Australian Government
Department of Health and Ageing
National Health and Medical Research Council

MINISTRY OF HEALTH
MANATŪ HAUORA

Nutrient Reference Values for Australia and New Zealand

Evidence Appendix 2006

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EVIDENCE APPENDIX FOR THE 2006 NUTRIENT REFERENCE VALUES FOR AUSTRALIA AND NEW ZEALAND

ENDORSED BY THE NHMRC ON 9 SEPTEMBER 2005

UPDATES: In 2017, the fluoride AI and UL for infants and young children (0-8 years) and the sodium SDT and UL for adults were updated. For further information see the NHMRC Guidelines and Publications Page.

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This document is a general guide. The recommendations are for healthy people and may not meet the specific nutritional requirements of all individuals. They are designed to assist nutrition and health professionals assess the dietary requirements of individuals and groups and are based on the best information available at the date of compilation.

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Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes. A risk assessment model for establishing upper intake level for nutrients*. Washington, DC: National Academy Press, 1998.

Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.

Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes. Applications in dietary assessment*. Washington, DC: National Academy Press, 2000.

Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. Washington, DC: National Academy Press, 2001.

Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (macronutrients)*. Washington, DC: National Academy Press, 2002.

Food and Nutrition Board: Institute of Medicine. *Dietary Reference Intakes for water, potassium, sodium, chloride and sulfate*. Panel on the dietary reference intakes for electrolytes and water. Washington, D.C: National Academy Press, 2004.

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BACKGROUND

This Appendix details the evidence base for the 2006 Nutrient Reference Values for Australia and New Zealand (NHMRC 2005). The NRVs were developed with the assistance of a team of expert reviewers each of whom were asked to review one or more of the recently developed US:Canadian Dietary Reference Value to assess their suitability for use in Australia and New Zealand. To do this, reviewers were asked to look at the evidence base of the relevant US:Canadian review, using as a guide the NHMRC Levels of Evidence; to assess recommendations for other key countries and bodies such as the World Health Organization and to assess the relevance of any new data that had been published since these reviews.

THE ASSESSMENT PROCESS

Reviewers were asked to complete a pro forma that asked them to assess the suitability of the US:Canadian DRI recommendations for adoption in Australia and New Zealand taking into consideration:

- the completeness and currency of the evidence base
- the interpretation of the evidence
- the selection of indicators for estimating requirements
- the justifiability of recommendations for various age and gender categories
- whether the needs of special groups were considered, including vegetarians, formula fed vs breast fed babies; cultural and racial groups; cigarette smokers; oral contraceptive users; those with high alcohol use or drug use, athletes, tropical dwellers or any other special group
- interactions with other nutrients or non-nutrients including the issue of bioavailability
- whether the effect of other factors had been considered (socio-economic status of study populations, customary intake of other competing nutrients, or interfering/enhancing factors, lifestyle characteristics (eg physical labour) prevalence of disease, climatic effects etc)
- whether differences in dietary patterns of Australia and New Zealand were so markedly different from those of the US:Canada as to affect any of the recommendations (particularly relevant to the AI and AMDR recommendations)
- whether the upper level of intake (UL) was adequately addressed and whether it was appropriate for Australia/New Zealand
- whether there was evidence for a chronic disease protective effect of higher than RDI levels of intake
- whether they had any other considerations that they wished to raise that would affect recommendations for Australia and New Zealand
- recommendations from other countries such as the UK, European countries or bodies such as the FAO:WHO or European Commission

They were asked to provide an evidence based assessment of the key papers used in the US:Canadian review of DRIs to derive the recommendations and to provide an analysis of any key, missing papers or key papers published since the DRI review of that nutrient, using, where possible or relevant, the NHMRC levels of evidence (next page).

Finally, they were asked to state whether they felt that Australia/New Zealand should adopt, adopt with minor changes, adopt with substantial changes or reject the US:Canadian recommendation as unsuitable for use in Australia/New Zealand and to summarise their overall recommendations.

The evidence tables and rationales for variation from the recommendations of the US:Canadian DRI reviews are summarised in this Appendix.

THE EVIDENCE BASE

The National Health and Medical Research Council (NHMRC) has released a guidebook entitled "*How to use the evidence assessment and application of scientific evidence*". This guidebook, however, relates to evidence assessment in relation to clinical practice. In many cases the development of evidence-based guidelines for clinical practice deals with evidence in relation to a specific disease and a specific therapeutic agent. Similar criteria are not easily used for assessment of evidence related to the level of nutrient intake required for sustenance and avoidance of deficiency disease.

There are a number of initiatives underway around the world to try to develop an evidence-based approach to nutrition and health issues, but this has generally been in response to the need for "proof" in relation to health claims for food components. In Australia, a set of proposed levels of evidence for food or health claims has been developed by Food Standards Australia and New Zealand (FSANZ), which is similar to, but somewhat broader in scope than the NHMRC approach for clinical guidelines. These are primarily intended to assess evidence related to the efficacy of individual nutrients or food components in relation to chronic disease outcome rather than avoidance of deficiency disease.

However, the NHMRC designation of levels of evidence for clinical practice were still considered useful in relation to the scientific data discussed in this document.

The NHMRC Levels of Evidence are outlined below.

I	Evidence obtained from a systematic review of all relevant randomised controlled trials.
II	Evidence obtained from at least one properly-designed randomised controlled trial.
III-1	Evidence obtained from well-designed pseudorandomised controlled trials (alternate allocation or some other method).
III-2	Evidence obtained from comparative studies (including systematic reviews of such studies) with concurrent controls and allocation not randomised, cohort studies, case-control studies, or interrupted time series with a control group.
III-3	Evidence obtained from comparative studies with historical control, two or more single arm studies, or interrupted time series without a parallel control group.
IV	Evidence obtained from case series, either post-test or pretest/post-test.

Source: *A Guide to the Development, Implementation and Evaluation of Clinical Practice Guidelines* NHMRC 1999.

Six levels of evidence are designated by NHMRC, Level I being based on a systematic review of all relevant randomised control trials with Level II being on evidence obtained from at least one properly designed randomised control trial. With perhaps the exception of calcium, there are few Level I or Level II nutrient intervention trials assessing adequacy of nutrient intake in relation to deficiency states, although a number of nutrient-supplement trials have been undertaken in relation to chronic disease aetiology.

Some of the studies used to set nutrient requirements fall within Level III or Level IV, the levels of evidence which include study designs such as cohort studies, case-control studies and comparative ecological studies with historical controls or case series. However, much of the evidence comes from animal or human experimental studies that do not fall within these categories, or observational or cross-sectional survey data (eg all the recommendations for infants are based on the composition of milk from healthy mothers and a significant amount of the evidence for the ULs comes from individual case-reports of excessive intakes related to accidentally high intakes or under special conditions such as parenteral feeding).

Because of the nature of the Nutrient Reference Values, the background papers were developed as a result of a process of comprehensive, rather than systematic, review of the literature.

The NHMRC states that "a decision should be made about what is feasible and appropriate in a given situation and the extent to which reasonable standards have been met by the available body of evidence".

Although the recommendations were, where possible, evidence-based, there are generally very limited data on which to base recommendations. When assessing the literature, life-stage and gender are considered to the extent possible, but for many nutrients and for many age/gender/physiological categories, requirements have to be estimated from one age/gender category (on the basis of metabolic body weight, energy requirements, potentially decreased absorptive capacity, activity levels, additional needs for foetal growth or production of breast milk etc) rather than derived directly from experimental data. For infants, all recommendations are based on the composition of breast milk from healthy mothers.

The data on which the reference values are based are often scanty or drawn from studies that have substantial limitations. Apart from studies of frank deficiency disease, there are few studies that address the effects of inadequate intake on specific health indicators. While the recommendations are often given as single rounded numbers, it is acknowledged that these values may imply a precision not fully justified by the available human data. Nevertheless they represent our best attempt to identify the requirements of the various age/gender and life-stage groups.

ENERGY

The recommendations for energy were derived from an assessment of the evidence base and methods used by the US:Canadian Government review of DRIs of 2002 (FNB:IOM 2002) and the D.A.CH recommendations of the German, Austrian and Swiss Nutrition Societies (German Nutrition Society 2002) together with the FAO:WHO:UNU (2004) and current recommendations of other key countries.

After review, the approach of the US:Canadian report was used for infancy, but concerns with allocation of the limited physical activity levels (PALs) used in the US report led to retention of the approach used in setting the earlier Australian/New Zealand RDIs (NHMRC 1991) which is similar to the approach of both the German/Austrian/Swiss Nutrition Societies (2002) and FAO:WHO:UNU (2004).

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. DERIVATION AND DESIGNATION OF PHYSICAL ACTIVITY LEVELS (PALs)

The US:Canadian DRI report (2002) gathered data from studies of total energy expenditure (TEE) using doubly labelled water (DLW) and basal metabolic rate (BMR), and derived PAL by dividing TEE by BMR. The data were then grouped into four categories of PAL that were later labelled with a description of activity level (see Table 1). This PAL grouping is somewhat arbitrary, especially as there was no attempt to match the US category of activity with any description provided by the study that generated the data. For example, analysis of the data for adults in the healthy weight range listed in Appendix I of the US report showed that the average TEE for each activity group was approximately as listed in Table 1.

TABLE 1. PAL CATEGORIES USED IN THE US: CANADIAN DRI REPORT AND NORMAL WEIGHT ADULT PAL MEANS CALCULATED FROM DATA IN APPENDIX I OF THE FNB:IOM REPORT (2002)

Description of activity	US PAL categories used	Actual US Means[a]
Sedentary	PAL = 1.000–1.399	PAL = 1.24 ± 0.11 (n=54)
Low active	PAL = 1.400–1.599	PAL = 1.51 ± 0.06 (n=271)
Active	PAL = 1.600–1.899	PAL = 1.74 ± 0.08 (n=162)
Very active	PAL = 1.900–2.499	PAL = 2.07 ± 0.15 (n=109)

[a] calculated from data listed in the Appendix of the report

The selection of activity level groupings in the US report may not be the most appropriate or representative of western lifestyles. Firstly, a PAL of 1.24 (ie the US sedentary category) reflects little more than bed rest. Secondly, Black et al (1996) have shown that for more than 500 working adults with predominantly sedentary activity, PAL averaged 1.55–1.65. This straddles the US low active and active groups.

In children, there is very little information about activity level in relation to growth, development and present or future health, or about PALs associated with different lifestyles. Torun et al (1996) shows that 6–18 year-old children living in urban settings in industrialized countries have an average PAL of 1.5–1.8 which straddles the low active and active ranges of the US:Canadian report. Means for the children/adolescent database in the US:Canadian report show that a disproportionate number of the sample is in the sedentary or low active groups. Thus, the same concerns about arbitrary assignment of PAL cut off points exist for children and adolescents as for adults.

The method chosen to determine the energy requirements of children, adolescents and adults is based on energy expenditure (EE) data obtained from DLW studies. These suggested higher values for EE than previous methods based on factorial estimates alone.

There is limited information about the comparability of factorial estimates of EE and DLW estimates, except that DLW estimates tend to be higher. Although EE data obtained from DLW studies have been derived from subjects with a wide range of age, weight, height and PALS, there is no information about the extent to which the activity levels observed are representative of adults in the western world. Thus, although the average EE from all the DLW results included in the US:Canadian DRI report (FNB:IOM 2002) is about 1.7 x BMR (1.72 ± 0.29), it is not known to what extent this reflects a population average or a bias due to characteristics of subjects volunteering for study. As noted above, data from Black et al (1996) reported in the D.A.CH report (2002) suggest that in working adults with predominantly sedentary activity, PAL averages 1.55–1.65. This is lower than the US 'average'.

B. THE METHOD ADOPTED FOR ENERGY REQUIREMENTS IN CHILDREN, ADOLESCENTS AND ADULTS

The method used in this report for estimating energy requirements for children, adolescents and adults is similar to that used by the D.A.CH (German Nutrition Society 2002) report. It has the advantage of estimating EE at any PAL, but is limited by the choice of equation used to calculate BMR. It is also limited by uncertainty regarding the exact level of PAL to use, especially with children. However, this method is similar to that used to derive the previous Australian recommendations for energy intake that were adopted in New Zealand (NHMRC 1991) and the publication of the FAO:WHO:UNU (2004) described in the chapter on Energy in the Nutrient Reference Value report. An example is given below.

Worked example for estimating energy requirement:

Prediction of the food energy required to maintain energy balance in a group of non-pregnant, non-lactating women aged 45 years, with a mean body weight of 60 kg and a light activity level.

(a) Predicted BMR is 5.6 MJ/day (from equations of Schofield 1985; see Table 2)

(b) Energy expenditure in light activity is 1.6 x BMR (see Table 3 in main NRV Energy Chapter)

(c) Daily energy expenditure = BMR x activity level
= 5.6 x 1.6
= 8.96 MJ/day

(d) No adjustments are necessary for growth, pregnancy or lactation in this case

(e) No correction is needed to the energy requirement obtained if the women are consuming a 'typical' Australian/New Zealand diet containing about 20 g dietary fibre

TABLE 2. EQUATIONS FOR ESTIMATING BMR IN MJ/DAY FROM BODY WEIGHT IN KG

Gender	Age (years)	Equation (from Schofield 1985)
Males	3–10	BMR = 0.095.(kg body weight) + 2.110
	10–18	BMR = 0.074.(kg body weight) + 2.754
	18–30	BMR = 0.063.(kg body weight) + 2.896
	30–60	BMR = 0.048.(kg body weight) + 3.653
	>60	BMR = 0.049.(kg body weight) + 2.459
Females	3–10	BMR = 0.085.(kg body weight) + 2.033
	10–18	BMR = 0.056.(kg body weight) + 2.898
	18–30	BMR = 0.062.(kg body weight) + 2.036
	30–60	BMR = 0.034.(kg body weight) + 3.538
	>60	BMR = 0.038.(kg body weight) + 2.755

If correction of the energy requirement for the composition of the diet is needed, the procedure described in Annex III (FAO:WHO 2002) can be used as follows:

The metabolisable energy (ME) of the diet can be obtained using the following general conversion factors:

Protein	16 kJ per g
Fat	37 kJ per g
Available carbohydrate *(expressed by difference; minus dietary fibre)*	16 kJ per g
Total dietary fibre (in mixed diets)	8 kJ per g
Alcohol	29 kJ per g

For 'typical' Australian diets, which contain 10–20% energy from protein, 0–6% energy from alcohol, and 1–3% of energy from fibre, no correction is necessary as the error will be less than 2.5%. For diets very high or low in protein, or high in dietary fibre (or high in alcohol or other components not used traditionally in foods) the following corrections can be applied.

- For every 1% of ME from protein consumed above the amount that provides 15% of ME in the diet, increase the requirement by 0.2% (or add 3.4 kJ for each g of protein consumed above the amount that provides 15% of energy). Decrease (or deduct) this amount when protein intake is less than 15% of ME
- For every 1% of ME from alcohol consumed, increase the requirement by 0.1% (or add 3 kJ per g of alcohol to the energy requirement)
- For every 1% of ME from dietary fibre (natural and intact in plant foods) consumed above the amount that provides 2% of ME in the diet, increase the requirement by 0.25% (or add 2 kJ for each g of fibre above the amount that provides 2% of energy). Decrease (or deduct) this amount when dietary fibre intake is less than 2% of ME
- For other components when present in foods or diets, including those not traditionally added (eg non-digestible and fermentable carbohydrates, medium and short-chain triglycerides, lactic acid etc) add the difference between the metabolisable and net metabolisable energy values (kJ/g). Sets of both values are given in the FAO:WHO report (2002)

Worked example of adjustment for diet type:

For an energy requirement estimated at 8,000 kJ/day and a diet containing 30% of ME from protein, 5% of ME from fibre and no alcohol.

- For the extra protein (15% of energy above 15% ME for a total of 30%), increase the requirement by 3% (15 x 0.2%)
- For the extra fibre (3% ME above 2% ME for a total of 5%), increase the requirement by 0.75% (3 x 0.25%)
- In total, increase the energy requirement of this diet by 3.75% (3% for extra protein, 0.75% for extra fibre), for a total of 8,300 kJ/day (8,000 x 1.0375)

EVIDENCE BASE

Databases: PubMed and cross referencing to papers in US:Canadian DRI review (FNB:IOM 2002), the D.A.CH Reference values report (German Nutrition Society 2002) and COMA report on Dietary Reference Values (Department of Health 1991).

Search terms: doubly labelled water, doubly labelled water, doubly-labelled water, pregnancy, lactation, children, total energy expenditure, adolescents, infants.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to establish the energy content of breast milk		
Survey data	Butte et al (1984a)	Human milk intake and composition and infant growth measured longitudinally in 45 exclusively breast-fed infants monthly for 4 months. 37–41 datasets available at each time period. Babies were born to predominantly white, well-educated mothers. No information on sampling or refusal rates for participation. Methods used are appropriate.
Survey data	Dewey & Lonnerdal (1983)	Dietary intake, milk composition, growth and activity monitored monthly for 20 breast-fed infants from 1–6 months. Mothers were highly educated and not defined in terms of race. 18–20 datasets available at each time period. No information provided on sampling or refusal rates for participation. Methods seem appropriate. Activity assessed by diary.
Survey data	Dewey et al (1984)	Human milk intake and composition. Milk samples collected longitudinally from 46 women during months 7–20 of lactation (119 samples) and compared with composition of samples collected at 4–6 months of lactation (101 samples). Study compared data from Dewey & Lonnerdal (1983) from 20 mothers with additional data obtained from 12 of these mothers and another 62 mothers providing milk samples from 4–20 months of lactation. No information provided on sampling or refusal rates for participation. Methods seem appropriate.
Survey data	Ferris et al (1988)	Human milk intake and composition. Milk from 12 mothers collected longitudinally from 2–16 weeks post-partum. No information provided on sampling or refusal rates for participation. Methods seem reasonable.
Survey data	Lammi-Keefe et al (1990)	Human milk intake and composition. Milk from 6 mothers collected at 0600, 1000, 1400, 1800 and 2200 hours to determine differences in composition over the day. No information provided on sampling or refusal rates for participation (readers are referred to a previous paper). Methods seem reasonable.
Papers used to assess the energy cost of growth		
Review of survey data	Butte et al (1989)	Review of studies looking at energy cost of growth and components of growth. Compares energy balance data with theoretical body composition data and finds agreement between both methods.
Survey data	Butte et al (2000a)	Body composition of 76 children followed prospectively at 0.5, 3, 6, 9, 12, 18 and 24 months of age. 40 formula-fed, 36 breast-fed for first 4 months, primarily Caucasian. Sampling details and refusal rates are not given. Methods seem reasonable. Multi-compartment model used. Zero to 4 measures are missing at each time period. First longitudinal study to document prospective incremental growth rates partitioned into body composition segments. Effectively replaces Fomon et al (1982) for first 2 years due to greater accuracy and use of same children prospectively.
Survey data	Butte et al (2000b)	Pivotal study for on growth for the current review. 76 children studied [likely to be the same as in Butte et al (2000a)], 72 completed the study. TEE, sleeping metabolic rate and body composition measured at 3, 6, 9 and 12 months of life. Provides revised estimates of TEE and energy deposition data for first 2 years of life. Data provide the basis for growth estimates in the US report. No information provided on sampling or refusal rates for participation.

Level of evidence	Reference	Study type, issues addressed and key findings
Survey data	Fomon et al (1982)	Examines the body composition of children 0–12 years on which theoretical costs of growth are calculated. Several data sets are used, referenced to other papers or previous studies. Sampling information is referred to other papers or studies. Methods seem reasonable, although many assumptions are made to adjust calculations for adults to children. Extensive referencing to previous work makes assessing the research population difficult. Concludes that assumptions about adult body composition are not valid in children and provides reference values.
Re-analysis of published survey data	Roberts & Young (1988)	Data were from 4 published studies with ≥10 low birth weight infants in whom nutrient balance studies had been conducted to measure ME and nitrogen balance and calorimetry had been used to measure TEE. Sampling details and refusal rates not given but the reader is referred to source papers. Use of multiple regression appears reasonable and data are consistent with animal models.
Papers used to assess physical activity levels (PALs) in children and adolescents		
Review and re-analysis of published data	Torun et al (1996)	Pivotal study of PAL in children and adolescents for current. Reviews previous studies assessing total energy expenditure and PAL in children 1–18 years including DLW, heart rate monitoring and exercise diary studies. Presents data as raw means of combined studies, however states that weighted means were similar. Formal meta-analytic procedures are not employed. Data were acquired from a number of different population groups in 396 subjects for the DLW data. A larger sample size is given when the DLW data are combined with the other measures. As discussed in the notes for children, this use of the two tables results in inconsistencies in the US report.
Papers used to assess the additional energy requirements pre and post pregnancy		
Survey data	Forsum et al (1992)	DLW-TEE and energy intake (self-reported). Determined in 22 women at weeks 0, 16–18 and 30 of gestation and in 19 women at week 36. TEE decreased during early pregnancy because of reduced activity, and then increased substantially. Under-reporting of energy intake by participants in pre-pregnant state was substantial.
Survey data	Goldberg et al (1991)	DLW-TEE, energy intake (self-reported) and BMR. Determined in 10 women at 36 weeks of gestation. Daily TEE was about 600 kJ higher at 36 weeks than pre-pregnancy and energy cost of activity was about 900 kJ lower.
Survey data	Goldberg et al (1993)	DLW-TEE, BMR, (self-reported) energy intake and changes in body composition. Determined in 12 women pre-pregnancy and then at 6-week intervals during gestation. Individual variation in response of TEE to pregnancy was considerable. Mean total energy cost was higher than the recommendations at the time. Self-reported food intake was substantially less than the objectively-determined additional energy requirement to account for the observed weight gain and TEE.
Survey data	Koop-Hoolihan et al (1999)	DLW-TEE, RMR, diet-induced thermogenesis, activity, energy expenditure and energy intake measured in 10 women pre-pregnancy, at 8–10 weeks, 24–26 weeks and 34–36 weeks of gestation. Response of TEE to pregnancy varied widely (from –105 to 3,421 kJ/day). Major conclusion: "The use of a single recommendation for increased energy intake in all women is not justified."
Papers used to assess the energy costs of lactation		
Survey data	Allen et al (1991)	Milk composition and secretion rates. Composition of human milk measured during 6 months of exclusive breast feeding in 13 women.
Survey data	Anderson et al (1981)	Human milk intake and composition. Milk samples collected longitudinally during the first 29 days of lactation. 10 mothers with term births and 17 mothers with premature births.
Survey data	Anderson et al (1983)	Human milk intake and composition – milk samples collected longitudinally during the first 14 days of lactation in 9 mothers with term births and 14 mothers with premature births.
Survey data	Butte et al (1984b)	Human milk intake and composition –milk samples collected longitudinally during the first 4 months of lactation (n=45).
Survey data	Heinig et al (1993)	Human milk intake and composition – intakes of human and formula milk in infants up to 12 months old (73 breast fed, 46 formula fed infants).

Level of evidence	Reference	Study type, issues addressed and key findings
Survey data	Nommsen et al (1991)	Human milk intake and composition – milk samples collected longitudinally at 3, 6, 9 and 12 months post-partum (n=46 out of initial 92 were studied for the full 12 months).
Human experimental data	Butte et al (2001)	DLW-TEE lactation studies and weight change during lactation. TEE studied in 24 women at 3 months lactation.
Human experimental data	Forsum et al (1992)	DLW-TEE lactation studies and weight change during lactation. TEE studied in 23 women at 2 and 6 months lactation.
Human experimental data	Goldberg et al (1991)	DLW-TEE lactation studies and weight change during lactation. TEE studied in 10 women at 1, 2 and 3 months of lactation.
Human experimental data	Koop-Hoolihan et al (1999)	DLW-TEE lactation studies (mostly pregnancy but included TEE studied in 10 women at 1 month post-partum).
Human experimental data	Lovelady et al (1993)	DLW-TEE lactation studies and weight change during lactation. TEE studied in 9 women at 3–6 months lactation.
Review	Black et al (1996)	Lifestyle associated with different PALs. Reviews 574 DLW measures of TEE combined with descriptions of activity level.
Review	Butte & Hopkinson (1998)	Changes in weight and body composition during lactation (main finding was variability in weight change).

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MACRONUTRIENTS AND WATER

PROTEIN

The recommendations for protein were derived from an assessment of the evidence base used by the US:Canadian Government Dietary Reference Intake review of DRIs (FNB:IOM 2002) and consideration of additional key papers missing from that review or published since it was released and current recommendations of other key countries and organisations.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The US:Canadian DRI recommendations were adopted for use in Australia and New Zealand with a number of amendments as outlined below.

A. INFANTS

The data used to derive the average protein content of human milk for this age group differ in the analytical procedure used to determine the protein content. In the Butte et al (1984a) study, 'true' protein was measured based on the difference from Kjeldahl analysis of total and non-protein nitrogen (NPN), whereas protein was determined by the Lowry method in the other three data sets. The study of Heinig et al (1993), which measured milk protein content by both methods, found that the Lowry method gives values for protein that are essentially equivalent to total N x 6.25 or "crude" protein as obtained by Kjeldahl analysis. (the mean difference between Kjeldahl and Lowry values was 25.5%). Since a considerable proportion of the NPN in human milk is utilised, it seems more appropriate to use values for crude rather than true protein to estimate the AI for this age group. This approach was also used in the FAO:WHO:UNU 1985 report. If the Butte et al (1984a) data are converted to crude protein, then the estimated average protein content of human milk for this age group is 12.7 g/L and the intake is 1.65 g/kg/day. Data from an additional paper from Australia on the protein concentration of breast milk was also included in the estimate.

B. METHOD FOR CHILDREN 4 YEARS OF AGE AND OVER, ADOLESCENTS AND ADULTS

Gender differences in body composition develop during childhood and result in a gender difference in protein requirement for maintenance when expressed per kg body weight per day. The current US maintenance requirements are estimated to be 110 mg/kg/day for all children up to the age of 13 years based on aggregated nitrogen (N) balance data from 95 children (79 of whom were aged between 2 and 5 years). In the absence of N balance data for adolescents aged 14–18 years, the maintenance requirement is based on the average adult estimate (105 mg/kg/day) derived by Rand et al (2003). Changes in both the proportion of fat free mass (FFM) during growth and differences between boys and girls suggest that the assumption of the same N requirement per kg body weight throughout the age range and for both genders may not be appropriate.

The US adult protein requirement was derived primarily from a meta-analysis of 19 short-term N balance studies, with similar protocols (UNU 1979), from which balance data for individuals were available at three or more levels of intake in the range 50 to 200 mg N/kg/day (Rand et al 2003). Analysis of the data from these studies on 235 individuals (181 males and 54 females) resulted in a median N requirement for balance of 105 mg N/kg/day [0.66 g protein/kg/day (N x 6.25) and a CV of 12%. A loss of 5 mg N/kg/day was included in the temperate studies and 11 mg N/kg/day in the tropical studies to allow for dermal and miscellaneous N losses.

The data on which the Rand et al (2003) adult median requirement estimate is based differ from those used for the 1985 WHO:FAO:UNU estimate (on which the current Australian/New Zealand RDI was based) in four ways. They are:

- more representative of short-term N balance data for adults of both sexes and some older individuals
- based on studies of mixed as well as single source proteins
- inclusive of a different allowance for dermal and miscellaneous losses under temperate and tropical conditions
- derived from an analysis of individual, rather than aggregated estimates of N balance

A significant difference with gender was found (109 versus 91 mg N/kg) but was not used in deriving the US:Canadian EAR:RDI because the difference disappeared when the estimate was based on grouped data from the same studies. Rand et al (2003) explained this result on the grounds of skewness and variability in the data for the men. They also noted, however, that the observed gender difference might be expected on the basis of differences in body composition and commented that this warranted consideration of how the protein requirement is expressed. Given that there are clear physiological differences in body composition with gender, it would seem more appropriate to use the separate values for adult males and females.

An alternative approach that accounts for variance in body composition with growth and gender is based on relativity to fat free mass. Gender differences in body composition develop during childhood and result in a gender difference in protein requirement for maintenance throughout childhood and adulthood when expressed per kg body weight per day.

In view of the very limited data available for adult women and children aged 4 years and over, the N maintenance requirements for these groups were based on body composition and the primary estimate of 109 mg N/kg body weight for young men derived by Rand et al (2003) which, when expressed per kg FFM, is 128 mg/kg (assuming an FFM of 85% in the mainly young adult males studied). The N maintenance requirements of adult women and children expressed per kilogram body weight can then be derived from the value of 128 mg/kg FFM and the body composition data of Butte et al (2000) and Ellis et al (2000) that are already used to derive estimates for protein accretion during growth. This approach has the advantage of allowing for racial differences in body composition when appropriate. The EARs and RDIs for Australia and New Zealand were based on this approach.

C. OLDER ADULTS

The individual data analysed by Rand et al (2003) gave a 25% higher (but not statistically significant) requirement estimate in the 14 adults aged 50 years and over for whom data were available (130 mg N/kg/day versus 104 mg N/kg/day). This was apparently due to a significantly lower efficiency of utilization in the older individuals (31% versus 48%). Since the data came from only one estimation study, Rand et al (2003) concluded that there was insufficient information to make different recommendations for older people. In a 14-week study of 10 men and women, aged 55–77 years who consumed diets containing 0.8 g protein/kg/day, Campbell et al (2001) observed that decreased urinary nitrogen excretion over time was associated with loss of mid-thigh muscle and concluded that an RDA (RDI) for protein of 0.8 g/kg may not be adequate to meet the needs of the vast majority of older people. Morse et al (2001) have also assessed the adequacy of the RDA (RDI) of 0.8 g/kg/day in 11 women aged 70–81 years by nitrogen balance at three levels of protein intake ranging from 0.5 to 1.0 g/kg/day. At week 2, the mean protein requirement for N balance was 0.70 g/kg/day and at week 3 it was 0.56g/kg/day. Morse et al (2001) concluded that short-term nitrogen (N) balance studies are inadequate to establish protein requirements in older women and that further research using alternative criteria measures was needed.

Although the data are sparse, given these concerns, it was felt that it would be prudent to recommend a somewhat higher RDI for adults over 70 years. An additional 25% was added to requirements in line with the data of Rand et al (2003). This level of intake is equivalent to the 40–50th percentile of current intakes at this age in Australia and New Zealand and is consistent with mean protein intakes of

1.02–1.06 g/kg/day in a large sample of free-living elderly men and women, without overt debilitating disease and without any evidence of protein inadequacy (Munro et al 1987).

D. PREGNANCY

The additional requirement in pregnancy is lower than that recommended by the US:Canadian DRI review as it was deemed more relevant to use the increase in weight to the middle of each trimester, rather than to the end in estimating the additional requirements in each trimester and to use the protein utilisation rates from Rand et al (2003).

E. LACTATION

Using a factorial approach, the additional requirement in pregnancy has been estimated as 21.2 g/day (FNB:IOM 2002) assuming that all N in breast milk is provided by extra protein. This was the figure used by the US:Canadian Committee. However, about 20–25% of the N in milk is non-protein and can be provided by the unused portion of the maintenance protein intake. On this basis, the additional need is about 18 g/day or 0.30 mg/kg body weight/day.

EVIDENCE BASE

Databases: PubMed, Medline Biological Abstracts and search of cross-references and references in FNB:IOM (2002).

Search terms: Recommended protein requirement or reference intake; protein and requirement; protein or nitrogen and requirements; diet and bone or osteoporosis; protein and bone or osteoporosis.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the protein requirements for infants		
Human physiological data	Allen et al (1991)	Milk volume by test weighing in 13 subjects during exclusive breastfeeding in the first 6 months of lactation adjusted for insensible loss.
Human physiological data	Butte et al (1984)	True protein content of human milk in 13 exclusively breast-fed infants from 4–12 weeks. Milk sampled at second feed of the day and total N and NPN measured by Kjeldahl.
Human physiological data	Butte et al (2000)	Body protein increase, from total body potassium (TBK) in 76 mainly Caucasian infants aged 0.5–24 months.
Human physiological data	Dewey et al (1983)	Breast milk composition in 20 women at monthly intervals from 1– 6 months. Milk sampled at second feed of the day and protein content measured by Lowry method.
Human physiological data	Dewey et al (1984)	Milk composition at 7–11 months in 46 women who breastfed >6 months. Milk sampled at second feed of the day and protein content measured by Lowry method. The influence of weaning on nutrient content was examined.
Human physiological data	Heinig et al (1993)	Milk volume by test weighing corrected for insensible loss in 73 women breastfeeding at 3–12 months.
Human physiological data	Mitoulas et al (2002)	Australian study of human milk composition during 12 months of lactation (n=≤17). True protein in milk decreased from 10.5 g/L at 1 month to about 8.0 g/L at 6 and 12 months.
Human physiological data	Nommsen et al (1991)	Longitudinal study of human milk composition in 92 subjects over 18 months. Milk protein concentration was negatively related to milk volume at 6 and 9 months and positively related to nursing frequency at 6 months.

Level of evidence	Reference	Study type, issues addressed and key findings
Review paper	Dewey et al (1996)	Review of basis for recommendations in the 1985 FAO:WHO:UNU report. Revised estimates of protein intake were provided from data for milk intake from Butte et al (1984) and Heinig et al (1993). Depending on age, the revised values are 10–26% (0.20–0.46 g/kg/day) less than those listed in the 1985 report.
Papers used to assess the protein requirements for children and adolescents		
Human physiological data	Ellis et al (2000)	Body protein content, from TBK, in 856 children aged 5–18 years from three ethnic groups in North America.
Human experimental data	King et al (1973)	Efficiency of protein utilisation (retention/intake) in 6 pregnant adolescents receiving between 0.98 and 2.38 g protein/kg/day during second half of pregnancy.
Papers used to assess the protein requirements in adults		
Level II-1	Morse et al (2001)	18-day N balance data for elderly women at 3 levels of N intake suggest that short-term N balance is inadequate to establish protein needs of elderly. Mean requirement at week 1 was 0.7 g/kg/day compared with 0.56 g/kg at week 3 (n=11).
Meta-analysis of human experimental data	Rand et al (2003)	Meta-analysis of short-term N balance estimates of requirement based on 3 levels of intake, using a common protocol in 235 adults.
Papers used to assess protein requirements in pregnancy and lactation		
Human physiological data	Carmichael et al (1997)	Median pregnancy weight gain of 16 kg based on >2,500 women with BMI in the normal range and good pregnancy outcome. Note: weight gain for 49% of these women exceeded the range of 11.5–16.0 kg recommended by IOM in 1990.
Human physiological data	DeSantiago et al (1995)	N balance study in 7 healthy lactating Mexican women at intakes of 0.8, 1.0 and 1.2 g protein/kg/day (70% vegetable sources) designed to resemble the habitual diet. Equilibrium balance was at 1.1 g protein/kg per day.
Human physiological data	Motil et al (1998)	Longitudinal study of 10 lactating women showed lean body mass preserved throughout 6 months of exclusive breastfeeding. Protein intakes (1.4 g/kg/day) were sustained during weaning as milk protein output declined by 32%. Suggests metabolic needs of milk protein production can be met solely by the maternal diet.
Papers used to assess potential adverse effects of high or higher intakes of protein		
Level III-2	Chow et al (1994)	Case-control study to assess dietary risk factors for renal cell cancer in US subjects. Risk increased with protein intake (ORs of 1.2, 1.4 and 1.9 in the second, third and fourth quartiles). Quartile 4 intake >87.5 g/day for men & >72.3 g/day for women.
Level III-2	Decarli et al (1997)	Case-control study in Italy on 2,569 incident cases of breast cancer and 2,588 controls. Negative association between total protein and breast cancer (OR 0.90 for additional 100 kcal/day from protein).
Level III-2	De Stefani et al (1999)	Case-control study in Uruguay to examine the risk of cancer of the upper digestive tract (oral cavity, pharynx, larynx and oesophagus) associated with nutrient intake. Strong positive association with protein intake (OR 2.5 highest to lowest tertile). Mean protein intake 90.9 g/day in cases.
Level III-2	Meyer et al (1997)	Elevated risk of fracture in women with high intake of protein from non-dairy sources in presence of low calcium intake after 11-year follow-up of 20,000 men and women.
Level III-2	Sellmyer et al (2001)	High ratio of animal to vegetable protein associated with >risk of femoral neck fracture over 7 years in post menopausal women with low energy of about 5,000 kJ/day and low protein of 58 g/day but not low calcium intake (1,124 mg/day) (n=1,035).

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Shu et al (1993)	Case-control study in Shanghai found dietary intakes of animal fat (OR 3.5 highest vs lowest quartile) and animal protein (OR 3.0) were associated with an increased risk of endometrial cancer. After adjustment for total energy, positive associations were seen with consumption of meat, fish and eggs.
Level III-2	Toniolo et al (1994)	Prospective cohort study of 14,291 New York women. Positive association between red meat but not protein intake (RR 1.87 highest vs lowest quintile) and risk of breast cancer. Quintile 5 protein intake of about 108 g/day versus 32 g/day in quintile 1.
Human physiological data	Brandle et al (1996)	Long-term protein intake of widely varying amounts is correlated with endogenous creatinine clearance and is a crucial control variable for the glomerular filtration rate in subjects with healthy kidneys.
Human physiological data	McClellan et al (1930)	No adverse clinical effects of prolonged self-selected meat-only diet (15–24% PE) but negative calcium balance (n=2).
Human physiological data	Poortmans & Dellalieux (2000)	No negative effects on renal function was determined in athletes consuming long-term protein intakes of up to 2.8 g/kg body weight.
Review	Bell & Whiting (2002)	An adequate intake of protein in elderly women is important for preservation of bone mass.
Review	Clinton (1993)	Unable to quantitate with certainty the contribution of dietary protein to the risk for any human malignancy.
Review	Giovannucci & Willett (1994)	No published reports from epidemiological studies of diet and colon cancer have shown a significant association with intake of protein from sources other than red meat.
Review	Heaney (1998)	Excess protein will not harm the skeleton if calcium intake is adequate. A dietary calcium:phosphorus ratio of ≥20:1 (mg:g) probably provides adequate protection for the skeleton.
Review	Jackson (1999)	More sensitive measures needed to assess possible adverse effects of high protein intake.
Review	Kerstetter (2003)	No definitive intervention studies for adverse effect of a high protein diet on bone, but some evidence that on reducing protein intake to 0.7 g/kg, calcium absorption falls & serum parathyroid hormone and calcitriol increase for at least 2–4 weeks.
Review	Metges & Barth (2000)	In view of insufficient evidence to set protein ULs, intakes above those consumed habitually in well-nourished populations inadvisable.
Review	Parnaud & Corpet (1997)	A positive association between meat intake and colon cancer incidence is supported in most case-control studies (22 of 29) but in only 2 of 5 prospective cohort studies. Data from 6 experimental studies do not support an effect.
Papers used to assess benefits for chronic diseases from higher protein intake		
Level II	Schurch et al (1998)	Protein supplementation of (20 g/day for 6 months) to increase protein from about 0.75 to 1.05 g/kg/day) in elderly patients with recent hip fracture attenuated proximal femur bone loss and increased IGF-1 (n=30 per group).
Level III-1	Dawson-Hughes et al (2004)	Increasing protein intake with meat from 0.78 to 1.55 g/kg/day in adults >50 years, while leading to increased urinary excretion of calcium (expressed as % of intake) also resulted in favourable changes in serum IGF-1 and N-telopeptide (n=16 per group).
Level III-1	Farnsworth et al (2003)	Increasing energy from protein from 16% to 27% had beneficial effects and no adverse effects on bone turnover or calcium excretion in persons with fasting insulin >12 mU/L (n=28 and 29 per group).
Level III-1	Gannon et al (2002)	An increase from 15% to 30% PE in persons with type 2 diabetes improved glucose control (n=12 crossover).

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-1	Layman et al (2003a, b)	Increasing energy from protein from 15% to 28% had positive effects on body composition, blood lipids, blood glucose, insulin and satiety during weight loss on a diet of 7,100 kJ (n=12 women per group).
Level III-1	Parker et al (2002)	Increasing energy from protein from 16% to 28% in persons with type 2 diabetes resulted in greater reduction in total and abdominal fat mass in women and greater LDL reduction in both sexes (n=27 and 28 per group).
Level III-1	Roughead et al (2003)	Calcium retention the same for diets with either 12 or 20% energy from protein provided by extra meat for 8 weeks (n=15).
Level III-2	Barbone et al (1993)	Case-control study in US women found no statistically significant direct associations between any food item and endometrial cancer but OR of 0.7 for highest (>74.7 g) versus lowest (<62.5 g) tertile of protein intake.
Level III-2	Franceschi et al (1999)	Case-control study in Italy to assess oral and pharynx cancer risk associated with energy and macronutrient intake. Inverse association with protein intake (OR 0.8 for 100 kcal/day added protein).
Level III-2	Gao et al (1994)	Case-control study in Shanghai found inverse association with oesophageal cancer risk in men (OR 0.6 highest versus lowest quartile) but not for women (OR 1.7).
Level III-2	Hannan et al (2000)	BMD loss at femoral neck and lumbar spine over 4 years in Framingham cohort significantly decreased from Q1 to Q4 of protein intake equivalent to >13.5%E, 0.71 g/kg/day and 51 g/day (n=615).
Level III-2	Munger et al (1999)	Risk of hip fracture negatively associated with protein intake above 17% energy from protein after 2-year follow-up in >30,000 postmenopausal women.
Level III-2	Wengreen et al (2004)	Protein intake (≥14.0–30.8% energy) associated with significantly reduced risk of hip fracture in men and women 50–69 but not those >70 years (n=1,167 cases).
Level IV	Lei et al (1996)	Retrospective case-control study in Guangzhou involving all lung deaths registered in 1986. Controls were selected from the same year of death. Home interviewers surveyed consumption of selected food items categorised as never, weekly or daily. No association between high protein food items and incidence of lung cancer.
Level IV	Rapuri et al (2003)	Positive associations between quintile of protein intake as % energy (≥18%) and BMD (not significant for hip) at baseline (n=473) but no association with bone loss over 3 years in 96 postmenopausal women.
Level IV	Whiting et al (2002)	Protein, potassium and phosphorus intake are significant predictors of TB-BMD and LS-BMD in adult men (n=57).
Review	Key et al (2004)	No evidence for protein intake *per se* as factor in cancer risk and evidence for preserved meat classified as probable rather than convincing.
Review	Prentice (2004)	No firm evidence on which to base recommendations about optimal protein intake for bone growth or the prevention of osteoporosis.
Review	Swinburn et al (2004)	Protein probably not an important influence on obesity (limited range of intake) but increasing intake may be beneficial for some for weight control.

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FATS & FATTY ACIDS

The recommendations for dietary fats and fatty acids were derived from an assessment of the evidence base used by the US:Canadian Government review of DRIs (FNB:IOM 2002) and consideration of additional key papers missing from those reviews or published since they were released, and current recommendations of other key countries.

After review, the approach of the US:Canadian review to the setting of AIs based on population intakes was adopted.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The AI recommendations vary from those of the US:Canada (FNB:IOM 2002) because of differences in population intakes. The lack of specific data in pregnancy and lactation meant that these recommendations had to be derived by adding an allowance for the additional needs to support foetal and placental tissues or the fatty acids secreted in milk. The US:Canadian report did not make specific recommendations for long chain (LC) n-3 fats, but stated that docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) could account for up to 10% of the αlinolenic acid (ALA) recommendation. However, it was felt that there was sufficient indication in the literature that LC n-3 fatty acids were as important as ALA (if not more so) in maintaining adequate nutritional status. Separate AI recommendations for total LC n-3 that include DHA, EPA and DPA were made based on current population intakes. The potential benefits of higher than AI intakes are discussed in the 'Chronic disease' section of the NRVs.

EVIDENCE BASE

Databases: PubMed, Cochrane library and Medline plus search of cross-references and references in FNB:IOM (2002).

Search terms: essential fatty acids, linoleic, αlinolenic, n-3, n-6, omega-3, omega-6, DHA, EPA, DPA requirement, intake, children, adults, randomised controlled trial, chronic disease, coronary heart disease, human studies.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the required level of essential fatty acids to prevent deficiency		
Level IV	Fleming et al (1976) Holman et al (1982) Jeppersen et al (1998) Riella et al (1975)	Patients on essential fatty acid-free total parenteral nutrition, then re-fed. Most studies looked at time course and clinical signs. Little dose-response data. In one study, inclusion of ALA at 0.54% energy could reverse symptoms of n-3 deficiency.
Experimental studies	Burdge et al (2003) Emken (2003) Pawlosky et al (2001)	Conversion of αlinoleic acid (ALA) to EPA/DHA is limited and varies according to the intakes of other fatty acids.
Survey data	Bang et al (1990)	Inuit n-3 intake comprises EPA/DHA/DPA and negligible ALA, without obvious signs of essential fatty acid deficiency.
Papers used to assess chronic health benefits of LC n-3 fats		

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Burr et al (1989)	RCT with a factorial design to examine the effects of dietary intervention in the secondary prevention of myocardial infarction (MI). 2,033 men who had recovered from MI. The subjects advised to eat fatty fish had a 29% reduction in 2-year all-cause mortality compared with those not so advised.
Level II	GISSI-Prevenzione Investigators (1999)	11,324 patients surviving recent MI randomly assigned supplements of n-3 PUFA, vitamin E or both or none (control, n=2,828) for 3.5 years. Endpoints were death, non-fatal MI and stroke. Dietary supplementation with n-3 PUFA led to a clinically important and statistically significant benefit.
Level III-2	Albert et al (1998)	Prospective cohort study – US Physicians' Health Study of 20,551 men. After controlling for age, fish intake associated with a reduced risk of sudden death, with an apparent threshold effect at a consumption level of 1 fish meal per week. Estimated dietary n-3 fatty acid intake from seafood also associated with a reduced risk of sudden death but no significant trend across increasing categories of intake. Neither dietary fish consumption nor n-3 fatty acid intake was associated with a reduced risk of total MI, non-sudden cardiac death or total cardiovascular mortality.
Level III-2	Albert et al (2002)	Prospective, nested case-control analysis followed up for 17 years – Physicians' Health Study. Shows base-line blood levels of LC n-3 fatty acids inversely related to the risk of sudden death.
Level III-2	Dolecek (1992)	Data from controls in Multiple Risk Factor Intervention Trial (MRFIT) RCT in coronary heart disease (CHD). Primary prevention involving 12,866 middle-aged men determined to be at high risk of CHD. No significant associations with mortality detected for linoleic acid (LA) the predominant dietary PUFA.
Level III-2	Hu et al (1999)	As part of prospective cohort study – during 10 years of follow-up. A higher intake of ALA was associated with a lower relative risk (RR) of fatal IHD. For nonfatal MI, there was only a modest, non-significant trend toward a reduced risk when extreme quintiles were compared. Study supports the hypothesis that a higher intake of ALA is protective against fatal IHD.
Level IV	Dallongeville et al (2003)	Cross-sectional analysis was conducted of 9,758 men, age 50 to 59 years, without CHD, compared across 4 categories of fish consumption. Triglycerides, systolic blood pressure and diastolic blood pressure were lower and HDL cholesterol levels were higher in fish consumers than in non-consumers. Similarly, heart rate decreased across the categories of fish intake. DHA content of erythrocyte phospholipids was inversely correlated with heart rate.
Level IV	Djousse et al (2001)	National Heart, Lung, and Blood Institute Family Heart Study of 4,584 participants in a cross-sectional design. A higher intake of either ALA or LA was inversely related to the prevalence OR of CAD. ALA and LA had synergistic effects on the prevalence OR of CAD.
Level IV	Pischon et al (2003)	Investigated habitual dietary n-3 fatty acid intake and its interaction with n-6 fatty acids in relation to the plasma inflammatory markers in 405 healthy men and 454 healthy women. n-6 fatty acids do not inhibit the anti-inflammatory effects of n-3 fatty acids. Combination of both types of fatty acids associated with the lowest levels of inflammation.
Expert reviews	Mori et al (1999) Simopoulos (1999)	Suggests that DHA is the primary mediator of cardiovascular benefits, influencing gene expression of key metabolic regulators, particularly in endothelial cells.
Papers used to assess any adverse effects related to fatty acid ratios		
Level I	SanGiovanni et al (2000)	Meta-analysis of RCTs: Studies of infants born at term with different LC n-3 PUFA intake. Includes RCT and non-randomised studies. Studies published in or prior to 1998, RCTs. Improved cardiac acuity at only 2 months of age. No other differences. Non-randomised studies: LC n-3 PUFA improved cardiac acuity at 2 and 4 months and improved visually evoked potential (VEP) acuity at 4 months.
Level I	Simmer (2002)	Meta-analysis of RCTs: Trials of infants born at term involving an LC n-3 PUFA intervention. Trials published in or prior to 1999 included. Growth: No difference between groups. Visual development: No analysis conducted. Developmental quotient: No difference between groups.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Jensen et al (1997)	RCT. Infants fed formulas containing an LA:ALA ratio of 4.8:1 had lower arachidonic acid concentrations and impaired growth compared to infants fed ratios of 9.7:1 or above.
Level II	Jensen et al (1997) Makrides et al (2000) Ponder et al (1992)	Randomised trial with varying ratios of n-6 and n-3 essential fatty acids, LA and ALA. No adverse effect on growth or visual acuity.
Level III-3	Becker et al (1983)	Effects of dietary fats on plasma cholesterol, serum lipoproteins and apoproteins (Apos), A-1, B and CIII. Shows that changes in dietary fat affect serum lipids, lipoproteins and apoproteins even when consumed on a cholesterol-free diet, that omega-6 PUFA lowers LDL cholesterol, total cholesterol and Apo B to a greater extent than monounsaturated or saturated fat and that consumption of a cholesterol-free formula diet results in significant decreases in the concentrations of total and LDL cholesterol in plasma when compared to values obtained on a mixed food home diet containing approximately 300 mg cholesterol/day.
Level IV	Arntzenius et al (1985)	Relationship between diet, serum lipoproteins and the progression of coronary lesions in 39 patients with stable angina pectoris. Intervention of 2-year vegetarian diet, polyunsaturated:saturated fatty acids of at least 2 containing <100 mg of cholesterol per day. Dietary changes associated with a significant increase in LA content of cholesteryl esters and a significant lowering of body weight, systolic blood pressure, serum total cholesterol and total cholesterol:HDL cholesterol. Angiograms after 24 months were assessed visually (with blinding) and by computer-assisted image analysis. Both types of assessment indicated progression of disease in 21 of 39 patients but no lesion growth in 18. Coronary lesion growth correlated with total cholesterol:HDL cholesterol ($r = 0.50$, $p = 0.001$) but not with blood pressure, smoking status, alcohol intake, weight or drug treatment. Disease progression was significant in patients who had values for total cholesterol:HDL cholesterol greater than the median of 6.9 throughout the trial period. No coronary lesion growth was observed in patients who had values for total cholesterol:HDL cholesterol of less than 6.9 throughout the trial or who initially had values greater than 6.9 that were significantly lowered by dietary intervention.
Cross-sectional survey data	Sonneburg et al (1996)	Examined relationships between macronutrients and plasma triglycerides, HDL, and the total/HDL cholesterol ratio in 695 premenopausal and 727 postmenopausal women in Framingham Offspring/Spouse Study. Plasma triglycerides were inversely related to polyunsaturated fat and directly related to saturated fat and oleic acid. Direct relationship between dietary fat and HDL cholesterol was limited to postmenopausal women.
Expert review	Crawford et al (2000)	Summary of international workshop on the role of plant-derived omega-3 fatty acids in human nutrition in Milan in 2000 based on updated scientific evidence presented and discussed at the workshop.
Survey data	Howe et al (2005)	Data on dietary intake of LC omega-3 polyunsaturated fatty acids using data from 1995 NNS for Australia.

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CARBOHYDRATE

The recommendations for carbohydrate were derived after consideration of the FNB:IOM (2002) review of DRIs for the US:Canada, taking into account recommendations from other key countries and health authorities. AIs were set only for infancy and were based on mean breast milk composition and volume.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The US:Canadian DRI report presented an AI for infants that was adopted and also EARs and RDIs for other age groups and genders. Given that the prime role of carbohydrates is the provision of energy, particularly for the brain, and that energy can also be provided from other dietary sources, such as protein and fat (and alcohol), it was considered more appropriate to assess the needs for carbohydrate in relation to fat and protein intakes rather than in isolation. The range of carbohydrate intakes relative to fat and protein that is consistent with health is discussed in the 'Chronic disease' section of the NRVs.

EVIDENCE BASE

Database: Medline and cross-referencing and review of key references in FNB:IOM (2002).

Search terms: Behaviour and sugar or sucrose; dental caries and carbohydrate or sugar or sucrose; plasma lipids and carbohydrate or sugar or sucrose or fructose; triacylglycerols and carbohydrate or sugar or sucrose or fructose; coronary heart disease and carbohydrate or sugar or sucrose; insulin sensitivity and carbohydrate or sugar or sucrose or fructose; diabetes and carbohydrate or sugar or sucrose; obesity and carbohydrate or sugar or sucrose; cancer and carbohydrate or sugar or sucrose; blood pressure and carbohydrate or sugar or sucrose; bone density and sugar or sucrose; nutrient density and sugar or sucrose; energy regulation and carbohydrate or sugar or sucrose; carbohydrate and Australia or New Zealand.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess infant carbohydrate requirements		
Survey data	Allen et al (1991)	From 21–180 days, lactose is 63–69 g/L; glucose is 0.27–0.32 g/L.
Survey data	Anderson et al (1981)	From 2–29 days, lactose content is 51–65 g/L.
Survey data	Anderson et al (1983)	From 3–14 days, total carbohydrate is 62–67 g/L.
Survey data	Coppa et al (1993)	From 4–120 days, total carbohydrate is 78–84 g/L; lactose is56–69 g/L.
Survey data	Dewey and Lonnerdal (1983)	From 1–6 months, total lactose is 70–78 g/L.
Survey data	Dewey et al (1984)	From 4–20 months, lactose is 77–72 g/L.
Survey data	Ferris et al (1988)	From 2–16 weeks, lactose is 63–70 g/L.
Survey data	Lammi-Keefe et al (1990)	At 8 weeks, lactose is 73–80 g/L; glucose is 0.26–0.33 g/L.
Survey data	Neville et al (1984)	From 33–210 days, lactose is 72 g/L and glucose is 0.27 g/L mid feed.
Survey data	Nommsen et al (1991)	From 3–12 months, lactose is 73–74 g/L.

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DIETARY FIBRE

The recommendations for dietary fibre were derived from an assessment of the evidence base used by the US:Canadian Government review of DRIs (FNB:IOM 2002) and consideration of additional key papers missing from that review or published since it was released, and current recommendations of other key countries and organisations. AIs were set for all age and gender groups on the basis of maintaining adequate laxation. No recommendations were made for infancy as breast milk contains no dietary fibre and there is no known physiological need for fibre at this age. No UL was set.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

1. DEFINITIONS

In the US:Canadian DRI report (FNB:IOM 2002), an AI was set for total fibre, defined as the sum of dietary fibre plus functional fibre. The following definitions were used in that report:

Dietary fiber: non-digestible carbohydrates and lignin that are intrinsic and intact in plants (includes inulin, oligosaccharides eg raffinose, stachyose, verbacose, fructans, polydextrose, methylcellulose, resistant maltodextrins; includes some RS that is natural and inherent in foods or created in normal processing of food such as flaking eg RS1 and 2).

Functional fiber: isolated, non-digestible carbohydrates that have beneficial effects in humans (includes synthetic manufactured or naturally occurring oligosaccharides and manufactured RS eg RS3 and 4; also modified but natural polysaccharides or oligosaccharides).

Non digestible monosaccharides, disaccharides and sugar alcohols were considered as sugars, not fibre.

However, the report also states that food tables do not generally include pectin and gums and that synthetic RS is very recent addition to the food supply, thus estimates of fibre intake to date generally represent dietary fibre.

This definition of dietary fibre differs from that currently used in Australia and New Zealand and for legislative purposes. As such, it has not been adopted in this report.

2. BASIS OF AI RECOMMENDATION

The US:Canadian DRI process used cardiovascular risk reduction as the sole marker for requirement, even for children, and excluded laxation and gut function. The figure for fibre/unit energy from the highest quintile of intake in cohort studies for CHD in adults in the US and Finland of 3.34 g/1,000 kJ was taken and applied to other age and gender categories in proportion to median energy intakes in those groups to derive an AI for total fibre. In line with the approach used to set recommendations for other nutrients, the Australian/New Zealand AIs were set in relation to their laxation effect, rather than for minimisation of chronic disease risk. The potential for the latter is discussed in the 'Chronic disease' section. With the lack of experimental data, the AI was set on the basis of median intakes from the highest consuming adult male and female age groups from the combined National Nutrition Surveys of Australia and New Zealand. As the nutrient database for the Australian surveys did not include some 60% of RS in the food supply and the Englyst method used for the New Zealand database measures no RS, an additional amount equivalent to 4.2 g/day for men and 2.7 g/day for women was added to the dietary fibre figure and rounded up. The RS estimates were based on the analysis of Baghurst et al (1996) which showed that men in Australia and New Zealand consumed 4.0 g RS/100 g starch and women consumed 4.7 g RS/100 g starch.

EVIDENCE BASE

Databases: Medline, PubMed and search of cross-references and references in FNB:IOM (2002).

Search terms: fibre, resistant starch, dietary fibre, laxation, coronary heart disease, dietary intakes, Australia and New Zealand.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the median dietary fibre intakes in Australia and New Zealand at different ages		
Survey data	ABS (1998) re-analysed in appropriate age groups	Median intakes of Australians range from 12.1 g/day at age 2 years to 16.7 g at 4–8 years and 24.6 g at ages 19–30 years for males and from 12.3 g at 2 years, to 14.9 g at 4–8 years to 18.9 g at 19–30 years for females. Highest median intakes were at ages 19–69 years for men and at 50–69 years for women.
Survey data	Baghurst et al (1996)	RS intakes for Australia and New Zealand are 4.0 g/100 g starch for men and 4.7 g/100 g starch for women.
Survey data	MOH (1999) (re-analysed in appropriate age groups)	Median intakes of New Zealanders adult males ranged from 23.8 g at ages 19–30 years to 24.3 g at 31–50 years, 22.0 g at 51–70 years and 20.8 g over 70 years. Median intakes for women ranged from 16.9 g at 19–30 years to 17.8 g at 31–50 years, 18.5 g at 51–70 years and 17.9 g over 70 years. For adolescents aged 15–18 years, median intakes were 23.4 g for boys and 16.0 g for girls.
Survey data	MOH (2003)	Median intakes for NZ children range from 16.7 g in 5–6 year-old boys, 18.8 g at 7–10 years, 21.4 g at 11–14 years. Among girls, median intakes were 14.5 g for 5–6 year-olds, 16.5 g at 7–10 years and 17.2 g at 11–14 years.
Papers used to assess the role of dietary fibre in laxation		
Level III-2	Astrup et al (1990)	Fibre supplementation, weight loss study. Those on isolated plant fibre supplement had higher numbers of bowel movements than those without the supplement.
Level III-3 and IV	Burkitt et al (1972) Cummings et al (1978) Kelsay et al (1978) Lupton et al (1993)	Consumption of dietary fibre improves laxation to ameliorate constipation.
Level IV	Baghurst et al (1985)	Intervention in elderly people in a nursing home with varying levels of dietary fibre showed reduction in constipation and less use of aperients, with no adverse effects on mineral status.
Survey data	Birkett et al (1997)	Strong positive correlation between intake of fibre and daily faecal weight and negative correlation with transit time.
Survey data	Morais et al (1999)	Children with chronic constipation have lower fibre intakes than controls.

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WATER

The recommendations for water were derived after consideration of the FNB:IOM (2004) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The approach taken by the US:Canadian DRI of setting recommendations for total water requirements as AIs based on median population intakes was adopted, as was the AI for infants based on breast milk consumption and volume. However, the figures for children, adolescents and adults differed from those of the US:Canadian DRI as they were based on intake data for the Australian National Nutrition survey of 1995 (ABS 1998). The percentage of water ingested from foods versus fluids at various ages, used in calculating fluid requirements from total water requirements, was also slightly different from the US:Canadian figures as outlined in the Water chapter. No national data were available for New Zealand.

The increment for pregnancy was based proportionally on the variation between median intakes in pregnancy vs non-pregnancy from the US (FNB:IOM 2004) as no national data for pregnancy were available for Australia and New Zealand. The increment for lactation was determined by adding the volume of water lost through breast milk to the AI for adult, non-lactating women, as no data are available for median intakes during lactation in Australia and New Zealand.

EVIDENCE BASE

Databases: Medline and PubMed plus cross-referencing and review of key references in FNB:IOM DRI (2004), the review used to set values.

Search terms: water, fluids, hydration.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess relationships between water consumption and health outcome		
Level II	Borghi et al (1996) Iguchi et al (1990)	Show relationship between water consumption and urinary volume and occurrence of kidney stones
Level III	Curhan et al (1996, 1998) Embon et al (1990) Hughes & Norman (1992)	Show relationship between water consumption and urinary volume and occurrence of kidney stones
Level III	Gopinathan et al (1988)	Shows relationship between dehydration and mental performance
Level III	Hiatt et al (1996)	Shows relationship between fluid consumption and cancer of the urinary tract
Level III	Michaud et al (1999)	Shows relationship between water intake and colon cancer
Level III	Shannon et al (1996)	Shows relationship between water intake and colon cancer
Level III	Ship & Fischer (1997)	Shows relationship between fluid intake, and water in particular, for bladder cancer
Level III	Wilkens et al (1996)	Shows relationship between total fluid intake, and tap water in particular, for lower urinary tract cancer in women

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VITAMINS

VITAMIN A

The recommendations for vitamin A were derived from an assessment of the evidence base used by the US:Canadian Government review of DRIs (FNB:IOM 2001) together with consideration of additional key papers missing from that review or published since it was released and current recommendations of other key countries and organisations such as the FAO:WHO.

The FNB:IOM (2001) used a factorial approach based on the amount of dietary vitamin A required to maintain a given body pool size in well-nourished subjects. For infants, recommendations were based on the concentration in breast milk of healthy mothers and an estimate of usual milk volume. While this approach was generally applied, some components of the estimations were varied in determining requirements for Australia and New Zealand. Some nomenclature changes were also made.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. CONVERSION RATE OF CAROTENOIDS TO RETINOL EQUIVALENCE

Summary

There has been some discussion in the literature about the conversion factors used to derive retinol equivalence for carotenoids. The US:Canadian DRI review suggested a Retinol Activity Equivalence Activity (RAE) of 12 µg for ß-carotene and 24 µg for αcarotene and αcryptoxanthin. However, this was based on experimental absorption studies using the less well-absorbed green leafy vegetables as the predominant source of carotenoids. In Australia and New Zealand, the predominant source of these carotenoids in the diet are carrots, pumpkins, sweet potato (kumara), other root and fruiting vegetables and fruit. This, together with the recent position taken by the FAO that the evidence base for a change at this time is insufficient (FAO:WHO 2001), led to retention of the equivalence conversion factor of
6 µg for αcarotene and 12 µg for αcarotene and αcryptoxanthin.

The term Retinol Equivalents (REs) has been retained in favour of the new RAE terminology used by the US:Canadian review. The factor of 6 used to estimate the bioefficacy of αcarotene is derived from imperfect yield after cleavage of αcarotene (1 mole αcarotene yields 0.5 mole retinol) and an estimate that only one-third of the αcarotene is absorbed (available) in a mixed diet. A factor of 12 is used to estimate the bioefficacy of αcarotene and cryptoxanthin, owing to lower yield from intestinal cleavage. When first proposing these factors, the FAO:WHO noted that they were averages for a mixed diet but that individual foods varied (FAO:WHO 1967).

Detailed rationale

The US:Canadian DRI report (FNB:IOM 2001) recommended reducing the αcarotene to retinol conversion ratio from 6:1 to 12:1 based on three considerations:

- results of a study showing that "the relative absorption of αcarotene from the mixed vegetable diet compared to αcarotene in oil is only 14 per cent. Based on this finding, approximately 7 µg of dietary αcarotene is equivalent to 1 µg of αcarotene in oil" (van het Hof et al 1999b)
- that the conversion factor for dark-green leafy vegetables is one-half that from "orange fruits and some yellow tubers, such as pumpkin squash" (de Pee et al 1998)
- that there is a "low proportion of dietary αcarotene that is consumed from fruits compared to vegetables in the United States" (Chug-Ahuja et al 1993)

The mix of vegetables and fruits contributing to vitamin A intake in Australia and New Zealand is very different from that used in the experimental study of van het Hot et al (1999b) or that generally consumed in the US and Canada and used as the basis for their recommendations. Table 1 shows the contribution of foods to the provitamin A intake in adult Australian women for comparison (ABS:DHAC 1995). Also shown is the relative absorption of the food groups compared to absorption from synthetic αcarotene. The New Zealand data groups all vegetables together (72% of provitamin A), with other contributions to provitamin A being fruit (5%), butter and margarine (5%), soups and stocks (4%), bread-based dishes (2%) and non-alcoholic beverages (2%).

TABLE 3. CONTRIBUTION OF FOODS TO PROVITAMIN A INTAKE FROM THE 1995 NATIONAL NUTRITION SURVEY OF AUSTRALIA AND EXPERIMENTAL RELATIVE ABSORPTION OF FOOD GROUPS

Source of vitamin A	Contribution to provitamin A carotenoid intake in Australian women (%)	Absorption relative to αcarotene in supplement (%)
Carrots and roots	44.4	19–26
Other fruiting vegetables	12.2	28[a]
Fruit products (not including fruit in pastries etc)	6.6	28[a]
Fruit and vegetable juices and drinks	4.3	44[b]
Tomatoes and products	3.0	0
Peas and beans	2.2	96
Dairy products	2.1	100[c]
Leaves and stalks	2.0	5
Fats and oils	1.6	100[c]

[a] Based on de Pee et al (1998) finding mango/papaya/pumpkins had more than twice the retinol generation of leaves and carrots, and using van het Hof et al (2000) 14 % for a mix of leaves and carrots

[b] Calculated from Torronen et al (1996), carrot juice result using method of van het Hof et al (2000)

[c] Assuming the absorption of αcarotene from butter, margarine and dairy products is the same as from capsules

Based on Table 3, it is evident that the 14% proposed by van het Hof et al (2000) and adopted by the US:Canadian DRI committee is not appropriate for Australia and New Zealand. Green leaves are not an important contributor to provitamin A in our diet and carrots, 'other' fruiting vegetables and fruit are important. There appear to be no studies in the literature testing the absorption from dairy products, fruit, fruit juice, sweet potato (kumara) or pumpkins. Hence, use of the 14% absorption factor and the 12:1 conversion factor proposed by the US:Canadian DRI review, which is heavily influenced by the low absorption from spinach, to the whole Australian and New Zealand diet is not supported.

Given these considerations, the FAO approach of retaining the 6:1 factor for αcarotene and 12:1 factor for other provitamin A carotenoids was adopted until more definitive data become available.

B. BREAST MILK CONTENT OF VITAMIN A

Recommendations in relation to figures used for the vitamin A content of breast milk have been varied.

Infants 0–6months

In determining the AI for infants, data not considered by the US:Canadian DRI review were taken into account. Canfield et al (2003) reported a study conducted with 50 women in each of 9 countries, including Australia and the US.

TABLE 4. RETINOL CONCENTRATION IN BREAST MILK

Country	N	Age	Retinol concentration		
		(days)	(nmol/g lipid)	(μmol/L)	(μg/L)
Australia	53	121	0.028	1.086	310
Canada	55	129	0.030	1.188	339
Chile	51	87	0.051	1.242	355
China	52	74	0.025	1.043	298
Japan	51	98	0.036	1.230	351
Mexico	50	124	0.040	1.321	377
Philippines	60	67	0.038	1.624	464
United Kingdom	50	74	0.029	1.052	301
USA	49	116	0.044	1.227	351

The women included in the study would meet the criteria of a population with no apparent deficiency and so could be used to set an AI. They were women with a singleton infant, visiting a paediatrician, with a pregnancy weight gain of at least 8.5 kg (to exclude those on a restricted diet), non-smokers, not taking supplements of vitamin A or αcarotene, parity <6, consuming at least three serves of fruits and vegetables (combined).

According to the Australian figure from Canfield et al (2003), the concentration of retinol in breast milk is 310 μg/L which yields 242 μg/day assuming 0.78 L/day for consumption of breast milk. Hence the AI should be 242 μg/day (or 250 μg/day with rounding), not 400 μg/day as recommended by the US:Canadian DRIs.

Infants 7–12 months

The AI for infants 7–12 months is derived from the AI for younger infants with respect to breast milk, so the recommendation for 7–12 months was adjusted accordingly. The US:Canadian figure for the contribution from complementary foods was used due to the lack of relevant national dietary information in Australia and New Zealand.

C. UNITS FOR THE AI FOR INFANTS 0–6 MONTHS AND 7–12 MONTHS

The US:Canadian DRI review document states that the conversion of carotenoids from breast milk is not certain in infants, as a result of which 'vitamin A' in this context refers only to retinol. However, the units for the AI are RAE, which could imply that carotenoids in the diet are also included in the estimation. Because of the uncertainty around carotenoid conversion in breast milk, carotenoids were not considered in setting the AI for Australia and New Zealand, which has been expressed in units of μg/retinol as retinyl esters.

For older infants, the US:Canadian DRIs included carotenoids in the non-breast milk phase of the diet in the estimation, but not the carotenoids in breast milk. However, as some carotenoid activity has been included, the recommendations are expressed as RE.

D. PREGNANCY RDI

The EAR for pregnancy has been set at the same level as the US:Canadian recommendations (FNB:IOM 2001), however, the RDIs are slightly higher. The US:Canadian review rounded up to the nearest 10 μg for pregnancy but to the nearest 100 μg for all other age and gender groups and for lactation. As this seems inconsistent, and as no explanation was given in the text, rounding has been to the nearest 100 μg for all groups.

EVIDENCE BASE

Databases:. PubMed, Cochrane database and review of papers in FNB:IOM (2001) and cross-referencing.

Search terms: fruit, vegetable, vitamin A, carotene; clinical trials bioavailability, human, vitamin A conversion, vitamin, vitamin and lactation.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess bioavailability, bioefficiency and needs of vitamin A in adults		
Level II	Edwards et al (2001)	Subjects fed 4 test meals: 5.6 mg αcarotene as raw carrots or 6.2 mg as raw spinach with 1 g or 20 g oil. Midday meal contained same amount of oil, except in 1 subject who was changed from 1 g to 20 g fat. Over 8.5 hours, corrected to 6 mg dose, 0.3 mg retinol was absorbed in the high fat tests and there was no consistency in the low fat tests. In 1 subject given low fat then high fat, serum retinol had not returned to baseline at end of testing. In most instances, more retinol than αcarotene was absorbed (n=3).
Level II	Edwards et al (2003)	Over 19 weeks, subjects given baseline diet and randomised intervention periods of 3 weeks of 2.5 mg or 5 mg αcarotene/day as watermelon juice or 0.6 mg/day as tomato juice with 2-week washouts. At 1 week, increments in plasma αcarotene were 27%, 34% and 4% in low, high watermelon and tomato juice, respectively, vs controls. At 3 weeks the increments were 70%, 100% and –6%, respectively, vs –12% in controls (n=22).
Level II	Huang et al (2000)	A single test meal was used, not repeated daily intake. 12 mg αcarotene from various sources examined. Compared to beadlets in a capsule, absorption was 37% for fried sweet potato balls, 33% for stir-fried shredded carrots, 26% for stir-fried water convolvulus leaves and 65% for beadlets given with stir-fried daikon (n=10).
Level II	John et al (2002)	UK adults without chronic illness in 2 general practices randomised to advice from nurse or not. Unequal exclusions applied in analysis. After 6 months, the intervention group increased intake of fruit and vegetables from 3.4 to 4.9 serves and the control to 3.5 serves. Plasma αcarotene and αcarotene both increased by 7% in the intervention group (n=690).
Level II	Rock et al (1992)	While following low (<5 mg/day) αcarotene diet, tested with 25 mg beta-carotene in a capsule with or without 12 mg citrus pectin. 3 weeks between tests. Plasma αcarotene was much higher after a meal without pectin up to 8 days later (last measurement). At 48 hours, response was about half in the group given pectin (n=7).
Level II	Rock et al (1998)	Low (<5 mg αcarotene) background diet with additional 9.3 mg αcarotene as either 113 g each of processed/pureed spinach and carrots or 54.9 g raw carrot and 39 g spinach (3.4 mg αcarotene during processed diet and 4.5 mg αcarotene during raw). Processing increased all-*trans* αcarotene by 105% and αcarotene by 87% compared with 38% and 79%, respectively, for raw vegetables (not significant). Note different relative quantities of the two foods in each phase (n=8).
Level II	Smith-Warner et al (2000)	RCT to test increased fruit and vegetable consumption in US adults with previous diagnosis of adenomatous polyp. The intervention group increased intake from 7.3 to 11.9 serves/day as 1.2 serves veg, 2.5 serves fruit and 1.1 serves juice (mainly orange). Slight decline in control group. Carrots tripled intake; apples, oranges and bananas doubled intake; broccoli, lettuce and grapefruit increased intake by 50%. Plasma levels were increased by 50% for αcarotene, 30% for αcarotene, 20% for cryptoxanthin and <11% for lutein and lycopene. No change in controls (n=201).
Level II	van den Berg & van Vliet (1998)	Short term feeding trial: responses of carotenoids and retinyl palmitate in the triacylglycerol-rich lipoprotein (TRL) fraction after a separate 15-mg beta-carotene dose compared with those after a dose of 15 mg beta-carotene combined with 15 mg lycopene or lutein. Study showed that lutein, but not lycopene, negatively affected beta-carotene absorption when given simultaneously with beta-carotene but apparently had no effect on beta-carotene cleavage (n=12).

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	van het Hof et al (1999a)	Test meals for 4 days: low αcarotene control, 300 g of broccoli (2.4 mg αcarotene), green peas (1.7 mg αcarotene), whole leaf spinach (24.6 mg αcarotene), chopped spinach (23.8 mg αcarotene), 33.3 mg synthetic αcarotene. Broccoli and peas yielded higher plasma αcarotene levels, despite lower dose, calculated as 74% and 96%, respectively, of equivalent dose of synthetic αcarotene (n=28–67 for different arms).
Level II	Zino et al (1997)	NZ adults with intake ≤3 serves (mean 2.2) of fruit and vegetables/day, randomised to 8 serves or usual diet (1 serve=1 piece fruit or ½ cup of cooked veg). Analysis not adjusted for baseline differences. The intention to treat analysis is not presented, although the change, but not its correct p value, can be assessed. After 8 weeks, plasma αcarotene increased by 53% and αcarotene increased by 43% in the intervention group compared to almost no change in controls. Dietary αcarotene not reported. The intervention group increased fruit intake by 177 g, juice by 341 g, vegetables by 104 g (n=87).
Level II or III-1 – allocation method is not described	Castenmiller et al (1999)	6 arms: 3-week low carotenoid diet then control diet vs 20 g spinach as whole blanched leaves, minced, liquefied or liquefied plus pectin (about 10 mg αcarotene/day) vs supplement of 0.5 mg/day and fibre. Compared to the αcarotene supplement, estimated that 5% of whole leave spinach was absorbed and >5% of the other types (n=70).
Level II or III-1 – allocation method is not described	de Pee et al (1998)	4-arm trial in anaemic Indonesian children with untreated parasites. 6 days/week for 9 weeks, comparing 556 μg/day RE from retinol-rich foods (eggs, liver, margarine), 509 μg/day RE from papaya, mango and pumpkin, 684 μg/day RE from green leaves and carrots and low diet (44 μg/day RE). Serum retinol increased with all interventions. With fruit, the increase was 50% that with retinol-rich foods. With vegetables, the increase was 33% that with retinol-rich foods. Shows that fruit has a conversion factor of 12:1 and vegetables a factor of 26:1 (n=238).
Level II or III-1 – allocation method is not described	van het Hof et al (1999b)	Dutch people (n= 54). On a high vegetable diet (490 g/day including 185 g cooked green beans, broccoli, spinach, green peas, Brussel sprouts or a vegetable mix in rotation plus vegetable-based salad and soup) containing 5.1 mg αcarotene, 22 on a low vegetable diet (130 g/day) containing 1.5 mg αcarotene and 10 on the low vegetable diet supplemented with αcarotene and lutein (total 7.2 mg αcarotene/day) for 4 weeks. After correcting for differences in dose, the relative response of plasma carotene to the high vegetable diet was 14% of the response to αcarotene supplement.
Level III-1	Torronen et al (1996)	After 10 days on a low αcarotene diet, subjects consumed 12 mg αcarotene as either raw carrots, juice or capsules for 6 weeks. Compared to baseline (end of low diet phase) serum αcarotene levels trebled in capsule group, doubled in juice group and increased by 60% in raw group at 6 weeks (n=38).
Level III-1	Micozzi et al (1992)	Adult US men. Compared to the effect on plasma αcarotene of 30 mg αcarotene as a supplement, 30 mg as carrots raised plasma αcarotene by only 18% and 3 mg as broccoli by 2.4% after 6 weeks (n=30).
Level III-2	de Pee et al (1995)	Diet group not randomised but matched to wafer consumers from other villages. Compared enriched wafer and 3.5 mg αcarotene as stir-fried leaves or carrots. Serum αcarotene or retinol or breast milk retinol in breastfeeding Indonesian women with parasites was not altered (n=175).
Level III-3	McEligot et al (1999)	Trial of telephone advice to increase vegetable juice consumption. 63 in study, but they broke randomisation and matched juice drinkers to non-drinkers and on carotenoid intake and compared serum levels – but only 5–18 in the analyses (or 10–36 – not clear if n is people or matched sets). αcarotene in juice drinkers is 3 times higher than non-drinkers, but αcarotene is lower (not significant). Given n and non-use of data, the value of the results and the p values is questionable.
Level IV	Haskell et al (1997)	28 Bangladeshi patients undergoing surgery. Goal of study was to develop a method to estimate liver concentration. Liver storage rate was also calculated.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Hume & Krebs (1949)	Among 16 people entering the trial, only 3 became depleted. A dose of 1300 IU (390 μg) retinol/day for 6 months yielded plasma level of 65 IU/dL (19.5 μg/dL) at 6 months (n=1). A dose of 1,250 IU (750 μg) αcarotene yielded plasma retinol of 63 IU/dL (18.9 μg/dL) at 1 month but eye signs had become worse although plasma levels had improved, then 6 months on 2500 IU (1,500 mg αcarotene) yielded a plasma level if 83 IU (24.9 μg) (n=1). The 3rd person was given 2,500 IU αcarotene for 3 weeks and achieved a plasma level of 96 IU (28.8 μg/dL).
Level IV	Parker et al (1999)	Using radiolabels, raw carrot [5 mg (9.8 μmol) αcarotene] and 20 g fat in a meal yielded 0.4 mg retinol as retinyl ester in chylomicrons over 7 hours. The quantity of unchanged αcarotene was much less (n=1).
Level IV	Sauberlich et al (1974)	8 subjects depleted for 359–771 days (depending on when their deficiency signs appeared) then repleted using 8 different schedules with either αcarotene or retinol. In 4 subjects (weighing 60–98 kg) given incremental doses, 150 μg/day retinol corrected dark adaptation. In 2 subject given αcarotene of unknown *cis:trans* ratio, 300 μg/day corrected dark adaptation. 2 other subjects were given 2,400 μg/day. Dosages were 75, 150, 300, 600 and 1,200 μg/day. However, the US EAR is based on the amount to achieve a serum retinol level of 20 μg/dL. In the men receiving retinol, this was achieved with 300 (n=1) or 600 μg/day (n=2), 4th person not tested; and with 1,200 μg αcarotene (n=2).
Level IV	Tang et al (2000)	Single dose of 6 mg or 126 mg labelled all-*trans* αcarotene. Over 21 days, 6 mg dose yielded 1.6 mg retinol and the 126 mg dose, 2.3 mg retinol, ie conversion ratios of 3.8:1 and 55:1 (n=1).
Human experimental data	Yeum et al (1996)	This study finds that plasma carotenoids increase 36-fold in young men and women given a 3-day rotating diet containing 6 mg αcarotene, among other things. But there is no baseline information on carotenoid intake. Broccoli was added at one point but only the effect on lutein was measured. This was actually a vitamin K trial.
Survey data	de Pee et al (1998)	Cross-sectional survey of 24-hour recall and serum retinol in Indonesian women with young children. Using the conventional factors, the Spearman correlation between serum retinol and total A intake was 0.15. Recalculation of total vitamin A assuming that only 16% of αcarotene is converted increased the Spearman correlation to 0.21. This was interpreted as supporting the idea that the 6:1 conversion factor is wrong. (It may be that in developing countries, a single 24-hour recall does not assess usual intake etc. Both correlations round to 0.2).
Expert opinion	Paul & Southgate (1978)	Comments that divisor of 6 is for the total diet and that the 1969 UK RDI report includes the assumption that carotenoids from milk and milk products should be divided by 2 not 6, which changes the intake of RE by 100 μg/day (p33).
Papers used to assess requirements or bioavailability of vitamin A in children		
Level I	Glasziou & Mackerras, (1993)	A meta-analysis of all RCTs. Adequate supply of vitamin A, either through supplementation or diet, has a major role in preventing morbidity and mortality in children in developing countries. In developed countries, vitamin A may also have a role in those with life-threatening infections such as measles and those who may have a relative deficiency, such as premature infants.
Level II	Edwards et al (2002)	While on baseline diet, subjects given 18.6 mg dose of αcarotene as pureed baby food carrots or boiled mashed carrots or raw grated carrots plus labelled retinyl acetate and 5 g oil with test meals. Over 8.5 hours post-ingestion, 0.53 mg retinol, 0.44 mg αcarotene and 0.26 mg αcarotene from puree and 0.44 mg retinol, 0.16 mg αcarotene, 0.09 mg αcarotene from boiled-mashed carrots (n=9) and 0.43 mg retinol, 0.38 mg αcarotene and 0.19 mg αcarotene from raw grated carrot (n=6).
Level II (if 'randomly assigned' as stated, but not described how this was done)	Jalal et al (1998) sub study 1	Factorial 4-arm study in Indonesian children 3–6 years with ascaris infestation. Compared to basic meal group (baseline serum retinol 0.66 μmol/L), serum retinol increased by >0.15 μmol/L in 15 g fat plus levamisole group, by 0.2 μmol/L in carotene group (750 μg RE, 80% from red sweet potato) and 0.35 μmol/L in combined group (all $p<0.05$) (n=166).

Level of evidence	Reference	Study type, issues addressed and key findings
Level II (if 'randomly assigned' as stated, but not described how this was done)	Jalal et al (1998) sub study 2	Factorial 4-arm study in Indonesian children 3–6 years with ascaris infestation. Compared 750 RE (80% from red sweet potato) supplemented with 15 g fat or levamisole or both. Serum retinol increased by about 0.1 μmol/L with no difference between groups (n=169).
Level II (if 'randomly assigned' as stated, but not described how this was done)	Persson et al (2001)	8–12 year-old Bangladeshi children dewormed and fed 3 different diets: dark green leafy vegetables (4.4 mg αcarotene/day) sweet pumpkin (1.5 mg αcarotene/day) or low carotene vegetables (0.03 αcarotene/day). Fat for all diets was 8 g/day. Children fed 6 days/week for 6 weeks. Baseline mean retinol was low–normal. There was no difference in the change in retinol in the 3 groups. Serum αcarotene rose significantly more in the dark green leafy vegetables group than the control group; serum αcarotene rose somewhat in pumpkin group but ns compared to both dark green leafy vegetables and control groups (n=110).
Level II or III-1 (allocation method not described)	Bulux et al (1994)	Guatemalan children of 7–12 years, 4 arm trial: placebo 3/week vs 1,000 μg/day RE as palmitate 5/week vs 720 μg αcarotene as carrots plus 10 g fat/day vs 6.3 mg as αcarotene capsule + food/day. Children with parasites were treated at start. Baseline retinol was low–normal. Serum retinol did not change in any group. Plasma αcarotene only changed in group receiving αcarotene capsule (n=65).
Level II or III-1 (allocation method not described)	Drammeh et al (2002)	Gambian children of 2–7 years in a 4-arm trial. Placebo capsule (once) vs 60,000 RE retinol (once) vs 150 μg RE /day as reconstituted dried mango + 5 g oil vs mango for 5 days/week for 4 months. Compared to placebo, there was a small but significant increase in plasma retinol with the vitamin A capsule and mango + fat groups that was not different between the groups. The mango alone group had a non-significant increase. Assuming 40% of a high dose is stored, the effective capsule dose was 24,000 μg RE, whereas total dose from mangoes was 12,000 μg/day RE (n=176).
Level II or III-1 (allocation method not described)	Takyi (1999)	Ghanaian preschool aged children, 20% prevalence of worms. Compared to those receiving 10 RE/day from pounded, homogenised cassava and kapok leaves, serum retinol was significantly higher in those receiving 400 RE/day from leaves plus 10 g fat, or 400 RE/day from leaves plus 10 g fat plus mebendazole or 400 RE/day supplemental αcarotene plus fat. There was no significant difference between the last 3 groups and prevalence of deficient serum vitamin A declined from 19.6% to 5.9%. Serum retinol was not significantly increased in those who received 400 RE/day from leaves alone compared to 10 RE from leaves (n=416).
Level III-2	Faber et al (2002)	Home gardening program to promote yellow and dark-green vegetables, South Africa. Serum retinol in children 2–5 years increased in experimental village and decreased in control (<0.05). Poor baseline recruitment in control (n=2 villages).
Level III-2	Tang et al (1999) Tang et al (2000) Yin & Qin (1998)	Middle-income Chinese children 5–6.5 years, all dewormed, given high carotene vegetables (4.6 mg αcarotene) vs usual low carotenoid vegetables for the season (0.7 mg αcarotene). After 10 weeks, serum retinol and αcarotene were significantly lower in the low group but maintained in high group. Body stores in a sub group also lower in low group (n=41 or 82).
Level III-2	Vuong (2000)	Anaemic Vietnamese children (31–70 months) given rice, rice with gac fruit (3.5 mg αcarotene) or 5 mg αcarotene powder. Serum αcarotene rose more in gac fruitgroup than powder group, both significantly more than rice group. Serum retinol increased to same extent in all 3 groups. NB different quantities of αcarotene. (n=193).
Level III-3	Ribaya-Mercado et al (2000)	First study: Albendazole plus orange fruit and vegetables (mango, melon, papaya, squash, sweet potato and carrots, 13 mg αcarotene) with fat given for 12 weeks instead of usual school foods. Serum retinol increased significantly (from 0.68 to 1.06 μmol/L). Undernourished school-aged Filipino children selected because they had low retinol, so finding that improvement was greatest in those with lowest baseline may be regression to the mean (n=27). Second study: Albendazole and non-vitamin A-containing foods given for 12 weeks instead of usual school foods. Serum retinol increased significantly (from 0.66 to 0.86 μmol/L). Undernourished school-aged Filipino children (n=25).

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	van Lieshout et al (2001)	Indonesian children (8–11 year), 60% prevalence of parasites (not treated): using 80 μg/day retinol + 80 μg αcarotene with different radiolabels for 3–10 weeks. The bioefficacy was 2.4 μg αcarotene to 1 μg/day retinol. The *cis/trans* ratio was 3/1 for αcarotene, so postulated that bioefficacy of *trans* isomer may be as high as 1.5 μg to 1 μg/day retinol (n=35).
Papers used to assess needs or bioavailability of vitamin A in lactation		
Level II (if 'randomly assigned' as stated, but not described how this was done)	Ncube et al (2001)	Lactating women in Zimbabwe with baseline retinol level of 0.9 μmol/L. Compared to no change in placebo group, serum retinol increased by 0.3 μmol/L in group given 6 mg αcarotene in capsule, by 0.2 μmol/L in those given 6 mg αcarotene as pureed papaya, by 0.3 μmol/L in those given 6 mg as grated carrot for 6 days. All received additional 10 g oil. However, the carrot group had slightly lower baseline and so the decline in per cent deficient was not as large in this group as the capsule and papaya groups. Retinol dose-response test in subset indicated that women receiving capsule or papaya had an increase in liver retinol stores (n=196).
Papers used to assess the infant requirements and breast milk concentrations of vitamin A		
Level II	Rice et al (1999)	3-arm trial comparing supplements of 60 mg RE (once) vs 7.8 mg αcarotene daily vs placebo given to lactating Bangladeshi women not treated for parasites. After 6 months, serum retinol for infants was better in retinol group (p=0.06) and liver stores were highest in the retinol group and somewhat higher in the αcarotene group than the placebo group. 28% of children in the αcarotene group and 24% of those in the placebo group had serum retinol level <0.07 μmol/L, vs 17% in retinol group (n=220).
Level III-2	Canfield & Kaminsky (2000)	A review of 3 trials published elsewhere. States that αcarotene as beadlets or red palm oil given to lactating women increases the serum retinol levels of the infants. (Original papers not obtained).
Survey data	Canfield et al (1997)	Used for AIs. Mean retinol content of breast milk, mean age 5.8 months (n=12).
Survey data	Canfield et al (1998)	Used for AIs. Mean retinol content of breast milk, mean age 9.3 months (n=3).
Survey data	Canfield et al (2003)	Used for AIs. 9 countries, mean retinol content of breast milk, mean age 3.3. months (n=417).
Papers used to assess UL for beta-carotene		
Level II	ATBC (1994)	Randomised, double-blind placebo-controlled study. A total of 29,133 Finnish male smokers randomly assigned to one of four treatment groups: 50 mg dl-αtocopherol/day (equivalent to 55 IU), 20 mg/day αcarotene, both αtocopherol and αcarotene, or placebo. Follow-up continued for 5–8 years. Lung cancer incidence not affected by αtocopherol treatment, but the incidence of prostate cancer was reduced. αTocopherol had no apparent effect on total mortality but was associated with increased mortality from haemorrhagic stroke. In contrast, deaths from ischaemic stroke and IHD were reduced in the αtocopherol group. Subsequent analysis of the risk factors for stroke indicated that vitamin E increased the risk of sub-arachnoid haemorrhage and decreased the risk of cerebral infarction in hypertensive men but had no effect on normotensive men. Showed an 11% increase in risk of IHD with αcarotene and an 18% increase in lung cancer.
Level II	Omenn et al (1996)	The CARET trial on lung cancer produced an increased risk with 30 mg αcarotene administered together with retinyl palmitate, as well as an increase in total mortality.

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THIAMIN

The recommendations for thiamin were derived after consideration of the FNB:IOM (1998) review of RDIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the US:Canadian DRI review for thiamin (FNB:IOM 1998) were adopted unchanged. These figures are higher than the former RDIs based on new evidence and more emphasis on the need for thiamine saturation of tissues. Assessment of population intakes in Australia indicate that these higher levels will be met through continued reliance on mandatory thiamin enrichment of bread-making flour to achieve adequate status. This may not be the case in New Zealand where thiamin enrichment is not mandatory.

EVIDENCE BASE

Databases: PubMed, Medline and a search of cross-references and references in FNB:IOM (1998) and recent reviews of thiamin by other expert committees.

Search terms: Vitamin B_1, thiamin.

Abbreviations used in the following summary: T, thiamin; TU, thiamin in urine; ETKA, erythrocyte transketolase activity; E-TPP, erythrocyte thiamin pyrophosphate; E-T, erythrocyte thiamin; NA, not available.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the requirement for thiamin for infants aged 0–12 months		
Level III-3	Dick et al (1958)	Basal diet served to 8 boys (calculated mean of 514 mcg T, analysed mean of 570–637 mcg T, mean 3,582 kcal/day) for 69 days. TU was measured for the second 10 days of the first 20-day period on the low T diet. Then stepwise increments T at regular intervals.
Level IV	Nail et al (1980)	The content of human milk is 1.7 mg T/day for well-nourished mothers with (n=7) and without (n=5) multi-vitamin supplements. Measured T in breast milk and 24-hour TU at intervals post-partum. Dietary T intakes for 1 day before (24-hour recall) and 3 days during expressed milk collection (3-day records). Observed increase in T in breast milk over time, but no difference between groups. TU rose more in supplemented women.
Survey data	Committee on Nutrition (1985)	The mean concentration of T in human milk is 0.21 mg/L and the mean volume for intake of human milk is 0.78 L/day. This gives an AI for T of 0.16 mg T/day for infants aged 0–6 months (rounded to 0.2 mg T). For reference infant weight of 7 kg, this corresponds to 0.03 mg T/kg/day.
Survey data	Wyatt (1991)	323 children (up to 18 months) in three groups by age. Total blood T (various fractions) was highest in children under 3 months (mean 258 nmol/L), lower in the next group at 312 months (mean 214 nmol/L), lower in the group age 12+ months (mean 187 nmol/L). Lower T levels due to phosphorylated form.
Papers used to assess the requirements for thiamin in children and adolescents		
Survey data	Bailey et al (1994)	T/day (calculated) lower than T/day (analysed), and well correlated with T/day but not T/1,000 kcal. Estimated T intake was above 0.4 mg T/1,000 kcal (normal). 12% of females and 17% of males had high ETKA-coefficients (indicating deficiency). Recommends further investigation of T standards.
Papers used to assess the requirements for thiamin in adults		

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Brin (1962)	8 healthy medical students divided into two groups. In 4 men, T intake was limited in to 0.190 mg/day for 8 weeks and was effective in achieving T depletion assessed by TU and ETKA in 4 T-depleted students. A random selection of 4 controls took the same diet and an additional 2 mg T/day supplement. No measured effect of T depletion on ill health (subjective feelings, physical findings, nerve conduction studies, ECG, EEG – but results not given for scrutiny). Indicates that intake of 0.190 mg T/day is below the T requirement, as measured by TU.
Level II	Wood et al (1980)	Australian double-blind study of partial T restriction designed to develop biochemical standards for T depletion. Medical students. Random allocation to each of 2 groups – 19 followed the same low T diet, including 10 randomly selected controls who took an additional supplement of 5 mg T/day. Intake of 0.175 mg T/1,000 kcal (0.500 mg T/day, calculated/ analysed) for 4-5 weeks was effective in achieving T depletion assessed by TU and ETKA. No measured effect of T depletion on ill health (subjective feelings, physical findings, psychological testing, nerve conduction studies, work performance). This study indicated that intake of 0.175 mg T/1,000 kcal (0.500 mg T/day) is below the T requirement, as measured by TU. Objective measurements of signs and symptoms of T deficiency were conducted and did not lead to signs of deficiency in the time of the study.
Level III-2	Hoorn et al (1975)	33 male and 120 female geriatric Dutch patients compared to 54 healthy blood donors. Vitamins B_1, B_2 and B_6 activation coefficients returned to normal after 12 days (on 20 mg T/day, plus vitamins B_2 and B_6).
Level III-2	Oldham (1962)	Metabolic unit study of 10 active elderly women and 8 young women controls (18–21 years). T depletion for 13 days (0.33 mg T/day) for older women and for 16–21 days (0.39 mg T/day) for younger women. Both groups then given test dose 1,200 mg T, followed by stepwise repletion of about 0.5 mg T/day for 12–13 day. Measured TU after various T intakes.
Level III-3	Bamji (1970)	Study 1: 26 healthy and 39 with T deficiency (appears to have been regarded as peripheral neuropathy – which is not specific for thiamin deficiency). ETKA/TU measured before and after administration of 1 mg TP. Study 2: 4 males and 4 females, no age given, no controls. Low T intake (0.1 mg T/1,000 kcal for 2–3 weeks), then stepwise addition of T supplements. Minimum requirement for young men measured by TU. T-EAR men: 0.313–0.359 mg/1,000 kcal T-EAR women: 0.214–0.263 mg/1,000 kcal.
Level III-3	Ziporin et al (1965)	8 healthy young men (no age given, no controls) on low TD (0.11–0.18 mg T/day) for three periods: 1. Control: 9 days (low T intake + 1.6 mg T supplement) 2. Depletion: 39 days (low T intake) 3. Repletion: 12 days (low T intake and stepwise T supplement) Minimum requirement for young men (measured by TU). No observations made of clinical T deficiency.
Level IV	Anderson et al (1986)	Total T intake 1.2 mg (21 men) and 1.0 mg (21 women) (ages 25, 55 and 75 years) determined using criteria of maximum ETKA. T intake estimated by 7–day food recall (by calculation).
Level IV	Elsom et al (1942)	9 women studies for 28–120 day; T intake on repletion ranged from 0.2–0.77 mg/day. Normal urinary excretion found only above 0.65 mg/day.
Level IV	Foltz et al (1944)	4 men studied over 1 month. T intake on repletion over 0.95 mg/day gave urine 50% of normal; above 1.44 mg/day normal urinary excretion.
Level IV	Henshaw et al (1970)	39 women studied over 3–7 days. T intake in repletion/maintenance at 0.82 mg/day associated with 50% abnormal ET activity and urinary levels. Repletion intake of 1.02 mg/day associated with 29% abnormal ET activity and 95% abnormal excretion.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Horwitt et al (1948)	24 subjects over 3 years. T intake on repletion/maintenance at 0.2 mg/day gave abnormal metabolism and clinical signs. At 0.4 mg/day, results varied. At 4.0 mg/day normal metabolism and clinical signs.
Level IV	Kraut et al (1966)	4 men, 2 women studied over 9–10 months. T intake during repletion/maintenance was 2.0–2.5 mg/day, which gave normal TU.
Level IV	Reuter et al (1967)	After a fasting cure, 6 obese women (26–68 years) on (normal) T intake 0.42 mg/1,000 kcal, then increased T stepwise to 2.46 mg/1,000 kcal (measurement of 24-hour TU and ETKA). Results suggest a much higher T requirement. Approximately 0.7 mg T intake/day (0.4 mg T/1,000 kcal) met minimum requirement based on measurements of 24-hour TU and ETKA. Estimated minimum T requirement approximately 04 mg T/day. Achievement of maximum ETKA required 1.1 mg/1,000 kcal (2.0–2.5 mg T/day).
Level IV	Sauberlich et al (1979)	Longitudinal study of 7 young men in two groups. Measurements of T intake (by analysis), ETKA, TU. Concluded that 0.30 mg T/1,000 kcal (approximately 1 mg T/day) met the minimum requirement (measured by TU and close to that for normal ETKA). Carefully controlled study in a metabolic unit, but no control subjects.
Papers used to assess the requirements for thiamin in pregnancy		
Level IV	Chong & Ho (1970)	Six population groups (n=36) where ETKA was similar in pregnant women to other groups. 36% of 103 pregnant Malaysian women had TPP effect >25% – a higher percentage than found for males and non-pregnant women.
Level IV	Daum et al (1948) Hathaway & Strom (1946) Oldham et al (1946)	Studies of intake/excretion ratios in non-pregnant women used as comparative data for pregnancy.
Level IV	Heller et al (1974)	One measurement of ETKA in 556 pregnant German women at various stages of gestation. Mean activation coefficient of 1.13 compared to 1.05 in 300 reference blood donors. Standard of 1.20 in non-pregnant adults. 26% of women with uncomplicated pregnancies and 21% with complications had activation coefficients >1.20 and were classified as abnormal.
Level IV	Lockhart et al (1943)	16 pregnant women studied in 10th lunar month. Three times as much T (diet+supplements) was needed by 16 pregnant women to achieve TU peak (as needed by non-pregnant women).
Level IV	Oldham et al (1950)	Strong correlation between total T intake and excretion, but no consistent decrease in T excretion in pregnancy.
Level IV	Slobody et al (1949)	48 pregnant women and 35 of their newborn infants (5 days old). Mean free T concentration was higher in cord blood than newborn infants, and both were higher than in maternal blood.
Level IV	Tripathy (1968)	33 pregnant women in low socio-economic group (no vitamin supplements). TKA in cord blood tended to be proportional to maternal blood and was higher in blood of pregnant than in non-pregnant women.

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RIBOFLAVIN

The recommendations for riboflavin were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. The AIs for younger infants were based on breast milk concentrations together with an estimate of the mean volume of breast milk. The requirements for older infants were extrapolated from a consideration of those of younger infants on a body weight basis and from adults on a metabolic body weight basis (FNB:IOM 1998). For adults, the EAR was based on results of depletion-repletion studies (Sebrell et al 1941, Williams et al 1943, Brewer et al 1946, Davis et al 1946, Roe et al 1982, Belko et al 1983, Kuizon et al 1992, Boisvert et al 1993, Keys et al 1944, Horwitt et al 1949,1950, Bessey et al 1956, Boisvert et al 1993, Madigan et al 1998, FNB:IOM 1998) plus consideration of additional needs in pregnancy or lactation. These were extrapolated to children and adolescents on a metabolic body weight basis including an allowance for growth.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 1998) for use in the US and Canada were adopted for Australia and New Zealand, except for older adults for whom the EARs were increased on the basis of studies by Boisvert et al (1993) and Madigan et al (1998) showing that this group had increased needs to sustain riboflavin status.

EVIDENCE BASE

Databases: Australasian Medical Index, Medline, Current contents, Science Citation Index and search of cross-references and references in FNB:IOM (1998).

Search terms: riboflavin, human, vitamin B_2.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the dietary riboflavin intake requirement in adults		
Level IV	Belko et al (1983)	Repletion study for 12 weeks, with a period of exercise, 49 subjects. 0.8 mg/1,000 kcal gave a normal EGRAC in the non-exercise period and ≥1.2 mg/1,000 kcal was normal in the exercise period.
Level IV	Bessey et al (1956)	Repletion study for 8 or 16 months, 57 subjects. 2.4 mg/day gave normal erythrocyte riboflavin.
Level IV	Boisvert et al (1993)	Case series. Titration of riboflavin-deficient male and female subjects on a western diet with increasing riboflavin intakes. Urinary excretion of riboflavin is low with intakes up to 1.0–1.1 mg/day, but is much higher with intakes of ≥1.3 mg/day. The average EGRAC is above 1.4 with intakes below 1.3 mg/day, but below 1.4 with intakes of ≥1.3–1.4 mg/day.
Level IV	Brewer et al (1946)	12-day metabolic study of 14 women. Level of riboflavin intake for normal urinary excretion is ≥1.04 mg/day.
Level IV	Davis et al (1946)	Repletion study, 12 men over 8 months. 0.66 mg/1,000 kcal gave normal urinary excretion and 0.49 mg/1,000 kcal did not.
Level IV	Horwitt et al (1949)	Repletion study for 9–10 months or 3 months, 39 patients. 1.1 mg/day gave normal urinary excretion.
Level IV	Keys et al (1944)	Repletion study for 5 months, 6 men. 0.99 mg/day gave normal urinary excretion.
Level IV	Kuizon et al (1992)	Repletion study for 40 days, 7 subjects. Intakes ≥0.5mg/1,000 kcal gave normal EGRAC.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Madigan et al (1998)	Case series, observational. Assessment of riboflavin intakes and riboflavin status among elderly. Intakes at or above levels currently recommended are associated in the elderly with inadequate riboflavin status.
Level IV	Roe et al (1982)	Repletion study for 10 weeks, baseline 1.69 mg/day riboflavin, 10 subjects. 0.6. 0.8 and 1.0 mg/1,000 kcal all gave abnormal EGRA of 50%, 20% and 10%, respectively.
Human experimental data	Horwitt et al (1950)	Subjects kept on controlled diets for extended periods. Clinical deficiency symptoms occur with riboflavin intake of 0.55 mg/day but not ≥0.75 mg/day. Urinary excretion of riboflavin is low with intakes of 0.55–1.1 mg/day, but is much higher with intakes of ≥1.6 mg/day, implying a discontinuity (saturation) above 1.1 mg/day.
Human experimental data	Sebrell et al (1941)	Subjects kept on controlled diets for extended periods – shows deficiency symptoms are common with riboflavin intake of 0.5 mg/day.
Human experimental data	Williams et al (1943)	Subjects kept on controlled diets for extended periods – shows deficiency symptoms do not occur with riboflavin intake of 0.8 mg/day.
Expert opinion	Marriage et al (2003)	Detailed literature review on riboflavin megadoses in managing mitochondriopathies.
Expert opinion	Powers (2003)	Detailed literature review of the role of riboflavin in health and disease.

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NIACIN

The recommendations for niacin were derived from an assessment of the evidence base used by the US:Canadian Government DRI review (FNB:IOM 1998) and consideration of additional key papers missing from those reviews or published since they were released, and current recommendations of other key countries.

The EAR for adults was based on a number of studies of niacin intake and urine N_1-methylnicotinamide (Goldsmith et al 1952, 1955, Horwitt et al 1956, Jacob et al 1989) and extrapolated on a body weight basis, including an allowance for growth and energy needs. The recommendations for infants were based on breast milk concentrations of niacin.

After review, the FAO:WHO and US:Canadian recommendations (FNB:IOM 1998) were adopted with the variations outlined below.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The only variation was to add a separate UL for nicotinamide, based on the fact that it does not produce the flushing response elicited by nicotinic acid and appears to have some benefit in relation to diabetic management.(Pozilli et al 1995, Lampeter et al 1998). The recommendations of the European Commission were adopted (EC 2002).

EVIDENCE BASE

Databases: Medline, Current Contents, Science Citation Index and Australasian Medical Index as well as search of cross-references and references in FNB:IOM (1998) and recent reviews of niacin by other expert committees charged with setting recommendations for requirements in the UK, German-speaking nations, the European Community and for the FAO:WHO.

Search terms: niacin, nicotinamide, human studies.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess what intake of niacin equivalents (NE) results in adequate niacin status		
Level IV	Jacob et al 1989	Male subjects on controlled diets (each subject on 3 different intakes) for extended periods. From primary data, on average, NE intakes of 11.3 mg/day achieved niacin adequacy.
Human experimental data	Goldsmith et al 1952	Female subjects kept on controlled diets with low NE, for extended periods. From primary data for a subset of participants, on average, NE intakes of 12.6 mg/day achieved niacin adequacy.
Human experimental data	Goldsmith et al 1955	Female subjects on controlled diets with low NE for extended periods. From primary data for a subset of participants, on average, NE intakes of 10.9 mg/day achieved niacin adequacy.
Human experimental data	Horwitt et al 1956	Male subjects kept on controlled diets, most with low NE, for extended periods. From primary data for a subset of participants, on average, NE intakes of 11.5 mg/day achieved niacin adequacy.
Paper used to assess what level of niacin intake causes flushing		
Level III-2	Sebrell & Butler 1938	3 groups of adult females, on identical diets except for supplementation with different levels of nicotinic acid. At 50 mg/day, most subjects had flushing. There was no flushing at lower doses.
Papers used to assess any benefits or adverse effects of high dose nicotinic acid or nicotinamide		

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Pozzili et al 1996	Meta-analysis of RCT (and other trials) showing high-dose nicotinamide (4–100 mg/kg) preserves residual αcell function in recent-onset type 1 diabetes.
Level II	Berge et al 1991	Review of Coronary Drug Project. In post-coronary patients, nicotinic acid (3 g/day) lowered incidence of non-fatal MI after 6 years and all-cause mortality after 15 years.
Level II	Lampeter et al 1998	The Deutsche Nicotinamide Intervention Study (DENIS), evaluated the clinical efficacy of high doses of nicotinamide in children at high risk for type 1 diabetes. The group receiving nicotinamide exhibited decreased first-phase insulin secretion in response to intravenous glucose. No other side effects were observed.

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VITAMIN B_6

The recommendations for vitamin B_6 were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. The AIs for younger infants were based on breast milk concentrations (West & Kirksey 1976) and an estimate of the mean volume of breast milk. The requirements for older infants were extrapolated from that of younger infants on a metabolic body weight basis (FNB:IOM 1998). The EAR for adults was based on results of depletion-repletion studies (Baker et al 1964, Yess et al 1964, Miller & Linkswiler 1967, Linkswiler 1978, Miller et al 1985, Selhub et al 1993, Brown et al 1975, Kretsch et al 1995, Huang et al 1998, Hansen et al 1996a,b, Hansen et al 1997, FNB:IOM 1998), plus consideration of additional needs in pregnancy or lactation. Requirements for adults 51 years and over are higher than those for younger adults. These were extrapolated to children and adolescents on a metabolic body weight basis including an allowance for growth.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

With the exception of the UL, the recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 1998) were adopted for Australia and New Zealand. These recommendations are almost identical to those of the FAO:WHO (FAO:WHO 2001), but are higher than the previous Australian/New Zealand RDIs (Coles-Rutishauser 1990). For the UL, a UF of 4 instead of 2 was used because of the uncertainty about the length of the experimental studies. Most subjects were studied for only 4–5 months. One longer term retrospective study had indicated that symptoms may take longer than 1–2 years to develop and was preferred by the European Commission when setting its recommendations. However, the same study also had a number of design issues so it was decided to retain the two experimental studies of Bernstein & Lobitz (1988) and Del Tredici et al (1985) as the basis of the NOAEL, but apply the greater UF because of the findings of Dalton & Dalton (1987).

Codes used in the following summary of evidence	
Vitamin B_6	B_6
Erythrocyte alpha-aspartate aminotransferase	EAST
Erythrocyte alpha-alanine aminotransferase	EALT
4-Pyridoxic acid	4-PA
Plasma homocysteine	PHC
Pyridoxine	PN
Pyridoxine hydrochloride	PN.HCl
Tryptophan	T
Tryptophan excretion	TE
Xanthurenic acid excretion	XAE
NA	Not available

Note: See also supplementary information at the end of the evidence tables

EVIDENCE BASE

Databases: PubMed, Medline and search of cross-references and references in FNB:IOM (1998).

Search terms: Vitamin B_6, pyridoxine.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the requirements of vitamin B_6 for infants aged 0–6 months		
Level III-3	Borschel & Kirksey (1986)	B_6 intakes (calculated from 1-day food record of mothers and infants on day milk samples collected) in breast-fed (2 groups supplemented with 2.5 or 15 mg PN.HCl/day) and formula-fed (1 group) infants at 1, 2, 4 and 6 months. Infant B_6 intakes highest in formula-fed infants and lowest for breast-fed infants whose mothers received 2.5 mg PN.HCl/day. PLP lowest in infants breast-fed at each time interval and lowest in infants of women supplemented at 2.5 mg B_6/day.
Level III-3	Kang Yoon et al (1995)	6 groups of neonates given varying amounts of PN.HCl and mothers with varied B_6 intake (24-hour diet recall, calculated) over 28-day experimental period. Lower B_6 in milk of mothers of pre-term infants than term infants, even when mothers had a supplement of 27 mg B_6/day. Infant plasma PLP was related to their B_6 intake. In infants of mothers supplemented with 2 mg day/day of PN, plasma PLP fell to 32 nmol/L by 1 month and was 270 nmol/L in infants with additional direct supplement of 4 mg B_6/day. Formula-fed infants had much higher plasma PLP values (about 200 nmol/L). B_6 adequacy is questionable for unsupported breastfeeding of infants of mothers in 2 mg B_6 supplemented groups.
Level IV	West & Kirksey (1976)	13 healthy lactating women (24–42 years) B_6 intake (Burke diet record, calculated). Mean B_6 of 0.13 mg/L in human milk at maternal B_6 intakes of less than 2.5 mg/day and 0.24 mg/L at intakes of 2.5–5 mg/day. However, milk B_6 variable between subjects at similar B_6 intake.
Survey	Andon et al (1989)	30 lactating women (selected, no supplements) had mean B_6 intake of 8.63 nmol/day and mean breast milk B_6 of 733 nmol/L.
Review	Borschel (1995)	Concluded that increasing the B_6 intake of lactating women to >10 mg/day may improve the B_6 status of breast-fed infants.
Case report	Kirksey & Roepke (1981)	PLP of 15 nmol/L in full term infants with B_6 responsive convulsions.
Abstract only available	Chang & Kirksey (2002)	Studied B_6 status (PLP, growth) of 47 breast-fed infants (B_6 intake determined, 4 levels of maternal B_6 supplementation (4, 7.5 and 10 mg PN.HCl/day) in first 6 months. A maternal supplement of 2.5 mg PN.HCl/day provided sufficient B_6 in breast milk (0.15 mg/day) for B_6 status and growth.
Papers used to assess the vitamin B_6 requirements for children and adolescents		
Level III-3	Heiskanen et al (1995)	198 children followed up at age 2 months to 11 years (multiple B_6 status indicators).
Survey	Driskell et al (1985)	583 adolescent girls (12, 14 and ears): 20% with marginal B_6 status and 13% with B_6 deficiency by abnormal EALT ratios. B_6 intake (24-hour food recall) averaged about 1.2 mg. Half of the subjects <66% B_6-RDA (NRC 1974).
Survey	Driskell et al (1987)	112 adolescent girls follow-up study (at 12, 14,16 years). 20% B_6 deficiency and almost 30% marginal deficiency with average intake 1.25 mg B_6, as assessed by EALT ratios.
Survey	Fries et al (1981)	Investigation of B_6 status in 35 healthy preschool children (3 and 4 years): Unsupplemented children, B_6 intake (24-hour food recall, 2-day food record, estimated, including supplements)0.9–1.3 mg B_6/day and plasma PLP average 58–78 nmol/L, higher in supplemented children.
Survey	Kirksey et al (1978)	127 (12–14 year-old) female adolescents: 13% considered to have inadequate B_6 status on basis of EALT ratios >1.25. Average diet B6 estimated from 24-hour recall was 1.24 mg B_6.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Chang et al (2002)	168 healthy children (7–12 years, anthropometry available) studied in Taiwan. B_6 status indicators (PLP, EALT-AC, EAST-AC) strongly correlated with B_6 intake (two x 3-day food recall, calculated). Adequacy of these indicators (including cut-off point PLP >20 nmol/L) was used to determine the EARs of 0.84 mg/day (1.01 RDA) and 0.75 mg/day (0.89 RDA) for boys and girls, respectively.
Papers used to assess the vitamin B_6 requirement for men aged 19–50 years		
Level III-3	Baker et al (1964)	8 healthy young men (18–22 years) on identical B_6-free formula diet (0.06 mg B_6/day) divided into 2 groups (30 and 100 g protein/day, respectively). Baseline period 1 week + 4 mg B_6.HCl. Then B_6 depletion until 80% in each group showed minimum XAE 200 mg/day following 10 g T load. Vitamin B_6 depletion rate was directly related to protein intake. Then repletion with 1 mg B_6/day for 2 weeks followed by step-wise increase of 0.5 to 1.5 mg/day. Group on low protein diet required 1.25 mg PN/day (equivalent to 1.5 mg B_6 in food) to return TE to baseline after a 10 g T challenge. Subjects on 100 g protein/day needed 1.5 mg PN/day (equiv to 1.9 mg B_6 in food). Concluded that optimal B_6 requirement (as B_6.HCL) appeared to be 1.25–1.5 mg/day (at 30 g protein/day) and 1.75–2.0 mg/day (at 100 g protein/day).
Level III-3	Miller & Linkswiler (1967)	Studied effect of dietary protein on T metabolism during B_6 depletion. 11 men (20–31 years) on basal diet including 0.16 mg PN/day and 54 or 150 g protein/day. Baseline period (this diet plus 1.5 mg B_6) when T metabolite excretion low. Then depletion on basal diet showed slow increase in excreted T metabolites over a few weeks. Higher protein level reduced the time taken to show increase in T metabolite excretion.
Level III-3	Miller et al (1985)	Study of the effect of dietary protein on metabolism of B_6. 8 men (21–31 years) on diets containing a constant 1.6 mg B_6/day (from food and food fortified with PN) and stepwise 0.5 (low), 1.0 (medium) or 2.0 (high) g protein/kg (5 days at each level). Reports for the last half of each period – UB_6, 4-PA, plasma total B_6, PLP. As protein increased, slow decrease in 4-PA and B_6. Results indicate increased protein intake resulted in retention of B_6 for increased catabolism of amino acids. Recommends consideration of protein intake when evaluating B_6-EAR.
Level III-3	Yess et al (1964)	6 men on diet including 0.16 mg PN/day and 100 g protein/day for 55 days. Investigated various tryptophan metabolites after a 2 g T load. Supplementation with 0.6 or 0.9 mg PN/day normalised T catabolites in nearly all subjects. Suggests an EAR of less than 0.9 mg PN/day (equivalent to <1.0 mg adjusted for food B_6).
Survey	Selhub et al (1993)	Cross-sectional study: 1,160 free living adults, 67–96 years, from the Framingham Study, adjusted for folate status, gender, age. Half of the subjects on freely-selected diets with average 1.3 mg B6 had plasma homocysteine levels similar to subjects with much higher intakes of B_6.
Papers used to assess the vitaminB_6 requirement for women aged 19–50 years		
Level III-1	Kretsch et al (1995)	8 women (21–30 years) in metabolic ward on high protein diet (1.55 g/kg/day). Depletion diet <0.05 mg B_6 for 11–28 days (2 subjects developed EEG abnormalities). Stepwise repletion with 0.5, 1.0, 1.5 and 2.0 mg PN/day (in periods of 14–21 day). Between 1.0 and 1.5 mg PN/day restored PLP to baseline and 1.5–2.0 mg B_6 restored XAE to baseline values. Plasma PLP in the baseline period averaged 25 nmol/L and dropped to <10 nmol/L during depletion. PLP values averaged 20 nmol/L plasma at intake of 1 mg B_6/day, suggesting an EAR of about 1 mg/day (1.25 mg food B_6). Normalisation of AST activation factors occurred with intakes 1.5–2.0 mg B_6/day but lagged behind the diet changes.
Level III-1	Hansen et al (1997)	Authors suggest RDA >0.16 mg B_6/g protein/day for women for adequate B_6 status. Investigated B_6 requirements of 10 young women on 85 g protein/day. Basal diet adaptation 1.03 or 0.84 mg B_6, fed for 15 days. Subjects then received 3 or 4 levels of B_6 repletion for periods of 10–12 days.
Level III-3	Brown et al (1975)	Depleted 15 women (average age 22) on oral contraceptives and 9 controls (no contraceptives) on diet with low B_6 (0.19 mg/day) and 78 g protein/day for 28 days. They were then repleted with 0.8, 2.0 or 20 mg PN.HCl for 28 days. During depletion, PLP fell from about 50 to 14 nmol/L. PLP increased to about 24 and 60 nmol/L on repletion.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Hansen et al (1996a)	Crossover design 9 women (mean 29 years). Crossover design, 18 day each. Studied effects of diets low and high in PN glucoside on status indicators. 10 women; two food-based diets of 1.5 mg B_6 with 9% or 27% present as the naturally occurring B_6 glucoside. Lower bioavailability with glucoside and possibly increased faecal excretion. On low B6-glucoside diet, mean PLP concentration >30 nmol/L, suggesting EAR <1.5 mg.
Level III-3	Hansen et al (1996b)	9 women, three periods of 14days in which effects of protein intake on B6 status was studied in women of 19–38 years. Diet 1.25 mg PN, protein intake 0.5, 1.0, or 2.0 g/kg body weight. At the two lower levels, PLP remained above 30 nmol/L. On highest protein intake, PLP dropped to 20–30 nmol/L. Decrease in plasma PLP with increase in protein intake accompanied by decrease in urinary-PA and urinary B6 indicates that more B6 is retained by tissues.
Level III-3	Hansen et al (2001)	7 premenopausal women (no supplements), 3-day food records prior to commencement of study. 1 mg B_6/day and 1.2 g protein/kg body weight. 7-day adjustment and 3 successive 14-day experimental periods (PN supplement raised B_6 intake-analysed to 1.5, 2.1 and 2.7 mg/day for the 3 experimental periods, respectively). Recommendations for B_6 intake assessed on the effect of B_6 intake and B_6/protein ratio on B_6 status indicators. Used USA:CAN DRI Committee method to determine EAR and RDA. Data suggest B_6-EAR of 1.1–1.2 mg/day, similar to those of the USA:Canada 2000 recommendations and an RDA of 1.5–1.7 mg/day for young women (slightly higher than USA:Canada Report 2000).
Level III-3	Huang et al (1998)	The authors concluded that current B_6-RDA of 1.6 mg/day based on 0.16 mg/g protein is not adequate and may require re-evaluation. Depletion-stepwise repletion study of B_6 requirements in 8 women (28–34 years) in a metabolic unit, fed high protein diet (1.55 g/kg body weight). Baseline diet for 9 days and depletion diet for 27 days. Repletion diets given as PN.HCl daily over several weeks. For baseline intake, 1.5-2 mg PN/day normalized plasma PLP and 4-PA urine to baseline values. No haematological abnormalities in depletion. Plasma PLP was above 20 nmol/L at end of depletion. At intake of 1.26 mg B_6, mean PLP was 38 nmol/L. On basis of plasma PLP, suggested EAR is between 0.45 and 1.26 mg/day, probably <1mg. If suggested cut-offs for EAST are applied to ratios for alpha-EAST and alpha-EALT in the study, EAR would be around 1.3 mg based on alpha-EAST or above 0.7 mg based on alpha-EALT.
Survey	Driskell et al (1989)	Investigation of B_6 status of 15 obese black adult women of 21–51 years. Dietary B_6 intake of these women and controls (15 non-obese) = 1.18 mg (two day diet recall, calculated). PLP = 60 (obese) and 63 nmol/L (controls). No apparent effect of obesity on B_6 status.
Papers used to assess the vitamin B_6 requirement for adults aged 51 years and over		
Level III-3	Ribaya-Mercado et al (1991)	B_6 requirements of elderly men and women about 1.96 and 1.90 mg/day. 12 men and women >60 years, protein intakes 0.8 or 1.2 g/kg body weight. Depletion/repletion B_6 intakes based on protein intake; averaged 0.15, 1.20, 1.80, 2.60 mg in men and 0.1, 0.9, 1.3, and 1.9 in women. Baseline studies on self-selected diets (average 1.6 mg B_6/day men and 1.4 mg/day women). XAE (after T load) close to baseline for half the men and all of the women at food B_6 intake of 1.6 mg/day. About 1.9 mg/day B_6 required to reach baseline values of plasma PLP and urinary 4-PA consuming 1.2 g protein/kg body weight. Higher levels of B_6 required to normalise EAST activation factors.
Cross-sectional	Selhub et al (1993)	1,160 adults (67–96 years) in the Framingham Heart Study, homocysteine levels studied. Data adjusted for age, gender, folate, B_{12}. At plasma PLP of 20 nmol/L, homocysteine concentration averaged 13 µmol at a dietary intake of about 1.3 mg B_6. Inverse relationship observed between homocysteine levels and PLP or B_6 intake. B_6 intakes as high as 1.92 mg/day were associated with high homocysteine levels.
Level IV	Meydani et al (1991)	8 healthy elderly (4 male, 4 female) on B_6 deficient diet 3 µg/kg body weight/day for up to 20 days (approx 0.17 mg/day for men and 0.10 mg/day for women) showed impairment in interleukin-2 and lymphocyte proliferation. Restoration of cell-mediated immunity parameters required >22.5 mg/ kg body weight/day. Not possible to determine a requirement from these data.
Papers used to assess the vitamin B_6 requirements in pregnancy		

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Barnard et al (1987)	Compared PLP and PL in 30 pregnant women (stage of pregnancy not stated) and 27 non-pregnant controls (17–23 years, middle income, no oral contraceptives, vitamin supplements in either group). Mean PLP was lower (16.65 nmol/L) in pregnant women than in controls (30.51 nmol/L). The authors considered that PLP was not a good indicator of B_6 status in pregnancy.
Level III-3	Hamfelt & Tuveno (1972)	58 pregnant women divided into 3 groups receiving 0, 2 or 10 mg B_6/day) and assessed three times during pregnancy. B6 status of mother and child related at delivery. A supplement of 2–10 mg B_6/day is necessary to keep mean PLP constant during pregnancy. Recommended 10 mg B_6/day supplement during pregnancy.
Level III-3	Lumeng et al (1976)	33 pregnant women on baseline diet assigned to one of 3 groups receiving 2.5 or 4.0 or 10.0 mg B_6/day (in multivitamin-mineral preparation). Maternal and foetal PLP studied. Results indicated that subjects consumed <2 mg /B6/day from food and required B_6 supplementation >4mg/day to maintain B_6 status.
Level III-3	Schuster et al (1981)	127 pregnant women (37 adolescent and 90 adults, no supplements) assessed at initial visit and at 30 weeks pregnancy . Measured EALT ratios in mothers during pregnancy and Apgar scores for infants at birth. Results indicated mothers with B_6 deficiency during pregnancy (as EALT ratio elevated) associated with lower Apgar scores in their infants.
Survey	Cleary et al (1975)	Effect of two levels of B_6 supplements (2.0–2.5 mg/day, 10 mg/day) during pregnancy on maternal/fetal PLP at term, with 58 non-pregnant controls (no supplements). Results suggest that supplement of >2.0–2.5 mg B_6/day is required to restore B_6 status in mother, and perhaps foetus.
Papers used to assess the vitamin B_6requirements in lactation		
Level III-3	Borschel et al (1986)	Studied B_6 intakes (calculated from 1-day food record mothers and infants on the day milk samples were collected) in breast-fed (2 groups supplemented with 2.5 or 15 mg PN.HCl/day) and formula-fed (1 group) infants at 1, 2, 4 and 6 months. PLP was lowest in infants breast fed at each time interval and lowest in infants of women supplemented at 2.5 mg B_6/day.
Level IV	West et al (1976)	19 women at 3 weeks to 30 months of lactation. Total B_6 (including supplements) 1.3–12.5 mg/day. 6/19 had B_6 intakes of <2.5 mg B_6/day and had lower B_6 levels in milk.
Papers used to set Upper Level of Intake of vitamin B_6		
Survey data	Bernstein & Lobitz (1988)	70 diabetic patients, most studied for no more than 5 months at 100–150 mg/day. No adverse effects reported.
Survey data	Dalton & Dalton (1987)	Retrospective assessment of symptoms in a treated cohort with PMT. Mean B_6 levels in those with symptoms were the same as in those without, but those with symptoms had been on pills for 2.9 years compared with 1.6 years for those without.
Survey data	Del Tredici et al (1985)	Carpel tunnel patients. No adverse effects seen at 4 months at 100–300 mg/day.

SUPPLEMENTARY INFORMATION

Compound	Units of measurement		Description
Pyridoxine (PN)	1mmol = 170 mg	1g = 5.9 mmol	Three naturally interconvertible forms present in the tissues
Pyridoxal (PL)	1mmol = 167 mg	1g = 6.0 mmol	
Pyridoxamine (PM)	1mmol = 168 mg	1g = 6.0 mmol	
Pyridoxal-5-phosphate (PLP)	1mmol = 247 mg	1g = 4.1 mmol	Principal active form

Compound	Units of measurement		Description
4-Pyridoxic acid (4-PA)	1mmol = 183 mg	1g = 5.5 mmol	Principal excretory form
Pyridoxine hydrochloride (PN.HCl)	1mmol = 206 mg	1g = 4.9 mmol	Usual form of supplements

Aspect of B_6 metabolism	Conversion factors for units of measurement
Dietary vitamin B_6 equivalent	(Food vitamin B_6) + (Supplementary vitamin B_6 × 1.27) (Food and Nutrition Board: Institute of Medicine, 2000-DRI Reference intakes for vitamin B_6)
Vitamin B_6 losses due to food storage and processing	Varies by food type – see Table 3 in Coles-Rutishauser (1991) Bioavailability in a mixed diet is approximately 75% [Tarr et al (1981)] – USA/CAN RDI Report (2000), p165: 1 mg food vitamin B_6 = 1.27 × mg synthetic B_6
Vitamin B_6 absorption	Efficient. No conversion factors are indicated (USA:Can DRI 2000)

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VITAMIN B_{12}

The recommendations for vitamin B_{12} were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. EARs and RDIs were set based on experimental data in adults that was extrapolated on a body weight basis, taking into account growth needs, to all other age groups except infants. For infants, the AI was derived by multiplying the concentration in breast milk by average daily intake of breast milk. Pregnancy recommendations were based on the additional needs of the foetus and lactation recommendations took into account the additional vitamin B_{12} secreted in milk. RDIs were derived from EARs assuming a CV of 10%.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations for Australia and New Zealand are the same as those published for the US and Canada (FNB:IOM 1998) with the exception of the recommendation that all adults over 51 years get most of their requirement from fortified foods or vitamin B_{12} supplements. Whilst recognising that there are substantial numbers of older adults who suffer from atrophic gastritis which can affect absorption of vitamin B_{12}, it was not considered necessary to recommend that everyone over 51 years should consume fortified foods or supplements to overcome the effects of a medical condition affecting a minority of this age group.

Studies of atrophic gastritis and vitamin B_{12} status to date have generally been carried out in convenience samples of older people and the estimates of the prevalence of atrophic gastritis have varied greatly, even when similar diagnostic criteria have been used. Measurement of circulating concentrations of pepsinogens (PGs) I and II or gastrin is often used to determine the prevalence of atrophic gastritis in population groups. In the largest study, 31.5% of 359 free living and institutionalised older volunteers (>60 years) living in Boston had serum PG concentrations of <20 µg/mL that are indicative of atrophic gastritis (Hurwitz et al 1997). However, in a study of 248 male and female volunteers (>65 years) living in Kansas City, less than 10% had PG concentrations indicative of atrophic gastritis (Krasinski et al 1986).

In New Zealand, Green et al (2005) recently examined serum PGs I and II as markers of atrophic gastritis and serum vitamin B_{12} in elderly (>65 years) participants (n=466) in the 1997 National Nutrition Survey (MOH 1999). Atrophic gastritis was present in 6.7% (n=27) of the participants for whom PG I:PG II was ≤2.9; 3.1% had severe and 3.5% had mild-moderate atrophic gastritis. Marginal (148-21 pmol/L) and deficient (<148 pmol/L) serum vitamin B_{12} concentrations were found in 28% (n=130) and 12% (n=56), respectively, of the participants.

The presence of atrophic gastritis increased the risk of having a deficient or marginal serum vitamin B_{12} by 20.0 fold (95% CI 6.0–67.0) and 5.1 fold (95% CI 1.4–19.1), respectively. Despite increasing individual risk, the prevalence of atrophic gastritis in this population was not high enough to fully explain the extent of low serum vitamin B_{12}. Consistent with reports from other countries, this study suggests that the prevalence of low serum vitamin B_{12} (<150 pmol/L) is around 10% in people over 65 years in New Zealand and provides further evidence that atrophic gastritis is associated with lower serum cobalamin concentrations. However, the prevalence of atrophic gastritis in this survey at 6.6% was much lower than the 10–30% reported in other surveys (used by the DRI committee). This study did not include institutionalised elderly who may have a higher rate of atrophic gastritis. Finally, this study indicated that atrophic gastritis does not explain the majority of low serum cobalamin concentrations. There are no recent Australian studies of representative populations.

There is no evidence to suggest that those individuals between 50 and 65 years are at increased risk of low serum vitamin B_{12} concentrations compared to younger populations. There are also few data to suggest that this group is at increased risk of atrophic gastritis. Accordingly, the basis of the recommendation that this age group consume most of their vitamin B_{12} from fortified foods or supplements is particularly difficult to justify.

Currently, fortification of foods with vitamin B_{12} is not permitted in Australia and New Zealand, with the exception of soy-based beverages. Despite this, vitamin B_{12} intakes in Australia and New Zealand are not

markedly different from those in the US and Canada (Baghurst et al 2000, MOH 1999,2003, NHANES III as detailed in FNB:IOM 1998).

EVIDENCE BASE

Databases: Medline, searches of *Proceedings of New Zealand Nutrition Society, Nutrition & Dietetics, Australian Journal of Nutrition and Dietetics* and the *Journal of the New Zealand Dietetic Association*, plus cross-referencing and review of key references in FNB:IOM (1998), used to set values.

Search terms: Vitamin B_{12}, requirements, Australia; New Zealand.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the vitamin B_{12} requirements of infants		
Survey data	Trugo & Sardinha (1994)	9 well- nourished Brazilian mothers had had breast milk B_{12} concentrations of 0.42 and 0.34 μg/L at 2 and 3 months, respectively. Used to calculate the EAR for infants 0–6 months. Also used to calculate the additional B_{12} required for lactation.
Papers used to assess the vitamin B_{12} requirements in adults		
Level II	Seal et al (2002)	RCT (n=31) of patients (mean age 81.4 years) with low serum B_{12} (100–150 pmol/L at screening) but no haematological abnormalities or other evidence of pernicious anaemia. Patients were randomised for 1 month to 10 μg oral cyanocobalamin, 50 μg cyanocobalamin, or placebo. Mean serum B_{12} increased by 51% in the 50 μg group ($P<0.05$ vs placebo), 34.4% in the 10 μg group (not significant versus placebo) and 11.7% in the placebo group. The authors conclude that 50 μg, but not 10 μg, improves serum B_{12}. This study suggests that the RDA of 2.4 μg/day for persons 70 years or older may be insufficient to meet the vitamin B_{12} needs of people with suboptimal B_{12} status arising from protein bound vitamin B_{12} malabsorption. Relevance to the EAR set for chronic consumption of vitamin B_{12} is limited as the study was too small and only 1 month's duration.
Human experimental data	Baker & Marhan (1981)	4 East Indian subjects with B_{12} deficiency anaemia resulting from dietary inadequacy. No indication of dietary intake. Oral vitamin B_{12} between 0.3–0.65 μg/day was sufficient to give a satisfactory haematological response but serum B_{12} was ≤74 pmol/L in all. Used with other studies to provide evidence that >1 μg B_{12}/day is needed to maintain serum B_{12} concentrations.
Human experimental data	Barstrup-Madsen et al (1983)	62 patients with pernicious anaemia were given slow-release intramuscular B_{12} (1 mg) every three months for at least 8 years. Assuming 15% retention of B_{12} this corresponds to an intramuscular dose of 1.7 μg day. All patients had B_{12} values above 180 pmol/L and all had complete haematological response. Given as evidence that the minimum intra-muscular requirement for B_{12} is 1–2 μg/day.
Human experimental data	Best et al (1956)	6 subjects with pernicious anaemia given 2 μg oral dose of B_{12} Co^{60} with intrinsic factor. With 70% absorption of the oral dose, 1.4 μg would be absorbed. 4/6 subjects had adequate haematological response. Used as evidence that 1.4 μg of absorbed vitamin B_{12} meets the needs of most people with pernicious anaemia.
Human experimental data	Bozian et al (1963)	Used with other papers to estimate extra loss of B_{12} from bile by a person with pernicious anaemia in excess of that lost by a healthy individual at 0.5 μg/day.
Human experimental data	Darby et al (1958)	Key study in setting EAR. 4 of 7 patients with pernicious anaemia achieved maximum erythropoiesis with 1.4 μg cyanocobalamin/day. 50% of patients with pernicious anaemia achieved adequate haematological response on a dose of 1.4 μg/day.
Human experimental data	El Kohlty et al (1991)	8 cholecystectomised patients excreted an average 1.4 μg B_{12}/day in bile. Assuming 50% reabsorption of B_{12} in bile, the average loss of biliary B_{12} in the stool would be 0.7 μg/day. Used with other papers to estimate extra loss of B_{12} from bile by a person with pernicious anaemia in excess of that lost by a healthy individual at 0.5 μg/day.

Level of evidence	Reference	Study type, issues addressed and key findings
Human experimental data	Hansen & Weinfeld (1962)	Given as evidence that 3 μg/day of B_{12} produces a reticulocyte response that does not respond to more B_{12}.
Human experimental data	Heyssel et al (1966)	Used with other 3 papers to estimate extra loss of B_{12} from bile by a person with pernicious anaemia in excess of that lost by a healthy individual at 0.5 μg/day. Also about 11% of vitamin B_{12} in liver is absorbed and about 65% of vitamin B_{12} in mutton is absorbed. Used collectively with other absorption data to estimate average fractional absorption of 50%.
Human experimental data	Lindenbaum et al (1990)	Given as evidence that 0.8–1 μg B_{12}/day (given as boluses) will maintain normal haematological and serum metabolite status in half of the individuals and 1.7 μg will maintain all individuals.
Human experimental data	Reizenstein (1959)	Used with other papers to estimate extra loss of B_{12} from bile by a person with pernicious anaemia in excess of that lost by a healthy individual at 0.5 μg/day.
Human experimental data	Sullivan & Herbert (1965)	Given as evidence that 0.1 μg/day of either cyanocobalamin or coenzyme B_{12} intramuscularly for 15 days (n=40) was not sufficient to treat PA and maintain adequate B_{12} status.
Human experimental data	Will et al (1959)	10 μg B_{12} given intramuscularly every 2 weeks or 20 μg given monthly was not sufficient to maintain serum cobalamin concentrations in any subjects (n=40). Used to provide evidence that 0.7 μg/day vitamin B_{12} is not sufficient.
Case report	Jathar et al (1975)	7 east Indian lactovegetarians consuming 0.3–0.8 μg B_{12}/day from milk. 50% had B_{12} concentrations below 74 pmol/L. Used to provide evidence that >1 μg day B_{12} is needed to maintain serum B_{12} concentrations.
Case report	Winawer et al (1967)	1 64 year-old strict vegan woman with megaloblastic anaemia, atrophic gastritis, normal stomach acidity, no intrinsic factor antibodies and a serum B_{12} <74 pmol/L. Given 1 μg B_{12} orally for 80 days, which increased serum B_{12} to 64 pmol/L. Used to provide evidence that 1 μg/day is not sufficient to maintain serum B_{12} concentrations at 150 pmol/L.
Survey data	Narayanan et al (1991)	10 subjects with low serum B_{12} (<120 pmol/L) not caused by vegetarianism or disease. Mean intake of B_{12} was 1.5 μg/day (range 0.6–1.9). 6/10 had either a megaloblastic bone marrow neuropathy that responded to a B_{12} injection, an MCV that decreased with injection, or a red blood cell count that improved with B_{12} injection. Given as evidence that the EAR should be greater than 1.5 μg/day.
Papers used to assess the absorption of Vitamin B_{12} from food		
Human experimental data	Doscher-holmen et al (1975, 1978, 1981)	About 24–36% of B_{12} from eggs is absorbed, 25–47% of B_{12} from trout is absorbed and about 60% of B_{12} from chicken is absorbed. Used collectively with other absorption data to come up with average fractional absorption of 50%.
Case report	Stewart et al (1970)	1 Hindu woman with megaloblastic anaemia consuming a diet containing 0.5 μg B_{12}/day. 1 μg oral dose for 55 days. Serum B_{12} rose to 121 pmol/L and haemoglobin stabilised at 107 g/L. She then stopped the supplement and began consuming one pint of fresh milk for 45 days containing 1 μg B_{12} for a total of 1.5 μg/day. Her haemoglobin rose to 134 g/L and her serum B_{12} fell to 100 pmol/L. Given as evidence that 1.5 μg B_{12}/day as a supplement may not be sufficient to maintain serum levels on a diet containing 0.5 μg B_{12}/day.
Papers used to assess the extent of atrophic gastritis in the elderly		

Level of evidence	Reference	Study type, issues addressed and key findings
Survey data	Green et al (2005)	A high prevalence of low serum vitamin B12 concentrations in the elderly has been attributed to atrophic gastritis because it leads to decreased stomach acid secretion and malabsorption of food-bound B12. To examine this relation, PGs I and II were measured as markers of atrophic gastritis, and serum B12 in elderly (> 65 yrs) participants (n=466) in the 1997 National Nutrition Survey of New Zealand. Atrophic gastritis was present in 6.6% (n=27) of the participants (PG1: PGII ≤2.9); 3.1% had severe (PG I <20 μg/L) and 3.5% had mild-moderate (PG I ≥20 μg/L) atrophic gastritis. Marginal (148–221 pmol/L) and deficient (< 148 pmol/L0 serum B12 concentrations were found in 28% (n=130) and 12% (n=56), respectively, of the participants. The presence of atrophic gastritis increased the risk of having a deficient or marginal serum B12 by 20.0 fold (95% CI: 6.0, 67.0) and 5.1 fold (95% CI: 1.4, 19.1) fold, respectively. Despite increasing individual risk, the prevalence of atrophic gastritis in this population was not high enough to fully explain the extent of low serum B12. Suggests that the prevalence of low serum B12 (<150 pmol/L) is about 10% in New Zealand older persons (> 65 years). Provides further evidence that atrophic gastritis is associated with lower serum cobalamin concentrations. However, the prevalence of atrophic gastritis in this survey at 6.6% is much lower than the 10–30% reported in other surveys (used by the DRI committee). This study did not include institutionalised elderly who may have a higher rate of atrophic gastritis. Indicates that atrophic gastritis does not explain the majority of low serum cobalamin concentrations. Findings consistent with overseas studies.
Survey data	Hurwitz et al (1997)	Of 248 male and female volunteers (>65 years) living in Kansas City, 10% had PG concentrations indicative of atrophic gastritis. Used with other papers to indicate a rate of atrophic gastritis of 10–30% in older populations.
Survey data	Krasinski et al (1986)	Of 359 free living and institutionalised elderly (>60 years) Bostonians, 31.5% had atrophic gastritis based on low serum PGs. Used with other papers to indicate a rate of atrophic gastritis of 10–30% in older populations.
Papers used to assess the population Vitamin B_{12} status in Australia and New Zealand		
Survey data	Barber et al (1989)	A cross-sectional study of 100 elderly subjects (>70 years) residing in geriatric wards or rest homes within the Auckland region. Sampling technique not defined. Using a cut-off point of 101 pmol/L, 23% had low serum B_{12} concentrations, of whom 48% had haematological findings consistent with megaloblastosis. Shows that a high proportion of institutionalised NZ elderly has suboptimal B_{12} concentrations and a significant number of these show haematological signs of B_{12} deficiency. Findings consistent with overseas studies.
Survey data	Davis et al (1975)	Cross-sectional survey of 293 Western Australian Aborigines. No information given on sampling frame. No participant had a plasma B_{12} less than 150 pmol/L. No evidence of low plasma B_{12} among Australian Aborigines living in Western Australia.
Survey data	De Jong et al (2003)	Representative cross-sectional study (46% response rate) of 103 elderly women aged 70–80 years living in Dunedin, New Zealand. Using a cut-off point of 150 pmol/L, 13% of women had suboptimal plasma B_{12} concentrations. No participant had megaloblastic anaemia. Consistent with overseas studies, this study shows a high rate of suboptimal B_{12} status among a representative group of elderly women living in a NZ community.
Survey data	Hanger et al (1991)	Cross-sectional survey of 257 elderly subjects (>65 years) randomly selected from the patient roll of a Christchurch medical centre. Using a cut-off point of 114 pmol/L, 7.3% of the group had sub-optimal serum B_{12} concentrations. Only one patient had an elevated MCV. Study suggests that even using a conservative cut-off point for serum B_{12} (114 vs 150 pmol/L) a high percentage of this healthy group of Christchurch seniors had suboptimal vitamin B_{12} concentrations. Findings consistent with overseas studies.
Survey data	Hokin & Butler(1999)	As part of cross-sectional surveys conducted in 1992, 1994, and 1997, the serum B_{12} status of 245 lacto-ovo-vegetarian or vegan and 53 non-vegetarian predominately male Australian Seventh-day Adventist ministers (mean age 46 years) who did not take supplements was assessed. Using a cut-off of 150 pmol/L, 30% of lacto-ovo-vegetarian or vegan and 0% of non-vegetarian participants had suboptimal plasma B_{12} concentrations. Dual-isotope Schillings test results in 36 lacto-ovo-vegetarians with abnormally low B_{12} concentrations indicated that dietary deficiency was the cause in 70% of cases. Indicates a high prevalence of suboptimal B_{12} status amongst Australian vegetarians. Indicates that inadequate intake is responsible for most of the low B_{12} concentrations observed.

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FOLATE

The recommendations for folate were derived from an assessment of the evidence base used by the US:Canadian Government DRI review of 2000 (FNB:IOM 1998) and consideration of additional key papers missing from that review or published since it was released and current recommendations of other key countries and organisations such as the FAO:WHO.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The US:Canadian DRI recommendations were adopted for use in Australia and New Zealand for all ages and genders. Although there have been many publications relating to folate since publication of the US:Canadian recommendations (FNB:IOM 1998), they do not provide information that would support altering the recommendations. The key new papers are included in the evidence table.

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in FNB:IOM (998).

Search terms: folate, folic acid, nutrition, recommended dietary intake, estimated average requirement, upper intake limit.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the folate requirement of infants		
Human experimental data	Asfour et al (1977)	Intake of 4.3 µg folate/kg required in infants to prevent folate deficiency. 3 dose groups, each with 4 infants.
Human experimental data	Ek & Magnus (1982)	Formula milk containing 78 µg folate/L prevents folate deficiency in infants. Folate intake per day not provided.
Human experimental data	Salmenpera et al (1986)	35 µg folate/L in formula milk inadequate to maintain folate status in infants compared to breast milk.
Human experimental data	Smith et al (1983)	Breast milk containing 45–50 µg folate/L adequate for prevention of folate deficiency in infants.
Human experimental data	Smith et al (1985)	Breast or formula milk with ≥85 µg folate/L sufficient to prevent folate deficiency in infants.
Papers used to assess the folate requirements of non-pregnant adults		
Level II	Melse-Boonstra et al (2004)	Parallel RCT over 12 weeks. Shows that one of the dietary forms of folate (heptaglutamyl folic acid) has a bioavailability of 64–68% based on plasma folate and red cell folate and a bioefficacy of 106% based on reduction of plasma homocysteine. These results suggest that the polyglutamyl chain of folates reduces their bioavailability by about 35%, however given enough folate intake, a maximal reduction in homocysteine can be achieved (n=60 per group).
Level II	Tucker et al (2004)	Double blind RCT. Shows that daily folic acid intake together with B_{12} and B_6 at RDA levels in supplemented cereal decreased homocysteine in healthy 50–85 year-olds from 7.9 to 7.5 µmol/L (n=93 per group).

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	van Oort et al (2003)	Parallel RCT over 12 weeks. Shows that the minimum folic acid supplementation required for 90% optimal reduction in plasma homocysteine in healthy adults aged 50–75 years was 400 µg/day. Investigated doses of folic acid of 50–800 µg/day. Dietary intake of folate for the participants not provided (n=38–52 per group).
Level II	Venn et al (2002)	RCT over 4 weeks, healthy volunteers of 50–70 years. Increasing dietary folate from 263 µg/day to 618 µg/day significantly increased serum folate by 37% and decreased homocysteine from 12 µmol/L to 11 µmol/L over a 4-week period (n=14–20).
Level II	Venn et al (2003)	RCT over 24 weeks. Healthy subjects aged 40–60 years. Supplementation with either 100 µg folic acid/day or 100 µg L-5-methyltetrahydrofolate (MTHF)/day resulted in significant increments in plasma folate (52% and 34%, respectively) and red cell folate (31% and 23%, respectively) and a significant reduction in plasma homocysteine (–9.3% and –14.6%, respectively). MTHF was significantly more effective than folic acid in reducing plasma homocysteine (n=50–53 per group).
Level III-2	Rimm et al (1998)	Prospective cohort study within the Nurses' Health Study. Cohort size of 80,000 and number of CHD cases 939. Shows that those women with folate intakes in the top quintile (median 696 µg folate/day) had 31% reduction in risk of developing CHD compared to those in the bottom quintile (158 µg folate/day). The strongest effect was a reduction in risk of 73% in those women who consumed more than 1 alcoholic drink per day.
Level III-2	Zhang et al (1999)	Prospective cohort study within the Nurses' Health Study. Cohort size of 122,000 and number of breast cancer cases was 3,483. Results indicate that folate intake at levels greater than 300 µg/day is associated with a 25% reduction in breast cancer risk in those women who consume at least 15 g of alcohol per day.
Human experimental data	Jacob et al (1994)	Metabolic study, 10 men, 33–46 years, depletion-repletion. 100 µg folate/day was inadequate to normalise homocysteine after folate depletion period.
Human experimental data	Milne et al (1983)	Metabolic study, 40 men, 19–54 years. 200 µg folate/day resulted in decrease in haematocrit and blood folate over 2–8 months.
Human experimental data	O'Keefe et al (1995)	Metabolic study, 17 women, 21–27 years, dose-response 200, 300 and 400 µg folate/day. Homocysteine was minimised at 400 µg/day and 200 µg/day was inadequate to maintain folate stores in all subjects.
Human experimental data	Sauberlich et al (1987)	Metabolic depletion-repletion study, 10 non-pregnant women, 21–40 years. 300 µg dietary folate/day required to maintain folate stores in erythrocytes.
Survey data	Bates et al (1980)	Observational study, 21 elderly men and women. Mean folate intake of 135 µg/day, no clinical folate deficiency but 40% of subjects had RBC folate of <305 nmol/L.
Survey data	Garry et al (1984)	304 Caucasian men >60 years. 75% of non-supplement users had folate intakes <250 µg/day but only 3% had RBC folate <305 nmol/L.
Survey data	Jagerstaad (1977) Jagerstaad & Westesson (1979)	Observational study. 37 men and women aged 60–70 years. Folate intake estimated to be 100–150 µg/day and RBC folate 175–350 nmol/L.
Survey data	Koehler et al (1996)	44 men and women, 60–70 years. Average folate intake, 300 µg/day; RBC folate, 1.035 nmol/L; plasma homocysteine, 11.2 µmol/L.
Survey data	Ortega et al (1993)	72 men and women aged 65–89 years in Spain. Folate deficiency was prevalent with 85% <327 nmol/L folate in erythrocytes. Intake approx 214 µg/day.
Survey data	Selhub et al (1993)	1,160 men and women, 67–80 years, Framingham study. Folate intake at >350 µg/day required to normalise plasma homocysteine <13 µg/day.
Papers used to assess folate needs in pregnancy		

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Colman et al (1975)	Controlled intervention studies. 122 pregnant women with 0, 300, 500 and 1,000 µg folic acid/day. No folate depletion in supplemented subjects.
Level II	Czeizel & Dudas (1992)	RCT. 4,156 pregnant women randomised to mineral supplement or vitamin supplement containing 800 µg folic acid for 1 month before conception and during the 1st trimester. Congenital malformations significantly lower in the vitamin group. There were no NTD-affected pregnancies in the vitamin group compared to 6 cases in the mineral group.
Level II	Wald et al (1991) MRC Vitamin Study Research Group	RCT in women with previous neural tube defect (NTD)-affected pregnancy. 4,000 µg folic acid (with or without other B vitamins) versus placebo 1 month before conception and 1st trimester. Shows 72% reduction in risk.
Level II	Willoughby & Jewel (1966)	350 pregnant women randomised to 0, 100, 300, 400 µg folic acid/day. The folic acid supplement prevented deficiency in 72%, 84% and 94%, respectively.
Level III-1	Lowenstein et al (1966)	Controlled intervention study, 311 pregnant women. Supplementation with 500 µg folic acid/day prevented erythrocyte folate deficiency in 10–20% compared to 40–60% of controls.
Level III-2	Bower & Stanley (1989)	Case-control study, 77 NTD-affected cases and 154 controls. Effect of folate intake and multivitamin use before and during early pregnancy. 75% reduction in NTD-affected pregnancy risk in top quartile of folate intake versus lowest quartile.
Level III-2	Kirke et al (1992)	Randomised trial, 354 women with previous NTD-affected pregnancy treated with 360 µg folic acid (with or without multivitamins) or placebo 2 months before conception and 1st trimester. Trial prematurely terminated after 1st NTD birth which occurred in placebo group.
Level III-2	Milunsky et al (1992)	Prospective cohort, 22,000 women completing pregnancy. RR of NTD-affected pregnancy in women who used folic acid-containing multivitamins during the first 6 weeks of pregnancy was 0.27 compared with never users.
Level III-2	Mulinare et al (1988)	Case-control study. 347 NTD-affected cases and 2,829 controls. Effect of multivitamin use containing 0–800 µg folic acid 1month before and during 1st trimester. Shows 60% reduction in NTD risk.
Level III-2	Smithells et al (1983)	Non-randomised controlled multicentre trial. 454 pregnant women with prior NTD-affected pregnancy who were either supplemented with 360 µg folic acid plus multivitamins or 519 pregnant women who were not supplemented from 1 month before conception through to 1st trimester. Recurrence rate was 0.5% in treated group compared to 4.7% in control group.
Level III-2	Thomson et al (2001)	Epidemiological case-control study. Shows for the first time that supplementation with folate during pregnancy reduces the risk of acute lymphocytic leukaemia in the child by 60%.
Level III-2	Vergel et al (1990)	Controlled intervention in 81 women with previous NTD-affected pregnancy given 5,000 µg folic acid compared to 114 women who became pregnant without folic acid supplements. No NTD in treatment group and 4 NTD in control group.
Level III-2	Werler et al (1993)	Mothers of 436 cases with NTDs and 2,615 controls with other major malformations. For daily use of multivitamins containing folic acid in the periconceptional period (28 days before through 28 days after the last menstrual period), the RR was 0.4, 95%CI: 0.2, 0.6.
Human experimental data	Boddie et al (2000)	Study, using stable isotope technique. Suggested a significantly reduced capacity to absorb monoglutamyl and polyglutamyl folate in women with a history of NTD vs unaffected controls. Results suggest that women with a history of NTD may have a higher requirement for folate than normal.
Survey data	Dawson (1966)	20 pregnant women. 150 µg folic acid supplement did not prevent low serum folate in 40%.
Papers used to assess whether folate supplementation in pregnancy has any harmful effects		
Level III-2	Zhu et al (2003)	Study in China of 126,000 women who took 400 µg folic acid/day before and during early pregnancy and 114,000 women who did not supplement during pregnancy. Shows that there was no effect of folic acid on the rate of multiple births.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Picciano et al (2004)	31 young women were fed low-folate diets on a 4-day rotation with or without cow milk for 8 week. The inclusion of cow milk in the diet enhanced the bioavailability of food folate as assessed by the changes in erythrocyte folate and plasma total homocysteine concentrations but not in plasma folate concentrations.
Survey data	Gindler et al (2001)	Study in China of more than 20,000 pregnant women who consumed 400 µg folic acid/day during early pregnancy. No evidence of an increased risk for miscarriage relative to women who did not take the supplement (n=1,820).

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PANTOTHENIC ACID

The recommendations for pantothenic acid were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs were set for all age and gender groups, rather than EARs and RDIs. The AIs were based on breast milk concentrations for infants aged 0–6 months and extrapolated on a metabolic body weight basis for infants of 7–12 months, and on median population intakes for Australia and New Zealand for all other ages (Baghurst & Record 2004, LINZ 1992). Additional needs for pregnancy and lactation were estimated from the mother's requirement plus those for gain in body weight, and breast milk, respectively.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The AIs vary from those of the US:Canadian DRI review as they are based on median intakes in Australia and New Zealand for each age group. (The adult 19–50 year US:Canadian AIs were based on several small studies of dietary intake in US adults and adolescents. With the exception of infants, AIs for other age groups were extrapolated from the AIs for adults on a metabolic body weight ratio basis).

EVIDENCE BASE

Databases: Medline plus cross-referencing and review of key references in FNB:IOM (1998), used to set values.

Search terms: Pantothenate, pantothenic acid, Australia or New Zealand, diet survey, nutrient intake.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the pantothenic acid requirements in adults		
Level III-3	Fox et al (1961)	8 women (18–24 years) consumed their normal diets for 5 days. Mean intake of pantothenate derived from diet records was 3.4–10.3 mg/day. They were then put on defined diets containing 2.8 mg pantothenate/day. 4 women were then put on 7.8 mg pantothenate/day for 10 days, 12.8 mg pantothenate/day for another 10 days. The other 4 were put on 12.8 mg pantothenate/day for 10 days, then 7.8 mg pantothenate/day for another 10 days. The mean daily urinary pantothenate excretion was 3.2, 4.5, and 5.6 mg on 2.8, 7.8, and 12.8 mg pantothenate/day, respectively. From the regression equation given relating intake to urinary excretion, a pantothenate intake of approximately 4 mg/day would result in a similar urinary excretion of pantothenate. Used as supporting evidence for dietary intake data that 5 mg is adequate.
Papers used to assess the dietary intake of pantothenic acid		
Survey data	Baghurst & Record (2004)	Pantothenate intakes for the National Nutrition Survey of 1995, 1996. Shows a median intake of 3.5 mg in 2–3 year-olds, 3.9 mg in 4–8 year-olds, 5.0 mg in 9–13 year-old males and 4.1 mg in females, 6.1 mg for 14–18 year-old boys and 4.3 mg in girls, 6.3 in 19–30 year-old men and 4.3 mg in women, 5.6 mg in 31–49 year-old men and 4.2 mg in women, 4.9 mg in 50–69 year-old men and 4.1 mg in women, 4.7 mg in men over 70 years and 3.9 mg in women.
Survey data	LINZ (1992)	Shows median intakes of 5.8 mg in 15–18 year-old boys and 3.7 mg in girls, 5.4 mg in 19–24 year-old men and 3.7 mg in women, 5.5 mg in 25–44 year-old men and 3.6 mg in women, 4.9 mg in 45–64 year-old men and 3.5 mg in women, 4.1 mg in men over 65 years and 3.3 mg in women.

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BIOTIN

The recommendations for biotin were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As data were limited, no EARs and RDIs were set. The AI for younger infants was based on breast milk concentrations together with an estimate of the mean volume of breast milk. As there are limited experimental or intake data for older infants, children, adolescents and adults, the AIs for these groups were extrapolated from those of younger infants on a metabolic body weight basis (FNB:IOM 1998), taking into consideration population intake data from New Zealand for people over 15 years of age (LINZ 1992). The AIs for pregnancy were based on those for non-pregnant women with an additional allowance for foetal and placental tissue. In lactation, an additional allowance was made for the biotin secreted in milk.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 1998) for use in the US and Canada were adopted for infants and younger children. Gender-specific AIs for ages 14 years and upward were estimated based on the standard body weights with reference to population intake data from New Zealand. The US:Canadian recommendations used one estimate for both males and females throughout.

In pregnancy, an additional allowance was made for all three age groups based on additional foetal and placental tissues. The US:Canadian review did this only for the adolescent age group. The increases recommended in lactation are the same as those of the US:Canadian report.

EVIDENCE BASE

Databases: PubMed, Medline, Current Contents and search of cross-references and references in FNB:IOM (1998).

Search terms: Biotin, nutrition, estimated average requirement, recommended dietary intake, upper intake limit, adequate intake, toxicity.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the intake of biotin needed for infants		
Survey data	Hirano et al (1992) Paul & Southgate (1978) Salmentera et al (1985)	Key survey data used for AI's. Longitudinal variations in biotin in human milk. Average level is 6µg/L.
Papers used to assess the intake of biotin needed in pregnancy		
Level II	Mock et al (2002a)	Increased excretion of 3-hydroxyisovaleric acid occurs frequently in pregnancy and reflects reduced biotin status. This raises concern about potential teratogenicity.
Level II	Mock et al (2002b)	Validation of 3-hydroxyisovaleric acid as an early biomarker of biotin deficiency.
Level IV	Mock & Stadler (1997)	Evidence that biotin status decreases during pregnancy.
Expert opinion	Zempleni & Mock (2000)	Review of evidence that marginal biotin deficiency is teratogenic.
Papers used to assess adverse effects at high doses of biotin		

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Zempleni et al (2001)	Evidence that pharmacological doses of biotin decrease *ex vivo* mitogen-induced lymphocyte proliferation and cytokine production.

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CHOLINE

The recommendations for choline were derived after consideration of the FNB:IOM (1998) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. Several papers relating to choline have been published since the FNB:IOM review, but none provide data that would substantially alter the conclusions from that review (Zeisel et al 2003, Hongu & Sachan 2003, Allard 2002, Niculescu & Zeisel 2002, Compher et al 2002). As there are limited data, AIs were set for all age and gender groups rather than EARs and RDIs. The AIs were based on breast milk concentrations for solely breast-fed infants aged 0–6 months and for all other age groups on experimental data from studies in adult males extrapolated on a body weight basis, including a factor for growth. Additional needs for pregnancy and lactation were estimated from the mother's requirement plus needs for the foetus and products of pregnancy and breast milk, respectively.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The only variation is the lower recommendation for 14–18 year-old pregnant and lactating mothers, for which the degree of rounding up in the estimation was to the nearest 25 mg rather than 50 mg, in line with the degree of rounding up used for the older mothers.

EVIDENCE BASE

Database: Medline, Ovid Technologies plus cross-referencing and review of key references in the review used to set values (FNB:IOM 1998).

Search terms: choline, nutrition, Australia, New Zealand, diet survey, nutrient intake, recommended dietary intake.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess what level of dietary choline causes deficiency		
Level III-1	Zeisel et al (1991)	Healthy male volunteers fed choline-deficient diet supplemented with 500 mg choline/day for 1 week. Randomly divided into two groups – 1 continued to receive choline and the other received none for next 3 weeks. In week 5, all subjects received choline. In choline-deficient group, plasma choline and phosphatidylcholine concentrations decreased 30% during the 3-week period of a choline-deficient diet and plasma and erythrocyte phosphatidylcholine decreased 15%. No such changes in the control group. In the choline-deficient group, serum alanine aminotransferase activity increased from a mean of 0.42 mukat/L to a mean of 0.62 mukat/L during the 3-week period of the choline-deficient diet. There was no change in the control group. Tests of liver and renal function unchanged in both groups. Serum cholesterol decreased 15% in the deficient group and did not change in the control group.
Papers used to assess potential cognitive benefits of choline		
Level I	Fioravanti & Yanagi (2004)	Cochrane Review: some evidence that CDP choline has positive effect on memory and behaviour, at least in the short term. Evidence of benefit from global impression is stronger, but still limited by the duration of the studies. Some evidence that the effect of treatment is more homogeneous for patients with cognitive impairment secondary to cerebrovascular disorder.
Expert review	McDaniel et al (2003)	For most of the 'brain-specific' nutrients phosphatidylserine, phosphatidylcholine, citicoline, piracetam, vinpocetine, acetyl-L-carnitine and antioxidants, particularly vitamin E, some mildly suggestive effects found in preliminary controlled studies using standard psychometric memory assessments or more general tests designed to reveal cognitive impairment.
Papers used to assess adverse effects at high doses of choline		

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Lawrence et al (1980)	Double blind trial with control. 14 patients with a variety of ataxic disorders given choline chloride at 0.2 g/day for 3 weeks and 9 g/day for 3 weeks. At 150 mg/kg body wt, 5/14 had a fishy odour and 12/15 had nausea and diarrhoea.
Level III-3	Growdon et al (1977a)	Double-blind crossover trial. 20 patients with stable buccal-lingual-masticatory movements. Oral choline at 9 g/day in week 1and 12 g/day in week 2. Mild cholinergic toxicology of blurred vision, anorexia and diarrhoea.
Level IV	Boyd et al (1977)	Non-blind pilot study in 7 patients treated for dementia. Adverse symptoms above 7.5 g choline/day.
Level IV	Gelenberg et al 1979)	Non-blind trial in 5 men with mild to severe tardive dyskinesia. 8–9 g/day as choline chloride for 6–8 weeks. Fishy odour and gastrointestinal irritation in 5/5.
Level IV	Growden et al (1977b)	10 patients with Huntington's disease took choline orally (8–20 g/day) over 2–17 weeks. Fishy body odour in 10/10. At 250–300 mg/kg b wt, lacrimation, anorexia, vomiting and diarrhoea.

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VITAMIN C

The recommendations for vitamin C were derived after consideration of the FNB:IOM (2000) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 2000) for use in the US and Canada were not adopted as they were based on the antioxidant potential of vitamin C using measures of neutrophil ascorbate concentration, rather than prevention of deficiency disease. For consistency, the recommendations here are based on the intakes thought to protect against scurvy with a safety margin in recognition of the limited data available across the various age bands. This approach is consistent with that of FAO:WHO (2002) and the United Kingdom (COMA 1991) as well as previous recommendations for Australia and New Zealand (NHMRC 1991).

EVIDENCE BASE

Databases: Medline, PubMed plus cross-referencing and review of key references in FNB:IOM (2002), German Society review (2002), Expert Group on Vitamins and Minerals (2003) and FAO:WHO (2002) which were used to set the values.

Search terms: vitamin C, ascorbic acid, ascorbate, human.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the vitamin C requirement in infancy		
Survey data Case reports	Goldsmith (1961) Rajalaksmi et al (1965) van Eekelen (1953)	Clinical signs of scurvy occur at intakes of ≤7–8 mg/day.
Survey data	Bates et al (1982) Byerley and Kirsey (1985) Karra et al (1986) Salmenpera (1984) Sneed et al (1981) Thomas et al (1979,1980) Udipi et al (1985) WHO (1998)	Human breast milk contains 34–120 mg vitamin C/L.
Papers used to assess the vitamin C requirement in adults		
Human experimental data	Baker et al (1969)	Study of experimental scurvy. Ascorbate catabolism in adult males is 2.9%/day, SD = 0.6.
Human experimental data	Baker et al (1969,1971) Kallner et al (1979)	Turnover studies with labelled ascorbic acid show that whole body content of vitamin C is 20 mg/kg or 1,500 mg.

Level of evidence	Reference	Study type, issues addressed and key findings
Human experimental data	Kallner et al (1979)	Shows 70–90% of usual intake is absorbed, falling to ≤50% with increasing doses above 1 g/day.
Human experimental data	Newton et al (1983)	Study in elderly women of intake vs plasma concentrations showing sigmoidal relationship.
Survey data Case reports	Baker et al (1969)	Clinical signs of scurvy occur at intakes below 10 mg/day in men.
Survey data Case reports	Goldsmith (1961) Rajalakshmi et al (1965) van Eekelen (1953)	Clinical signs of scurvy occur at intakes of ≤7–8 mg/day. Basis of extra need in pregnancy.
Paper used to assess the effects of smoking on vitamin C requirements		
Level III-3	Kallner et al (1981)	Time course of radioactivity in plasma and urine after oral administration of a single dose of (1-^{14}C) ascorbic acid. Healthy male volunteers smoking more than 20 cigarettes per day. 40% higher intake required in smokers to reach equivalent steady state and body pools.
Level III-2	Lykkersfeldt et al (2000)	Double-blind, placebo-controlled design with 272 mg vitamin C, 31 mg all-rac-α-tocopheryl acetate and 400 µg folic acid in a population of smokers and non-smokers. Only ascorbic acid was significantly depleted by smoking per se ($p < 0.01$). After the 3-month supplementation period, ascorbic acid was efficiently repleted in smokers ($P < 0.001$).
Papers used to assess adverse effects at high doses of vitamin C		
Level II	Gokce et al (1999)	Randomised double-blind trial, placebo controlled. 21 CHD patients; single supplementary dose of 2,000 mg vitamin C followed by 30 days on 500 mg/day. No reported adverse effects in treatment group but not clear whether this was specifically addressed.
Level III-2	Mai et al (1990)	20 patients with multiple sclerosis randomised to receive supplements of 2,000 mg vitamin C, 6 mg sodium selenite and 480 mg vitamin E, or placebo, for 5 weeks. No increase in symptoms over placebo.
Level III-2	Morton et al (2001)	Retrospective cohort study of 994 women including 227 regular users of vitamin C supplements of 100–5,000 mg/day, mean 745 mg/day. Average duration of use 12.4 years. No side effects reported but not specifically measured.
Level III-3	Urivetsky et al (1992)	15 patients with renal stones given 100, 500, 1,000 or 2,000 mg supplements to assess effects on urine oxalate. Increase was significant above 500 mg.
Level III-3	Wandzilak et al (1994)	15 healthy volunteers given 1,000, 5,000 or 10,000 mg for 5 days, each separated by 5 days with no supplements. Dose-dependent increase in urinary oxalate, but clear trend not apparent when non-enzymic ascorbate to oxalate conversion taken into account. Some diarrhoea at 10,000 mg. Study not blind.
Level IV	Auer et al (1998)	10 healthy males given 4,000 mg/day for 5 days. No significant increase in oxalate excretion.
Level IV	Cameron & Campbell (1974)	Stepped study. Healthy volunteers received increasing doses of vitamin C by 1,000 mg steps. Frequent gut effects at 3,000–4,000 mg/day.
Level IV	Cook et al (1984)	17 adults given 2,000 mg vitamin C with meals for 16 weeks. Assessment of dietary iron absorption and assimilation on body stores. 9 continued for 2 years. No subjective side effects.
Human experimental data	Levine (1996, 1999)	7 healthy subjects. Measured steady state plasma ascorbate, urinary ascorbate and urinary oxalate. At 1,000 mg vitamin C/day, there were statistically significant increases in urinary oxalate (in the normal range) and uric acid excretion.
Single case report	McLaran et al (1982)	Describes rapidly fatal cardiomyopathy in a young man; ingested large amounts of ascorbic acid for 12 months and was admitted with severe heart failure; died after eight days. Idiopathic haemochromatosis was diagnosed at autopsy.

Level of evidence	Reference	Study type, issues addressed and key findings
Expert review	George & Powell (1997)	Haemochromatosis is common, occurring in approximately 1 in 300 people in Caucasian populations, and untreated can cause serious morbidity and early death.

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VITAMIN D

The recommendations for vitamin D were derived after consideration of the FNB:IOM (1997) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. An evidence-based review of several international recommendations was also available to the committee (Flight & Baghurst 2003).

AIs were set for adults and infants assuming little exposure to sunlight in the belief that the best indicator of adequacy currently available is the intake level that allows an individual to maintain normal serum 25(OH)D levels. For older people, effects on maintenance of bone and effects on fracture rates were also considered. The adult recommendations were adopted for children and adolescents as there were little available data. Some additional recommendations are made for high risk groups such as the elderly institutionalised and dark-skinned, veiled women whose exposure to sunlight may be minimal. The recommendations of the US:Canadian DRIs (FNB:IOM 1997) were adopted with the exceptions described below.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. HIGH RISK GROUPS

A special note has been added that it may be necessary to consider supplementation for groups such as the elderly institutionalised or dark-skinned, veiled women, especially during and after pregnancy.

B. UPPER INTAKE LIMIT

Since publication of the US:Canadian DRIs, two studies have shown that intakes above that recommended in the US:Canadian review are apparently safe. To this end, the NOAEL for adults was set at 100 µg/day with a UF of 1.2 to account for variability in findings and the small numbers in studies. This gave a UL of 80 µg/day which was also used for children and adolescents and in pregnancy and lactation.

EVIDENCE BASE

Databases: Australasian Medical Index, Pub Med, cross-referencing and review of key references in FNB:IOM (1997), used to set the values.

Search terms: vitamin D, humans, deficiency.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the intake levels of vitamin D that infants need to maintain serum 25(OH)D and/or prevent rickets		
Level II	Greer et al (1982)	18, 9 breast-fed infants received 400 IU/day. Placebo group (n=9) had a significant decrease in bone mineralisation and in serum 25(OH)D concentrations compared to the vitamin D-supplemented group. Longer term follow-up.
Level III-1	Specker et al (1992)	Vitamin D supplementation (100, 200 or 400 IU/day) during the first 6 months of life. None of the infants had rickets at 6 months of age. Because of the low serum 25(OH)D concentrations, especially among infants in the north, it may be prudent to supplement the diet with vitamin D at a dose of 400 IU/day.
Level III-3	Leung et al (1989)	150 bottle-fed infants. Shows that the vitamin D intake from fortified milk and cereals was more than half of the recommended amount throughout the first 18 months and that the serum 25(OH)D level of the infants at 18 months was normal.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Specker et al (1987)	Infant 25(OH)D concentrations correlated with sunshine exposure when mothers had low (less than 35 ng/mL) or high (greater than 35 ng/mL) serum 25(OH)D concentrations. Estimates were made to determine sunshine exposure necessary to maintain serum 25(OH)D concentrations above the lower limit of the normal range (11 ng/mL). A conservative estimate would be 30 minutes per week wearing only a diaper or 2 hours a week fully clothed without a hat.
Level III-3	Specker et al (1985)	Infants wearing only a diaper require no more than 30 minutes of exposure to sunlight per week. Exposure of face required only 2 hours per week.
Papers used to assess the effect of vitamin D on bone status in children		
Level II	Du et al (2004)	A 2-year milk intervention trial of 757 10-year-old girls, in Beijing, randomised into three groups. Over the 2-year period, those receiving additional cholecalciferol compared with those receiving milk without added 25-hydoxycholecalciferol had significantly greater increases in the size-adjusted change in total body bone mineral content (2.4% vs 1.2%) and bone mineral density (5.5% vs 3.2%).
Papers used to assess the intake of vitamin D that adults need to maintain adequate vitamin D or bone status		
Level I	Bischoff-Ferrari et al (2004)	Meta-analysis of RCTs of vitamin D in elderly populations. Examined falls of clearly defined origin. Vitamin D supplementation appears to reduce risk of falls by more than 20% among ambulatory or institutionalised older individuals with stable health.
Level II	Chapuy et al (1992)	Supplements of 1.2 g calcium and 20 µg (800 IU, 20 µg/day) vitamin D_3. Hip fractures were 43% lower and the total number of non-vertebral fractures was 32% lower.
Level II	Dawson-Hughes et al (1997)	Supplements of 500 mg calcium plus 700 IU (17.5 µg/day) vitamin D_3 moderately reduced bone loss measured in the femoral neck, spine and total body and reduced the incidence of non-vertebral fractures over the 3-year study period.
Level II	Heaney et al (2003)	Shows oral input of 12.5 µg of vitamin D/day sustains autumn levels of serum 25(OH)D in winter at northern latitude 41.2°.
Level II	Lips et al (1996)	Shows no decrease in the incidence of hip fractures and other peripheral fractures in Dutch elderly persons after vitamin D supplementation. (3.5 years of supplementation with 400 IU or 10 µg/day).
Level III-1	Dawson-Hughes et al (1997)	In men and women 65 years of age or older who are living in the community, dietary supplementation with calcium and vitamin D moderately reduced bone loss measured in the femoral neck, spine, and total body over the three-year study period and reduced the incidence of nonvertebral fractures.
Level III-1	Dawson-Hughes et al (1991)	Women on 400 IU (10 µg/day) had less bone loss in the spine, but not at the femoral neck. 17.5 µg/day required to prevent bone loss from femoral neck. At latitude 42 degrees, healthy postmenopausal women with vitamin D intakes of 100 IU daily can significantly reduce late wintertime bone loss and improve net bone density of the spine over one year by increasing their intake of vitamin D to 500 IU daily. A long-term benefit of preventing vitamin D insufficiency in the winter seems likely although it remains to be shown. Observed changes in bone as well as in fat and lean tissue appear to be related to season.
Level III-2	Flicker et al (2003)	Prospective cohort of 952 elderly in Australia. Shows link between vitamin D status and falls.
Level III-3	Holick (1994)	Experiment briefly described in a paper based on a lecture. Submariners with no access to sunlight tested with 15 µg supplement did not fully maintain vitamin D status.
Level III-3	Kinyamu et al (1997)	3 groups of women young, elderly (free living) and elderly (nursing home). Mean serum 25-hydroxyvitamin D (calcidiol) was 10.8 +/- 4.4 nmol/L in nursing home elderly, 11.3 +/- 4.2 nmol/L in the young and 11.5 +/- 3.2 nmol/L in free-living elderly. Vitamin D deficiency, defined as serum calcidiol <4.8 nmol/L, occurred in 8% of women in nursing homes, 6% of the young women, and 1.6% of the free-living elderly women. Calcitriol corrects the malabsorption of calcium.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Krall et al (1989)	Women with mean age of 58 years had elevated PTH levels during winter on 5.5 µg/day of vitamin D. There was no seasonal variations in PTH when they consumed more than 5 µg/day of vitamin D.
Level III-3	Lamberg-Allart et al (1993)	Adults 19–50 years obtain most of their vitamin D from exposure to sunlight. Intake of (3.3 µg/day) considered to keep serum 25(OH)D at acceptable levels. Serum 25(OH) D concentration was lower and PTH higher in vegetarians. Low serum 25(OH)D concentrations accompanied by high serum intact parathyroid hormone concentrations, which are also affected by a low calcium intake.
Level IV	Kimlin et al (2003)	Non-comparative longitudinal study of effects of latitude on production of cutaneous vitamin D showed that latitude effects varied. More vitamin D is produced in equivalent southern latitudes.
Cross-sectional survey study	Green et al (2004a, b)	National Survey data in New Zealand showed that 2.8% of New Zealanders 15 years or over had 25(OH)D levels indicative of deficiency and 28% had levels indicative of insufficiency. In children, 4% of 5–14 year-olds had vitamin deficiency and 31% had insufficiency.
Cross-sectional survey study	Mowe et al (1999)	2-armed study in Norway, 246 men and women. Shows physiological role for calcidiol in muscle function.
Cross-sectional survey study	Pasco et al (2001)	861 Australian women. Adequate vitamin D cannot be obtained from solar radiation alone in winter.
Cross-sectional survey study	Pasco et al (2004)	Observational, in Australia, well-defined community of 27,203 women ≥55 years old. 287 randomly selected from electoral rolls, 1,635 with incident fractures and 1,358 presenting to a university hospital with falls. A fall in 25(OH)D in winter is accompanied by increases in (1) PTH levels, (2) bone resorption, (3) the proportion of falls resulting in fracture, and (4) the frequency of hip and wrist fracture.
Papers used to assess the upper limit of intake for vitamin D		
Level II	Vieth et al (2001)	Randomised trial. 100 µg/day does not cause hypercalcaemia.
Level II	Vieth et al (2004)	Randomised trial. 100 µg/day does not cause hypercalcaemia.
Level III-2	Narang et al (1984)	Non-randomised trial. Control arm supplemented at 60 µg/day or 95 µg/day. 95 µg/day, but not 60 µg/day, caused hypercalcaemia. No independent measure of vitamin D intake.
Animal experiment	Taura et al (1979)	Coronary atherosclerosis developed in normolipemic swine fed a basal ration supplemented with 125,000 IU, 62,500 IU and 31,250 IU of vitamin D3/kg of diet for 3 months and subsequently only the basal ration for the following 3 months. The incidence of atherosclerotic lesions was proportional to the vitamin D3 doses and had many features resembling those in coronary arteries from humans.
Animal ecxperiment	Toda et al (1985)	Ultrastructural studies conducted on the coronary arteries of 6 week-old piglets from sows which received diets containing either 25 micrograms or 3.7 micrograms of vitamin D3 per pound of basal ration. Results suggested that an excess dietary intake of vitamin D by pregnant animals may have potential angiotoxic effects on the coronary arteries of their offsprings.

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VITAMIN E

The recommendations for vitamin E were derived after consideration of the FNB:IOM (2000) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

There were three areas of variance.

A. THE STRENGTH OF THE EVIDENCE BASE FOR SETTING AN EAR

The US:Canadian DRI report (FNB:IOM 2000) recommended an EAR (and thus an RDA) based on the experimental work of Horwitt in adult males (Horwitt et al 1960, 1963). However, the Australian:New Zealand NRV Committee did not feel that the available experimental evidence was sufficiently clear in terms of dose-response for use as the basis of an EAR. An AI has therefore been adopted for Australia and New Zealand, based on median intakes of the two populations.

The DRI 2000 committee relied on the *in vitro* erythrocyte haemolysis test as the indicator of vitamin E requirement. They interpreted the results of Horwitt et al (Horwitt 1960, 1962, Horwitt et al 1963) as indicating that a plasma tocopherol concentration higher than 12 µmol/L was required to prevent more than 12% erythrocyte haemolysis in 50% of participants (n=6). The amount of vitamin E needed to return serum tocopherol concentrations in depleted individuals (n=5) to 12 µmol/L was used as the criterion to define vitamin E adequacy. Twelve mg of RRR αtocopherol was required to achieve the effect (Horwitt 1960). The CV was set at 10% of the EAR, leading to an RDA of 15 mg RRR αtocopherol (EAR plus twice the CV).

The only other relevant study was conducted in children with cystic fibrosis. Some degree of erythrocyte haemolysis occurred when serum tocopherol concentrations were below 9 µmol/L (Farrell et al 1977). As noted in the main Chapter on vitamin E, there is some doubt about the relevance of erythrocyte haemolysis for setting vitamin E requirements. Even if its validity was accepted, there is a significant gap in the dose-response data that makes it difficult to assess adequacy with any degree of certainty.

Fewer than 5% of the participants in the US NHANES III met the new RDA for vitamin E and fewer than 25% when supplement use was included. Notwithstanding the low percentage of the US population meeting the EAR or RDA for vitamin E, more than 99% of the NHANES III participants had serum tocopherol concentrations above 12 µmol/L – the concentration above which the 2000 committee considered vitamin E status to be adequate. Thus, there is a discrepancy between the dietary intake results that indicate a high prevalence of low vitamin E intake in the US and the serum results that indicate good vitamin E status in the population. The 2000 committee argued that energy and fat intake were underreported in the NHANES surveys. As a result, vitamin E intakes, which are associated with fat intake, were also underestimated (Briefel et al 1997, Mertz et al 1991). The committee provided little evidence to quantify the extent to which underreporting of fat intake leads to an underestimation of vitamin E intake.

An analysis of vitamin E intakes in Australia and New Zealand (ABS 1998, MOH 1999) shows them to be very similar to those reported in the US (NHANES III). Therefore, adoption of the US EAR and RDA (as EAR and RDI) would have implied that the majority of Australians and New Zealanders do not ingest enough αtocopherol. It would be difficult for New Zealanders and Australians to meet the US RDA without taking supplements, assuming that vitamin E intakes are not greatly underestimated.

The authors of the key papers on which the US:Canadian EAR was set, and others, have said the following about the erythrocyte haemolysis test:

"*To date, no one has proved that the test in question is anything more than an* in vitro *assay of the antioxidant titer of the red blood cell, a test which could possibly have no direct relationship to the stability of the erythrocyte* in vivo." (Horwitt et al 1956)

"Much of the present information on human requirements for tocopherol rests on interpretations of the peroxide hemolysis test. I have never considered this test to be more than an indication of the rate at which erythrocyte fatty acids can be oxidized." (Horwitt 1960)

"the concentration of the hydrogen peroxide, the rate at which it is added to the erythrocyte suspension, the temperature, and the elapsed time of incubation all can have large effects on the hemolysis obtained… one can easily choose conditions at which all normal blood would hemolyze or, conversely, where no deficient blood would hemolyze." (Bieri 2002)

B. THE VALIDITY OF CONSIDERING ONLY αTOCOPHEROL AS CONTRIBUTING TO VITAMIN E ACTIVITY

The US:Canadian DRI committee decided to limit recommendations for vitamin E to αtocopherol only. The Australian:New Zealand NRV Committee felt that αtocopherol equivalents should continue to be used to set nutrient reference values for vitamin E, because it is premature to state that gamma (γ)-tocopherol, the other major tocopherol in foods, has no biological activity. The US:Canadian committee excluded αtocopherol in setting DRIs because a specific binding protein that maintains serum concentrations has not been identified. However, little is known about the exact biological functions of αtocopherol, αtocopherol or other forms of vitamin E. Gamma-tocopherol is a commonly consumed component of the human diet – almost 70% of vitamin E in the US diet (McLaughlin & Weihrauch 1979) – and changes readily with dietary intake (Lemcke-Norojarvi et al 2001, Liu et al 2003). All forms of naturally occurring vitamin E appear to be equally well absorbed and incorporated into chylomicrons. Plasma αtocopherol concentrations are influenced by dietary intake and range from 5–20% of αtocopherol concentrations despite the absence of a specific transport protein for αtocopherol. Moreover, there is evidence that αtocopherol is not inert, but has biological effects or is associated with disease risk in humans (Huang et al 2003, Jiang & Ames 2003, Jiang et al 2001, Liu et al 2003, White et al 2001).

C. UPPER LIMIT OF INTAKE

The emphasis of the US:Canadian DRI decision was on animal experiments. The Australian:New Zealand NRV committee decided that there was sufficient human experimental evidence, supported by clinical trial evidence, to set a UL based on the studies of Meydani et al (1998), in line with the approach of the European Commission (European Commission Scientific Committee on Food 2003).

EVIDENCE BASE

Databases: Medline plus cross-referencing and review of key references in the review used to set values (FNB:IOM 2000) and other reports from key health authorities.

Search terms: vitamin E or tocopherol or tocotrienol and one of the following: absorption or bioavailability; deficiency; pentane or ethane and breath or respire; human and milk; isoprostane; safety; erythrocyte or "red blood cell" and lysis or "hydrogen peroxide" or fragility or hemolysis; Australia or New Zealand; systematic review or meta-analysis; clinical trial or randomised controlled trial or follow-up study or cohort study or case-control study or dietary intervention or diet therapy or supplement; trial or supplement; study or dietary supplement; or diet supplement; or prospective study or prospective trial or diet; therapy or diet; treatment and one of the following: heart disease or cardiac death or ischaemic heart disease or cardiovascular disease or coronary heart disease or arrhythmia or stroke or cerebrovascular disease or atherosclerosis or angina or occlusive vascular disease or coronary angioplasty or thrombosis or heart attack or myocardial infarction or myocardial disease; diabetes; cancer; cataract.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess what levels of plasma αtocopherol are associated with erythrocyte haemolysis		
Level III-2	Horwitt et al (1956,1963) Horwitt (1960,1962, 1974)	All papers by Horwitt are based on the results of 1 vitamin E depletion-repletion study in human participants. Briefly, one group of 19 participants was fed a basal diet containing 3–4 mg tocopherol/day. Another group (n=9) received the same diet plus a supplement of 15 mg RRR-a-tocopheryl acetate/day, and a third group (n=10) was fed the regular hospital diet. The vitamin E-deficient diet was consumed for 54 months, after which individuals were given a different dose of vitamin E supplements. A new polyunsaturated fat content of the basal diet was adopted at several times during the study. Serum tocopherol, lipids (cholesterol, triacylglycerol), in vitro erythrocyte haemolysis and in vivo survival, erythrocyte fatty acid composition of erythrocytes and adipose tissue were measured.
Level IV	Farrell et al (1977)	Evidence that children with cystic fibrosis who had serum tocopherol concentrations of less than 9 µmol/L had increased erythrocyte haemolysis scores and reduced in vivo erythrocyte survival. Vitamin E supplementation (n=6) corrected erythrocyte survival time. Results for changes in erythrocyte haemolysis were not reported.
Papers used to assess if dietary αtocopherol has a physiological function and a role in health		
Level III-1	Lui et al (2003)	Results indicate that αtocopherol play a role in protein kinase C activation, nitric oxide release in platelets and endothelial cell nitric oxide synthase activation.
Level III-2	Huang et al (2003)	Cohort study. Results suggest potential chemoprotective effects of αtocopherol on prostate cancer risk.
Level III-2	Lemcke-Norojarvi et al (2001)	Results indicate that consuming foods rich in αtocopherol can increase serum αtocopherol concentrations.
Review of evidence	Jiang et al (2001)	Review of evidence that αtocopherol may contribute to human health.
Survey	White et al (2001)	Evidence from 1,047 females that dietary intake of αtocopherol affects serum αtocopherol concentrations.
Animal study	Jiang and Ames (2003)	Findings provide strong evidence that αtocopherol shows anti-inflammatory activities *in vivo* that may be relevant to biological function and disease prevention in humans.
Papers used to assess what level of vitamin E causes toxicity		
Level II	GISSI-Prevenzione Investigators (1999)	11,324 patients who had survived recent MI were randomly assigned supplements of n-3 PUFA (1 g daily), or 300 mg vitamin E (as synthetic αtocopherol) or both or neither for 3.5 years. The primary combined efficacy endpoint was death, non-fatal MI and stroke. Smokers and ex-smokers were evenly distributed through the groups. Vitamin E treatment had no effect on the combined or separate endpoints.
Level II	Heart Protection Study Collaborative Group (2002)	A randomised placebo-controlled trial of antioxidant vitamin supplementation. 20,536 high risk adults (ie with CHD, other occlusive vascular disease or diabetes) were given a daily supplement of 600 mg dl-a-tocopherol (equivalent to 660 IU), 250 mg vitamin C and 20 mg αcarotene or placebo for 5 years. No difference in all cause mortality was revealed. Compliance was 83% on average in each treatment group, adverse experiences were sought at each follow up visit (every 4 months for the first year and every 6 months thereafter) and no significant side effects were reported. It is unclear whether minor side effects were reported or investigated. There was no significant difference in the number having a haemorrhagic stroke. Current and ex-smokers were evenly distributed between groups.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Leppälä et al (2000)	ATBC study: The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group (1994). Randomised, double-blind placebo-controlled study. 29,133 Finnish male smokers randomly assigned to one of 4 treatment groups: 50 mg dl-αtocopherol/day (equivalent to 55 IU), 20 mg/day αcarotene, both αtocopherol and αcarotene, or placebo. Follow-up continued for 5–8 years. Lung cancer incidence was not affected by αtocopherol treatment, but the incidence of prostate cancer was reduced. αtocopherol had no apparent effect on total mortality, but was associated with an increase in mortality from haemorrhagic stroke (7.8 deaths per 10,000 person years in the αtocopherol group versus 5.2 deaths per 10,000 person years in the no αtocopherol groups). In contrast, deaths from ischaemic stroke and ischaemic heart disease (IHD) were reduced in the αtocopherol group. Subsequent analysis of the risk factors for stroke indicated that vitamin E increased the risk of sub-arachnoid haemorrhage (RR, 2.45, 95%CI: 1.08, 5.55) and decreased the risk of cerebral infarction (RR, 0.7, 95% CI: 0.55, 0.89) in hypertensive men, but had no effect on normotensive men.
Level II	Meydani et al (1998)	A double-blind placebo-controlled study of 88 healthy subjects (aged >65 years) to assess the effect of supplementation on general health. 4 groups received placebo, 60, 200 or 800 IU dl-αtocopherol/day (34, 134 or 537 mg d-αtocopherol equivalents) for four months (n=17, 19, 18 and 19 subjects, respectively). No side effects reported. Supplementation had no effect on plasma concentration of other antioxidant vitamins and minerals, glutathione peroxidase, superoxide dismutase or total cysteine. There was no significant effect of vitamin E on serum non-specific immunoglobulin concentrations or anti-DNA and anti-thyroglobulin antibodies. The cytotoxic ability of neutrophils against *Candida albicans* was not compromised. Vitamin E had no effect on body weight, plasma total proteins, albumin, glucose, plasma lipids or the lipoprotein profile, total bilirubin, serum liver enzymes, blood count, platelet number, bleeding time, haemoglobin, haematocrit, urinary or serum creatine levels. Supplementation had no detrimental effect on health.
Level II	Yusuf et al (2000)	HOPE study: A total of 9,541 subjects (6,996 men, 2,545 women) aged 55 year and over at high risk for cardiovascular events were enrolled in a trial with a 2 x 2 factorial design. The participants received either 400 IU vitamin E (from natural sources – no further details provided) or placebo and either ramipril or matching placebo for a mean of 4.5 years. The primary endpoint was a combination of MI and stroke and death from cardiovascular causes. The secondary outcomes included unstable angina, congestive heart failure, revascularisation or amputation, death from any cause, complications of diabetes and cancer. There were no significant differences in the numbers of deaths from cardiovascular causes (RR 1.05, 95% CI: 0.9–1.22) between those receiving vitamin E or placebo or in any of the secondary outcomes. There were no differences in adverse effects between the vitamin E and placebo group or in the numbers of patients who stopped taking the study medication. There was also no difference in the incidence of haemorrhagic stroke between the groups.
Level II	Primary Prevention Project (2001)	In a controlled open 2 x 2 factorial trial, 4,495 people were randomised to receive low dose aspirin (100 mg/day) or no aspirin, and vitamin E (300 mg/day as synthetic αtocopherol), or no vitamin E, to investigate the prevention of cardiovascular events in people with 1 or more major cardiovascular risk factors (hypertension, hypercholesterolaemia, diabetes, obesity or family history). The mean follow up period was 3.6 years. The main combined endpoint was the cumulative rate of cardiovascular death, non-fatal MI and non-fatal stroke. Predefined analyses included cardiovascular deaths, total deaths, and total cardiovascular events. Smokers and ex-smokers were evenly distributed through the groups. Vitamin E had no effect on any pre-specified endpoint.

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VITAMIN K

The recommendations for vitamin K were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs rather than EARs and RDIs were set for all age and gender groups. The AIs were based on breast milk concentrations for infants and on median population intake data for other groups. The data used to derive the recommendations for Australian and New Zealand NRVs for Vitamin K were based on a re-analysis of the intake data collected in the National Nutrition Survey for Australia (1995). As vitamin K data are not available for Australian foods, the nutrient data used were from the USDA food database.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The population intakes and therefore the AIs for Australia and New Zealand vary from those of the US. Results from the NHANES III survey (FNB:IOM 2001) was used to set the US AIs, but it seems to give much higher figures for vitamin K intake than other recent large-scale surveys in the US. In NHANES III, median intakes for various age bands varied from 89–117 µ/day for men (mean intakes 97–125 µg/day) and 79–88 µg/day for women (mean intakes 94–100 µg/day). This study and others from the US and UK have shown that mean intakes can be from 10–20% higher than median intakes as there is a big skew to the right in intake distribution data.

Two other large scale US studies give much lower values than the NHANES III survey. The US Food and Drug Administration Total Diet Study of nearly 3,000 subjects using diet recall and record (Booth et al 1996) gave a mean intake of only 71 µg/day for men and women aged 25–45 years, and 80 µg/day in those over 60 years. The other survey using 14-day diaries in 4,000 US men and women with a demographic profile similar to the US census also gave mean intakes of 70–80 µg/day (Booth et al 1999b).

Estimates from the UK (Thane et al 2002) also give lower figures, with median intakes of 70 µg/day for men and 61 µg/day for women over 65 years (means 84 µ/day for men, 73 µg/day for women). Mean intakes of 84 µg/day for men and 75 µg/day for women have been reported for adults in Ireland (Duggan et al 2004).

The highest adult age group median intake in Australia, as assessed by re-analysis of the National Nutrition Survey using the US database, was 66 µg/day for men, and 60 µg/day for women. Intakes were highest in the 50–69 year age group for men and in the 70+ age group for women, and lowest in the 19–30 year age group for both genders. This finding of somewhat higher intakes in older adults has been shown in several studies in western countries and probably reflects higher intakes of green leafy vegetable in these groups. There are no national data for New Zealand. Intakes of dark-green leafy vegetables, the major source of vitamin K, also appear to be somewhat lower in Australia and New Zealand than in the US.

The FAO:WHO (2001) recommends vitamin K intakes of 65 µg/day for men and 55 µg/day for women, based on experimental evidence that 1 µg/kg body weight is an adequate intake. The UK also recommended 1 µg/kg body weight as safe and adequate. The German/Austrian/Swiss Nutrition Societies recommend 70 µg/day and 60 µg/day for younger men and women on the same basis (German Nutrition Society 2002), but an additional 10 µg/day for those over 50 years as a precaution against low absorption although it is acknowledged that there is no evidence for this.

EVIDENCE BASE

As the recommendations for Vitamin K are based on median population intake, the evidence analysis below is restricted to the data relating to the toxicity of Vitamin K.

Databases: PubMed and Cochrane database plus cross-referencing and review of key references in FNB:IOM (2001).

Search terms: vitamin K, randomised controlled trial, phylloquinone, osteocalcin, Vitamin K, clinical trial, prothrombin.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the dietary intake level of vitamin K that affects prothrombin time (PT)		
Level II	Binkley et al (1999, 2000)	Prothrombin time (PT) did not change in younger and older subjects given 1 mg/day vs placebo in addition to normal diet for 2 weeks (n=219).
Level IV	Allison et al (1987)	RCT of effect of 10 different antibiotics. Depletion diet (2–5 µg/day) for 13 days, and antibiotics. PT increased within the normal range. Level IV because RCT tested differences between antibiotics, not vitamin K intake. (n=33).
Level IV	Bach et al (1996)	Describes the effect of 1 mg/day vitamin K in reversing biochemical changes induced by warfarin. Not relevant for describing effect of dietary change in healthy people
Level IV	Booth et al (1999)	15 young adults depleted for 15 days (10 µg/day) then repleted at 206 µg/day for 10 days. PIVKA-II and % undercarboxylated osteocalcin (%ucOC) measured in only 4 subjects, were restored to baseline levels.
Level IV	Ferland et al (1993)	PT unchanged during a 13-day depletion diet of 10 µg/day compared with baseline of 80 µg/day. Also no changes in PT during various levels of repletion (5, 15, 25, 45 µg/day for 4 days each) using a formula drink. (n=32).
Level IV	Frick et al (1967)	Patients who could not eat were depleted using parenteral glucose and nutrients except vitamins K, A and E and antibiotics for 30–32 days, a single IV dose of 0.03 µg/kg or more vitamin K increased PT for more than 24 hours. The highest dose tested was 0.5 µg/kg (in one person, once). Predicts that a single dose of 1.5 µg/kg would have corrected PT back to normal, assuming a logarithmic association. (n=5).
Level IV	Olson et al (1984, 2002)	PT not changed after depletion diet (8 µg/day) for 3–8 weeks. (n=7).
Level IV	O'Reilly (1971)	PT changes, but not out of normal range, after depletion diet for 4 weeks. (Although diet stated to be 25 µg/day, the new assays using HPLC yield much lower levels than the old assays, so diet was certainly much less). (n=4).
Level IV	Suttie et al (1988)	No change in PT on depletion diet (50 µg/day for 21 days) or repletion with either additional 50 or 500 µg/day for 12 days (n=10), (Note: although the repletion phase was randomised, the 2 groups were apparently combined in the analysis, hence not an intention-to-treat analysis).
Level IV	Udall (1965)	A depletion diet for 3 weeks increases PT, but not to haemorrhagic levels. (n=10).
Papers used to assess what effect vitamin K has on markers of bone health		
Level II	Binkley et al (1999, 2000)	%ucOC decreases in both younger and older subjects given 1 mg/day vs placebo in addition to normal diet for 2 weeks. (n=219).
Level II	Braam et al (2003)	In Dutch women 50–60 years and at least 2 year postmenopausal. Those receiving 500 mg calcium+8 µg vitamin D+10 mg zinc+1 mg vitamin K had 1.7% higher BMD at femoral neck compared to placebo at 3 years, whereas those receiving calcium + vitamin D + zinc had a non-significant increase of 0.4%. No difference between groups at the lumbar spine. (n=181).
Level II, III-1 or III-2 (Allocation method not described)	Knapen et al (1993), study 2	Women aged 50–80 stratified as fast or normal losers based on urinary calcium. Given 1 mg vitamin K/day or placebo within each stratum, for 3 months. Bound, and therefore total, OC increased in the fast losers receiving vitamin K. No effect in normal losers. (n=58 fast losers, 53 normal losers).

Level of evidence	Reference	Study type, issues addressed and key findings
Level II, III-1 or III-2 (Allocation method not described)	Schaafsma et al (2000), normal BMD group	Postmenopausal women with normal BMD. Compared to the placebo group, those receiving 80 µg vitamin K and 1,000 mg calcium with or without 400 IU vitamin D had decreased %ucOC from about 33% to about 28% after 1 year (2 arms with different vitamin D supplementation were combined in the analysis). (n=96).
Level II, III-1 or III-2 (Allocation method not described)	Schaafsma et al (2000), low BMD group	Postmenopausal women with low BMD. No difference in the decrease in %ucOC in those receiving 350 IU vitamin D + 80 µg vitamin K + 1,000 mg calcium vs 400 IU vitamin D + 1,000 mg calcium after 1 year. (n=45).
Level II, III-1 or III-2 (Allocation method not described)	Binkley et al (2002), sub-study B	5-arm study, placebo and 250, 375, 500 or 1,000 µg/day vitamin K for 2 weeks. Compared to no change from baseline in placebo (7% ucOC), doses of 250, 375 & 500 µg/day reduced %ucOC by approx 50% and 1,000 µg/day by approx 75%. (n=100, 23–31 year-olds).
Level II, III-1 or III-2 (Allocation method not described)	Sokoll et al (1997)	Subjects received both diets in randomised order. On 420 µg diet, %ucOC decreased from 22% to 13% but did not change on 100 µg diet. (n=9, 20–33 year-olds).
Level III-2	Booth et al (2000)	Dietary vitamin K intake assessed in 335 men and 553 women in the Framingham Heart Study. Cohort study. Individuals in the highest quartile of vitamin K intake (median, 254 µg/day) had a significantly lower fully adjusted relative risk of hip fracture than did those in the lowest quartile of intake (median, 56 µg/day). There were no associations between vitamin K intake and BMD in either men or women. Low vitamin K intakes were associated with an increased incidence of hip fractures. Low vitamin K intake was not associated with low BMD.
Level III-2	Douglas et al (1995)	Giving 1 mg vitamin K or vitamins K and D has the same effect on %ucOC after 6 weeks. No change in control phase.
Level III-2	Feskanich et al (1999)	Nurses' Health Study cohort. Women in quintiles 2–5 of vitamin K intake had a significantly lower age-adjusted RR of 0.70 (95% CI: 0.53, 0.93) for hip fracture than women in the lowest quintile (<109 µg/day). Risk did not decrease between quintiles 2 and 5 and risk estimates were not altered when other risk factors for osteoporosis, including calcium and vitamin D intakes, were added to the models. Low intakes of vitamin K may increase the risk of hip fracture in women.
Level IV	Binkley et al (2002), sub-study A	Subjects given increasing doses for 1 week at each level: 7% ucOC in weeks on placebo and 250 µg compared to approx 2% ucOC with 500, 1,000 and 2,000 µg vitamin K/day.
Level IV	Booth et al (1999)	15 young adults depleted for 15 days (10 µg/day) then repleted at 206 µg/day for 10 days. %ucOC was restored to baseline levels with repletion for the 4 subjects measured.
Level IV	Booth et al (2003)	Women of 60–80 years fed diet containing 18 µg/day and 5 levels of supplement added: 72 µg (28 days) then for 14 days each: 0, 68, 182 and 432 µg/day: %ucOC rose from 40% to 60% during baseline diet. No significant differences in %ucOC at end of supplementation and all were significantly higher (40%) than before the trial. (n=21).
Level IV	Knapen et al (1989)	1 mg/day for 2 weeks decreases %ucOC in postmenopausal women but not premenopausal women.
Level IV	Knapen et al (1993), study 1	Compared to baseline, giving 1 mg/day to women for 2 weeks causes total OC to increase in those over 64 years because bound OC increases, no effect on free OC. No effect in younger women. (n=145).

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MINERALS

CALCIUM

The recommendations for calcium were derived from an assessment of the evidence base used by the FAO:WHO (2001) and the US:Canadian Government DRI review of 1997 (FNB:IOM 1997) together with consideration of additional key papers missing from those reviews or published since they were released and current recommendations of other key countries. An evidence-based review of calcium recommendations from a number of countries was also available to the committee (Flight & Baghurst 2003).

The estimates of needs, except for those for infants, were made using a calcium balance approach.

After review of the FAO:WHO and US:Canadian recommendations, the FAO:WHO recommendations were adopted with the variations outlined below. Whilst the US:Canadian review used a different approach to setting recommendations, the resulting AIs were generally very similar to the recommended nutrient intake (RNI) set by FAO:WHO. The US set AIs rather than EARs and RDIs because of concerns about the uncertainties in the methods inherent in the balance studies used to determine desirable balance estimates (and their nutritional significance), lack of concordance between observational and experimental data and lack of longitudinal data on the effect of calcium on bone loss and fracture.
The Australian/New Zealand committee was in agreement with the approach taken by FAO:WHO which was also the method used to set the Australian/New Zealand RDIs of 1991 (NHMRC 1991).

VARIATION(S) FROM THE FAO:WHO RECOMMENDATIONS

A. AIs FOR INFANTS

To maintain consistency across nutrients for infants, an AI based on the calcium content of breast milk was adopted from the US:Canadian DRI review (FNB:IOM 1997).

B. EARs AND RDIs FOR CHILDREN

The FAO:WHO recommendations used different age bands for children than those used for the Australian/New Zealand NRVs. For the 4–8 year category, the requirement was estimated from calcium accretion rates, obligatory losses and body size, and assuming, as for the FAO:WHO, that net absorption is 1 SD above that of adults and that the CV is 15%. After rounding, this gave a figure similar to that for 7–9 year-olds in the FAO:WHO report. As calcium and bone mineral accretion at ages 9–11 years is more like that of 4–8 year-olds than 12–18 year-olds (FAO:WHO 2001, pp152), an additional age category was formed specifically for calcium recommendations for 9–11 year-olds, with an intermediate requirement estimated as for the younger age group. (FAO:WHO 2001, pp152). For 12–13 year-olds and 14–18 year-olds, the recommendation for 10–18 year-olds from the WHO:FAO was adopted.

EVIDENCE BASE

Databases: PubMed plus search of *Journal of Bone and Mineral* (key journal for bone) and search of cross-references and references in FAO:WHO (2001) and FNB:IOM (1997).

Search terms: calcium and calcium requirement, human.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess what factors can affect calcium losses		
Level IV	Heaney et al (1999) Marshall (1976) Nordin (1988)	From the obligatory minimum, urinary calcium increases with a slope of about 5–10%.
Level IV	MacFadyen et al (1965)	Decrease in plasma water calcium of only 9.17 mg/100 mL is sufficient to account for decrease in urinary calcium of 63 mg in 27 subjects changed from normal to low-calcium diet.
Human experimental data	Charles et al (1983) Hasling et al (1990)	Daily insensible calcium losses are 40–80 mg and unrelated to calcium intake.
Papers used to assess whether higher calcium intakes or calcium supplements improve markers of bone health		
Level I	Cumming & Nevitt (1997)	A systematic review of effectiveness of calcium supplements and/or dietary calcium for the prevention of osteoporotic fractures in postmenopausal women. 4 randomised trials of calcium supplements. RR reductions between 25% and 70%. Meta-analysis of 16 observational studies – not consistent. OR of 0.96 per 300 mg/day increase in calcium intake.
Level I	Mackerras & Lumley (1997)	Meta-analysis of 9 RCTs of the effect of calcium supplementation on bone density in post menopausal women. Shows that the rate of bone loss was less in supplemented women in the first, but not the second, year of treatment.
Level I	Shea et al (2003, 2004)	Cochrane review. Trials considered for inclusion randomised postmenopausal women to calcium supplementation or usual calcium intake in the diet and reported BMD of the total body, vertebral spine, hip, or forearm or recorded the number of fractures, and followed patients for at least 1 year were. Calcium supplementation alone has a small positive effect on BMD. Data show a trend toward reduction in vertebral fractures. Unclear if calcium reduces the incidence of non-vertebral fractures.
Level I	Welken et al (1995)	A meta-analysis of 27 cross-sectional, two longitudinal and four intervention studies, assessing the effect of calcium intake on bone mass in young and middle-aged females and males. Shows evidence that calcium intakes were positively associated with bone mass in premenopausal women, although calcium intake alone did not account for a large amount of the variance in bone mass.
Level II	Bonjour et al (1997)	Double-blind placebo-controlled trial in 149 prepubertal girls (mean age, 7.9 years). Subjects given food products containing calcium for 1 year. BMD gains were greater at radial and femoral neck. Greater effect appeared to be observed in girls with calcium intake <880 mg/day.
Level II	Chapuy et al (2002)	610 women aged 64–99 year randomised to either calcium-vitamin D_3 fixed combination, calcium and vitamin D3 separately or placebo. In a sub-group of 114, femoral neck BMD decreased in placebo group but not in treatment groups. RR of hip fracture also higher in placebo.
Level II	Chevalley et al (1994) Recker et al (1996) Reid et al (1995)	Individual RCTs relating calcium supplementation to bone loss and bone density.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Dawson-Hughes et al (1990) Elders et al (1994) Prince et al (1995) Reid et al (1993)	Randomised trials showing that calcium supplements are effective in slowing bone loss in older women.
Level II	Grant et al (2005)	5,292 people randomly assigned 800 IU daily oral vitamin D3, 1,000 mg calcium, oral vitamin D_3 + calcium or placebo. The findings do not support routine oral supplementation with calcium and vitamin D_3, either alone or in combination, for the prevention of further fractures in previously mobile elderly people.
Level II	Nowson et al (1997)	A co-twin RCT of calcium supplementation on bone density in 42 twin pairs aged 10–17 years over 18 months. A significant within-pair difference at the spine and hip at 6 months but no significant effects after 6 months.
Level II	Riggs et al (1998)	4-year RCT in 236 postmenopausal women (mean age 66 years) randomised to either calcium or placebo. Significant treatment effect at 1 year at lumbar spine, proximal femur and total. At 4 year, no effect at spine, small effect at femur ($p<0.015$).
Level II	Specker & Binkley (2003)	Randomised trial of activity and calcium supplementation of 239 children. 74% completed study. Randomised to either gross motor or fine motor activity and within each activity group either placebo or control. Significant interaction between supplement & activity in corticol thickness and area, suggesting calcium modifies the bone response to activity.
Level IV	Abrams et al (2000)	Longitudinal study of 34, multiethnic girls of 7–8 years supplemented with 1,200 mg calcium/day. Calcium kinetics, BMC and bone markers every 6 months. No control group and small numbers.
Level IV	Iulano-Burns et al (2003)	Randomised intervention of exercise and calcium in 66 girls, mean age 8.8 years over 8.5 months. Calcium supplementation (with food) was blinded. Bone mineral increased 2–4% more at the humerus and radius sites in the calcium-supplemented groups. There was an exercise-calcium effect at the femur. Twin model is a tight design for BMD studies.
Papers used to assess the rate of mineral accretion at different ages		
Level IV	Bailey et al (1999)	6-year longitudinal study allows for differences in maturation rate. Data are presented for peak bone mineral accrual and total bone mineral accrual 1 year post-peak.
Level IV	Bailey et al (2000)	Study to estimate the magnitude and variability of peak calcium accretion rates. Shows peak accrual higher than previous cross-sectional analysis (Martin et al 1997).
Papers used to assess the relationship between protein intake and bone loss		
Level IV	Geinoz et al (1993)	74 patients hospitalised for various medical indications – divided into two groups according to their dietary protein intakes, evaluated during the first 28 days in hospital while on a regular diet. Patients in the group with the higher protein had higher BMD at the level of the femoral neck – men in this group also had higher lumbar spine BMD. After 4 weeks in hospital, women with higher protein intake had significantly enhanced bicipital and quadricipital muscle strength and better performance.
Level IV	Hannan et al (2000)	Relationship between protein intake and change in BMD over 4 years in 391 women and 224 men aged 68–91 years, from the Framingham Osteoporosis study. Shows that those in the lowest quartile of protein intake had greatest bone loss. Suggests importance of protein for bone health in elderly.
Cross-sectional survey	Cooper et al (1996)	Examined relationship in 72 premenopausal women among 6 key nutrients thought to affect bone metabolism and BMD in the axial and appendicular skeleton using data in population-based sample. Positive association between protein intake and bone mineral in the distal radius and proximal femur. No such relationship found in 218 postmenopausal women. Suggests that dietary protein intake may be a determinant of the peak bone mass attained by premenopausal white women.

Level of evidence	Reference	Study type, issues addressed and key findings
Expert review	Kerstetter et al (2003a)	High dietary protein intakes increase urinary calcium excretion that will result in hypercalciuria. Majority of calcium balance studies have not detected an effect of dietary protein on intestinal calcium absorption or serum PTH but found that dietary protein intakes at and below 0.8 g/kg were associated with a probable reduction in intestinal calcium absorption enough to cause secondary hyperparathyroidism. Long-term consequences of these low-protein diet-induced changes in mineral metabolism not known. Also, several epidemiologic studies demonstrate reduced BMD and increased rates of bone loss in individuals habitually consuming low-protein diets.
Papers used to assess the bone mineral content of children		
Not applicable	Specker et al (2001)	Cross-sectional analysis of bone mineral content in 239 children aged 3–5 years. Useful due to limited bone density data in this age group.
Paper used to assess calcium needs in pregnancy and lactation		
Expert review	Prentice (2003)	Pregnancy and lactation associated with physiological adaptive changes in mineral metabolism independent of maternal mineral supply within normal dietary intakes. These processes provide minerals necessary for fetal growth and breast milk production without requiring an increase in maternal dietary intake or compromising maternal bone health in the long-term. May not apply to pregnant women whose mineral intakes or sunlight exposure are marginal.
Papers used to assess calcium status of vegetarians		
Level III-3	Kohlenberg-Mueller & Raschka (2003)	7 women and 1 man, 19–24 years, received a vegan diet based on plant foods and calcium-rich mineral water in the first 10 days and a lactovegetarian diet during the following 10 days. Concludes that a well-selected vegan diet maintains calcium status, at least for a short-term period.
Survey data	Larsson & Johansson (2002)	Compared the dietary intake and nutritional status of young Swedish vegans and omnivores. Vegans had dietary intakes lower than the average requirements of calcium.
Expert review	New (2004)	Literature analysis through a combination of observational, clinical and intervention studies assessed in relation to bone health for lacto-ovo-vegetarian and vegan diets versus omnivorous, predominantly meat diets, consumption of animal versus vegetable protein, and fruit and vegetable consumption. No differences in bone health indices between lacto-ovo-vegetarians and omnivores. Conflicting data for protein effects on bone with both high and low protein consumption being detrimental to the skeleton. Growing support for a beneficial effect of fruit and vegetable intake on bone, with mechanisms of action currently unknown. From data available vegetarians appear to have normal bone mass.

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CHROMIUM

The recommendations for chromium were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs, rather than EARs and RDIs, were set for all age and gender groups. The AIs for infants were based on breast milk concentrations (Anderson et al 1993) and on estimates of median daily intakes. The estimations for adults involved multiplying the concentration of chromium in diets designed for adults (Anderson et al 1992), and the median population intake of energy for adults in Australia and New Zealand derived from the National Nutrition Surveys (ABS 1998, MOH 1999).
The AIs for children and adolescents were derived from the adult AI on a body weight basis, taking into account growth needs.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations for Australia and New Zealand are the same as those published for the US:Canada in 2001 (FNB:IOM 2001). Although estimation of adult needs (and thus estimates for other age groups and for pregnancy and lactation) used the Australian and New Zealand median energy intakes, these were not sufficiently different from the US energy intake data to change the AI substantially.

EVIDENCE BASE

Databases: Medline plus cross-referencing and review of key references in FNB:IOM (2001).

Search terms: Chromium, recommended intakes.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the requirements for chromium of infants 0–6 months		
Survey data	Anderson et al (1993)	Key data for review. 17 lactating women, 60 days post-partum.
Survey data	Casey & Hambidge (1984)	Key data for review. 45 lactating women at 5 different stages of lactation (0–14 day to 7+ months).
Survey data	Casey et al (1985)	Key data for review. 11 lactating women at 4 stages of lactation (0–31 days).
Survey data	Mohamedshah et al (1998)	Key data for review. 6 lactating women, 90-day period.
Paper used to estimate requirements of children, adolescents and adults		
Survey data	Anderson et al (1992)	Chromium content of 22 daily well-balanced diets, ranged from 8.4 to 23.7 micrograms/1000 cal with a mean +/- SEM chromium content of 13.4 +/- 1.1 micrograms/1000 cal.
Papers used to estimate UL		
Expert review	Flodin (1990)	Review examines the toxicity and drug interactions of the thirteen vitamins and the trace elements chromium, selenium, and zinc.
Expert review	Hathcock 1997	Many widely discussed putative adverse effects of vitamin C, vitamin E, and trivalent chromium have little factual basis.

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COPPER

The recommendations for copper were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs, rather than an EARs and RDIs, were set for all age and gender groups. The AIs for infants were based on breast milk concentrations and for other groups, on median population intake data. The data used to derive the recommendations for Australian and New Zealand NRVs for copper were based on a re-analysis of the intake data collected in the National Nutrition Survey for Australia, 1995 (ABS 1998) and the National Surveys of New Zealand in 1997 and 2002
(MOH 1999, 2003). As copper data are not available for Australian foods, the nutrient data used were from the USDA food data. The median intakes in Australia and New Zealand for each age band were almost the same.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The FNB:IOM recommendations for the US and Canada were based on three studies with a total of 31 participants – one in young men, one in men of mixed ages and one in post-menopausal women. These are high quality publications and they provide a good level of evidence. However, they are limited by the age and gender of the experimental subjects. The UK DRV position was similar, but the committee did not set an EAR. For this reason the recommendations for Australia and New Zealand take the form of AIs based on median population intakes in the two countries for all groups except for infants where copper concentrations of breast milk and weaning food were used to determine the AI.

EVIDENCE BASE

Databases: Medline and search of cross-references and references in the relevant report (FNB:IOM 2001).

Search terms: Copper, human, bioavailability, toxicity.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess factors affecting the bioavailabilty of copper		
Level III-1	Hunt & Vanderpool (2001)	Copper bioavailability is lower in lacto-ovo-vegetarian (LOV) (33%) compared to non-vegetarian (NV) (42%) diets. In contrast LOV diets contains more copper than NV diets and net copper absorption is therefore greater in LOV diets.
Level III-1	Olivares et al (2002)	In infants, copper bioavailability is high (78–84%) but not affected significantly by age (1 month vs 3 months). Copper absorption correlates inversely with faecal copper excretion.
Level III-1	Turnlund et al (1983)	Copper bioavailability is greater from animal than plant protein. Copper absorption is increased during pregnancy only when plant protein is consumed.
Level III-1	Turnlund et al (1998)	Copper homeostasis in men is regulated by the extent of endogenous copper excretion.
Papers used to assess what intakes of copper are needed for balance		
Level III-1	Turnlund et al (1990)	Copper intake of 0.8 mg/day is sufficient to maintain adequate copper status for 42 days in young men.
Level III-1	Turnlund et al (1997)	Copper requirement in young men is 0.4–0.8 mg/day.
Level III-1	Widdowson & Dickerson (1964)	Whole body copper content: full term baby (body weight 3.5 kg), 4.7 mg; young boy, 3.3 mg; adult 1.7 mg. (n=1 in each case).

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Shike et al (1981)	In patients receiving total parenteral nutrition, age 15–71 years, copper requirement was 0.3 mg/day. In presence of increased fluid loss, requirement increases to 0.4–0.5 mg/day.
Paper used to assess what are good markers of copper status		
Level III-1	Milne & Nielsen (1996)	Sensitive biomarkers of copper status include cytochrome c oxidase activity and copper in platelets.
Papers used to assess what levels of copper intake could be harmful		
Level III-1	Davis (2003)	A low copper (0.6 mg/day) diet is associated with more faecal free radical production and low alkaline phosphatase activity – both putative risk factors for colon cancer.
Level III-1	Davis et al (2000)	High (3 mg/day, n=7) vs low (1 mg/day, n=4) copper intake exacerbates the effect of zinc on amyloid precursor protein in women.
Level III-1	Harvey et al (2003)	Men fed 6.0, 1.6 and 0.7 mg copper/day for 8 weeks. No significant effect on endogenous copper excretion or on biomarkers of copper status. No data on urinary output. Study design (kinetics) not clear.
Level III-1	Pratt et al (1985)	Supplementation with 10 mg copper/day (as gluconate) for 12 weeks produced no side effects compared with placebo. (n=7).
Level III-1	Turley et al (2000)	Multicentre double-blind cross over RCT with 3 mg and 6 mg copper. No effect on LDL oxidation and no reported adverse effects with intakes up to 7 mg copper/day.

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FLUORIDE

Note: The body of evidence and recommendations (AI and UL) for infants and young children (0-8 years) were updated in 2017. For further information see the NHMRC Guidelines and Publications Page.

The 2006 recommendations for fluoride were derived after consideration of the FNB:IOM (1997) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the US:Canadian DRI review were adopted unchanged.

EVIDENCE BASE

Databases: Medline and Cochrane database plus cross-referencing and review of key references in FNB:IOM (1997), which was used to set values.

Search terms: fluoride, fluoridation, fluorosis, caries, cancer fracture, osteoporosis, teeth.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess what factors influence assessment of fluoride needs in infancy and early life		
Level III-2	Esala et al (1982)	Only marginally higher fluoride levels in breast milk from females in high fluoride area compared with low fluoride area.
Level IV	Ekstrand et al (1981)	No evidence of an increase in fluoride concentration in breast milk after fluoride supplement.
Level IV	Ekstrand et al (1984)	Only slight increase in fluoride concentration in breast milk after high doses of fluoride.
Survey	Dabeka et al (1986)	Average concentration of fluoride in breast milk is 0.013 mg/L.
Human experimental data	Buzalaf et al (2001)	Brazilian infant formulas. When reconstituted in deionized water, only soy-based formula provides >0.07 mg/kg/day. When formula is reconstituted in drinking water with fluoride, daily fluoride intake is 0.097–0.249 mg fluoride/kg/day.
Human experimental data	Buzalaf et al (2002)	Whether or not fluoride is present in water supply, there was no significant variation in fluoride concentration among 3 different dates of manufacturing for all Brazilian infant formulas.
Human experimental data	Silva & Reynolds (1996)	Australian infant formula reconstituted with fluoridated water results in >0.1 mg fluoride/kg/day.
Human experimental data	Spak et al (1982)	72% of all fluoride in milk and 65% of all fluoride in water-diluted infant formula were absorbed in adults.
Human experimental data	Zohouri & Rugg-Gunn (2000)	Fluoride retention in 4 year-old children is much lower than previously assumed. Mean fluoride intake from all sources, 0.426 mg/day.
Papers used to assess what factors affect fluoride status or fluorosis		
Level III-2	Clark et al (1994)	Cohort study. Infant formula and parental education are associated with dental fluorosis, but both had little predictive ability.
Level III-2	Heller et al (1997)	Cohort study. A suitable trade-off between caries and fluorosis at 0.7 ppm fluoride in water.
Level III-2	Osuji et al (1988)	Case-control. Early tooth brushing (<25 months) and prolonged use of infant formula (7–13 months) associated with increased risk of fluorosis.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Pendrys et al (1994)	Mild to moderate fluorosis associated with infant formula, frequent brushing and inappropriate supplement use.
Expert opinion	Fomon & Ekstrand (1999)	Fluoride intakes increased by change in infant feeding practices to more extended formula feeding from mid 1970s into 1990s.
Expert opinion	Krishnama-chari (1986)	Age, sex, calcium intake in the diet, dose and duration of fluoride intake and renal efficiency in fluoride affect skeletal fluorosis outcome
Expert opinion	Mascarenhas (2000)	Review. Identifies 4 major risk factors for fluorosis: use of fluoridated water, fluoride supply, fluoride toothpaste and infant formula before age of 6 years.
Papers used to assess whether fluoridation improve dental or bone health		
Level II	Riggs et al (1990)	Four-year prospective clinical trial in 202 postmenopausal women with osteoporosis and vertebral fractures who were randomly assigned to receive sodium fluoride or placebo. All received a calcium supplement. Treatment group had increases in median BMD of 35% in the lumbar spine, 12% in the femoral neck, and 10% in the femoral trochanter, but the BMD decreased by 4% in the shaft of the radius. The number of new vertebral fractures was similar in the treatment and placebo groups, but the number of non-vertebral fractures was higher in the treatment group. Conclude that fluoride therapy increases cancellous, but decreases cortical BMD and increases skeletal fragility. Under conditions of this study, the fluoride-calcium regimen was not effective treatment for postmenopausal osteoporosis.
Level III-2	Kroger et al (1994)	BMD of spine and femoral neck measured in a random stratified sample of 3,222 perimenopausal women of whom 969 had used fluoridated drinking water (1.0–1.2 mg/L) for over 10 years; compared with 2,253 women with low levels of fluoride in drinking water (<0.3 mg/L). BMD of the spine was significantly higher in the fluoride group. Femoral neck BMDs did not differ between the groups. When the BMD values were adjusted for confounding factors, differences between the groups increased. No significant difference between the groups in the prevalence of self-reported fractures sustained during 1980–1989. Conclude fluoridation of drinking water has a slight increasing effect on axial BMD in women in low fluoride areas.
Level III-2	Selwitz et al (1998)	Cohort study. Water fluoridation still beneficial and no difference in dental fluorosis between fluoridated and non-fluoridated communities.
Level III-2	Slade et al (1995)	Negative linear relationship between dental caries and per cent lifetime exposure to fluoride in drinking water.
Level III-2	Slade et al (1996)	Caries rate significantly lower in fluoridated water area.
Level III-2	Sowers et al (1991)	Study of bone mass and fractures in 827 women aged 20–80 years in 3 rural Iowa communities selected for the fluoride and calcium content of water supplies. Control community had calcium content of 67 mg/L and a fluoride content of 1 mg/L. Higher-fluoride community had 4 mg fluoride/L of water. Residence in the higher-fluoride community was associated with a significantly lower radial bone mass in premenopausal and postmenopausal women, an increased rate of radial bone mass loss in premenopausal women and significantly more fractures among postmenopausal women. 5-year RR of any fracture was 2.1 in women in the higher-fluoride community compared with women in the control community. 5-year RR of wrist, spine, or hip fracture in the higher-fluoride community was 2.2.
Level III-2	Stephen et al (2002)	Cohort study. Caries benefit of naturally fluoridated water (1 ppm) and results in borderline mild fluorosis.
Systematic review of public water fluoridation *Effect on dental caries* IV (mainly)	McDonagh et al (2000)	Best available evidence suggests fluoridation of drinking water does reduce caries prevalence.

Level of evidence	Reference	Study type, issues addressed and key findings
Dental fluorosis III-2 (experimental) *Bone fractures* III-2, III-3 and IV Cancer studies (18/26 studies)		The prevalence of fluorosis at a water fluoridation of 1.0 ppm was estimated to be 48% and for fluorosis of aesthetic concern, it was 12.5%. At 0.1 ppm the prevalence of fluorosis was 15% and with fluorosis of aesthetic concern, 6%. Meta-regression of bone fracture studies found no association with water fluoridation. No clear association between water fluoridation and incidence or mortality of bone cancers, thyroid cancer or all cancers.
Cross-sectional survey	Sowers et al (1986)	Study of bone mass of women in 3 rural communities with differing mineral content of the water supply. Shows no protective effect with higher fluoride intake. Older women from the high fluoride community reported significantly more fractures. No observed community difference in young women's bone mass or fracture history. Young women in the high fluoride community consuming calcium and vitamin D in excess of 800 mg/day and 400 IU/day, respectively, had significantly better bone mass ($p < 0.05$) than their peers.
Papers used to assess any health problems associated with higher fluoride intakes		
Level I	Hauselmann & Rizzoli (2003)	In treating postmenopausal osteoporosis, fluoride increases BMD in lumbar spine, but does not result in a reduction of vertebral fractures. With increasing fluoride, the risk of non-vertebral fractures and gastrointestinal side effects increases. There is no effect on the vertebral fracture rate.
Level II	Leverett et al (1997)	No effect of prenatal fluoride supplement on the development of caries and fluorosis in deciduous teeth.
Level III-2	Dean & Elvove (1937)	Water fluoride not exceeding 1 ppm does not result in significant fluorosis.
Level III-2	Demos et al (2001)	Studies tend to indicate that the addition of fluoride to drinking water of about 1 ppm does not increase the incidence of fracture or decrease BMD compared with non-fluoridated water.
Level III-2	Griffin et al (2002)	Cohort: 2% of US schoolchildren may experience perceived aesthetic problems attributed to fluoride in drinking water. May be an over estimation of effect of fluoride water as no data on fluoride toothpaste or diluted fluoride infant formula consumption.
Level III-2	Holtgrave et al (2002)	Cohort study. Fluoride supplement in childhood resulted in extensive pulp calcification in caries free primary molars with subsequent obstruction of the pulp cavity.
Level III-2	Leone et al (1954)	Difference in CVD between 2 towns due to multiple statistical tests. No real significance.
Level III-2	Leone et al (1955)	10–15% osteosclerosis (x-ray evidence) in individuals living in high fluoride area (8 mg/L), but no clinical symptoms.
Level III-2	Li et al (2001)	Cohort: Long-term fluoride exposure from drinking water >4.32 ppm increased risk of overall fracture as well as hip fracture.
Level III-2	McCauley & McClure (1954)	Drinking water of ≥0.5 ppm fluoride leads to no evidence of ossification in radiographs of the hand and wrist of children aged 7–14 years.
Level III-2	Schlesinger et al (1956)	No difference in x-rays of children after 10 years of fluoridated water (1.2 ppm) compared with controls (fluoride-free water).
Level III-2	Sowers et al (1986)	Greater fluoride intake (4 mg/L) was not association with increased bone mass.
Level III-2	Teotia et al (1998)	Cohort study. Dental caries caused by high fluoride and low dietary calcium intake.
Level III-3	Larsen et al (1988)	Increase in fluorosis of primary teeth in children fed milk formula compared with cow's milk.
Level IV	Stevenson & Watson (1957)	Small number of cases (23/170,000) of osteosclerosis identified from x-ray examination in patients living with high fluoride content (4–8 mg fluoride/L) in their drinking water.

Level of evidence	Reference	Study type, issues addressed and key findings
Systematic review (mixed types)	Truman et al (2002)	Community water decreased dental caries experience among children aged 4–17 years by a median of 50.7% during 3–12 years of follow-up. Stopping community water fluoride may lead to median 17.9% increase in caries.
Expert opinion	Fomon et al (2000)	In the US, fluoride intakes increased in infants in last 30 years in both fluoridated and non-fluoridated communities Intakes are likely to continue to increase and to be associated with further fluorosis.

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IODINE

The recommendations for iodine were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.
An expert review of recommendations relevant to Australia and New Zealand (Thomson 2002) was also available to reviewers. EARs and RDIs were set for most age and gender groups based on results of experimental studies.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. AI FOR INFANTS

The infant AI was based on the concentration of iodine in breast milk. Breast milk iodine is highly variable and dependent on maternal intakes. The US:Canadian recommendations were based on data from the US, which generally give higher results than found in many other countries, including most European countries. The AI for Australia:New Zealand was based on the FAO:WHO (2001) assessment of mean breast milk iodine content that was consistent with the findings from one New Zealand study (Johnson et al 1990).

B. EAR FOR ADULTS

The EAR set for adults (100 µg/day) was higher than that of 95 µg/day set by the US on the basis of some data from New Zealand relating urinary iodide to thyroid volume (Thomson et al 2001). However, with rounding, this resulted in the same RDI for both sets of recommendations.

C. EAR FOR LACTATION

The EAR for lactation was lower than that set by the US:Canada as the amounts secreted in breast milk were judged to be lower.

EVIDENCE BASE

Databases: Medline, PubMed plus cross-referencing and review of key references in FNB:IOM (2001) and expert review by Thomson (2002).

Search terms: Iodine, thyroid, goitre, hypothyroid, hyperthyroid, cretinism, growth, goitrogens, human.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the concentration of iodine in breast milk		
Level IV	Delange et al (1984)	Iodine retention in full-term infants is 6.7 µg/kg/day, retention 22 µg/day. Iodine balance studies on full-term infants aged 1 month, fed 20 µg/kg/day iodine: total excretion was 12.7 µg/kg/day, iodine retention 7.3 µg/kg/day. Mean body weight at 6 months of 7 kg, child excretes 90 µg/day if in positive iodine balance.
Survey	Gushurst et al (1984)	24 women, 21–36 years, lactation state 14 days–3.5 years. Median iodine concentration of US women consuming non-iodised salt was 113 µg/L and of women consuming low or high amounts of iodised salt was 143 or 270 µg/L. Median intake for all was 146 µg/L. Estimated iodine intake of infants, 114 µg/day.
Survey	FAO:WHO (2001)	Review. Breast milk provides mean of 90 µg/day (115 µg/L).
Survey	Johnson et al (1990)	Wellington data showed 145 µg/L <30 days after delivery, then 126 µg/L at <60 days and 50 µg/L at >60 days.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the iodine requirement in children		
Level IV	Ingenbleek & Malvaux (1974)	4-day balance study, 7 children, 1.5–2.5 years, previously malnourished and rehabilitated. Median iodine intake on rehabilitation, 63.5 µg/day, iodine balance, +19 µg/day (CV 20%)
Level IV	Malvaux et al (1969)	Balance study. 8 year-old children consuming 20–40 µg/day in negative iodine balance (–23 to –26 µg/day). Average requirement estimated as 65 µg/day (40+26). Balance study. 16 boys and girls, 9–13 years, average intake 31 µg/day, average balance –24 µg/day, giving requirement of 55 µg/day (31+24). 10 children, 14–16 years, average iodine balance –24 µg/day, consumed an average of 34 µg/day giving a requirement of 58 µg/day (34+24).
Survey data	Delange et al (1997)	Key data used for setting AIs. Prevalence of goitre in children 6–15 years in Europe. Compared prevalence with median urinary iodine. Level of urinary iodine where there is only 2% prevalence was 100 µg/L – 98th centile (equivalent to RDI).
Papers used to assess the iodine requirement of adults		
Level IV	DeGroot (1966)	4 normal subjects assessed by 3 methods for uptake. Absolute iodine uptake, 21–97 µg/day; thyroid hormone excretion by two methods, 69–171 µg/day and 49–147 µg/day, respectively.
Level IV	Fisher & Oddie (1969a)	Turnover studies in euthyroid subjects. Accumulation of radioiodine by thyroid for 18 men and women aged 21–48 years was 96.5 µg/day (CV of 40%).
Level IV	Fisher & Oddie (1969b)	Uptake/turnover study. 274 euthyroid subjects in US. Uptake and turnover was 91.2 µg/day.
Level IV	Harrison (1968)	Provided 100 µg/day to 13 subjects, giving a slight positive balance (13 µg).
Level IV	Vought & London (1967)	Obligatory excretion of iodine 57 µg/day.
Survey data	Thomson et al (1977, 2001)	189 subjects (102 males, 87 females) recruited from Dunedin and 144 (77 males, 77 females) from the Waikato, November 1993–June 1994. Greater than for Otago Median urinary iodide excretions – 60 and 76 µg/day for Dunedin and Waikato, respectively. Thyroid hormone levels within normal ranges.
Survey data	Thomson et al (2001)	233 residents of Otago, New Zealand collected two 24-hour urine samples for assessment of iodine status. Significant correlations found between urinary iodide excretion and thyroid volume and thyroglobulin. Multiple regression analysis of data for subjects divided into 3 groups according to 24–hour urinary iodide excretion (<60, 60–90, >90 µg iodide/day) or iodide/creatinine ratio (<40, 40–60, >60 µg creatinine/g) showed significant differences in thyroid volume ($p=0.029$ and $p=0.035$, respectively) and thyroglobulin ($p=0.019$ and $p=0.005$, respectively) among the groups. Physiological requirement is between 85 and 100 µg/day.
Papers used to assess the iodine requirement in pregnancy		
Level IV	Dworkin et al (1966)	Balance study. 5 pregnant, 4 non-pregnant women. Balance estimated at 160 µg/day.
Level IV	Glinoer (1998)	Pregnant women, initial urinary iodine of 36 µg/L, treated with 100 µg/day. Median urinary iodine increased to 100 µg/L at 33 weeks, thyroid volume increased 15% compared to 30% in controls. Thus 100 µg supplement bringing total to 150 µg/day was insufficient to prevent increased thyroid size.
Level IV	Pedersen et al (1993)	54 pregnant women, 200 µg/day supplement as potassium iodide drops from 2nd trimester. Urinary iodine increased from 55 to 105 µg/L, thyroid volume increased 15.5%, TSH and thyroglobulin levels did not change. Controls showed increases of 31% in thyroid volume, 75% in serum thyroglobulin and 21% in serum TSH. Thus 250–280 µg/day prevented goitre in pregnancy.
Level IV	Romano et al (1991)	Pregnant women in iodine-deficient area given iodised salt to add 120–180 µg iodine/day. Urinary iodine rose from 37 to 154 µg/day in 2nd trimester and was 100 µg/day in 3rd trimester. Little change in untreated controls. Total iodine intake of 200 µg prevented goitre.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the levels of dietary iodine that may cause adverse effects		
Level III-3	Gardner et al (1988)	TSH in 30 adult males, 22–40 years receiving supplements of 500, 1,500 or 4,500 µg/day for 2 weeks. Baseline urinary iodine excretion, 287 µg/day, giving a LOAEL of 1,800 µg/day.
Level III-3	Paul et al (1988)	9 men, 26–56 years, 23 women 23–44 years iodine supplements of 250, 500 or 1,500 for 14 days. Iodine intake of about 1,700 µg/day increased TSH secretion.

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IRON

The recommendations for iron were derived from an assessment of the evidence base used by the US:Canadian Government DRI review of 2001 (FNB:IOM 2001) together with consideration of additional key papers missing from that review or published since it was released and current recommendations of other key countries and organisations such as the FAO:WHO.

The estimates of needs, apart from those for infants, were made using a factorial modelling approach.

After review, the US:Canadian recommendations published by the Food and Nutrition Board of the Institute of Medicine were adopted with the exceptions described below.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. BREAST MILK CONCENTRATION

The US:Canadian DRI review used data from nine studies and averaged these. Three of the nine were of relatively low quality (Garza et al 1983, Anderson 1993, Lemons et al 1982) involving studies with small numbers and one was limited to the first month of life (Mendelson et al 1982). Recalculation of the average iron concentration of milk reported in the remaining five studies gave an average concentration of 0.26 mg/L and an AI of 0.2 mg/day instead of 0.27 mg/day. The lactation requirements are thus also slightly less than the US:Canadian DRI recommendations.

B. IRON ABSORPTION RATES FOR CHILDREN AGED 1-3 YEARS.

In the US:Canadian DRI review, an iron absorption of 10% was set for infants aged 12 months based on the assumption that the primary source of iron at this age is infant cereal which is most often fortified with low bioavailable iron. However, for all other ages, an iron absorption of 18% was assumed based on the studies of Hallberg & Rossander-Hulten (1991) and Cook et al (1991).

The 18% figure was derived from the results of these studies by:

1. correcting to a serum ferritin concentration of 15 μg/L (the level at which iron stores were assumed to be just adequate)
2. assuming the bioavailability of non-haem iron to be 16.8% and that of haem iron to be 25%
3. assuming that haem iron constitutes 10–15% of iron in the diets of both adults and children.

Data from the NNS 1995 survey in Australia (Baghurst et al 2000) show that haem iron constitutes about 10% of total iron in diets of 4–15 year-olds and about 15% in older adolescents and adults. However, for younger children aged 2–3 years, haem was only 7% of total iron. There were no data for 1 year-olds, however data from New Zealand (Soh et al 2004) showed that haem iron is only 3.8% of total iron for 1year-olds, giving an overall figure of approximately 5.5% haem iron for the 1–3 year-old age group.
A bioavailability figure of 14% was therefore adopted for the 1–3 year-olds to give a higher EAR and RDI for this age group.

C. UPPER INTAKE LIMITS

For infants, the US:Canadian DRI review set a figure of 40 mg/day based on supplementation studies using gastrointestinal symptom as the adverse event. As data were consistent, a UF of 1 was applied, such that the LOAEL became the NOAEL. Since the DRI review, two relevant RCTs have been published. One of these (Dewey et al 2002) assessed growth outcomes as well as gastrointestinal symptoms and found adverse effects on aspects of growth in infants with good iron status. Swedish infants were given supplements at 1 mg/kg body weight/day. Gains in length and head circumference were significantly lower in those who

received iron than in those given placebo between 4 and 9 months. The same effect on length was seen in infants from Honduras, but only at 4–6 months among those with initial haemoglobin (Hb) ≥110 g/L. There was no significant effect of iron supplementation on morbidity, nor any significant interaction between iron supplementation and site, but for diarrhoea (with both sites combined), there was an interaction between iron supplementation and initial Hb. Among infants with Hb <110 g/L at 4 months, diarrhoea was less common among those given iron than in those given placebo from 4–9 months, whereas the opposite was true among those with Hb ≥110 g/L ($p<0.05$). Routine iron supplementation of breast-fed infants may therefore benefit those with low Hb, but may present risks for those with normal Hb.

In contrast, the second study (Friel et al 2002) found no adverse symptoms at supplement levels of 7.5 mg/day and the authors stated that there may even be benefits.

Given this uncertainty, a UF of 3 was applied to bring the UL down to 20 mg/day for infants (10 mg naturally from food and milk and up to 10 mg for supplements and additives).

EVIDENCE BASE

Databases: Medline and search of cross-references and references in FNB:IOM (2001).

Search terms: iron, human milk and intakes; iron and basal losses; blood volume expansion and children; blood, iron content and haemoglobin; iron and bioavailability; iron, menstruation, blood loss and women; iron, pregnancy and breast feeding; iron, supplementation and side effects; iron, cancer and diet; iron, interactions, copper and zinc; iron, cardiovascular disease and diet; malabsorption syndromes, coeliac disease, gastroenteritis, enteropathy, irritable bowel syndrome and iron requirements; alcoholics, drug abuse, alcoholism and iron requirements; smoking and iron requirements; vegetarianism and iron requirements; dietary iron requirements.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the iron requirements for infants		
Survey data		Iron intakes of breast fed infants
	Anderson (1993)	Iron intake = 0.20 mg/day, milk iron = 0.26 mg/L (n=6).
	Butte et al (1987)	Iron intake = 0.15 mg/day, milk iron = 0.2 mg/L, milk intake = 735 g/day (n=45).
	Dewey & Lonnerdal (1983)	Iron intake = 0.13–0.21 mg/day over time, milk iron = 0.2–0.31 mg/L, milk intake = 673–896 mL/day, increasing with age (n=20).
	Garza et al (1983)	Iron intake = 0.03 mg/day, milk iron = 0.03 mg/L (n=6).
	Lemons et al (1982)	Iron intake = 0.66 mg/day, milk iron = 0.84 mg/L (n=7).
	Lipsman et al (1985)	Iron intake = 0.26 mg/day, milk iron = 0.33 mg/L (n=25).
	Mendelson et al (1982)	Milk iron = 0.8–1.1 mg/L across 1st month lactation. No difference between term and preterm milks. (n=10 prem; 8 term).
	Picciano & Guthrie (1976)	Iron intake = 0.18 mg/day, milk iron = 0.23 mg/L (n=50).
	Vaughan et al (1979)	Iron intake = 0.27 mg/day, milk iron = 0.42 mg/L (n=38).
Human experimental data	Abrams et al (1997)	14.8% iron in breast milk taken with food was incorporated into erythrocytes compared with 11.0% from non-meal dose of ferrous sulphate. Hence, well absorbed even after foods added to diet. (n=16).
Human experimental data	Davidsson et al (2000)	Compared absorption of ferrous fumarate and ferric pyrophosphate in fortified infant cereals with 25 or 50 mg ascorbic acid. Ferrous fumarate, 4.1% vs 1.3% erythrocyte incorporation. Ascorbate had no effect. (n=20).

Level of evidence	Reference	Study type, issues addressed and key findings
Human experimental data	Garby et al (1964)	Estimated basal losses 0.03 mg/kg/day. (n=3).
Expert opinion/ review	Dallman (1986)	Assumes approx 12% total iron in stores, based on 300 mg in a woman. 50 mg store and 420 mg total iron at 12 months. Iron store expressed per kg body weight = (5 mg/kg).
Expert opinion/ review	Hawkins (1964)	Blood volume 70 mL/kg body weight, calculated from formulas based on height and weight developed in 1930s and 1940s.
Expert opinion/ review	Smith & Rios (1974)	Iron content of Hb = 3.39 mg/g, non-storage iron in tissue, 0.7 mg/kg body weight.
Key survey data	Dibley et al (1987)	Mean body weight 9.0 and 8.4 kg for boys and girls, respectively, CV 10%, and growth rates of 13 g/day for 6–12 months. (n=720).
Key survey data	Skinner et al (1997)	Food pattens of 1–3 year-olds. Shows 6–24 month-olds eat little meat. (n=98).
Papers used to assess the iron requirements for children and adults		
Level II-2	Nilsson & Solvell (1967)	Double blind crossover with no placebo to study menstrual losses. Compared 4 oral contraceptives for 1 cycle each, repeated three times. Length of cycle decreased. Days of heavy losses decreased and those of light losses increased. Blood loss reduced from 31 to 14 mL. (n=19).
Human experimental data	Barrett et al (1994)	Absorption of non-haem iron at 12, 24 and 36 weeks was 7%, 36% and 66%, respectively, compared with 11% post-partum. (n=12).
Human experimental data	Green et al (1968)	Radio isotope studies of men (USA, S Africa, Venuezuela). Heights not given, so used data on mean losses per day and heights from elsewhere. Losses were 0.9–2.42 mg/day. Total sample size for estimates not clear; possibly 41.
Expert opinion	Dallman (1986)	See above for 7–12 month-olds.
Expert opinion	Hallberg et al (1991)	Provides centiles for observed menstrual blood losses in menstruating women. Losses for the 50th, 75th, 90th and 95th centile were 30, 52.4, 83.9 and 118 mL, respectively, from study below (Hallberg et al 1966b).
Expert opinion	Hawkins (1964)	See above for 7–12 month-olds.
Expert opinion	Smith & Rios (1974)	See above for 7–12 month-olds.
Expert opinion/ review	Beaton (2000)	For every 10 g/L increase in Hb, need 175 mg absorbed iron.
Expert opinion/ review	Bothwell et al (1979)	Median iron needs for pregnancy, 500 mg/day. Highly variable. Depends on whether Caesarean birth (greater loss) and primipara (greater loss).
Expert opinion/ review	Bothwell (2000)	Total iron needs in pregnancy, 580 mg/day.
Expert opinion/ review	FAO (1988)	Iron in Hb expansion for a 55 kg woman is 500 mg/day.
Expert opinion/ review	Hytten & Leitch (1971)	Total iron needs in pregnancy, 600 mg/pregnancy.
Key survey data	Beaton et al (1970)	Mean menstrual cycle, 27.8 days. Mean menstrual losses, 0.44 mg iron/day (highest, 5.9 mg/day). 55% <0.53 mg/day, 13% >1.3 mg/day. Women with high menstrual losses may not meet iron needs from diet (mean iron intake, 12.4 mg/day).
Key survey data	Beaton et al (1989)	Nutrition Canada survey of children of 0–13 years. Developed regression equation to give Hb by based on year of age. (n=2,148).
Key survey data	Beaton et al (1989)	See above.

Level of evidence	Reference	Study type, issues addressed and key findings
Key survey data	Cole et al (1971)	Estimates of menstrual losses (n=348). Mean and median were 37.5 and 27.6 mL. Shows increased losses with parity but not age. Mean on pill, 12.7 mL (n=23) and with coil, 56.3 mL. (n=25).
Key survey data	Hallberg et al (1966a)	Estimates of menstrual losses (n=137). Mean and median of 34 and 26 mL, respectively. 95% women <87 mL. Losses less in women <25 years (n=46) and older women. (n=5).
Key survey data	Hallberg et al (1966b)	Estimates of menstrual losses (n=476). Centiles (10th–90th) at different ages. Median, 75th and 90th centiles for 15 year-olds of 28.4, 44.5, 65.1 mL/cycle, respectively Losses to 50 year-olds (median, 75th, 95th centile) were 36.4, 69.5 and 133.1 mL. (n=476) Mean menstrual losses (n=85) were 37.5 mL/cycle and median was 27.6 mL/cycle. Mean on pill, 12.7 mL/cycle (n=23) and with coil, 56.3 mL. (n=25).
Key survey data	Haycock et al (1978)	Developed equation to estimate body surface area from cross-sectional survey of 34 measures of people (infants to adults) to give body surface area by ages based on height and weight. (n=81).
Key survey data	Tanner et al (1966)	To estimate variability in growth rates of children. From graphs, CV about 25–30% for 1-year, adjusted to 40% because of greater variability for shorter period (ie 6-month is 30% higher).
Papers used to assess the bioavailability of iron		
Human experimental data	Cook et al (1974)	Radio iron study (n=83). Shows inverse relationship (–0.74) for ferritin and inorganic iron absorption.
Human experimental data	Cook et al (1991)	Enhancing and inhibiting factors. Shows much larger differences in iron absorption for single meals (2.5–13.5% absorption) than 2 weeks of meals (3.2–8.0% absorption). Iron status is most important determinant of iron absorption. (n=45).
Human experimental data	Hallberg et al (1998)	Western diets should cover iron requirements of most women. The high prevalence of iron deficiency probably reflects low bioavailability.
Human experimental data	Hallberg & Rossander-Hulten (1991)	Requirements for absorbed iron, 2.84 mg/day in adult women and 3.21 mg/day for adolescent girls, which translate to iron uptake per day of 18.9 mg and 21.4 mg, respectively. Using a reference dose absorption of 60% (ferritin 15 µg/L), 25% of population has low iron status (low ferritin), estimated diet iron intakes of 10.5 mg/day after adjustment for lower bioavailability of fortified iron and under-reporting. Calculated that iron absorption from USA diet is 16.6%.
Human experimental data	Hallberg & Hulten (2000)	Based on many previous radio iron studies. Algorithm to predict iron absorption, adjusted to a ferritin of 23 µg/L (40% reference dose absorption).
Human experimental data	Reddy et al 2000	9 radio iron studies (n=86). Algorithm developed to predict non-haem iron absorption based on animal tissue, vitamin C and phytate, adjusted to ferritin of 30 µg/L.
Human experimental data	Whittaker et al (1991)	Iron absorption during pregnancy. Shows increases that suggest diet can meets iron demands.
Human experimental data	Whittaker et al (2001)	Absorption and utilisation of iron during early and late pregnancy.
Key survey data	Raper et al (1984)	Using Monsen's model and dietary intakes, bioavailable iron estimated in US diet. Depending on age, haem iron is 6–12% of iron intakes, vitamin C range is 42–60 mg/day and estimated available iron is 6.5–8.7%. (n=9,547).
Papers used to assess adverse effects of high iron intake		
Level II	Burman (1972)	Blood donors, 12% side effects vs placebo, 7% for 10 mg iron/day as colloidal ferric hydroxide in infants of 3–24 months. Drop out rate due to side effects for 25% of blood donors and 16% for placebo. Questioned monthly about symptoms.
Level II	Dewey et al (2002)	Routine supplementation (1 mg/kg/day) may present risks for infants with normal iron status.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Friel et al (2003)	Iron supplementation (7.5 mg/day) of breast-fed infants appears safe and might have beneficial effects.
Level II	Frykman et al (1994)	Crossover design, 1-month treatment. Minor side effects, especially increased obstipation, for 25% of blood donors supplemented with 60 mg iron as fumarate vs 14% placebo. Recorded daily symptoms. (n=100).
Level II	Fuerth (1972)	Double blind intervention for 12 months. No strong side effects of 30 mg/day iron as ferrous sulphate vs placebo. Questionnaire re side effects. (n=602).
Level II	Hallberg et al (1966c)	More side effects with supplements vs placebo for 222 mg iron/day as ferrous sulphate, iron gluconate, iron fumarate and iron glycine sulphate. 12.4%–13.9% vs 23%–32%. Questionnaire. (n=1,496).
Level II	Reeves & Yip (1985)	6% vs 9% for vomiting/diarrhoea and 9% vs 1% for constipation with 3 mg iron/kg body weight as ferrous sulphate on empty stomach for 3 months in 1 year-olds (n=278). Questionnaire.
Level II	Rybo & Solvell (1971)	Crossover design with pregnant women, 2 weeks per treatment, 200 mg iron/day as fast and slow release ferrous sulphate vs placebo in 2 studies. Blood donors. Side effects of constipation and diarrhoea in placebo vs supplement were 8% vs 11% and 4% vs 7%, respectively (n=778). 1–39% side effects vs 24% in placebo for pregnant women (n=117). Questionnaire.
Level III-1	Blot et al (1981)	French post-partum women, questionnaire after birth, no placebo. 16% minor side effects and 14% severe side effects in. 6% drop-outs. 105 mg iron and vitamin C vs 105 mg iron and vitamin C + folate.
Level III-1	Brock et al (1985)	Single blinded, no placebo, 56 days, diary. 34% moderate to severe side effects and 14% could not complete course of 50 mg iron as ferrous sulphate (wax vs conventional matrix) on empty stomach. (n=602).
Level III-1	Coplin et al (1991)	Double blind crossover, no placebo, diary. Mild to moderate side effects in 67% women, 32% GI effects on 50 mg iron (ferrous sulphate vs bis-glycino iron II) on empty stomach. (n=40).
Level III-1	Farquhar (1963)	No effect on height or weight of 5 mg iron as ferric pyrophosphate in a multivitamin given to infants from 0–12 months of age compared to multivitamin without iron. No placebo. No record of side effects.
Level III-1	Liguori (1993)	Italy, questionnaire, no placebo. Fewer side effects with iron-protein succinylate (60 mg iron) vs slow release ferrous sulphate (105 mg iron) on empty stomach – 11.8% vs 26.3%. (n=1,095).
Level III-2	Flood et al (2003)	Risk of colorectal cancer not associated with meat intakes.
Level III-2	Levi et al (2000)	Risk of colorectal cancer associated with iron intakes.
Level III-2	Terry et al (2002)	Iron supplements associated with increased risk of endometrial cancer.
Expert opinion	Casanueva & Viteri (2003)	Iron supplementation of non-anaemic pregnant women, 60 mg/day increased risks whereas 120 mg iron 1–2/per week did not.
Expert opinion	Nelson (2001)	Benefits of iron supplementation need to be measured against the long-term risks of colorectal cancer.

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MAGNESIUM

The recommendations for magnesium were derived after consideration of the FNB:IOM (1997) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. Some papers relating to magnesium have been published since the FNB:IOM review, but none provides data that would substantially alter the conclusions from that review.

EARs and RDIs were set on the assumption that the best indicator of adequacy currently available is the level that allows an individual to maintain total body magnesium over time. This, together with extrapolation on a body weight basis for age and gender groups for whom there were no experimental data, was used to set all recommendations except those for infants, for whom an AI was set based on the mean volume and magnesium content of breast milk and complementary foods.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 1997) for use in the US and Canada were adopted for use in Australia and New Zealand.

EVIDENCE BASE

Databases: Medline plus cross-referencing and review of key references in FNB:IOM (1997), used to set values.

Search terms: diet, magnesium.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess factors affecting the bioavailability of magnesium		
Level III-2	Andon et al (1996)	6 mg/kg body weight/day adequate for 26 female adolescents. Calcium intake had no effect on availability of magnesium.
Level III-2	Schwartz et al (1984)	Organic magnesium more readily absorbed than inorganic in 8 males, 48–62 years.
Level III-3	Schwartz et al (1973)	High protein diet increases magnesium retention in 6/11 14 year-old males.
Human experimental data	Lakshmanan et al (1984)	Fibre, protein, calcium and phosphate intake each influence magnesium balance (16 males, 18 females).
Paper used to assess suitable methods for measuring magnesium status		
Level III-2	Shils & Rude (1996)	Shows balance studies acceptable to study magnesium status.
Papers used to assess needs for magnesium of the elderly and postmenopausal women		
Level III-2	Elwood et al (1996)	Caerphilly cohort. of 2,172 men (45–59 years) where those with IHD had higher occurrence of previous low magnesium intake.
Level III-2	Stendig-Lindberg et al (1993)	Magnesium increases or stabilises bone density in 27/31 mostly magnesium-deficient post menopausal females.
Paper used to assess the needs for magnesium of adolescents		
Human experimental data	Abrams et al (1997)	6 mg/kg body weight/day inadequate for some adolescents (13 male, 13 female).

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess adverse effects of magnesium		
Level II	Marken et al (1989)	Supplemental magnesium does not alter lipid profile (12 males, 23 females).
Level II	Paolissa et al (1992)	Elderly (6 males, 6 females) are magnesium deficient and supplemental magnesium improves their insulin sensitivity.

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MANGANESE

The recommendations for manganese were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs rather than EARs and RDIs were set for all age and gender groups.
The AIs were based on breast milk concentrations for infants and on median population intake data for other groups. The data used to derive the recommendations for Australian and New Zealand were based on a re-analysis of the intake data collected in the National Nutrition Survey for Australia, 1995 (ABS 1998) and the National Surveys of New Zealand of 1997 and 2002 (MOH 1999, 2003). As manganese data are not available for Australian foods, the nutrient data used were from the USDA food data. The median intakes in Australia and New Zealand for each age band were almost the same.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

As the AIs are based on population intakes, the AIs for Australia and New Zealand are some 40-50% higher than those for the US and Canada. In addition, no increased requirement for lactation was included in the Australian and New Zealand recommendations, as the additional needs to cover losses in milk are less than 3 µg/day.

The FNB:IOM (2001) established a UL of 11 mg/day for adults of manganese from food, *based on the highest reported intake level* presented in a review by Greger (1999). The UL for children and adolescents was derived from the adult value on a body weight basis. As no adverse effects have been reported in people of any age from dietary intake this was not considered to be an appropriate approach to setting a UL.

EVIDENCE BASE

As the recommendations for manganese are based on median population intake, the evidence analysis below is restricted to the data relating to the toxicity of manganese.

Databases: Medline, Ovid Technologies plus cross-referencing and review of key references in FNB:IOM (2001), used to set values.

Search terms: manganese; human.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the levels of manganese intake related to toxicity		
Level III-1	Davis & greger (1992)	Managanese supplements (15mg) for 124 days resulted in elevated serum manganese concentration and activity of manganese superoxide dismutase activity, indicative of LOAEL.
Level III-1	Finley et al (2003)	Manganese intake in the range 0.8-20. mg for 8 weeks shows no effect of deficiency or toxicity.
Level IV	Woolf et al (2002)	Case report. Manganese toxicity through 5-year consumption of contaminated well water produces adverse effects on cognitive function.
Expert opinion	Greger (1999)	Review of intake studies, biomarkers and toxic effects of manganese.

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MOLYBDENUM

The recommendations for molybdenum were derived after consideration of the FNB:IOM (2001) review of DRIs for the US and Canada and recommendations from other key countries and health authorities.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations of the Food and Nutrition Board of the Institute of Nutrition (FNB:IOM 2001) for use in the US and Canada were adopted for use in Australia and New Zealand.

EVIDENCE BASE

Databases: Medline, PubMed plus cross-referencing and review of key references in the FNB:IOM DRI review (2001), the European Commission SCF report (2000) and the UK Expert Group on Vitamins and Minerals (2003).

Search terms: Molybdenum, human, English.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the molybdenum requirement in men		
Human experimental data	Turnlund et al (1995a)	Well-controlled, 120-day balance study, 4 healthy men. Intake of 22 µg/day provides average balance of 0.3 µg/day over 102 days, after a period of 0–48 day adaptation. Shows very efficient absorption over wide range of intakes, and retention and turnover regulated by urinary excretion.
Human experimental data	Turnlund et al (1995b)	Absorption 88–93%, most efficient at highest intake. Urinary excretion regulates retention, mechanism to conserve molybdenum at low intakes. NOAEL, 1,488 µg/day for 24 day.
Human experimental data	Turnlund & Keyes (2000)	Same subjects and protocols as Turnlund (1995). No effect of molybdenum intakes of 22–1,500 µg/day on copper absorption, urinary excretion, retention or serum levels.
Papers used to assess the concentration of molybdenum in breast milk		
Survey	Anderson (1992)	Milk concentration 17.0 µg/L (7 women up to 5 months).
Survey	Aquilio et al (1996)	Milk concentration 4.1 µg/L (14 women at 21 days).
Analysis of milk samples	Biego et al (1998)	Milk concentration 4 µg/L (17 samples mature milk).
Survey	Bougle et al (1988)	Mature human milk 2.6 µg/L (6 women, 1 day to 2 months).
Survey	Krachler et al (1998)	Same subjects and samples as Rossipal (1998). Reports overall mean concentration of human milk of 3.7 µg/L based on samples from 5 stages: 1–3 days, 4–17 days, 42–60 days, 66–90 days and 97–293 days.
Survey	Rossipal & Krachler (1998)	Mature human milk levels, 1.4–1.8 µg/L.
Papers used to set the UL		
Expert review	Brewer (2003)	Because of its rapid action, potency, and safety, tetrathiomolybdate (TM) is proving to be a very effective drug for initial treatment of acutely ill Wilson's disease patients. Beyond this, TM has antiangiogenic effects, because many proangiogenic cytokines require normal levels of copper.

Level of evidence	Reference	Study type, issues addressed and key findings
Expert review	Goodman et al (2004)	Disruptions of copper homeostasis are rare and they cause serious disorders such as Wilson's disease and Menkes disease.
Animal experimental data	Fungwe (1990)	LOAEL 1.6 µg/kg/day and NOAEL 0.9 µg/kg/day. Use of intraspecies UF of 10 compared to UF of 3 in the US review.

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PHOSPHORUS

The recommendations for phosphorus were derived after consideration of the FNB:IOM (1997) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. Some papers relating to phosphorus have been published since the FNB:IOM review, but none provides data that would substantially alter the conclusions from that review.

EARs and RDIs were set for adults on the assumption that the best indicator of adequacy currently available was the level of dietary intake that supported a selected serum P_i level. In the absence of data on serum P_i or phosphorus balance in children from 1–8 years, estimation of body accretion for these age groups was used on known tissue composition and growth rates. For 9–13 year-olds, longitudinal data and a large cross-sectional database were available, allowing estimation of phosphorus requirement from tissue accretion data using a factorial approach (FNB:IOM 1997) that was then also adopted for the 14–18 year-olds. For infants, an AI was set based on an assessment of the amount of phosphorus available in breast milk and complementary foods.

The recommendations of the US:Canadian review of Dietary Reference Intakes (FNB:IOM 1997) for infants and children were adopted but those for adults and pregnancy and lactation were varied as outlined below.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

ESTIMATION OF ADULT RDI

In the US:Canadian recommendations, data on the relationship between serum P_i and dietary intake of phosphorus, derived from Nordin (1976, 1989), were mathematically transformed. An estimate of the dietary intake required to reach the bottom end of the normal range of serum P_i (2.5 centile, 0.87 mmol/L) was then assigned as the EAR for adults (580 mg). The authors note that, by definition, this dietary intake would only be sufficient for 50% of the population to reach the bottom end of the normal serum P_i range. They then assumed a relatively small CV of 10% to reach an RDA (the US:Canadian equivalent of the RDI) for adults of 700 mg.

Based on the relationship between serum P_i and dietary intake of phosphorus, the RDI has been set to cover the needs of most of the population at the level of intake required to achieve a serum P_i of 1 mmol (3.1 mg/day), the level attained by most people in the population (Nordin 1976, 1989). This is consistent with previous approaches used in Australia and New Zealand to set the RDI for adults (Nordin 1989). This would equate to an estimated CV for the EAR of 35%. This higher RDI for adults is then reflected in recommendations for pregnancy and lactation.

EVIDENCE BASE

Databases: PubMed, search of *J Bone Mineral Research* plus cross-referencing and review of key references in review used to set values (FNB:IOM 1997).

Search terms: phosphorus, requirements, human trials.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess factors determining the phosphorus requirements of infants		
Level III	Specker at al (1997)	To determine if varying calcium and phosphorus intake affected bone mass accretion. 1st phase, 61 randomised to low vs moderate mineral formula + group of 34 breast-fed infants; 2nd phase, after 6 months, all randomised to moderate, high mineral of whole cow milk. DRI document reported the intake of phosphorus solids was 151 mg at 9 months and 255 mg at 1 year. A mean figure of 200 mg combined with 75 mg from milk gives an AI of 275 mg. Total phosphorus intake reported at 6, 9 and 12 month by group (eg 606 mg/day for moderate-mineral group at 12 months) but not clear how the figure for solids was derived.
Level IV	Allen et al (1991)	13 exclusively breast-feeding women studied for 0–6 months. Concentration and secretion rate of milk components analysed throughout the study period. Milk volume ranged from 717-801 mL/day. Free phosphate showed little change over 6 months.
Level IV	Butte et al (1984)	45 expectant mothers planning to breastfeed. No other comparative group. Documents breast milk intake and growth.
Level IV	Dewey et al (1984)	Milk intake assessed as 600 mL/day. Assuming milk phosphorus concentration of 124 mg/L , gives intake from human milk of 75 mg/day. Reports the breast milk composition of samples collected by 46 women from 7–20 months of lactation. Phosphorus analysis not done. Milk volumes reported as 782 mL/day for full lactation, 405 mL/day for partial weaning and 163 mL/day for weaned. 600mL/day appears to be the average for full and partial lactation.
Human experimental data	Atkinson et al (1995)	Shows phosphorus concentration in human milk after 1 month of lactation of 124 mg/L. Using intake of 780 mL/day, AI set at 100 mg/day.
Papers used to assess factors determining the phosphorus requirements of children		
Level III	Lutwak et al (1964)	Balance study on 18 girls aged 10 years allocated to 2 different diets – one supplemented with a high calcium and phosphorus bread (not clear if randomised from description). Used to estimate absorption efficiency at 60–80% from balance data.
Level IV (as reported)	Slemenda et al (1994)	3-year longitudinal study of 6–14 year-old children (n=90). RCT of calcium supplementation on bone mineral (previously reported). Reports changes in anthropometric data for the whole group. Estimates tissue accretion of phosphorus using both lean and osseous tissue gains from the data of Martin et al (1997) and Durenberg et al (1990). The use of whole body DXA data to estimate body composition from the same data set is preferable but such data are not available.
Human experimental data	Deurenberg et al (1990)	Cross-sectional study used to derive gains in lean mass, assuming a phosphorus content of 0.23%. Body composition of 208 females and 170 males aged 7–20 years assessed using underwater weighing (body density) and skin folds. Per cent body fat was subtracted from body weight to estimate lean mass. Not clear if corrected for bone mineral as this would fall into the lean tissue by underwater weighing. The DRI document refers to pubertal girls aged 10.5 years (who should, in fact, be pre-pubertal
Human experimental data	Ellis et al (1997)	Used to derive known increments in BMC on 313 multiethnic females aged 3–18 years with DXA. Value of 19% by weight used as the phosphorus content of bone. Daily accretion of phosphorus in bone calculated from cross-sectional measures of whole body BMC in children.

Level of evidence	Reference	Study type, issues addressed and key findings
Human experimental data	Fomon et al (1982)	Phosphorus content of body tissue from body composition data. Values of 19% and 0.23% by weight used as the phosphorus content of bone and lean tissue, respectively. Based on reference data using total body water and total body potassium and 50th percentile figure for height and weights from NCHS National Center for Health Statistics.
Human experimental data	Greger et al (1978)	Balance study used to estimate absorption efficiency. Examined mineral balance in 14 girls 12.5–14.5 years fed 2 different levels of zinc and soy with 1.07 g calcium.
Human experimental data	Martin et al (1997)	Assumes a phosphorus content of bone mineral of 19%. Cross-sectional analysis of the peak bone mineral content (320 g/year for boys and 240 g/year for girls). The BMC was measured once a year and the values represent those at peak accrual only.
Papers used to assess factors determining the phosphorus requirements of adults		
Level IV	Grimm et al (2001)	Crossover design. Assessed the affects of a 6-week high phosphorus diet (3,008 mg/day) on bone markers in 10 young women. No changes in serum phosphorus level but supplementation caused intestinal distress, mild diarrhoea. No changes in renal function or bone markers.
Human experimental data	Heaney & Recker (1982)	Reference for the absorption efficiency of 60–65% for phosphorus. According to the DRI text the variation in absorptive performance in adults is narrow. Balance data on 170 pre-menopausal women are presented for calcium but not phosphorus. Therefore it appears the reported value for absorption efficiency is either an estimate or the authors provided the original data. Shows that increasing phosphorus levels had little effect on calcium balance.
Human experimental data	Wilkinson (1976)	Phosphorus absorption efficiency is 60–65%.
Human experimental data	Stanbury (1971)	Phosphorus absorption efficiency is 60–65%.
Papers used to assess the phosphorus requirements in pregnancy and lactation		
Level III-3	Chan et al (1982)	Partially randomised. Used for evidence that serum phosphorus levels are higher in lactating women. Reports effects of calcium and bone mineral status of lactating mothers vs mothers who were formula feeding. Breast-fed infants randomised to vitamin D or control. Standard formula used. No difference in phosphate levels between groups and no changes during lactation.
Level III-3	Kalkwarf et al (1996)	Effects of differing calcium levels on intestinal calcium absorption during lactation. 96 women divided into 24 lactating and 24 non-lactating at each of 4.6 and 9.6 months and followed for 6 months. Half of the women in each feeding group received calcium vs placebo. Serum phosphorus was significantly higher in lactating vs non-lactating women. No differences between groups after weaning.
Level III-3	Lopez et al (1996)	Longitudinal study of 30 women to examine changes in BMD and bone turnover during and after lactation. Controls were 26 non-nursing women. Serum phosphorus significantly higher in lactating vs non-nursing women but returned to normal 6 months post-weaning.
Level III-3	Specker et al (1991)	Prospective comparison of 26 lactating and 32 non-lactating post-partum controls over 1st year post-partum. Over 1st 6/12 months, serum phosphorus and parathyroid hormone decreased with time, but levels were similar among women who were weaning vs those who had not weaned, and higher than those who had never lactated. Serum phosphorus not associated with phosphorus intake.
Level IV	Byrne et al (1987)	Compares calcium status in 8 older lactating women at 2 and 6 weeks with 8 nulliparous women. Shows serum phosphate levels were higher in lactating women. Note: The DRI document states currently no evidence that lactation increases phosphorus requirements.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Cross et al (1995)	Reference used to confirm that serum phosphorus levels during pregnancy are within the normal range at intakes of 1,550 mg/day (assessed by 6-day food records). Followed 10 women and examined calcium regulatory hormones and bone markers. Post-weaning levels of 1.03 mmol/L were reported in 8 lactating women followed throughout pregnancy. Reports serum phosphorus levels.
Level IV	Dobnig et al (1995)	Relationship between parathyroid hormone and serum markers of bone turnover and BMD in 35 women post-partum until 6 months. Data on those beastfeeding to 3 and 6 months compared to those who stopped. Serum phosphorus level differed at 3 months of lactation from control only. Longer term lactation (6 months) led to a significant decrease in radial cancellous bone density and to elevations in serum markers of bone resorption and formation.
Level IV	Kent et al (1990)	Shows significantly higher plasma P_i in 40 lactating vs 40 control women, associated with renal P_i conservation. Postweaning levels returned to normal. Shows increased bone turnover despite renal conservation of calcium and P_i. Longitudinal weaning data for 26 women shows a normalisation of fasting plasma P_i.
Level IV	Kent et al (1991)	The fractional absorption of calcium was measured in 26 control women, 49 women in the last trimester of pregnancy and 31 of these women in established lactation. Compared with controls, the fractional absorption of calcium was significantly elevated in late pregnancy, but not in established lactation.
Human experimental data	Heaney & Skillman (1971)	Calcium balance and kinetics on 15 pregnant women (during and after pregnancy) and 9 controls. Phosphorus balance significantly correlated with stage of pregnancy. DRI paper reported that net phosphorus absorption was 70% and phosphorus absorption increases by about 10% during pregnancy. These data are not reported in the paper but the authors may have provided the raw data to allow these calculations.

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POTASSIUM

The recommendations for potassium were derived after consideration of the FNB:IOM (2004) review of DRIs for the US and Canada, taking into account recommendations from other key countries and health authorities. As there are limited data, AIs rather than EARs and RDIs were set for all age and gender groups. The AIs were based on breast milk concentrations for infants and on median population intakes for other age and lifestyle stages.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The FNB:IOM used experimental data in men in relation to sodium blunting and occurrence of renal stones to set their AI for adults and extrapolated this to other age groups. The data used by the FNB:IOM to assess the optimal level for the sodium blunting effect was primarily related to African American males with high sodium sensitivity (Morris et al 1999) and therefore considered insufficient to set requirements for all age and gender groups in Australia and New Zealand. Thus the AIs for adults, children and adolescents were based on the population intake in Australia and New Zealand. For adults, the intake of the highest consuming age band was used. Data relating to renal stone formation also used by the FNB:IOM in setting the AI were included in the consideration for the SDT for reduction of chronic disease.

EVIDENCE BASE

Databases: Medline and search of cross-references and references in the relevant FNB:IOM report (FNB:IOM 2004).

Search terms: Potassium, human, blood pressure, toxicity.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the effect of potassium on sodium blunting		
Level I	Whelton et al (1997)	Meta-analysis of 33 RCTs. Potassium lowers blood pressure and "blunts" the effect of sodium chloride on blood pressure, mitigating salt sensitivity and lowering urinary calcium excretion.
Level III-3	Morris et al (1999)	Metabolic study of 38 men. Basal diet of low potassium, then supplementation with potassium. Salt sensitivity, measured before and after, was blunted at 4.7 g/day in African American men and 2.7 g/day in white males.
Papers used to assess the effect of low potassium on mineralisation of bone		
Level III-2	New et al (1997)	The association between dietary intake and BMD investigated in 994 healthy premenopausal women. In those with higher intakes of potassium, lumbar spine BMD was significantly higher. High long-term intake of potassium may be important to bone health, possibly because of beneficial effect on acid-base balance.
Level III-2	Tucker et al (1999)	Investigated associations between dietary potassium and BMD in elderly subjects. Greater potassium intake was significantly associated with greater BMD at 4 sites for men and 3 sites for women. Greater intakes of potassium were also each associated with less decline in BMD at 2 hip sites. There were no significant associations between baseline diet and subsequent bone loss in women. Results support the hypothesis that alkali-producing dietary components, including potassium, contribute to maintenance of BMD.

Level of evidence	Reference	Study type, issues addressed and key findings
Level IV	Maurer et al (2003)	Studied effects of neutralisation of dietary acid load in 9 healthy subjects under metabolic balance conditions on calcium balance, bone markers and endocrine systems relevant to bone. Neutralisation for 7 days induced a significant cumulative calcium retention and significantly reduced the urinary excretion of deoxypyridinoline, pyridinoline, and n-telopeptide. No significant effect was found on free IGF-1, PTH/1,25(OH)(2) vitamin D, or thyroid hormones. Concludes that an acidogenic western diet results in mild metabolic acidosis in association with a state of cortisol excess, altered divalent ion metabolism and increased bone resorptive indices.
Level IV	Sebastian et al (1994)	Administered oral potassium bicarbonate to 18 post-menopausal women on a constant diet of calcium and protein for 18 days in doses that nearly completely neutralised the endogenous acid. Potassium bicarbonate improved calcium and phosphorus balance, reduced bone resorption and increased the rate of bone formation.

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SELENIUM

The recommendations for selenium were derived after consideration of the FNB:IOM (2000) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. An expert review of recommendations relevant to Australia and New Zealand (Thomson & Paterson 2001) and an evidence-based review of several international recommendations (Flight & Baghurst 2003) were also available to the committee. EARs and RDIs were set for most age and gender groups based on results of the experimental studies of Duffield et al (1999) in New Zealand.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

The recommendations in this report differ from those of the US: Canadian review in a number of ways detailed below.

A. AI OF INFANTS

The US:Canadian recommendations are based on the concentration of selenium in breast milk in the US (averaging 18 µg/L). The data used for the Australian:New Zealand recommendations were based on three studies undertaken in Australia and New Zealand. (Daniels et al 2000, Cumming et al 1992, Dolamore et al 1992) which give lower estimated values of 11–15 µg/L for Australia and New Zealand.

B. EARs AND RDIs OF ADULTS

The US:Canadian recommendations were based on consideration of two intervention studies, one in China (Yang et al 1987) and one in New Zealand (Duffield et al 1999), that assessed the effects of supplementation on plasma GP_X activity. Both studies supplemented subjects (about 10 per group) with varying levels of selenomethionine for 5 and 8 months and assessed at what intake (diet + supplement) the plasma GP_X plateau was reached (Table 5).

TABLE 5. INTERVENTION STUDIES USED TO DERIVE EARS IN THE US:CANADIAN DRI REVIEW

Study	Subjects	Dietary intake (µg/day) & design	Seleno-methionine (µg/day)	PI GP_X Plateau	Intake used for EAR estimation (µg/day)
Yang et al 1987 China	Males: 18–42 years n=8–9/group allocation unknown	11 Observer record, direct measure local food	10, 30, 60 and 90 8 months	30, 60 and 90 converged at 4 (7?) months α30 µg/day gives plateau (Baseline GP_X 35% plateau values after suppl + 200 µg/day inorganic, ie depleted)	11 + 30 = 41
Duffield et al 1999 NZ	Males: 17 Females: 35 19-59 years n=10–11 random allocation	28 Duplicate portion method	10, 20, 30 and 40 20 weeks	Authors reported plateau at 40 µg/day US re-analysis suggested plateau at 10µg/day	28+40 = 68 28+30 = 58

The Australian/New Zealand committee decided to disregard the Yang et al (1987) data for the following reasons:

- The study is not published in a peer reviewed journal
- The quality of the paper is not high
- Total n=40–45 males with 8–9 per group, no details of selection or allocation, no indication of blinding
- No clear description of methods
- No statistical analysis reported. Results are based on visual inspection of figures that contain no error bars (which suggest that there is a stronger case for a plateau for erythrocyte GP_X than plasma levels, although the author concludes the opposite)
- The figures present a data point at each month, but it is not clear what these represent
- Subjects are from a low selenium area of China with a 70% cereal subsistence diet. This population is very different to those of Australia and New Zealand in terms of selenium status. There is a large range of potential confounders and the generalisability of the data is likely to be poor
- The US:Canadian DRI review claims that the groups converge at 4 months while the authors claim 7 months. The control and + 10 µg/day group do not appear to be clearly separated and less than the other three supplement groups as claimed.

The study by Duffield et al (1999) was the only remaining good quality study, but it was subject to interpretational difficulties. A number of authors suggest that the GP_X plateau is reached when the correlation between blood and plasma selenium and GP_X drops. Table 6 shows the Duffield et al (1999) correlations between plasma and blood selenium and GP_X. The plasma data suggest that the plateau occurs at around +20 to +30 µg/day. The whole blood data suggest it occurs at around 10 µg/day. Duffield et al (1999) reported that plasma GP_X was only 5% of blood GP_X.

TABLE 6. PLASMA AND BLOOD SELENIUM AND GP_X

Selenium:GP_X ratio	Selenomethionine supplement (µg/day)				
	0	10	20	30	40
Plasma selenium:GP_X	0.88	0.28	0.29	0.08	0.08
Blood selenium:GP_X	0.63	0.64	0.33	0.26	0.35

[From Duffield et al (1999)]

The US:Canadian DRI review claimed that the plateau occurs at a supplemental level of +10 µg/day, the level consequently used for their estimates. The US:Canadian DRI report gives no details of the statistical analysis undertaken on the Duffield et al (1999) data other than to say regression analysis was used and *'the increase at the lowest level tested could not be statistically differentiated from the increase at the highest level tested'*. Ten µg/day was said to be chosen 'conservatively'. It was not confirmed that the GP_X plateau occurred statistically.

For estimating the EAR, the Australian/New Zealand committee decided to use the level of + 25 µg/day, a value at which both the plasma and blood selenium to GP_X ratios would have plateaued. The Selenoprotein P data from Duffield et al (1999) also support a plateau at about 25–30 µg/day. Selenoprotein P accounts for 44–70% of total selenium in plasma (Hill et al 1996, Patching & Gardiner 1999) and correlates very strongly with GP_X in depleted subjects (the correlation is about 0.9) (Hill et al 1996) but appears to be saturated at lower selenium concentrations than GP_X (Duffield et al 1999).

The 25 µg/day of supplemental selenium in the Duffield study plus the 28 µg/day of selenium from food gives a total intake of 53 µg/day which was adjusted to 55 µg/day for men and 44 µg/day for women to account for body weight differences between subjects in the study (mean weight 73.7 kg) and standard body weights for men and women of 76 kg and 61 kg, respectively.

A well designed study by Xia et al (2005) in China of people with pre-existing low selenium status showed that full expression of GP_X was achieved with a selenium supplement of 37 µg/day in the form of selenomethionine and with 66 µg/day supplement as selenite, with a background dietary intake of 11 µg/day for men and 9 µg/day for women. Thus, using selenomethionine, a total intake in this group of about 47 µg/day was necessary for expression of GP_X. Using selenite, which is much less bioavailable, 76 µg/day was necessary. Body weights in this study averaged 58.4 kg for males and 53.3 kg for women. Adjusting for the reference body weights of 76 kg for men and 61 kg for women, this would give a value of 61 µg/day for men and 53.8 µg/day for women for selenomethionine, slightly greater than the corresponding data from New Zealand. However, full expression of selenoprotein P was not achieved at the highest dose of either form (61 µg as selenomethionine or 66 µg as selenite). The authors suggested that selenoprotein P may be a better marker for selenium status, but the validity of this is unclear as yet.

A lower quality study from China (Yang et al 1987) gave a requirement adjusted to a 76 kg body weight of 52 µg/day which is very close to that of Duffield et al (1999), as interpreted above.

Taking into account the studies of Duffield et al (1999) and Xia et al (2005), an EAR of 58 µg/day is estimated for men (average of 55 and 61 µg/day) and 49 µg/day for women (average of 44.0 and 53.8 µg/day). This was rounded to 60 µg/day for men and 50 µg/day for women.

The RDI was then set assuming a CV of the EAR of 10% to give an RDI after rounding of 70 µg/day men and 60 µg/day for women.

C. EAR AND RDIs OF CHILDREN AND ADOLESCENTS

The recommendations for EARs and RDIs for children and adolescents vary from the US:Canadian DRI values as they were extrapolated from the adult recommendations on a metabolic body weight basis.

D. EARs AND RDIs IN PREGNANCY

The US:Canadian DRI review used the data of Schroeder et al (1970) to estimate a need for an additional 4 µg/day in pregnancy due to foetal needs, based on a muscle selenium content of 240 µg/kg wet weight. Three other papers were not considered in the US review. One New Zealand study by Casey et al (1982) gave a lower figure for selenium in muscle of 79 µg/kg wet weight. Oster et al (1988) reported a figure of 111 µg/kg wet weight in skeletal muscle from studies in Germany and a study by Zachara et al (2001) from Poland gave a muscle content of 51 µg/kg. Poland is a low selenium area with reported intakes of 40 µg/day, similar to the median intake of 46 µg/day in New Zealand (National Nutrition Survey 1999). From Market Basket Surveys (Fardy et al 1989), the Australian intake is thought to be about 70 µg/day. The US intake is thought to be between 80 and 206 µg/day (Pennington and Schoen 1996, NHANES III as reported in FNB:IOM 1997). Dietary selenomethionine is non-specifically incorporated into skeletal muscle after saturation of selenoproteins, therefore the higher content seen in the US study probably does not reflect requirement, but simply reflects their higher intakes. Using total body selenium burden µg/kg data (rounded up) from the New Zealand, German or Polish studies to extrapolate to a 4 kg foetus and distributing across 270 days of pregnancy would give an additional daily requirement of between 1.1 µg/day or 1.5 µg/day. Accordingly, 2 µg/day was added to the EAR for non-pregnant women and rounded up to the nearest 5 µg. Several countries assume that any additional requirement in pregnancy can be met by an adaptive increase in absorption (Scientific Committee EU 1993, UK COMA 1991, Netherlands Food and Nutrition Council 1989).

In developing EARs and RDIs, the US report also claimed greater susceptibility in women to selenium deficiency, based on Chinese data (Ge et al 1983, Cheng & Qian 1990). This is a narrative review of epidemiological studies in China with no details of methodology. Ge et al (1983) actually cite children below 10 years as being most susceptible and women of childbearing age being 'involved'. Even if the prevalence were demonstrably lower in men, there may be a range of cultural and social gender-based issues around food access, and hence differences in selenium intake that may explain the differences in prevalence.

In the last 20 years, cases reported in China have been limited to children only, with no differences between boys and girls (Cheng & Qian 1990). This paper provides prevalence data in a population with no gender or age data. It also reports data from a study of only "children" for which no gender data are given. The study ended in 1986 and there are no adult or gender data, so the claim that women have greater susceptibility does not appear to be supported.

There may be gender effects at very low intakes, but there is no evidence that women are more susceptible at current intakes. Thus no increased female susceptibility was assumed in setting the Australian/New Zealand requirements which were based on relative body weights. Note that in terms of cancer susceptibility, men may have a higher requirement for selenium and obtain greater benefit from selenium supplementation (Waters et al 2004).

E. LACTATION

As a different figure was used from the US:Canadian DRI for breast milk concentration, this changed the estimate of lactation requirements.

EVIDENCE BASE

Databases: Medline plus cross-referencing and review of key references in FNB:IOM DRI review, the expert report prepared for the NZ Ministry of Health (Thomson & Patterson 2001) and an evidence-based review of international recommendations prepared for the Australian Nutrition Trust (Flight & Baghurst 2003).

Search terms: Selenium, human, English.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the selenium requirements of infants		
Survey data	Cumming et al (1992)	Selenium concentration in human milk in Australia, 12 µg/L. Term infants, 6–12 weeks lactation (n=20).
Survey data	Daniels et al (2000)	Selenium concentration in human milk in Australia, 13 µg/L. 388 pooled expressed breast milk samples, range of lactation stages, from 68 mothers of preterm infants (samples collected in 1992). [also Daniels (unpublished data)]11µg/L. Term infants, 16 weeks lactation, 2002 (n=39).
Survey data	Dolamore et al (1992)	Selenium concentration in human milk in South Island NZ, 13.4 µg/L. Suggest a level of 15 µg/L to account for higher levels in the North Island.
Survey data	Levander et al (1987)	Selenium concentration in human milk in US, 15–20 µg/L.
Survey data	Mannan & Picciano, (1987)	Selenium concentration in human milk in US, 15–18 µg/L.
Survey data	Smith et al (1982)	Selenium concentration in human milk in US, 15–18 µg/L.
Survey data	Traflikowska et al (1996)	Selenium concentration in human milk in Poland,9.2 µg/L. 3–4 weeks lactation (n=16).
Survey data	Zachara & Pilecki (2001)	Selenium concentration in human milk in Poland, 10.5 µg/L. 14–58 days lactation (n=352).
Papers used to assess the relationship between dietary intake of selenium and plasma/blood GP_X		
Level II	Duffield et al (1999)	Gives the total intake of selenium (supplement + diet) required to give plasma GP_X plateau. Plateau difficult to define, but level of +25 µg (total of 53 µg/day) covers both blood and plasma data.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Xia et al (2005)	Supplemental trial in China. People with pre-existing low selenium status. Full expression of GP_X achieved with 37μg selenium/day supplement as selenomethionine and 66 μg/day as selenite. Background dietary intake of 11 μg/day for men and 9 μg/day for women. Full expression of selenoprotein P was not achieved at the highest dose of either form (61 μg as selenomethionine or 66 μg as selenite). The authors suggested that selenoprotein P might be a better marker for selenium status.
Level III-3	Xia et al (1989)	Men GP_X 37% in Keshan disease (KD) area compared to non-KD area. Max plasma GP_X as criterion.
Level III-2	Yang et al (1987) Study B	Total intake (supplement + diet) giving a plasma GP_X plateau, men 18–42 years, selenium intake 11 μg. Added 0, 10, 30, 60 and 90 μg for 8 months. With weight adjustment would give requirement of 52 μg/day for 76 kg body weight.
Expert review	Thomson (2004a)	Most countries base their recommendations on measures of GP_X and other blood measures, in response to varying intakes of selenium.
Papers used to assess gender differences in selenium requirements		
Level III-2	Cheng and Qian (1990)	Cases of KD over last 20 year limited to children. US DRI review claims greater susceptibility of women based on this, but claim not supported by data.
Expert Review	Waters et al (2004)	Results from cohort studies conducted in seven countries (Belgium, China, Finland, Japan, The Netherlands, Norway and the US) were used to assess the strength of association between low selenium status and the incidence of all cancers, sex-specific cancers and cancers at particular anatomic sites. In general, the available data support the hypothesis that cancer risk in men is more influenced by selenium status than is cancer risk in women.
Papers used to assess the selenium content of muscle for estimating foetal accretion		
Survey	Casey et al (1982)	Dry weight selenium concentration of skeletal muscle to extrapolate to whole body selenium and then to selenium content and accretion of a 4-kg foetus. Selenium in muscle of 79 μg/kg wet weight.
Survey	Oster et al (1988)	Total body selenium in healthy adult cadavers, Germany. Used for extrapolation to selenium content of foetus. Reports on selenium content of 13 different tissues with a figure of 111 μg/kg wet weight in skeletal muscle.
Survey	Schroeder et al (1970)	Selenium content of muscle tissue used as basis to estimate total body selenium content and selenium accretion of 4-kg foetus during pregnancy. Body selenium content of 240 μg/kg.
Survey	Zachara et al (2001)	Total body selenium in healthy adult cadavers, Poland. Used for extrapolation to selenium content of foetus. Muscle content of 51 μg/kg.
Papers used to assess potential adverse effects of selenium		
Survey	Longnecker et al (1991)	Used to estimate NOAEL; based on endpoints of hair and nail brittleness and loss. Confirms no clinical evidence of toxicity with highest intake of 789 μg/day (Average 240 μg/day) in high selenium area of US (n=142).
Survey	Yang & Zhou (1994)	Used to support NOAEL; based on endpoints of hair and nail brittleness and loss. NOAEL of 600 μg/day.
Level I	Duffield-Lillico et al (2003)	Double-blind, placebo-controlled RCT to test whether selenium could prevent non-melanoma skin cancer in 1,312 patients who had previously had this disease. Results showed that selenium supplementation is ineffective at preventing basal cell carcinoma and that it increases the risk of squamous cell carcinoma and total non-melanoma skin cancer.
Paper used to assess at what level of selenium Keshan Disease appears		
Survey	Yang & Xia (1995)	Minimum intake to prevent KD, 16.2 μg/day × 1.3 (safety factor) = 21 μg/day.
Paper used to assess current intake and selenium status in Australia and New Zealand		
Expert Review	Thomson (2004b)	The selenium status of New Zealanders has increased, but remains low compared with populations of many other countries and may still be considered marginal, although the clinical consequences of the marginal selenium status are unclear. There are no recent reports of blood selenium levels in Australians, but earlier reports indicate that they are generally greater than those of New Zealanders.

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SODIUM

Note: The body of evidence and recommendations (UL and SDT) for adults were updated in 2017. For further information see the NHMRC Guidelines and Publications Page.

The 2006 recommendations for sodium were derived after consideration of the FNB:IOM (2004) review of DRIs for the US and Canada and recommendations from other key countries and health authorities. As there are limited data, AIs rather than EARs and RDIs were set for all age and gender groups.
The AIs were based on breast milk concentrations for infants and on experimental and epidemiological data in adults related to physiological needs and, for the UL, blood pressure outcomes.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

In setting the AIs, it was felt that the data available indicated somewhat lower requirements than those identified by the US:Canadian DRI review, which itself recognised the interpretational difficulties in setting an AI for sodium. The AI for sodium was set to cover basic physiological needs and take into account temperature variations and, given the widespread of sodium in the basic food supply, nutrient adequacy issues for the diet as a whole. The UL was the same as that for the FNB:IOM recommendations (2,300 mg or 100 mmol/day). However, an additional value of 1,600 mg or
70 mmol/day was given as an SDT for maintenance of low blood pressure throughout life for the population as a whole and in particular for older and overweight members of the population with hypertension who may derive immediate benefits from further reductions in salt intake below the UL.

In terms of the feasibility of being able to consume diets conforming with the AI range of 460–920 mg/day (20–40 mmol/day) in adults, data from the Hobart salt study showed that a systematic sample of Hobart people (n=94) recruited from the electoral role and instructed in 24-hour urine collection (without mentioning salt) included two women and one man who excreted between 20 and 40 mmol of sodium in samples of normal urine volume and normal potassium and creatinine content (Beard et al 1997). A diet limited to low salt foods by the definition of the Australian:New Zealand Food Code (sodium ≤120 mg/100 g) provides sodium excretion rates of <50 mmol/day for men and <40 mmol/day for women. Both sexes sometimes excreted less than 20 mmol in one of their collections (Beard et al 1997 and unpublished data).

Most foods in their natural state have a sodium content of well under 100 mg/100 g. However, it is recognised that current intakes in Australia and New Zealand greatly exceed both the AI and UL. Achieving a reduced sodium intake in the population will be an incremental process, requiring changes in individual behaviour towards salt consumption, increased intake of foods such as vegetables and fruits which are relatively low in sodium, and replacement of high salt foods with acceptable lower
salt versions.

EVIDENCE BASE

Databases: Medline, PubMed and search of cross-references and references in the relevant report (FNB:IOM 2004).

Search terms: sodium, salt, blood pressure, cardiovascular disease

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the level of sodium in breast milk		
Survey data	Dewey & Lonnerdal (1983)	20 women. 1 month post-partum, 0.23 g/L; 2 months post-partum, 0.26 g/L; 3 months post-partum, 0.18 g/L; 4 months post-partum, 0.18 g/L; 5 months post-partum, 0.17 g/L; 6 months post-partum, 0.13 g/L.

Level of evidence	Reference	Study type, issues addressed and key findings
Survey data	Gross et al (1980)	18 women, 1 month post-partum 0.20 g/L.
Survey data	Keenan et al (1982)	14 women, 3.5–6 weeks post-partum, 0.18 g/L. 14 women, 8.5–18 weeks post-partum, 0.11 g/L. 12 women, 20–32 weeks post-partum, 0.12 g/L.
Survey data	Lemons et al (1982)	7 women, 1 month post-partum, 0.16 g/L. 13 women, 1.5 months post-partum, 0.20 g/L-preterm. 9 women, >2 months post-partum, 0.16 g/L-preterm.
Survey data	Morriss et al (1986)	52 women 3 weeks post-partum, 0.17 g/L.; 5 month post-partum 0.11 g/L.
Survey data	Picciano et al (1981)	26 women. 1 month post-partum, 0.15 g/L; 2 months post-partum, 0.12 g/L; 3 months post-partum, 0.13 g/L.
Paper used to assess the levels of sodium consumed by healthy populations		
Survey data	Rose et al (1988)	Intersalt study showed intakes in "healthy" populations (0–1% hypertension) from 0.02 mmol (Yanomama people) to 27 mmol (Asaro people) beyond which population blood pressure rose. At 51 mmol/day (Rambugu and Ndori people) hypertension was 5–6% and in populations at 90–250 mmol/day (most of sample), 8–35%. Median of about 20%. The relationship was exponential suggesting that at 40 mmol/day hypertension prevalence would be about 2%.
Papers used to assess the sodium intakes affecting blood pressure in controlled trials		
Level II	Sacks et al (2001)	Analyses from the DASH-Sodium Trial: Effect of sodium level on systolic and diastolic blood pressure in 412 normotensive and hypertensive participants. Sodium levels defined as higher, 3.5 g/day (150 mmol/day); intermediate, 2.3 g/day (100 mmol/day) and lower, 1.2 g/day (50 mmol/day). Drop in blood pressure about twice as much in transition from intermediate to lower compared to higher to intermediate.
Level III-2	Bruun et al (1990)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day). Each sodium level was provided daily for 4 days: 1.2 g (50 mmol); 4.1 g (180 mmol); 8.7 g (380 mmol). 10 normotensive men and women, no significant differences and 12 hypertensive men and women, no significant differences.
Level III-2	Ferri et al (1996)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day) among 61 hypertensive men. Each sodium level was provided daily for 2 weeks: 0.46 g (20 mmol); 3.2 g (140 mmol); 7.4 g (320 mmol). Significant difference ($p<0.05$) from highest to intermediate, not significant from intermediate to lower intake.
Level III-2	Fuchs et al (1987)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day). Among 11 normotensive men and women with a family history of hypertension, each sodium level was provided daily for 9 days: 3.7 g (16 mmol); 2.6 g (111 mmol); 5.5 g (239 mmol). No significant differences. Among 6 normotensive men and women without family history of hypertension each sodium level was provided daily for 9 days: 0.18 g (8 mmol); 2.4 g (103 mmol); 5.6 g (245 mmol). No significant differences.
Level III-2	He et al (1999)	Prospective cohort study (14,407 participants), including 2,688 overweight and 6,797 non-overweight persons. Concluded that high sodium intake is strongly and independently associated with an increased risk of CVD and all-cause mortality in overweight persons.
Level III-2	Johnson et al (2001)	Blood pressure (mmHg) according to sodium dose in g/day (mmol/day). Each sodium level was provided daily for 2 weeks: 0.92 g (40 mmol), 2.1 g (90 mmol), 3.2 g (140 mmol), 5.5 g (240 mmol) 7.8 g (340 mmol). ANOVA simultaneously comparing the 4 pair-wise blood pressure differences between the lowest sodium level (baseline) and each of the 4 higher sodium levels. Among 8 systolic diastolic hypertensive elderly subjects, systolic, significant ($p<0.01$); diastolic, significant ($0.01\leq p<0.05$). In 15 isolated systolic hypertensive elderly subjects, systolic significant ($0.001\leq p<0.01$); diastolic, significant ($0.01\leq p<0.05$). In 17 normotensive elderly subjects, systolic, significant ($0.001\leq p<0.01$); diastolic, significant ($0.01\leq p<0.05$).

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Kirkendall et al (1976)	Mean supine blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day) among 8 normotensive men. Each sodium level was provided daily for 4 weeks: 0.23 g (10 mmol); 4.8 g (210 mmol); 9.4 g (410 mmol). No significant differences.
Level III-2	Luft et al (1979)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day) among 14 normotensive men. Each sodium level was provided daily for 3 to 7 days. Significant difference between 10 and 800 mmol/day ($p<0.05$).
Level III-2	MacGregor et al (1989)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day) among 20 hypertensive men and women. Each sodium level was provided daily for 4 weeks 1.1 g (50 mmol); 2.3 g (100 mmol); 4.6 g (200 mmol). Significant ($p<0.01$) from highest to intermediate and from intermediate to lower intakes.
Level III-2	Roos et al (1985)	Blood pressure (mmHg) according to dietary sodium intake; 8 normotensive men and women. Each sodium level was provided daily for 5 days: 0.46 g (20 mmol); 4.6 g (200 mmol); 25.9 g (1,128 mmol). Not significantly different.
Level III-2	Sullivan et al (1980)	Blood pressure (mmHg) according to dietary sodium intake in g/day (mmol/day) among 6 normotensive subjects at risk of hypertension. Each sodium level was provided daily for 4 days: 0.23 g (10 mmol); 4.6 g (200 mmol); 9.2 g (400 mmol). Significant drop from highest to median level, no other significant drops.

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ZINC

The recommendations for zinc were derived from an assessment of the evidence base used by the US:Canadian Government DRI review of 2001 (FNB:IOM 2001) and consideration of additional key papers missing from that review or published since it was released, and current recommendations of other key countries and organisations such as the FAO:WHO and IZiNCG (International Zinc Nutrition Consultative Group).

A factorial approach was used in FNB:IOM (2001) to estimate requirements for absorbed zinc (ie physiological requirements) for all age groups except infants aged 0–6 months, for whom an AI was set by the FNB:IOM based on observed mean intake of zinc from infants fed only human milk.

The physiological requirement for zinc was defined in FNB:IOM (2001) as the minimal amount of zinc that must be absorbed to offset the amount of endogenous zinc lost from both non-intestinal and intestinal sites. Also required were data on the additional zinc required for growth of children, accretion of zinc for maternal and foetal tissues during pregnancy and secretion in breast milk during lactation.

One major review of zinc metabolism and requirements was published by the International Zinc Nutrition Consultative Group (IZiNCG 2004) after completion of the FNB:IOM review. This review agreed with the approach taken by the FNB:IOM but provided new estimates of the relative absorption fractions for men, women and children. It also provided some new estimates of endogenous losses based on a consideration of a wider range of human studies that included men and women and assessed the relationship between total absorbed zinc and intestinal losses. For some of these estimates, studies that used formulated rather than real foods were excluded. The new IZiNCG estimates, appropriately adjusted for reference body weights, were used in deriving the new Australian:New Zealand recommendations as outlined below.

VARIATION(S) FROM THE FNB:IOM DIETARY REFERENCE INTAKE RECOMMENDATIONS FOR THE US AND CANADA

A. ABSORPTION FRACTIONS

The FNB:IOM used factors of 41% and 48% for absorption of zinc from a US and Canadian mixed diet for men and women, respectively. Note that a figure of 27% was used for pregnancy and lactation, despite a statement that zinc absorption in pregnancy and lactation did not differ from that of non-pregnant women. These absorption fractions were derived from 10 data sets based on whole diets from studies on men only. All of the diets used had a relatively low range of phytate:zinc molar ratios. Moreover, three of them were based on formula diets prepared from purified ingredients. The IZnICG (2004) calculated new and lower zinc fractional absorption estimates for both men and women derived from studies of whole diet only. These revised zinc absorption estimates are markedly lower, at 24% for men and 31% for women, at body weights approximating the standard NRV body weights and for diets with phytate:zinc molar ratios of 4–18 which encompass the Australian and New Zealand diet. The estimates of dietary zinc required to achieve a set level of absorbed iron have thus been amended in the calculations for the Australian and New Zealand NRVs for zinc for men and women, including pregnant women and for children and adolescents 14 years of age and over. As there is evidence of increased absorption in lactation, an additional 10% (as suggested by both FNB:IOM and IZiNCG) was added to the absorption fraction in lactation, bringing it to 41%.

B. VARIATION IN ESTIMATES OF ENDOGENOUS LOSSES

i Deletion of menstrual zinc loss from derivation for absorbed zinc

The figure used by FNB:IOM for menstrual loss of zinc was deemed to be too high by IZiNCG (2004), based on the data published in the paper of Hess et al (1977). In fact, the authors report a negligible zinc content for menstrual fluid loss for both users and non-users of oral contraceptive agents (OCAs). Hence, the contribution of menstrual zinc loss was ignored for the calculation of the EAR for adolescent girls and premenopausal women. This reduced the corresponding estimates for the requirements for absorbed zinc made by the FNB:IOM for these two life-stage groups by 100 µg/day, and, in turn, reduced both of the corresponding estimates for the EAR and RDI for zinc.

ii Intestinal losses

When reference body size differences were accounted for, the IZiNCG review (IZiNCG 2004) which included a wider range of studies than the FNB:IOM (FNB:IOM 2001) gave similar figures for non-intestinal losses but much lower estimates of intestinal losses. For men, the intestinal losses were estimated at 1.54 mg/day (vs 2.57 mg/day for the FNB:IOM) and, for women, 1.06 mg/day (compared to 2.30 mg/day for the FNB:IOM). As these data were extrapolated to intestinal losses for children and adolescents on a body weight basis, this variation in estimates affected all of the EARs and RDIs. For infants, the AI is based on the average zinc content of breast milk.

C. FOR FUTURE CONSIDERATION –UPPER LEVEL OF INTAKE FOR INFANTS

There is some concern that the ULs for infants aged 7–12 months (ie 5 mg zinc/day) and children aged 1–3 years (ie 7 mg zinc/day), estimated from supplementation studies, overlap with current dietary intakes in Australia and New Zealand. Lind et al (2003), in a double-blind RCT, showed that plasma copper does not differ between infants receiving 10 mg zinc/day or placebo. However, Bhandari et al (2002) reported lower plasma copper levels in 6–12 month-olds given 10 mg zinc/day and in 1–2.5 year-olds given 20 mg/day over 4 months.

In the Australian NNS 1995 survey reports, data were provided for children aged 2–3, 4–7, 8–11 and 12–15 years. The 50th, 75th and 90th centiles for adjusted zinc intakes (from diet alone) for children aged 2–3 years were 6.9, 7.9 and 9.1 mg/day, respectively, indicating that a large proportion of Australian children in this age group had zinc intakes greater than the UL. In New Zealand, one survey of 362 children with a mean age of 49±8 months, reported a mean zinc intake of 6.1 mg/day. Centile data were not provided. A study based on a random sample of New Zealand infants aged 6.0–8.9 months and 9.0–11.9 months from three cities in the South Island of New Zealand (Ferguson and Gibson unpublished data) reported mean zinc intakes for non-breast-fed infants of 4.8 mg/day at 6.0–8.9 months and 4.7 mg/day at 9.0–11.9 months compared to the UL for infants of 7–12 months of 5 mg zinc/day.

The form in which the zinc is consumed (supplemental vs diet) may underpin these apparent anomalies, but this possibility needs further research.

EVIDENCE BASE

Databases: Medline and search of cross-references and references in the relevant FNB:IOM report (FNB:IOM 2001).

Search terms: Zinc, absorption, human, men, women.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess the relationship between total absorbed zinc and intestinal losses of endogenous zinc		
Level III-3	Turnlund et al (1984)	Single-arm cohort crossover design. 4 men for 63 days. Zinc absorption and intestinal losses of endogenous zinc via faecal monitoring of ^{67}Zn excretion on 15 mg zinc/day without added phytate or cellulose. Absorption of 5.1 mg/day and losses of 2.7 mg/day from semi-purified formula.
Level III-3	Turnlund et al (1986)	Single-arm cohort crossover design. 6 young men for 12 weeks. Zinc absorption and intestinal losses of endogenous zinc via faecal monitoring of ^{67}Zn and ^{70}Zn excretion on15 mg/day. Absorption of 5.0 mg/day, losses of 3.8 mg/day from semi-purified formula.
Level III-3	Wada et al (1985)	Single-arm crossover design. 6 young men, 75 days. Mixed diets of 16.4 and 5.5 mg/day. Measured zinc absorption (4.1 and 2.7 mg/day, respectively) and intestinal losses of endogenous zinc (1.9 and 1.9 mg/day, respectively). Faecal monitoring of ^{67}Zn and ^{70}Zn excretion.
Level IV	Hunt et al (1992)	Single-arm cohort. ^{65}Zn isotope study of zinc absorption (3.1 mg/day), retention, and intestinal endogenous losses (1.6 mg/day) in mixed diet (14.0 mg zinc/day) in 14 men for 9 weeks. (Subjects lost weight).
Level IV	Jackson et al (1984)	Zinc absorption of 3.4 mg zinc/day and intestinal losses of endogenous zinc of 3 mg zinc/day when 1 male was fed a mixed diet (7.2 mg zinc/day). Via faecal monitoring of ^{67}Zn excretion.
Level IV	Lee et al (1993)	Single-arm cohort. Measured zinc absorption (2.4 mg/day) and intestinal losses of endogenous zinc (1.8 mg/day) when 8 subjects fed soy-protein-based diet (4.1 mg/day zinc) for 6 months. Phytate:zinc, 21) via faecal monitoring of ^{70}Zn excretion.
Level IV	Taylor et al (1991)	Single-arm cohort. 5 young men. Semi-purified formula (5.6 mg and 0.9 mg zinc/day). Measured zinc absorption (2.2 and 0.9 mg/day, respectively) and intestinal losses of endogenous zinc (1.9 and 0.8 mg/day, respectively) by faecal monitoring of ^{67}Zn excretion.
Papers used to assess the non-intestinal endogenous zinc losses, by source of loss		
Human experimental/ survey data	Hunt et al (1992)	Seminal zinc losses: 0.1 mg/day derived for 11 men from Johnson et al (1993). No change in seminal losses over 1.4–10.3 mg zinc/day. Ejaculate volume only decreased at lowest zinc intake (1.4 mg/day).
Human experimental/ survey data	Johnson et al (1993)	IOM figure (0.54 mg/day) derived for integumental plus sweat zinc losses in 11 men (from 1 study, 28–35 days). No change in losses over zinc intakes that encompass physiological needs (1.4 to 10.3 mg/day).
Expert opinion	FNB:IOM (2001) IZiNCG (2004)	17 studies. Urinary zinc losses for men of 0.63 mg/day based on intakes of 4–25 mg/day.
Papers used to assess the zinc requirement of adult women via factorial approach		
Human experimental/ survey data	Hess et al (1977)	Menstrual zinc losses. Data suggest losses are only 5 μg/day over menstrual cycle based on 5 non-OCA users and five OCA users. (No difference between OCA and non-OCA users). Note IOM used 0.1 mg/day based on these results. This figure is erroneous.
Human experimental data	Johnson et al (1993)	Integumental plus sweat zinc losses 0.46 mg/day for women. Based on figure for men (0.54 mg/day) and adjusted for body surface area of women (ie multiplied by 0.86).

Level of evidence	Reference	Study type, issues addressed and key findings
Expert opinion	FNB:IOM (2001) IZiNCG (2004)	Urinary zinc losses of 0.44 mg/day based on 10 studies of women.
Expert opinion	FNB:IOM (2001) IZiNCG (2004)	7 studies used were of men on the assumption that there are no gender differences. Intestinal excretion of endogenous zinc.
Papers used to assess the relationship between total absorbed zinc and intestinal losses of endogenous zinc		
Level III-3	Hunt et al (1995)	Single-arm cohort. 14 women. Measured zinc absorption (2.0 vs. 3.6 mg zinc/day) and intestinal losses of endogenous zinc (0.4 vs 0.9 mg zinc/day) on low meat (6.7 mg zinc/day) and then high meat (13 mg zinc/day) in a crossover design for 7 weeks. Phytate:zinc was 15 in all groups. Retention of ^{65}Zn measured by whole body counter.
Level III-3	Hunt et al (1998)	Single-arm cohort. 21 females fed a lacto-ovo-vegetarian diet (9.1 mg zinc/day) and then a mixed diet (11.1 mg zinc/day) in crossover design for 8 weeks. Measured zinc absorption (2.4 vs 3.7 mg zinc/day) and intestinal losses of endogenous zinc (0.8 vs 1.4 mg zinc/day). Phytate:zinc of 14 vs 5. Absorption via whole body counting of ^{65}Zn.
Level III-3	Knudsen et al (1995)	Single-arm cohort. 5 males, 3 females. Measured zinc absorption (3.1 mg zinc/day) and intestinal endogenous losses (2.6 mg zinc/day) on high fibre mixed diet (10.7 mg zinc/day). Faecal monitoring of ^{67}Zn and ^{70}Zn excretion vs whole body retention using ^{65}Zn for comparison.
Level III-3	Sian et al (1996)	Cohort with parallel design. Chinese women, 10 fed plant-based diets and 10 fed mixed diets (5.2 and 8.1 mg zinc/day, respectively). Measured zinc absorption (1.6 vs 2.8 mg zinc/day) and intestinal losses of endogenous zinc (1.3 vs 2.3 mg zinc/day). Phytate:zinc molar ratios of 11 vs 10). Faecal monitoring of oral ^{65}Zn excretion. IV ^{70}Zn also given.
Level IV	Lowe et al (1997)	Single-arm cohort. 6 women fed a mixed diet (7 mg zinc/day). Measured zinc absorption (2.0 mg zinc/day) and intestinal losses of endogenous zinc (2.0 mg zinc/day). Faecal monitoring of oral ^{65}Zn excretion. IV ^{70}Zn also given.
Papers used to assess the zinc requirements of infants aged 0–6 months		
Level II	Dewey et al (1999)	RCT (non-blinded) 4–6 months. Weight and length gain of term low birth weight infants exclusively breast-fed (n=59) between 4–6 months were the same as those given breast milk plus complementary foods (in jars) (n=60) 2 x daily from 4–6 months. Thus, amount of zinc in human milk is sufficient to support normal growth in low birth weight infants up to 6 months of age.
Level II	Krebs et al (1995)	RCT, double-blind. Provide data for zinc content of human milk at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 9 months post-partum (n=71). Not affected by zinc supplementation.
Level II	Walravens & Hambidge (1976)	RCT, double blind. Length and weight gain for male infants (n=19) at 6 months receiving zinc-supplemented infant formula (5.8 mg/L) significantly greater than males fed unsupplemented formula ie controls (n=18) (1.8 mg/L). No difference for females. IOM estimated zinc intake for controls was 1.4 mg zinc/day. Note: AI set at 2 mg/day.
Level II	Walravens et al (1992)	RCT. Growth rate of immigrant infants supplemented with zinc (5 mg/day) for 3 months was higher than placebo. Infants were not exclusively breast fed. Complementary foods may have displaced breast milk and/or interfered with zinc absorption from breast milk.
Level II or III-1	Heinig et al (1998)	RCT, limited detail available. No difference in growth patterns of exclusively breast-fed US term infants given placebo or zinc supplement from 4–6 months of age. Thus zinc intake from breast milk (plus any additional zinc from pre-existing hepatic stores) is adequate to support growth from 4–6 months of age.
Level III-1	Cohen et al (1994)	RCT, non-blinded, from 4–6 months. Breast-fed Honduran infants (n=141) fed complementary foods (rice-cereal, chicken and fruit from jars; low zinc ratio) twice daily between 4 and 6 months of age received twice as much zinc as those exclusively breast-fed. No significant difference in weight and length gain between groups. Data suggest that AI for zinc for 4–6 months is adequate for growth.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Krebs et al 1994)	Designed as RCT but data for two groups combined so became one-arm cohort study. Data on zinc intakes at 2 weeks (2.3 mg/day) and 3 months (1.0 mg/day) for exclusively breast-fed term infants (n=71) from test-weighing and zinc analysis of human milk. FNB:IOM assumed mean breast milk volume 0.78 L/day to calculate zinc intakes at 1, 2, 3 and 6 months.
Level IV	Krebs et al (1996)	Derived factorial estimates of zinc requirements at 1 month and 5 months for infants that were consistent with AI for infants of 0–6 months via measurements of zinc intake from human milk, fractional absorption and endogenous losses in 9 breast-fed infants aged 2–5 months. Used calculated losses for integumental and urine losses.
Expert review	FNB:IOM (2001)	12 studies provide data on zinc content of human milk up to 12 months of lactation. Shows that zinc content of human milk declines rapidly during first 6 months (Krebs et al 1985, 1994, 1995, Moser & Reynolds 1983)
Expert opinion	Krebs & Hambidge (1986)	Urinary zinc excretion rate of 10 μg/kg for full-term infants based on data from very low birth weight infants at 2 months postnatal age. Estimate confirmed by measurements on 3 breast-fed infants at 4, 7, 9 months. Integumental zinc losses of 10 μg/kg body weight based on adult data due to lack of data for infants. IOM used these 2 estimates.
Papers used to assess factors affecting the AI for zinc of infants aged 7–12 months and children aged 1–3 years		
Level II	Heinig et al (1998) Abstract	RCT. No difference in growth patterns of exclusively breast-fed US term infants given placebo or zinc supplement from 4–6 months of age. Provides evidence that zinc intake from breast milk (plus any additional zinc from pre-existing hepatic stores) is adequate to support growth.
Level II	Walravens et al (1992)	RCT. Growth rate (linear and ponderal growth) of immigrant infants 4–9 months of age (n=57) supplemented with zinc (5 mg/day) for 3 months was higher than placebo. Infants were not exclusively breast fed. Dietary zinc intakes and food sources of zinc not recorded. Complementary foods may have displaced breast milk and/or interfered with zinc absorption from breast milk.
Level III-2	Krebs et al (1995)	Designed as RCT but data for two groups combined so, it became single-arm cohort. Data on zinc intakes from human milk at 5 and 7 months. At 7 months zinc intake from human milk only 0.52 mg/day for breast-fed term infants (n=71). Based on data from test-weighing and zinc analysis of human milk. Therefore human milk alone inadequate to meet AI after 6 months.
Level III-3	Jalla et al (2002)	Parallel cohort study. Fractional absorption of zinc tended to be higher (but not significantly) for beef vs iron-fortified rice cereal CF (41 vs 36%) fed to 18 breast-fed male infants aged 7 months. Measured by faecal monitoring of isotope (^{70}Zn or ^{67}Zn) excretion.
Level IV	Abrams et al (1996)	Single-arm cohort. Fractional absorption of zinc from breast milk is 49.5% based on 14 breast-fed infants 5–7 months (also fed solid food) via urinary excretion of oral and IV isotopes, ^{70}Zn and ^{67}Zn.
Level IV	Davidsson et al (1996)	Single-arm cohort study. Fractional zinc absorption from wheat-based complementary food is 33.9% for 6 infants (18–30 weeks) fed cow milk formulas. Measured by faecal monitoring of stable isotope (^{70}Zn). FNB:IOM thus used 30% for fractional absorption of zinc from infants 6–12 month and 1–3 years.
Level IV	Fairweather-Tait et al (1995)	Single-arm cohort, crossover design. Fractional zinc absorption from vegetable-based complementary food is 28.6 and 31.1% without and with iron for 9-month-old infants (n=11) based on faecal monitoring of ^{67}Zn and ^{70}Zn excretion.
Human experimental & survey data	Krebs et al (1996)	Average intestinal excretion of endogenous zinc for infants 2–4 months fed human milk is 50 μg/kg/day. Measured endogenous faecal zinc on 7 infants by isotope dilution.
Survey	Widdowson & Dickerson (1964)	Growth. Tissue zinc content is 20 μg/g wet weight (lean tissue and fat) from chemical analysis of infants and adult cadavers. Mean weight of new tissue accretion is 13 g/day for 6–12 months and 6 g/day for 1–3 years.
Expert opinion	Krebs & Hambidge (1986)	Provides estimates for integumental and urine losses based on calculations.

Level of evidence	Reference	Study type, issues addressed and key findings
Papers used to assess EARs for zinc of adults and during pregnancy and lactation		
Level II	Caulfield et al (1999a)	RCT. Zinc supplement (15 mg/day) reduced decline in serum zinc at 28–30 and 37–38 weeks of gestation for Peruvian women (n=270) with zinc intake of 7 mg/day.
Level II	Caulfield et al (1999b)	RCT. Zinc supplement (15 mg/day) had no effect on birth size or gestational age in Peruvian women with mean zinc intake of 7 mg/day.
Level II	Goldenberg et al (1995)	RCT. Zinc supplementation (25 mg/day) of Afro-American women with mean zinc intake 13 mg/day (and plasma zinc < median) resulted in increases in both head circumference and birth weight, but predominantly in those with BMI <26.
Level II	Krebs et al (1995)	RCT double-blind study. Data on zinc concentrations of human milk (n=71) at 2 weeks (2.75 mg/L) and then at monthly intervals until 9 months. Data from zinc-supplemented and unsupplemented mothers. No effect of zinc supplement on zinc concentrations of milk.
Level III-1	Fung et al (1997)	Single-arm cohort study. Longitudinal with measurements made before conception and in lactation. Fractional zinc absorption increased during lactation from 14.6% at preconception to 25% 7–9 weeks post-partum in 13 women consuming 10 mg zinc/day based on urinary enrichments of dual-stable-isotope method (^{68}Zn and ^{70}Zn). If only include 9 women who did not take iron supplements, fractional zinc absorption was 15% vs 31% in 7–9 weeks of lactation.
Level III-2	Moser-Veillon & Reynolds (1990)	One-arm cohort study. Data on zinc concentrations of human milk (n=23) from 37-week post-partum through 6 months post-partum. No correlation between maternal zinc intake and breast milk zinc concentration.
Level III-2	Ortega et al (1997)	Prospective cohort study (n=57). Pregnant women (n=25) with zinc intakes <7.5 mg/day in 3rd trimester had lower zinc concentrations in mature human milk compared to those with intakes >7.5 mg/day (n=32) (28.7 vs. 33.1 µmol/L). Convenience sample.
Level III-2	Scholl et al (1993)	Prospective cohort study. Gravid women with dietary zinc intake ≤6 mg/day (n=116) had >3 fold increase in risk of preterm deliveries (<33 weeks).
Level III-3	Hambidge et al (1983)	Cohort with parallel design. Zinc supplement (15 mg/day) did not reduce decline in plasma zinc during pregnancy for US women consuming 13.1 mg zinc/day.
Level III-3	Sian et al (1996)	Cohort with parallel design. Fractional zinc absorption was 34% when 10 Chinese women fed mixed diet (8.1 mg zinc/day). Phytate:zinc molar ratio of 10. Measured by faecal monitoring of ^{70}Zn (IV) and ^{67}Zn (oral) excretion.
Level III-3	Turnlund et al (1991)	Single-arm cohort, crossover design. Fractional zinc absorption 27% when 8 young women fed rotating menu of animal-protein diet (10.9–12.3 mg zinc/day) or plant-protein diet (8.2–9.9 mg zinc/day) (adequate in vitamin B_6) measured by faecal monitoring of ^{67}Zn excretion.
Level IV	Fung et al (1997)	Single-arm cohort study. Fractional zinc absorption at preconception was 14.6% on average in 13 women consuming 10 mg zinc/day based on urinary enrichments of dual-stable-isotope method (^{68}Zn and ^{70}Zn).
Level IV	Fung et al (1997)	Fractional zinc absorption at 34–36 weeks gestation not significantly higher than preconception (19% vs 14%) in 13 women based on urinary enrichments of dual-stable-isotope method (^{68}Zn and ^{70}Zn).
Level IV	Hunt et al (1992)	Single-arm cohort study. Zinc absorption was 29% in 14 women fed a mixed diet (7.8 mg zinc/day) for 9 weeks. (Note: Subjects lost weight). ^{65}Zn isotope study, zinc retention measured.
Level IV	Hunt et al (1998)	Single-arm cohort study. Zinc absorption was 23% in 21 females fed a mixed diet (11.1 mg zinc/day) in crossover design for 8 weeks. Phytate:zinc of 5. Absorption via whole body counting (^{65}Zn).
Level IV	Kirksey et al (1979)	Single-arm cohort study. No correlation between maternal zinc intake and zinc concentrations of milk.
Human experimental data	August et al (1989)	Zinc absorption on zinc-adequate diet (2.8–5.0 mg + 10 mg supplement) was 39% in young subjects (n=9) measured by faecal monitoring using stable isotope methods.

Level of evidence	Reference	Study type, issues addressed and key findings
Exert opinion (Review)	Swanson & King (1987)	New estimates for mean rate of accumulation of zinc by maternal and embryonic and foetal tissues during four quarters of pregnancy as 1.2 (0.08 mg), 3.7 (0.53 mg), 8.1 (0.53 mg), and 11.2 (0.73 mg) μmol/day. Figures based on rate of tissue weight gain during gestation and tissue zinc concentrations.
Human experimental/ survey data	Sian et al (2002)	High fractional zinc absorption (53%) and low endogenous faecal excretion in 18 Chinese women at 2 months of lactation with zinc (7.6 mg/day). Measured by dual-isotope urine enrichment (IV ^{70}Zn and oral ^{67}Zn).
Survey data	Jackson et al (1988)	Increase in fractional zinc absorption in lactating women from the Amazon.
Survey data	Mares-Perlman et al (1995)	US nationally representative sample in 1975–1980. Mean zinc intake of Afro-American women from US NHANES II (1975–1980) reported as 8.5 mg/day (median = 7.2 mg/day). Pregnancy status not stipulated but not found to influence zinc intakes in other studies.
Survey data	Moser-Veillon et al (1996)	Abstract only. Increase in fractional zinc absorption in lactation in US women.
Survey data	Woodhouse et al (2000)	Abstract only. Higher fractional zinc absorption in lactating Brazilian women
Survey data	Yang et al (2000)	No relationship between maternal zinc intake and infant weight or height gain from birth in 1,956 pregnant and lactating Chinese women, despite low zinc intakes. Suggests that lactation performance of these women was sufficient to support infant growth, despite low zinc intakes. Not clear whether the situation is optimal.
Expert opinion	King and Turnlund (1989)	Estimate of amount of zinc available in lactation from zinc released from post-partum involution of uterus and decrease in maternal blood volume for first month (1 mg zinc/day).
Papers used to assess adverse effects of high zinc intake		
Level II	Bhandari et al (2002)	Double-blind RCT. Plasma copper reported to be lower in children given either 10 mg zinc/ day (for those 6–12 months), and 20 mg zinc/day (for those 1–2.5 years) for 4 months. Note: Results not separated by age group. Dietary zinc intake unknown.
Level II	Lind et al (2003)	Double-blind RCT. Plasma copper did not differ between infants receiving 10 mg zinc/day or placebo for 6 months. Note: Estimated zinc intake from supplement was 8.2 mg/day but dietary zinc intake not measured. Zinc supplement given between meals, possibly avoiding interference between supplemental zinc and absorption of copper from diet or endogenous intestinal losses.
Level II	Walravens & Hambidge (1976)	Double-blind RCT. No effect on serum copper or cholesterol concentrations of zinc-supplemented formula (5.8 mg vs 1.8 mg zinc/L) fed to 68 healthy full-term infants for 6 months. No other adverse effects. Note: Erythrocyte copper zinc superoxide dismutase (ESOD) not measured.
Level IV	Botash et al (1992)	Single case study. Clinical and biochemical features of copper deficiency induced by oral zinc supplement (16 mg zinc/day) for 6 months, and 24 mg zinc /day for 1 month) in a 13 month-old girl.
Level IV	Gibson et al (1989)	RCT. No effect of zinc supplement (10 mg zinc/day) ingested by 30 healthy Canadian boys (5–7 years) with height-for-age centiles <10th on serum copper or ESOD. Mean dietary zinc intake was 6.7 + 1.9 mg/day. No other adverse effects noted.

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NUTRIENTS AND CHRONIC DISEASE EVIDENCE BASE

MICRONUTRIENTS, DIETARY FIBRE & CHRONIC DISEASE

ANTIOXIDANT VITAMINS AND MINERALS

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in the relevant FNB:IOM report (FNB:IOM 2000).

Search terms: Vitamin C, ascorbic acid, vitamin A, retinol, αcarotene, vitamin E, tocopherol, selenium, antioxidants, coronary heart disease, cancer, diabetes, stroke, chronic disease.

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Eidelman et al (2004)	Assessed 7 large-scale randomised trials of the effectiveness of vitamin E in the treatment and prevention of CVD. Data available on myocardial infarction (MI), stroke, or cardiovascular death. 6 of the 7 trials showed no significant effect of vitamin E on CVD. In a meta-analysis, vitamin E had neither a statistically significant nor a clinically important effect on any important cardiovascular event or its components: nonfatal MI, or cardiovascular death The authors concluded that the ORs and CIs provide strong support for a lack of statistically significant or clinically important effects of vitamin E on CVD.
Level I	Ness et al (1999)	Meta-analysis of 3 trials with vitamin C supplements and CVD in western populations (total 1,034 subjects). No overall reduction in mortality with vitamin C supplementation (RR = 1.08).
Level I	Shekelle et al (2004)	Systematic review of placebo-controlled RCTs with a meta-analysis where justified. Concluded that there is good evidence that vitamin E supplementation does not beneficially or adversely affect cardiovascular outcomes. The ORs and CIs provide strong support for a lack of statistically significant or clinically important effects of vitamin E on CVD.
Level II	ATBC (1994)	Randomised, double-blind placebo-controlled study. A total of 29,133 Finnish male smokers randomly assigned to one of four treatment groups: 50 mg dl-αtocopherol/day (equivalent to 55 IU), 20 mg/day αcarotene, both αtocopherol and αcarotene, or placebo. Follow-up continued for 5–8 years. Lung cancer incidence not affected by αtocopherol treatment, but the incidence of prostate cancer was reduced. αTocopherol had no apparent effect on total mortality but was associated with increased mortality from haemorrhagic stroke. In contrast, deaths from ischaemic stroke and IHD were reduced in the αtocopherol group. Subsequent analysis of the risk factors for stroke indicated that vitamin E increased the risk of sub-arachnoid haemorrhage and decreased the risk of cerebral infarction in hypertensive men but had no effect on normotensive men. Showed an 11% increase in risk of IHD with αcarotene and an 18% increase in lung cancer.
Level II	Blot et al (1993)	The Linxian cancer intervention study included αcarotene with vitamin E and selenium and showed a 9% reduction in total mortality, a 123% reduction in cancer mortality and a 10% decrease in stroke with the supplement mix. Shows no beneficial effect of vitamin C on cancer mortality rates.
Level II	Blot et al (1995)	Human intervention trials in China. Used a mixed supplement including selenium. Significantly lower total mortality occurred among those receiving supplementation with αcarotene, vitamin E and selenium.
Level II	Chen et al (1988) DeCosse et al (1989) Greenburg et al (1994) Hofstad et al (1998) McKeown-Eyssen et al (1988)	5 trials show no secondary polyp preventive effect of vitamin E.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Clark et al (1996) Duffield-Lillico et al (2003)	Intervention study in the US. Shows no effect of supplements of 200 µg/day selenium on skin cancer risk but significant reduction in total cancer and cancers of prostate, lung and colon-rectum. However, a further analysis of this study (Duffield-Lillico et al 2003) found that supplementation actually increased risk of squamous cell carcinoma and total non melanoma.
Level II	Ernster et al (1985) London et al (1985)	Two small trials showing no effects of vitamin E on mammary dysplasia or breast disease.
Level II	GISSI-Prevenzione Investigators (1999)	11,324 survivors of recent MI randomly assigned supplements of n-3 PUFA (1 g daily), 300 mg vitamin E (as synthetic α-tocopherol), both, or neither, for 3.5 years. The primary combined efficacy endpoint was death, non-fatal MI and stroke. Smokers and ex-smokers were evenly distributed through the groups. Vitamin E treatment had no effect on the combined or separate endpoints.
Level II	Greenberg et al (1990)	Intervention study for skin cancer showed no effect of α-carotene
Level II	Greenberg et al (1994)	No effect of vitamin C in the Polyp Prevention Trial.
Level II	Greenberg et al (1994)	Intervention trial for polyp prevention with α-carotene showed no effect.
Level II	Heart Protection Study (2002)	Placebo-controlled RCT of antioxidant vitamin supplementation. 20,536 high risk adults (those with CHD, other occlusive vascular disease or diabetes) were given a daily supplement of 600 mg dl-α-tocopherol (equivalent to 660 IU), 250 mg vitamin C and 20 mg α-carotene or placebo for 5 years (Heart Protection Study Collaborative Group 2002). No difference in all cause mortality. Compliance 83% on average in each treatment group. Adverse experiences sought at each follow-up visit (every 4 months for the first year and every 6 months thereafter). No significant side effects reported.
Level II	Heart Protection Study Collaborative Group (2002)	Placebo-controlled RCR of antioxidant vitamin supplementation. 20,536 high risk adults given a daily supplement of 600 mg dl-α-tocopherol, carotene or placebo for 5 years. No difference in all cause mortality. No significant difference in the incidence of haemorrhagic stroke.
Level II	ATBC (1994) Heinonen et al (1998)	Heavy smokers. Shows no benefit for lung cancer with vitamin E, 34% lower incidence of prostate cancer.
Level II	Hennekens et al (1996)	No effect on CVD or cancer in men of 50 mg supplements on alternate days.
Level II	Lee et al (1999)	No effect on CVD or cancer in women of 50 mg supplements on alternate days.
Level II	Mackerras et al (1993)	Two Australian RCTs of α-carotene and cervical dysplasia [See Mackerras et al (1999)]. Show that α-carotene was not effective in slowing the progression of cervical dysplasia.
Level II	Mackerras et al (1999)	Double-blind, placebo-controlled, randomised, factorial study using a daily oral administration of 30 mg α-carotene and/or 500 mg vitamin C in 141 women with minor squamous atypia or cervical intra-epithelial neoplasia (CIN) I. Over approximately 2 years of follow-up, regression rate was slightly higher, but not significantly so, in those randomised to α-carotene compared to no α-carotene and slightly lower, but not statistically significant, for those randomised to vitamin C compared to no vitamin C. In a model with no interaction, the progression rate was slightly higher in those randomised to α-carotene and also in those randomised to vitamin C. Neither of these was statistically significant. However, there was some evidence of an interaction effect of the two compounds on the progression rate, with 7 of the progressed lesions occurring in those randomised to both vitamins compared to a total of 6 in the 3 other groups. Concluded that currently available evidence from this and other trials suggests that high doses of these compounds are unlikely to increase the regression or decrease the progression of minor atypia and CIN I.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Omenn et al (1996)	The CARET trial on lung cancer produced an increased risk with 30 mg αcarotene administered together with retinyl palmitate, as well as an increase in total mortality.
Level II	Primary Prevention Project (PPP) (2001)	Controlled open 2 x 2 factorial trial, 4,495 people randomised to receive low dose aspirin (100 mg/day) or no aspirin, and vitamin E (300 mg/day as synthetic αtocopherol) or no vitamin E, to investigate the prevention of cardiovascular events in people with one or more major cardiovascular risk factors. Follow-up period of 3.6 years. Vitamin E had no effect on cumulative rate of cardiovascular death, non-fatal MI and non-fatal stroke.
Level II	Teikari et al (1998)	Intervention for cataracts showed no effect of 50 mg/day αtocopherol.
Level II	Yusuf et al (2000)	HOPE study. 9,541 subjects aged 55 or over at high risk for cardiovascular events enrolled in a trial with a 2 x 2 factorial design. Received either 400 IU vitamin E or placebo and either ramipril or matching placebo for a mean of 4.5 years. The primary endpoint was a combination of MI and stroke and death from cardiovascular causes. The secondary outcomes included unstable angina, congestive heart failure, re-vascularisation or amputation, death from any cause, complications of diabetes and cancer. No significant differences in the numbers of deaths from cardiovascular causes, or in any of the secondary outcomes, between those receiving vitamin E and placebo. No differences in adverse effects between the vitamin E and placebo group or in the numbers of patients who stopped taking the study medication. Also no difference in the incidence of haemorrhagic stroke between the groups.
Level III-2	Andersson et al (1996)	Case-control study of prostate cancer. Shows no link with vitamin E.
Level III-2	Bandera et al (1997)	Vitamin C may be preventive for lung cancer in NY State Cohort of 25,544 men.
Level III-2	Broome et al (2004)	Double-blind study. 22 adults with relatively low plasma selenium concentrations received 50 or 100 μg selenium (as sodium selenite) or placebo daily for 15 weeks. Selenium supplements augmented the cellular immune response. Humoral immune responses were unaffected. Selenium-supplemented subjects also showed more rapid clearance of the poliovirus, and the poliovirus reverse transcriptase-polymerase chain reaction products recovered from the faeces of the supplemented subjects contained a lower number of mutations. Data suggest that the additional 100 μg selenium/day may be insufficient to support optimal function.
Level III-2	Brown et al (1999)	US male health professionals (n=36,644) in prospective cohort study. Modestly lower risk of cataract extraction in men with higher intakes of lutein and zeaxanthin but not of other carotenoids (αcarotene, αcarotene, lycopene, and αcryptoxanthin) or vitamin A. Lutein and zeaxanthin may decrease the risk of cataracts severe enough to require extraction, although this relation appears modest in magnitude.
Level III-2	Bueno de Mesquita et al (1991)	Dutch case-control study. Vitamin C preventive for men, not women, for pancreatic cancer.
Level III-2	Chasan-Taber et al (1999)	A prospective cohort of registered female nurses. During 761,762 person-years of follow-up, 1,471 cataracts were extracted. Those with the highest intake of lutein and zeaxanthin had a 22% decreased risk of cataract extraction compared with those in the lowest quintile. Other carotenoids (αcarotene, αcarotene, lycopene, and αcryptoxanthin), vitamin A and retinol were not associated with cataract in multivariate analysis. Lutein and zeaxanthin and foods rich in these carotenoids may decrease the risk of cataracts severe enough to require extraction.
Level III-2	Clarke et al (1996)	RCT of 200 mg selenium on cancer risk. Risk of prostate cancer 33% that of placebo group.
Level III-2	Comstock et al (1992) Knekt et al (1988)	Cohort studies showing no association between vitamin E and prostate cancer.
Level III-2	Comstock et al (1997)	Prospective cohort study showing a weak inverse relationship with lung cancer with vitamin E.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Eichholzer et al (1996)	Inverse association of vitamin E and prostate cancer.
Level III-2	Enstrom et al (1986)	Cohort of 3,119 non-institutionalised adult residents of Alameda County. No relation between category of intake of vitamin C estimated in 1974 and subsequent mortality from cancer, circulatory disease, all other causes, or all causes combined.
Level III-2	Enstrom et al (1992)	NHANES follow-up cohort, 11,000 adults. Vitamin C intake of 300 mg/day associated with reduced risk of CVD, 45% in men and 25% in women.
Level III-2	Eye Disease Case-Control Study Group (1993)	Compared serum levels of carotenoids, vitamins C and E, and selenium in 421 patients with neovascular age-related macular degeneration (AMD) and 615 controls. Persons with carotenoid levels in the medium and high groups, compared with those in the low group, had markedly reduced risks of neovascular AMD, with levels of risk reduced to 25% and 33%, respectively. No statistically significant protective effect was found for vitamins C or E or selenium individually, but an antioxidant index combining all 4 showed significant reductions of risk with increasing levels of the index.
Level III-2	Fontham et al (1988)	Vitamin C may be preventive for lung cancer at 140 mg/day in US.
Level III-2	Freudenheimn et al (1990)	Case-control study. Vitamin C may be preventive for rectal cancer.
Level III-2	Gale et al (1995)	Prospective cohort study. 730 elderly British men. Vitamin C above 45 mg/day compared to below 28 mg/day associated with 50% reduced stroke risk.
Level III-2	Ghadirian et al (1991)	Canadian case-control study. Vitamin C may be preventive for pancreatic cancer.
Level III-2	Giovannucci et al (1995a)	Prospective cohort study to examine the relationship between the intake of various carotenoids, retinol, fruits, and vegetables and the risk of prostate cancer. Findings support recommendations to increase vegetable and fruit consumption to reduce cancer incidence and suggest that tomato-based foods may be especially beneficial regarding prostate cancer risk.
Level III-2	Graham et al (1992)	New York State cohort. 18,000 postmenopausal women. No association of vitamin C with breast cancer.
Level III-2	Hankinson et al (1992)	Prospective cohort study. Dietary carotenoids (not necessarily αcarotene) may decrease the risk of cataracts severe enough to require extraction.
Level III-2	Hinds et al (1984) Le Marchand et al (1989)	2 case-control studies in Hawaii. No association between vitamin C and lung cancer.
Level III-2	Howe et al (1990)	Meta-analysis of case-controls. Vitamin C is preventive for breast cancer. Each 300 mg increase associated with a 37% decrease in risk in postmenopausal, but not premenopausal, women.
Level III-2	Howe et al (1992)	Collaborative pooled study of case-control studies. Shows vitamin C preventive for pancreatic cancer.
Level III-2	Hunter et al (1993)	Cohort study of Nurses' Health Study. No effect of vitamin C on breast cancer.
Level III-2	The Italian- American Cataract Study Group (1991)	Clinic-based case-control study of age-related cataracts. A total of 1,008 cases and 469 controls aged 45–79 years. Suggests that associations exist with educational status, cortisone use, sunlight exposure and handgrip strength. Other findings require further evaluation.
Level III-2	Jacques & Chylack (1991) Leske et al (1991) Robertson et al (1989)	Benefit of vitamin C on cataracts.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Khaw et al (2001)	4-year prospective study of the relation between plasma ascorbic acid concentrations and mortality due to all causes, and to CVD, IHD, and cancer in 19,496 men and women aged 45–79 years. Followed up for causes of death for about 4 years. Plasma ascorbic acid concentration inversely related to mortality from all-causes, and from CVD and IHD in men and women. Ascorbic acid inversely related to cancer mortality in men, but not women.
Level III-2	Knekt et al (1991)	Finnish cohort, 4,000 men. Vitamin C may be preventive for lung cancer.
Level III-2	Knekt et al (1994)	Prospective cohort study, 5,000 Finnish men and women. Vitamin C associated with reduced CVD risk in women but not men. Those with more than 91 mg/day had a lower risk than those with less than 61 mg/day.
Level III-2	Kushi et al (1996a)	Cohort study. Vitamin C preventive for breast cancer. Iowa Women's Health Study. 20% decrease with greater than 500 mg/day.
Level III-2	Kushi et al (1996b)	34,486 postmenopausal women with no CVD. Intake of vitamins A and C not associated with lower risks of dying from coronary disease in postmenopausal women. The intake of vitamin E from food was inversely associated with the risk of death from CHD.
Level III-2	Losonczy et al (1996)	11,178 persons aged 67–105 years from the Established Populations for Epidemiologic Studies of the Elderly in 1984-1993. Vitamin C alone had no effect, but use of vitamin E reduced the risk of all-cause mortality and risk of coronary disease mortality. Simultaneous use of vitamins E and C associated with a lower risk of total mortality and coronary mortality.
Level III-2	Lyle et al (1999)	Cohort study of adults in Beaver Dam Eye Study. Results of short-term follow-up consistent with a possible protective influence of lutein on the development of nuclear cataracts, but evidence weak.
Level III-2	Mares-Perlman et al (1994)	Cohort study of Nutritional Factors in Eye Disease Study (n=2,152). In persons without diabetes, regular use of multivitamin preparations 10 years in the past was associated with decreased risk for nuclear sclerosis and increased risk for cortical opacities. In persons with diabetes, past multivitamin use was not associated with nuclear sclerosis but was associated with decreased risk for cortical opacities. Multivitamin use was not cross sectionally or longitudinally related to posterior subcapsular cataract in persons with or without diabetes.
Level III-2	Mares-Perlman et al (1995)	Cohort study of 1,919 persons in the Beaver Dam Eye Study. In men, intakes of numerous nutrients in the highest versus lowest quintile were associated with 40–50% reduced odds of more severe nuclear sclerosis. Relations with some nutrients (vitamins A, C, and E, riboflavin, thiamin and niacin) were at least partly explained by previously identified inverse associations with multivitamin use. Relations with other nutrients (folate, αcarotene, and dietary fibre) appeared to reflect associations with intake of foods, particularly vegetables. Inverse associations with individual nutrients and foods were often weaker or nonexistent in women.
Level III-2	Nyyssonen et al (1997)	Prospective Swedish study. 1,605 men. Those with increased plasma itamin C had 60% decreased risk of CVD.
Level III-2	Ocke et al (1997)	Dutch cohort, 561 men. Vitamin C may be preventive for lung cancer.
Level III-2	Pandey et al (1995)	Prospective cohort study – Western Electric in Chicago. 1,556 middle-aged men. Greater than 113 mg vitamin C/day associated with 25% reduced CVD risk compared to less than 82 mg/day.
Level III-2	Rimm et al (1993)	39,910 US male health professionals. No effect of vitamin C on CHD, but evidence of an association between a high intake of vitamin E and a lower risk of CHD in men.
Level III-2	Romney et al (1985)	Case-control study. Vitamin C may be preventive for cervical cancer.
Level III-2	Sahyoun et al (1996)	725 elderly Americans. 62% lower CHD risk in those with vitamin C more than 388 mg/day compared to those less than 90 mg/day.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Seddon et al (1994)	The Multicenter Eye Disease Case-Control Study. 356 case subjects, 520 control subjects. Higher dietary intake of carotenoids associated with a lower risk for AMD. Among the specific carotenoids, lutein and zeaxanthin were most strongly associated with a reduced risk. Neither vitamin E nor total vitamin C consumption was associated with a statistically significant reduced risk for AMD.
Level III-2	Shekelle et al (1981)	US prospective study, 3,102 men. Vitamin C may be preventive for cancer.
Level III-2	van't Veer et al (1996)	Case-control study of vitamin E on breast cancer. Shows no effect.
Level III-2	Verhoeven et al (1997) Dorgan et al (1998)	Two prospective studies of breast cancer. No relationship with vitamin E status.
Level III-2	Vitale et al (1993) Hankinson et al (1992)	No benefit of vitamin C on cataracts. Benefit of vitamin C on cataracts in long term, not short term.
Level III-2	Wassertheil-Smoller et al (1981)	Case-control study. Vitamin C may be preventive for cervical cancer.
Level III-2	West et al (1994)	Cohort study of Baltimore Longitudinal Study of Aging. 976 participants. Logistic regression analyses suggested that αtocopherol was associated with a protective effect for AMD, adjusted for age, sex and nuclear opacity. An antioxidant index, including ascorbic acid, αtocopherol, and αcarotene, was also protective for AMD. The data suggest a protective effect for AMD of high plasma values of αtocopherol. An antioxidant index of plasma ascorbic acid, αtocopherol and αcarotene was also protective. The use of vitamin supplements to prevent AMD was not supported by these data, which showed no protective effect of vitamin use.
Level III-2	Yong et al (1997)	Vitamin C at greater than 133 mg/day may be preventive for lung cancer in NHANES follow-up cohort of 10,000.
Level III-2	Yong et al (1997)	US NHANES I Epidemiological Follow-up study showed an inverse association in smokers between E and cancer risk.
Level III-2	Yoshizawa et al (1998)	Nested case-control study in prospective cohort study of toenail selenium and prostate cancer risk showing higher selenium may reduce risk prostate cancer.
Level III-2	Zatonski et al (1991)	Polish case-control study. Vitamin C may be preventive for pancreatic cancer.
Level III-3	Kocyigit et al (2001)	Plasma selenium and the activity of glutathione peroxidase (GP_X) were measured in tobacco smokers and compared with those of non-smokers. Plasma selenium concentration and erythrocyte GP_X activities were significantly lower in tobacco smokers than in non-smokers. There were significant positive correlations between erythrocyte GP_X and plasma selenium levels and a negative correlation between plasma thiocyanate and selenium content in tobacco smokers. Suggests that antioxidative enzyme activities change, depending on their cofactor concentrations in tobacco smokers.
Level IV	Pak et al (2002)	Observations from RCT for finasteride. Prostate Cancer Prevention Trial confirmed that selenium supplementation was associated with marked reductions in risks to total (all-site except skin) carcinomas and to cancers of the prostate and colon-rectum.
Cross-sectional survey data	Hammond et al (1996)	Sex differences in macular pigment (MP) optical density (measured psychophysically) were examined. Males had 38% higher MP density than females despite similar plasma carotenoid concentrations and similar dietary intake. Dietary intakes of carotenoids, fat and iron, as well as plasma concentrations of lutein and zeaxanthin were positively related to MP density in males. Conversely, only plasma lutein and zeaxanthin was related to MP density for females, and dietary fat was negatively related to MP density. Sex differences in protection of the retina by MP and in the relationship between the retina, blood and diet could be a factor in the incidence of retinal diseases, especially ADM.

Level of evidence	Reference	Study type, issues addressed and key findings
Expert review	Combs (2005)	Reaching plasma selenium concentration of 120 µg/L would require supplemental selenium of about 100 µg/day in addition to diet intake of 75 µg/day for those on low-baseline.
Expert review	Snodderly (1995)	Epidemiologic data indicate that individuals with low plasma concentrations of carotenoids and antioxidant vitamins and those who smoke cigarettes are at increased risk for AMD. The combination of evidence suggests that carotenoids and antioxidant vitamins may help to retard some of the destructive processes in the retina and the retinal pigment epithelium that lead to AMD.
Expert review	Thomson (2004)	Intakes higher than those recommended and plasma selenium concentrations that might be protective for cancer or result in other additional health benefits have been proposed. There is an urgent need for more large-scale trials to assess any such beneficial effects and to provide further data on which to base more reliable estimates for intakes and plasma selenium levels that are protective. Current evidence suggests that a plasma level of about 120 µg/L may be optimal for protection against some cancers.
Expert review	Waters et al (2004)	In general, the available data support the hypothesis that cancer risk in men is more profoundly influenced by selenium status than cancer risk in women. Factors contributing to the apparent difference in the effects of selenium on cancer incidence in men and women may include sex-based differences in the metabolism and/or tissue distribution of selenium, as well as sex- or gender-related factors that influence tumour biology.
Expert review	Whanger (2004)	Information from both animal and human research indicates that 100–200 µg additional selenium/day is necessary for greatest reduction of cancer.

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FOLATE

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in the relevant FNB:IOM report (FNB:IOM 1998).

Search terms: folate, folic acid, nutrition, recommended dietary intake, estimated average requirement, upper intake limit, homocysteine, coronary heart disease, cancer.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-1	Ashfield-Watt et al (2002)	RCT, 126 subjects per group (42 of each of genotypes CC, CT and TT). Suggests that it may be necessary to increase dietary folate intake from 282 µg/day to between 400 and 600 µg/day in TT genotypes for them to experience plasma folate and homocysteine concentrations equivalent to the levels in CC and CT genotypes with intake levels of 282 µg/day. Even at 660–814 µg folate intake/day, TTs still had significantly higher homocysteine than CCs at folate intake levels of 282 µg/day, however the difference was small and unlikely to be biologically significant.
Level III-1	Fenech et al (1998)	Placebo-controlled intervention study. 6 months, 30 subjects per group, young Australian adults. Shows that intake of 700 µg folic acid with 7 µg vitamin B_{12} reduced chromosome damage rate in lymphocytes by 25% in those individuals with above average chromosome damage rate. No further protection was provided by increasing intake to 2,000 µg folic acid with 20 µg vitamin B_{12}. This study indicates that above RDI intake of folate is required to minimise chromosome damage (a risk factor for cancer) in 50% of the subjects studied who were otherwise not considered to be deficient by conventional criteria.
Level III-1	Melse-Boonstra et al (2004)	Parallel randomised placebo-controlled trial, 12 weeks, 60 subjects per group. Shows that one of the dietary forms of folate (heptaglutamyl folic acid) has a bioavailability of 64–68% based on plasma folate and red cell folate and a bioefficacy of 106% based on reduction of plasma homocysteine. Suggests that the polyglutamyl chain of folate reduces bioavailability by about 35%, however sufficient folate intake can achieve maximal reduction in homocysteine.
Level III-1	Pena et al (2004)	Randomised, double-blind, placebo-controlled crossover trial. Shows that 8 weeks of treatment of 5 mg folic acid improved endothelial cell function by 2.6% in children and adolescents with type I diabetes (n=35).
Level III-1	Schynder et al (2001)	Prospective, double-blind, randomised trial. Groups of 48 and 54 subjects. Plasma homocysteine reduced from 11 to 7 µmol/L and coronary stenosis significantly reduced (compared to controls) after daily supplementation for 6 months with 1000 µg folic acid, 400 µg vitamin B_{12} and 10 mg vitamin B_6 in patients who underwent percutaneous coronary angioplasty.
Level III-1	Tucker et al (2004)	Double-blind RCT. 93 healthy subjects aged 50–85 years per group. Shows that daily intake of folic acid with vitamins B_{12} and B_6 at RDA levels in supplemented cereal decreased homocysteine from 7.9 to 7.5 µmol/L. µ
Level III-1	van Oort et al (2003)	Parallel randomised placebo-controlled trial, 12 weeks, 38–52 subjects aged 50–75 years per group. Doses of folic acid of 50–800 µg/day. Minimum folic acid supplementation required for 90% optimal reduction in plasma homocysteine in healthy older adults was 400 µg/day. Dietary intake of folate for the participants not reported.
Level III-1	Venn et al (2002)	RCT. 4 weeks, 14–20 subjects aged 50–70 years. Showed that increasing dietary folate from 263 µg/day to 618 µg/day significantly increased serum folate by 37% and decreased homocysteine from 12 µmol/L to 11µmol/L over a 4 week period.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-1	Venn et al (2003)	Placebo-controlled RCT. 24 weeks, 50–53 subjects per group. Shows that supplementation of healthy subjects aged 40–60 years with either 100 µg/day folic acid or 100 µg/day L-5-methyltetrahydrofolate (MTHF) resulted in significant increments in plasma folate (52% and 34%, respectively) and red cell folate (31% and 23%, respectively) and a significant reduction in plasma homocysteine (–9.3% and –14.6%, respectively). MTHF was significantly more effective than folic acid in reducing plasma homocysteine.
Level III-2	McLean et al (2004)	Prospective cohort study with 12.3–15.0-year follow-up, 825 men and 1,174 women. Shows that men and women in the highest quartile of plasma homocysteine had a greater risk of hip fracture than those in the lowest quartile. The risk was almost 4 times higher for men and 1.9 times higher for women. These findings suggest that the homocysteine concentration is an important risk factor for hip fracture in older persons.
Level III-2	Rimm et al (1998)	Prospective cohort study within the Nurses' Health Study. Cohort size of 80,000 and number of CHD cases, 939. Shows that women with folate intakes in the top quintile (median 696 µg folate/day) had 31% reduction in risk of developing CHD compared to those in the bottom quintile (158 µg folate/day). The strongest effect of reduction in risk of 73% occurred in women who consumed more than 1 alcoholic drink per day.
Level III-2	Seshadri et al (2002)	Prospective cross-sectional study with 8-year follow up within the Framingham cohort, 1,092 subjects (667 women and 425 men). Shows an increased relative risk for dementia and Alzheimer's disease with increasing plasma homocysteine. The risk for Alzheimer's disease was double for those with plasma homocysteine greater than 14 µmol/L compared to those with <14 µmol/L. Increasing plasma homocysteine by 5 µmol/L increased the multivariate adjusted risk of Alzheimer's disease by 40%.
Level III-2	van Meurs et al (2004)	Prospective cohort study with follow up of 2.7–8.1 years. Number of subjects (older men and women): Rotterdam Study, 562 subjects in cohort 1 and 553 subjects in cohort 2; Longitudinal Aging Study Amsterdam, 1,291 subjects. Shows that a homocysteine level in the highest age-specific quartile was associated with a 1.9 fold increase in the risk of fracture (95%CI: 1.4, 2.6). The associations between homocysteine levels and the risk of fracture appeared to be independent of BMD and other potential risk factors for fracture. An increased homocysteine level appears to be a strong and independent risk factor for osteoporotic fractures in older men and women.
Level III-2	Zhang et al (1999)	Prospective cohort study within the Nurses' Health Study. Cohort size of 122,000 and number of breast cancer cases 3,483. Indicates that folate intake at levels greater than 300 µg/day is associated with a 25% reduction in breast cancer risk in women who consume at least 15 g of alcohol per day.
Survey	McNulty et al (2002)	Cross-sectional study in healthy subjects aged 19–63 years. 107 males, 179 females. Shows that low riboflavin is the main determinant of high homocysteine and the extent of folate-induced homocysteine lowering in MTHFR TT genotype subjects. There is no difference in homocysteine concentration of CC, CT and TT genotypes with adequate riboflavin status, possibly because activity of mutant MTHFR is normalised at adequate riboflavin concentration. Riboflavin status of a population should be taken into account when determining optimal intake levels of folate.

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CALCIUM & VITAMIN D

EVIDENCE BASE

For Calcium

Databases: PubMed plus search of *Journal of Bone and Mineral* (key journal for bone) and search of cross-references and references in FAO:WHO (2001) and FNB:IOM (1997).

Search terms: calcium and calcium requirement, human; osteoporosis, chronic disease

For Vitamin D

Databases: Australasian Medical Index, Pub Med, cross-referencing and review of key references in FNB:IOM (1997), used to set the values.

Search terms: vitamin D, humans, deficiency, osteoporosis, chronic disease

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Shaukat et al (2005)	Meta-analysis of RCTs. 3 trials, 1,485 subjects with previously removed adenomas randomised to calcium versus placebo supplementation. The study endpoint was recurrence of adenomas at the end of 3–4 years in 1,279 patients who completed the trials. Shows that recurrence of adenomas was significantly lower in subjects randomised to calcium supplementation. Suggests that calcium supplementation prevents recurrent colorectal adenomas.
Level I	Weingarten et al (2004)	Systematic review of RCTs of the effects of dietary calcium on the development of colonic cancer and adenomatous polyps. Concludes that although the evidence from two RCTs suggested that calcium supplementation may contribute somewhat to the prevention of colorectal adenomatous polyps, there was insufficient evidence to recommend the general use of calcium supplements to prevent colorectal cancer.
Level II	Baron et al (1999)	Randomised, double-blind trial of the effect of supplementation with calcium carbonate on the recurrence of colorectal adenomas. 930 subjects with a recent history of colorectal adenomas received either calcium carbonate daily or placebo. Calcium supplementation was associated with a significant, though moderate, reduction in the risk of recurrent colorectal adenomas.
Level II	Grant et al (2005)	RCT. 5,292 people aged ≥70 years who were mobile before developing a low-trauma fracture. Randomly assigned 800 IU daily oral vitamin D_3, 1,000 mg calcium, oral vitamin D_3 (800 IU/day) combined with calcium (1,000 mg/day), or placebo. Findings did not support routine oral supplementation with calcium and vitamin D_3, either alone or in combination, for the prevention of further fractures in previously mobile elderly people.
Level II	Holt et al (1998)	Single-blind RCT. 70 subjects with a history of polypectomy for colonic adenomatous polyps. Increasing daily intake of calcium by up to 1,200 mg via low-fat dairy food in subjects at risk for colonic neoplasia reduced proliferative activity of colonic epithelial cells and restored markers of normal cellular differentiation.
Level III-2	Cho et al (2004)	Pooled analysis of 10 cohort studies of 534,536 individuals. 4,992 incident cases of colorectal cancer were diagnosed between 6 and 16 years of follow-up. Milk intake was related to a reduced risk of colorectal cancer. Calcium intake was also inversely related to the risk of colorectal cancer. The RR for the highest versus the lowest quintile of intake was 0.86 for dietary calcium and 0.78 for total calcium (combining dietary and supplemental sources). The inverse association for milk was limited to cancers of the distal colon and rectum.
Level III-2	Rugg-Gunn et al (1984)	Cohort study conducted in 7 schools on children initially aged 11.5 years. Correlations between caries increment and dietary factors were low. Cheese was cariostatic.

Level of evidence	Reference	Study type, issues addressed and key findings
Expert review	Giovannucci (2005)	No epidemiologic studies have directly measured vitamin D concentrations or intakes on risk of total cancer incidence or mortality. Higher rates of total cancer mortality in regions with less UV-B radiation and in overweight and obese people are associated with lower circulating vitamin D and are compatible with a benefit of vitamin D on mortality. The only individual cancer sites examined directly in relation to vitamin D status are colorectal, prostate and breast cancers. Data are promising for breast cancer but are far too few to support a conclusion. The evidence is substantial that higher 25(OH)D levels through increased sunlight exposure or dietary or supplement intake inhibit colorectal carcinogenesis. The biologic evidence for an anti-cancer role of 25(OH)D is also strong for prostate cancer, but the epidemiologic data have not been supportive.
Expert review	Kashket & DePaola (2002)	Shows that milk and cheese could reduce the effects of metabolic acids and could help restore the enamel lost during eating. Postulated mechanisms involve buffering, salivary stimulation, reduction of bacterial adhesion, reduction of enamel demineralisation, and/or promotion of remineralization by casein and ionisable calcium and phosphorus.
Expert review	Moynihan & Petersen (2004)	Review of evidence for an association between nutrition, diet and dental diseases. Presents dietary recommendations for the prevention of dental diseases. Reports that cheese is cariostatic.
Expert review	Peterlik & Cross (2005)	In addition to skeletal disorders, calcium and vitamin D deficits increase the risk of malignancies, particularly of colon, breast and prostate gland, chronic inflammatory and autoimmune diseases (eg type 1 diabetes, inflammatory bowel disease, multiple sclerosis), and other metabolic disorders (metabolic syndrome, hypertension). Attenuation of signal transduction from the ligand-activated vitamin D receptor and calcium-sensing receptor seems to be the prime mechanism by which calcium and vitamin D insufficiencies cause perturbation of cellular functions in bone, kidney, intestine, mammary and prostate glands, endocrine pancreas, vascular endothelium, and, importantly, the immune system.
Animal experiment-ation	Bowen et al (1991)	Milk appears to have many of the physical properties of a good saliva substitute. Desalivated rats given 2% milk or lactose-reduced milk remained essentially caries-free. Results showed that milk and lactose-reduced milk can be used safely by hyposalivatory patients as a saliva substitute.
Animal experiment-ation	Reynolds & Johnson (1981)	Milk is cariostatic in rats.

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SODIUM

Note: The body of evidence and recommendations (UL and SDT) for adults were updated in 2017. For further information see the NHMRC Guidelines and Publications Page.

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in FNB:IOM (2004).

Search terms: Sodium, blood pressure, hypertension, coronary heart disease, stroke, mortality, morbidity, nutrition.

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Alam & Johnson (1999)	Meta-analysis of RCTs. For all trials together, chronic high NaCl diet significantly increased mean systolic and diastolic blood pressure. Data suggest that a chronic high NaCl diet in elderly patients with essential hypertension is associated with an increase in systolic and diastolic blood pressure. The association is significant for both, but more marked for systolic than diastolic. The effect is more pronounced with age and NaCl dose strongly predicts systolic blood pressure in older patients.
Level I	Cutler et al (1997)	Meta-analysis of RCTs. Effects on blood pressure of lowering sodium in hypertensive and normotensive subjects are –4.8/–2.5 and –1.9/–1.1 mmHg. Median differences in sodium excretion between sodium-reduction and control groups in these subgroups were –77 and –76 mmol/24 hour. The blood pressure reduction that would result from a substantial lowering of dietary sodium in the US population could reduce cardiovascular morbidity and mortality.
Level I	Ebrahim & Davey Smith (1998) Graudal et al (1998) Midgley et al (1996)	Meta analyses including short term trials and sodium loading trials. Concludes that decreases in blood pressure in response to sodium reduction are not sufficient to justify population-wide advice to lower salt intakes.
Level I	Hooper et al (2002)	Systematic review including meta-analysis of RCTs assessing long-term effects of advice to reduce dietary salt in adults with and without hypertension. 11 trials with follow-up from 6 months to 7 years. Shows significant reductions of 2.5/1.2 mmHg in systolic and diastolic blood pressures at 6–12 months but only a significant reduction for systolic blood pressure at 13–60 months. 24-hour sodium excretion was reduced by 1.1 g or 48 mmol sodium/day at 6–12 months and by 0.8 g or 35.5 mmol sodium/day at 13–60 months.
Level I	MacGregor & He (2002)	Meta-analysis of RCTs involving modest salt reductions and a duration of at least 4 weeks. 17 trials in hypertensives, 11 trials in normotensives. Significant reductions in systolic and diastolic blood pressure of 4.96/2.73 mmHg in hypertensives and 2.03/0.97 mmHg in normotensives. On a population-wide basis, a modest reduction in salt intake for a period of 4 or more weeks has a significant effect on blood pressure in hypertensive and normotensive individuals.
Level I	Mulrow et al (2002)	Meta-analysis of RCTs on diet advice and lowering of blood pressure. Concludes that weight-reducing diets in overweight hypertensive persons can affect modest weight loss in the range of 3–9% of body weight and are probably associated with modest blood pressure decreases of about 3 mmHg systolic and diastolic. Weight-reducing diets may decrease dosage requirements of persons taking antihypertensive medications.

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Xin et al (2001)	Meta analysis of RCTs to assess the effects of alcohol reduction on blood pressure. 15 trials (total of 2,234 participants) Overall, alcohol reduction was associated with a significant reduction in mean systolic and diastolic blood pressures of –3.31 mmHg (–2.52 to –4.10 mmHg) and –2.04 mmHg (–1.49 to –2.58 mmHg), respectively. A dose-response relationship was observed between mean percentage of alcohol reduction and mean blood pressure reduction. Effects of intervention were enhanced in those with higher baseline blood pressure. Authors suggest that alcohol reduction should be recommended as an important component of lifestyle modification for the prevention and treatment of hypertension among heavy drinkers.
Level II	Appel et al (1997)	Dietary Approaches to Stop Hypertension (DASH) trial. Assessed effects of dietary patterns on blood pressure in about 460 normotensive and hypertensive adults. Subjects received a control diet (low in fruit, vegetables and dairy products, with a fat content typical of the average US diet) for 3 weeks and were then randomised to receive one of 3 diets for 8 weeks: the control diet, a diet rich in fruit and vegetables, or a combination diet (the DASH diet), rich in fruit, vegetables, low-fat dairy products, and with reduced saturated and total fat. The trial showed a diet rich in fruits, vegetables, and low-fat dairy products, reduced mean blood pressure by 5.5/3.0 mmHg. The diet rich in fruit and vegetables reduced systolic blood pressure by 2.8 mmHg but did not reduce diastolic blood pressure. The DASH diet reduced blood pressure by 11.4/5.5 mmHg in hypertensives and by 3.5/2.1 mmHg in non-hypertensives.
Level II	Hunt et al (1998)	1,509 white male and female subjects participating in phase II of the Trials of Hypertension Prevention were genotyped at the angiotensinogen locus. Persons in the usual care group with the AA genotype at nucleotide position –6 had a higher 3-year incidence rate of hypertension compared with those with the GG genotype, RR of 1.4. In contrast, the incidence of hypertension was significantly lower after sodium reduction for persons with the AA genotype, but not for persons with the GG genotype. Decreases of diastolic blood pressure at 36 months in the sodium reduction group versus usual care group showed a significant trend across all 3 genotypes with greater net blood pressure reduction in those with the AA genotype than those with the GG genotype (+1.1 mmHg). A similar trend across the 3 genotypes for net systolic blood pressure reduction was not significant. Trends across genotypes for the effects of weight loss on hypertension incidence and decreases in blood pressure were similar to those for sodium reduction. Angiotensinogen genotype may affect blood pressure response to sodium or weight reduction and the development of hypertension.
Level II	John et al (2002)	RCT, 6 months, of a brief negotiation method to encourage an increase in consumption of fruit and vegetables to at least 5 daily portions. Shows that the effects of the intervention on fruit and vegetable consumption, plasma antioxidants and blood pressure would be expected to reduce CVD in the general population.
Level II	Margetts et al (1986)	Randomised crossover trial, 58 subjects aged 30–64 years with mild untreated hypertension. Allocated to either a control group eating a typical omnivorous diet or to one of 2 groups eating an ovo-lacto-vegetarian diet for one of two 6-week periods. Systolic blood pressure fell by about 5 mmHg during the vegetarian diet periods and rose correspondingly with resumption of a meat diet. The main nutrient changes with the vegetarian diet included an increase in the ratio of polyunsaturated to saturated fats and intake of fibre, calcium and magnesium, and a decrease in the intake of protein and vitamin B_{12}. There were no consistent changes in urinary sodium or potassium excretion or body weight. In untreated subjects with mild hypertension, changing to a vegetarian diet may bring about a worthwhile fall in systolic blood pressure.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Sacks et al (2001)	Assessed combined effect of the DASH diet and reduced salt intake. About 400 adults randomly assigned to the control or DASH diet for three months. Each subject consumed the diet for 30 days at each of three levels of salt: high, intermediate and low. The potassium intakes were greater on the DASH diet than in the controls, but were kept the same for all levels of salt intake. Reducing salt intake reduced blood pressure by 6.7/3.5 mmHg on the control diet and by 3.0/1.6 mmHg on the DASH diet. The combined effects on blood pressure of the DASH diet and low salt intake were greater than either of the interventions alone. With this combination, mean systolic blood pressure was 11.5 mmHg lower in participants with hypertension, and 7.1 mmHg lower in participants without hypertension. The effects were observed in those with and without hypertension, in both sexes and across racial groups.
Level II	Svetkey et al (2001)	The DASH trial was a randomised outpatient feeding study comparing the effects on blood pressure of 3 dietary patterns. The genotype of participants was also determined. There was no association between angiotensin converting enzyme (ACE) genotype and blood pressure response. Associations with angiotensin polymorphism were significant. Net systolic and diastolic blood pressure responses to the DASH diet were greatest in individuals with the AA genotype (–6.93/–3.68 mmHg) and least in those with the GG genotype (–2.80/0.20 mmHg). A similar relationship existed for the fruit and vegetable diet. Angiotensin genotype is associated with a blood pressure response to the DASH diet. The AA genotype confers excess risk of hypertension and is associated with increased responsiveness to diet.
Level II	TOHP Collaborative Research Group (1997)	Longitudinal study assessed effects of reduced salt intake and weight loss on blood pressure. At 6 months, sodium excretion was reduced by 1.8 g in the salt reduction group and by 1.5 g in the combined intervention group, achieving salt intake levels of 2.4 g and 2.9 g (124 mmol sodium), respectively. Systolic and diastolic blood pressures were significantly lowered compared to controls in the salt, weight loss and combined intervention groups. However, effects were less at 36 months. Systolic blood pressure reductions were small but significant for the salt reduction group and the weight loss group. Sodium excretion increased significantly over time and at 36 months, there were only small but significant differences from the usual care group.
Level II	Whelton et al (1998)	The Trial of Nonpharmacologic Interventions in the Elderly (TONE) assessed the effects of salt reduction to a target of 4.8 g (0.8 g or 80 mmol sodium) and weight loss, alone and combined, in older hypertensives whose blood pressures were controlled with one antihypertensive drug. Where both interventions were successful, more people were able to stop and remain off medication.
Level III-2	Poulter et al (1990)	Controlled longitudinal observational study, The Kenyan Luo study, of people from a low blood pressure population migrating to an urban area. Migrants studied as soon after migration as possible and followed up at 3, 6, 12, 18 and 24 months after migration. A cohort of controls living in a rural area matched for age, sex and locality were observed at the same periods. Urinary sodium:potassium ratio, pulse rate and weight are important predictors of increased blood pressure among migrants from a low blood pressure community and may also be implicated in the initiation of essential hypertension.
Observational survey	Intersalt Cooperative Research Group (1988)	Collected data on 24-hour urinary sodium excretion and blood pressure of over 10,000 adults in 52 groups from 32 countries. Significant positive associations found between sodium excretion and both systolic and diastolic blood pressures. However, when four centres that had very low salt intakes were removed from the analysis, the overall association was not statistically significant, although an association was found between salt intake and increase in blood pressure with age.
Observational survey – re-analysis	Davey Smith & Phillips (1997) Day (1997)	Re-analysis of (Intersalt Cooperative Research Group (1988) and Elliott (1993, 1994) papers shows that Elliot may have overestimated correction factors.
Observational survey – re-analysis	Elliott (1993, 1994, 1996)	Re-analysis of Intersalt study (Intersalt Cooperative Research Group 1988). Adjusting for regression dilution caused by measurement errors shows stronger associations.

Level of evidence	Reference	Study type, issues addressed and key findings
Cross-sectional survey	Chen et al (1995)	Community-based cross-sectional study. 2,865 residents aged 6 to 74 years (85.6% of the target population) in Canada. Age significantly modifies the relations of body mass index with mean diastolic blood pressure in both sexes, and with the prevalence of high blood pressure in females. The relations are stronger in children and young adults than in older persons.
Cross sectional survey	He et al (1991)	The relations of sodium, potassium, calcium and magnesium to blood pressure were investigated in 4 groups of men in China. In ecological correlation analysis, dietary and urinary sodium were significantly and positively correlated with both systolic and diastolic pressures, whereas serum sodium showed no relation with blood pressure. Potassium in diet, serum and urine was negatively related to systolic and diastolic pressures, whereas the sodium:potassium ratio showed a positive association. For calcium, only urinary excretion was significantly and positively related to blood pressure. No relation was found between magnesium and blood pressure. Analyses at the individual level confirmed the results for sodium and potassium seen at the ecological level, but in addition, dietary calcium and magnesium were significantly and negatively correlated to both systolic and diastolic pressures, and urinary magnesium was inversely related to diastolic pressure.
Cross-sectional survey	Marmot et al (1994)	From Intersalt study. The significant relation of heavy drinking (3–4 or more drinks/day) to blood pressure, observed in both men and women and younger and older men, was independent of, and additive with, the effect on blood pressure of body mass index and urinary excretion of sodium and potassium. Indicates the usefulness of targeting those at high risk as well as the general population to reduce the adverse effects of alcohol on blood pressure.
Expert review	Corvol et al (1999)	A large series of studies in humans and animals. The angiotensinogen gene appears to play a significant but modest role in human blood pressure variance. Too early to propose dietary recommendations and specific drug treatment according to genotypes of patients.

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POTASSIUM

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in FNB:IOM (2004).

Search terms: Potassium, blood pressure, mortality, morbidity, nutrition, chronic disease, renal stones.

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Whelton et al (1997)	Meta-analysis of 33 RCTs. Potassium lowers blood pressure and blunts the effect of sodium chloride on blood pressure, mitigating salt sensitivity and lowering urinary calcium excretion.
Level III-2	Curhan et al (1993)	Longitudinal prospective study of 45,000 men over 4 years. Shows the lowest rate of kidney stones in the highest quintile of intakes (≥4.0 g/day).
Level III-2	Curhan et al (1997)	Longitudinal prospective study of 91,000 women over 12 years. Shows the lowest rate of kidney stones in the highest quintile of intakes (≥4.7g/day).
Level III-2	Hirvonen et al (1999)	Finland, male smokers. Shows reduced stones at the second quartile of intake (at 4.6 g/day) with no further reductions at higher quartiles.
Level III-3	Morris et al (1999)	Metabolic study of 38 men. Basal diet of low potassium, then supplemented with potassium. Salt sensitivity, measured before and after, was blunted at 4.7 g/day in Afro-American men and 2.7 g/day in white males.

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DIETARY FIBRE

EVIDENCE BASE

Databases: PubMed, Medline and Current Contents plus search of cross-references and references in FNB:IOM (2002).

Search terms: dietary fibre, dietary fiber, resistant starch, recommended dietary intake, coronary heart disease, cancer.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	MacLennan et al (1995)	Partially double-blinded RCT. 390 subjects. Treatments were combinations of fat at 25% of total energy and supplements of 25 g of wheat bran daily and a capsule of αcarotene (20 mg/day). No statistically significant prevention of total new adenomas with any intervention. Patients on the combined intervention of low fat and added wheat bran had zero large adenomas at 24 and 48 months, a statistically significant finding ($p = 0.03$), but because only small numbers of patients were studied, authors warn this finding must be treated with caution.
Level II	Schatzkin et al (2000)	Randomly assigned 2,079 men and women to 1 of 2 groups: an intervention group given intensive counseling and assigned to follow a low fat, high fibre, high fruit and vegetables diet and a control group given a standard brochure on healthy eating and assigned to follow their usual diet. Diet for intervention group did not influence the risk of recurrence of colorectal adenomas.
Level III-2	Baghurst & Rohan (1994) Lee et al (1991) Lubin et al (1986) Van't Veer et al (1990)	Breast cancer case-control studies reporting decreased risks associated with fibre-rich diets.
Level III-2	Kushi et al (1992) Willett et al (1992)	Two cohort studies in the US. Show no inverse relationship between fibre and breast cancer.
Level III-2	Pietinen et al (1996)	Cohort, Finnish men. Highest consumption group (34.8 g/day) had an RR of 0.68 for CVD compared to the lowest consumption group (16.1 g/day).
Level III-2	Rimm et al (1996)	Health Professionals' Cohort. Difference in fibre intake of 16.5 g between the highest (28.9 g/day) and lowest (12.4 g/day) intake groups and RRs of 0.45 and 0.59 for fatal heart disease and total MI, respectively.
Level III-2	Rohan et al (1993)	Canadian cohort study. Shows a 32% reduction in breast cancer risk in the top quintile relative to the bottom quintile of fibre consumers.
Level III-2	Wolk et al (1999)	The Nurses' Health Study. Shows a difference between the highest (22.9 g/day) and lowest (11.5 g/day) consumption groups of 11.4 g fibre a day, RR of 0.77 for total CHD.
Level IV	King et al (1984)	Comparative study of 2 populations in Micronesia, 1 at high risk and 1 at low risk of developing type 2 diabetes and for which estimates of dietary fibre intake were of no predictive value in estimating the risk of subsequent diabetes.
Expert review	Alberts (2002)	Shows little benefit of fibre on colon cancer.
Survey data	Cassidy et al (1994)	International comparative study showing greater correlations between colon cancer and starch (and thus resistant starch) intake across countries than between colon cancer and dietary fibre.
Surveys & ecological studies	West & Kalbfleisch (1971) West (1974)	Show inverse association between fibre content of the diet and regional prevalence of diabetes.

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BALANCE OF MACRONUTRIENTS & CHRONIC DISEASE

PROTEIN

EVIDENCE BASE

Databases: PubMed 1995–2004 (Medline), Biological Abstracts 1985–1999 plus cross-checking of all references in FNB:IOM (2000).

Search terms: Recommended protein requirement or reference intake; protein and requirement; protein or nitrogen and requirements; diet and bone or osteoporosis; protein and bone or osteoporosis; protein requirements.

Note: Most of the additional papers were identified by links from recent publications rather than from the above searches.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Schurch et al (1998)	Protein supplementation of 20 g/day for 6 months to increase protein from about 0.75 to 1.05 g/kg in elderly patients with recent hip fracture. Shows attenuated proximal femur bone loss and increased IGF-1 (n=30 per group).
Level III-1	Dawson-Hughes et al (2004)	Increasing protein intake with meat from 0.78 to 1.55 g/kg/day in adults >50 years leads to increased urinary excretion of calcium (expressed as % of intake) also favourable changes in serum IGF-1 and N-telopeptide (n=16 per group).
Level III-1	Farnsworth et al (2003)	Increasing energy from protein from 16% to 27% had beneficial effects and no adverse effects on bone turnover or calcium excretion in persons with fasting insulin >12 mU/L (n=28 and 29 per group).
Level III-1	Gannon et al (2003)	An increase from 15% to 30% energy from protein in persons with type 2 diabetes improved glucose control (n=12 crossover).
Level III-1	Layman et al (2003a, b)	Increasing energy from protein from 15% to 28% had positive effects on body composition, blood lipids, blood glucose, insulin and satiety during weight loss on a diet of 7,100 kJ (n=12 women per group).
Level III-1	Morse et al (2001)	18-day N balance data for elderly women at 3 levels of N intake. Suggests that short-term N balance is inadequate to establish protein needs of elderly. Mean requirement at week 1, 0.7 g/kg/day compared with 0.56 g/kg/day at week 3 (n=11).
Level III-1	Parker et al (2002)	Increasing energy from protein from 16% to 28% in persons with type 2 diabetes resulted in greater reduction in total and abdominal fat mass in women and greater LDL reduction in both sexes (n=27 and 28 per group).
Level III-1	Roughead et al (2003)	Calcium retention the same for diets with either 12% or 20% energy from protein provided by extra meat for 8 weeks (n=15).
Level III-2	Barbone et al (1993)	Case-control study in US women found no statistically significant direct associations between any food item and endometrial cancer but OR of 0.7 for highest (>74.7 g) vs lowest (<62.5 g) tertile of protein intake.
Level III-2	Chow et al (1994)	Case-control study to assess dietary risk factors for renal cell cancer in US subjects. Risk increased with protein intake (ORs of 1.2, 1.4 and 1.9 in the second, third and fourth quartiles, respectively). Quartile 4 intake >87.5 g for men and >72.3 g for women.
Level III-2	Decarli et al (1997)	Case-control study in Italy of 2,569 incident cases of breast cancer and 2,588 controls. Negative association between total protein and breast cancer (OR 0.90 for additional 100 kcal/day from protein).
Level III-2	De Stefani et al (1999)	Case-control study in Uruguay to examine the risk of cancer of the upper digestive tract (oral cavity, pharynx, larynx and oesophagus) associated with nutrient intake. Strong positive association with protein intake (OR 2.5 highest vs lowest tertile). Mean protein intake 90.9 g/day in cases.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Franceschi et al (1999)	Case-control study in Italy to assess oral and pharynx cancer risk associated with energy and macronutrient intake. Inverse association with protein intake (OR 0.8 for 100 kcal/day added protein).
Level III-2	Gao et al (1994)	Case-control study in Shanghai found inverse association with oesophageal cancer risk in men (OR 0.6 highest vs lowest quartile) but not for women (OR 1.7 highest vs lowest quartile).
Level III-2	Hannan et al (2000)	BMD loss at femoral neck and lumbar spine over 4 years in Framingham cohort. Significant decrease from quartile 1 to quartile 4 of protein intake equivalent to >13.5% energy, 0.71 g/kg and 51 g/day (n=615).
Level III-2	Meyer et al (1997)	Elevated risk of fracture in women with high intake of protein from non-dairy sources in presence of low calcium intake after 11-year follow-up of 20,000 men and women.
Level III-2	Munger et al (1999)	Risk of hip fracture negatively associated with protein intake above 17% energy from protein after 2-year follow-up in >30,000 postmenopausal women.
Level III-2	Sellmyer et al (2001)	High ratio of animal to vegetable protein associated with increased risk of femoral neck fracture over 7 years in postmenopausal women with low energy intake of about 5 MJ/day and low protein of 58 g/day. Calcium intake, 1,124 mg/day (n=1,035).
Level III-2	Shu et al (1993)	Case-control study in Shanghai found dietary intakes of animal fat (OR 3.5 highest vs lowest quartile) and animal protein (OR 3.0 highest vs lowest quartile) were associated with an increased risk of endometrial cancer. After adjustment for total energy, positive associations were seen with consumption of meat, fish and eggs.
Level III-2	Toniolo et al (1994)	Prospective cohort study of 14,291 New York women. Positive association between red meat but not protein intake (RR 1.87 highest vs lowest quintile) and risk of breast cancer. Quintile 5 protein intake about 108 g vs 32 g in quintile 1.
Level III-2	Wengreen et al (2004)	Protein intake of 14.0–30.8% of energy associated with significantly reduced risk of hip fracture in men and women of 50–69 years, but not those >70 years (n=1,167 cases).
Level IV	Lei et al (1996)	Retrospective case-control study in Guangzhou involving all lung deaths registered in 1986. Controls were selected from the same year of death. Home interviewers surveyed consumption of selected food items categorised as never, weekly or daily. No association between high protein food items and incidence of lung cancer.
Level IV	Rapuri et al (2003)	Positive associations between quintile of protein intake as per cent energy (≥18%) and BMD (not significant for hip) at baseline but no association with bone loss over 3 years in 96 post-menopausal women (n=473).
Level IV	Whiting et al (2002)	Protein, potassium and phosphorus intakes are significant predictors of total bone BMD and lumbar spine BMD in adult men (n=57).
Human physiological data	Poortmans & Dellalieux (2000)	In athletes consuming long-term protein intakes of up to 2.8 g/kg body weight. Shows no negative effect on renal function.
Review	Bell & Whiting (2002)	An adequate intake of protein in elderly women is important for preservation of bone mass.
Review	Giovannucci & Willett (1994)	Among published reports from epidemiological studies of diet and colon cancer, none has shown a significant association with intake of protein from sources other than red meat.
Review	Jackson (1999)	More sensitive measures needed to assess possible adverse effects of high protein intake.
Review	Kerstetter et al (2003)	No definitive intervention studies for adverse effect of a high protein diet on bone, but some evidence that on reducing protein intake to 0.7 g/kg, calcium absorption falls and serum PTH and calcitriol increase for at least 2–4 weeks.
Review	Parnaud & Corpet (1997)	Positive association between meat intake and colon cancer incidence is supported in most case-control studies (22 of 29) but in only 2 of 5 prospective cohort studies. Data from 6 experimental studies do not support an effect.

Level of evidence	Reference	Study type, issues addressed and key findings
Review	Prentice (2004)	No firm evidence on which to base recommendations about optimal protein intake for bone growth or the prevention of osteoporosis.
Review	Swinburn et al (2004)	Protein probably not an important influence on obesity (limited range of intake), but increasing intake may be beneficial for some for weight control.

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FATS & FATTY ACIDS

EVIDENCE BASE

Note: Some of the references in the carbohydrate section are also relevant to dietary fats.

Databases: Medline plus cross-checking of all references in FNB:IOM (2000).

Search terms: very low fat diet and weight; low fat diet and weight; dietary fat and obesity low fat diet and weight; low fat diet and weight and Cochrane; low carbohydrate diet and obesity; low fat diet and hyperinsulinaemia; carbohydrate and fat and lipids; total fat and cancer.

Level of evidence	Reference	Study type, issues addressed and key findings
Total, saturated and *trans* fats, polyunsaturates and monounsaturates in general		
Level I	Astrup et al (2000)	A reduction in dietary fat without intentional restriction of energy intake causes weight loss (greater in heavier subjects).
Level I	Howell et al (1997)	Meta-analysis of effects of dietary fat and lipids. Predictions indicate that compliance with 30% of energy from fat, <10% from saturated fat and <300 mg cholesterol/day will reduce plasma total cholesterol by 5% compared with average American diet.
Level I	Mensink & Katan (1992)	Reviewed 27 controlled trials, published 1970–1991, that met specific inclusion criteria. All fatty acids elevated HDL cholesterol when substituted for carbohydrates, but the effect diminished with increasing unsaturation of the fatty acids.
Level I	Mensink et al (2003)	Meta-analysis of 60 controlled trials. Prediction equation that quantifies effects of carbohydrate/fat ratios (4–53% energy from fat) on lipids. Low fat, high carbohydrate diets have less favourable total cholesterol/HDL ratio but does not conclusively relate to risk of CVD.
Level I	Pirozzo et al (2002)	Fat-restricted diets are no better than calorie-restricted diets in achieving long-term weight loss.
Level I	Yu-Poth et al (1999)	Step I and Step II dietary interventions have multiple beneficial effects on CVD risk factors.
Level II	Brehm et al (2003)	Weight loss greater on low carbohydrate than low fat over 6 months.
Level II	Conrad et al (2000)	Suggests that 10% fat energy is an unrealistic target.
Level II	Foster et al (2003)	Weight loss greater on low carbohydrate, high fat diet than low fat over 6 months, but not different at 1 year.
Level II	Hill et al (1991)	Diet composition does not affect total daily energy expenditure.
Level II	Leibel et al (1992)	Diet composition does not affect total daily energy expenditure, even with extreme changes in the fat:carbohydrate ratio.
Level II	Ness et al (2002)	RCT. Former participants in the Diet and Reinfarction Trial. Assessed current fish intake and cereal fibre intake and compared to all-cause mortality, stroke mortality and coronary mortality. Shows no substantial long-term survival benefit. The early reduction in all-cause mortality observed in those given fish advice (unadjusted hazard 0.70 (95% CI: 0.54, 0.92)) was followed by an increased risk over the next 3 years (unadjusted hazard 1.31 (95% CI: 1.01, 1.70).
Level II	Samaha et al (2003)	Severely obese subjects with a high prevalence of diabetes or the metabolic syndrome lost more weight after 6 months on a carbohydrate-restricted diet than on a calorie- and fat-restricted diet.
Level II	Sharman et al (2004)	Weight loss greater on low carbohydrate, high fat diet than low fat over 6 weeks.
Level III-1	Turpeinen et al (1979)	Controlled intervention trial, in 2 mental hospitals near Helsinki between 1959 and 1971. The use of a serum cholesterol-lowering diet exerted a substantial preventive effect on CHD.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Boyd et al (2003)	The summary RR, comparing the highest and lowest levels of intake of total fat, was 1.13 (95% CI: 1.03, 1.25). Cohort studies (n=14) had a summary RR of 1.11 (95% CI: 0.99, 1.25) and case-control studies (n=31) had an RR of 1.14 (95% CI: 0.99, 1.32).
Level III-2	Bray & Popkin (1998)	Review. Low fat diets are hypocaloric.
Level III-2	Clarke et al (1997)	Meta-analysis of metabolic ward studies of solid food diets in healthy volunteers. In typical British diets, replacing 60% of saturated fats by other fats and avoiding 60% of dietary cholesterol would reduce blood total cholesterol by about 0.8 mmol/L (that is, by 10–15%), with 80% of this reduction being in LDL cholesterol.
Level III-2	Flood et al (2003)	Prospective cohort in the US, 45,496 women and 386,716 person-years of follow-up. 487 incident cases of colorectal cancer. No evidence of an association between fat and colorectal cancer incidence.
Level III-2	Harding et al (2004)	EPIC study. Increased dietary polyunsaturated:saturated fat ratio was associated with a reduced risk of diabetes, independent of age, sex, family history of diabetes and other lifestyle factors.
Level III-2	Hill et al (2000)	Review. Low fat diets are hypocaloric.
Level III-2	Huncharek & Kupelnick (2001)	A high total fat intake is associated with a 24% increased risk of development of ovarian cancer.
Level III-2	Laaksonen et al (2005)	Population-based cohort of 1,551 middle-aged men. Concluded that dietary polyunsaturated, and more specifically, linoleic, fatty acid intake may have a substantial cardioprotective benefit that is also reflected in overall mortality. Dietary fat quality seems more important than fat quantity in the reduction of cardiovascular mortality in men.
Level III-2	Neaton & Wentworth (1992)	316,099 men screened for the Multiple Risk Factor Intervention Trial (MRFIT) were examined. Strong graded relationships between serum cholesterol levels above 4.65 mmol/L (180 mg/dL), systolic blood pressure above 110 mmHg, and diastolic blood pressure above 70 mmHg and mortality due to CHD were evident. Systolic and diastolic blood pressure, serum cholesterol level and cigarettes per day were significant predictors of death due to CHD in all age groups.
Level III-2	Oh et al (2005)	Cohort study in the US. 78,778 women with 20 years of follow-up. Polyunsaturated fat intake was inversely associated with CHD risk whereas *trans* fat intake was associated with an elevated risk of CHD. The associations between intakes of polyunsaturated fat and *trans* fat with CHD risk were most evident among women younger than age 65 years. The inverse association between poly-unsaturated fat intake and CHD risk was strongest among women whose body mass index was ≥25 kg/m^2. Findings continue to support an inverse relation between polyunsaturated fat intake and CHD risk, particularly among younger or overweight women. In addition, *trans* fat intake was associated with increased risk of CHD, particularly for younger women.
Level III-2	Satia-Abouta et al (2003)	Case-control study. Incident cases of histologically confirmed colon cancer, aged 40–80 years (n=613) and matched controls (n=996). Total energy intake was consistently associated with colon cancer risk.
Level III-2	Sorkin et al (1992)	1,052 men, participants in the Baltimore Longitudinal Study on Aging. 3 age groups, 28–64, 65–74 and 75–97 years. In all groups, cholesterol was a significant risk factor for CHD.
Level III-2	Smith-Warner et al (2002)	280,419 female and 149,862 male participants followed for up to 6–16 years and 3,188 lung cancer cases were documented. Fat intake was not associated with lung cancer risk.
Level III-2	Stamler et al (1986)	356,222 men in MRFIT. For each 5-year age group, the relationship between serum cholesterol and CHD death rate was continuous, graded, and strong for cholesterol concentrations ≥4.65 mmol/L , with almost half the excess deaths in serum cholesterol quintiles 2–4. These data show that the relationship between serum cholesterol and CHD is not a threshold one, with increased risk confined to the two highest quintiles, but rather is continuously graded.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Weijenberg et al (1996)	Cohort of men aged 64–84 years from the Dutch town of Zutphen followed for 5 years. Total cholesterol was not significantly associated with the incidence of CHD, but for mortality the RR corresponding to a 1.00 mmol/L increase was 1.40. HDL cholesterol was not associated with mortality from CHD. The RR for the incidence of the disease, corresponding to a 0.26 mmol/L increase, was 0.80. For the ratio of HDL cholesterol to total cholesterol, the relative risk for CHD incidence corresponding to a 0.05 increase amounted to 0.70 (95% CI: 0.51, 0.95). Total cholesterol seems to be a stronger risk factor for mortality from the disease, whereas HDL cholesterol is more strongly associated with the incidence of a first CHD event.
Level III-3	Aro et al (1997)	80 healthy subjects consumed a dairy fat-based (baseline) diet for 5 weeks, then an experimental diet high in either *trans* fatty acids or stearic acid for another 5 weeks. High amounts of *trans* fatty acids had more adverse effects on lipoproteins than did equal amounts of stearic acid and dairy fat. Stearic acid reduced LDL cholesterol, did not affect LDL:HDL cholesterol, and increased lipoprotein(a), although to a lesser extent than did *trans* fatty acids.
Level III-3	Judd et al (1994)	Effects of *cis*- and *trans*-monounsaturated fatty acids (TFAs) and saturated fatty acids assessed in 29 men and 29 women consuming controlled diets. Subjects ate each diet for 6 weeks in a Latin square design. Compared with oleic acid, dietary TFAs raise LDL cholesterol, but to a slightly lesser degree than do saturates. High TFA concentrations may result in minor reductions of HDL cholesterol.
Level III-3	Judd et al (1998)	Controlled diet study, 23 men and 23 women. Effects of butter and 2 types of margarine on blood lipid and lipoprotein concentrations. Consumption of TFA-margarine or PUFA-margarine improved blood lipid profiles for the major lipoproteins associated with cardiovascular risk when compared with butter. Shows a greater improvement with PUFA-margarine than with TFA-margarine.
Level III-3	Louheranta et al (1999)	Randomised crossover design. 14 healthy women. Effects of diets high in TFAs and oleic acid [monounsaturated fat (MUFA) diet] on glucose and lipid metabolism. Subjects ate both experimental diets for 4 weeks. The TFA diet resulted in a higher total:HDL cholesterol ratio and an elevation in triglyceride and apo B concentrations, but had no effect on glucose and insulin metabolism compared with the MUFA diet.
Level III-3	Magarey et al (2001)	Fat intake a poor predictor of adiposity in children.
Level III-3	Muller et al (1998)	Randomised crossover design, 16 female normolipidaemic students. Diets with two test margarines for 14 days. Partially hydrogenated fish oil, with its unfavourable effects on plasma lipids, can be replaced by vegetable oils in margarine without appreciable loss of functional properties, but with significant improvement in the effects on plasma lipoproteins.
Level III-3	Nestel et al (1992)	Double-blind comparison of 4 separate diets, 27 mildly hypercholesterolaemic men. Effect of additional dietary TFAs (7% energy) on plasma lipids was assessed. Total and LDL cholesterol were significantly lower during the 3-week oleic acid-rich diet, and were similar during the other 3 diets. HDL cholesterol was significantly higher with the palmitic acid-rich diet, 42 mg/dL, compared with elaidic acid, 38 mg/dL, which in turn was not lower than with oleic acid, 38 mg/dL. Plasma elaidic acid concentration rose 7-fold with the TFA diet, but did not increase the vulnerability of LDL to oxidative change. The elaidic acid-rich diet led to significant elevations in the level of lipoprotein (a) compared to all the other test diets.
Level III-3	Noakes & Clifton (1998)	Compared the effects on plasma lipids of margarines containing either a TFA-free hard fraction (achieved through interesterification from primarily saturated fatty acids) or a partially hydrogenated hard fraction in 38 mildly hyperlipidaemic subjects. Compared with butter, TFA-free margarines may be as effective as, or more effective than, margarines containing TFA-free margarines in lowering LDL cholesterol.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-3	Seppanen-Laakso et al (1993)	The effects of zero erucic acid rapeseed oil and olive oil on plasma fatty acid composition and serum cholesterol were studied in margarine users (n=46). Shows a dose-dependent rise in ALA and oleic acid levels during rapeseed and olive oil substitutions, respectively. Rapeseed oil substitution increased the proportion of eicosapentaenoic acid in plasma phospholipids. A slight decrease in LDL cholesterol and an increase in HDL cholesterol led to a significantly higher HDL-C/total cholesterol ratio. Results suggest a marked competitive effect for ALA. No competitive action of polyunsaturated acids comparable to rapeseed oil was found during olive oil substitution.
Level III-3	Sundram et al (1997)	Crossover design of dietary TFAs specifically exchanged for *cis* 18:1, 16:0 or 12:0 + 14:0 in 27 male and female subjects on moderate fat, low cholesterol, whole food diets during 4-week diet periods. The *trans*-rich fat significantly elevated total cholesterol and LDL cholesterol relative to the 16:0-rich and 18:1-rich fats and uniquely depressed HDL cholesterol relative to all of the fats tested. TFAs also elevated lipoprotein (a) values relative to all dietary treatments.
Level IV	Gould et al (1995)	Very low fat diet, <15% fat energy, associated with atherosclerotic regression.
Cross-sectional survey	Lopez-Garcia et al (2005)	Cross-sectional study of 730 women from the Nurses' Health Study I cohort. C reactive protein (CRP) levels were 73% higher among those in the highest quintile of *trans* fat intake. IL-6, soluble (s) TNFR, E-selectin, sICAM-1 and sVCAM-1 levels were higher. TFA intake was positively related to plasma concentration of CRP, sTNFR-2, E-selectin. sICAM-1 and sVCAM-1. Suggests that higher intake of TFAs could adversely affect endothelial function.
Survey data	Jousilahti et al (1998)	Data from 5 independent risk factor surveys in eastern Finland. The association between cholesterol level and CHD risk and the prediction of the effect of different prevention strategies was estimated by use of logistic regression models. The risk of CHD death among subjects with cholesterol ≥8.0 mmol/L was approximately 5-fold that of individuals with cholesterol <5.0 mmol/L. The community-based population strategy in CVD prevention was effective in decreasing cholesterol levels among the entire population.
Expert review	Ascherio et al (1999)	Metabolic and epidemiological studies indicate an adverse effect of TFAs on the risk of CHD.
Expert review	Hegsted et al (1993)	Regression analysis of the combined published data on effects of dietary fatty acids and cholesterol on serum cholesterol and lipoprotein cholesterol in humans. Shows that saturated fatty acids increase, and are the primary determinants of, serum cholesterol. Polyunsaturated fatty acids actively lower serum cholesterol, monounsaturated fatty acids have no independent effect on serum cholesterol and dietary cholesterol increases serum cholesterol and must be considered when the effects of fatty acids are evaluated. More limited data on LDL cholesterol show that changes roughly parallel the changes in serum cholesterol. Changes in HDL cholesterol cannot be satisfactorily predicted from available data.
Expert review	Jequier (1999)	Lower limit of fat intake to meet the energy needs of adults is 10–15% energy.
Expert review	Kris-Etherton et al (2004)	Epidemiologic studies have shown a beneficial association between polyunsaturated fatty acid, specifically linoleic acid, intake and CVD morbidity and mortality. Clinical studies have shown that n-6 polyunsaturated fatty acids have the most potent cholesterol-lowering effects of the individual fatty acid classes. Emerging evidence suggests that they have favourable effects on postprandial lipaemia. However, some studies suggest that high intakes of linoleic acid may have adverse effects on proinflammatory cytokines and adhesion molecules.
Expert review	Weisburger (1988)	Populations consuming 10% fat energy maintain adequate health.
LC n-3 fatty acids (omega fatty acids)		
Level I	Beckles Willson et al (2002)	Omega-3 fatty acids (from fish oils) for cystic fibrosis. Cochrane Database Systematic Review. Results inconclusive: "The review of trials found that regular omega-3 supplements may provide some benefits for people with cystic fibrosis with relatively few adverse effects, although the evidence is insufficient to draw firm conclusions. There is insufficient evidence to recommend routine use of supplements of omega-3 fatty acids in people with cystic fibrosis."

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Hooper et al (2002)	Omega-3 fatty acids for prevention of CVD. The Cochrane Database Systematic Review. Inconclusive but incomplete.
Level I	Joy et al (2003)	Polyunsaturated fatty acid supplementation for schizophrenia. Cochrane Database Systematic Review. Results inconclusive: "The use of omega-3 polyunsaturated fatty acids for schizophrenia remains experimental and large well designed, conducted and reported studies are indicated and needed."
Level I	Kris-Etherton et al (2002)	Fish consumption, fish oil, omega-3 fatty acids and CVD. Concludes LC n-3 fatty acids reduce the incidence of CVD. Large-scale epidemiologic studies suggest that individuals at risk for CHD benefit from the consumption of plant- and marine-derived omega-3 fatty acids, although the ideal intakes are unclear. Evidence from prospective secondary prevention studies suggests that EPA+DHA supplementation ranging from 0.5 to 1.8 g/day (either as fatty fish or supplements) significantly reduces subsequent cardiac and all-cause mortality.
Level I	US FDA (2000)	Meta-analysis of all evidence – RCT, case-control and cohort, showing benefit of LC omega fatty acids.
Level I	Woods et al (2002)	Cochrane Database Systematic Review. Dietary marine fatty acids (fish oil) for asthma in adults and children. Results inconclusive: "There is little evidence to recommend that people with asthma supplement or modify their dietary intake of marine n-3 fatty acids (fish oil) in order to improve their asthma control. Equally, there is no evidence that they are at risk if they do so."
Level II	Bemelmans et al (2002)	Mediterranean αLinolenic Enriched Groningen Dietary Intervention (MARGARIN). Increased intake of ALA and group nutritional education on cardiovascular risk factors affected CVD risk. Increased ALA intakes decrease the estimated IHD risk to an extent similar to that found with increased linoleic intakes. Group nutritional education can effectively increase fish intake.
Level II	Burr et al (1989)	Effects of change in fat, fish and fibre intakes on death and myocardial reinfarction, the Diet and Reinfarction Trial (DART). A modest intake of fatty fish (2 or 3 portions per week) may reduce mortality in men who have recovered from MI.
Level II	de Lorgeril et al (1994)	Mediterranean ALA-rich diet helps secondary prevention of CHD. An ALA-rich Mediterranean diet seems to be more efficient than presently used diets in the secondary prevention of coronary events and death.
Level II	Dolecek (1992)	Provides evidence of relationships between dietary polyunsaturated fatty acids and mortality in the MRFIT. No significant associations with mortality were detected for linoleic acid.
Level II	GISSI-Prevenzione Investigators (1999)	Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after MI. Dietary supplementation with n-3 polyunsaturated fatty acids leads to a clinically important and statistically significant benefit.
Level II	Mori et al (1999)	Shows that DHA, but not EPA, lowers ambulatory blood pressure and heart rate in humans. Suggests that DHA is the principal omega-3 fatty acid in fish and fish oils that is responsible for their blood pressure lowering and heart rate lowering effects in humans.
Level II	Natvig et al (1968)	Controlled trial of the effect of ALA on incidence of CHD. Shows benefit.
Level II	Nilsen et al (2001)	Effects of a high-dose concentrate of n-3 fatty acids or corn oil introduced early after an acute MI on serum triglycerides and HDL cholesterol. No clinical benefit of a high-dose concentrate of n-3 fatty acids compared with corn oil, despite a favourable effect on serum lipids.
Level II	Thies et al (2003)	Shows association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques. Atherosclerotic plaques readily incorporate n-3 PUFAs from fish-oil supplementation, inducing changes that can enhance plaque stability. By contrast, increased consumption of n-6 PUFAs does not affect carotid plaque fatty acid composition or stability over the time course studied.
Level II	von Schacky et al (1999)	Dietary omega-3 fatty acids modestly mitigate the course of coronary atherosclerosis in humans.

Level of evidence	Reference	Study type, issues addressed and key findings
Level II	Wallace et al (2003)	Compared effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. Concludes that, with the exception of IL-6 production, a modest increase in intake of either ALA or EPA+DHA does not influence the functional activity of mononuclear cells. The threshold of EPA+DHA intake that results in decreased IL-6 production is between 0.44 and 0.94 g/day.
Level III-2	Albert et al (2002)	Assessed blood levels of LC n-3 fatty acids and the risk of sudden death. The n-3 fatty acids found in fish are strongly associated with a reduced risk of sudden death among men without evidence of prior CVD.
Level III-2	He et al (2002)	Assessed fish consumption and risk of stroke in men. Suggests that eating fish once per month or more can reduce the risk of ischemic stroke in men.
Level III-2	Hu et al (2002)	Assessed fish and omega-3 fatty acid and risk of CHD in women. Among women, higher consumption of fish and omega-3 fatty acids is associated with a lower risk of CHD, particularly CHD deaths.
Level III-2	Iso et al (2001)	Assessed intake of fish and omega-3 fatty acids and risk of stroke in women. Data indicate that higher consumption of fish and omega-3 polyunsaturated fatty acids is associated with a reduced risk of thrombotic infarction, primarily among women who do not take aspirin regularly, but is not related to risk of haemorrhagic stroke.
Level III-2	Mozaffarian et al (2003)	Cardiovascular Health Study. Cardiac benefits of fish consumption may depend on the type of fish meal consumed. Among adults aged ≥65 years, modest consumption of tuna or other fish broiled or baked, but not fried fish or fish sandwiches, is associated with lower risk of IHD death, especially arrhythmic IHD death.
Level III-2	Siscovick et al (1995)	Assessed dietary intake and cell membrane levels of LC n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. Dietary intake of n-3 polyunsaturated fatty acids from seafood was associated with a reduced risk of primary cardiac arrest.

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CARBOHYDRATE

EVIDENCE BASE

Note: Some of the references in the fats section are also relevant to carbohydrates.

Database: Medline plus crosschecking of all references in FNB:IOM (2000).

Search terms: behaviour and (sugar or sucrose); dental caries and (carbohydrate or sugar or sucrose); plasma lipids and (carbohydrate or sugar or sucrose or fructose); triacylglycerols and (carbohydrate or sugar or sucrose or fructose); coronary heart disease and (carbohydrate or sugar or sucrose); insulin sensitivity and (carbohydrate or sugar or sucrose or fructose); diabetes and (carbohydrate or sugar or sucrose); obesity and (carbohydrate or sugar or sucrose); cancer and (carbohydrate or sugar or sucrose); blood pressure and (carbohydrate or sugar or sucrose); bone density and (sugar or sucrose); nutrient density and (sugar or sucrose); energy regulation and (carbohydrate or sugar or sucrose); carbohydrate and (Australia or New Zealand).

Level of evidence	Reference	Study type, issues addressed and key findings
Level I	Anderson & Major (2002)	Meta-analysis of the effect of non-soya pulses on blood lipids. Pulse consumption raised fasting HDL cholesterol and lowered triglycerides and total and LDL cholesterol.
Level I	Yu-Poth et al (1999)	Meta-analysis of effects of the National Cholesterol Education Program Step I and II diets (reduce total and saturated fatty acids and by default increase carbohydrate) on CVD risk factors. Both Steps 1 and 2 lowered triglycerides, total and LDL cholesterol, and total cholesterol:HDL ratio. No change in HDL after Step 1, but reduction in HDL on Step 2 diet.
Level II	Obarzanek et al (2001)	High carbohydrate diet (58% of energy) lowered total, LDL and HDL cholesterol concentrations and had no effect on triglycerides compared with a lower carbohydrate diet (50% of energy).
Level II	Poppitt & Swann (1998)	Ad libitum energy intake of obese women receiving an appetite suppressant was greater (10.5 MJ/day) when consuming a high fat (50% of energy) diet than when consuming a low fat (25% of energy) diet (8.1 MJ/day). The high fat diet induced a positive fat and energy balance.
Level II	Poppitt et al (2002)	RCT over 6 months comparing a control diet (40% energy from fat) with 2 low-fat diets having simple or complex carbohydrate components. Mean weight loss was achieved in the complex carbohydrate group. Total cholesterol and triglycerides were higher in the simple carbohydrate group than the complex carbohydrate group.
Level II	Raben et al (2002)	RCT comparing the effect of artificial sweetener or sucrose consumption on energy intake and body weight over 10 weeks. Sucrose at 28% of energy intake was associated with increased energy intake, greater body fat and fat mass, and higher blood pressure than artificial sweeteners.
Level II	Turley et al (1998)	A high carbohydrate diet (from fruits, vegetables, grains and legumes) improved blood lipid profiles and led to a slight weight loss despite efforts to maintain equal energy intake compared with a high fat diet.
Level III-1	Anderson & Ward (1979)	70% carbohydrate diet from whole grains, vegetables and pulses fed to 20 diabetic men. Compared with a 43% carbohydrate traditional diabetic diet, the high carbohydrate diet resulted in lower or discontinued daily insulin dose, lower fasting and 3-hour postprandial blood glucose concentrations, lower serum total cholesterol and unaltered triglyceride concentrations.
Level III-1	Anderson et al (1980)	70% of energy carbohydrate diet from whole grains, vegetables and pulses fed to 14 diabetic men. Compared with a 43% of energy carbohydrate traditional diabetic diet, the high carbohydrate diet resulted in lower daily insulin dose, lower fasting blood glucose concentrations and lower serum total cholesterol and postprandial triglyceride concentrations.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-1	Anderson et al (1991)	70% of energy carbohydrate diet from whole grains, vegetables and pulses fed to 12 diabetic men and women. Compared with a 39% of energy carbohydrate diet, the high carbohydrate diet resulted in reduced basal insulin requirements, increased carbohydrate disposal, lower serum total and HDL cholesterol and unaltered triglyceride concentrations.
Level III-1	Antonis & Bersohn (1961)	70% of energy carbohydrate diet associated with lower or unchanged triglycerides compared with high fat diets. Transient increase in triglycerides with change from a 45% to a 70% of energy carbohydrate diet.
Level III-1	Borkman et al (1991)	High carbohydrate diet (>50% of energy) lowered fasting serum LDL and HDL cholesterol and raised triglycerides relative to a lower carbohydrate (<40% of energy) diet.
Level III-1	Brynes et al (2003)	Randomised crossover study to assess 4 dietary intervention strategies on blood glucose metabolism and lipids. The 4 diets were high fat (50% energy with 34% MUFA), low GI, high GI and high sucrose. The diets were eaten ad libitum for 24 days each. No difference among diets for fasting glucose, lipids, or homeostasis model assessment (HOMA). Increase in 6-hour triglyceride concentration between baseline and day 24 over the sucrose period. Change in postprandial HOMA between baseline and day 24 greatest in the high GI period. The low GI diet induced weight loss compared with the high sucrose diet.
Level III-1	de Roos et al (2001)	Randomised crossover study to assess the effect of diet (high MUFA compared with high carbohydrate) on vascular response. Flow-mediated vasodilation was the same for both diets, despite lower HDL and higher triglyceride concentrations during the high carbohydrate period.
Level III-1	Fukagawa et al (1990)	68% carbohydrate diet from whole grains, vegetables and pulses fed to 12 healthy men and women. Compared with a 43% carbohydrate free choice control diet, the high carbohydrate diet resulted in lower fasting blood glucose and insulin concentrations, lower fasting serum total cholesterol and unchanged triglyceride concentrations and increased glucose disposal rates.
Level III-1	Garg et al (1994)	In people with type 2 diabetes receiving glipizide therapy, a high carbohydrate diet (55% of energy) raised fasting plasma triglycerides, VLDL cholesterol and day-long triglycerides compared with a lower carbohydrate diet (40% of energy) with a high MUFA content. Total, LDL and HDL cholesterol not different between diets.
Level III-1	Glueck et al (1982)	Covert fat replacement with an indigestible synthetic compound reduces total caloric intake.
Level III-1	Grundy et al (1988)	A high carbohydrate diet (65% of energy) lowered fasting plasma total, LDL, and HDL cholesterol relative to a lower carbohydrate (45%) diet. A low carbohydrate (45% of energy), high MUFA diet did not lower HDL.
Level III-1	Kiehm et al (1976)	75% of energy carbohydrate diet from natural high fibre foods fed to 13 diabetic men. Compared with a 43% of energy carbohydrate American Diabetic Association diet, the high carbohydrate diet resulted in decreased or discontinued use of diabetic drugs (sulphonylureas and insulin), lower fasting plasma glucose and lower fasting serum total cholesterol and triglyceride concentrations.
Level III-1	Lawton et al (1993)	Ad libitum energy intake of obese women was 1,336 kcal on a high fat (>50%) dinner and 677 kcal on a high carbohydrate (> 50%) dinner.
Level III-1	Lissner et al (1987)	Two-week periods of consuming a low fat (15–20% of energy), a medium fat (30–35% of energy), and a high fat (45–50% of energy) diet. Compared with the medium fat diet, the low fat diet resulted in an 11% deficit, and the high fat diet a 15% increment in total energy intake. The high fat diet increased weight.
Level III-1	Marckmann et al (2000)	High carbohydrate diet (59% of energy) with low sucrose content (2.5% of energy) improves blood lipids and coagulant activity relative to a high carbohydrate diet (59% of energy) with higher sucrose content (23.2% of energy). High carbohydrate, low sucrose diet improved LDL cholesterol and non-fasting coagulant activity relative to a high fat diet.
Level III-1	Pereira et al (2002)	Randomised crossover study to assess the effect of refined or unrefined grain products on glucose metabolism. Measures of insulin sensitivity showed improvement on the whole grain diet.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Poppitt & Swann (1998)	The ad libitum energy intake of lean men was greater as fat content of a lunch meal increased incrementally from 20–60% of energy. No decrease in weight of food eaten as energy density increased.
Level III-1	Proserpi et al (1997)	48-hour whole body indirect calorimetry. Ad libitum energy intake of young men was 18.25 MJ/day on a high fat (61% of energy) diet and 14.41 MJ/day on a high carbohydrate (67% of energy) diet. Only the high fat diet induced a positive fat balance.
Level III-1	Thomas et al (1992)	1-week whole room indirect calorimetry. Ad libitum energy intake of lean and obese adults was 11.04 MJ/day on a high fat (52% of energy) diet and 10.67 MJ/day on a high carbohydrate (62% of energy) diet. Only the high fat diet induced a positive fat balance in lean participants.
Level III-1	van Stratum et al (1978)	Two-week periods of consuming mainly liquid formula diets, either low fat (24% of energy) or high fat (47% of energy) and having similar energy density. No difference in ad libitum energy intake between diets.
Level III-1	Vidon et al (2001)	High complex carbohydrate diet improves blood lipids and insulin sensitivity compared with a high fat diet (ratio of 1:1:1 for saturated:monounsaturated:polyunsaturated fatty acids.
Level III-2	Augustin et al (2001)	Case-control study assessing risk of breast cancer and carbohydrate intake in Italian women. Positive association between glycaemic index (OR highest vs lowest quintile 1.4) and glycaemic load (OR 1.3) and risk of breast cancer.
Level III-2	Augustin et al (2003)	Case-control study assessing risk of endometrial cancer and dietary glycaemic measures in Italian and Swiss women. Positive association between glycaemic index (OR highest vs lowest quintile 2.1) and glycaemic load (OR 2.7) and risk of endometrial cancer.
Level III-2	Janket et al (2003)	Prospective study of 39,345 US women assessing incident type 2 diabetes over years. No association between sugar, sucrose, fructose, glucose or lactose intake at baseline and incident diabetes.
Level III-2	Karjalainen et al (2001)	Prospective study assessing sugar intake and caries over 3 years in 3-year-old Danish children. Sucrose intake at 3 years was predictive of dental caries at 6 years of age. Children with caries had a mean sucrose intake of 40 g/day. Caries-free children ate <32.5 g/day ($p < 0.05$).
Level III-2	Liu et al (2000)	Prospective study of 75,521 North American women. Whole grain intake was protective against type 2 diabetes. The risk increased as the ratio of refined:unrefined grains increased.
Level III-2	Michaud et al (2002)	Prospective study of 88,802 US women assessing incident pancreatic cancer over 18 years. Carbohydrate and sucrose intake were not associated with overall risk of pancreatic cancer. Among sedentary, overweight women, a high glycaemic load and a high fructose intake were associated with increased relative risks.
Level III-2	Newby et al (2003)	Longitudinal study assessing dietary pattern on weight change. A diet high in white bread or refined grains increased waist circumference compared to a healthy diet.
Level III-2	Rodrigues & Sheiham (2000)	Prospective study assessing the effect of a sugar restrictive policy on caries in children attending Brazilian nurseries. The weight of sugar consumed at nurseries that adopted the policy was 22.9 g/day compared with 53.5 g/day in nurseries without guidelines. The OR for 1 year caries increment in children consuming >32.6 g of sugar during nursery attendance was 2.99 (95% CI: 1.82, 4.91).
Level III-2	Sacks & Katan (2002)	Meta-analysis suggesting that high carbohydrate diets lower HDL cholesterol and raise triglycerides, except when low glycaemic foods are used.
Level III-2	Saltzman et al (1997)	Ad libitum energy intakes were not different between diets that provided 20% or 40% energy as fat when the diets were matched for energy density.
Level III-2	Stubbs et al (1995)	Two-week periods of consuming a low fat (20% of energy), a medium fat (40% of energy) and a high fat (60% of energy) diet. Energy density and energy intake increased with increasing fat content.

Level of evidence	Reference	Study type, issues addressed and key findings
Level III-2	Stubbs & Harbron (1996)	Large differences in dietary macronutrient proportions (20–60% of energy as fat, 68–28% of energy as carbohydrate) do not lead to differences in energy intake if the diets are isoenergetic.
Level III-2	Terry et al (2003)	Prospective study of 49,124 Canadian women assessing incident colorectal cancer over 16.5 years. No association found between glycaemic load, total carbohydrate or total sugar intakes and incident colorectal cancer.
Level III-2	Tremblay et al (1989)	A high fat diet (assessed by food quotient) induces body fat accumulation.
Level III-2	Tremblay et al (1991)	A high fat diet (assessed by food quotient) induced a higher energy intake despite a smaller weight of food consumed due to greater energy density. When carbohydrate was ≤25% of energy intake, the effect was similar, but there was no longer a difference in weight of food consumed.
Level III-3	Hsing et al (2003)	Case-control study assessing risk of prostate cancer, insulin resistance and body fat in Chinese men. Insulin sensitivity was inversely associated, and insulin resistance positively associated, with risk of prostate cancer. Insulin resistance combined with high waist:hip ratio gave an OR of 8.2 (95% CI: 2.8, 23.7).
Level III-3	Kalkwarf et al (2003)	Retrospective study of milk intake and bone health in US women. Women with low milk intake during childhood and adolescence have less bone mass in adulthood and greater risk of fracture.
Key survey data	Liebman et al (2003)	Risk of overweight and obesity associated with sweetened drinks and super-sized portions.
Key survey data	Park et al (2003)	Analysis of dietary intake data from NHANES III. High carbohydrate intake (>60% of energy intake) was associated with increased odds of the Metabolic Syndrome.
Key survey data	Rodriguez-Artalejo et al (2003)	Sweetened beverage consumption associated with higher energy intake and lower calcium intake in Spanish children.
Key survey data	Tucker et al (2002)	Dietary pattern affects BMD. A high fruit and vegetable intake is associated with higher BMD and a high candy intake with lower BMD.
Key survey data	Yang et al (2003)	Analysis of dietary intake data from NHANES III. A low carbohydrate intake was associated with elevated serum C-peptide concentrations. Adjusting for total and added sugars strengthened the association in men. Suggests that the type of carbohydrate may affect glycaemic control.

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The National Health and Medical Research Council

The National Health and Medical Research Council (NHMRC) was established in 1936 and is now a statutory body within the portfolio of the Australian Government Minister for Health and Ageing, operating under the *National Health and Medical Research Council Act 1992 (*NHMRC Act*)*. The NHMRC advises the Australian community and the Australian Government, and State and Territory governments on standards of individual and public health, and supports research to improve those standards.

The NHMRC Act provides four statutory obligations:

- to raise the standard of individual and public health throughout Australia;
- to foster development of consistent health standards between the states and territories;
- to foster medical research and training and public health research and training throughout Australia; and
- to foster consideration of ethical issues relating to health.

The NHMRC also has statutory obligations under the *Prohibition of Human Cloning Act 2002* (PHC Act) and the *Research Involving Human Embryos Act 2002* (RIHE Act).

The activities of the NHMRC translate into four major outputs: health and medical research; health policy and advice; health ethics; and the regulation of research involving donated IVF embryos, including monitoring compliance with the ban on human cloning and certain other activities. A regular publishing program ensures that Council's recommendations are widely available to governments, the community, scientific, industrial and education groups. The Council publishes in the following areas:

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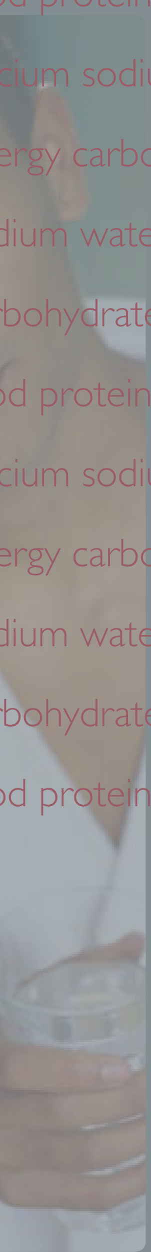

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