Review of Dietary Advice on Vitamin A

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Scientific Advisory Committee on Nutrition

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1 Summary

Background

1. In May 2003, the Expert Group on Vitamins and Minerals (EVM), an independent expert advisory committee, reported on safe intake levels of vitamins and minerals in food supplements and fortified foods sold under food law (EVM, 2003). Safe Upper Levels (SULs) were recommended when supported by adequate available evidence. A Guidance Level was set when the evidence base was inadequate to determine a SUL. Guidance Levels, are less secure than SULs because they have been derived from limited data.

2. The EVM set a Guidance Level for retinol intake of 1500 µg/day, for adults, based on evidence that intakes above this level may increase the risk of bone fracture. There were insufficient available data to set a Guidance Level for children.

3. Following findings from the 2000/1 National Diet and Nutrition Survey (NDNS) for adults aged 19 to 64 years (Henderson et al, 2003), that the diet of 9% of men and 4% of women may include retinol at levels above 1500 µg/day, the Scientific Advisory Committee on Nutrition (SACN) was asked by the Food Standards Agency (FSA) to reassess dietary advice to consumers on foods and supplements containing retinol.

Methodology

4. The studies on retinol and bone health previously considered by the EVM were reassessed from a nutritional perspective. The SACN framework for risk assessment (2002) was used as a template to identify and assess evidence published since the EVM report.

5. Data from the NDNS series were used to model the effect of reducing consumption of foods that are particularly rich sources of retinol, or supplements containing retinol, on the number of consumers exceeding the EVM Guidance Level of 1500 µg/day.

6. The effect of animal feeding practices on the liver retinol content of livestock and poultry was also considered.

Main findings from the review of evidence on retinol and bone health

7. Bone fractures, bone mineralisation, and biochemical markers of bone turnover, were the outcomes of interest assessed in the studies considered.

8. Five studies considered bone fracture as an outcome measure. Two of theses studies showed a significant association between retinol intake and bone fracture risk and 1 study found a significant association between serum retinol concentration and bone fracture risk.

9. Seven studies examined bone mineral as an outcome measure. Four of these were cross-sectional and three were prospective in design. One cross-sectional study found a significant association between retinol intake and lower BMD. One prospective study found no overall association between retinol intake and BMD but a significant negative association after stratification by supplement use for supplement users and a positive association for nonusers, for women only; 2 prospective studies found a significant association between retinol intake and an increase in BMC/BMD.

10. One intervention study found no relationship between supplementation with very high doses of retinol and serum markers of skeletal turnover.

Dietary intakes of retinol in Great Britain

11. Data from the NDNS for adults aged 19-64 years (Henderson et al, 2003) indicate that 9% of men and 4% of women have retinol intakes exceeding the Guidance Level of 1500 μg/day. The NDNS of people aged 65 years and over (Finch et al, 1998) suggest that 11% of men and 10% of women have retinol intakes exceeding 1500 μg/day.

12. Liver was a major source of retinol for those with retinol intakes greater than 1500 µg/day, contributing 70% of the total retinol intake for adults aged 19-64 years (Henderson et al, 2003) and 83% of the total retinol intake for adults aged 65 years and over (Finch et al, 1998).

13. Dietary supplements (including fish liver oils) also make an important contribution to retinol intakes of consumers that exceed 1500 µg/day, contributing 16-17% of the total retinol intake for adults aged 19-64 years (Henderson et al, 2003) and 8% of the total retinol intake for adults aged 65 years and over (Finch et al, 1998).

14. Whilst population intakes of retinol from consumption of liver have decreased since 1986/7, there has been an increase in retinol intakes from supplements. The retinol content of liver has also fallen over this time period. Overall, population intakes of retinol are less than they were in 1986/7.

Retinol content of liver and animal feeding practices

15. An adequate retinol intake is essential for maintaining productivity, reproduction and the immune status of poultry and livestock. The veterinary implications of lower levels of retinol supplementation would need to be determined if a reduction in retinol supplementation of poultry and livestock is considered as part of a strategy to reduce retinol intake by human consumers.

The potential impact of dietary change to reduce retinol intakes

16. The scenario modelling for adults aged 19-64 years, and adults 65 years and over, suggests the most effective way to prevent the occurrence of any retinol intakes above 1500 µg/day would be by removing both main sources of retinol, i.e. liver and supplements.

17. For adults aged 19-64 years, this scenario may lead to an unacceptable increase in the proportion of the population with intakes falling below the LRNI for total vitamin A intake. For adults 65 years and over, this scenario does not have any major effects on the proportion of the population with intakes falling below the LRNI for total vitamin A intake.

Conclusions

18. The evidence for an association between high intakes of retinol and poor bone health is inconsistent. There are some epidemiological data to suggest that retinol intakes of 1500 µg/day and above are associated with an increased risk of bone fracture. Data published since the EVM report do not strengthen the evidence for an association between retinol intake and bone health.

19. In the UK, liver is the major source of retinol for those with retinol intakes exceeding 1500 µg/day. Supplements also make an important contribution to retinol intakes of those consuming more than 1500 µg/day of retinol. Overall, retinol intakes in the UK were less in 2000/1 than in 1986/7.

Recommendations

20. There is currently insufficient evidence on the association between bone health and retinol intakes above 1500 µg/day to justify a change in dietary advice to all consumers regarding consumption of foods or supplements containing retinol.

21. As a precaution, however, it may be advisable for regular consumers of liver (once/week or more) not to increase liver intakes or take supplements containing retinol (including those containing fish liver oil).

22. It may also be advisable for population subgroups at increased risk of osteoporosis, such as postmenopausal women and older people, not to consume more than 1500 µg/day of retinol. This could be achieved by limiting intakes of liver and limiting intakes of supplements containing retinol (including those containing fish liver oil).

23. Further research is required on the relationship between retinol intake and fracture risk. Prospective studies, with more reliable and long-term assessments of retinol intake, are necessary to clarify whether high intakes have a detrimental effect on bone.

24. A reduction in retinol content of poultry and livestock feed as part of a strategy to reduce the retinol intake of regular consumers of liver should be explored further. The implications of lower levels of retinol supplementation for the welfare and productivity of poultry and livestock would need to be determined if such a strategy is considered.

25. Consideration should also be given to reducing the levels of retinol in supplements.

2 Background

Terminology

26. There are two forms of vitamin A: preformed vitamin A (retinol), is found in foods of animal origin and in some fortified foods such as margarine; provitamin A plant carotenoids, are precursors of vitamin A and can be converted to retinol in the body. Provitamin A carotenoids are less biologically active than retinol. The total vitamin A content of a food, i.e., the sum of retinol and provitamin A carotenoids, is measured relative to the activity of retinol and is expressed as micrograms (µg) of retinol equivalents (RE) (see paragraph 43).

Advice issued by the Expert Group on Vitamins and Minerals on Vitamin A Intake

27. In May 2003, the Expert Group on Vitamins and Minerals (EVM), an independent expert advisory committee, reported on safe intake levels of vitamins and minerals in food supplements and fortified foods sold under food law (EVM, 2003). (See Annex 1 for information on current legislation regarding food supplements and food fortification.)

28. The EVM carried out a detailed review, which included both toxicological and nutritional considerations. The EVM terms of reference are set out in Annex 2. Safe Upper Levels (SULs) were recommended when supported by sufficient data. The SUL represents an intake that can be consumed daily over a lifetime without significant risk to health and is based on adequate available evidence. A Guidance Level was set when the evidence base was inadequate to determine a SUL. Guidance Levels represent an approximate indication of levels that would not be expected to cause adverse effects. They are less secure than SULs because they have been derived from limited data.

29. The EVM set a Guidance Level for vitamin A intake of 1500 µgRE/day, for adults, based on evidence that intakes above this level may increase the risk of bone fracture. Although the Guidance Level was set for vitamin A, it refers specifically to retinol. A Guidance Level was not set for children as there were no data that allowed specific conclusions to be drawn. Children have an increased requirement for vitamin A, relative to body size (Department of Health, 1991), which also adds uncertainties.

Terms of reference

30. Following findings from the 2000-1 National Diet and Nutrition Survey (NDNS) for adults aged 19 to 64 years (Henderson et al, 2003), that the diet of 9% of men and 4% of women may include retinol at levels above 1500 µgRE/day, the Scientific Advisory Committee on Nutrition (SACN) was asked by the Food Standards Agency (FSA) to reassess dietary advice to consumers on foods and supplements containing vitamin A. A subgroup on vitamin A was established, with the following terms of reference:

- To review the current advice to consumers on vitamin A intakes and consumption of liver;

- To consider other strategies that might reduce the retinol intake of higher consumers.

Current Government recommendations on vitamin A intake

31. In 1991, the Committee on Medical Aspects of Food Policy (COMA) revised the dietary reference values (DRVs) for food energy and nutrients in the UK (Department of Health, 1991). The Reference Nutrient Intake (RNI) is the amount of a nutrient that is considered sufficient to meet the requirements of 97.5% of the population. The Lower Reference Nutrient Intake (LRNI) represents the amount of a nutrient which is likely to meet the needs of 2.5% of the population. For adults, the RNI for total vitamin A was set at 700 µgRE/day for men and 600 µgRE/day for women; the LRNI was set at 300 µgRE/day for men and 250 µgRE/day for women.

32. The RNIs set for infants and children are: 350 µgRE/day for 0-12m; 400 µgRE/day for 1-6y; 500 µgRE/day for 7-10y; 600 µgRE/day for 11-14y. For girls 11y and above and boys 15y and above, the RNIs are the same as those for adults. The LRNIs were set at: 150 µgRE/day for 0-12m; 200 µgRE/day for 1-6y; 250 µgRE/day for 7-14y. For girls 11y and above and boys 15y and above, the LRNIs are the same as those for adults.

33. The Department of Health (DH) recommends that pregnant women, or women who might become pregnant, should not take supplements containing vitamin A unless advised to do so by their general practitioner, or eat liver or liver products (DH, 1990). The advice is based on the teratogenic[1] risks associated with retinol. The threshold level for this effect is unclear but the lowest supplemental dose associated with teratogenic risk is 3000 µg/day (Rothman et al, 1995). The EVM (2003) also considered the teratogenic risks associated with retinol and endorsed 3000 µg/day as the threshold level for teratogenicity.

34. Retinol intakes that have been associated with bone fracture (1500 µg/day) are lower than those associated with teratogenesis.

Current recommendations on vitamin A intake in the USA and Europe

35. In 2001, the Food and Nutrition Board (FNB) of the Institute of Medicine in the USA, established a set of Dietary Reference Intakes for vitamins and minerals to replace previously published Recommended Dietary Allowances (RDAs) and Recommended Nutrient Intakes (RNIs) for the USA and Canada (FNB, 2001). In determining the Tolerable Upper Intake Level[2] (UL) for retinol, the possible risks of bone fracture associated with high intakes were considered. The FNB concluded that the relevant studies were conflicting and were not used to determine the UL for retinol.

1 A teratogen is an agent or substance that can cause malformations of an embryo or fetus.
2 The Tolerable Upper Intake Level represents the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

36. For adults, 3000 µg/day was established as the UL for retinol. For women of reproductive age, the UL was based on evidence for teratogenicity; for all other adults, the UL was based on evidence for liver abnormalities. ULs were also established for infants and children. For infants, case reports of hypervitaminosis A were used to derive the UL. The UL for children was extrapolated from the value of 3000 µg/d for adults, on the basis of relative body weight (see Table 1, Annex 3).

37. In September 2002, as part of its review of the upper intake levels of individual vitamins and minerals unlikely to pose adverse health risks, the European Scientific Committee on Food (SCF) established a UL for retinol intakes of 3000 µgRE/day for adults, based on the teratogenic risks (SCF, 2000). The SCF noted that findings on bone density and risk of fracture were reported at lower daily intakes than other adverse effects but concluded that the available data did not provide sufficient evidence of causality and were not appropriate for establishing a UL. As the UL may not adequately address the possible risk of bone fracture in particular vulnerable groups, postmenopausal women, who are at greater risk of osteoporosis and fracture, were advised to restrict their intake to 1500 µgRE/day.

38. ULs for children were also established, based on the value of 3000 µgRE/day for adults, with correction for differences in basal metabolic rate compared to adults (See Table 2, Annex 3).

3 Introduction

39. Vitamin A is a fat-soluble vitamin and is required for vision, embryogenesis, growth, immune function, and for normal development and differentiation of tissues (Garrow et al, 2000).

40. Vitamin A is a generic term, which refers to a number of different compounds known as *retinoids*, with varying degrees of biological activity. Dietary vitamin A can be obtained in two forms:

- Preformed vitamin A (retinol) is found only in foods of animal origin and in fortified foods such as margarine.[3] The parent compound of this group is all-*trans* retinol, which is the alcohol form of vitamin A. Retinyl esters are fatty acid ester derivatives of all-*trans* retinol, retinal is its aldehyde form, and retinoic acid is the acid form. The main sources of retinol are liver, dairy products, eggs, butter and margarine.

- Provitamin A carotenoids in plant foods are retinol precursors and can be converted to retinol in the body. Approximately 50 out of the 600 carotenoids found in nature, can be converted into retinol. Provitamin A carotenoids commonly found in food are β-carotene, α-carotene and β-cryptoxanthin. β-carotene is the most important of the provitamin carotenoids in terms of its relative provitamin A activity and quantitative contribution to the diet. Provitamin A carotenoids are found in the yellow-orange pigments of vegetables and fruits, such as carrots and sweet potatoes, and dark green vegetables, e.g., spinach.

3 *The Spreadable Fats (Marketing Standards) (England) Regulations 1999 (Statutory Instrument 1999/2457)(Regulation 4):* Any margarine sold by retail is required to be fortified with vitamin A at levels comparable with or higher than butter, i.e., not less than 800µg and not more than 1000µg per 100g margarine.

41. β-carotene and other provitamin A carotenoids undergo oxidative cleavage in the intestinal mucosa to retinaldehyde, which is subsequently reduced to retinol. Some carotenoids are also absorbed into the blood intact. Retinol is absorbed from the intestine and transported in chylomicrons to the liver, where it is stored in the form of retinyl esters. Retinol is mobilised from the liver, normally bound to retinol-binding protein (RBP), and released into the plasma. When it reaches target cells, retinol is converted to retinoic acid, which exerts its effects by binding to specific nuclear receptors.

42. The absorption efficiency of retinol is high, between 70-90% (Sivakumar and Reddy, 1972). The bioavailability of provitamin A carotenoids, i.e., the amount available for utilisation (Jackson, 1997), is lower, ranging from less than 5% to 50% (Garrow et al, 2000). It is influenced by a number of factors including: type of carotenoid; molecular linkages; amount of carotenoid consumed, food matrix; effectors of absorption (e.g. presence of fat) and conversion; nutrient status; genetic factors; host-related factors; and interactions between these variables (de Pee & West, 1996).

43. To take account of the contribution from provitamin A carotenoids, the total vitamin A content of the diet is usually expressed as micrograms (μg) of retinol equivalents (RE): 1 μg RE = 1 μg retinol = 6 μg β-carotene = 12 μg other carotenoids with provitamin A activity (WHO, 1967).

44. In 2001, the Food and Nutrition Board of the Institute of Medicine in the USA, introduced the term Retinol Activity Equivalent (RAE) to express the activity of provitamin A carotenoids in terms of retinol (FNB, 2001). Based on data showing that retinol activity of purified β-carotene in oil is half that of retinol (Sauberlich et al, 1974) and that the retinol activity of dietary β-carotene is 1/6 rather than 1/3 that of β-carotene in oil (Van het Hof, 1999), the conversion factor was revised so that 1 μg RAE = 1μg retinol = 2 μg β-carotene in oil = 12 μg dietary β-carotene = 24 μg of other dietary provitamin A carotenoids.

45. The term *vitamin A* is used to describe any compound with the biological activity of the vitamin, including preformed *vitamin A* (retinol and its active metabolites), and provitamin A carotenoids. In published literature, the terms *vitamin A*, *preformed vitamin A*, and *retinol*, are often used interchangeably. In this report: a distinction is made between preformed vitamin A (retinol) and provitamin A carotenoids; the term *total vitamin A* refers to the sum of retinol and provitamin A carotenoids.

4 Methodology

46. The studies on retinol and bone health previously considered by the EVM were reassessed from a nutritional perspective. The SACN framework for risk assessment (2002) was used as a template to identify and assess evidence published since the EVM report.

47. The quality of the data on the relationship between retinol intake and bone risk was assessed by considering: study design; methods used for assessment of total vitamin A and retinol intakes; and allowance for the main confounding factors, with particular consideration of nutrients and other factors affecting bone.

48. Information on population intakes of retinol was obtained from the British National Diet and Nutrition Survey (NDNS) series. The NDNS data were also used to model the effect of reducing consumption of foods such as liver, that are particularly rich sources of retinol, or supplements containing retinol, on the number of consumers exceeding the EVM Guidance Level of 1500 μgRE/day. Additionally, the effect of such reductions on the proportion of the population whose intakes fall below the LRNI for total vitamin A and other nutrients (iron, zinc, folate, vitamin B_{12}) was considered.

49. The potential for reducing the liver retinol content of livestock and poultry was also explored. Since a detailed review of animal feed issues is not within the remit of SACN, this matter is only considered briefly in this report.

5 Review of the evidence on Retinol and bone health

Dietary assessment of retinol

50. Natural sources of retinol are foods of animal origin, dairy products, and fish. Liver and liver products are particularly rich sources of retinol. Fortified foods (especially margarine) and supplements[4] (including fish liver oils[5]) are also important sources of retinol. The inter- and intra-individual variance of retinol intake is high since retinol is found in very high concentrations in a limited number of foods, such as liver, which may be consumed infrequently (Willett, 1998). This makes it difficult to obtain reliable estimates of average consumption and to identify consumers of high levels of retinol in a population. Food Frequency Questionnaires (FFQs) can focus on particular nutrients with few food sources and may be useful for supplementing information available from limited numbers of daily records of consumption (Bates et al, 1997).

Biochemical measures as an index of retinol exposure

51. Under normal physiological conditions, a 1:1 complex between retinol and RBP accounts for 90% of the total retinol present in plasma; retinyl esters, incorporated in chylomicrons, accounts for approximately 8%; small amounts of retinoic acid and other metabolites are also present (Olson, 1984).

4 All 'vitamin A' supplements in the UK contain retinol.
5 Fish liver oils are particularly rich sources of retinol.

52. Plasma retinol concentration has been used as a biochemical measure of habitual intake (retinol exposure). Plasma retinol levels are homeostatically controlled, between 1.8-2.02 μmol/L in the UK (Ruston et al, 2004), over a wide range of liver reserves and normal levels of consumption are usually unrelated to plasma levels (Krasinski et al 1989; Willett et al, 1983). Mean plasma retinol values fall when liver stores are exhausted and increase at liver concentrations above 300 μg/g (Olson, 1984). When the capacity for storage of retinol in liver is exceeded or the rate of intake is greater than the rate it can be removed by the liver, there is a marked increase in plasma levels. Plasma retinol concentrations, therefore, are insensitive indicators of intake or body reserves unless they are very high or very low.

53. Plasma retinol levels are also depressed by impaired or inadequate formation of RBP, for example with inadequate intakes of protein and zinc (Olson, 1984) and liver disease (DeLuca, 1979). Additionally, plasma retinol concentrations are reduced during the inflammatory response accompanying conditions such as fever and infection as a consequence of decreased concentrations of RBP, which is a negative acute phase reactant (Stephensen and Gildengorin, 2000).

54. Fasting levels of plasma retinyl esters have also been used as a marker of high retinol intakes because they are found in the plasma when intakes exceeds the capacity of the liver to store retinol or produce RBP (Olson 1984; Krasinski et al, 1989). Although total vitamin A intake has not been found to correlate with plasma retinyl ester levels, there is some evidence that long-term use of retinol supplements (≥ 5y) is associated with higher fasting blood levels of circulating retinyl esters in elderly subjects (Krasinski, 1989).

Assessment of bone health

55. Bone is a metabolically active tissue consisting principally of a collagen protein framework, which is strengthened by crystalline bone mineral, largely composed of calcium and phosphates. It comprises two distinct layers: an outer dense shell of cortical compact bone; and an inner latticed layer of trabecular bone. Approximately 80% of the skeleton is made up of cortical bone and 20% of trabecular bone.

56. Skeletal turnover is a regulated process in which bone is constantly being broken down by osteoclast cells (resorption) and constantly being deposited by osteoblast cells (formation). Osteocytes, which are derived from osteoblasts, are found within the mineralised bone matrix and play a role in controlling the extracellular concentration of calcium and phosphate. The maintenance of a normal, healthy, skeletal mass depends on interactions between osteoblasts, osteocytes, osteoclasts, and constituents of the bone matrix, to keep the process of bone resorption and formation in balance. Net bone loss occurs when bone resorption exceeds bone formation either as a result of increased resorption or decreased formation rates. Bone mineral loss is associated with increased fracture risk in both men and women. During childhood and adolescence, skeletal mass increases and net bone deposition continues at a greater rate than resorption until peak bone mass (maximum bone mass achieved at skeletal maturity) is reached. Net loss of bone begins within a few years of achieving peak bone mass. Rates of net loss are initially slow but, in women, accelerate rapidly after the menopause.

57. Both genetic and environmental factors influence bone health. Genes determine the size and shape of the skeleton and make an important contribution to the differences in bone status between healthy individuals. Bone health is also influenced by body composition, hormonal and reproductive factors, physical activity and nutritional intake (DH, 1998). Research on the relationship between nutrients and bone health has focused mainly on calcium, which is a main bone forming mineral, and vitamin D, which is required for calcium absorption. Both calcium and vitamin D have been positively associated with bone health. Other nutrients that have been positively associated with bone health include phosphorus, magnesium, potassium, fluorine, vitamin K and vitamin C.

Higher intakes of protein have been negatively and positively associated with bone health. Dietary constituents which have been negatively associated with bone health include sodium, alcohol, and caffeine. All these factors can be potential confounders in studies of bone health.

58. Osteoporosis is a systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, resulting in greater bone fragility and a consequent increased risk of bone fracture (WHO, 1994). The amount of bone per unit volume is decreased, but the composition remains unchanged. The most common fractures that occur as a result of osteoporosis are of the wrist, hip and spine. Osteoporosis becomes a major health problem with increasing age and although it is most common in postmenopausal women it is also a health problem for men. The lifetime risk of any fracture has been estimated to be 53% at age 50 years among women, and 21% at the same age among men (van Staa et al, 2001).

59. In studies of factors influencing bone health, the most clearly defined outcome of interest is bone fracture following minimal trauma. Most studies, however, use intermediate outcome measures of bone mineralisation and metabolism to assess bone status.

60. Low bone mineral mass is a risk factor for fragility fractures (WHO, 1994). Bone mineral density (BMD) or bone mineral content (BMC) measurement is a diagnostic test, which measures the amount of mineral in bone. It is used as an indicator of bone strength and risk of future fracture. It has been estimated, from prospective studies, that the risk of osteoporotic fracture increases 1.5-3 times with each standard deviation (SD) decrease in BMD (WHO, 1994).

61. Absorptiometry has generally been used to measure bone mineral in epidemiological studies of bone health. Dual energy X-ray absorptiometry (DXA) can be used to assess BMD in the spine, hip, forearm, and whole body. It has high reproducibility and involves low doses of radiation. Single energy X-ray absorptiometry (SXA) can only measure peripheral sites such as the heel and forearm. In earlier absorptiometry techniques,

single photon absorptiometry and dual photon absorptiometry, gamma rays were used as the photon source.

62. Since bone strength depends on both bone density and microscopic bone structure, bone density measurement cannot provide a complete assessment of bone strength. Techniques to measure BMD and BMC only determine the amount of mineral contained in the bone envelope per unit area scanned and do not provide a measure of the volumetric density of the bone or the mineralised tissue within the bone (Prentice, 1995). This means that BMC and BMD measurements are influenced by the size, shape, and orientation of the bone. As a consequence, the usefulness of cross-sectional studies examining the association between nutrition and bone health is limited unless adjustment has been made for the confounding influence of size (DH, 1998). Bone mineral measurements are more useful in prospective studies, where changes can be assessed over time.

63. There are also limitations with studies that have measured BMD/BMC at only one skeletal site. This may not adequately represent the mineralisation of the skeleton overall, as there can be differences in BMD/BMC between different skeletal sites within an individual.

64. Biochemical markers of skeletal turnover have also been used to assess bone loss. Plasma levels of osteocalcin, procollagen carboxypeptide, procollagen amino peptide, and bone specific alkaline phosphatase, reflect osteoblast activity and are validated indices of bone formation (DH, 1998).

65. Bone fractures, bone mineralisation, and biochemical markers of bone turnover, were the outcomes of interest assessed in the studies considered.

Animal Studies

66. Evidence from animal studies has clearly shown that acute toxic exposure or chronic high doses of retinol can have adverse skeletal effects (Binkley & Krueger, 2000). For example, young rats fed high doses of retinyl acetate (12,012 µg/day) developed limb fractures within 14 days (Moore & Wang, 1945) and growing rats and guinea pigs given doses of 3003-15015 µg/day of retinol sustained fractures of long bones (Wolbach, 1947). Administration of 7,508-22,523 µg/day of retinol to growing rats for 17 days caused bone lesions and bone thinning (Leelaprute et al, 1973). Hough et al (1988) treated growing rats with 3003-7508 µg/day of retinyl palmitate for 21 days and observed an increase in bone resorption and a reduction in bone formation. Spontaneous limb fractures and increased skeletal turnover were also observed.

67. The available data suggest that the adverse skeletal effects of retinol observed in animals occur as a result of increased bone resorption and decreased bone formation (Binkley & Krueger, 2000). These studies have, however, exposed young growing animals to very high intakes of retinol. It cannot be assumed that similar responses occur in humans habitually consuming large amounts of retinol.

Human Studies

68. Details of all the studies considered, including the allowance made for the risk factors associated with bone health, are tabulated in Annex 4.

Studies which considered bone fractures as endpoints

Cross-sectional studies

69. **Sowers and Wallace** (1990) examined the relationship between total vitamin A from supplements, serum retinol levels, radial bone mass, and fracture history in 246 postmenopausal women (aged 55-80y) in the USA. Fracture history was obtained by interview. Serum retinol levels were measured within 6 months of blood sample collection. Nutrient intake was assessed from an interview regarding supplement intake and a 24-hour food recall. Thirty-six percent of this population reported taking a

supplement containing vitamin A on a continuing basis within the previous year. Among supplement users, 54% had intakes less than 1000 µgRE/day, 38% had intakes between 1000-2000 µgRE/day, and 8% had intakes greater than 2000 µgRE/day. The mean intake of total vitamin A from food and supplements was 903 µgRE/day.

70. No association was found between increased fracture risk and serum retinol level or supplement use. There was no association between radial bone mass and intake of total vitamin A from supplements or from supplements and food sources combined. There was no relationship between radial bone mass or serum retinol levels. Mean bone mass of women in the highest tertile of serum retinol levels was not significantly different to that of women in the lowest tertile.

71. This study has a number of limitations: one 24-hour food recall was used for assessment of total vitamin A from food, which would not provide reliable estimates of intake (see paragraph 50); serum retinol levels are under homeostatic control and are not reliable markers of status (see paragraph 52); the sample size and power were adequate to test the overall association of supplement use with bone mass but not to test any association with doses exceeding 2000 µgRE/day; bone fractures were self-reported; bone mass was examined at only one site, which may not reflect bone mass at other sites; the analysis did not control for any other nutrients or important confounders such as physical activity and family history of osteoporosis.

Case-control studies

72. **Melhus** *et al* (1998) examined the association between total vitamin A intake and hip fracture in a nested case-control study in Sweden. Women (aged 40-76y) who had a first hip fracture within 2-64 months of entering the study were defined as cases (n=247) and were matched (by age and county) to four controls (n=873). A questionnaire was used to estimate usual intake of 60 foods during the previous 6 months. Subjects were sent a second questionnaire requesting information on potential confounders, including supplement intake and physical activity level. For cases, the mean total vitamin A intake from foods was 1760 µgRE/day and the mean

retinol intake from foods was 960 μg/day. For controls, the mean total vitamin A intake from foods was 1630 μgRE/day and the mean retinol intake from foods was 880 μg/day.

73. A graded increase in relative risk (RR) of hip fracture was found with increasing dietary retinol intake (p for trend = 0.006). The risk of hip fracture was doubled when dietary retinol intakes greater than 1500 μg/d were compared with intakes of less than 500 μg/d (Odds Ratio 2.1, 95% CI: 1.1-4.0). Dietary intakes of β-carotene were not associated with fracture risk.

74. This study was a reanalysis of data from a previous study of diet and hip fracture risk (Michaëlsson et al, 1995a) which did not originally consider the association of retinol intakes with hip fracture risk. High intakes of iron, magnesium, and vitamin C were found to be risk factors for hip fracture in the previous study. After adjustment for intakes of iron, magnesium, and vitamin C, the association of retinol intake with risk of hip fracture decreased but remained significant. High intakes of iron, magnesium, and vitamin C remained as statistically significant risk factors for hip fracture (Michaëlsson, 2000).

75. The main limitations of this study are: the possibility of information bias as cases were questioned about covariates such as physical activity and supplement intake after the occurrence of hip fracture and may have changed their dietary habits or physical activity as a result of the fracture; data on possible confounding factors, such as thyroid hormone therapy and family history of osteoporosis, were not available; dietary intake data were obtained for the previous 6 months which may not be a reliable reflection of long-term habitual intakes.

Prospective studies

76. **Feskanich** *et al* (2002) examined the relationship between total vitamin A intake and risk of hip fracture among 72,337 postmenopausal women (aged 34-77y) in the USA, followed for 18 years in the Nurses' Health Study. The total dietary intake from food and supplements was assessed every 4 years using a FFQ. Mean cumulative intake data, from 5 FFQs, were obtained for

total vitamin A, retinol, and β-carotene. The mean long-term intake, from food and supplements, was 2265 μgRE/day for total vitamin A and 1212 μg/day for retinol.

77. The RR of hip fracture for women in the highest quintile of total vitamin A intake from food and supplements (≥3000 μgRE/day) compared to those in the lowest quintile (<1250 μgRE/day) was 1.48 (95% CI: 1.05-2.07). The risk of hip fracture was increased with retinol intake from food and supplements: the RR of hip fracture for women in the highest quintile of retinol intake (≥ 2000 μg/d) compared to those in the lowest quintile (<500 μg/day) was 1.89 (95% CI: 1.33-2.68). No increase in risk was observed with β-carotene intake. The RR for women with retinol intakes greater than 1500 μg/day, from food and supplements, compared with women consuming less than 500 μg/day was 1.64 (95% CI: 1.14-2.35).

78. Although hip fractures in this study were self-reported with no further identification of fracture site, accurate reports would be expected from a study cohort of nurses.

79. **Michaëlsson** *et al* (2003) investigated the association between serum retinol concentrations and risk of fracture in 2047 men (aged 49-51y) in Sweden, who were followed for 30 years. Blood samples were obtained from subjects at baseline and serum concentrations of retinol and β-carotene were measured 13-16 years later. A dietary assessment (including supplement intake) using a 7-day dietary record was made for 1138 men, 20 years after commencement of the study.

80. The overall risk of fracture increased by 26% for each 1 SD increase in serum retinol concentration. This increment was non-linear and mainly a feature of the highest quintile of serum retinol concentration. Multivariate analysis of the risk of fracture in the highest quintile for serum retinol compared with the middle quintile was 1.64 (95% CI, 1.12-2.41) for any fracture and 2.47 (95% CI, 1.15-5.28) for hip fracture. It is unclear why the middle quintile of serum retinol levels was used as the reference quintile. Serum β-carotene levels were not associated with risk of fracture.

81. In the group of men where dietary data were available, no significant association was found between retinol intake, from food and supplements, and fracture risk. No significant association was found between dietary retinol intake and serum retinol concentration, 20 years earlier. Dietary β-carotene intake was not associated with the risk of fracture.

82. The limitations of this study concern the methods used to assess retinol status: serum retinol concentrations were measured from one blood sample, which was analysed 13-16 years after it had been obtained at baseline; serum retinol concentration is not a sensitive indicator of retinol status (see paragraph 52); it is unlikely that one measurement of serum retinol concentration, obtained 30 years previously, would provide a reliable marker of habitual intake or reflect any changes in diet that may have occurred over time.

83. **Lim** *et al* (2004) examined the relationship between fracture risk and total vitamin A and retinol intake from food and supplements in 34,703 postmenopausal women (aged 55-69y) in the USA, who were followed for 9.5 years. Total vitamin A intake at baseline was assessed using a semi-quantitative FFQ. Thirty-five percent of participants reported using supplements containing retinol or β-carotene. Mean total vitamin A intake of subjects was 4333 μgRE/day from food and supplements.

84. Following multivariate adjustment, a small non-significant increase in risk of hip fracture was found for supplement users compared to nonusers (RR=1.18, 95% CI 0.99-1.41) but risk of all fractures was not increased in supplement users. There was no significant dose-response relationship between hip fracture or all fracture risk and intake of total vitamin A or retinol from supplements, foods and supplements combined, or from food sources alone.

85. Limitations of this study include: definition and accuracy of outcome measures, since fractures were self reported and no distinction was made between fractures due to high trauma events and those due to low/moderate events; and assessment of intakes of total vitamin A and retinol from one FFQ at baseline, which is unlikely to provide a reliable indication of habitual consumption over longer periods of time.

Studies which considered bone mineral as endpoints

86. Whilst most of the studies in this section measured bone mineral density as outcome measures, Freudenheim et al (1986) measured bone mineral content and Sowers and Wallace (1990) measured bone mass. The bone mineral assessment techniques used in these studies can be found in Table 2, Annex 4.

Cross-sectional studies

87. **Sowers and Wallace** (1990) investigated whether total vitamin A from supplements or serum retinol levels were associated with radial bone mass in postmenopausal women in the USA (see paragraph 69 for further details of this study). No relationship was found between total vitamin A from the diet and supplements, or serum retinol, with radial bone mass. The limitations of this study are described in paragraph 71.

88. **Melhus** *et al* (1998) investigated the association of dietary retinol intake with BMD of 175 women (aged 28-74y) in Sweden. This study was a reanalysis of a previously published cross-sectional study of diet and BMD (Michaëlsson et al, 1995b) in which the dietary intake of retinol was not originally analysed. Diet was assessed from four 1-week dietary records and BMD was measured at the lumbar spine, total body, and at 3 regions of the proximal femur. The mean total vitamin A intake from foods was 1513 μgRE/day and the mean retinol intake from foods was 780 μg/day.

89. Dietary retinol intake was not significantly associated with BMD in univariate analysis. In multivariate analysis no significant changes were found in BMD at intakes up to 1500 μg/day. When subjects with intakes in excess of 1500 μg/d were compared with those with intakes less than 500 μg/day, BMD was found to be significantly less at all 5 sites in the groups with the higher levels of consumption.

90. **Ballew** *et al* (2001a) analysed the association between fasting serum concentration of retinyl esters and BMD in the third National Health and Nutrition Examination Survey, 1988-1994 (NHANES III) in the USA. A high prevalence of fasting serum concentration of retinyl esters, greater than 10% of the serum concentration of total retinol, had previously been

noted for subjects in this survey (Ballew et al, 2001b). Data on fasting serum retinyl levels were available for 5790 men and women (aged 20 to over 90y). BMD was measured for total hip and four sites at the hip: femoral neck, trochanter, intertrochanter, and Ward's triangle. Participants reported dietary supplement usage over the previous 30 days. A 24-hour dietary recall was taken to check that subjects had not consumed excessive amounts of retinol on the day before being tested.

91. The median total vitamin A intake from food and supplements was 2193 µg/day for men, 1837 µg/day for premenopausal women, and 2096 µg/day for postmenopausal women. Overall, 25.6% of subjects were taking supplements containing retinol. The median intake obtained from supplements was 1495 µgRE/day. Serum retinyl ester levels were within normal limits for all subjects who reported supplement usage. No significant association was found between fasting serum retinyl ester concentrations and BMD at any site in multivariate analysis.

92. A limitation of this study is that serum retinyl esters have not been found to correlate with total vitamin A intake (see paragraph 54). Although elevated levels of serum retinyl esters indicate total vitamin A intakes in excess of the liver storage capacity, they may reflect recent excess intake rather than long-term intake.

93. **Sigurdsson** *et al* (2001) examined the association between high dietary intake of total vitamin A and BMD in 232 Icelandic women (aged 70y). BMD was measured for the total skeleton, lumbar spine, femoral neck, trochanter, intertrochanter, and total hip. Dietary intake was assessed using a semi-quantitative FFQ reflecting the previous 3 months of intake. Mean retinol intake, from food and supplements, was 2300 µg/day, with more than half this amount obtained from cod liver oil and multivitamins.

94. Categorisation into quintiles of retinol intake showed that women in the lowest quintile had retinol intakes less than 1000 µg/day and those in the highest quintile had intakes greater than 3700 µg/day. There were no significant differences in mean or median values of BMD at any measured site according to quintiles of retinol or β-carotene intake and no significant

association was found between retinol or β-carotene intake and BMD at any site in univariate or multivariate analysis.

95. The main limitations of this study are: the lack of long-term assessment of total vitamin A intake, which was only estimated for the previous 3 months; a number of important confounding factors were not considered.

Prospective studies

96. **Freudenheim** *et al* (1986) investigated the association amongst usual intakes of 14 nutrients, including vitamin A (it is not clear if this refers to total vitamin A or retinol), with loss of BMC from the arm, in 99 healthy adult white women (aged 35-65y) in the USA. This study was part of a 4-year clinical trial to determine the effect of diet and calcium supplementation on bone loss. Dietary intake of all foods and supplements was estimated from seventy-two 24-hour dietary records collected for each subject over 3 years. BMC measurements were made of the radius, ulna, and humerus arm bones. For premenopausal women, the mean vitamin A intake was 2718 μgRE/day for those who had not received calcium supplements and 1912 μgRE/day for those who had received calcium supplements. The mean vitamin A intake for postmenopausal women who had not received calcium supplements was 2590 μgRE/day and 2280 μgRE/day for those who had received calcium supplements.

97. In the calcium supplemented postmenopausal women (n=34) a negative correlation was found between rate of change in ulna BMC and vitamin A intake. This association was largely influenced by one subject with a high supplemental intake (average vitamin A intake of 4392 μgRE/day) and was not significant when this subject was excluded. In the premenopausal women who had not received calcium supplements (n=9), a significant positive correlation, i.e., a slower rate of bone loss, was found between vitamin A intake and the rate of change in humerus BMC.

98. The limitations of this study concern: outcome measure, since bone mass was only examined at one site; the sample size, which was small in each of the 4 groups (pre- and postmenopausal women by calcium supplement status); and statistical analysis, since multivariate analysis was not carried out.

99. **Houtkooper** *et al* (1995) assessed the relationship between rates of change in BMD for total body and at four bone sites with nutrient intakes, body composition, and exercise. Sixty-six healthy premenopausal women (aged 28-39y) in the USA were followed for 12 months. The subjects were part of a randomised trial on the effects of resistance exercise on BMD of previously sedentary women. All subjects were supplemented with calcium (500 µg/d) in order to prevent the possibility that calcium might be a limiting nutrient and enable investigation of the role of other nutrients and exercise. Dietary and supplement intake was assessed from diet records for 4 randomly assigned days in the month prior to each 5 and 12 month testing period. The mean daily nutrient values used in the analyses were based on intake only from food (except for calcium, which included intake from food and supplements). Intakes from supplements were not included in the analyses as supplement usage was found to be irregular and intakes for some participants were very large. The mean intake for vitamin A (not clear if this refers to total vitamin A or retinol) was 1220 µgRE/day and the mean intake for carotene was 595 µgRE/day. The average calcium intake of subjects was 1326 µg/day (300-500 µg higher than the recommended levels for this age group, in the USA).

100. In multiple regression analysis, a significant positive association was found between vitamin A intake and rate of change in total body BMD, i.e. vitamin A intake was significantly associated with an annual rate of increase in total body BMD and, therefore, with less bone loss. No association was found between calcium and BMD change.

101. The limitations of this study concern: the sample population, which was relatively young and premenopausal; the sample size, which was small; assessment of vitamin A intake, since supplement intake was not included in the analyses; and statistical analysis, as a number of important confounding factors were not considered in multivariate analysis.

102. **Promislow** *et al* (2002) examined associations of retinol intake with BMD and BMD change in a free-living elderly population in the USA. The cohort comprised 570 women and 388 men (aged 55-92y at baseline) who were followed for 4 years. Dietary intake was assessed once at baseline using a dietary questionnaire. BMD at total hip, femoral neck, and lumbar

spine, was measured at baseline and after 4 years. In this population, 50% of women and 39% of men were taking retinol-containing supplements. The mean retinol intake from diet and supplements was 1248 μg/day for women and 1244 μg/day for men.

103. When supplement users and nonusers were considered together, and after adjusting for age, no relationship was found between retinol intake and BMD at baseline or change in BMD after 4 years. After stratification by supplement use, associations of retinol intake with BMD, and BMD change, were found to be negative for supplement users and positive for nonusers at all sites, for women only. For non-supplement users these associations were significant for BMD at the hip and femoral neck; for supplement users, the association was significant for change in BMD at the hip.

104. In multivariate analysis the association between retinol intake and BMD and BMD change remained negative for women taking retinol supplements and positive for women not taking retinol supplements. For supplement users, a significant negative association was found between retinol intake and BMD at the femoral neck and spine and for non-supplement users a significant positive association was found between BMD at the hip and femoral neck. For supplement users significant negative associations were observed for change in BMD at the hip and femoral neck.

105. A limitation of this study was that dietary intakes of retinol were assessed from one dietary questionnaire at baseline and may not be an accurate reflection of habitual intakes.

Studies which considered serum markers of skeletal turnover as endpoints

Intervention studies

106. **Kawahara** *et al* (2002) evaluated the effect of retinol supplementation on skeletal turnover in 80 healthy men (aged 18-58y) in the USA. The study was a single-blind, placebo controlled, 6-week trial, in which subjects consumed either 7576 µg/day retinol palmitate or placebo. Serum markers used to assess skeletal turnover were bone specific alkaline phosphatase (BSAP), N-telopeptide of type I collagen (NTx) osteocalcin (Oc). Serum BSAP and NTx were measured at 2, 4 and 6 weeks of supplementation and serum Oc was measured at baseline and after 6 weeks of supplementation.

107. No change was found in levels of BSAP, NTx or Oc in either the placebo or supplemented group.

108. The limitations of this study concern: the outcome measure, since the sample size allowed 90% power to detect BSAP changes of 2.5 units/litre but smaller changes in bone turnover could contribute to bone loss over a longer time period; the short duration of the intervention, meant that long-term effects of high levels of retinol supplementation could not be assessed; and the study population, as subjects were young to middle-aged men, who are less susceptible to bone fracture.

Summary and Conclusions

109. The Guidance Level set by the EVM for retinol intake, of 1500 µgRE/day, was based on evidence that intakes above this level may increase the risk of bone fracture.

110. Animal studies have shown that high doses of retinol have adverse effects on bone and provide possible mechanisms for the effects of retinol on bone. Most of the animal studies have used retinol doses considerably greater than those consumed by humans.

111. In human studies the majority of the data on the relationship between retinol intake and bone health is derived from epidemiological evidence

and suffers from the inherent limitations of observational research. There are few available studies and most were not specifically designed to examine the relationship between retinol intake and bone health.

112. Retinol intakes are difficult to measure since retinol is found in very high concentrations in a limited number of foods, such as liver, which may not be consumed on a regular basis. The dietary assessment methods used in most of the studies have been inadequate to obtain reliable estimates of total vitamin A/retinol intakes. Dietary habits or supplement use may change over a long period of time resulting in misclassification of intake.

113. In the retrospective studies, the possibility exists of reverse causality, i.e., individuals may have increased their intake of supplements containing retinol as a consequence of suffering a fracture or being at increased risk of fracture.

114. The reporting of total vitamin A or retinol intakes have not been consistent across the different studies, making comparisons difficult: some studies have reported only on total vitamin A intake without distinguishing between retinol and provitamin A carotenoids; other studies have not made it clear whether the term *vitamin A* refers to total vitamin A or retinol. The estimates of mean intakes for total vitamin A and retinol have varied widely between different studies in the same country.

115. A number of nutrients, as well as many other factors, have been associated with bone health. Studies vary in the account taken of these so that confounding cannot be excluded. Additionally, the clustering of nutrients and the strong correlation between many nutrients makes it difficult to separate their effects.

116. Findings from the studies which have examined the association between retinol intakes and bone health are inconsistent; some studies have shown an association while others have not.

117. Bone fracture was used as an outcome measure in 5 studies. Two studies showed a significant association between retinol intake and bone fracture risk and 1 study found a significant association between serum retinol

concentration and bone fracture risk. Of the 2 studies which found an association between retinol intake and bone fracture risk, only the prospective study by Feskanich et al (2002) was well designed with a lengthy follow-up period of 18 years, cumulative estimates of long-term dietary intake, and allowance for the main confounding factors.

118. Seven studies examined bone mineral as an outcome measure. Four of these were cross-sectional in design and could therefore only compare intakes with bone status at one time point. One cross-sectional study found a significant association between retinol intake and lower BMD; 1 prospective study found no overall association between retinol intake and BMD but a significant negative association after stratification by supplement use for supplement users and a positive association for nonusers, for women only; 2 studies found a significant association between retinol intake and an increase in BMC/BMD.

119. One intervention study found no relationship between supplementation with very high doses of retinol (7576 µg/day) and serum markers of skeletal turnover. As this was a short-term study, the long-term effects of retinol supplementation were not assessed.

120. Whilst the available evidence on the relationship between retinol intake and bone health provides a basis for concern, the observational nature of the evidence makes it difficult to establish causality.

Interaction between retinol and vitamin D

121. Cholecalciferol, or vitamin D_3, is the natural form of vitamin D and is synthesised by the action of sunlight on the skin. Endogenous production of vitamin D is quantitatively a more important source of vitamin D than diet. Vitamin D is metabolised to the active hormone, dihydroxycholecalciferol ($1,25(OH)_2D_3$), which carries out all its functions (Jones et al, 1998).

122. Vitamin D plays an important role in bone mineralisation and in the control of plasma calcium concentration. The active metabolite, $1,25(OH)_2D_3$, stimulates absorption of dietary calcium from the intestine and resorption

of calcium from bone. High intakes of vitamin D can be toxic, causing calcium to be deposited in soft tissues, including the kidneys, and may cause damage (EVM, 2003). When dietary calcium is limited or vitamin D status is poor, secretion of parathyroid hormone (PTH) is increased. PTH stimulates the production of $1,25(OH)_2D_3$ which promotes calcium absorption in the intestine and calcium release from bone. The secondary hyperparathyroidism caused by poor vitamin D status has been linked with an increased risk of osteoporosis and fracture in the elderly (DH, 1998). Prolonged vitamin D deficiency results in a poorly mineralised skeleton, leading to rickets and osteomalacia (DH, 1998).

123. Groups at risk of poor vitamin D status include those with little exposure to sunlight, e.g. older people who are housebound or live in institutions, and people from ethnic communities wearing concealing clothing (DH, 1998). The amount of melanin in the skin also affects the ability to synthesise vitamin D: the darker the skin colour, the longer the exposure to sunlight required to synthesise equivalent quantities of vitamin D (DH, 1998). Others at risk of inadequate intakes of vitamin D are those with increased metabolic demands, e.g. pregnant and lactating women, and infants. As vitamin D is synthesised in the skin from sunlight, COMA did not consider a dietary intake was necessary for adults living a normal lifestyle (DH, 1991). An RNI of 10 µg/day was set for adults at particular risk. For infants and children, an RNI of 8.5 µg/day was set for those aged 0-6m and of 7 µg/day for those aged 7m-3y.

124. Evidence, mainly from animal studies, suggests that retinol and vitamin D have antagonistic effects. In rats, high doses of retinyl palmitate were found to reduce the effects of excessive doses of vitamin D (Clark & Bassett, 1962) and, conversely, vitamin D was shown to prevent retinol toxicity (Vedder & Rosenberg, 1938). In poultry, adverse effects of retinol (at levels exceeding 13,514 µg per kg of basal diet) were only found to occur at marginal levels (12.5 µg/kg) of dietary vitamin D (Aburto & Britton, 1998).

125. A dose-response study in rats on the effects of increasing amounts of retinyl acetate (0-8621 µg/day) and increasing amounts of vitamin D (0-0.645 µg/day) on bone mineralisation, showed that interactions between the

vitamins occurred at all vitamin D doses but were more evident as the amount of vitamin D decreased (Rohde et al, 1999). Administration of 0.005 µg/day vitamin D was associated with an increase in serum calcium concentration (from 1.37 mmol/L to 2.34 mmol/L) which was not observed at retinyl acetate dose levels of 3448 µg/day. If retinol was exerting an effect by increasing bone resorption, higher dosage levels would be expected to raise serum calcium levels. This was not observed, indicating antagonism of vitamin D effects in the intestine and bone.

126. Only one study has examined the interaction between retinol and vitamin D in humans. Johansson and Melhus (2001) examined the acute effects of single doses of retinol and vitamin D on calcium homeostasis and bone resorption in a double-blind crossover study (n=9). All subjects received either 15,000 µg retinyl palmitate, 2 µg $1,25(OH)_2D_3$, 15,000 µg retinyl palmitate plus 2 µg $1,25(OH)_2D_3$, or placebo, on four separate occasions. The retinyl palmitate dose was representative of the amount in a standard serving of liver. There was a significant increase in serum levels of vitamin D and retinyl esters corresponding to intakes. The combined intake of both vitamins resulted in serum levels that were not significantly different from separate intakes of the vitamins. A significant increase in serum levels of calcium was found in response to vitamin D intake and a significant decrease after intake of retinyl palmitate. The rise in serum calcium concentration was significantly lower after the combined intake of the vitamins compared to the increase after vitamin D alone. None of the vitamin preparations had an effect on serum levels of C-telopeptide of type I collagen, which is a marker of bone resorption.

127. The mechanism for the interaction between retinol and vitamin D is not known. The active metabolites of both vitamins achieve their effects by regulation of gene expression through nuclear receptors. *In vitro* studies have suggested the potential for interactions between the vitamins at the ligand binding sites of the nuclear receptors (Thompson et al, 1998). At high concentrations, retinol metabolites might interfere or compete *in vivo* with the normal binding of vitamin D to the nuclear receptor.

Summary and conclusions

128. Evidence for the interaction between retinol and vitamin D is based largely on animal studies. These studies have been conducted over short periods of time and there are no data from longer-term studies. The available data indicate that the antagonistic effects of retinol on vitamin D occur mainly at low dietary levels of vitamin D.

129. Only one study has examined this interaction in humans. As this was an acute study with a small sample size, it is not possible to reach clear conclusions concerning the antagonistic effects of retinol on vitamin D in humans.

130. The vitamin D status of certain population subgroups, e.g. older people confined indoors and people from ethnic communities wearing enveloping clothing, may be poor. High intakes of retinol might be of greater concern in such populations.

6 Dietary intakes of retinol and Provitamin A Carotenoids in Great Britain

131. Information on retinol and provitamin A carotenoid intakes of the British population has been obtained from the National Diet and Nutrition Survey (NDNS) series.

Dietary methodology

132. In the NDNS series, diet was assessed from weighed records of all foods consumed over a period of 7 consecutive days (4 days for adults aged 65y and over). As the recording of food intake is restricted to a short continuous time period, the habitual intake of rarely consumed foods may be over- or underestimated at an individual level (although estimates of population mean intake should to be reliable). The retinol content of a few rarely consumed foods, i.e., liver and liver products, is particularly high. Consumption, or lack of consumption, of such foods during the recording period will have a substantial impact on estimates of habitual retinol intake; as a consequence, retinol intake may appear to be atypically high for some individuals and atypically low for others. This would be more pronounced for shorter rather than longer periods, e.g., when food intake has been recorded for 4 rather than 7 days. If data were available for longer time periods the mean level of retinol intake would be expected to be more evenly distributed amongst the population.

Dietary sources of retinol and provitamin A carotenoids

133. The main dietary sources of retinol and provitamin A carotenoids and the contribution to overall population intakes of total vitamin A, for men and women, are shown in Table 1. The data are from the 2000/1 NDNS of adults aged 19-64 years (Henderson *et al*, 2003) and the 1994/5 NDNS of people aged 65 years and over (Finch et al, 1998).

134. The major dietary source of retinol is liver, which is an extremely rich source of retinol for those who consume it. Concentrations of retinol range from 10,500 µg/100g in chicken liver to 25,200 µg/100g in calf liver (FSA, 2002). Liver consumption is unevenly distributed across the population.

135. Dietary supplements also make an important contribution to retinol intake. Fish liver oils are particularly rich sources of retinol; for example, the retinol content of cod liver oil is 18000 µg/100g (FSA, 2002). During the manufacturing process for food supplements some of the active components of the product can be destroyed. To compensate for this loss, the usual practice employed by supplement manufacturers is to over-formulate so that the product fulfils the claim for content stated on the label at the point of sale and throughout its shelf life. Over-formulation expressed as a percentage of the amount declared on the label can range from 30-65%[6].

6 Data based on information provided by the Council for Responsible Nutrition and the Health Food Manufacturers' Association (2005).

Table 1: Mean retinol and carotene intakes for adults aged 19-64y (NDNS 2000/1) and over 65y (NDNS 1994/5) with percentage contributions from some key food groups

Contribution of retinol and carotenes to total vitamin A intake	Adults aged 19-64 yrs (NDNS 2001)		Adults over 65 years (NDNS 1994/5)	
	Men (n=766)	Women (n=958)	Men (n=632)	Women (n=643)
Retinol (µg/day)	**674**	**472**	**937**	**805**
% contribution from food sources:				
Liver[7]	35.3	21.5	46.0	43.5
Oily fish	0.6	0.8	0.5	0.3
Dairy (excl. butter)	17.4	19.6	15.1	15.7
Butter/spreads/ margarines	12.1	11.1	13.5	13.3
Egg & Dishes[8]	6.2	6.2	4.2	3.5
Supplements	15.3	25.5	9.6	13.2
Carotenes[9] (µgRE/day)	**344**	**327**	**325**	**271**
% contribution from food sources:				
Dairy (excl. butter)	2.7	2.4	3.8	3.9
Butter/spreads/ margarines	3.6	2.5	6.1	5.7
Fruit & juice	2.4	4.1	2.4	2.3
Vegetables	58.5	64.1	66.9	68.3
Supplements	1.1	2.6	0.0	0.3
TOTAL (µgRE/day)	**1018**	**799**	**1262**	**1076**

7 In the NDNS analyses, the term 'liver' also includes liver sausage and liver pate.
8 Figures are based on broad groupings; the category 'Egg & Dishes' does not capture the contribution of eggs in many dishes where egg is not the primary ingredient, e.g. cakes.
9 Sum of β-carotene, and half the amount of α-carotene and β-cryptoxanthin.

Overall contribution of retinol and provitamin A carotenoids to population intakes for men and women by age

136. There was a wide range of intakes within age and sex groups and the distributions were skewed, reflecting the limited distribution of retinol in foods. Median intakes were 20-50% lower than mean intakes.

Adults Aged 19-64 Years

137. Based on the 2000/1 NDNS of adults (Henderson *et al*, 2003), 9% of men and 4% of women had intakes of retinol exceeding 1500 μg/day. The data show an age related trend: 1% of men and 3% of women in the 19-24y age group exceeded this level compared to 14% of men and 8% of women in the 50-64y age group.

138. Liver was a major source of retinol for those with retinol intakes greater than 1500 μg/day, contributing 70% of the total retinol intake for men and women. The average retinol intake of male liver consumers was 2540 μg/day, with 2061 μg/day from liver alone; the average retinol intake of female liver consumers was 1920 μg/day, with 1514 μg/day from liver alone. Consumers of liver comprised 10% of the NDNS sample, of which 57% of men and 41% of women had retinol intakes that exceeded 1500 μg/day. Liver consumption also shows an age related trend: liver contributed 31% of retinol intakes for men in the 19-49y age group, compared to 42% in the 50-64y age group; for women, liver contributed 18% of the retinol intake in the 19-49y age group and 27% in the 50-64y age group.

139. The contribution of dietary supplements to retinol intakes of those exceeding 1500 μg/day was 17% for men and 16% for women, which is less than the contribution from liver. Overall, dietary supplements containing retinol were consumed by 20% of the NDNS sample and among these 24% men and 9% of women had retinol intakes that exceeded 1500 μg/day.

140. The contribution of supplements containing fish oils (mainly fish liver oils) to retinol intake was 41 µg/day compared to 77 µg/day obtained from other supplements. For supplement consumers, fish oils contributed 431 µg/day of total retinol intake and other supplements contributed 341 µg/day.

141. Of those people who consumed neither liver nor supplements containing retinol, none had retinol intakes greater than 1500 µg/day.

Adults Aged 65 Years and Over

142. Data from the NDNS of people aged 65 years and over (Finch *et al*, 1998) suggest that 11% of men and 10% of women (excluding those in residential care) have retinol intakes above 1500 µg/day. For both men and women with retinol intakes over 1500 µg/day, liver contributed over 83% of intake. Among those who consumed liver, 78% of men and 79% of women had retinol intakes that exceeded 1500 µg/day.

143. Dietary supplements also contribute to higher intakes of retinol in this group accounting for approximately 8% of the retinol intakes of men and women with intakes exceeding 1500 µg/day. Among those taking dietary supplements containing retinol, 25% of men and 18% of women had intakes that exceeded 1500 µg/day.

144. The contribution of supplements containing fish oils (mainly fish liver oils) to retinol intake was 70 µg/d compared to 29 µg/d obtained from other supplements. For supplement consumers, fish oils contributed 631 µg/d of total retinol intake and other supplements contributed 179 µg/d.

145. Of those people who consumed neither liver nor supplements containing retinol, none had retinol intakes greater than 1500 µg/day.

Trends in Retinol Intake

146. Data from the 2000/1 NDNS of adults aged 19-64y (Henderson et al, 2003) show that mean retinol intakes are considerably less than they were in the 1986/7 survey (Gregory et al, 1990). For men, mean intakes were 673 µg/day in 2001 compared to 1277 µg/day in 1986/7; for women, mean retinol intakes were 472 µg/day in 2000/1 and 1133 µg/day in 1986/7.

147. The mean liver consumption (g/wk) of adults (19-64 years) in 2000/1, adults (16-64 years) in 1986/7, and adults aged 65 years and over in 1994/5, is shown in Table 2.

Table 2: Mean liver consumption (g/wk)

SURVEY	CONSUMERS OF LIVER						TOTAL NDNS SAMPLE
	Men (g/wk)	% who ate	Women (g/wk)	% who ate	All (g/wk)	% who ate	(g/wk)
Adults 2000/1 (19-64y)	133 (100)	12	97 (80)	7	119 (86)	9	11
Adults 1986/7 (16-64y)	127 (106)	23	114 (90)	24	120 (100)	24	29
Adults 1994/5 (65y+)*	185 (143)	11	171 (158)	10	177 (156)	10	18

Median values are given in parentheses

* free-living

148. Overall, mean liver consumption of adults aged 19-64 years was higher in 1986/7 (29g/wk, 4g/day) than in 2000/1 (11g/wk, 1.6g/day). The equivalent retinol intake would depend on the type of liver consumed; based on calf liver, which contains the highest levels, retinol intake would correspond to 7308 µg/wk (1044 µg/day) in 1986/87 and 2772 µg/wk (396 µg/day) in 2001.

149. The percentage consumers of liver was higher in 1986/7 (24%) than in 2000/1 (9%). For male consumers of liver, mean intakes were slightly higher in 2000/1 (133g/wk or 19g/day) than in 1986/7 (127g/wk, 18g/day); based on calf liver, this would be equivalent to retinol intakes of 33,516 µg/wk (4788 µg/day) in 2000/1 and 32,004 µg/wk (4536 µg/day) in 1986/7. For female consumers of liver, consumption was lower in 2000/1 (97g/wk,

14g/day) compared to 1986/87 (114g/wk, 16g/day); the equivalent retinol intakes (based on calf liver) would be 24,444 µg/wk (3528 µg/day) in 2000/1 and 28,728 µg/wk (4032 µg/day) in 1986/7.

150. Food composition data suggest that the retinol content of liver has decreased over time. A comparison of liver retinol content of different animal livers from the fifth edition of *McCance and Widdowson's The Composition of Foods* (MAFF, 1991) and the sixth edition (FSA, 2002) is shown in Table 3.

Table 3: Retinol content of liver based on food composition data from McCance and Widdowson's Composition of Foods

Type of Liver	Retinol content (µg/100g)	
	Fifth Edition (1991)	Sixth Edition (2002)
Calf (fried)	39,780	25,200
Chicken (fried)	12,230	10,500
Lamb (fried)	22,680	19,700
Ox (stewed)	20,100	17,300
Pig (stewed)	22,820	22,600

151. The percentage contribution of supplements to total vitamin A intake is shown in Table 4.

Table 4: Percentage contribution of supplements to total vitamin A and retinol intake

SURVEY	Total Vitamin		Retinol	
	Male	Female	Male	Female
Adults 2000/1 (19-64y)	10	15	15	25
Adults 1986/7 (16-64y)	3	5	4	7
Adults (65y+)* 1994/5	7	10	10	13

* free-living

152. The percentage contribution of supplements to total vitamin A and retinol intake in adults was notably higher in 2000/1 than in 1986/7. For men, supplements contributed 10% to total vitamin A intake in 2001 and 3% in 1986/7; for women, the contribution of supplements to total vitamin A intake was 15% in 2000/1 and 5% in 1986/7. The percentage contribution from dietary supplements to retinol intake was 4% in 1986/7 compared to 15% in 2000/1 for men, and 7% in 1986/7 compared to 25% in 2000/1 for women.

153. It has not been possible to assess trends in liver and supplement intake for adults over 64 years as there are no data since the 1994/5 NDNS of people aged 65 years and over (Finch et al, 1998).

Comparison of diets in the UK, USA, and Sweden

154. Evidence suggesting that retinol intakes above 1500 µg/day may be detrimental to bone is based on data from the USA and Sweden. To consider the relevance of these findings to the UK population, a comparison was made of food consumption and intakes of nutrients important to bone health in the UK, US, and Sweden.

155. Comparison of mean food consumption, based on surveys from different countries, can be misleading because of a number of factors. There may be differences in: dietary assessment methods; definitions used, e.g., in the classification of particular foods or food groups, and in the categorisation of adult age groups. There may also be variation in response rates and surveys may have taken place in different years. All surveys also have a certain level of sampling error but this is not always stated.

156. Data from national dietary surveys in the UK, USA, and Sweden, were used to compare diets between the three countries. These were the: 2000/1 NDNS of adults aged 19-64y, for the UK (Henderson et al, 2003); 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) (United States Department of Agriculture, 1997), for the USA; and Riksmaten 1997-98 Survey of Dietary Habits and Nutrient Intake in Sweden (Becker and Pearson, 2002).

157. Large differences in dietary assessment methodologies employed by these surveys may make any observations unreliable. The NDNS used weighed records of all foods consumed over 7 consecutive days, the CSFII used 24-hour recalls for two separate non-consecutive days, and the Riksmaten Survey used an unweighed pre-coded 7-day diet diary. There are also differences in age groups between the surveys and there may be differences in food composition between the countries.

158. For food groups, the available data are not comparable because of a number of differences between the three countries in classification of the foods under particular food categories.

159. A comparison of the mean daily intakes of total vitamin A, retinol, and nutrients important to bone health (vitamin D, calcium, potassium, phosphorus, magnesium) in the UK, USA, and Sweden, are set out in Annex 5. The data are only for food sources of these nutrients and do not include intake from supplements.

160. The main differences suggested by the available information are summarised below:

- *Total vitamin A:* Mean intakes are much higher in the USA and Sweden than in the UK. Compared to the USA, intakes in Sweden are 16% and 19% higher for men and women respectively. Compared to the UK, total vitamin A intakes are 44% higher for men and 64% higher for women in Sweden.

- *Retinol:* Mean retinol intakes are much higher in Sweden than in the USA and UK. Compared to the USA, intakes are 70% higher for men and 84% higher for women in Sweden; compared to the UK, intakes are 75% higher for men and 120% higher for women in Sweden.

- *Vitamin D:* Mean intakes of both men and women are highest in Sweden. Intakes in Sweden are 11% higher for men and 17% higher for women than in the USA and 68% higher for men and 75% higher for women than in the UK.

- *Calcium:* The highest mean intakes are in Sweden. For men, intakes are similar in Sweden and the UK; for women, intakes are 19% higher in Sweden than in the UK. Compared with the USA, intakes in Sweden are 21% greater for men and 44% greater for women.

- *Potassium:* The highest mean intakes of potassium are found in Sweden. Compared with the UK, mean intakes in Sweden were 5% higher for men and 15% higher for women. Compared with the USA, intakes in Sweden were 11% higher for men and 31% higher for women.

- *Phosphorus*: For men, mean intakes are similar in Sweden, USA, and UK; for women, intakes in Sweden are 16% higher than in the UK and 27% higher than in the USA.

- *Magnesium:* For men, mean intakes are similar in Sweden and the USA; for women intakes are 26% higher in Sweden than the USA. Compared to the UK, mean intakes are 11% and 27% higher in Sweden for men and women respectively.

161. The available information indicates that there are dietary differences between these countries in terms of nutrients related to bone health. Data from these surveys indicate that mean intakes, from food sources, of total vitamin A, retinol, and all the nutrients important for bone health are highest in Sweden. Retinol intakes appear to be much higher in Sweden than in the USA. This is inconsistent with assessments of mean retinol intake in the studies on retinol intake and bone health (see Annex 4), which show that mean retinol intakes are generally higher in the USA studies than for the study based in Sweden.

162. The dietary data from the surveys does not include information on nutrient intakes from supplements, which can make a substantial contribution to nutrient intakes of those who consume them. Without this information, assessment of retinol intakes and other nutrients important for bone health are unlikely to be reliable estimates.

Summary and Conclusions

163. The NDNS data suggest that a 4-11% of the population may have retinol intakes exceeding 1500 µg/day. Older age groups are more likely to exceed this level than younger age groups of the population.

164. High proportions of those people with retinol levels exceeding 1500 µg/day are consumers of liver. Dietary supplements (including fish liver oils) also make an important contribution to retinol intakes of consumers that exceed 1500 µg/day.

165. Liver consumption is greatest in the older age groups. This is of particular concern as older age groups are more vulnerable to bone fracture risk (Cummings et al, 1995). High levels of retinol intake might be compounding this risk.

166. Whilst population intakes of retinol from consumption of liver have decreased since 1986/7, there has been an increase in retinol intakes from supplements. The retinol content of liver has also fallen over this time period. Overall, population intakes of retinol are less than they were in 1986/7.

167. Data from national dietary surveys indicate that there are differences in nutrient intake between the UK, USA, and Sweden. Retinol intakes (from food sources) are much higher in Sweden than in the UK and USA. The relevance of these differences between the countries is difficult to ascertain because of varying methodologies. Furthermore, the dietary survey data do not include information on nutrient intakes from supplements, which can be an important source of retinol.

7 Retinol content of liver and animal feeding practices

168. As a detailed review of animal feed issues is outside the remit of SACN, they are only considered briefly in relation to retinol.

169. The retinol content of liver is high and varies widely between animals, both within and between species (Scotter et al, 1992). Calf liver contains the highest level (25,200 µg/100g) closely followed by pig liver (22,600 µg/100g) and lamb liver (19,700 µg/100g); lowest retinol concentrations are found in chicken liver (10,500 µg/100g) (FSA, 2002).

170. Concentrations of retinol in animal products (milk, meat, eggs) are strongly correlated with the level of retinol in the diets of the animals producing them.

171. For many non-ruminant livestock, diets are supplemented with synthetic retinol (usually as retinyl acetate). The maximum content of feeding stuffs for fattening farm animals in the EU is set by the European Commission (Directive 70/524/EEC, as amended).

172. In ruminants, provitamin A carotenoids in grass and other forages are converted to retinol. However, because both the concentration of carotenoids in forages and the efficiency of conversion to retinol are variable, supplementation of ruminant diets is common. Being fat-soluble, levels of retinol in milk closely follows the milk fat content and as a result, there are clear seasonal differences in the retinol content of bovine milk. These differences, however, may be modified by changes in the concentrations of retinol or provitamin A carotenoids in the diets of dairy cows. No maximum content has been set for the diet of lactating dairy cows.

173. On many farms, the young, recently weaned calf is the animal at greatest risk of developing retinol deficiency. Furthermore, retinol concentrations in the livers of pre-ruminant calves decline rapidly when the diet contains low levels of retinol (Swanson et al, 2000). For this reason, maximum

permitted contents of retinol in milk replacers for feeding calves are higher than for other feed materials. The higher levels of retinol in calf livers may therefore be the result of both higher fetal exposure and higher retinol concentrations in milk replacer powders.

174. Data from analyses of feeding stuffs by local authorities (FSA, 2005) show that the retinol content of animal feed may exceed the maximum content in some cases. Although this analysis was not carried out on representative samples from across the UK, it provides a useful indication of the situation. The practice of adding nutrients to products at levels higher than those stated on the label is known as *overage*. It is employed by some feed manufacturers to ensure that products contain at least the amount stated on the label throughout their shelf life, which may be up to 3 years.

Summary and Conclusions

175. The wide range of retinol content in animal products reflects the wide variation in livestock production systems in the UK.

176. Retinol concentrations in animal products are closely related to retinol concentration in feed. Lowering retinol supplementation of feeds would be expected to result in a reduction in the retinol concentrations in animal products.

177. An adequate retinol intake is essential for maintaining productivity, reproduction and the immune status of poultry and livestock. The veterinary implications of lower levels of retinol supplementation would need to be determined if a reduction in retinol supplementation of poultry and livestock is considered as part of a strategy to reduce retinol intake by human consumers.

8 The potential impact of dietary change to reduce intakes of Retinol

178. The impact of dietary change on retinol intakes was investigated by modelling data from the 2000/1 NDNS of adults aged 19-64 years (Henderson et al 2002) and the 1994/5 NDNS of people aged 65 years and over (Finch et al, 1998). The purpose of the modelling was to explore the effect of advice to reduce the proportion of the population with retinol intakes above 1500 µg/day on the proportion of the population with intakes below the LRNI. As liver is a rich source of other micronutrients, the effect of reducing liver intakes on the intakes of iron, zinc, folate, and B12 was also considered. The following scenarios were considered:

- Status quo based on NDNS data.
- Status quo based on adjusted NDNS estimates using information from the Oxford cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) Study.[10]
- Reduction of liver retinol levels by 25% through control of animal feed formulation.[11]
- Setting a 25g/week upper limit for liver consumption.[12, 13]
- Zero liver consumption.[13]

10 EPIC is an ongoing multicentre prospective study investigating the relationship between nutrition and cancer. It was initiated in 1992 and involves over 500,000 participants in 10 European countries, who will be followed for cancer incidence and cause-specific mortality for several decades. EPIC-Oxford is one of two EPIC cohorts in the UK, the other being EPIC-Norfolk.

11 An assumption has been made that this will not reduce retinol levels in other animal produce, particularly eggs and dairy. The retinol content of eggs and milk is influenced by concentrations in feed, but effects are variable depending on other feeding practices.

12 The following nutrient composition has been assumed for 25g liver, based on an approximate average of different liver codes in the NDNS nutrient databank: 5000 µg retinol, 3µg iron, 3.5µg zinc, 15?g vitamin B_{12}; 40µg folate.

13 Where this has resulted in reduced consumption for some consumers, the removed liver has not been substituted by other food.

- Elimination of retinol from all dietary supplements (except for intrinsic retinol, e.g. in cod liver oil, where it cannot be removed).

- Zero liver consumption and removal of retinol from all dietary supplements.

179. An explanation of how EPIC-Oxford data were used to adjust NDNS estimates of retinol intake can be found in Annex 6. The adjustments attempt to overcome two limitations of short-run dietary records with respect to habitual intake of rarely consumed foods: failing to capture some people who would be identified as liver consumers if assessed over a longer time period; and overestimating typical weekly consumption for those liver consumers who are captured.

180. The percentage of consumers whose intakes fall above or below the Guidance Level of 1500 µg/day and below LRNIs, under the different scenarios, can be found in Annex 7.

Scenario modelling for adults aged 19-64 years

181. The 2000/1 NDNS data indicate that for adults aged 19-64 years, the proportion of the population who currently have retinol intakes above 1500 µg/day is 8.8% for men and 3.9% for women. These data are comparable with those obtained from the EPIC-Oxford study where retinol intakes above 1500 µg/day were 9.3% for men and 4.2% for women.

182. Results from the different scenarios are given below:

- *Reduction of liver retinol levels by 25%* would result in a slight decrease in the percentage of men with intakes above 1500 µg/day, from 8.8% to 8%, but would result in no change to the percentage of women with intakes above 1500 µg/day.

- *Decrease in liver consumption to 25g/week* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 3.6% and 1.8% respectively.

- *Complete removal of liver from the diet* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 2.3% and 1.5% respectively.

- *Complete removal of retinol from supplements* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 6.1% and 2.4% respectively. This scenario would result in an increase in the proportion of people with total vitamin A intakes falling below the LRNI for total vitamin A from 5.7% to 6.7% for men and from 7.5% to 9.0% for women.

- *Complete removal of liver from the diet and complete removal of retinol from supplements* would reduce the percentage of men and women with retinol intake levels above 1500 µg/d to 0%. This scenario would not have any major effects on intakes of iron, zinc, folate, and vitamin B12 but would result in an increase in the proportion of people with total vitamin A intakes falling below the LRNI for total vitamin A from 5.7% to 7% for men and from 7.5% to 9.2% for women.

Scenario modelling for adults aged 65 years and over

183. The NDNS of people aged 65 years and over (Finch et al, 1998) indicates that in 1994/5, the proportion of the population with retinol intakes above 1500 µg/day was 10.6% for men and 9.5% for women. Results from the scenario modelling show that the estimate for retinol intakes above 1500 µg/day, based on EPIC-Oxford data, are 14.9% for men and 13.4% for women.

184. Results from the different scenarios are given below:

- *Reduction of liver retinol levels by 25%* would reduce the percentage of men and women with intakes above 1500 µg/day to 9.5% and 9.4% respectively.

- *Decrease in liver consumption to 25g/week* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 3% and 2.6% respectively.

- *Complete removal of liver from the diet* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 2.3%.

- *Complete removal of retinol from supplements* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 8.3% and 7.1% respectively.

- *Complete removal of liver from the diet and complete removal of retinol from supplements* would reduce the percentage of men and women with retinol intake levels above 1500 µg/day to 0%.

185 None of the above scenarios would affect the proportion of people with total vitamin A intakes below the LRNI or affect intakes of iron, zinc, folate, and vitamin B_{12}.

Summary and Conclusions

186. The scenario modelling for adults aged 19-64 years, suggests that decreasing liver consumption to 25g/week, complete removal of liver from the diet, or complete removal of retinol from supplements, would all lead to substantial reductions in retinol intakes. The most effective way to reduce intakes would be by removing both main sources of retinol, i.e. liver and supplements, which would prevent the occurrence of any retinol intakes above 1500 µg/day. Although this scenario does not have any major effects on intakes of iron, zinc, folate, and vitamin B_{12}, it may lead to an unacceptable increase in the proportion of the population with intakes falling below the LRNI for total vitamin A intake.

187. The scenario modelling for adults 65 years and over suggests that the NDNS for people aged 65 years and over (Finch et al, 1998) may underestimate the proportion of adults in this age group with retinol intakes above 1500 μg/day. In this population, decreasing liver consumption to 25g/week or complete removal of liver from the diet would lead to substantial reductions in retinol intake. Complete removal of retinol from supplements would have a smaller effect. Removing both liver and supplements would prevent the occurrence of any retinol intakes above 1500 μg/day. This scenario does not have any major effects on intakes of iron, zinc, folate, and vitamin B_{12}, or on the proportion of the population with intakes falling below the LRNI for total vitamin A intake.

9 Conclusions

188. The EVM set a Guidance Level for retinol intake of 1500 µg/day, for adults, based on evidence that intakes above this level may increase the risk of bone fracture (EVM, 2003). By setting a Guidance Level, the EVM recognised that the data on retinol intake and bone fracture risk were not robust enough to set a Safe Upper Level. Guidance Levels represent an approximate indication of levels that would not be expected to cause adverse effects but have been derived from limited data and are less secure than SULs.

189. The EVM did not set a Guidance Level for retinol intakes for children because of insufficient data. Advice on total vitamin A intakes for children remains unchanged (Department of Health, 1991, see paragraph 32).

190. The evidence for an association between high intakes of retinol and poor bone health is inconsistent. There are some epidemiological data to suggest that retinol intakes of 1500 µg/day and above are associated with an increased risk of bone fracture. Only one intervention study has examined this relationship. This study showed no effect of short-term supplementation using a very high dose of retinol (7576 µg/d) on plasma markers of bone turnover in healthy men; the long-term effects of high dose supplementation, however, are unknown.

191. Data published since the EVM report, do not strengthen the evidence for an association between retinol intake and bone health. The available data reflect the inherent difficulties of conducting large scale studies of lengthy duration which are necessary in order to observe long-term risks and the problems of isolating effects of retinol intake from all the other factors associated with bone health.

192. The evidence on the relationship between retinol intake and risk to bone health originates mainly from the USA and Sweden. Although there are differences in the intake of nutrients important for bone health between the UK, the USA, and Sweden, the relevance of these differences is difficult to ascertain because of the limitations in comparing dietary data from different countries.

193. Data from the NDNS for adults aged 19-64 years (Henderson et al, 2003) indicate that 9% of men and 4% of women have retinol intakes exceeding the Guidance Level of 1500 µg/day. The NDNS of people aged 65 years and over (Finch et al, 1998) suggest that 11% of men and 10% of women have retinol intakes exceeding 1500 µg/day.

194. In the UK, liver is the major source of retinol for those with retinol intakes exceeding 1500 µg/day. Supplements also make an important contribution to retinol intakes of those consuming more than 1500 µg/day of retinol.

195. Consumption of liver was lower in 2000/1 than in 1986/7 and the retinol content of liver has also decreased over this time period. The intake of supplements containing retinol was higher in 2000/1 than in 1986/7. Overall, retinol intakes in the UK were less in 2000/1 than in 1986/7.

196. There is very limited evidence, from animal studies, that inadequate intakes of vitamin D might increase the adverse effects of high retinol levels on bone. In the UK, vitamin D status is poor in some subgroups of the population (e.g. older people confined indoors, some ethnic communities) who may potentially be at more risk from high retinol intake.

10 Recommendations

197. There is currently insufficient evidence on the association between bone health and retinol intakes above 1500 µg/day to justify a change in dietary advice to all consumers regarding consumption of foods or supplements containing retinol.

198. As a precaution, however, it may be advisable for regular consumers of liver (once/week or more) not to increase liver intakes or take supplements containing retinol (including those containing fish liver oil).

199. It may also be advisable for population subgroups at increased risk of osteoporosis, such as postmenopausal women and older people, not to consume retinol at intakes greater than 1500 µg/day. This could be achieved by limiting intakes of liver, either by consuming smaller portions[14] or eating liver less often, and limiting intakes of supplements containing retinol (including those containing fish liver oil). As vitamin A (retinol/provitamin A carotenoids) is essential for health at all ages, it is important to ensure that consumption should not be reduced to levels below the reference nutrient intake for total vitamin A intake (700 µgRE/day for men and 600 µgRE/day for women).

200. Based on evidence for the teratogenic risk of retinol, pregnant women or women planning to become pregnant are still advised not to consume liver, liver products, or supplements containing retinol (DH, 1990).

201. Further research is required on the relationship between retinol intake and fracture risk, as the lack of reliable data makes it difficult to reach clear conclusions. Ethical considerations would not permit a long-term intervention study on the effects of increasing retinol intakes on bone health; prospective studies, with more reliable and long-term assessments of retinol intake, are necessary to clarify whether high intakes have a detrimental effect on bone. Emerging evidence regarding the relationship between retinol intake and bone fracture should also be monitored.

[14] The average portion size of liver for consumers of liver is 100g (Ministry of Agriculture Fisheries and Food, 1997)

202. A reduction in retinol content of poultry and livestock feed as part of a strategy to reduce the retinol intake of regular consumers of liver should be explored further. The implications of lower levels of retinol supplementation for the welfare and productivity of poultry and livestock would need to be determined should such a strategy be considered.

203. Consideration should also be given to reducing the levels of retinol in supplements. Dietary supplements can contain 30-65% more retinol than the amount stated on the label. This is due to the practice of *over-formulation* employed by the food supplements industry to compensate for any loss of product during the manufacturing process and to ensure that products contain no less than the amount stated at the point of sale and throughout their shelf-life.

204. Monitoring of trends in retinol consumption would be advisable.

205. A clearer understanding of the interactions between retinol and vitamin D is required. Future prospective studies, in humans, on the association between high retinol intake and fracture risk should also assess vitamin D exposure.

206. As previously recommended by COMA (DH, 1998), groups that may be at risk of poor vitamin D status should be made aware of the importance of achieving adequate vitamin D status.

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Annex 1

Current Legislation regarding food supplements and food fortification

Food supplements

In Great Britain most products described as dietary or food supplements are regulated as foods and subject to the general provisions of the Trade Descriptions Act 1968, the Food Safety Act 1990 and the Food Labelling Regulations 1996 (as amended). The Food Safety Act makes it an offence to sell food that is not safe for consumption, not of the nature, substance or quality demanded by the consumer or that is falsely or misleadingly described or labelled.

The Food Labelling Regulations lay down general labelling requirements and prohibit the use of medicinal claims. The Regulations contain specific requirements that must be met if a supplement claims to be a source of vitamins and minerals including that it is rich in, or an excellent source of such substances. The Regulations also prohibit any food, including a supplement, from making a claim that it has the property of preventing, treating or curing a human disease or any reference to such a property.

Food supplements, like other foods, are not required to demonstrate their efficacy before marketing, nor are they subject to prior approval unless they are genetically modified or are "novel". It is the responsibility of the manufacturer, importer or distributor to ensure that their product complies with the necessary legislation.

A product presented for treating or preventing disease, or which may be administered with a view to restoring, correcting or modifying physiological function in humans, falls within the definition of a medicinal product. Such products normally require a licence before they can be sold or supplied and are subject to the requirements of the Medicines for Human Use (Marketing Authorisation) Regulations 1994. Control of medicinal products is the responsibility of the Medicines and Healthcare products Regulatory Agency (MHRA).

The EU Directive 2002/46/EC, harmonising European Community legislation on food supplements, defines the term 'food supplements', contains a list of vitamin and mineral sources that may be used in the manufacture of food supplements, sets out labelling requirements and provides a framework for maximum and minimum levels for vitamins and minerals in food supplements to be set in the future. The Directive came into force on 12 July 2002 and was implemented in UK law in July 2003. The Food Supplements (England) Regulations 2003 came into force on 1 August 2005.

Food fortification

Fortification of foods with nutrients is subject to general safety controls provided for in the Food Safety Act 1990. No specific maximum limits are laid down in the Act or anywhere else in UK legislation. Responsibility for ensuring that products are safe and properly labelled lies with manufacturers. Voluntary fortification of foods is permitted in the UK providing that the final foodstuff is safe and appropriately labelled. In the UK, margarine and some types of flour are subject to mandatory fortification.

The European Commission adopted a draft proposal on the addition of vitamins and minerals to food in November 2003 to harmonise Community rules on the voluntary addition of nutrients to food. The aims of the Regulation are to facilitate the free circulation of such products and to provide a high level of consumer protection across the Community by ensuring that the products concerned do not present any risk to public health. The proposal is only concerned with the *voluntary* addition of vitamins and minerals to foods and will not affect national rules on mandatory fortification.

Annex 2

Expert Group on Vitamin and Minerals

TERMS OF REFERENCE

- Establish principles on which controls for ensuring the safety of vitamin and mineral supplements sold under food law can be based;
- Review the levels of individual vitamins and minerals associated with adverse effects;
- Recommend maximum levels of intakes of vitamins and minerals from supplements if appropriate;
- Report to the Food Standards Agency board (previously until 2001 recommendations were presented to the Food Advisory Committee).

Annex 3

Tolerable Upper Intake Levels of preformed Vitamin A

Table 1: Food and Nutrition Board, Institute of Medicine, USA

Age	Tolerable Upper Intake Level (UL) for preformed vitamin A (µg/day)
0-12 months	600
1-3 years	600
4-8 years	900
9-13 years	1,700
14-18 years	2800
Adults	3000

The UL for children and adolescents was based on the adult UL of 3000 µg/d, adjusted on the basis of relative body weight using the formula: $UL_{child} = UL_{adult}$ X $Weight_{adult}/Weight_{child.}$

Values for reference weights are based on data collected from 1998-1994 as part of the Third National Health and Nutrition Examination Survey (NHANES III).

Table 2: European Scientific Committee on Foods

Age (years)	Tolerable Upper Intake Level (UL) for preformed vitamin A (retinol and retinyl esters) (µg RE/day)
1 – 3	800
4 – 6	1100
7 – 10	1500
11 – 14	2000
15 – 17	2600
Adults	3000

The UL for children is based on the value of 3000 µgRE/day for adults, with correction for differences in basal metabolic rate compared to adults using scaling according to body surface area (body weight $^{0.75}$)

Annex 4

Human studies of retinol and bone health

Table 1: Studies of retinol and bone fracture

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Sowers & Wallace (1990) *Retinol, supplemental vitamin A and bone status* USA	Cross-sectional	246 women Postmenopausal Age: 55-80y *Inclusion criteria:* No bone mass associated disease Able to climb 3 stairs	Interview re intake of nutritional supplements & 24-hour food recall	Serum retinol	Self-reported by interview	Total vitamin A from food & supplements: 903 µgRE From: Supplements: 309 µgRE Food: 594 µgRE Separate retinol and β-carotene intake not provided.	Age, body size, smoking status, oestrogen use, thiazide drug use humeral muscle area	No association found between increased probability of, fracture and serum retinol or supplement use
Melhus et al (1998) *Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture* SWEDEN	Case-Control	1120 women Age:40-76 years 247 cases/873 controls	FFQ of usual intake of 60 foods during previous 6 months	N/A	Hospital discharge records	Total vitamin A from food: Cases: 1760 µgRE Controls: 1630 µgRE Retinol from food: Cases:960 µg Controls: 880 µg β-carotene from food: Cases: 800 µgRE Controls: 750 µgRE	Energy intake, BMI, age at menopause, lifetime physical activity, smoking status, hormone replacement therapy, diabetes mellitus, oral contraceptive or cortisone use, previous osteoporotic fracture, iron, vitamin C, calcium.	Odds Ratio = 2.1 with retinol intake >1500µg/d compared to ≤500µg/d (95% CI, 1.1-4.0) After adjustment for intakes of iron, magnesium, vitamin C and calcium: Odds Ratio = 1.54 with retinol intake >1500 µg/d compared to ≤500µg/d (p = 0.02) No increased risk observed with β-carotene

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Feskanich et al (2002) *Vitamin A intake and hip fractures among postmenopausal women* USA	Prospective Study 18y follow-up	72,337 women Age: 34-77y *Exclusion criteria:* Previous hip fracture Diagnosis of cancer, heart disease, osteoporosis, stroke	FFQ every 4 years. Mean intake values determined from the mean of 5 FFQs over 18 years.	N/A	Self-reported by questionnaire	Total vitamin A from food and supplements: 2265 µgRE Retinol from food and supplements: 1212 µg β-carotene from food and supplements: 771 µgRE	Age, follow-up cycle, BMI, postmenopausal hormone use, smoking status, physical activity, thiazide drug use, intakes of calcium, protein, vitamins D and K, alcohol use, caffeine.	Relative Risk = 1.48 with total vitamin A intake ≥3000 µgRE/d compared to <1250 µgRE/d (95% CI, 1.05-2.07) Relative Risk = 1.89 with retinol intake ≥2000 µg/d compared to <500 µg/d (95% CI, 1.33-2.68) No association found between β-carotene and fracture risk
Michaëlsson et al (2003) *Serum retinol levels and the risk of fracture* SWEDEN	Prospective Study 30y follow-up	2047 Men Age: 49-51y	7-day dietary assessment in subgroup of 1138 men, 20 years after entry into study	Serum retinol and serum β-carotene at baseline	Hospital discharge register	Not provided.	Age, weight, height, serum β-carotene, serum calcium, serum albumin, smoking status, physical activity, alcohol use.	Rate Ratio *(5th quintile of serum retinol concentration compared to 3rd quintile)*: For any fracture = 1.64 (95% CI, 1.12-2.41) For hip fracture = 2.47 (95% CI, 1.15-5.28) No association between serum β-carotene levels and risk of fracture.

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Lim et al (2004) *Vitamin A intake and the risk of hip fracture in postmenopausal women: the Iowa Women's Health Study* USA	Prospective Study 9.5y follow-up	34,703 Women Postmenopausal Age: 55-69y *Exclusion criteria:* Premenopausal Energy intakes <600 kcal or >5,000 kcal History of cancer	FFQ at baseline	N/A	Self-reported by questionnaire	Total vitamin A from food and supplements: 4333 µgRE Total vitamin A from food and supplements Supplement users: 5699 µgRE Non-users: 3584 µgRE Separate retinol and β-carotene intake not provided	*For hip fracture:* Age, BMI, waist-to-hip ratio, diabetes mellitus, physical activity, steroid medication, oestrogen replacement, energy intake. *For all fractures:* As above (excl. waist-to-hip ratio) – plus alcohol use, cirrhosis, thyrotropic, sedative, antiepileptic, or diuretic medication.	No association found between total vitamin A or retinol intake from supplements only, food and supplements, or food only and risk of hip or all fractures

Table 2: Studies of retinol and bone mineral assessment

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Sowers & Wallace (1990) *Retinol, supplemental vitamin A and bone status* USA	Cross-sectional	246 women Postmenopausal Age: 55-80y *Inclusion criteria: No bone mass associated disease* Able to climb 3 stairs	Interview re intake of nutritional supplements & 24-hour food recall	Serum retinol	Bone mass Raidal bone SPA	Total vitamin A from food and supplements: 903 µgRE From Supplements: 309µgRE Food: 594mgRE Separate retinol and β-carotene intake not provided	Age, body size, smoking status, oestrogen use, thiazide drug use, humeral muscle area.	No association between radial bone mass and vitamin A supplement and dietary intake or serum retinol levels.
Melhus et al (1998) *Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture* SWEDEN	Cross-sectional	175 women Pre & postmenopausal Age: 28-74y	Four 1-week dietary records	N/A	BMD Lumbar spine Total body 3 regions of proximal femur DXA	Retinol from food: 780µg β-carotene from food: 733 µgRE	Age, energy intake, BMI, smoking status, exercise, cortisone use, diabetes mellitus, menopausal status, HRT, oral contraceptives, exercise, previous fracture	At retinol intakes >1500µg/d compared to <500µg/d significant reductions in BMD: 14% at lumbar spine (p=0.001) 6% for total body (p=0.009) Proximal femur: 10% at femoral neck (p=0.05) 13% at Ward triangle (p=0.01) 9% at trochanter (p=0.03)

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Ballew et al (2001) *High serum retinyl esters are not associated with reduced bone mineral density in the Third National Health & Nutrition Examination Survey, 1988-1994* USA	Cross-sectional	2888 men 2902 women Age: 20-90y *Exclusion criteria:* Use of anti-seizure or corticosteroid medication, cancer or thyroid disease, elevated c-reactive protein, acute hepatitis or chronic liver disease, lactating	24-hour recall	Serum retinyl ester	BMD Total hip Femoral neck Trochanter Intertrochanter Ward's triangle DXA	Median total vitamin A from food and supplements: ♀ (premenopausal): 1837 μgRE ♀ (postmenopausal): 2096 μgRE ♂ 2193 μgRE Separate retinol intake and β-carotene intake not provided.	Sex, age, BMI, race/ethnicity, smoking status, alcohol use, diabetes, physical activity, supplemental intakes of vitamin A, D & calcium, oral contraceptives, oestrogen replacement use, menopausal status, parity	No association found between serum retinyl esters and BMD at any site
Sigurdsson et al (2001) *A lack of association between excessive dietary intake of vitamin A and bone mineral density in seventy-year-old Icelandic women* ICELAND	Cross-sectional	232 women Age: 70y` *Exclusion criteria:* Hyperparathyroid-ism Oestradiol use Bisphosphanates use Glucocorticoid use	One FFQ for previous 3 months of intake	N/A	BMD Total skeleton Lumbar spine Femoral neck Trochanter Intertrochanteric region DXA	Total vitamin A from diet and supplements: 2500 μgRE Retinol: 2300 μg β-carotene: 143 μgRE	Total energy intake, body weight/body composition, calcium intake	No association found between retinol or ß-carotene intake and BMD at any site

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Freudenheim et al (1986) *Relationships between usual nutrient intake and bone-mineral content of women 35-36 years of age: longitudinal and cross-sectional analysis* USA	Cross-sectional & Prospective 4y follow-up	99 women Pre & postmenopausal Age: 35-65y *Exclusion criteria:* Acute/chronic disease, history of immobility or osteoporosis, use of oestrogen or oestrogen like compounds, corticosteroids, fluoride, diphosphonates., calcitonin	Seventy-two 24h-dietary records collected for each participant over 3 years	N/A	BMC Arm bones: Radius Humerus Ulna SPA	Vitamin A (not clear if total vitamin A or retinol) from food and supplements: Premenopausal ♀ Non-Ca supplemented: 2718 μgRE Ca supplemented: 1912 μgRE Postmenopausal ♀ Non-Ca supplemented: 2590 μgRE Ca supplemented: 2280 μgRE β-carotene intake not provided.		*Cross-sectional analysis:* No association between vitamin A and arm bone loss. *Longitudinal analysis:* In postmenopausal calcium supplemented group, negative correlation between vitamin A and rate of change in ulna BMC – correlation not significant when 1 subject with very high vitamin A supplemental intake omitted. In premenopausal non-calcium supplemented, total vitamin A intake associated with increase in humerus bone (p=0.07).

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	**Results**
Houtkooper et al (1995) *Nutrients, body composition and exercise are related to change in bone mineral density in premeno-pausal women* USA	Prospective 1y follow-up	66 women Premenopausal & taking calcium supplements Age: 28-39y *Exclusion criteria:* Pregnant or lactating, use of oral contraceptives or medications affecting bone metabolism, regular exercise, history of anorexia, bulimia, cancer, diabetes, thyroid disease, or myocardial infarct	Dietary records over 8 randomly assigned days	N/A	BMD Total body Lumbar vertebrae 2-4 Femoral neck Ward's triangle Trochanter DXA	Vitamin A (not clear if total vitamin A or retinol) from food only: 1220 µgRE β-carotene: 595 µgRE	Fat mass at baseline and rate of change in fat mass over 1 year, exercise status	Vitamin A intake significantly associated with increase in total body BMD (R^2=0.31, p=0.02) For β-carotene intake R^2=0.28, p=0.07
Promislow et al (2002) *Retinol intake and bone mineral density in the elderly: The Rancho Bernardo Study* USA	Cross-sectional & Prospective 4y follow-up	570 women 388 men Age: 55-92y at baseline	Diet assessment questionnaire	N/A	BMD Total hip Femoral neck Lumbar spine DXA	Retinol from food and supplements: ♀ = 1248 µg ♂ = 1244 µg food only: ♀ = 497 µg ♂ = 624 µg β-carotene intake not assessed	Age, weight change, BMI, calcium intake, diabetes status, menopausal status, exercise, smoking status, alcohol use, thiazide drug use, thyroid hormone use, steroid use, oestrogen use, supplemental retinol	No association between retinol intake and BMD or BMD change. *For ♀ supplement users only:* A significant negative association was found between retinol intake and BMD at the femoral neck (p=0.02) and total spine (p=0.03) and for BMD change at femoral neck (p=0.05) and total hip (p=0.02).

Study	Design	Study sample	Dietary assessment	Biomarker	Bone mineral measurement, site & method	Mean daily total vitamin Aretinol/ β-carotene intake	Other factors considered in analysis	Results
Promislow et al (2002) *Retinol intake and bone mineral density in the elderly: The Rancho Bernardo Study* USA	Cross-sectional & Prospective 4y follow-up	570 women 388 men Age: 55-92y at baseline	Diet assessment questionnaire	N/A	BMD Total hip Femoral neck Lumbar spine DXA	Retinol from food and supplements: ♀ = 1248 µg ♂ = 1244 µg food only: ♀ = 497 µg ♂ = 624 µg ß-carotene intake not assessed	Age, weight change, BMI, calcium intake, diabetes status, menopausal status, exercise, smoking status, alcohol use, thiazide drug use, thyroid hormone use, steroid use, oestrogen use, supplemental retinol	No association between retinol intake and BMD or BMD change. *For ♀ supplement users only:* A significant negative association was found between retinol intake and BMD at the femoral neck (p=0.02) and total spine (p=0.03) and for BMD change at femoral neck (p=0.05) and total hip (p=0.02).

BMD=bone mineral density; BMC=bone mineral content; SPA=single photon absorptiometry; DXA=dual energy x-ray absorptiometry; Ca=calcium

Table 3: Studies of retinol and skeletal markers of bone turnover

Study	Design	Study sample	Supplemental dose of retinol	Biochemical Measure	Mean vitamin A intake	Other Factors Considered	Results
Kawahara et al (2002) *Short-term vitamin A supplementation does not affect bone turnover in men* USA	Randomised single-blind trial 6 weeks	80 Men Age: 18-58y *Exclusion criteria:* Renal or hepatic disease, history of malabsorption, use of compounds that interfere with fat absorption	7576 µg retinol palmitate/d	Osteocalcin, Bone specific alkaline phosphatase, N-telopeptide of type-1 collagen.	Not given		No change in any measured marker of skeletal turnover

Annex 5

Comparison of diets in UK, US, and Sweden

Average daily intakes of nutrients important to bone health in the UK, US, and Sweden *(does not include intake from supplements)*

NUTRIENTS	NDNS 2001 (19-64y)		USDA 1994-96 Continuing Survey of Food Intakes by Individuals (20y & over)		Riksmaten Swedish Dietary Survey 1997-98 (18-74y)	
	Men	Women	Men	Women	Men	Women
Total Vitamin A (µgRE)	911	671	1133	930	1310	1110
Retinol (µg)	571	352	589	422	1000	775
Vitamin D (µg)	3.7	2.8	5.6*	4.2*	6.2	4.9
Calcium (µg)	1007	777	886	643	1070	925
Potassium (µg)	3367	2653	3198	2332	3540	3060
Phosphorus (µg)	1493	1112	1474	1019	1570	1290
Magnesium (µg)	308	229	326	234	345	295

*Figures for vitamin D obtained from CDC (NHANES data 1988-94) (20-59yrs)
Note:
The surveys took place in different years. There are also great differences in methodologies, numbers of participants and response rates between each of the surveys.
The NDNS used 7-day weighed food diaries
The USDA survey used 24h recalls for 2 non-consecutive days
The Riksmaten Survey used unweighed pre-coded 7-day record book

Annex 6

Adjustment of NDNS estimates of retinol intake using data from EPIC-Oxford

In the 2001 NDNS of adults aged 19-64y, diet was assessed from weighed records of all foods consumed over 7 consecutive days. For the 1994/5 NDNS of adults aged 65y and over, diet was assessed from weighed records of all foods consumed over 4 consecutive days.

Food frequency questionnaire (FFQ) data from EPIC-Oxford suggest that of the people who consume liver at least once per month, only 20% do so at least once per week. Assuming this to be the case and further assuming that the remaining 80% consume liver at an average rate of once per month, then the two different levels of liver consumers have the following probabilities of materialising as liver consumers in the 7-day NDNS survey: high-level (100%) and low-level (25%). This means that only 40% of all liver consumers in the NDNS would be expected to be captured and for half of these, their liver consumption has probably been over-estimated (once per month consumers masquerading as once per week consumers). To attempt to recover the "true" picture, the following adjustments were made to the NDNS data:

(a) The level of liver consumption for a randomly selected 50% of NDNS-captured liver consumers was divided by a factor of 4;

(b) The frequency of low-level consumers was multiplied by a factor of 4, by adding low-level liver consumption to the dietary records of a randomly-selected subset of a size equal to 1.5 times the number who actually reported liver consumption during the 7-day NDNS food diary [as only 40% of consumers have been captured and it has been assumed that all those not captured are low-level consumers (see above)].

For the NDNS of adults aged 65 years and over, an extra step was required prior to the two adjustment steps described above. This was necessary to reflect the altered balance of probabilities of capturing liver consumers

when a 4-day food diary is used in place of a 7-day food diary. The number of liver consumers was randomly increased by a factor of 7/4 by adding liver consumption to the dietary records of a randomly-selected subset equal to $^{3}/_{4}$ times the number who actually reported liver consumption in the 4-day NDNS food diary. At the same time, the mean daily amount of liver consumed was reduced by a factor of 7/4. This assumes that each observed liver consumer would not consume liver again during that week, but that 3/7 of people who would have consumed liver in the week studied were missed due to incomplete coverage of the week.

Annex 7

Percentage of consumers whose intakes fall above/below Guidance Level[1] and LRNI[2] under different scenarios

Adults 19-64 years

Scenario	Vitamin A intakes		Other vitamins (< LRNI)			
	>1500 µg retinol only	<LRNI (µgRE)	Fe	Zn	Folate	B12
Status Quo	8.8	5.7	0.9	3.7	0.4	0.4
	3.9	7.5	28.9	3.8	2.1	0.7
Adjusted status quo	9.3	4.9	0.6	3.6	0.4	0.3
	4.2	6.8	28.5	3.7	2.0	0.6
25% less retinol in liver[3]	7.6	5.7	0.9	3.7	0.4	0.4
	3.6	7.5	28.9	3.8	2.1	0.7
Reduced liver (25g/w)	3.6	5.7	0.9	3.7	0.4	0.4
	1.8	7.5	29.3	3.8	2.1	0.7
No liver	2.3	6.0	0.9	3.7	0.4	0.4
	1.5	7.7	29.4	3.8	2.2	0.7
No retinol in supplements	6.1	6.7	0.9	3.7	0.4	0.4
	2.4	9.0	28.9	3.8	2.1	0.7
No liver and no retinol	0.0	7.0	0.9	3.7	0.4	0.4
in supplements	0.0	9.3	29.4	3.8	2.1	0.7

Males (top entry in each cell) and Females (bottom entry).

1 Guidance Level = 1500 µg/d
2 The LRNI represents the amount of a nutrient that is likely to meet the needs of 2.5% of the population.
3 Assumes that it is possible to reduce the level of retinol in liver (by controlling animal feed fortification) without also reducing the level in eggs and dairy products. Data are not presently available to support this assumption.

Adults aged 65 years and over

Scenario	Vitamin A intakes		Other vitamins (< LRNI)			
	>1500 µg retinol only	<LRNI (µgRE)	Fe	Zn	Folate	B12
Status Quo	10.6	4.7	0.7	8.0	0.6	0.4
	9.5	3.4	5.2	4.9	5.1	1.3
Adjusted status quo	14.9	2.9	0.6	7.3	0.5	0.3
	13.4	2.4	4.6	4.6	4.2	1.0
25% less retinol in liver[3]	9.5	4.7	0.7	8.0	0.6	0.4
	9.4	3.4	5.2	4.9	5.1	1.3
Reduced liver (25g/w)	3.0	4.7	0.7	8.0	0.6	0.4
	2.6	3.4	5.2	4.9	5.1	1.3
No liver	2.3	4.9	1.0	8.4	0.6	0.4
	2.3	3.7	5.2	5.1	5.2	1.3
No retinol in supplements	8.3	4.7	0.7	8.0	0.6	0.4
	7.1	3.9	5.2	4.9	5.1	1.3
No liver and no retinol in supplements	0.0	4.9	1.0	8.4	0.6	0.4
	0.0	4.3	5.2	5.1	5.2	1.3

Males (top entry in each cell) and Females (bottom entry).

3 Assumes that it is possible to reduce the level of retinol in liver (by controlling animal feed fortification) without also reducing the level in eggs and dairy products. Data are not presently available to support this assumption.