism (Basu and Dickerson, 1996 and references therein; Christian and West, 1998 and references therein). However, 10 cross-sectional studies have failed to establish any consistent relationship between zinc and vitamin A in humans (reviewed, Christian and West, 1998).

Drugs

30. Minocyclin, prescribed together with vitamin A, is a well-established treatment for acne vulgaris. Both medications have been implicated as a possible cause of pseudotumour cerebri. There is a concern that the combination might potentiate the ability of each to cause symptoms at lower doses and/or after a shorter duration of treatment. Moskowitz *et al.* (1993) reported the case of a young female who developed symptoms after 6 weeks when prescribed minocyclin with 30,000 μg RE/day vitamin A. Cases of benign intracranial hypertension have been reported in persons taking tetracycline plus vitamin A in amounts ranging from 12,000-45,000 μg RE/day (Hathcock *et al.*, 1990 and references therein).

31. Drugs such as colchicine and cholestyramine, antacids and mineral oil laxatives may inhibit vitamin A absorption (Anonymous, 1998).

32. In animals, co-administration of cytochrome P-450 inhibitors, ketoconazole and liarazole, causes a significant increase in the half-life of retinoic acid (Collins and Mao, 1999 and references therein).

Iron

33. Vitamin A may aid absorption of iron, by preventing the inhibitory effects of phytate. There is an association between vitamin A status and anaemia. The underlying mechanism for this remains obscure but Vitamin A deficiency may impair the re-utilisation of iron for erythropoiesis (Bloem, 1995 and references therein). In Tanzanian schoolchildren (9-12 years) vitamin A (5000 IU) alone or with iron (200 mg) for 12 weeks significantly increased haemoglobin by 13.5 g/l (vitamin A) and 22.1 g/l (iron and vitamin A) compared to 3.6 g/l with placebo (Mwanri *et al.*, 2000).

34. An antagonistic interaction between vitamin A and vitamin D has been suggested and animal studies have indicated that vitamins A and D may lessen the toxic effects of each other (Hathcock *et al.*, 1990 and references therein).

Vitamin D

35. Vitamins A and D are often consumed together and it may be difficult to separate the symptoms of the two hypervitaminoses. Some of the symptoms common to both include weakness, fatigue, vomiting, lassitude, headache, nausea, diarrhoea, polydipsia, anorexia, weight loss and conjunctivitis (Bendich and Langseth, 1989 and references therein).

Vitamin C

36. There is a dearth of information in the recent literature concerning any vitamin A-vitamin C interaction. However, earlier reports suggested that hypervitaminosis A may decrease tissue storage of vitamin C (Bauernfeind, 1980 and references therein)

Vitamin E (alpha-tocopherol)

37. Co-ingestion of vitamins A and E, in very high quantities (60,000 µg RE and 500 mg, respectively) in children, resulted in increased absorption of vitamin A (estimated from the excretion of a radioactive tracer) (Kusin *et al.*, 1974). Dietary vitamin E also influences tissue distribution of retinyl esters (Napoli *et al.*, 1984) and may serve as a non-competitive inhibitor of retinyl ester hydrolysis in liver, kidney and intestine (Napoli and Beck, 1984). In some cases, vitamin E has been found to ameliorate some of the toxic manifestations of excessive vitamin A (Bauernfeind, 1980 and references therein).

Vitamin K

38. Studies in humans and animals have shown that chronic hypervitaminosis A can lead to hypothrombinaemia. Antagonism of vitamin K by excess vitamin A was suggested when clotting times were restored by feeding supplementary vitamin K (Bauernfeind, 1980 and references therein). Vitamin K can also serve as a non-competitive inhibitor of retinyl ester hydrolysis in liver, kidney and intestine (Napoli and Beck, 1984).

Corticosteroids and Stress

39. Circulating concentrations of retinol may be subject to transient downward fluctuations brought along by physical or pathological stress. It is thought that secretion of corticosteroids reduces tissue vitamin A by enhancing elimination (Basu and Dickerson, 1996 and references therein).

Effect on thyroid hormone levels

40. Literature concerning the effect of hypervitaminosis A on the thyroid gland have not been consistent, with both vitamin A-induced thyroid hypertrophy and atrophy having been reported in rats (Nieman and Obbink, 1954 and references therein; Petenusci, 1980). There seems less conjecture over the influence of vitamin A status over thyroid hormone levels. Vitamin A deficient rats have been reported to exhibit increases in plasma total thyroxine (T4) and triiodothyronine (T3), and free thyronine (Morley *et al.*, 1978). In contrast, high levels of vitamin A in rats have been shown to cause decreased plasma total T4 and T3, decreased T4 half-life and increased dialysable T4 and T3 and T3 distribution space, in the absence of any changes in thyroid stimulating hormone levels or response to thyroid releasing hormone (Morley *et al.*, 1970; Garcin *et al.*, 1984). Similar, although transient, changes in thyroid hormones, in the absence of changes in RBP or pre-albumin, were reported in normal (vitamin A sufficient) human subjects, following administration of therapeutic doses (9,000 μg RE/day) of vitamin A (Morley *et al.*, 1981). Thyroxine has been shown to accelerate the conversion of β-carotene to retinol (Aktuna *et al.*, 1993). The toxicological significance of these findings, if any, is not clear.

Absorption, distribution, metabolism and excretion

(Reviewed by Blomhoff *et al.*, 1991; Basu and Dickerson, 1996; Olson, 1996; IARC, 1998).

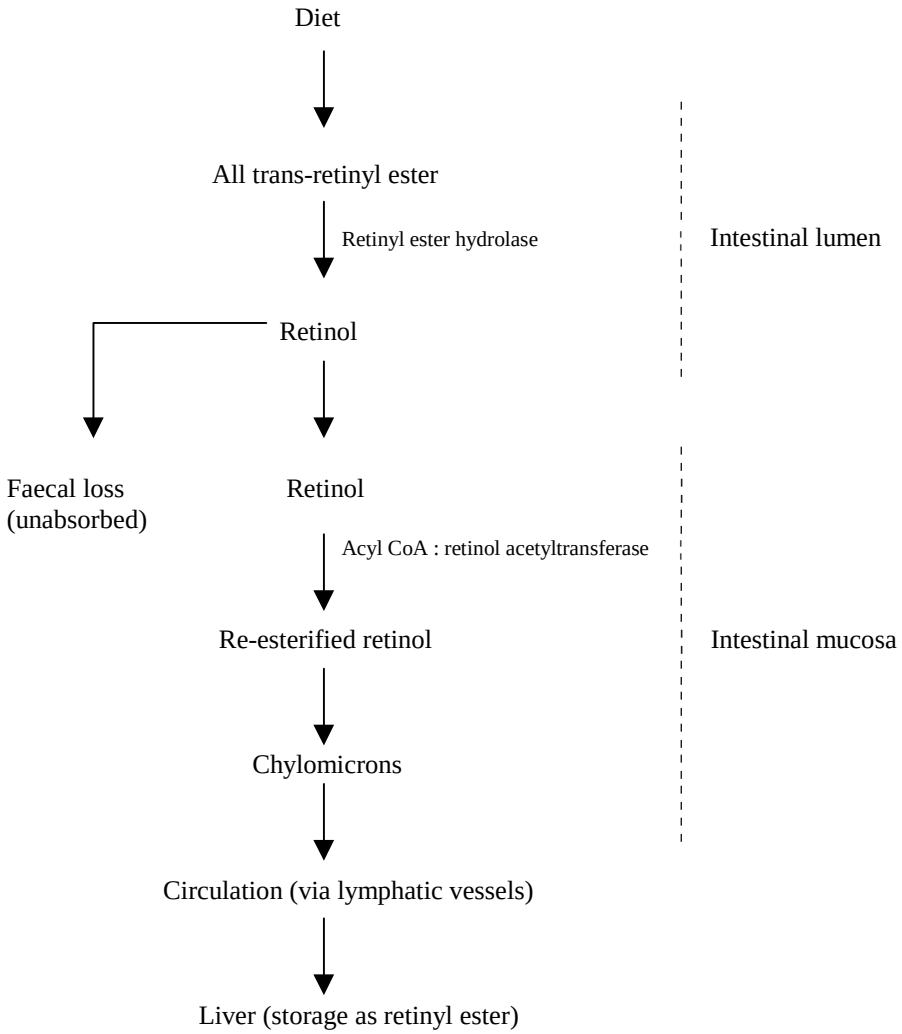
Absorption

41. Dietary preformed vitamin A is mainly in the form of long-chain fatty acid esters. Retinyl esters and provitamin A carotenoids are released from ingested food by proteolytic digestion in the stomach and tend to aggregate with other lipid material. They are then hydrolysed by the bile and pancreatic esterases, present in the small intestine. Retinol esters can also be hydrolysed by two enzyme systems located on the intestinal brush-border membranes (BBM); a pancreatic-origin lipase, responsible for short-chain retinyl esters, and an enzyme intrinsic to BBM which hydrolyses long-chain retinyl esters. Retinol and carotenols are then transported, together with hydrocarbon carotenoids, in micellor form, across the plasmalemma of the absorptive epithelial cells (enterocytes) of the intestinal villus. A proportion (dependent on vitamin A status) of carotenols is converted to retinol, primarily within the intestinal mucosa. Newly absorbed and newly synthesised retinol is then re-esterified, mainly with palmitic acid, before it is incorporated, together with triacylglycerols, into chylomicra which are released into the circulation via the lymph (Figure 2).

42. In plasma, the chylomicra are rapidly hydrolysed by plasma lipoprotein lipase. The chylomicron remnants and associated retinyl esters are then taken up, largely but not exclusively, by the hepatic parenchyma. Lysosomal enzymes within hepatocytes degrade the remnants and the retinyl esters are hydrolysed at the plasma membrane. Retinol is then transferred to the stellate cells (also known as fat storing cells, Ito cells or lipocytes) in the free form where it is re-esterified and stored as long-chain saturated retinyl esters. In the well-nourished state, approximately 90% of the body's vitamin A are stored within the stellate cells of

the liver. Other tissues, such as kidney, lungs, adrenals, retina and intraperitoneal fat, contain ~9% and the remaining 1% is contained within plasma.

Figure 2 : Vitamin A absorption. Adapted from Basu and Dickerson (1996).



Distribution

Retinol is recycled between liver and peripheral tissues, via the blood and lymph. Mobilisation of vitamin A from the liver requires the de-esterification of retinyl esters back to the alcohol form, retinol. This process is regulated by hepatic retinol hydrolase. Free retinol is then bound to retinol-binding protein (RBP) synthesised and secreted by the hepatocytes. The interaction between retinol and RBP is thought to maintain solubility of the vitamin within plasma and provide protection against oxidative damage. The retinol-RBP complex, known as holo-RBP, is then released into the circulation. The plasma concentration of holo-RBP is maintained under homeostatic control, the mechanism of which is not well defined.

43. The retinol-RBP forms complexes 1:1 with transthyretin (TTR or pre-albumin), which is thought to stabilise the binding of RBP and retinol and increase its half-life (holo-RBP $t_{1/2}$ = 4 hours, holo-RBP-TTR $t_{1/2}$ = 12 hours) by preventing loss via glomerular filtration. It has been estimated that approximately 95.5 % of plasma retinol is present as the ternary TTR complex, 4.4% as holo-RBP and <0.1% as free retinol. Administered vitamin A equilibrates with the body reserves within approximately two weeks.

44. Many extrahepatic tissues can take up and store retinol. Tissues such as adipose, kidney, testes, lungs and bone marrow all contain significant amounts of vitamin A and some have been shown to possess the enzymes required for retinol esterification and retinol ester hydrolysis. Uptake of protein-bound retinol into extra-hepatic tissues is thought to be receptor-mediated. Specific membrane receptors recognise the binding-proteins but not retinol itself. However, there is no concomitant uptake of the binding protein with retinol. After retinol is delivered to the cell, RBP loses affinity for TTR, returns to the plasma as apo-RBP and is eliminated by glomerular filtration.

45. Other vitamin A compounds that circulate in the plasma are normally present in much lower concentrations and include all-*trans*-retinyl ester (<0.2 μmol/l, ~10% that of retinol), all-*trans*-retinoic acid, 13-*cis*-retinoic acid, all-*trans*-4-oxoretinoic acid, 13-*cis*-oxo-retinoic acid, all-*trans*-retinyl β-glucuronide, all-*trans*-retinoyl β-glucuronide and 13-*cis*-retinoyl β-glucuronide (concentrations between 2-20 nmol/l, ~0.1-1% that of retinol). The regulation of these compounds in the circulation and their uptake by cells is poorly understood. Unlike holo-RBP, plasma concentrations of total carotenoids and the relative amounts of the individual components are dependent on amounts ingested in the diet.

46. Within cells, retinol, retinal and retinoic acid are usually complexed with cellular binding-proteins. For example, retinol is bound to CRBP (CRBP II in the intestine). Other intracellular binding proteins with different retinoid specificities have been identified in various different tissues; these include cellular retinoic acid-binding protein (CRABP), cellular retinal-binding protein (CRALB), which is principally present in the retina of the eye, and inter-photoreceptor retinol-binding protein (IRBP), present in the extracellular space of the retina. These binding proteins possibly act as intracellular receptors which facilitate the transfer of specific retinoids to the nucleus, where they participate in the control of gene expression for differentiation and growth.

Metabolism

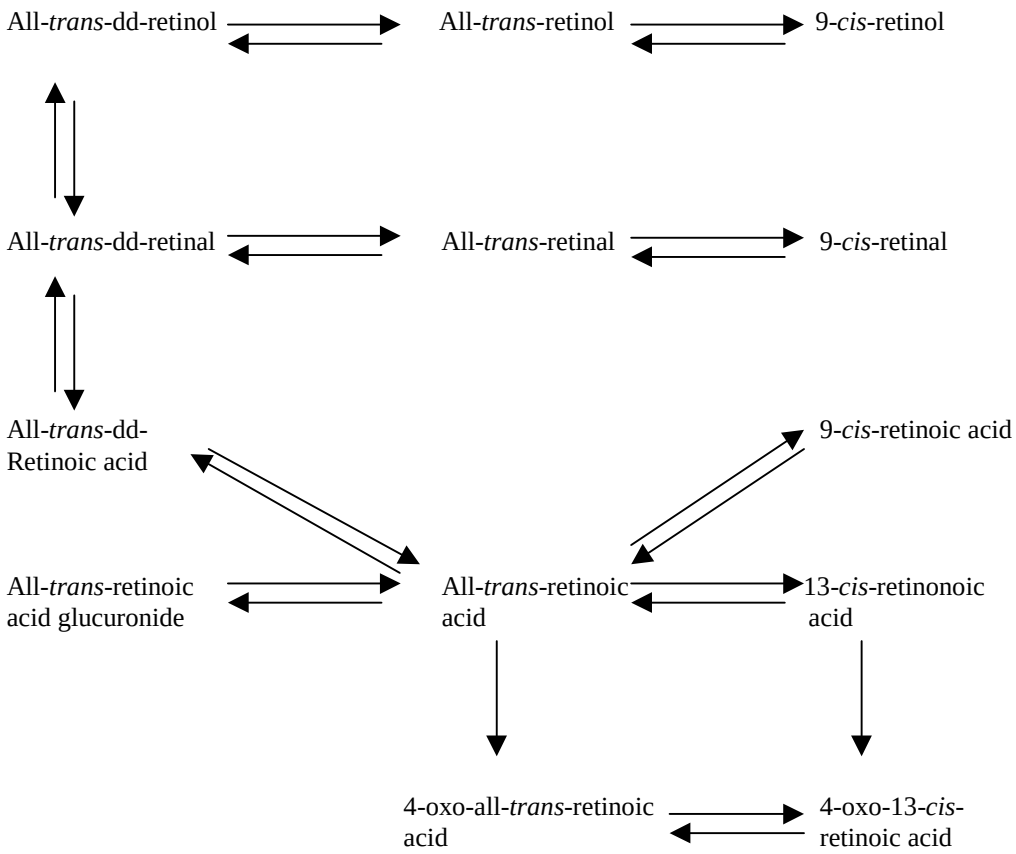
47. Retinol can undergo a series of enzyme catalysed oxidative biotransformations, interconversions and conjugations, most of which are reversible (Figure 5). The cellular binding proteins appear to facilitate and direct retinoid metabolism, allowing interaction of the retinoid substrate with enzymes by a direct protein-protein mechanism. Sequential oxidations of retinol, first to retinal, then to retinoic acid, are catalysed by alcohol (retinol) and aldehyde dehydrogenase enzymes, respectively. In addition, CYPs 1A1, 1A2, 2B4, 2C3 and 2J4 are also purported to be involved in the oxidation of various forms of retinaldehyde to

retinoic acid (Collins and Mao, 1999). Isomerisation of all-*trans* to 11- *cis* retinoids is mediated by a specific isomerase in retinal pigment epithelial cells and takes place at the level of retinol, not retinal. Less is known of how 9-*cis*-retinoid isomerisation occurs, but reaction is also thought likely to be at the retinol level. Both retinol and retinoic acid, by reaction with uridine diphosphoglucuronic acid, form retinyl β-glucuronide and retinoyl β-glucuronide, respectively. Retinol is esterified by transfer of fatty acid from the α-position of phosphotidyl choline to form retinyl esters, the major forms being palmitate and stearate. Retinoic acid can also be hydroxylated at several positions including C-4, C-16, and C-18 and converted to the 5,6-epoxide and cleaved at various points in its conjugated chain to form a variety of oxidation products. Hydroxy retinoic acid can be oxidised irreversibly to its 4-oxo derivative.

48. The catabolism (4-oxidation) of retinol and retinoic acid is CYP-mediated. In human liver, the isoform thought to be involved in this reaction is CYP2C8 (Leo *et al*, 1989). Other CYPs capable of catalysing the 4-hydroxylation of retinoic acid include CYP 1A2, 2A4, 2B1, 2B6, 2C3, 2C7, 2D6, 2E1, 2E2, 2G1, 3A4, 3A6, 26 (Collins ands Mao, 1999)

49. Biological activity is usually retained to a greater or lesser extent. For example, all-*trans*-and 13-*cis* isomers are readily interconvertible, with the 13-*cis* form generally being less biologically active than the all-*trans* and 9-*cis* forms. Within target cells, retinol is primarily oxidised to retinal. Retinal can undergo Schiff's base formation with the ε-amino group of lysine in proteins, the most functionally important interaction being that which occurs between 11-*cis*-retinal and opsin in the eye. A small proportion of retinal is irreversibly oxidised further to retinoic acid, which interacts with nuclear receptors and is important in control of gene expression and cell differentiation. Hydroxylations, epoxidations, dehydrations and oxidative carbon-carbon bond cleavage reactions generally result in complete inactivation of vitamin A, although the 4-oxo derivatives of retinoic acid have some biological activity in humans.

Figure 3: The metabolic pathway for vitamin A (adapted from Creech Kraft and Willhite, 1997)



Excretion

50. Most inactive oxidised products are excreted in the urine. Glucuronide conjugates, formed in the liver, undergo biliary excretion (Figure 2). Most biliary excreted metabolites are eliminated via the faeces, but a proportion are reabsorbed from the gut and transported back to the liver. This enterohepatic recirculation aids the conservation of the body's vitamin A reserves but may also contribute to its cumulative toxicity when taken in excess. In general, approximately 10-20% of ingested vitamin A remains unabsorbed, approximately 20% is excreted in the faeces via the bile, 17% is eliminated in urine, 3% is expired as CO_2, and 40-50% is retained by the body, mainly in the liver. (Basu and Dickerson, 1996 and reference therein; Olson, 1996 and references therein).

Homeostasis of vitamin A

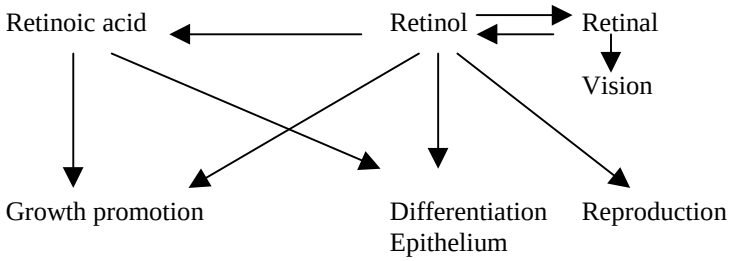
51. Except in times of severe deficiency or toxicity, plasma concentrations of vitamin A are tightly regulated. Liver reserves have to be depleted to <20 μg/g before plasma levels fall below 20 μg/100 ml. Only when storage concentrations of retinol are >300 μg/g is homeostatic control lost. The underlying mechanism of control is unclear but appears to be mediated by processes that also control the

rates of synthesis and/or secretion of hepatic RBP. When vitamin A status is low, levels of plasma RBP are reduced. However, this is not due to decreased synthesis since apoRBP accumulates within the liver. Administration of vitamin A, in order to alleviate deficiency, results in a decrease in hepatic apoRBP following rapid secretion of retinol-RBP (holo-RBP) into plasma (Basu and Dickerson, 1996 and reference there in; Olson, 1996 and references there in; Blomhoff *et al.*, 1991; DH, 1991). Consequently, dietary intakes of vitamin A of up to 7,500 µg RE/day have little or no effect on steady-state plasma retinol concentrations in normal vitamin A-sufficient individuals. In contrast, small increases in baseline plasma retinol (<10%) have been observed in groups who routinely use commercial vitamin A supplements of >1,500 µg RE/day. However, studies in subjects receiving oral supplements of >5,100 µg RE have shown that the most marked changes are in plasma concentrations of retinyl esters, all-*trans* and 13-*cis* retinoic acids and all-*trans* and 13-*cis* oxoretinoic acid (IARC, 1998 and references therein).

Function

52. Vitamin A is essential to the processes of vision, reproduction, embryonic development, growth and cellular differentiation (Figure 4). Requirements for vitamin A have also been implicated in the immune response, taste, hearing, and maintenance of appetite. Failure of cell division and differentiation can affect stem cells, and for example, result in impaired haematopoiesis. Lack of vitamin A results in keratinisation of mucus secreting ciliated epithelium and other epithelial changes. The tissues most affected by this include the trachea, skin, salivary gland, cornea and testes.

Figure 4: The functions of retinoic acid, retinol and retinal (adapted from Miller *et al.*, 1998)



53. Most of the above processes are directly or indirectly dependent upon cellular differentiation and control of gene expression, with the majority of effects of vitamin A explained by the existence of complex signal transduction pathways, retinoid receptors, binding-proteins with bioactive vitamin A metabolites serving as physiological ligands. There is also a hypothesis that vitamin A is involved as a cofactor in the biosynthesis of cell surface glycoproteins that act as antigenic determinants, viral receptors, and markers of cellular identity (Gerster, 1997 and references therein; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

Control of gene expression, cell differentiation, growth and development

54. Retinoic acid is now recognised as an important signalling molecule that, as a ligand to its nuclear receptors, alters gene expression at the level of transcription. Two sets of retinoic acid nuclear receptors have been identified, known as RAR and RXR, each having α, β and γ subgroups. The RARs and RXRs belong to a family of hormonal regulatory proteins, which include those for steroids, thyroid hormones and vitamin D. Each receptor consists of six domains: an amino-terminal activation domain, a DNA-binding domain, a hinge region, a ligand-binding domain, and a carboxy-terminal involved in heterodimerisation. The RAR receptors bind either all *trans*- or 9-*cis*-retinoic acid, whereas the RXR receptors bind only 9-*cis*-retinoic acid. RXR receptors form dimers with themselves as well as with RAR, the vitamin D receptor, the T3 receptor, PXR, PPAR, βCAR etc. Binding of these dimers to specific response elements usually results in increased gene transcription, although inhibitory interactions do occur (Gerster, 1997 and references therein; Evans, 1996; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

Embryonic development

55. Retinol and retinoic acid are essential for morphogenesis in embryonic development and may be involved in control of *Hox* gene expression, vital for correct sequential development (Marshall *et al.*, 1996).

The visual cycle (non-genomic mechanism)

Vitamin A is required for vision in the dark and for colour perception. The active form of vitamin A in this function is retinal. In the retinal pigment epithelial cells, all-*trans*-retinol undergoes enzymatic isomerisation to 11-*cis*-retinol. 11-*cis*-retinol associates with IRBP and is transported to the rod outer segment to be oxidised to 11-*cis*-retinal by retinol (alcohol) dehydrogenase. The 11-*cis*-retinal forms a Schiff's base with a specific lysyl residue in the membrane bound protein, opsin. The resultant rhodopsin, when exposed to light, isomerises to form a transoid intermediate. Thereafter follow several more protein conformational changes. The intermediate meta-rhodopsin II, interacts with a G protein, transducin, and activates phospodiesterase resulting in the hydrolysis of GTP to GMP via the formation of cGMP. cGMP maintains the opening of sodium channels in the rod outer segment. As the level of cGMP falls, sodium entry decreases and the rod cell- membrane hyperpolarises. Changes in membrane potential are transmitted to and integrated by the brain. Return to the basal state occurs by the reconversion of meta-rhodopsin II to opsin and all-*trans*-retinal. All-*trans*-retinal is then reduced to the corresponding isomer of retinol, and consequently isomerised back to 11-*cis*-retinol.

56. During this cycle, not all vitamin A is conserved and must be replaced from the circulation. A similar sequence of events is involved in the process of colour sensing in cone cells (Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

Deficiency

57. Vitamin A deficiency poses a major health problem in the developing world, with pre-school age children and lactating women being the most widely affected groups. Common signs of clinical vitamin A deficiency, associated with serum levels <0.35 mmol/l, include night blindness, xerophthalmia (dryness of the eye), and Bitots spots. Damage to the eye can be permanent. Deficiency can also lead to growth retardation, weight loss, diarrhoea, thickening of bone shafts, impaired hearing, taste and smell, wasting of testicles, reduced sperm count, improper tooth formation and gum disease. Prolonged deprivation can result in death. Vitamin A inadequacy, in the absence of signs of acute deficiency, is associated with increased child mortality rate and higher incidence of severe infections. Inadequate vitamin A status is often a consequence of febrile disease, protein-calorie malnutrition, and low-fat intake or lipid malabsorption. Mild deficiency can lead to patchy keratinisation of the epithelial lining of the gastrointestinal, respiratory, and urogenital tracts and also skin. Changes in gut morphology can lead to loss of appetite. It is suggested that alterations to the epithelial barrier may be responsible for increased susceptibility to infection (Anonymous, 1998 and references therein; Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

58. Maternal insufficiency of vitamin A during pregnancy can result in a spectrum of malformations in the offspring, including ocular defects such as anophthalmia or microphthalmia, and defects of the lung, cardiovascular system and urogenital system. Extreme deficiency results as well in forelimb abnormalities and cleft face, but the embryos are not viable (Moriss-Kay and Sokolova, 1996).

59. Vitamin A deficiency is rare in the developed world and is usually limited to those who have absorption difficulties, liver disease or those who consume a lot of alcohol. Vitamin A deficiency is common in alcoholics and may contribute to skin problems, cirrhosis of the liver and susceptibility to infections. Episodes of opportunistic infections in HIV infected individuals may also precipitate vitamin A depletion.

Overview of reported beneficial effects

Prevention and treatment of deficiency.

60. Large-scale vitamin A supplementation has resulted in decreased rates of childhood morbidity and mortality from infectious diseases such as diarrhoea, measles, and respiratory disease, and non-infectious diseases such as anaemia, particularly in under-developed countries. These beneficial effects are probably entirely due to correction of deficiency. Typically, doses of 30,000-60,000 μg RE (dependent on age) are administered intermittently (Sommer, 1997).

Skin disorders

61. Vitamin A can be beneficial in the treatment of skin disorders including acne, psoriasis and ichthyosis. Severe cases of acne, which do not respond to antibiotics or other treatments, may respond to high doses of synthetic retinoids such as tretinoin, isotretinoin and etretinate. In the treatment of psoriasis, vitamin A-derivative drugs act by reducing the increased growth, turnover and keratinisation of skin cells associated with the disease.

Treatment of cancer

62. There have been reports that vitamin A may be beneficial when used as a chemo-preventative or adjuvant agent in the treatment of some cancers. For example, 90,000 μg RE/day decreased reoccurrence of tumours in a group of lung cancer patients (Pastorino *et al.*, 1993), 7,500 μg RE/day reduced the rate of mesothelioma incidence in a group of former asbestos workers (Musk *et al.*, 1998; de Klerk *et al.*, 1998) and 90,000 – 270,000 μg RE/day for 5 weeks resulted in remission or partial remission in 20/20 patients treated for leukoplakia, a precancerous change in mucous membranes, often related to smoking (Issing *et al.*, 1997). However, the Committee on Medical Aspects of Food and Nutrition Policy found that there was insufficient evidence to conclude that vitamin A protects against the development of various cancers.

Others

63. There has been a report that lung function was improved in chronic obstructive pulmonary disease patients supplemented with 1,000 μg RE vitamin A per day (Paiva *et al.*, 1996). Claims have been made that vitamin A may be useful in the treatment of hypogonadism in alcoholics. However, Worner *et al*.. (1988) reported no improvement in sexual function and possible hepatotoxicity, following 3,000 μg RE/day for 4 months. Vitamin A is used as a treatment for abnormal dark-adaptation and eye drops containing vitamin A have been used in the treatment of blurred vision, cataracts, glaucoma, conjunctivitis and dry eyes. Other disorders where vitamin A has been tried as treatment include asthma, sebaceous cyst, fibrocystic breast disease and pre-menstrual syndrome. It has been suggested that high intakes of vitamin A, from either supplements or food, may be helpful in the prevention of ulcers (Anonymous, 1998 and references therein). It has also been suggested that vitamin A should be used as an adjunct treatment (with zinc) to improve the effectiveness of iron in the treatment of anaemia (see Kolsteren *et al.*, 1999; Bloem, 1995; Suharno *et al.*, 1993). In addition, improved vitamin A status may protect against the harmful effect of iron supplementation in environments where infections are highly prevalent and pathogenic bacteria compete effectively for iron in the circulation (Ribaya-Mercado, 1997). Although vitamin A is commonly regarded to be beneficial to the immune systems of children, it has been less extensively studied in adults. A randomised controlled trial of supplementation with zinc (25 mg), vitamin A (800 μg) or both for 3 months in a home for the elderly in Rome found no beneficial effect of vitamin A alone on various markers of immune function and numbers of CD3+ and CD4+ T-cells were significantly reduced (Fortes *et al*., 1998).

Oral toxicity in humans

Acute Toxicity

64. Acute adverse effects from dietary sources of vitamin A are rare. Exceptional cases concerned Arctic explorers, who ingested large quantities of the vitamin by eating polar bear liver which contains approximately 6,000 μg (RE) of vitamin per g. Symptoms included nausea, vomiting, headache and vertigo (Description by arctic explorer Kane in Knudson and Rothman, 1953).

65. Acute hypervitaminosis A, as a result of accidental ingestion of high potency vitamin A preparations, is a more common occurrence (Basu and Dickerson, 1996 and references therein) and is associated with ingestion of doses greatly in excess of 100 times and 20 times the Recommended Daily Allowance or Reference Daily Intake, in adults and children, respectively (Olson, 1996 and references therein). Characteristic symptoms in adults include abdominal pain, anorexia, vomiting, blurring of vision, headache, vertigo, drowsiness, hypercalcemia, irritability, muscle weakness, peripheral neuritis and skin desquamation. In infants and young children, symptoms include anorexia, fontanelle bulging, drowsiness, increased intracranial pressure, irritability and vomiting. Symptoms are usually transient and disappear within a few days. However, there have been at least two fatalities reported; one concerning a neonate who ingested >60 times the suggested daily vitamin A dose for 11 days (Bush *et al.*, 1984) and the second, in a 56 year old man through self-medication, (up to and > 90,000 μg retinol/day with an estimated total intake of approximately 60,000,000 μg over the final 3 months of his life) (Leitner *et al.*, 1975).

66. In a placebo-controlled trial, where neonates (< 1 month) were treated for vitamin A deficiency, a single oral dose of 15,000 μg RE was well-tolerated (West *et al.*, 1992). In a similar study, a single 15,000 μg RE dose resulted in a small proportion of infants showing increased intracranial volumes, but without increases in intracranial pressure or incidence of bulging fontanelle (Agoestina *et al.*, 1994). Placebo-controlled studies reported by De Francisco *et al.* (1993) and Baqui *et al.* (1995, 1996) showed increased episodes of bulging fontanelle in infants following administration of 15,000 μg RE at 6,10, 14 weeks of age and 7,500 μg RE at approximately 6, 12 and 17 weeks of age, respectively, in conjunction with immunisation. Both studies showed a tendency towards a cumulative dose effect. In contrast, older children would appear to be more tolerant. Stabell *et al.* (1995) did not observe any increased incidence in bulging fontanelle in infants administered 30,000 μg RE at both 6 months and 9 months.

67. In a double blind, randomised, placebo controlled clinical trial, children (3 months-10 years) hospitalised with pneumonia and given vitamin A supplements (15,000-60,000 μg RE, dependent upon age) on two consecutive days following admission, had lower blood oxygen saturation, higher prevalence rates for retraction, and were more likely to require supplemental oxygen, than placebo controls (Stephensen *et al.*, 1998). However, a similar study showed no effect of vitamin A supplementation (60,000-120,000 μg RE, on 2 consecutive days, in

children 6-59 months) on the immediate outcome of a pneumonia episode (Nacul *et al.*, 1997).

Chronic toxicity

68. Worldwide incidence of chronic hypervitaminosis A is becoming more frequent. The reasons being (i) the increasing use of vitamin A supplements in children of developing countries where vitamin A deficiency is commonplace (ii) the increased use of vitamin A in the treatment of skin disorders such as acne and (iii) the increased number of people taking excessive doses of vitamin A for supposed beneficial health effects.

69. Over 500 individual cases of hypervitaminosis A have been reported over the last 40 years (Basu and Dickerson, 1996). These included a spate of cases in the 1950s, possibly relating to two potent pharmaceutical preparations, administered mainly to infants, containing 105,000-120,000 μg (RE) /dose. In the 1970s, a further peak of case reports occurred, possibly relating to increased use of vitamin A for skin disorders around this time.

70. Symptoms of chronic vitamin A toxicity include dry thickening skin, itching, fissure and peeling, cracking of lips, brittle nails, conjunctivitis, erythmatous eruptions, alopecia, anorexia, bone and joint pain, reduced bone mineral density, exacerbation of soft tissue rheumatism, muscular stiffness, hepatomegaly, hepatotoxicity, liver, spleen and lymph gland enlargement, polydipsis, polyuria, chronic headache and nausea, intracranial hypertension/pseudotumor cerebri (Hathcock *et al.*, 1990; Romano, 1995; Basu and Dickerson, 1996; Olson, 1996; Friedland and Burde, 1996; Wettstein and O'Neill, 1998; Melhus *et al.*, 1998). Laboratory findings may include elevated serum enzymes indicative of hepatotoxicity, prolonged prothrombin time (PT), hypercalcaemia, hypertriacylglycerolemia, elevated erythrocyte sedimentation and periosteal calcification on x-ray (Sibulesky *et al.*, 1999; HSDB, 1999). Most symptoms are rapidly reversible on cessation of treatment, although damage to eyes, bone, liver may be permanent (Basu and Dickerson, 1996 and references therein; Olson, 1996 and references therein).

71. Chronic hypervitaminosis A has been generally attributed to total daily intakes of >7,500-15,000 μg RE (>10 times RNI) over periods of weeks, months or years, the development of toxic manifestations being both dose and duration dependent. Most evidence for this comes from reported individual cases. However, one randomised double blind controlled trial (Wald *et al.*, 1985) revealed minor symptomatic and physical changes affecting the skin and mucous membranes in individuals receiving supplements of 3,000 μg RE/day for 6 months. Infants and children, on the whole, seem to be less tolerant than adults.

72. Reports of adverse effects associated with lower doses of vitamin A supplements (summarised in Annex 1) have not generally taken into account the vitamin A contribution made by the diet. Dietary intake is likely to be an important determinant of the tolerance to excess vitamin, since it will influence the degree of saturation of hepatic storage capacity. Where the information was available,

Hathcock *et al.* (1990) gave estimations of total intake of preformed vitamin A from both diet and supplements. In some cases, the dietary contribution was considerable (see table 2, below). It is possible, therefore, that adverse effects observed at the lower end of the toxic dose range may be a reflection of high dietary vitamin A. It would seem, however, that confounding factors, relating to diet, pre-existing disease, and exposure to drugs or alcohol abuse, may also be significant.

Table 2. Cases of chronic, low-dose vitamin A toxicity in adults in which some data on dietary intake is also available (Hathcock *et al.*, 1990):

Intake per day	**Age, sex**	**Duration**	**Symptoms**	**Other conditions**
5,000 + diet = 40-50,000	29, male	Unknown	Fatigue	Bilirubinaemia, anorexia
25,000 + diet = 50-75,000	42, male	10 years	Headache, desquamation, hypercalcaemia	Viral hepatitis
20,000 + diet = 50,000	62, male	7 years	Pedal oedema	Wasting, PEM

73. Signs of vitamin A toxicity have been noted in children presumed to have ingested much lower total amounts of vitamin A (Schurr *et al.*, 1983; Carpenter *et al.*, 1987). It has been suggested that this extreme vitamin A intolerance may have a genetic component (Carpenter *et al.*, 1987). However, the precise metabolic defect awaits elucidation.

74. Several population studies have examined effects of long-term vitamin A supplementation:

Population studies

(i) 284 women (aged 40-70), were classified into users (n=24) and non-users (n=260) of vitamin A supplements, and followed for 2 years. Dietary intake of vitamin A was similar in both groups. Supplemental intake, in the user group, was 925±8 μg RE/day (range 250-5,000). Serum retinol, retinyl esters and RBP concentrations neither correlated with vitamin A intake in either group nor differed between the users and non-users, over the 2 year period. However, concentrations significantly increased over time in both groups ($p<0.03$). Levels of serum alkaline phosphatase, aspartate aminotransferase and bile acids were neither related to concentrations of serum retinol, retinyl esters and RBP nor did they differ between users and non-users (Johnson *et al*, 1992).

(ii) Sibulesky *et al.* (1999) assessed the safety of long-term vitamin A supplementation in adults (18-54 years) suffering from retinitis pigmentosa. 146 otherwise healthy patients were treated with doses of 4,500 μg RE (15,000 IU)/day, for up to 12 years. A control group (n=149) received a trace supplement of 23 μg RE/day. Mean total vitamin A consumptions were 5,583 and 1,053 μg RE/day in the test and control groups, respectively. The test group showed an 8% increase in mean serum retinol at 5 years and 18% at 12

years ($p<0.001$). No plasma retinol concentrations exceeded the upper normal limit of 3.48 μM. Mean serum retinyl esters were increased approximately 1.7-fold at 5 years but thereafter remained stable. There were no clinical signs of liver toxicity attributable to vitamin A excess, although serum triacylglycerols tended to increase.

(iii) In a median 28 month follow-up to a clinical trial designed to evaluate the efficacy of high-dose vitamin A as an adjuvant treatment for resected stage I lung cancer, 283 patients were evaluated. Of these, 138 patients had received vitamin A supplements of 90,000 μg RE per day, in the form of retinol palmitate aqueous emulsion, for at least 12 months. The remaining 145 served as observational controls. Vitamin A-related symptoms of skin dryness and desquamation were observed in 60% of treated patients. Less than 10% reported other symptoms, such as dyspepsia, headache, nosebleeds and mild hair loss, and which were self terminating. Four patients had treatment interrupted due to viral hepatitis in one, vitamin A-related exacerbation of pre-existing atopic dermatitis in two, and haemorrhagic proctis in the fourth. At 24 month, serum gamma-GT levels were abnormally elevated in 69% of vitamin A-treated patients vs. 39% of controls (mean 149 vs. 58 IU/l $p<0.05$) and serum triglycerides were raised above 150 mg/dl in 74% of treated patients vs. 43% of controls (mean 283 vs. 179 mg/dl, $p<0.05$) (Pastorino et al, 1991).

(iv) Krasinski *et al.* (1989) showed that fasting plasma retinyl ester levels in a group of elderly vitamin A supplement users (60-98 years, n=31, supplements >1,500 μg RE/day) were significantly increased ($p<0.05$) compared to a similarly aged group (n=81) of non-users. Greater plasma retinyl ester levels were associated with long-term supplement use (> 5 years). Elevated serum aspartate aminotransferase activities were more prevalent in those subjects with plasma retinyl esters ≥380 nmol/l ($p<0.05$). However, it is not possible to determine from this study whether hepatotoxicity could be attributed to vitamin A supplementation.

(v) Stauber *et al.* (1991) found a dose-response relationship between supplemental vitamin A intake and plasma retinyl ester concentration in a 5 year study of 116 healthy elderly people (64-88 years, supplemental intake range 0-14,100 μg RE/day, dietary intake range approximately 750 - 7,000 μg RE/day). This relationship did not increase with length of supplementation. There was no evidence of abnormal liver function, although a significant correlation was found between aspartate aminotransferase levels and retinyl ester levels in women. There were also significant associations between retinyl ester levels and triglycerides, cholesterol, and high-density-lipoprotein; parameters that may be associated with vitamin A transport.

Association between vitamin A intake and reduced bone mineral density

75. It is claimed that hypervitaminosis A causes skeletal toxicity. As noted in paragraphs 67-72 symptoms of vitamin A toxicity include bone pain, reduced bone mineral density, and hypercalemia. Hypercalemia is associated with suppressed

serum parathyroid hormone (PTH) concentrations which Binkley and Krueger (2000) claimed suggests direct stimulation of bone resorption i.e. osteoclastic activity, by vitamin A. A number of epidemiological studies have evaluated the potential association between vitamin A intake and bone mass/fracture risk.

76. Sowers and Wallace (1990) evaluated vitamin A intake, serum retinol concentrations, radial bone mass and fracture history in 246 postmenopausal women from a single community in Iowa. More than 36 % of the population used a vitamin A supplement, with 8% using a supplement containg >2000 μg RE/day. No relationship was observed between radial bone mass and fracture history and vitamin A intake or serum retinol. However, Binkley and Krueger (2000) noted that this study had inadequate power to test an association between bone mass and vitamin A intakes >2000 μg RE/day (since only 8% of the study population had intakes exceeding this level); 36% of the population were aged < 60 years of age and were therefore likely to be hetergenous with regards to oestrogen depletion bone loss; and the site where bone mass was measured, i.e., central radius, is considered less responsive to change.

77. In a 4 year clinical trial, the effect of usual energy and intake of 14 nutrients on single-photon absorptiometric measurement of mineral content in arm bones was assessed in 99 women aged 35-60 given either a calcium supplement or placebo (Freudenheim *et al.*, 1986). In the post-menopausal women of the treatment group, there was an inverse correlation between vitamin A intake and the rate of change in ulna bone mineral content. In a single, highly supplemented patient (average intake 14, 624 IU/day, equivalent to 4392 μg RE) bone loss was very rapid for no other apparent reason.

78. The relationships between total energy intake, nutrient intake, body composition and exercise group status with rates of change in bone mineral density was measured in 66 premenopausal women taking calcium supplements (Houtkooper *et al*., 1995). Nutrients were not significant variables in regression models predicting bone mineral density slopes at any femur site. Significant variables in predicting total body bone mineral density slopes included retinol.

79. A cross-sectional study, involving 175 randomly selected females (28-74 years) and a nested control study, involving 247 women (40-60 years), who had a first hip fracture 2-64 months after enrolment, and 873 age-matched controls (selected from a mammography study cohort) was reported by Melhus *et al.*, (1998). Retinol intake was estimated from dietary records and a food-frequency questionnaire. In multivariate analysis, intake of preformed retinol was negatively associated with bone mineral density. For every 1 mg increase in daily intake, risk of hip fracture increased by 68% (95% CI 18-140%, P=0.006). For intake greater than 1.5 mg /day, compared with less than 0.5 mg /day, bone mineral density was reduced by 10% at the femoral neck (P=0.05), 14% at the lumber spine (P=0.001) and 6% for the total body (P=0.009). The risk for hip fracture was apparently doubled (Odds ratio 2.1 (CI 1.1-4.0). However no P value was given for this. Binkley and Krueger (2000) noted that concordant with this effect, epidemiological studies have reported that a 10% reduction in bone mass is associated with a 2 fold increased risk of fracture. Smoking was found to be a confounding factor. The authors

suggested that their study was limited by the possibility of information bias resulting from questioning case-patients after hip fracture had occurred, as was done for some covariates such as physical activity. In addition, data on thyroid hormone therapy and family history of osteoporosis were not available. Furthermore, the authors could not rule out confounding influences of an unidentified dietary factor but suggested that the possibility of a high degree of random error in the assessment of retinol intake would lead to an underestimation of the true risk of hip-fracture associated with high levels. However, how a random error would necessarily result in an underestimation is unclear.

80. The rationale for the Melhus study was based on the observation that in Europe, hip fracture rates varied 11 and 7 fold for women and men respectively with the highest rates occurring in Northern Europe particularly in Sweden and Norway (Johnell *et al*., 1992; Melton, 1995). Fracture rates were higher in Scandavia than in comparable populations in North America (Melton, 1995). The difference in European rates was sufficiently marked that fractures rates were higher in Swedish men than in Swiss or English women (Johnell *et al*., 1992). The authors further noted that the difference in incidence was higher between countries than between sexes suggesting that an important genetic or environmental factor was involved. Known risk factors were not thought to explain the finding. When dietary patterns in Europe were compared (Cruz *et al*., 1991) retinol intakes were found to be 6 fold higher in Scandanavia, compared to Southern Europe. It was further noted that animal data and enecdotal reports supported the view that retinol affected bone strength.

81. It was recently concluded at a consensus meeting of expert dermatologists that acne treatment with retinoid isotretinoin has no effect on bone growth in teenagers (Ortonne, 1997). Binkley and Krueger (2000) however questioned this conclusion, citing reports that up to 15% of patients treated with isotretinoin report symptoms of arthralgia and myalgia. They noted that existing studies on any potential skeletal toxicity are conflicting and are to small to draw any definite conclusions regarding the effects of systemic retinoid therapy on bone mass.

82. As part of the Nurses' Health Study, the relationship between high vitamin A intake from food and supplements and the risk of hip fracture was assessed (Feskanich *et al*., 2002). The study included 72337 post-menopausal women aged 34-77 and the period 1980 to 1998 was included in the analysis. During this period, 603 incident hip fractures occurred resulting from low or moderate trauma. After controlling for confounding factors, women in the highest quintile of vitamin A intake (≥ 3000 μg/day μg RE) had a significantly elevated relative risk (RR) for hip fractures (RR, 1.48; 95% CI 1.05 to 2.07; P for trend 0.03) compared to women in the lowest quintile of intake (<1250 μg/day RE). The increased risk was primarily attributable to retinol (RR, 1.89; 95% CI, 1.33-2.68; P for trend <0.001 comparing ≥ 2000 μg/day to < 500 μg/day. The association of retinol with hip fracture was reduced in women taking postmenopausal oestrogens. β-Carotene did not contribute to fracture risk. Women currently taking a specific vitamin A supplement had a non-significant increased risk of hip fracture (RR, 1.4; 95% CI 0.99-1.99) compared to those not taking the supplement. Among women not taking supplements, retinol from food was significantly associated with fracture

risk (RR, 1.69, 95% CI 1.05-2.74; P for trend= 0.05 comparing ≥ 1000 μg/day to < 400 μg/day. Overall the authors concluded that long term intake of diet high in retinol may promote the development of osteoporotic hip fractures in women and that the amount of retinol in supplements and fortified foods may need to be reassessed. Information from the food frequency questionaire indicated that multivitamins were the primary contributor to total retinol, with liver being the primary food source. However, the contribution from liver declined between 1980 and 1994 while the percentages from milk and breakfast cereals increased. Multivitamins were used by 34% of the cohort in 1980 increasing to 53% by 1994. Over the same period, use of vitamin A supplements remained between 3 and 5%. The authors noted that *in vitro* data suggest that vitamin A may have a direct effect on osteoblasts and osteoclasts, reducing bone formation and increasing bone resorption and suggested that if this occurred *in vivo*, long term exposure to high levels of viamin A could result in reduced bone density. The antagonistic effect of vitamin A on vitamin D was also noted. The authors further noted that the study cohort was largely white women and that the findings were not necessarily applicable to other ethnic/racial groups.

83. Relevant animal data is summarised in paragraph 125. On the body of human and animal evidence Binkley and Krueger (2000) concluded that excessive vitamin A increases bone resorption and decreases bone formation. They further hypothesised that 'subclinical' hypervitaminosis A may contribute to the development of osteoporosis though they were unable to identify a safe level of intake, recommending that studies of skeletal turnover and bone mass in animal models and humans are required before such a level can be set.

Association between Vitamin A intake and certain forms of cancer

84. Epidemiological studies have generally indicated an inverse correlation between the development of cancer and consumption of β-carotene-rich foods. Most observational studies have shown no evidence to suggest that vitamin A in the diet leads to an increase in overall cancer incidence (IARC, 1998). However, some studies have shown positive associations between high retinol intake and certain types of cancer (see paragraphs 87-91 and reviews by Hong and Itri, 1994 and IARC, 1998), although the data are not consistent. Furthermore, vitamin A was not necessarily the only or primary risk factor identified and it is difficult to determine whether any association between vitamin A and cancer risk is causal rather than spurious as many food groups that are rich sources of vitamin A are themselves also associated with increased risk of cancer at the same sites. IARC (1998) have concluded that there is no convincing evidence to suggest that retinol is carcinogenic.

85. Findings from several case-control and prospective studies, regarding the association between total and/or preformed vitamin A intake and prostate cancer, are inconsistent.Three studies have found significant positive associations in elderly men (Graham *et al.*, 1983; Kolonel *et al.*, 1988 and Giovannucci *et al.*, 1995). In contrast, Heshmat *et al.* (1985) and Hsing *et al.* (1990) observed trends towards elevated risk (P<0.05) only in younger men. Paganini-Hill (1987) showed an overall elevation of risk in the highest tertiles for total vitamin A and, in

particular, supplemental vitamin A intake. However, other studies have shown no association (Ross *et al.*, 1987) or have shown a positive trend that is not significant since the 95% CI ranges include 1 (West *et al.*, 1991; Andersson *et al.*, 1996). Ohno *et al.* (1988) showed a negative association between vitamin A intake and prostate cancer risk; this was significant for older men (70-79 years).

86. Studies by DeCarli *et al* (1987), Tuyns *et al* (1987) and Graham *et al* (1990a) revealed that diets rich in retinol were associated with an increased risk of cancer of the oesophagus (heavy consumers, intake not specified, RR=2.27, CI=1.13-4.52, P=0.02; intake >45,000 μg RE/month (>1,600 μg RE/day), RR=2.06, CI=1.47-2.99; intake >20,000 μg RE/month (>700 μg RE/day), RR=2.4, CI=1.28-4.5, P=0.0003, each study respectively). However, in these studies, risk of oesophageal cancer was far more strongly associated with cigarette smoking and alcohol consumption. In another study (Graham *et al.*, 1990b), retinol intakes >19,000-22,400 μg RE/month (>700-800 RE/day) were associated with increased risk of gastric cancer (OR 3.04 [CI 1.66, 5.56] P<0.01 males, OR 2.08 [CI 1.05, 4.14] P<0.05 females).

87. In a case-control study, D'Avanzo *et al.* (1997) found that retinol intake had a direct association with thyroid carcinoma risk with an odds ratio of 1.52 (CI=1.0-2.3, P<0.05) in the highest quartile for consumption (>1802 μg RE/day). To date, no other studies showing positive associations between retinol intake and thyroid cancer risk have been found.

88. Combined analysis of twelve case-control studies (Howe *et al.*, 1990), found little evidence for any association between intake of retinol and risk of breast cancer. Eight case-control and three prospective studies, discussed by Willett and Hunter (1994), found either no significant association or a slight inverse association (RR<1) between preformed vitamin intake and breast cancer. These are summarised in table 3 below. In another prospective study (Hunter *et al.*, 1993) among women in the highest quintile intake for total preformed vitamin A (≥ 2238 μg RE/day), the relative risk for breast cancer was 0.80 (CI 0.68 - 0.95, P=0.003), compared to the lowest quintile. Furthermore, women with the lowest intakes of total vitamin A from dietary sources but with the highest intakes from supplements, had a risk of breast cancer that was half that in the lowest quintile that did not take supplements (P=0.03). Verhoeven *et al.* (1997) found a weakly positive association between retinol intake and breast cancer in a cohort study involving 62,573 women (RR/lowest quintile = 1.24, CI 0.83-1.83), but this was not statistically significant. Bertone et al (2001) reported that the results of a population based case control study indicated that intakes of vitamin A (both dietary and supplemental) were unrelated to the risk of ovarian cancer.

Table 3. Studies of vitamin A intake and breast cancer (adapted from Willett and Hunter, 1994):

Study design	**Study population**	**N**	**Relative risks - high vs low intake categories**		
			Total vitamin A	**Preformed vitamin A**	**Carotenoid vitamin A**
Case-control	New York State	2024	0.8		

	Italy	1108		0.9	0.8
	Greece	120	0.5	0.6	0.6
	Italy	214		0.7	1.2
	Australia	451		1.2	0.8
	Italy	250		1.2	1.0
	Denmark	1474			1.2
	New York State	83	0.7		0.8
	Holland	133			0.6
	Australia	99		1.0	0.8
	Singapore	109			0.3
	Russia	81	0.2	0.5	0.2
	New York State	439			0.6
	Boston	313		0.7	0.6
Prospective	California	123	0.8		0.8
	United States	1439	0.8	0.8	0.9
	New York State	359	1.0	0.9	0.9
	Canada	519	0.8	0.8	0.8

89. Data on the association between preformed vitamin A intake and colon cancer are more sparse and inconsistent but have shown no significant association.

Supplementation trials

90. A lung cancer prevention trial (β-**Ca**rotene **R**etinol **E**fficacy **T**rial – CARET) was halted when interim results indicated that combined supplementation with retinol (7,500 μg RE/day) and β-carotene (30 mg/day) gave no benefit and may have caused some harm, particularly in male smokers, former smokers and men exposed to asbestos. The results showed 28% more lung cancers and 17% more deaths in participants taking retinol and β-carotene (Omenn, 1998). However, other studies (ATBC, 1994; de Klerk *et al.*, 1998) would seem to implicate β-carotene, rather than retinol, as the culpable agent, if any.

91. In a chemo-prevention study, former blue asbestos workers were randomly assigned to take either 7,500 μg RE retinol/day or 30 mg/day β-carotene. Subjects (512 per group) were followed up through to death (up to 260 weeks). Four cases of lung cancer and 3 cases of mesothelioma were observed in the retinol group, compared to 6 and 12 cases, respectively, in the β-carotene group. The relative rate of mesothelioma for those given retinol, compared to those receiving β-carotene, was 0.24 (CI 0.07-0.86). Furthermore, rates of death from all other causes were significantly lower in the retinol group, with the exception of the death rate from ischaemic heart disease, which was relatively higher in this group. However, this was not significant [1.72 (CI 0.50-5.86)]. Forty-five subjects taking retinol switched treatments due to abnormal liver function tests and one person taking retinol suffered from headache, attributed to benign intracranial hypertension. It not possible to say whether the results of this study reflect a retinol-associated chemoprevention effect or whether β-carotene was harmful, since no placebo group was included in the trial. However, the authors claim that the incidence of mesothelioma in the retinol group was lower than would be otherwise expected (de Klerk *et al.*, 1998).

92. In a study in the US, 525 adults with a history of basal call carcinoma or squamous cell carcinoma of the skin were randomly assigned to receive 7,500 μg RE retinol per day, placebo or isotretinoin, orally for 3 years. New skin cancers were more common in the retinol treated group than in those receiving placebo but the difference was not significant (Levine *et al.*, 1997).

93. The EUROSCAN (European study on chemoprevention with vitamin A and N-acetylcysteine) randomised clinical trial, involving a total of 2592 patients, administered 300,000 IU/day (90,000 μg RE/day) of retinyl palmitate for 1 year followed by 150,000 IU/day for the 2nd year to patients with either head and neck cancer or lung cancer (patients were also administered 600 mg acetylcysteine with or without retinyl palmitate). None of the supplementation regimes had any significant effect on survival, event-free survival or time to secondary primary tumour (median follow-up of 49 months). Side effects were reported by 45% of the patients receiving retinyl palmitate with or without acetylcysteine; typical side effects were mucocutaneous, e.g. dryness, itching, bleeding, and hair loss).

Developmental toxicity (teratogenicity)

94. The retinoid, isotretinoin (Roaccutane; 13-*cis*-retinoic acid prescribed for certain skin disorders, such as cystic acne) was confirmed as a teratogen in 1985, although there had been reports of treatment-associated embryopathy two years earlier. By 1993, there were 94 cases confirmed in the US. Characteristic features included severe motor deficit malformations of the heart, thymus, face, jaw, ears, palate and brain. The maternal oral dose associated with an elevated risk of isotretinoin-related malformations (approximately 25% among fetuses surviving 20 weeks of gestation) ranges between 0.5 and 1.5 mg/kg/day. A 3-day exposure, during the first month of human gestation, is sufficient to induce embryopathy. Most malformations of the head and neck, induced by retinoids, originate during migration of the neural crest and formation and development of the branchial apparatus (pharyngeal arches), which occur throughout weeks 2-5 following conception. By 1984, the aromatic retinoid, etretinate was also recognised as a human teratogen (Creech Kraft and Willhite, 1997 and references therein).

95. Isomerisation of 13-*cis*-retinoic acid (tretinoin, Retin-A) results in the formation of all-*trans*-retinoic acid, which is thought to be the likely ultimate retinoid teratogen. Since all-*trans*-retinoic acid is also a metabolite of retinol, there is concern that high intake of preformed vitamin A in the diet and/or through dietary supplements during pregnancy may carry a risk of fetal malformation.

96. The issue as to whether retinol, in sufficiently large doses, is teratogenic in humans has proved difficult to study. Human data available for the risk assessment of vitamin A-associated congenital abnormalities consist of case reports, clinical trials involving high vitamin A exposure, retrospective case-control studies and data collected prospectively from clinical trials with prenatal diagnosis. More recently, there has been a preliminary report of some comparative pharmacokinetic data in an effort to aid extrapolation of animal data for human risk assessment.

Case reports

97. Published anecdotal case-reports of congenital malformations occurring in association with maternal vitamin A consumption are shown in table 4, below (Miller *et al.*, 1998). Many of the defects reported bear similar characteristics to those reported for isotretinoin, although no single case has reported the same constellation of effects. Miller *et al* pointed out that the experience with isotretinoin may have prompted the reporting of some cases which may have otherwise not been reported. Details of exposures to other vitamins were not included in every case.

Table 4. Human case reports of congenital malformations associated with exposure to vitamin A during pregnancy (adapted from Miller *et al.*, 1998):

Organ system affected			
Face/head	**Cardiac**	**Genito-urinary**	**Other**
Tiny ear canals, "dysmorphic face", high palate	Transposition	Left double colecting system, ectopic insertion	Ectopic neurons
Absent right ear, cleft lip and palate	Hypoplastic left ventricle[c]	Urinary tract anomalies	Cranial nerve palsy[b]
Bilateral cleft palate	Heart defect[a]	Large single kidney, abnormal genitalia[d]	Small adrenals, multiple CNS[c]
Microcephalus			Partial sirenomelia[d]
Absent right ear and canal			Multiple bony abnormalities[e]
Absent left auditory canal, VATER syndrome			Spina bifida
Hypoplastic left ear			Club foot
Low set deformed ears, micrognathia, microopthalmia			Turner's syndrome
Cleft lip and palate, "cheek/jaw" abnormalities, left anophthalmia			Hydrocephalus
Cleft palate[a]			
Goldenhars-preauricular appendage, atresia of ear canals, henifacial atrophy[b]			
Microcephaly[c]			
Low set ears, dysmorphic face[d]			
Low set ears, micrognathia[e]			

[a-e]Abormalities of multiple organ sytems reported

98. The case reports relating birth defects and vitamin A consumption are of limited value due to selective reporting, lack of denominator data, and reports being prompted by previous associations. No vitamin A-related cases have shown the spectrum of defects associated with isotretinoin embryopathy. Only one case has reported a vitamin A exposure of <7,500 µg RE.

High-dose exposure

99. Two small trials (Zuber *et al.*, 1987; Conway *et al.*, 1958) have been conducted in which women received large doses of vitamin A throughout pregnancy. In one study 27 women received 7,500 μg RE/day (although one women received 15,000 μg RE/day and another, 4,500 μg RE/day) through the first trimester and some through the second and third trimesters also. No abnormalities were reported (Zuber *et al.*, 1987). The second study was a clinical trial of 39 women (59 pregnancies) to prevent the recurrence of cleft lip and palate by taking multivitamin supplements. Vitamin A intake from the supplements was 3,750 μg RE/day. No recurrence of cleft lip and palate occurred; other defects were not discussed (Conway, 1958). The value of the data from these small supplement trials is restricted by the size of the studies and the limited information provided regarding the assessment and evaluation of malformations.

Epidemiology studies

100. Seven epidemiology studies (5 case-control and two prospective cohort studies) have been reported between 1990-1999 (see Annex 2). Two of the case-control studies (Martinez-Frias *et al.*, 1990 and Werler *et al.*, 1990) have suggested that exposure to vitamin A supplements containing >15,000 μg RE may be associated with an increased frequency in birth defects. Although odds ratios (ORs) were >1, the CI values included 1, indicating that the level of risk was not statistically significant. Consequently, the results from these studies are also consistent with a "no effect" interpretation. In contrast, Rothman *et al.* (1995) suggested that data obtained from their prospective study showed an increased risk of vitamin A-associated teratogenesis following relatively modest supplemental doses of 3,000 – 9,000 μg RE. However, the authors' interpretation of the data has been widely challenged (Correspondence, 1996). In particular, there have been questions regarding the impact of some mis-classifications of exposure and category of defect upon the study outcome. Werler *et al.* (1996) commented that the weaknesses of the Rothman study made it inappropriate to identify a threshold dose of vitamin A-associated increased frequency of birth defects. The studies of Khoury *et al.* (1996), Shaw *et al.* (1996), Mills *et al.* (1997) (all case-control studies) and Mastroiacovo *et al.* (1999) (a prospective cohort study) showed no association between birth defect and vitamin A exposure >3000 μg RE. However, in the studies of Khoury and Shaw, the information on vitamin A exposure was limited.

101. The available epidemiological data are consistent with the suggestion that consumption of less than 3,000 μg RE/day from vitamin supplements is safe. However, a threshold for any teratologic effects remains to be identified, although results from the Mastroiacovo *et al.* (1999) study seem to suggest that it may be much higher than the Rothman *et al.* (1995) study has claimed. A general comment may be that none of these studies were of sufficient size to answer the questions asked of them.

Neural tube defect studies

102. Several observational studies have evaluated the effect of vitamin supplements on the risk of neural tube defects (NTDs) when taken periconceptionally. Most

provide evidence for a decreased risk of NTD in groups using multivitamins (Mulinare *et al.*, 1988; Milunski *et al.*, 1989; Shaw *et al.*, 1996). It is generally accepted that the protective effect can be attributed to folate. The studies were variable in their reporting of vitamin A intake. However, where the data were available (Shaw *et al.*, 1995, 1996, 1997; Miller *et al.*, 1989, 1997), no association was made between vitamin A and increased risk of NTD. Clinical trials with multi-vitamins, which included >1,000 μgRE vitamin A, to prevent NTDs also have made no association between vitamin A intake and NTD (see Annex 3). However, the NTD observational studies and clinical trials may have contained too few subjects to rule out risks of other malformations.

Comparative pharmacokinetic study

103. Measurement of maternal plasma retinoid concentrations is considered a better reflection of embryonic exposure than maternal vitamin A intake. Concentrations of vitamin A metabolites are determined by vitamin A intake and source (food or supplement), formulation of supplement, duration of supplementation, simultaneous intake with other food items, and also by metabolite elimination. When considering retinoid distribution in the mother and embryo, it is important to remember that different vitamin A metabolites possess different pharmacokinetic profiles. For example $t_{1/2}$ values for all-*trans*-retinoic acid and 13-*cis*-retinoic acid in human are 0.9 and ~20 hrs respectively. Furthermore, elimination of all-*trans*-retinoic acid is rate limited by its inter-conversion to the 13-*cis* form (Wiegand *et al.*, 1998 and references therein).

104. Miller *et al.* (1998) and Wiegand *et al.* (1998) have examined systemic exposure to retinol and some of its metabolites in pregnant women and women taking vitamin A supplements in an as yet partially completed multi-centre study. Retinoid concentrations have been measured in the plasma of 85 (so far, out of 160) pregnant women in the first trimester (weeks 5-12) and compared with concentrations found in women of childbearing age consuming vitamin A supplements (3,000 or 9,000 μg RE/day for 3 weeks, n=36 and 12, respectively) and in men and women having undergone therapy with isotretinoin (30 mg/day, n=30). Details of diet were also recorded. Plasma concentrations of vitamin A metabolites in the 85 pregnant women (nto supplemented) are shown in Table 5, below.

Table 5. Plasma concentrations of vitamin A metabolites in 85 pregnant women (Wiegand *et al*., 1998)

Metabolite	**Mean concentration (range) ng/ml**
All-*trans*-retinoic acid	1.33 (0.68-2.18)
4-oxo-all-*trans*-retinoic acid	0.34 (0.26-2.04)
13-*cis*-retinoic acid	1.41 (0.72-4.72)
oxo-*cis*-retinoic acid	2.44 (0.84-7.72)
Isotretinoin	Data not available
oxo-isotretinoin	(0.97-7.86)*

n=85

105. An assumption was made that endogenous concentrations in pregnant women must be non-teratogenic since all 160 women gave birth to healthy babies without birth defects. Concentrations of metabolites in vitamin-supplemented women were elevated only slightly above baseline and were similar to those observed during early pregnancy, which resulted in the birth of healthy babies. AUC analysis reflected a similar relationship. In contrast, the levels of metabolites observed in individuals undergoing isotretinoin therapy (30 mg/day, equivalent to 0.5 mg/kg bw/day), a dose known to be potentially teratogenic, were substantially raised compared to those observed during the ingestion of 9,000 μg RE supplements.

106. Based on the current hypothesis that retinoic acid is ultimately responsible for the teratogenicity of vitamin A, the authors suggested that the increases in plasma retinoic acid levels, following 3 weeks of daily dosing with 9,000 μg RE/day, seemed too small to cause a teratogenic effect. However, the interpretation of this study is strongly dependent on whether or not maternal plasma retinoic acid is a good index of fetal exposure to teratogenic risk. The divided dose studies of Tembe *et al.* (1996), in rats and rabbits, which divorced teratogenic potency from maternal plasma levels, would suggest that this is not necessarily so in all species. Furthermore, a question mark remains as to whether it is appropriate to extrapolate from non-pregnant women to pregnant women or pertinent to compare data from pregnant women with data from a mixed-sex group.

Adverse Drug Reactions

107. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting" of reactions. The small number of reactions reported for vitamin A mainly relate to multi-constituent products, and may not therefore be attributable to vitamin A. A few cases of congenital abnormality have been reported, but none were characteristic of vitamin A teratogenicity.

Potentially Vulnerable groups or Genetic Variations

Genetic basis to Vitamin A intolerance

108. No genetic defects in vitamin A handling have been identified, as yet. However, any such defects would probably be incompatible with survival. It has been suggested that there may be a genetic component to some cases of vitamin A intolerance (Carpenter *et al.*, 1987). However, the basis of this has not been defined.

The unborn child

109. Retinol is teratogenic, particularly following maternal exposure during the first trimester of pregnancy.

The young

110. Hathcock *et al.* (1990) have suggested that the apparent increase in susceptibility to vitamin A toxicity in children is partly, if not totally, due to smaller body size.. Table 6, below, summarises some cases of chronic, low-dose vitamin A toxicity in children.

Table 6. Cases of chronic, low-dose vitamin A toxicity in children (adapted from Hathcock *et al.*, 1990)

Dose (IU/day)	**Age, sex**	**Duration (months)**	**Symptoms**	**Other conditions**
6338	31, M	5	Xerosis, cortical thickening in femur, high alkaline phosphatase, irritability	None
2361	26, M	6	Irritability, dermatitis, some alopaecia	Pica of paint chips
2700-3300	29, F	28	Irritability, photophobia, pseudoparesis, seborrheic plaques	None
1689	48, F	24	Pain in feet and ankles, papillidema, dermatitis	None
3247	12, F	9	Irritability, vomiting	None
4085	30, M	12	Anorexia, ataxia, lethargy	Health food use

The elderly

111. The elderly appear to have quite variable tolerances to vitamin A (Hathcock *et al.*, 1990 and references therein) but it is uncertain whether low tolerance is more related to confounding factors, such as pre-existing liver disease and poor diet, rather than to age *per se*. However, in the absence of chronic disease, retinol stores within the liver tend to increase with age, probably due to increased absorption, or decreased clearance (Ward *et al.*, 1996 and references therein).

Diabetics

112. Type II diabetics suffer from a defect in hepatic retinol transport resulting in raised hepatic and decreased plasma retinol levels with increased plasma retinyl ester concentration. It has been suggested that this may be associated with zinc deficiency.

Protein-energy malnourished

113. See paragraphs 21 and 39.

Chronic renal failure and haemodialysis patients

114. Serum vitamin A and RBP are known to accumulate in chronic renal failure and haemodialysis (HD) patients in the absence of supplementation, due to impaired tubular metabolism and glomerular filtration. Following kidney transplantation, circulating levels of vitamin A can take approximately 2 years to normalise, implying vitamin A overload in the tissues. In confirmation of this, stores of vitamin A in the livers of chronic renal patients, at autopsy, have been

found to be high. As an extra complication, predialysis dietary manipulations in HD patients, used to avoid complications of uremia, may result in a reduced zinc intake. As a consequence, retinol metabolism, by zinc-dependent dehydrogenase, may be impaired (Russell, 1997; Komindr *et al.*, 1997 and references therein).

115. Typical diets for patients undergoing maintenance dialysis are frequently below the recommended dietary allowance for water-soluble vitamins, especially when protein intake is low. Replacement of these vitamins may be with multi-vitamin preparations, which may also include high levels of vitamin A, may provide some cause for concern (Komindr *et al.*, 1997 and references therein).

Liver compromised patients (drugs, alcohol, disease)

116. In acute liver disease, serum vitamin A levels can become elevated due to release of retinyl esters from liver stores and there are cases of hypervitaminosis A exacerbated by acute liver cell destruction (Russell, 1997).

117. In chronic liver disease, the storage capacity of the liver is reduced and the ability to synthesise RBP and transthyretin is impaired. Decreased transport of vitamin A from the liver could result in elevated liver levels and local tissue intoxication.

Oral toxicity in animals

Acute toxicity

118. Acute LD50 values for retinol and related compounds, given orally (oral intubation) are shown in Table 7, below. Acute lethal doses generally result in progressive weakness, difficulty in breathing, coma, loss of simple reflex and death, sometimes preceded by convulsions (Armstrong *et al.*, 1994 and references therein).

Table 7. Oral LD50 values for retinol and related compounds (cited, Hathcock *et al.*, 1990)

	Species	**mg/kg**	**IU/kg**	**µg RE/kg**
retinol	mouse rat	2,570	$8.6x10^6$	$2.57x10^6$
retinyl palmitate	rat	7,910	$14.4x10^6$	$4.44x10^6$
all-*trans*-retinoic acid	mouse rat	1,100-4,000 2,000		
13-*cis*-retinoic acid	mouse rat	3,389-26,000 >4,000		
etretinate	mouse rat	>4,000 >4,000		

Sub-chronic/chronic toxicity

119. The subchronic toxicity of vitamin A was extensively reviewed in the 1950s by Nieman and Obbink (1954) and available data are shown in Tables 8a and b, below. To summarise briefly, the first appearance of toxic signs was dependent upon species and age of animal, dose, dose formulation, duration of treatment and the specific toxic manifestation in question. Hypervitaminosis A resulted in anorexia, weight loss, anaemia, cachexia and finally, death. Frequently observed toxic events included skin effects (hair loss, localised erythema and thickened epithelium), internal organ effects (fatty infiltration of the liver and fatty changes in the heart and kidney, testicular changes, degeneration of myocardial fibres), blood effects (decreased haemoglobin, transient increases in circulating lipids and serum cholesterol, elevated serum triglycerides), skeletal effects (limping gait, spontaneous fractures, hind leg paralysis, increased osteoclast activity, decreased osteoblast activity, bone elongation without thickening, reduced formation of dentine and atrophy of odontoblasts), subcutaneous and intramuscular haemorrhage, hypothrombinaemia, internal haemorrhage, and inflammation of nasal passage, gut, and conjunctiva (reviewed by Hathcock *et al*, 1990; Nieman and Obbink, 1954). Nieman and Obbink (1954) concluded that oral chronic toxicity in the adult rat begins at approximately 25,000 IU/day per rat (approximately 30,000 µg RE/kg/day, based on a body wt of 250 g) and the effects become lethal at about 50,000 IU/day (approximately 60,000 µg RE/kg/day). Younger animals are more susceptible, with chronic toxicity beginning at a dose of approximately 3,000 – 3,750 µg RE/day with effects becoming lethal at 6,000 – 7,500 µg RE/day.

Table 8a. Summary of data on sub-chronic and chronic vitamin A toxicity in animals (oral)

Species	**Compound**	**Dose/duration**	**Effect**	**Reference**
Rat	Retinyl palmitate	up to 15,500 µg RE/kg/d x 10 mo	**no adverse effects**	Kamm *et al*, 1984
Rat	Retinol	33,000 µg RE/d	elevated serum triglycerides	Setty and Misra, 1975; Ahuja and Misra, 1975
Rat	Retinyl palmitate	310,000 – 620,000 µg RE/d	elevated serum triglycerides, reversible on discontinuation of treatment	Bayerle *et al*, 1973
Hamster	Retinyl acetate	~7,000 µg RE/kg/d	Gallstones	Cardenas *et al*, 1996
Mouse			bone abnormalities	Takase *et al*, 1999
Dog	Retinyl palmitate	up to 7,750 µg RE/kg/d x 10 mo	**no adverse effects**	Kamm *et al*, 1984

Table 8b. Summary of studies on oral chronic hypervitaminosis in experimental animals (adapted from Nieman and Obbink, 1954)

Animal	**Vitamin A-preparation**	**Dose (IU/day)**	**Toxicity observed**						
			A	**B**	**C**	**D**	**E**	**F**	**G**
Young rat		10-50,000	X	X		X	X	X	
Young rat		Up to 25,000	X	X		X			
Mature rat		60-120,000	X	X		X		X	
Mouse		24,000	X	X				X	
Young rat		300,000		X				X	
Young rat		60,000	X	X	X				
Young rat		120,000	X	X	X				
Young rat		120,000	X	X					X
Young rat		72,000	X	X	X				

Young rat		60,000	X	X	X			X	X
Young rat		30,000	X	X				X	
Young rat		120,000	X	X	X	X			X
Young rat		60,000	X	X	X				
Young rat		60,000	X	X					
Guinea pig		60,000	X						
Young rat		60,000	X			X			
Rabbit		180,000	X						
Rat		20,000		X					X
Young rat		60,000	X	X	X				X
Young rat		20,000	X	X	X				
Young rat		66,000	X		X	X			
Guinnea pig			X	X		X			
Guinnea pig							X	X	
Young rat		13,500	X	X					
Mature rat		26,500	X	X					
Guinea pig								X	X
Mature rat		80,000	X	X			X	X	
Young rat		30,000						X	
Rat and chicken		50-60,000						X	
Young rat		30-40,000	X	X	X				
Mature rat		30,000	X	X	X	X			X
Young rat		25-50,000			X				
Mature rat		45,000				X			
Guinea pig		60,000					X	X	
Mature rat		18-40,000							X
Young rat		24,000	X		X				
Rat		~60,000			X	X			
Young rat		10-40,000			X				
Young rat		50,000			X				
Dog (and rat)		1,500,000			X	X		X	X
Young rat		40,000		X	X				
Young rat		40,000			X				
Rat		15,000							
Several		50-100 IU/g bw	X	X	X	X	X	X	X
Young rat		43,000							X
Several animals		300-800 IU/g bw	X	X	X	X		X	X
Rat		Idem	X	X	X	X		X	X
Young rat		40,000							X
Chickens		50-75,000			X				
Ducks		100-600,000			X				
Young rat		50,000	X	X	X		X	X	
Young rat		50,000	X	X	X	X			X

Key:

A – general toxic symptoms
B – skin lesions
C – skeletsl lesions
D – haemorrhage and inflammation of mucous membranes
E – action on endocrine glands
F – degeneration of organs (mostly histologic)
G – chemical and morphologiv blood picture

120. More recently, there have been reports that high doses of vitamin A can have an antithyroid effect. Petenusci *et al.* (1980) demonstrated that high levels of vitamin A (4,500 µg RE/day for 10 days, as retinyl palmitate, administered intraperitoneally) in rats induced thyroid atrophy, which reversed when treatment was withdrawn for a further 20 days. Morley *et al.*, 1980) reported that retinol, administered orally to give intakes of ≥90,000 µg RE/week, resulted in decreased thyroid weights in rats. Furthermore, intakes of 225,000 µg RE/week lead to decreases in plasma total thyroxine (T4) and triiodothyronine (T3), decreased T4 half-life and increases in dialysable T4 and T3 and T3 distribution space. Similar

thyroid hormone changes were reported by Garcin *et al.* (1984) in rats receiving 6,750 µg RE/day (~47,000 µg RE/week) in the diet for 15 days.

121. Chronic administration of retinyl acetate to hamsters (equivalent to the order of 7,000 RE/kg b wt/day) resulted in development of gallstones (Cardenas *et al.*, 1996). In mice, a similar dose has been shown to cause progressive ossification in extra-osseous tissues, proliferation of sub-chondral bone, histopathological abnormalities in chondrocytes near osteochondral junctions and inappropriate expression of osteocalcin and type I collagen in metaplastic chondrocytes (Takase *et al.*, 1999).

122. Gross bone lesions characterised by resorption of parts of the pelvis, fibulae, and scapulae with bone thinning were observed in growing rats treated with 25,000-75,000 IU/day retinol as palmitate or retinol (vitamin A alcohol) for 17 days (Leelaprute *et al.*, 1973). Soft tissue calcification also occurred. The animals were given the doses either orally or via i.p. injection. The vitamin A palmitate was not toxic when given i.p. However retinol was more toxic when administered i.p.

123. Daily administration of 10000-50000 IU vitamin A produced fractures in the long bones in growing rats and guinea pigs; a dose-related increase in the number of osteoclasts was also observed (Wolbach, 1947). Frankel *et al.* (1986) administered growing rats 15000 RE as retinyl palmitate 3 times/week for 6 weeks after which proximal tibial histomorphometry was performed. An approximate 2-fold increase in osteoclast number and a similar reduction in osteoid surface compared to controls was observed. Similarly, Hough *et al*. (1988) treated young rats with 10,000 or 25,000 IU/day retinyl palmitate for 21 days; tibial histomorphometry revealed increased bone resporption (increased osteoclast size and number) and reduced bone formation. Spontaneous limb fractures and increased skeletal turnover (as measured by serum alkaline phosphatase and urinary hydroxyproline excretion) were also observed in the top dose group. Circulating levels of the potent bone resorbers, pTH, 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D were comparable in the treated animals and the controls suggesting that vitamin A was having a direct effect on bone. Reviewing the animal data Binkley and Kruegar (2000) concluded that high-dose vitamin A intake induces osteopenia and fractures in laboratory animals.

124. A single oral dose of 30 mg retinol equivalent given to adult rats, had no effect on biologically active parathyroid hormone (bioactive PTH) concentrations (Frankel *et al.*, 1984). Secretions of bioactive PTH were not altered by incubation of rat thyroparathyroid complexes with retinol *in vitro*. In rats given 15 mg RE, 3 times a week for 6 weeks serum bioactive PTH was not detectable and serum 25-hydroxyvitamin D was significantly lower than in controls. In acutely intoxicated rats (given 60 mg RE/day for 2 days) serum bioactive PTH levels were significantly lower than in controls. Lower doses of retinol (7.5 mg RE, 3 times a week for 3 weeks) suppressed serum bioactive PTH to undetectable levels but had no effect on serum 25-hydroxyvitamin D. Serum calcium and 25-hydroxyvitamin D were lower in vitamin D intoxicated rats who were also given 7.5 mg RE, 3 times a week.. The authors considered that the skeletal changes caused by high

levels of vitamin A (see paragraph 124) were independent of the effects on PTH but could be caused by the changes in vitamin D metabolites. However, these pathological changes could be modified by secondary changes in calcium metabolism and in the metabolism of calcium-regulating hormones.

125. Chronic toxicity data are limited. Studies cited by Kamm *et al.* (1984) showed no adverse effects in rats and dogs administered retinyl palmitate at doses 250 times higher, on a body weight basis, than the human RDA, for 10 months. However, it has been noted (Hathcock *et al.*, 1990) that the complexities of extrapolation between species do not allow these data to be construed as an indication that an intake 250 times the RDA is safe for humans.

Carcinogenicity

126. Several studies in rodent models have shown that natural and synthetic retinoids, when given in sub-toxic doses, are highly effective in inhibiting chemically-induced tumours of the mammary gland, skin, bladder, forestomach, or eosophagus (reviewed by Moon *et al.*, 1994). On the other hand, a minority of studies have reported apparent potentiation of chemically-induced tumour incidence by retinoids, although Moon *et al.* (1994) have suggested that such outcomes were most likely a result of overt toxicity, rather than a direct promotional effect of the retinoid.

Developmental toxicity (teratogenicity)

127. Retinoids, including retinol, have been conclusively shown to cause congenital malformations in animals (Hathcock *et al.*, 1990 and references therein). The nature and incidence of the malformations depend mainly upon dose, at what stage of gestation exposure occurs and to a much lesser extent, upon animal species and strain. Retinoids differ in their teratogenic potency e.g. in the rat, all-*trans*-retinoic acid > retinol > 13-*cis*-retinoic acid (isotretinoin) (Tembe *et al.*, 1996). Furthermore, the potency of a particular retinoid is species-dependent e.g. rabbits and monkeys are more susceptible than rats and mice to the teratogenic effects of isotretinoin (citations by Tzimas *et al.*, 1997) . The malformations that occur in animals are analogous to some of those that occur in human, including anencephaly, spinabifida, cleft lip, cleft palate, micronathia, microphthalmia, several types of malformation to the ear, teeth, salivary glands, and aortic arch, ventricular septal defects, imperforate anus, omphalocele, renal agenesis, polycystic kidney, hydronephrosis, phocomelia, digit malformation, certain defects of the genitalia, pituitary, thyroid, thymus, skull, vertebrae, ribs, and muscles and situs inversus. Table 9, below summarises the developmental toxicity studies in animals.

Table 9. Studies on the developmental toxicity of vitamin A and related compounds (adapted from Hathcock *et al.*, 1990)

Species	**Dosing**	**Results**
Hamster	All-*trans*-retinylidene methyl nitrone (50, 75 or 100 mg/kg)	% malformed fetuses and severity of malformations increased with increased dose. Malformations of eyelids with exophthalmos common at ≥50 mg/kg. Caudal nregression

		syndrome, characterised by occult spina bifida (sacral rachischisis occulta with myelocele or menigomyelocyte), imperforate anus and aplastic ot hypoplastic tail predominated at higher doses).
Monkey	Retinoic acid (7.5-10 mg/kg), oral, from g.d. 20-44	Craniofacial abnormalities in all fetuses, nearly all bones of craniofacial complex affected – zygomatic bone and mandible most severely affected. Abnormal specimens resembled Teacher-Collins syndrome in humans.
Mouse	All-*trans*-retinol or all-*trans*-retinylidene methyl nitrone (75 mg/kg) administered by gavage on day 7, 8, 9, 10 or 11 of gestation	Treatment on day 7, 8 or 9 with retinol induced malformations of head; treatment on day 8 with either retinoid produced highest *in utero* death rate. Malformations induced by administration of either retinoid were similar but retinol was associated with a higher frequency of malformed offspring.
	All-*trans* or 13-*cis* retinoic acid (100 mg/kg) on day 11.5 or 12.0 of gestation	Single oral dose of all-*trans* retinoic acid on either day was maximally effective. >90% of treated embryos developed reduction defects of limb bones and an equally high % also had cleft palate. Under identical conditions treatment with 13-*cis*-retinoic acid produced no apparent teratogenic effects.
	Retinoic acid (0, 5, 10, 20, 40, 60 or 80 mg/kg) administered to dam on day 8 of gestation	Neural tube defects produced by retinoic acid; females were preferentially affected. With increasing doses proportion of severely affected females increased then declined.
	Retinol palmitate (1.6 mg) to pregnant mice during neurulation	Most embryos became exencephalic. Changes in neuroepithelial cells were apparent within hours after maternal treatment. Early cellular alterations apparently led to disruption of architecture of neuroepithelium so that neural folds failed to meet and close. Tissues may survive intitial insult but continue to grow in everted manner characteristic of exencephaly.
Rat	Vitamin A palmitate (0.55, 5.5 or 22.0 mg) per animal by gavage on days 6-15 of gestation	Maternal toxicity evidenced by decreased body-weight gain, decreased food and water consumption at 22.0 mg. Malformations in surviving fetuses include cleft palate, exencephaly, microphthalmia, anophthalmia, hydronephroses, brachygnathia, pinna abnormalities and great vessel and heart anomalies.
	Vitamin A (30-45 mg) orally on days 6-15 of gestation	Fetuses collected on day 21 had anomalies of the eyes, eg open eyelids, exophthalimia, cataractous lens and retina. Eye malformations were stage dependent and mostly found in groups treated on days 8 and 9 of gestation.
	Retinyl acetate (55 mg) during the last third of gestation	In lung sections, expansion of lung either did not occur or was not uniform. Apparently hypervitaminosis A during fetal period affects normal lung maturation
	Retinyl acetate (41.3 mg) final dosage on day 15 of gestation	90% incidence of cleft palate. Increased DNA and glycoproteins synthesis found in fetal palate.
	Vitamin A palmitate (44 mg) during g.d. 5-7, 8-10, 11-13, 14-16 and 17-19	Animals dosed on days 8-10 were hyperactive. Animals dosed on days 11-13 acquired T-maze skills more slowly than did controls. Animals dosed on days 11-13 were significantly lighter than controls and all other test groups.
	Vitamin A (28.5 mg) gastric intubation on days 17 and 18 of gestation	Offspring showed slower rates of response than controls during discrimination training in adulthood; learning ability was not impaired.

128. Hypervitaminosis A has been associated with the development of permanent learning disabilities in the offspring from rats exposed during pregnancy. Furthermore, Adams *et al.* (1993) have suggested the possibility that the CNS may be more sensitive to vitamin A than other developing systems. In Fischer 344 rats, behavioural changes were observed in the offspring of dams exposed to doses down to 3,000 µg RE/kg, which is ten times lower than the lowest teratogenic dose reported in rats (Adams, 1993). However, the F344 rat may be particularly sensitive compared to other strains of rats, with doses that produce behavioural

effects in Sprague Dawley and Wistar rats being fatal in the F344. For example, 100,000 IU/day given between days 8 to 10 of gestation caused alterated behaviour in the Biel maze test in Sprague-Dawley rats and in the puzzle box maze and Biel maze tests in Wistar rats but pre- and post-natal death in Fischer F344 rats.

129. Dostal and Soukupova (1992) reported that intraperitoneal administration of 1,500 µg RE/day vitamin A, in the form of retinoleum palmiticum in aqueous solution, to pregnant ICR mice, resulted in abnormal delayed-type hypersensitivity (DTH) in female offspring. However, the data from these studies are interesting in that a single administration on gestation d16 resulted in an increased DTH whereas repeated administration, on d15-17, resulted in a depressed DTH.

130. Interspecies differences in sensitivity to the teratogenicity of retinol (summarised in table 10, below) and other retinoids may be explained by differences in pharmacokinetics, protein binding, maternal and foetal metabolism, transplacental pharmacokinetics, receptor and enzyme induction and toxicodynamics. Consequently, the interpretation of data from animal models, for purposes of human risk assessment, may be very complex and requires information on concentration-time relationships of the retinoid concerned and its active metabolites in both the maternal circulation and embryo during the most susceptible phase.

Table 10. Lowest reported teratogenic doses of retinol (or equivalent from retinyl esters) after oral administration in laboratory animals

Species	**Retinol (µg RE/kg)**
Rat*	45,000
Rat**	35,000
Mouse***	75,000
Mouse*	25,000
Hamster*	15,000
Monkey****	6,000
Rabbit*	2,500

*Data cited by Armstrong *et al*. (1994)
**Cohlan (1953)
*** Data cited by The Teratology Society (1987)
****Hendrickx *et al*. (1997)

131. Comparative metabolism and kinetic data suggest that mouse and rat are not good models for human. The rabbit, on the other hand, exhibits similar plasma retinoid patterns to humans, under both background and administration conditions. AUCs for all-*trans*-retinoic acid, following an embryotoxic dosing regimen in rabbits and vitamin A supplementation in humans, are also similar. Nonetheless, some metabolic differences exist in terms of the major *cis*-metabolite formed. Furthermore, the rabbit exhibits embryo-lethality rather than malformations following administration of excess vitamin A during organogenesis, although it is not possible to preclude that pregnancy loss does not occur in humans just because it has not yet been detected (Dolk *et al.*, 1999 and references therein).

132. Studies have suggested that the cynomolgus monkey is similar to human in terms of isotretinoin (13-*cis*-retinoic acid) -induced teratogenic effects. Furthermore, doses required to elicit teratogenicity are within an order of magnitude of that required in humans (humans 0.5-1.5 mg/kg/day – monkey 2.5-5 mg/kg/day) and the metabolic and pharmacokinetic profiles are comparable (Dolk *et al.*, 1999 and references therein).

133. A study of the teratogenicity of retinol in cynomolgus monkeys, reported by Hendrickx *et al.* (1997a,b), Wiegand *et al.* (1998) and Miller *et al.* (1998), showed dose-related increased rates of spontaneous abortion and malformations when administered oral doses, during early pregnancy (gestation d 16-27), of 0, 2250, 6,000, 12,000 and 24,000 μg RE/kg/day retinyl palmitate. Incidences of malformation were 1/21 and 5/11 at doses of 6,000 and 24,000 μg RE, respectively (intermediate dose not reported). The higher dose affected structures derived from the cranial neural crest. These malformations were similar to those observed with isotretinoin (13-*cis*-retinoic acid) in both monkey and women. However, the spectrum of defects differed slightly to that observed in isotretinoin syndrome in that there were more adversely affected craniofacial structures, thymus and heart defects were less severe and there were no brain malformations. Maternal toxicity was observed (nature not specified). The NOAEL and LOAEL determined in this study were 2250 RE/kg and 6,000 RE/kg, respectively.

134. On the basis of similarities between the cynomolgus monkey and human, following exposure to 13-*cis*-retinoic acid, in terms of malformations and defect patterns, most sensitive time of exposure, dose required, and metabolism and pharmacokinetics during early pregnancy, the authors (Miller *et al.*, 1998) assumed it reasonable to extrapolate these data, with appropriate adjustments, to humans. Thus, the NOAEL established in this study was, assuming 4 kg animal body weight, 10 x the intake of vitamin A required to fulfil physiological need (900 RE/day). Extrapolating these data to the human situation, 8,000 μg RE/day (10 times the human RDA = 10 x 800 = 8,000 μg RE/day) may represent a safe level of exposure in pregnant women. Alternatively, using the experimental NOAEL and allowing a safety factor of 10, for a 50 kg woman, 11,250 RE/day (NOAEL x 50/10 = 2,250 RE/kg x 50 kg/10=11,250 RE) may be considered a safe dose.

135. Biesalski *et al.* (1996) administered high levels of dietary retinyl palmitate, up to 52.5 x 10^3 μg RE/kg diet, for 8 months (approximately 3,000 μg RE/kg b wt/day), to female rats, in order to achieve periconceptional steady-state plasma retinyl ester concentrations of >1,525 nmol/l (>10 times normal). Periconceptional plasma retinol concentrations were approximately doubled. Concentrations of other retinoid metabolites, including retinoic acid, were not measured. There were no fetal malformations. The authors indicated that the dose given in this study corresponded to approximately 500 μg RE/kg (27,000 μg RE) in human females, taking into account body surface area and metabolism (the basis of this calculation was not made clear) and that in humans, a substantial increase in retinyl esters would only occur after chronic supplementation of >4,000 μg RE/day or the intake from 100g of liver. The authors would not exclude

the possibility that a single high dose of vitamin A or chronically elevated plasma retinyl esters might possess a teratogenic risk during early pregnancy.

Neonatal developmental toxicity

136. Neonatal exposure to high doses of retinol palmitate, by subcutaneous or intraperitoneal injection, has resulted in defective learning and depressed heat-pain responses and reduced sexual activity in adult rats (Kihara *et al.*, 1995; Csaba and Gaal, 1997)

Mutagenicity and genotoxicity

137. The majority of data from a variety of *in vitro* and *in vivo* genotoxicity tests (endpoints including reverse mutations in *Salmonella typhimurium* and *Saccharomyces cerevisiae*, DNA single-strand breaks and unscheduled DNA synthesis in primary rat hepatocytes, mutation in Chinese hamster ovary cells and V79 cells, sister chromatid exchange, chromosome aberrations, chromosome instability, and morphological differentiation in a variety of mammalian cells, DNA binding, and micronuclei formation), is consistent with the view that vitamin A (in the form of either retinol or retinyl esters) is not genotoxic (see reviews by De Flora *et al.*, 1999 and IARC, 1998). On the contrary, many studies have demonstrated a protective role for this nutrient. However, one isolated study reported retinol to be mutagenic in the bacterial Ames test with Salmonella strain TA104, in the presence of metabolic activation. Retinol was also found to cause "petite" mitochondrial mutations in *Saccharomyces cerevisiae strain 6-81*, but was not found to be mutagenic to nuclear genes. Generally, retinol has not been found to cause sister chromatid exchanges (SCE) in a number of mammalian cell types, however there has been a report that of a small but significant increase in SCE in cultured human lymphocytes. After extended treatment periods, retinyl palmitate has been shown to cause increased chromosomal aberrations in mouse bone marrow cells and spermatocytes (see reviews by De Flora *et al.*, 1999 and IARC, 1998).

Mechanisms of toxicity

138. The chronic toxicity of vitamin A is accumulative. When administration of excess vitamin A is prolonged, the limits of hepatic storage and RBP binding capacity are exceeded. Retinyl ester, rather than retinol, is mobilised from the liver and made far more available to membranes than would be usual. It has been considered that the amphiphatic nature of retinyl ester and detergent-like action may be responsible for the labilisation of various organelle membranes, resulting in the release organelle contents or alterations in membrane function. However, it is generally accepted that surfactant detergent-like activity is probably relevant only at extremely high concentrations. The more recent concept is that most retinoid-induced toxicities result from nuclear receptor (RXR, RAR)-mediated interaction and ensuing altered gene expression. Furthermore, the toxic moiety is thought likely to be the retinoic acid metabolite (Armstrong *et al*, 1994 and references therein).

Mechanism of teratogenesis

139. Retinoic acid plays an important role in the control of expression of many genes, including *Hox* genes, which are vital for correct positional and sequential development of the embryo (Marshall *et al.*, 1996). The morphogenic action of retinoic acid is most probably mediated through the actions of the RAR and RXR bound material on nuclear retinoic acid response elements (Mendelsohn *et al.*, 1994). The most sensitive exposure period, in both humans and animals, coincides with development and differentiation of the facial neural crest and formation of the branchial apparatus (pharyngeal arches), structures from which most retinoid-associated birth defects are derived. The evidence remains circumstantial, but there is a strong suggestion that retinoid teratogenicity may be the outcome of an exaggerated normal response, resulting from abnormal, inappropriate or prolonged transcriptional activation of critical genes in target embryonic cells. It appears that the primary receptors involved belong to the RAR subfamily (Creech Kraft and Willhite, 1997 and references therein).

140. Although there is some disagreement in the literature, much of the evidence so far indicates the teratogenic effects of retinol and other naturally occurring retinoid compounds are due to their conversion to all-*trans*-retinoic acid (Creech Kraft and Willhite, 1997 and references therein). It has been suggested that maternal AUC of all-*trans*-retinoic acid, rather than C*max*, may be an appropriate pharmacokinetic marker of embryonic exposure and embryopotency of all-*trans*-retinoic acid and that the duration of exposure may be the major determinant of embryotoxic outcome for retinoids in general (Tzimas *et al.*, 1997). However, the uncertainty of identity of the ultimate teratogen, the complexities of metabolism within both mother and embryo, the differing efficiencies with which metabolites undergo transplacental transfer and possible modulations of metabolising enzymes, binding proteins and receptors on prolonged or repeated exposures, may prevent a clear picture of fetal exposure being obtained from the maternal kinetic profile. More importantly, interspecies differences make comparisons of teratogenic potency and extrapolation for human risk assessment difficult.

Mechanism of bone toxicity

141. It has been suggested that vtamin A may have a direct effect on bone resulting in osteoporotic effects, but that a secondary interaction with vitamin D could also occur.

Regulatory considerations

142. Both Infant Formula and Follow-on Formula Regulations 1995 and the Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations specify a maximum level of 180 µg RE/100kcal. The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations specify that a whole meal replacement must provide at least 700 µg RE/day and a meal replacement 210 µg RE per meal. The Margarine Regulations make it compulsory for manufacturers to add 800 - 1000 µg RE/100g of margarine.

Guidance on high intakes

143. COMA (DH, 1991) recommended that regular intakes of vitamin A (REs) should not exceed 9,000 RE/day in adult men or 7,500 μg RE/day in adult women. Regular daily intakes in infants and children should not exceed 900 μg RE/day (<1 year), 1,800 μg RE/day (1-3 years), 3,000 μg RE/day (4-6 years), 4,500 μg RE/day (6-12 years) or 6,000 μg RE/day in adolescents. They added, "therapeutic doses may exceed these limits, but only under medical supervision". The Food and Nutrition Board of the Institute of Medicine has set tolerable upper intake levels for preformed vitamin A of 600μg/d for 3 years and below, 900μg/d for 4-8 years, 1700μg/d for 9-13 years, 2800μg/d for 14-18 years, and 3000μg/d for adults, including pregnancy and lactation (Trumbo *et al.*, 2001).

144. Advice given by the UK Chief Medical Officer (DH, 1990) states that retinol is teratogenic and that there is a suggested relationship between the incidence of birth defects in infants and high vitamin A intake (>3,300 μg RE/day) during pregnancy. As a precautionary measure women in the UK who are, or might become, pregnant were advised not to take supplements containing vitamin A unless advised to do so by a doctor or antenatal clinic. Following the issuing of this advice a voluntary agreement was reached with industry such that products containing more than the RDA of vitamin A should carry a warning label outlining the above advice.

145. The vitamin A content of liver is very high. The advice of the Chief Medical Officer (DH. 1990) also recommended that as a matter of prudence, women who are, or might become, pregnant should not consume liver or liver products. This advice was later reconsidered, but not changed, in the light of data showing differences in the pharmacokinetic handling of vitamin A from food and supplements.

Recommendations on maximum supplementation levels

146. A position paper by the Teratology Society (1987) recommended that women in their reproductive years should be informed that the excessive use of vitamin A, before and during pregnancy, could be harmful to their babies, that women at risk of becoming pregnant should consider their dietary intake of vitamin A before taking supplements and that supplementation of 8,000 IU (2,400 μg RE)/day should be considered the recommended maximum dose prior to, or during, pregnancy until further evaluations could be performed within the human population.

147. The American College of Obstetricians and Gynaecologists Committee (ACOG, 1995 & 1998) published the following opinion: that the US dietary intake of vitamin A is adequate to meet the needs of most pregnant women throughout gestation; routine supplementation during pregnancy is not recommended; in cases where dietary intake of vitamin A may not be adequate, e.g. strict vegetarians, dietary intake should be supplemented; carefully supervised supplementation may

be desirable for some pregnant women, such as recent emigrants from countries where vitamin A deficiency is endemic; supplementation with 5,000 IU (1,500 μg RE) should be considered the maximum intake prior to and during pregnancy.

148. A statement issued by the IVACG (1998) confirmed recommendations made in 1986 that "It is safe to give fertile women, independent of their vitamin A status, as much as 3,000 μg RE daily at any time during pregnancy". "No benefits have been demonstrated from taking a supplement during gestation where habitual vitamin A intakes exceed approximately 3 times the RDA (approximately 2,400 μg RE) from sources rich in provitamin A". " For supplementation of women residing in endemically deficient areas "a weekly supplement of up to 8,500 μg RE is a safe alternative to daily supplementation during pregnancy. A single high-dose supplement of up to 60,000 μg RE to breast-feeding women is safe up to 8 weeks post-delivery. For non-breast-feeding women, a single high-dose supplement of up to 60,000 μg RE is safe up to 6 weeks post-delivery". Furthermore, the 1998 statement went on to say that "Fortified food products can safely ingested during pregnancy and lactation, and vitamin A-rich natural foods, such as animal liver, consumed occasionally also can be safely ingested".

149. Participants of a World Health Organisation committee concluded that, on the basis of available data, there is no teratogenic risk from preformed vitamin A supplements of 3000 μg RE given to pregnant women who habitually consume less than the RDA. However, there is no justification for daily supplements at a level above 2,400 μg RE for pregnant women who habitually consume vitamin A at the level of the RDA or above" (WHO, 1998).

150. The Council for Responsible Nutrition, a UK trade association recommends a maximum level of 2300 μg vitamin A for long term supplementation and 7500 μg for short term supplementation (CRN, 1991).

Summary

Chemistry and nomenclature

151. The term "vitamin A" is often used loosely to refer to a group of fat-soluble compounds known as "the retinoids". Generally, their structure consists of a β-ionone ring, a conjugated isoprenoid side chain and a polar terminal group. The parent compound all-*trans*-retinol and its fatty acid ester derivatives, are referred to as preformed vitamin A, as opposed to the proform vitamin A precursors, such as β-carotene.

Occurrence in food, food supplements and licensed medicines

152. Foods rich in preformed vitamin A (retinol, retinyl esters) include dairy products, fortified margarine, liver and fish oils. Other sources of exposure include single- or multi-vitamin supplements and synthetic forms prescribed for therapeutic purposes, e.g. certain skin disorders.

Intake

153. Mean intake in the UK is approximately 1,800 and 1,600 μg RE/day (retinol equivalents/day[1]), in adult males and females, respectively. This is more than twice the adult Reference Nutrient Intake values of 700 and 600 μg RE/day. Most of the UK population would appear to have substantial vitamin A reserves.

Bioavailability

154. The extent of absorption of dietary preformed vitamin A is ~80% but this may be reduced if diets are low in fat or individuals are suffering from fat malabsorption syndrome. Bioavailability of hepatic stores of vitamin A may be decreased if protein nutritional status is low. The rate of absorption of vitamin A from supplements is influenced by the form of preparation, with aqueous dispersions and emulsions being absorbed at a faster rate than oily solutions. The pharmacokinetic handling of retinol from foods, such as liver, differs to that obtained from supplements, with the former exhibiting considerably lower plasma C*max* (maximum concentration) and AUC (area under the curve) values for retinol metabolites, particularly all-*trans*-retinoic acid.

Interactions

155. Alcohol may potentiate vitamin A-induced hepatotoxicity. In addition, competitive inhibition of alcohol dehydrogenase may lead to decreased synthesis of retinoic acid, resulting in functional vitamin A deficiency, which may be involved in fetal alcohol syndrome. itamin A may potentiate the development of intracranial hypertension when taken in combination with tetracycline and minocyclin type antibiotics. Drugs such as ketoconazole, which inhibit cytochrome P-450, can significantly increase the half-life of retinoic acid due to the inhibition of retinoid metabolism. Vitamins A and D are often taken together in high doses. Attributing cause of toxicity may be difficult since some symptoms are common to both hypervitaminoses. Hypervitaminosis A may decrease vitamin C tissue storage. Vtamin A/vitamin K antagonism in blood clotting function. itaminosis A may have an anti-thyroid effect. Deficiency in zinc may adversely affect mobilisation of vitamin A from hepatic stores and absorption of vitamin A from the gut. Vitamin A deficiency may be implicated in impaired iron absorption and decreased utilisation for erythropoiesis.

Absorption, distribution, metabolism and excretion

156. Dietary retinyl ester is released from food by proteolytic digestion and hydrolysed to retinol in the gut. The retinol is taken up into enterocytes in micellor form, undergoes re-esterification and is incorporated into chylomicra, which are released into the circulation via the lymph. Following the breakdown of chylomicra by serum lipases, the retinyl esters are released, taken up by hepatocytes and re-hydrolysed. The resulting retinol is transferred to the stellate

[1] 1 μg RE = 1 μg retinol

(fat storing) cells and stored in the form of long-chain fatty esters. Approximately 90% of the body's vitamin A is stored in the liver this way.

157. Plasma retinol is usually maintained under tight homeostatic control and concentrations do not alter significantly unless hepatic stores are severely depleted. If hepatic storage capacity is exceeded, plasma levels of retinyl ester increase, but plasma levels of retinol itself do not. Mobilised retinol is transported in plasma bound to retinol-binding protein and transthyretin. Uptake into the extra-hepatic tissues occurs via a receptor-mediated process. Once inside the cell, retinol undergoes a complex series of metabolic conversions, mainly oxidations and isomerisations. Intracellular binding proteins facilitate transport of specific vitamin A metabolites, such as retinoic acid, into the nucleus of the cell, where they interact with the retinoid nuclear receptors (RARs and RXRs) and exert their influence on gene expression.

158. Most vitamin A is recycled between the tissues and the liver. However, a proportion of oxidised products is not regenerated and is excreted in the urine or conjugated to glucuronide and excreted in the bile. Most biliary excreted conjugates are eliminated via the faeces, although a proportion is reabsorbed and returned to the liver.

159. In general, ~10-20% of ingested vitamin A remains unabsorbed, ~20% is excreted in the faeces via the bile, 17% is eliminated in urine, 3% is expired as CO_2, and 40-50% is retained by the body, mainly in the liver.

Function

160. Vitamin A is essential to the processes of vision, reproduction, embryonic development, morphogenesis, growth and cellular differentiation. With the exception of the visual process, most processes are related to the control of gene expression, with vitamin A metabolites, such as retinoic acid, acting as nuclear receptor-ligands.

Deficiency

161. Vitamin A deficiency is a problem largely in developing countries. It can lead to night blindness and xerophthalmia, growth retardation, keratinisation of epithelia, impaired hearing, taste and smell, increased susceptibility to infection, increased child mortality and reduced male fertility. In pregnancy, vitamin A deficiency can result in malformations in offspring. Deficiency in the developed world is usually limited to those with absorption difficulties, those with increased susceptibility to opportunistic infections, chronic liver disease patients and alcoholics.

Oral toxicity in humans

162. **Acute toxicity -** Symptoms of acute vitamin A toxicity include abdominal pain, anorexia, vomiting, blurred vision, irritability, headache, and in children, bulging of fontanelle. Acute toxicity is associated with doses well in excess of

100x and 20x the RDA, in adults and children, respectively. In trials, infants of < 6 months have been shown to develop acute symptoms following a single dose of 7,500-15,000 μg RE, whereas a dose of 30,000 RE appears to be well-tolerated in older children.

163. **Chronic toxicity -** Symptoms of chronic toxicity include dry thickening of skin, cracking of lips, conjunctivitis, erythematous eruption, alopecia, bone joint pain, chronic headache, intracranial hypertension and hepatotoxicity. On-set and severity of toxic manifestations are dependent on dose, duration and the manifestation in question. Chronic toxicity in adults is generally attributed to supplemental doses of >7,500-15,000 μg RE/day, over weeks, months or years. However, there have been cases of toxicity associated with lower doses of ~1,500-3,000 μg RE/day. Determination of a threshold dose for chronic toxicity may be confounded by pre-existing disease, alcohol abuse, drug therapy and limited knowledge of dietary contribution. Children appear to be more susceptible than adults. Extreme vitamin A intolerance may have a genetic basis, although the precise metabolic defect has not yet been elucidated.

164. Epidemiology data have shown that the risk of hip-bone fracture may be doubled when retinol intake is >1500 μg RE/day, compared to an intake of 500 μg RE/day. Epidemiology studies have generally shown a negative association between vitamin A intake and cancer. However, positive associations have been made with incidence of oesophageal cancer, gastric cancer, cancer of the thyroid and prostate cancer, the latter particularly in older men.

165. **Developmental Toxicity (Teratogenicity) -** There are a number of case reports of vitamin A-associated birth defects. However, only one case involved a vitamin A exposure of <7,500 μg RE. Since 1990, seven epidemiology studies (five retrospective case studies and two prospective cohort studies) have been reported. Two studies suggested a possible teratogenic effect of vitamin A supplements at very high doses. However, a lack of statistical significance meant that the data were also consistent with a "no effect" interpretation. Four studies, including one prospective study, failed to establish any association between vitamin A supplementation and increased risk of birth defects. Three of these studies demonstrated that there was no association between vitamin A supplementation of >3,000 μg RE and increased risk of birth defects. In contrast, one prospective study suggested a teratogenic effect of vitamin A at relatively modest doses of 3,000-9,000 μg RE. The data available are consistent with the suggestion that consumption of less than 3,000 μg RE/day from vitamin supplements is safe. However, a threshold for any teratologic effects remains to be identified.

Potentially vulnerable groups

166. Groups potentially vulnerable to vitamin A toxicity include those with a possible genetically determined intolerance, the young, the elderly, women suffering from osteoporosis, diabetics, the protein-malnourished, chronic renal failure and haemodialysis patients and individuals with compromised liver

function. Retinol may also represent a teratogenic danger to the unborn child, particularly within the first trimester of pregnancy.

Oral toxicity in animals

167. **Acute and chronic toxicity -** Vitamin A toxicity in animals is dependent upon dose, formulation, duration, species, and age. Oral LD50 values in rat and mouse are > 2.5 g/kg (2.5 x 10^6 µg RE). Chronic toxicity in the adult rat begins at about ~ 3,000 µg RE (3 mg) /kg/day). Younger animals may be more susceptible.

168. Hypervitaminosis A causes anorexia, weight loss, anaemia, acachexia and ultimately death. Chronic manifestations include effects to skin (hair loss, localised erythema and thickened epithelium), effects to internal organs (fatty infiltration of the liver and fatty changes in the heart and kidney, testicular changes, degeneration of myocardial fibres), blood effects (decrease haemoglobin, transient increases in circulating lipids and serum cholesterol, elevated serum triglycerides), skeletal effects (limping gait, spontaneous fractures, hind leg paralysis, increased osteoclast activity, decreased osteoblast activity, bone elongation without thickening, reduced formation of dentine and atrophy of odontoblasts), subcutaneous and intramuscular haemorrhage, hypothrombinaemia, internal haemorrhage, and inflammation of nasal passage, gut, and conjunctiva. Hypervitaminosis results in the development of gallstones in hamster.

169. **Developmental Toxicity -** Retinol has been shown to be teratogenic in laboratory animals in the following order of increasing susceptibility; rat and mouse, hamster, monkey, and rabbit. Lowest teratogenic doses reported in rat, monkey and rabbit are 35,000, 6,000 and 2,500 µg RE/kg, respectively. Malformations are largely analogous to those associated with synthetic retinoid teratogenicity in humans. Permanent learning disabilities have been seen in F344 rats born to dams exposed ≥ 3,000 µg RE/kg.

Mutagenicity, genotoxicity, carcinogenicity

170. The weight of evidence suggests that neither retinol nor retinyl ester is genotoxic. There is no evidence to suggest that retinol or retinyl ester is carcinogenic in laboratory animals and at sub-toxic doses, vitamin A can be highly effective in preventing chemically-induced tumours.

Mechanisms of toxicity

171. Most vitamin A- and retinoid-induced toxicities are now thought to result from nuclear receptor-mediated interaction and inappropriately altered gene expression.

Guidance on high intakes and recommendations on maximum supplementation levels

172. The Committee on the Medical Aspects of Food Policy (COMA) (DH, 1991) recommended that regular intake of vitamin A should not exceed 9,000 μg RE/day or 7,500 μg RE/day in adult men and women, respectively. Recommended maximum intakes for infants and children were lower, depending on age. Women of child-bearing potential have been advised by the UK Chief Medical Officer (DH, 1990) not to consume liver or liver products or to take vitamin A containing supplements except on the advice of a doctor or antenatal clinic. The Teratology Society (1987) recommended that supplements of 2,400 μg RE/day should be considered a maximum. The American College of Obstetricians and Gynaecologists (1995 & 1998) opined that routine supplementation during pregnancy was not recommended; in cases where dietary intake of vitamin A may not be adequate, e.g. strict vegetarians, dietary intake should be supplemented; carefully supervised supplementation may be desirable for some pregnant women, such as recent emigrants from countries where vitamin A deficiency is endemic; supplementation with 5,000 IU (1,500 μg RE) should be considered the maximum intake prior to and during pregnancy. The IVACG (1998, 1986) recommended that it was safe for fertile women, independent of vitamin A status, to be given as much as 3,000 μg RE/day at any time during pregnancy.

GLOSSARY

AUC	area under the curve
BBM	brush border membrane
bwt	body weight
cGMP	cyclic guanosine monophospate
cGTP	cyclic guanosine triphosphate
C_{max}	maximum concentration
COMA	UK Committee on Medical Aspects of Food and Nutrition Policy
CRABP	Cellular retinoic acid-binding protein
CRBP	cellular retinol-binding protein
DH	UK Department of Health
er	endoplasmic reticulum
FAO	Food and Agricultural Organisation
FDA	Food and Drugs Administration (US)
GTP	guanosine triphosphate
IRBP	Inter-photoreceptor retinol-binding protein
IU	International Unit
IVACG	International Vitamin A Consultative Group
LOAEL	lowest observed adverse effect level

LD50	Lethal Dose 50
NRC	National Research Council (US)
NTD	neural tube defect
PML	promyelocytic leukaemia
RAR	nuclear retinoic acid receptor
RXR	nuclear retinoic acid receptor
RDA	recommended daily allowance (US)
RDI	reference daily intake (FAO/WHO)
RE	retinol equivalent
RNI	reference nutrient intake (UK)
RBP	retinol-binding protein
T3	triiodothyronine
T4	thyroxine
TTR	transthyretin
WHO	World Health Organisation

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ANNEX 1

Table summarising cases of low-dose vitamin A toxicity in humans

Toxicity	Dose/intake of preformed vitamin A μg RE	Comments	Reference
Chronic toxicity in children	>720/kg/d x weeks, months or years	65 cases; age range 49 days –144 months; vitamin A intake from food mostly not specified; in many instance, preparation also contained high levels of vitamin D	Bauernfeind, 1980
Chronic toxicity in adults	>15,000/d (majority >30,000/d) x weeks, months or years	75 cases; vitamin A intake from food mostly not specified	Bauernfeind, 1980
Chronic toxicity in adults	3,000-15,000/d x weeks or years	10 low-dose cases, lower range may reflect high, but unspecified, vitamin A intake from food	Hathcock *et al*, 1990
Chronic toxicity in children	~500-1,900 /kg/d x months or years	6 low-dose cases, vitamin A intake from food was not specified	Hathcock *et al*, 1990
Chronic toxicity	0 (placebo)-10,800/d x 6 months	Randomised double blind controlled trial, vitamin A supplements, n=376, minor symptomatic and physical changes affecting skin and mucous membranes with supplements ≥3000 RE/d; vitamin A intake from food not specified	Wald *et al*, 1985
Chronic hepatotoxicity	~7,500-15,000/d x weeks or years	Lower range may reflect liver compromised patients	Leo *et al*, 1988
Chronic hepatotoxicity	6,000-13,500/d 7-10 years	3 members within same family history of supplement taking; hepatitis could not be explained by infectious, metabolic or immunologic disorder	Minuk *et al*, 1988
Chronic hepatotoxicity (irreversible)	7,500-≥30,000/d 1-10 years	41 cases, mean age ~49 (range 7-76), intake uncertain in 12 cases	Geubel *et al* , 1991
Chronic hepatotoxicity (reversible)	1,500/d x 10 years	Single case, adult, history of alcohol abuse, jaundice, hepatitis and blood transfusions, vitamin A intake from food was not specified	Oren and Ilan, 1992
Chronic hepatotoxicity	7,500/d >6 yrs	Single case, adult, history included hypertension, diabetes mellitus, ischemic cardiomyopathy and hypothyroidism and appropriate treatments, vitamin A intake from food was not specified	Kowalski *et al*, 1994 and references therein
Chronic hepatotoxicity	~10,000/d x 8 years	Single case, adult	Bergenss and Roels, 1965
Toxicity in vitamin A-intolerant individual	1,500/d x 5 years	Single case, child, 10.5 yrs, vitamin A from multi-vitamin supplement, intake from diet unspecified	Schurr *et al*, 1983
Toxicity in vitamin A-intolerant individuals	~1,500-3,000/d,	Two male siblings, 2yrs old and 5 mo old, the eldest died, vitamin A mainly from chicken liver spread	Carpenter *et al*, 1987
Pseudotumor cerebri	6,000/d	Single case, adult, contribution from diet unspecified	Friedland and Burde, 1996
Intracranial hypertension	Supplements ≥ 3,000/d	Single case, adult, vegetarian, vitamin A intake from food not specified	Wettstein and O'Neill, 1998

ANNEX 2

Table summarising case-control and cohort studies from 1990 to present on periconceptual vitamin A exposure and birth defects

Study design	N	Preformed vitamin A Exposure (RE/d) and outcome	Comments	Reference
Nested (unmatched) case-control within a surveillance system, monitoring malformations in >1000,000 births in 58 hospitals in Spain	11,293 non-chromosomal malformation cases, 11,193 controls	For vitamin A exposures (as retinyl palmitate alone or combined with other vitamins) >3,000 RE: OR = 1.1(CI 0.5, 2.5) For vitamin A exposures (alone or combined with other vitamins) >12,000 RE: OR = 2.7 (CI 0.8, 11.7) For vitamin A exposures (alone): OR = 9.9 (CI 1.4, 430.1) P=0.006 For vitamin A exposures (alone) <12,000 RE: OR = 0.5	Large screening programme, some malformations of doubtful relevance e.g. hip instability, capillary angioma. No pattern of malformation found to be similar to isotretinoin embryopathy Mothers asked "open-ended" questions about brands of drugs taken during pregnancy; some women may not have considered vitamins as drugs Only 16 cases and 14 controls exposed to ≥ 3,000 RE Only 11 cases and 4 controls exposed to ≥12,000 RE Some exposures may have occurred too late to be of relevance to the birth defects discovered **Comments in review by Miller *et al* (1998) (Writers note: no data on diet?)**	Martinez-Frias and Salvador, 1990
Case-control within a multi-hospital surveillance system, birth defects identified from hospital records	2,658 cranial neural crest-related defects, 2,609 controls with other malformations	Exposure 1st lunar month; OR = 2.5 (CI 1.0, 6.2, 15 exposed cases) Exposure 2nd lunar month ; OR = 2.3 (CI 0.9, 5.8)	Women asked specifically if and when they had taken vitamins, folic acid or iron; No data collected on dose, number of supplements or diet. However, supplements of 1,500-7,500 were known to be available during the study Assumption made that cranial neural crest defects were most likely vitamin A-related, and therefore not included in the control group Risk in those taking vitamin A alone higher than in those taking multivitamins (but not statistically significant) Strength of study was classification of malformation **Comments in review by Miller *et al* (1998), Dolk *et al* (1999)**	Werler *et al*, 1990
Prospective, from open pre-natal screening programme	22,748 pregnancies, 339 with malformations	Total intake >4,500 RE - Prevalence ratio (vs ≤ 1,500 RE): Cranial neural crest defects 3.5 (CI 1.7, 7.3) All defects 2.2 (CI 1.3, 3.8) Supplement exposure >3,000 RE – prevalence ratio vs ≤ 1,500 RE: Cranial neural crest defects 4.8 (CI 2.2, 10.5) All defects 2.4 (CI 1.3, 4.4) Smoothed regression used to calculate that risk in highest exposure category exceeded risk in the lowest category by one defect in 57 births (no CI given) 7 babies in high exposure category reported to have had neural crest defects, coincided with reported exposure in first month of pregnancy and before conception	Study originally designed to evaluate relationship between neural tube defects and folic acid (Milunsky et al, 1989), but included data on <u>total</u> vitamin A intake 3 mo either side last menstrual period (date self reported); information on retinol supplementation not available for 201 pregnancies, and the authors chose to estimate No geographical/population base and no denominator population; report contains no discussion of study design biases Identification of malformations was by physician in 76.5% of cases: Mothers supplied information on the remainder, considered a poor source of information on birth-defect diagnosis Some defects were subject to misclassification; malformations of the central nervous system, eye and heart were included in cranial neural crest defects but experimental studies have not shown a contribution of cranial neural crest cells to these abnormalities; it is impossible to determine effect of misclassification upon risk estimate	Rothman *et al*, 1995

			Increased risk of cranial neural crest defects based on 7 cases in the higher dose-group category, however, some cases may have been misclassified, in terms of exposure and/or diagnosis Rate of malformations considered low **Comments from Miller *et al* (1998); Werler *et al* (1996)** ~1.4% of the study population averages >3,000 RE/d from supplements	
Case-control within a population-based surveillance system	malformation cases, n=4,918 controls, n=3,029	Mulivitamins alone: All defects OR = 0.94 (CI 0.86, 1.03) Cranial neural crest defects OR = 0.86 (CI 0.76, 0.97) Multivitamins and vitamin A tablet: All defects OR = 0.60 (CI 0.38, 1.29) Cranial neural crest defects OR = 0.69 (CI 0.24, 1.91)	High power to defect differences; No data on vitamin A intake **Comments in review by Miller *et al* (1998)** Data contained in letter form only, not a full paper	Khoury *et al*, 1996 – letter,
Case-control within a population-based surveillance system	Clefts study 925 cases, 871 controls Heart study 254 cases, 561 controls	Presumed exposures > 3,000 RE Clefts: OR = 0.55 (CI 0,21, 1.5) Heart: OR = 0 (CI 0, 2.2)	Women interviewed an average of 3.5 yrs after delivery; only estimated data on vitamin A intake Data contained in letter and abstract form only, not a full paper	Shaw *et al*, 1996 – letter Lammer *et al*,1996-abstract
Geographically based case control	Neural tube defects, n=548 Major malformations other than neural tube defects, n=387 Normal controls, n=573	Major malformations; <1,500 reference >2,400 OR 0.79 (CI 0.40, 1.53) >3,000 OR 0.73 (CI 0.27, 1.96) results for NTDs similar to major malformations cranial neural crest defects <1,500 reference >2,400 OR 0.76 (CI 0.22 2.56) >3,000 OR 0.1.09 (CI.0.24, 4.98)	Data from National Institute of Child Health and Human Development of Neural Defects study Data on periconceptional exposure from supplements and fortified cereals collected by telephone between 1-5 months after birth defect was detected Further analysis showed no increased risk due to vitamin A exposure from supplements alone. Furthermore there was no evidence of teratogenic effect associated with organ meat consumption no evidence of retinoid embryopathy in subjects with intakes of 3,000- 15,000 RE) Power of study reduced by the small number of women taking very high doses of vitamin A (>1.5% of subjects exposed to >3,000 RE)	Mills *et al*, 1997
Multi-centred prospective control	n=311	Exposure during the first 9 weeks of gestation to >3,000 RE - median dose 15,000 (range 3,000-90,000) Prevalence rate of major malformations *vs* internal control group exposed to high vitamin A later in pregnancy 0.28 (CI 0.06, 1.23) Prevalence rate of major malformations *vs* internal control group undergoing non-teratogenic agent exposure 0.50 (CI 0.14, 1.76)	cohort of high vitamin A exposed women who directly or through their doctors, contacted European Teratology Information Services; exposure data obtained by detailed interview with follow up (with women and/or their doctors) 3-6 weeks post-delivery; ascertainment of major malformation was not obtained by the investigators. 3 infants born with major malformations, 2/152 exposed to <12,000RE/d, 1/159 exposed to >12,000RE/d, 0/120 exposed to >15,000RE/d power; 80% to detect an increased risk higher than 2.76 (alpha 0.10) using a 1:2 ratio of cases:controls and a control prevalence of 1.96%.	Mastroiacovo *et al*, 1999

OR = odds ratio; CI = 95% confidence interval

ANNEX 3

Clinical trials testing the effect of multi-vitamin supplements, containing vitamin A, on the prevention of NTDs

Study design	Preformed vitamin A supplementation (RE/d)	Outcome	Comment	Reference
Non-randomised trial	1,200	Reduction in recurrence of NTDs	Supplement taken from 28d prior to conception to date of second missed period Supplement contained folate	Schorah and Smithells, 1991
Randomised trial in high risk women Groups : Folate alone (n=298) *vs* Folate + Multivitamin (containing vitamin A, n=295) *vs* Multivitamin (containing vitamin A, n=302) alone *vs* Virtual placebo (n=300)	1,200	Recurrence of rate NTD in groups receiving folic acid reduced; folic acid 72% protective, RR = 0.28 (CI 0.12 - 0.71) Miller et al (1998) suggested that design allowed examination of vitamin A (multi-vitamins without folate) compared to no vitamins at all. NTD recurrence rate in group receiving vitamin A = 2.6% NTD recurrence rate in the group not receiving vitamin A = 4.3%	Study primarily concerned with prevention of NTDs by supplementation with folate Supplementation from pre-conception to12th week of gestation Miller et al,(1998) interpreted results as evidence that modest doses of vitamin A does do not increase risk of NTDs No detail on dietary exposure	MRC, 1991 Wald, 1992
Groups: Exposure to mulivitamins (containing vitamin A and folic acid) n = 1203 *vs* Control non-exposed group (n = 1510)	1,800	NTD rate significantly lower in multivitamin group the multivitamin group showed no significant increase in rate of any other category of malformation *vs* the expected Hungarian rate or control group population No retinoid syndromes cases were observed; Cleft lip (with or without cleft palate) occurred in 4 exposed pregnancies *vs* expected 1.2 (not statistically significant.); Cardiovascular defects were less common that expected	Exposure at l east 1 mo pre and 3 mo post-conception NTD reduction attributed to folate Cohort size too small to identify any teratogenic effect of vitamin A at such a low dose No detail on dietary exposure	Dudas and Czeizel, 1992
Case-control surveillance study No defects (n=35727 of which vit A (alone or multi-vit) exposed =3399) Defects (n=20830 of which vit A exposed =1642)	generally 1,800-2,700 but up to 30,000	11/1642 women using vitamin A gave birth to babies with congenital abnormalities rate of total congenital abnormalities in vitamin A treated group significantly lower ($P<0.001$) than non-treated; rates for most deformation groups lowered but significant only for pyloric stenosis ($p<0.05$) lower rate of vitamin A treatment during pregnancy and in the first trimester in most congenital abnormality groups	time and duration of supplementation varied, but generally started within first trimester one case of cleft lip born to mother using 4200 RE/d in mo 2. 4 women in control (no defects) group used >15000 RE/d within first trimester No detail on dietary exposure except corresponded to European level	Czeizel and Rockenbauer, 1998

ANNEX 4 TO EVM/00/02/P.REVISEDAUG2001

INTAKES OF RETINOL, β-CAROTENE, TOTAL CAROTENE AND TOTAL VITAMIN A (RETINOL EQUIVALENTS) FROM FOOD AND SUPPLEMENTS

The data presented on intakes of the above are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years[1][2][3][4][5]. As a report of the findings of the survey of young people aged 4 – 18 years has not yet been published these data have been deleted from this annex. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

Total intakes of retinol, total carotene, β-carotene and total vitamin A (retinol equivalents)

Tables 1-3 provide information on the median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively), of retinol (ie pre-formed retinol), β-carotene and total vitamin A (retinol equivalents[6]) by the British population, classified by age and sex. Both absolute and bodyweight adjusted intakes are given. In table 2, where data for β-carotene intakes were unavailable (in infants aged 6-12 months and adults aged 16-64 years) data for intakes of *total* carotene are provided. Sources of total carotene in foods are similar to those for β-carotene.

Absolute intakes for retinol decreased with age for pre-school children and increased with age for adults. Intakes at the 97.5%ile were 3-5 times the median in infants, pre-school children and young people but up to 16 times the median in adults aged 16-85 years and over. This again may reflect the relatively higher consumption of offal, including liver and liver products, by some adults. Retinol intakes adjusted for body weight showed a trend to decrease with age for pre-school children, young people and increase with age for females aged 16-64 years.

[1] Food and nutrient intakes of British infants. 1986
[2] National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3
[3] National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8
[4] Dietary and nutritional survey of British adults. 1986/7
[5] National Diet and Nutrition Survey of people aged 65 years and over. 1994/5
[6] Total vitamin A (retinol equivalents) is a measure of the overall potency of vitamin A and relates to the lower biological efficiency of carotenoids compared with retinol. 6μg β-carotene or 12μg of other active carotenoids (data on α-carotene and β-cryptoxanthin intakes were collected in NDNS surveys) are equivalent to 1μg retinol. Hence μg retinol equivalents = μg retinol + (μg β-carotene equivalents ÷ 6).

Total carotene intakes for adults aged 16-64 years apparently increased with age, and were generally higher in adults aged 16-64. This may be in part due to improvements in analytical methodology during the 1980s resulting in subsequent NDNS surveys (for pre-school children, young people and older people aged 65 years and over) using new analytical data for fruit and vegetables with lower carotenoid levels. The relatively high median intake of total carotene by infants (829μg/day) was predominantly provided by vegetables, commercial infant foods and meat dishes, which include dishes with carrots. (47%, 20% and 17% respectively). Intakes at the 97.5%ile were 2-5 times the median across all groups. β-carotene intakes adjusted for body weight showed a general trend to decrease with age for pre-school children, whereas corresponding intakes of total carotene increased with age for adults aged 16-64 years. This may reflect differences in the contribution of vegetables to intakes of total carotene in adults, young people and pre-school children (70%, 57% and 56% respectively).

Absolute median intakes of total vitamin A (retinol equivalents) were above the Reference Nutrient Intakes for each population group.

Sources of retinol and β-carotene in the diet

Tables 4 and 5 indicate the contribution made by different types of food to average intakes of retinol and β-carotene by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices.

The main source of retinol in this age group is milk and milk products, notably cheese excluding cottage cheese, followed by fat spreads, the main source of which was butter. As stated previously, the contribution of retinol from liver and liver products is low in this young age group and may also reflect a overall decreasing trend in the consumption of liver by the general population (1986 -1996, National Food Survey data).

The main source of β-carotene in this age group is vegetables, namely cooked carrots, followed by meat and meat products (which includes meat dishes containing vegetables) and beverages. β-carotene is frequently used as a colourant in certain products such as soft drinks.

UK legislation requires that vitamin A is added as a fortificant to margarine, which can be added as such or as its esters, β-carotene, or other biologically active carotenoids. Vitamin A is often also added to reduced fat spreads, and some breakfast cereals are also fortified.

Retinol and β-carotene intakes from supplements

For infants, only data on intakes of total vitamin A (retinol equivalents) from supplements are available. This provided around 8% of population average intakes

for this nutrient. For the other groups, dietary supplements provided around 2-19% of population average intakes of retinol. Dietary supplements made a negligible contribution to population average intakes of β-carotene in older people aged 65 years and over. Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately.

Table 6 shows the number of consumers of dietary supplements containing retinol in each age group (except for infants where only data on retinol equivalents is available), together with the median, range and upper level intakes of retinol from supplements for those who consumed them. The highest prevalence of vitamin A supplement use was in the infant group. Over 40% of infants took vitamin supplements containing retinol equivalents, obtaining 17% of their total intake from this source. At the time of this survey, the Panel on Child Nutrition of COMA[7] recommended supplementation with vitamins A, C and D for all infants from six months up to at least two years and preferably five years.

The high intakes of retinol from supplements in adults and older people free-living in the community was due in part to the use of cod liver oil supplements. Many multivitamins used were also a source of vitamin A. It should be borne in mind that the data for adults aged 16-64 years was collected in 1986/87 and use of supplements may have changed since then. The range of intakes from supplements was wide with maximum intakes from this source around 3,000μg per day.

Only a very small proportion of older people free-living in the community took supplements containing β-carotene as shown in table 7. For both males and females median intake of β-carotene from supplements was 125.0μg per day (range 93.8 - 1425.0μg per day), from cod liver oil with added β-carotene, and multivitamin and mineral supplements. Where data was available, none of the dietary supplements taken by other groups provided any β-carotene.

[7] Department of Health and Social Security (1988). Present day practice in infant feeding: third report. Report on Health and Social Subjects No 32. London: HMSO.

Table 1: Total intakes of retinol

	Absolute retinol intake (μg/day)			Bodyweight adjusted retinol intake (μg/kg bwt /day)[8]		
Age/sex	*intakes from food and supplements*					
	2.5%ile	Median	97.5%ile	2.5%ile	Median	97.5%ile
Infants (1986)						
6-12mths/M&F	157	449	1707	14.3	46.0	167.1
Pre-school children (1992/3)						
1½-2½ yrs/M&F	76.5	317	997	5.9	25.7	130.5
2½-3½ yrs/M&F	79.2	277	1056	5.4	18.6	107.4
3½-4½ yrs/M	89.7	277	1144	5.2	16.9	75.4
3½-4½ yrs/F	67.3	269	1388	3.7	16.6	90.2
Young people (1997/8)[9]						
4-6 yrs/M	81	238	1040	3.5	11.0	51.9
4-6 yrs/F	81	251	1015	4.0	12.5	45.0
7-10 yrs/M	88	259	1162	2.9	8.8	40.9
7-10 yrs/F	85	235	1004	2.7	7.5	34.9
11-14 yrs/M	74	270	990	1.3	5.9	20.0
11-14 yrs/F	59	214	895	1.0	4.6	18.3
15-18 yrs/M	89	293	996	1.2	4.4	12.3
15-18 yrs/F	60	232	940	0.9	3.9	16.5
Adults (1986/7)						
16-24 yrs/M	163	494	5788	2.0	6.9	73.6
16-24 yrs/F	119	401	5896	1.5	6.6	86.8
25-34 yrs/M	194	600	6671	2.3	8.0	97.6
25-34 yrs/F	106	456	6056	1.6	7.2	90.3
35-49 yrs/M	232	647	7510	3.2	8.5	87.3
35-49 yrs/F	136	519	5699	2.1	8.0	93.0
50-64 yrs/M	212	683	7405	2.9	8.4	88.0
50-64 yrs/F	192	562	5487	2.3	8.8	90.6
Older people free-living in the community (1994/5)						
65-74 yrs/M	153	444	5979	1.8	6.3	76.6
65-74 yrs/F	109	372	6080	1.5	6.1	80.3
75-84 yrs/M	165	444	5950	2.0	5.9	71.3
75-84 yrs/F	130	397	5975	1.9	5.9	83.4
85 and over/M	180	449	3917	2.7	6.4	55.4
85 and over/F	128	399	3816	1.9	6.9	87.9
Older people living in institutions (1994/5)						
65-84 yrs/M	193	473	3723	2.8	7.3	45.1
65-84 yrs/F	171	517	3101	2.7	8.0	57.3
85 and over/M	215	535	4101	2.5	8.2	61.7
85 and over/F	229	456	3594	3.2	7.9	56.2

[8] Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

[9] The survey of young people aged 4-18 years used new analytical data for retinol in pasteurised milk with lower retinol levels. This may partly explain the lower intakes of retinol in the 4-6 year group compared with the survey of pre-school children and infants, and in the 15-18 year group compared with the adults survey.

Table 2: Total intakes of beta-carotene

	Absolute beta-carotene intake (µg/day)			Bodyweight adjusted beta-carotene intake (µg/kg bwt/day)[10]		
Age/sex	*intakes from food sources*[11]					
	2.5%ile	Median	97.5%ile	2.5%ile	Median	97.5%ile
Infants (1986)[12]						
6-12mths/M&F	225	829	3140	17.4	81.9	316.9
Pre-school children (1992/3)						
1½-2½ yrs/M&F	130	609	2071	10.7	49.0	172.2
2½-3½ yrs/M&F	163	594	2609	11.2	40.4	182.7
3½-4½ yrs/M	134	630	3335	8.9	39.2	189.3
3½-4½ yrs/F	158	586	2793	9.3	36.2	166.6
Young people (1997/8)						
4-6 yrs/M	190	832	3437	7.9	40.5	169.0
4-6 yrs/F	232	951	2539	12.0	46.3	118.5
7-10 yrs/M	235	974	3318	6.5	33.0	111.8
7-10 yrs/F	245	1064	2665	7.9	34.4	85.3
11-14 yrs/M	229	1011	3459	5.2	23.9	75.2
11-14 yrs/F	212	892	3486	4.0	19.3	76.8
15-18 yrs/M	270	1153	5903	3.7	17.6	73.5
15-18 yrs/F	300	1142	3839	3.7	18.4	69.3
Adults (1986/7)[12]						
16-24 yrs/M	175	1229	6488	2.6	17.9	92.7
16-24 yrs/F	166	1179	5671	2.9	19.3	88.2
25-34 yrs/M	212	1676	7971	2.6	22.5	89.1
25-34 yrs/F	173	1567	6449	2.6	24.6	106.2
35-49 yrs/M	332	1999	7563	4.7	27.1	97.4
35-49 yrs/F	353	1934	6746	4.6	30.7	107.5
50-64 yrs/M	421	2360	8361	4.9	30.5	114.6
50-64 yrs/F	214	1848	7121	3.1	29.3	137.5
Older people free-living in the community (1994/5)						
65-74 yrs/M	216	1493	4401	3.3	19.2	60.9
65-74 yrs/F	196	1113	4835	2.9	16.5	70.7
75-84 yrs/M	229	1482	5272	3.0	18.6	70.1
75-84 yrs/F	162	1006	4029	2.9	16.5	60.6
85 and over/M	149	1419	5037	2.2	20.8	72.7
85 and over/F	176	1048	4495	2.7	17.6	81.2
Older people living in institutions (1994/5)						
65-84 yrs/M	415	1712	4177	7.5	25.1	53.7
65-84 yrs/F	291	1538	3574	5.3	24.0	59.2
85 and over/M	197	1750	4497	3.0	25.1	61.4
85 and over/F	357	1369	3401	6.2	24.1	56.9

[10] Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.
[11] Absolute intakes presented for all groups are from food sources only as the contribution of supplements was negligible.
[12] Total carotene - data for β-carotene unavailable.

Table 3: Total intakes of total vitamin A (retinol equivalents)

	Absolute total vitamin A (retinol equivalents)[13] intake (μg/day)			Bodyweight adjusted total vitamin A (retinol equivalents) intake (μg/kg bwt/day)[14]		
Age/sex	*intakes from food and supplements*					
	2.5%ile	Median	97.5%ile	2.5%ile	Median	97.5%ile
Infants (1986)						
6-12mths/M&F	282	670	1873	25.0	65.4	181.6
Pre-school children (1992/3)						
1½-2½ yrs/M&F	146	441	1837	11.1	36.2	145.7
2½-3½ yrs/M&F	142	417	1816	10.1	28.4	114.8
3½-4½ yrs/M	144	436	1632	8.5	26.7	94.8
3½-4½ yrs/F	132	408	1971	7.7	24.9	109.9
Young people (1997/8)						
4-6 yrs/M	131	432	1314	5.6	21.1	66.0
4-6 yrs/F	166	437	1265	8.7	21.0	53.0
7-10 yrs/M	196	460	1451	5.5	16.0	49.5
7-10 yrs/F	174	460	1331	6.2	14.1	44.2
11-14 yrs/M	145	485	1277	2.3	10.7	29.7
11-14 yrs/F	126	380	1296	2.1	8.7	24.8
15-18 yrs/M	188	584	1579	2.5	8.3	23.6
15-18 yrs/F	156	475	1413	2.4	7.7	24.6
Adults (1986/7)						
16-24 yrs/M	224	805	5962	2.9	11.4	77.3
16-24 yrs/F	226	658	6159	3.1	11.1	91.7
25-34 yrs/M	279	995	7529	4.1	13.0	103.2
25-34 yrs/F	177	738	6700	2.3	11.9	99.7
35-49 yrs/M	389	1118	8136	5.0	14.6	98.3
35-49 yrs/F	309	926	6461	4.6	14.6	101.8
50-64 yrs/M	369	1144	7801	4.9	14.5	97.8
50-64 yrs/F	330	1024	5891	4.0	16.5	96.6
Older people free-living in the community (1994/5)						
65-74 yrs/M	258	795	6578	3.5	10.1	86.1
65-74 yrs/F	234	653	6495	3.1	10.7	84.0
75-84 yrs/M	226	787	6793	3.1	10.4	80.0
75-84 yrs/F	208	632	6069	3.5	9.5	90.4
85 and over/M	258	780	4125	3.5	11.5	60.0
85 and over/F	217	642	4303	3.1	11.0	97.0
Older people living in institutions (1994/5)						
65-84 yrs/M	357	818	4086	5.3	12.8	49.8
65-84 yrs/F	339	767	3464	4.9	12.5	62.6
85 and over/M	404	913	4487	5.8	13.1	67.5
85 and over/F	329	735	3938	6.1	12.7	61.9

[13] Total vitamin A (retinol equivalents) is a measure of the overall potency of vitamin A and relates to the lower biological efficiency of carotenoids compared with retinol. 6μg β-carotene or 12μg of other active carotenoids (data on α-carotene and β-cryptoxanthin intakes were collected in NDNS surveys) are equivalent to 1μg retinol. Hence μg retinol equivalents = μg retinol + (μg β-carotene equivalents ÷ 6).

[14] Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

Table 4[15]: Sources of retinol in the diet

Food Type	Contribution of food types to average daily intake of retinol ug/day	% of total
Cereal and cereal products	54	18
- of which buns, cakes and pastries	*17*	*6*
- of which pizza	*15*	*5*
Milk and milk products	93	31
- of which cheese (excluding cottage cheese)	*40*	*13*
- of which semi skimmed milk	*23*	*7*
Egg and egg dishes	22	7
Fat spreads	63	21
- of which butter	*15*	*5*
Meat and meat products	54	18
- of which liver and liver dishes	*37*	*12*
Fish and fish dishes	3	1
Vegetables, potatoes and savoury snacks	7	2
Fruits and nuts	0	0
Sugar, confectionery and preserves	3	1
Beverages	<1	<1
Miscellaneous	4	1
Total intake from food	**304**	**100**
Intake from dietary supplements	*16*	*5*
Total intake from food and supplements	**320**	**100**

Table 5[15]: Sources of β-carotene in the diet

Food Type	Contribution of food types to average daily intake of β-carotene ug/day	% of total
Cereal and cereal products	96	6
Milk and milk products	59	4
Egg and egg dishes	4	<1
Fat spreads	59	4
Meat and meat products	191	13
- of which beef, veal and dishes	*84*	*6*
Fish and fish dishes	5	<1
Vegetables, potatoes and savoury snacks	851	57
- of which carrots, cooked	*404*	*27*
Fruits and nuts	27	2
Sugar, confectionery and preserves	8	<1
Beverages	116	8
Miscellaneous	75	5
Total intake from food	**1492**	**100**
Intake from dietary supplements	*0*	*0*
Total intake from food and supplements	**1492**	**100**

[15] NDNS: young people aged 4-18 years. 1997/8. 15-18 year group

Table 6: Retinol intake from supplements

	Consumers of retinol supplements		Retinol intake from supplements (consumers only) (μg/day)	
Age/sex	*Number*	*%*	*Median*	*Range*
Infants (1986)[16]				
6-12 mths/M&F	213	44	143	*
Pre-school children (1992/3)				
1½-4½ yrs/M&F	245	15	375	14 - 2057
Young people (1997/8)				
4-6 yrs/M&F	56	16	414	57 - 800
7-10 yrs/M&F	51	11	332	57 - 800
11-14 yrs/M	16	7	221	57 - 750
11-14 yrs/F	7	3	468	214 - 750
15-18 yrs/M	8	4	371	171 - 750
15-18 yrs/F	9	4	164	57 - 800
Adults (1986/7)				
16-64 yrs/M	50	5	1029	34 - 2679
16-64 yrs/F	104	9	651	3 - 3150
Older people free-living in the community (1994/5)				
65 and over/M	73	12	750	17 - 2250
65 and over/F	96	15	750	1 - 1600
Older people living in institutions (1994/5)				
65 and over/M	4	2	315	35 - 800
65 and over/F	7	3	86	60 - 750

* No data available

Table 7: β-carotene intake from supplements[17]

	Consumers of β-carotene supplements		β-carotene intake from supplements (consumers only) (μg/day)	
Age/sex	*Number*	*%*	*Median*	*Range*
Older people free-living in the community (1994/5)				
65 and over/M	2	<1	125	125
65 and over/F	6	<1	125	94 - 1425

[16] Retinol equivalents - data for retinol unavailable.

[17] Data provided for older people aged 65 years and over free-living in the community only as none of the dietary supplements taken by other groups provided any β-carotene. (Note: corresponding data for infants is unavailable).

ANNEX 3 TO EVM/00/02/P.REVISEDAUG2001

Vitamin A: Summary table of selected nutrition related information and existing guidance

Unit of usage	**µg RE/day**		**Tg RE/100 kcal**	**Tg RE/100g**
	Male	Female		
UK DRV[18] for adults (19-50+) LRNI EAR RNI	 300 500 700	 250 400 600		
Mean adult UK dietary intake Adults (16-64)[19] 65 years and over[20] free living institutionalised	 1277 1173 1054	 1133 969 962		
EU labelling RDA[21]	800			
Supplemental doses	800 – 2250			
Regulations Infant formula[22] Cereal-based baby foods[23] Weight reduction[24] whole daily diet replacement meal replacement Spreadable fats[25]	 at least 700 at least 210 per meal		 60-180 60-180	 800 - 1000
Maximum total safe daily intake COMA 1991[1] EHPM 1997[26]	9000 7500 (short term) 3000 (long term)	7500		
Maximum supplement levels WHO 1998	2400 (in pregnant women habitually consuming vitamin A at a level of RDA or above)			

[18] Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.
[19] Dietary and nutritional survey of British adults. 1986/7
[20] National Diet and Nutrition Survey of people aged 65 years and over. 1994/5
[21] The Food Labelling Regulations 1996
[22] The Infant Formula and Follow-on Formula Regulations 1995
[23] The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1997.
[24] The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.
[25] The Margarine Regulations (Statutory Instrument, 1967).
[26] Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.