1 **DRAFT SCIENTIFIC OPINION**

2 Scientific Opinion on Dietary Reference Values for calcium[1]

3 **EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]**

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5 **ABSTRACT**

6 Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies
7 (NDA) derived Dietary Reference Values (DRVs) for calcium. These include Average Requirement (AR),
8 Population Reference Intake (PRI) and Adequate Intake (AI). For adults, EFSA analysed data from a number of
9 balance studies undertaken in North America and found that the mean value where calcium intake equals
10 excretion is 715 mg/day in adults ≥ 25 years. An allowance for dermal losses of calcium (not included in the
11 balance data) of 40 mg/day was added to derive an AR of 750 mg/day. The upper bound of the 95 % prediction
12 interval at the estimated population mean at null balance (which represents the 97.5 percentile of the distribution
13 of the individual predictions for each calcium intake level) was 904 mg/day, and when dermal losses are added
14 this gives a PRI of 950 mg/day for adults ≥ 25 years. For infants (7–11 months) an AI was derived by
15 extrapolating the average amount of calcium absorbed by exclusively breast-fed infants (120 mg/day) using
16 isometric scaling and assuming an absorption of 60 %, and the AI is 280 mg/day. The AR for children was
17 derived using the factorial approach. The total quantity of calcium required for bone accretion and replacement
18 of endogenous losses was adjusted for % absorption to derive PRIs for children aged 1–3, 4–10, and 11–17 years
19 of 450, 800 and 1 150 mg/day, respectively. The PRI for young adults (18–24 years), who are still accumulating
20 calcium in bones, is 1 000 mg/day. This is the intermediate value between children aged 11–17 years and adults.
21 Taking into consideration adaptive changes in calcium metabolism that occur during pregnancy and lactation, the
22 PRI for non-pregnant women also applies to pregnant and lactating women of the respective age group.

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25 **KEY WORDS**

26 calcium, factorial approach, balance, Average Requirement, Dietary Reference Value

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values for the European population, including calcium. These include Average Requirement (AR), Population Reference Intake (PRI) and Adequate Intake (AI).

Calcium is an integral component of the skeleton; approximately 99 % of total body calcium is found in bones and teeth as calcium hydroxyapatite, where it has a structural role. The remaining 1 % of calcium found in the body acts as an essential intracellular messenger in cells and tissues.

Intestinal calcium absorption occurs through both an active, saturable, transcellular process and a nonsaturable, passive process. Active transport is controlled by $1,25(OH)_2D_3$ and passive transport is paracellular. Calcium absorption varies considerably throughout the lifespan, being higher during periods of rapid growth and lower in old age. Calcium absorption is affected by vitamin D status and current data suggest that % calcium absorption reaches a maximum at 25(OH)D concentrations of 30–50 nmol/L in both children and adults. Unabsorbed dietary calcium is lost in the faeces. The main routes of obligatory (endogenous) calcium loss are urine, faeces and skin and sweat (dermal losses).

If the dietary supply of calcium is insufficient to meet physiological requirements, calcium is resorbed from the skeleton so as to maintain blood concentrations within the range required for normal cellular and tissue functions. This causes a reduction in bone mass, which leads to osteopenia and osteoporosis, and an associated increased risk of fracture.

Hypercalcaemia, defined by serum calcium concentrations > 2.75 mmol/L (11 mg/dL), is unlikely to occur with high intakes of calcium from the diet alone but can be caused by high dose calcium supplements, especially when accompanied by vitamin D supplements as these can increase calcium absorption.

The main dietary sources of calcium in European countries differ, although dairy products are generally the most important food group. Rich food sources of calcium include dairy products, dark green vegetables, legumes, nuts, fish with soft bones (e.g. canned sardines), and calcium-fortified foods. Hard water also makes a significant contribution to calcium intakes.

Evidence from human studies on the relationship between calcium intake and various health outcomes was reviewed and found to be inconsistent. The Panel concluded that measures of bone health (skeletal growth, bone mineral density and fractures) could not be used to derive DRVs for calcium. Similarly, evidence related to cardiovascular outcomes and cancer were not helpful for deriving DRVs for calcium.

Calcium balance data collected from a number of carefully controlled metabolic studies undertaken in North American adults aged 25 years and over were analysed to determine the value where calcium intake equals calcium losses via urine and faeces. The mean value where calcium intake equals excretion is 715 mg/day. An allowance for dermal losses of calcium, which were not included in the balance data, of 40 mg/day was added to derive an AR of 750 mg/day. The upper bound of the 95 % prediction interval at the estimated population mean at null balance (which represents the 97.5^{th} percentile of the distribution of the individual predictions for each level of calcium intake) was 904 mg/day, and when dermal losses are added this gives a PRI of 950 mg/day.

In infants aged 7–11 months an AI was derived by estimating the average amount of calcium absorbed by exclusively breast-fed infants (120 mg/day) and extrapolating upwards using isometric scaling. Assuming an absorption of 60 %, the AI is 280 mg/day.

In children aged 1–17 years a factorial approach was employed where the quantity of dietary calcium that is sufficient for calcium accretion in bone and for replacement of obligatory body losses in 50 % of the population was the criterion upon which the AR is based. ARs for children aged 1–3, 4–10, 11–

17 years are 390, 680, and 960 mg/day, respectively. Assuming a coefficient of variation (CV) of 10 % the PRIs for children aged 1–3, 4–10 and 11–17 years are 450, 800 and 1 150 mg/day, respectively.

The AR for young adults (18–24 years), who are still accumulating calcium in bones, is 860 mg/day. This is the intermediate value between children aged 11–17 years and adults. Assuming a CV of 10 % the PRI is 1 000 mg/day.

Taking into consideration adaptive changes in calcium metabolism that occur during pregnancy and lactation, the PRI for non-pregnant women also applies to pregnant and lactating women of the respective age groups.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and, if necessary, to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community.[4] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context, EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[5] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance, EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

- Protein;
- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

Calcium is an essential nutrient that must be provided by the diet. The adult body contains approximately 1 200 g (women) and 1 400 g (men), 99 % of which is found in the skeleton, where it has a structural role. The remaining 1 % is found in extracellular fluids, intracellular structures and cell membranes, where it is involved in vascular, neuromuscular and endocrine functions.

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on the nutrient and energy intakes for the European Community, in which Population Reference Intakes (PRIs) for calcium for all age groups from six months onwards were derived. For this, the factorial approach was used for children and adults, including lactating women, but such data were unavailable for infants. In addition, a Lowest Threshold Intake was proposed for adults.

2. Definition/category

2.1. Chemistry

Calcium is the fifth most abundant element in the earth's crust, sea water, and the human body. It has an atomic mass of 40.08 Da, and it belongs to the group of the alkaline earths elements. Calcium has two mobile free electrons in the 4s orbital, and forms a stable divalent cation. There are six naturally occurring stable isotopes of calcium, the most abundant being ^{40}Ca (96.97 % natural abundance). Calcium salts are generally soluble, with the exception of calcium sulphate, carbonate and phosphates.

2.2. Functions of calcium

2.2.1. Biochemical functions

Calcium is an integral component of the skeleton; approximately 99 % of total body calcium is found in bones and teeth where it is mainly present as calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. It has a structural role, and is needed for tissue rigidity, strength and elasticity. Bone is a reservoir for calcium and other inorganic nutrients and participates in whole body mineral homeostasis through the processes of bone formation and resorption. It is a dynamic tissue that is continuously remodelled throughout the life course under the control of osteocytes (Bonewald, 2011). Osteoblasts are responsible for the formation of new bone tissue and osteoclasts for bone resorption. In infants and children, the rate of formation exceeds that of resorption and new bone tissue is laid down as part of the process of growth, whereas in later life the rate of bone resorption exceeds formation, resulting in bone loss and microarchitectural changes that compromise bone strength and increase the risk of fracture. The rate of loss of bone is dependent on the combination of many environmental and lifestyle factors (Schulman et al., 2011), but menopausal status, use of hormone replacement therapy, genotype and frequency of load-bearing physical activity are of overriding importance (Ferrari, 2008; Riancho and Hernandez, 2012). A number of dietary constituents are associated with changes in calcium balance that can influence bone calcium content either positively (e.g. calcium, vitamin D, fruits and vegetables, vitamin K, moderate alcohol, protein, inulin) or negatively (e.g. sodium, phytate, high alcohol) (Bonjour, 2011; Fairweather-Tait et al., 2011; Falcone et al., 2011; Anderson et al., 2012; Weaver et al., 2012; Welch et al., 2012); epigenetic factors have also been implicated (Holroyd et al., 2012).

The central core of long bones (the marrow cavity) is a major site for the development of haematopoietic cells and is one of the functional sites of the immune system. Some of the cells involved in bone remodelling originate from the bone marrow. Recent advances in bone cell biology and genetic studies have improved our understanding of the essential signalling pathways that control bone remodelling and bone mass, such as how parathyroid hormone (PTH), Wnt/Ca^{2+} signalling (SCF, 2003) and growth factors may trigger anabolic effects in bone. Novel signalling pathways generated

by cell-matrix and cell-cell communications regulating bone remodelling have more recently been identified (Marie, 2012).

The remaining 1 % of calcium found in the body acts as an essential intracellular messenger in cells and tissues. It has a critical role in many physiological functions involved in the regulation of metabolic processes, including vascular contraction and vasodilation, muscle contraction, enzyme activation, neural transmission, membrane transport, glandular secretion and hormone function. Due to its ability to complex with anions such as citrate and bicarbonate, ionised calcium is the most common signal transduction element in the human body (IOM, 2011).

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

If the dietary supply of calcium is insufficient to meet physiological requirements, due to low intake and/or inefficient gastrointestinal absorption, calcium is resorbed from the skeleton so as to maintain blood concentrations within the range required for normal cellular and tissue functions. This causes a reduction in bone mass, which leads to osteopenia (lower than normal bone mineral density (BMD)) and osteoporosis, characterised by a very low BMD, and an associated increased risk of fracture.

Skeletal disorders include rickets, osteomalacia (adult rickets), osteoporosis and fractures. Rickets and osteomalacia are associated with suboptimal bone mineralisation and are caused by vitamin D deficiency. However, the cut-off value for serum 25(OH)D concentration that is associated with risk of rickets in children and other vitamin D-related skeletal disorders is uncertain. A low intake of calcium often co-exists with vitamin D deficiency and both can independently cause nutritional rickets (Abrams, 2010b). An inadequate supply of calcium for bone development leads to stunted growth and bowing of long bones. Older adults with osteomalacia will not present with deformed bones but will have a reduced bone mass which leads to impaired bone strength.

Osteoporosis is a disorder associated with ageing, low BMD, and greater risk of fracture. Women are particularly at risk after the menopause when there is an accelerated loss of bone, but older men also experience age-related bone loss although the higher risk of fracture occurs some five to ten years later than in women (IOM, 2011).

Bone loss is strongly related to genotype, with genetic factors reported to explain 44–56 % of the inter-individual variance in bone loss at femoral neck, lumbar spine, and forearm in postmenopausal Caucasian women (Zhai et al., 2009). However, when the effects of all polymorphisms of genes identified through genome-wide association studies are combined, they explain less than 10 % of the variation in bone mass (Riancho and Hernandez, 2012). A shared genetic aetiology is often assumed between fracture and low BMD, but is not always the case. In 6 570 female twins, the prevalence of wrist fractures was 3.3 % and heritability was 54 % (Andrew et al., 2005). However, when forearm BMD was included as a covariate in models testing for a shared genetic aetiology between wrist fracture and BMD the magnitude of the genetic influence on risk of fracture was reduced very little, suggesting that many/some of the genes involved in wrist fracture are different from those involved in BMD. Another twin study found that clinical vertebral fractures were largely explained by environmental influences and not by genetic factors (Wagner et al., 2012). The authors concluded that individual-specific environmental influences such as lifestyle become more important with increasing age. The Panel notes that BMD, bone loss and risk of fracture are site- and age-specific and affected by different environmental and genetic factors.

2.2.2.2. Excess

Hypercalcaemia is defined by serum calcium concentrations > 2.75 mmol/L (11 mg/dL) (EFSA NDA Panel, 2012). It is unlikely to occur with high intakes of calcium from the diet alone but can be caused by high dose calcium supplements, especially when accompanied by vitamin D supplements as these can increase calcium absorption. The most common causes of hypercalcaemia include malignant

tumours, hyperparathyroidism of different aetiology, and less frequently excessive calcium and/or vitamin D intakes. Clinical symptoms of persistent hypercalcaemia are fatigue, muscular weakness, anorexia, nausea, vomiting, constipation, tachycardic arrhythmia, soft tissue calcification, failure to thrive and weight loss. Hypercalcaemia can lead to hypercalciuria when the renal capacity of calcium re-absorption is exceeded, and to renal concentration defects resulting in polyuria through activation of the renal calcium-sensing receptor. Consequences of severe chronic hypercalcaemia are nephrolithiasis and impairment of kidney function, resulting in loss of the concentrating ability of the kidney (i.e., a decrease in salt and water reabsorption), and in volume and salt depletion. Chronic hypercalcaemia may also lead to calcification of soft tissues (e.g., nephrocalcinosis and vascular calcification), particularly when phosphorus concentrations in the blood are also high, as in renal insufficiency. The age-related decrease in renal function increases the sensitivity of older people to excess calcium intakes.

The SCF (2003) based the derivation of a Tolerable Upper Intake Level (UL) for calcium on the evidence of different intervention studies of long duration, some of which were placebo controlled, in which total daily calcium intakes of 2 500 mg from both diet and supplements were tolerated without adverse effects. Because of the abundance of data, the application of an uncertainty factor was considered unnecessary. A UL of 2 500 mg of calcium per day from all sources was proposed for adults, and for pregnant and lactating women. In 2012, the EFSA NDA Panel (2012) concluded that there were no new data supporting a revision of the UL for calcium for adults, including pregnant and lactating women, of 2 500 mg, and that no new data had become available which would allow the setting of a UL for infants, children or adolescents.

2.3. Physiology and metabolism

2.3.1. Intestinal absorption

Intestinal calcium absorption occurs through both an active, saturable, transcellular process and a nonsaturable, passive process. Active transport involves entry of calcium into the enterocyte and is controlled by 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$ or calcitriol). This is the hydroxylated form of vitamin D (25-hydroxy-cholecalciferol or calcidiol), the synthesis of which is regulated by PTH. It has been proposed that the epithelial calcium selective channel TRPV6 mediates $1,25(OH)_2D_3$-dependent uptake of calcium across the brush border (Christakos, 2012). Calcium is then moved to the interior of the enterocyte by calcium binding protein (CaBP), calbindin, the synthesis of which is dependent on $1,25(OH)_2D_3$. Finally, calcium is extruded from the basolateral membrane against a concentration gradient by the intestinal plasma pump, PMCA1b, again controlled by $1,25(OH)_2D_3$ and also by dietary calcium intake (Christakos, 2012). Passive transport is paracellular, taking place through the tight junctions and structures present within intercellular spaces throughout the entire length of the intestine, although it predominates in the more distal regions.

Digested food (chyme) travels down the lumen of the small intestine for approximately three hours, passing through the duodenum in a few minutes and taking 2–3 hours to travel through the distal half of the small intestine (Christakos, 2012). Transcellular (active) transport is the major route of calcium absorption, with paracellular (passive) transport being responsible for an estimated 8–23 % of total calcium absorbed (McCormick, 2002). However, when calcium intake is high, paracellular transport accounts for a higher proportion of absorbed calcium because CaBP is rate-limiting and down-regulated when exposed to high concentrations of calcium (Bronner, 2003). Although the efficiency of absorption is highest in the duodenum (Wasserman, 2004) most calcium is absorbed in the ileum presumably because the exposure time of the chyme is much longer than that in the proximal intestine. Calcium can also be taken up in the colon by passive absorption: with a habitual estimated intake of 620 mg/day, the % colonic absorption (i.e. absorption > 7 hours post-ingestion) was calculated to be 4.2 % (Barger-Lux et al., 1989) and at intakes of about 900 mg/day colonic absorption was 5.7 % (Abrams et al., 2007).

Fractional calcium absorption is inversely related to the concentration of calcium present in the gut lumen (Ireland and Fordtran, 1973) and dietary load (Heaney et al., 1990). For example, absorption from a meal containing 15 mg or 500 mg of calcium was 64 % and 28 %, respectively (Heaney et al., 1990). In order to obtain reproducible data for calcium absorption at different levels of intake a period of adaptation is required, which should be a minimum of one week's duration (Dawson-Hughes et al., 1993). In women adapted to a high (2 000 mg/day) calcium diet, whole body retention of calcium increased from 27 % to 37 % when they were given a low (300 mg/day) calcium diet for two weeks; this was accompanied by a decline in serum calcium and an increase in serum PTH and $1,25(OH)_2 D_3$ concentrations (Dawson-Hughes et al., 1993).

Calcium absorption varies throughout the lifespan, being higher during periods of rapid growth and lower in old age. It has been estimated that in children, 3–3.5 % of the variability in absorption appears to be associated with height (Abrams et al., 2005a), which presumably reflects the calcium requirement for bone growth. Table 1 shows results of studies that have used dual stable isotope techniques for assessing calcium absorption in children.

Table 1: Summary of results of calcium absorption studies carried out in children using the dual stable isotope technique

Age (years) Mean ± SD or range	Sex	Ethnicity	n	Mean usual calcium intake (mg/day), ± SD	Calcium dose (mg)	Mean % absorption ± SD	Reference
5–7 months	Male and female	White US	14	215 from breast milk plus 44 from weaning food	Not reported	61.3 ± 22.7	Abrams et al. (1997b)
30 ± 2 months	Male and female	Mixed US	28	551 ± 41	One third of usual intake	45.6 ± 2.5	Lynch et al. (2007)
6.1–9	Male and female	White US	27	912 ± 58; 699 ± 55 during study	Not reported	28.9; 30.8	Abrams et al. (2001)
7–8.9	Female	US Caucasian; Mexican	19	1 200 during study	~350	32 ± 2; 34 ± 2	Abrams et al. (1999)
7.7 ± 2.1; 10.9 ± 1.1; 15.2 ± 1.3	Female	US	21; 13; 17	907; 931; 955	One third of usual intake	27.7 ± 8.2; 34.4 ± 11.9; 25.0 ± 7.9	Abrams and Stuff (1994)
8.3 ± 0.7; 9.1 ± 0.9; 10.2 ± 0.8	Female [(a)]	Mixed US	26; 34; 34	1 200 during study	350	33.0 ± 7.4; 30.7 ± 9.9; 36.6 ± 8.7	Abrams et al. (2000)
10–13	Female	US	17	1 010; 1 300 during study	300	39 ± 9	Whisner et al. (2013)
11.8 ± 0.8	Female	Mostly Caucasian	29	1 200–1 300	400	32.3 ± 9.8	Griffin et al. (2002)
12 ± 1 [(b)]	Female	White US	10	1 880; 848	627; 283	41 ± 15 and 37 ± 11 (from diet)	Wastney et al. (2000)
9.2 ± 2.5 (premenarche); 15.4 ± 0.9 (postmenarche)	Female	White;	36; 15	916; 962	One third of usual intake	30 ± 10; 25 ± 8	Abrams et al. (1995)
11.5 ± 0.2; 10.9 ± 0.2	Female	White; black	28; 23	1 222	350	43.0 ± 2.2	Abrams et al. (2004)
11.7 ± 1.5	Male and female	US mixed	25	1 310 during study	One third of intake	27.4 ± 12.6 (boys); 24.5 (girls)	Abrams et al. (1997a)
15.3 (14–16)	Male	Dutch	12	1 267 during study	200	47.8 ± 16.4	van den Heuvel et al. (1999)

(a): early prepubertal; late prepubertal; pubertal (Tanner stage 2)
(b): Further details on the study design are given in the text below.

In infants aged 5–7 months given breast milk and weaning food, the majority of calcium was provided by the milk; mean % absorption was 61.3 ± 22.7 % (Abrams et al., 1997b). In children aged 30 months, absorption was 45.6 ± 2.5 % (Lynch et al., 2007). In 6–9 year-old children, absorption from either calcium-fortified cereal or milk was 31 % when the mean dietary intake was 699 ± 58 mg/day and 29 % when the intake was 912 ± 55 mg/day (Abrams et al., 2001). In 7–8 year-old children consuming diets containing 1200 mg calcium/day, absorption was 32 ± 2% (Abrams et al., 1999). The Panel notes that calcium absorption is high in infancy (absorption efficiency of about 60 %) and decreases during childhood, from around 45 % in children aged 1–3 years to 30 % in children aged about 6 years.

Absorption is affected by pubertal status. When longitudinal measurements of calcium absorption in girls adapted to a diet containing 1 200 mg calcium/day were undertaken, at 8 years of age absorption was 33.0 ± 7.4 % (n = 26), at 9 years 30.7 ± 9.9 % (n = 34), and at 10 years 36.6 ± 8.7 % (n = 34) (Abrams et al., 2000). In another study in girls aged 7, 10 and 15 years absorption values were 27.7 ± 8.2, 34.4 ± 11.9 and 25.0 ± 7.9 % respectively (Abrams and Stuff, 1994). In girls aged 12 years consuming either a low (848 mg) or high (1 880 mg) calcium diet, dietary absorption (as opposed to absorption from the test meal, which generally contains one third of the daily intake of calcium) was calculated using compartmental modelling and found to be 37–41 % (Wastney et al., 2000). In 10–13 year-old girls, Whisner et al. (2013) reported an absorption of 39 ± 9 %. In boys aged 14–16 years consuming approximately 1 200 mg calcium/day, absorption was 47.8 ± 16.4 % (van den Heuvel et al., 1999). The Panel notes that absorption values reported in the literature differ according to the study population, habitual calcium intake and stage of puberty. The Panel notes that absorption increases in line with skeletal growth: 35 % at 7–10 years, 40 % at 11–14 years, and 45 % in boys aged 15–17 years (van den Heuvel, 1999). In post-pubertal girls aged 15–17 years absorption is 35 %. The Panel notes that these absorption data were obtained from studies in children consuming dietary calcium from 800–1 800 mg per day.

In adults, dietary calcium absorption is approximately 25 % (Gibson, 2005) but it is lower in postmenopausal women (Heaney et al., 1989) and in men over 60 years of age (Nordin and Morris, 2011). This appears to be the result of a developing resistance to the action of $1,25(OH)_2D_3$; fractional calcium absorption from diets containing different levels of calcium was correlated with serum $1,25(OH)_2D_3$ concentration in young (28.7 ± 5.3 years) but not in elderly (72.5 ± 3.0 years) women (Pattanaungkul et al., 2000). The menopause is associated with a significant fall in calcium absorption, possibly due to lower oestrogen levels affecting receptors in the small intestine (Nordin et al., 2004). Data from early radioisotope studies show a continuous reduction in absorption from the age of 60 years in men and women (Bullamore et al., 1970). Using data from 189 women aged 35–45 years at the start and followed for 17 years Heaney et al. (1989) calculated an average fall in absorption efficiency of 0.21 % per year after the age of 40 years, and a one-time decrease of about 2.2 % at the time of menopause.

Absorption increases approximately two-fold during pregnancy, in conjunction with increased expression of CaBP (Cross et al., 1995; Ritchie et al., 1998), and because it occurs before the third trimester when fetal growth is greatest, it is assumed to be a physiological adaptation that is driven by the anticipated increased requirements for calcium and mediated through changes in $1,25(OH)_2D_3$ (Gertner et al., 1986). By two to three months post partum, calcium absorption returns to values close to those observed in early gestation or prior to conception (Ritchie et al., 1998).

There are differences in calcium metabolism that are related to ethnicity but these are not usually manifest as differences in absorptive efficiency (Bell et al., 1993; Kung et al., 1998). Similar levels of fractional ^{47}Ca retention were reported in black and white women adapted to low and high calcium diets, despite higher concentrations of $1,25(OH)_2D_3$ in blacks, indicating that blacks may be less responsive to the action of $1,25(OH)_2D_3$ (Dawson-Hughes et al., 1993). However, one study found that postmenarchal African American girls had a higher absorption efficiency of calcium than Caucasian girls (Abrams et al., 1996).

Absorption is also influenced by genotype, for example polymorphisms of the vitamin D receptor gene *Fok1* (Abrams et al., 2005).

There are a number of dietary constituents that affect % calcium absorption, although the total calcium content of the diet is usually the overriding determinant (IOM, 1997). Acute studies of single foods, generally undertaken using stable isotopes, do not provide global estimates of absorption from whole diets, nor do they provide information on the long-term effects of calcium bioavailability on bone health (Fairweather-Tait and Teucher, 2002). However, the % absorption of calcium in food groups that provide the majority of calcium in the diet, including milk and milk products, grains (IOM, 1997; Martini and Wood, 2002) and water (Heaney, 2006), is fairly similar. Calcium may, however, be

poorly absorbed from foods rich in oxalic acid (e.g. spinach and rhubarb). Similarly, absorption is low from high phytic acid foods (whole grains, legumes, nuts, seeds) (IOM, 1997), with the exception of soybeans where, for example, % absorption from calcium-fortified soymilk and cow's milk is similar (Zhao et al., 2005).

Absorption of calcium from food supplements depends on when they are consumed and the dose: smaller doses taken with meals are better absorbed (Heaney, 1991). The solubility, chemical form and particle size of calcium does not greatly affect absorption (Nowak et al., 2008; Elble et al., 2011), although there are reports of higher % absorption from calcium citrate malate (Reinwald et al., 2008) and from "nanonised" pearl powder (Chen et al., 2008). Individuals with achlorhydria absorb calcium poorly from less soluble forms of calcium, such as calcium carbonate, unless the supplement is taken with a meal (Recker, 1985).

Calcium absorption is affected by vitamin D status (Seamans and Cashman, 2009). The data currently suggest that % calcium absorption reaches a maximum at 25(OH)D concentrations of 30–50 nmol/L in both children and adults (IOM, 2011).

2.3.2. Transport in blood

Calcium is present in the blood in three different forms: as free Ca^{2+} ions, bound to protein (about 45 %), and complexed to citrate, phosphate, sulphate and carbonate (about 10 %). Calcium in the blood (and in extracellular fluid) is kept constant at 2.5 mmol/L (range 2.25–2.6 mmol/L), and ionised calcium (between 1.1–1.4 mmol/L) is controlled by the interrelated action of three hormones, namely PTH, $1,25(OH)_2D_3$ and calcitonin (Section 2.3.5).

2.3.3. Distribution to tissues

Calcium deposition into bone is an on-going process during periods of growth, with maximal accretion during the pubertal growth spurt (Matkovic et al., 1994).

Maternal and fetal calcium metabolism are different: in the fetus, serum calcium, phosphorus and ionised calcium are higher than maternal values, whilst PTH and $1,25(OH)_2D_3$ are lower (IOM, 2011). Fetal requirements for calcium are met through physiological changes in the mother, including increased efficiency of absorption and a decrease in maternal bone mineral, predominantly from trabecular bone; calcium is actively transported across the placenta from the mother to the fetus (Olausson et al., 2012). Maternal serum calcium concentrations fall due to plasma volume expansion (Pedersen et al., 1984) and higher $1,25(OH)_2D_3$ (Seely et al., 1997), but ionised serum calcium remains within the normal range (Seely et al., 1997).

2.3.4. Storage

The skeleton and teeth contain 99 % of total body calcium and bone provides a reservoir for other essential calcium-dependent functions in the body. There are two types of bone in the skeleton: 80 % is cortical bone, the outer part of the skeletal structures, which is dense and compact with a high resistance to impact and a slow turnover rate, and 20 % is trabecular bone which is found inside the long bones, vertebrae, pelvis and other large flat bones, which is less dense and has a higher turnover rate.

The amount of calcium taken up into bone is age- (and growth-) dependent. Abrams (2006) has summarised the retention data available from the literature for infants; for exclusively breast-fed infants retention is 94 mg/day according to the classical balance technique (Fomon et al., 1982), and 82 mg/day from an isotope balance study (Abrams et al., 1997b), whereas for exclusively formula-fed infants, retention is more variable but higher. Specker et al. (1997) reported that although there was a positive relationship between calcium intake during the first 6 months of life and BMC at 6 months, the difference had disappeared by 12 months of age.

There are very few data on bone calcium accretion in young children. Weaver (1994) proposed values for calcium accretion in bone of 80 mg/day at 0–2 years of age, and 50 mg/day from 6–8 years, based on calculations made by Peacock (1991). During periods of skeletal growth, absorbed calcium that is retained in the body is transported to the bone, therefore measures of calcium retention can be used as an indirect measure of bone calcium accretion. In 1–4 year-old children (n = 28, mean age 30 ± 2 months, mean weight 12.6 ± 0.4 (SEM) kg) mean calcium retention, determined using a stable isotope technique, was 162 ± 17 mg/day (median 142 mg/day) (Lynch et al., 2007). However, although endogenous urinary and faecal losses were accounted for in the calculation of retention, dermal losses were not measured. If these are assumed to be 20 mg/day the median value for calcium bone accretion is 120 mg/day.

There is a marked increase in calcium accretion during puberty; Abrams et al. (2000) observed an increase during the late pre-pubescent compared with the early pre-pubescent phase, 135 ± 53 vs 110 ± 45 mg/day, respectively. Martin et al. (1997) used dual-energy X-ray absorptiometry to monitor BMC for a period of four years in North American children and calculated from cross-sectional data that the mean daily calcium retention throughout puberty was 282 mg in boys and 212 mg in girls. Longitudinal data collected from 60 boys and 53 girls revealed higher values for bone calcium accretion in males (Bailey et al., 2000). The mean age of peak calcium accretion was 14.0 years in boys and 12.5 years in girls, at which time calcium accretion rates were 359 ± 82 (range 199–574) mg/day for boys and 284 ± 59 (range 171–458) mg/day for girls. These values were obtained from children consuming diets providing 1 140 ± 392 mg/day (boys) and 1 113 ± 378 mg/day (girls) of calcium.

Molgaard et al. (1999) measured the annual increase in bone mineral content in Danish girls (n=192) and boys (n = 140) aged 6.5–19.5 years and, assuming that 32.2 % of bone is calcium, they calculated bone calcium accretion. The 50th centiles (mg calcium/day) for girls at Tanner stages 1–5 on first examination were 98.9, 192.6, 220.1, 116.4, and 60.8, respectively. For boys the values were 107.6, 187.1, 316.7, 250.8, and 96.8, respectively. According to van Buuren et al. (2012) the age at which 50 % of European girls reach Tanner stages 2–5 (mean of pubic hair and breast indicators) are 10.6, 11.7, 12.7 and 13.9 years. For boys (mean of pubic hair and genital indicators) the ages are 11.6, 13.0, 13.9, and 15.0 years. The Panel notes that in both girls and boys the maximum rate of bone accretion occurs at Tanner stage 3, at the age of 11.7 years for girls and 13.0 years for boys.

Vatanparast et al. (2010) collected longitudinal data from Canadian Caucasian boys and girls aged 9–18 years (not every subject completed all seven years of data collection; numbers of children at each age are given in Table 2) with the aim of determining the average accumulation of calcium over these years in order to determine calcium requirements for bone growth. Total body BMC was determined from annual dual-energy X-ray absorptiometry scans of the whole body, with 0.6 % reproducibility. The total body BMC, unadjusted for body size, was calculated at defined age points. Annual calcium retention (g/year) was derived by assuming that the BMC was 32.2 % calcium. The daily amount of calcium retained in bone at each age is given in Table 2.

Table 2: Bone calcium accretion from 9 to 18 years according to Vatanparast et al. (2010)

Age (years)	Boys		Girls	
	Number of subjects	Calcium retained (mg/day)	Number of subjects	Calcium retained (mg/day)
9	19	119.3	34	87.7
10	32	100.6	53	99.3
11	53	127.5	65	144.5
12	75	154.2	78	189.7
13	88	204.4	92	234.7
14	89	296.3	95	164.1
15	79	261.7	86	107.3
16	66	235.8	61	67.0
17	51	143.1	45	49.5
18	36	111.1	34	74.4
Mean ± SD		175.4 ± 69.3		121.8 ± 59.7

The Panel considers that the longitudinal data generated by Vatanparast et al. (2010) provides the most comprehensive information on bone calcium accretion in boys and girls aged 9–18 years.

Bone mass increases substantially during the first two decades of life, reaching a plateau, referred to as peak bone mass (PBM), when BMD is stable. The precise timing of this is uncertain and the rate of bone accrual varies by site (Hui et al., 1999; Ohlsson et al., 2011). A longitudinal study in Canada reported that there was no increase in BMC at any site seven years after peak linear growth (peak height velocity); the latter occurred at 11.8 years in girls and 13.5 years in boys (Baxter-Jones et al., 2011), being related to the age of puberty (Darelid et al., 2012), and this equates to a PBM at 18.8 years in women and 20.5 years in men. However, another longitudinal study from Canada reported that although total hip PBM was attained at 16–19 years in women and 19–21 years in men, lumbar spine PBM occurred much later, at 33–40 years in women and 19–33 years in men (Berger et al., 2010). A cross-sectional study in women reported that by the age of 22.1 ± 2.5 years, 99 % of peak BMD is attained, and by the age of 26.2 ± 3.7 years, 99 % of peak BMC is attained (Teegarden et al., 1995), indicating that calcium continues to be accrued in bones in young adults, with males having a later PBM than females.

For estimating DRVs the Panel considers it prudent to make an allowance in young adults (up to the age of 25 years) for calcium accretion into bone tissue.

2.3.5. Metabolism

Serum concentrations of calcium are homeostatically regulated to remain within a narrow range of 2.25–2.6 mmol/L (ionised calcium 1.1–1.4 mmol/L) and concentrations of soft tissue calcium are maintained at the expense of bone. When insufficient calcium is provided from the diet to balance obligatory losses and requirements for growth, calcium is taken from the bone. This mechanism is achieved through the interaction of three major calcium regulating hormones, PTH, $1,25(OH)_2D_3$, and calcitonin. The latter two determine how much Ca^{2+} moves out of or into the body, whilst PTH determines how Ca^{2+} moves between the extracellular fluid and bone. A decrease in serum concentrations of Ca^{2+} induces the release of PTH via the calcium-sensing receptor (CaSR) which is located on the cell surface of the parathyroid glands. PTH stimulates $1,25(OH)_2D_3$ synthesis in the kidney, bone resorption, and renal reabsorption of calcium (Perez et al., 2008). Synthesis of $1,25(OH)_2D_3$ is also stimulated by low serum phosphorus concentrations and decreases with high phosphorus concentrations. An increase in serum concentrations of Ca^{2+} inhibits PTH secretion via the CaSR and $1,25(OH)_2D_3$ synthesis, and stimulates calcitonin secretion by the parafollicular C cells of the thyroid gland. Other locations of the CaSR include the intestine, kidney, thyroid gland, lung, brain,

skin, bone marrow, and osteoblasts. According to population-based genome-wide association studies, individual serum calcium concentrations within the normal range are influenced by some single-nucleotide polymorphisms of the CaSR gene (O'Seaghdha et al., 2010; Riccardi and Brown, 2010). Other hormones involved are oestrogen and testosterone which prevent bone loss by inhibiting the stimulatory effect of cytokines on osteoclasts (Adamova et al., 2009), adrenal steroids which decrease osteoblast function and bone formation and increase osteoclast number and activity, glucocorticoids which decrease calcium absorption and renal calcium reabsorption and augment renal excretion, growth hormone which facilitates intestinal absorption and renal excretion of calcium, and thyroid hormones (hypothyroidism and hyperthyroidism are both associated with an increased risk of fracture but the underlying mechanism for bone loss is incompletely understood).

Bone constantly undergoes remodelling, and virtually the entire adult skeleton is remodelled over a 10 year-cycle. Trabecular bone turns over more rapidly than cortical bone, and weight-bearing activities (mechanical loading of the bone) are an important determinant of rates of bone turnover and can promote bone formation in children. During bed-rest, bone formation is rapidly decreased in parallel with increased urinary calcium excretion; bone collagen synthesis is decreased and breakdown increases after a time lag of several weeks (Scheld et al., 2001).

Although the current consensus is that genetic factors predominate in determining the rate of bone turnover (IOM, 2011), diet also plays a key role. Calcium, phosphorus and magnesium are structural components of bone, and vitamin D is required for calcium and phosphorus absorption. Many other dietary constituents are involved both individually and in complex combinations at various stages of bone metabolism (Schulman et al., 2011).

2.3.6. Elimination

Unabsorbed dietary calcium is lost in the faeces. The main routes of endogenous calcium excretion are urine, faeces and skin and sweat (dermal losses).

2.3.6.1. Urine

Urinary excretion is a function of the balance between calcium load filtered by the kidneys and the efficiency of absorption by the renal tubules. Approximately 98 % of filtered calcium is reabsorbed; approximately 70 % is reabsorbed passively in the proximal tubule, and the rest is under homeostatic regulation by the calcium sensing receptor of the ascending loop of Henle. Urinary calcium comprises absorbed calcium that is lost from the body after the requirements for bone, dermal and endogenous faecal excretion have been met. In adults, a positive association has been reported between urinary calcium excretion and calcium intake (Matkovic et al., 1995), but higher calcium intakes (with daily intakes ranging from 700–1 800 mg/day) are associated with only small increases in urinary calcium (Taylor and Curhan, 2009) because of a lower calcium absorption.

In a controlled feeding study in 27 healthy postmenopausal women Hunt et al. (2009) found that urinary excretion was related to both calcium and protein intake: 127 mg/day with a low protein diet (10 % of energy) providing 675 mg calcium/day; 150 mg/day with a high protein diet (20 % of energy) providing 675 mg calcium/day; 203 mg/day with a low protein diet (10 % of energy) providing 1 510 mg calcium/day; and 226 mg/day with a high protein diet (20 % of energy) providing 1 510 mg calcium/day. Charles et al. (1991) examined balance data from Nordin et al. (1987) and estimated that the minimum obligatory renal loss of calcium was 116 mg/day in adults, but emphasised the high degree of inter-individual variation and the multiple effects of environmental, behavioural and nutritional factors on the ability of the kidney to respond to calcium-conserving stimuli.

In young children (aged 2–3 years), urinary calcium excretion was reported to be approximately 40 mg/day, and in older children (aged 7–12 years) it was around 80 mg/day and increased to much higher levels (approximately 160–240 mg) in 17 year-olds (Peacock, 1991). Lynch et al. (2007) used stable isotopes to measure urinary calcium excretion in eight children aged 26 ± 3 months (weight

12.5 ± 0.8 kg and calcium intake 563 ± 70 mg/day) and reported a mean of 2.2 ± 0.2 (median 1.1) mg/kg body weight per day. However, six individuals had values > 4 mg/kg body weight per day, the threshold used to define hypercalciuria; therefore, the Panel considers that the mean value cannot be taken as representative for healthy children aged 2–3 years.

Endogenous urinary excretion was measured using a stable isotope technique in five children aged 3–14 years, and individual data (age) were 2.8 (female, 19 kg, 3 years), 1.7 (male, 39 kg, 5 years), 2.0 (male, 57 kg, 12 years), 1.1 (male, 62 kg, 14 years), and 2.1 (male, 91 kg, 14 years) mg/kg body weight per day (Abrams et al., 1991). The Panel notes the high inter-individual variability and small numbers, and considers that these data cannot be used to derive urinary calcium losses.

The mean urinary calcium excretion in 370 girls (aged 10.85 ± 0.41 years, weight 39.92 ± 0.42 (SE) kg) consuming 948 ± 20 (SE) mg calcium/day was 82.4 ± 2.4 (SE) mg/day (Matkovic et al., 1995); dietary sodium intake was the most powerful predictor of urinary calcium excretion, and when combined with calcium and protein intakes, it explained 21.4 % of the variation in urinary calcium. The Panel notes that this study measured urinary calcium excretion, not obligatory losses in urine.

In children aged 9–14 years, consuming a diet containing 1 200 mg calcium/day for two weeks before measurements were made, urinary excretion was determined using an intravenous stable isotope of calcium and reported to be 93.9 ± 43.8 mg/day in girls (n = 13, mean age 12.3 ± 1.6 years, mean weight 48.0 ± 17.7 kg) and 66.9 ± 26.2 mg/day in boys (n = 12, mean age 10.9 ± 1.1 years, mean weight 35.7 ± 7.0 kg) (Abrams et al., 1997a). There was a marked effect of body weight on urinary calcium excretion (the 12 year-old girls, weighing 48 kg, excreted nearly 30 % more calcium than the 11 year-old boys, weighing 36 kg). The Panel notes that when the mean values were expressed in relation to mean body weight, the urinary calcium excretion was similar between boys and girls: 1.96 mg/kg body weight per day in girls and 1.87 mg/kg body weight per day in boys.

Welch et al. (1995) employed calcium stable isotopes and reported a mean urinary excretion of 2.4 mg/kg body weight per day in 38 female children aged 5–16 years, with a calcium intake of 31 ± 12 mg/kg body weight per day. However, in five girls, the excretion was > 4 mg/kg body weight per day, the threshold used to define hypercalciuria. Adjusted data for the group excluding these individuals was not provided, so the estimate of 2.4 mg/kg body weight per day may not be representative of healthy girls.

The Panel notes that during periods of rapid growth the principal determinants of urinary calcium excretion are body weight and age.

The Panel notes the difficulties in determining the minimum obligatory loss of calcium in urine. This is partly due to the effects of growth (body weight) and physiological responses to differing levels of habitual intake. Even with the use of stable isotope tracers and modelling to eliminate the effects of dietary intake on excretion, there are differences in estimated values for obligatory losses in urine in each population group. The Panel considers that a value of 2 mg/kg body weight represents daily obligatory urinary calcium losses in children.

2.3.6.2. Faeces

Faecal calcium is derived from a mixture of unabsorbed calcium, sloughed mucosal cells, and intestinal secretions. Endogenous (obligatory) losses vary according to body size (and possibly calcium intake), but are unrelated to age or sex (Charles et al., 1991). Stable isotope techniques have to be used to measure endogenous faecal losses of calcium and results expressed per kg body weight. In adults, early isotope studies indicate a mean loss of 2.1 mg/kg body weight per day (Heaney and Skillman, 1964). A study in 191 perimenopausal women (mean weight 63.4 ± 11.2 kg) reported an endogenous calcium excretion into the gastrointestinal tract of 140 ± 34 mg/day (Heaney and Recker, 1994). When adjusted for body weight, the Panel notes that this equates to a loss of 2.2 mg/kg body weight per day.

Endogenous faecal calcium excretion was measured in five children aged 3–14 years and the mean value was 1.4 mg/kg body weight per day (Abrams et al., 1991). Lynch et al. (2007) measured endogenous faecal calcium excretion in eight young children, aged 26 ± 3 months, weight 12.5 ± 0.8 kg, with a mean calcium intake of 563 ± 70 mg/day, and reported a mean value of 3.5 mg/kg body weight per day. The Panel notes that the intake of calcium is rather high for 2 year-olds (see Section 3.2) and this may increase endogenous losses of calcium.

In children aged 9–14 years, consuming a diet containing 1 200 mg calcium/day for two weeks before measurements were made, obligatory faecal excretion was reported to be 61.2 ± 27.2 mg/day in girls (n = 13, mean age 12.3 ± 1.6 years, mean weight 48.0 ± 17.7 kg) and 69.1 ± 28.9 mg/day in boys (n = 12, mean age 10.9 ± 1.1 years, mean weight 35.7 ± 7.0 kg) (Abrams et al., 1997a). This equates to an endogenous faecal loss of 1.28 and 1.94 mg/kg body weight per day in girls and boys, respectively. In 36 girls aged 11 years (mean weight approximately 43 kg) consuming a low calcium diet (~300 mg/day) endogenous faecal calcium was 57 ± 4 mg/day and with a high calcium diet (1 300 mg/day) it was 86 ± 4 mg/day (Abrams et al., 2004). The Panel notes that this equates to an endogenous faecal loss of 1.3 and 2 mg/kg body weight per day when consuming a low and high calcium diet, respectively.

Wastney et al. (2000) determined endogenous faecal excretion values of 109.6 ± 50 and 92.8 ± 40 mg/day in girls aged 12 (11–14) years (weight 53 kg) consuming 848 or 1 896 mg calcium/day, which equates to a faecal excretion of 2.06 and 1.75 mg/kg body weight per day on the low or high calcium diets, respectively. The Panel notes that these differences were not significantly different and the fact that the high calcium diet did not increase endogenous faecal calcium loss is not consistent with the findings of Abrams et al. (2004).

The Panel notes the limited and divergent data for endogenous faecal losses of calcium in children. Abrams et al. (1999) suggested typical values for endogenous faecal calcium excretion of 2–5 mg/kg body weight per day in older infants and small children and 1–2 mg/kg body weight per day in adolescents and adults. Peacock (1991) proposed values for different ages using radioisotope data from adults; these range from 30 mg/day at 2 years to around 120 mg at 16 years. The average values reported for adults are 136 mg/day (Charles et al., 1991) and 140 mg/day (Heaney and Recker, 1994), which equates to a daily endogenous faecal loss of around 2 mg/kg body weight. In the absence of concordant data, the Panel considers that a value of 1.5 mg/kg body weight per day represents endogenous faecal losses of calcium in children.

2.3.6.3. Skin and sweat

Sweat contains calcium but the concentration is affected by the volume secreted and losses via this route are very variable, depending on the climate and level of physical activity. Calcium loss in sweat has been measured in small groups of volunteers or patients, sometimes under conditions that induce sweating, using a variety of techniques e.g. plastic bags to collect sweat (Consolazio et al., 1966; Isaksson et al., 1967), skin washing and weight recording (Mitchell and Hamilton, 1949), cotton suits (Palacios et al., 2003) and skin patches (Rianon et al., 2003). In one study in healthy adults in which sweat loss was measured for 24 hours using skin patches, the estimated loss was 35 ± 4 mg/day (mean ± SE) (Rianon et al., 2003), but in another study using cotton suits and with variable activity levels it was 103 ± 22 mg/day (Palacios et al., 2003). Hunt and Johnson (2007) used results from 19 balance studies to estimate calcium requirements and two of these (young men and young overweight women) included measurements of whole body surface losses of calcium (data unpublished). These were obtained over a 2-day-period by skin washing and extraction of calcium from cotton suits. The reported values for dermal losses of calcium were 3 mg/day in young men and 17 mg/day in young overweight women.

The wide inter-individual and inter-study variations presumably reflect inaccuracies in the methods used (e.g. sweat collections not being representative of losses from the whole body, incomplete calcium extraction from cotton suits, and/or calcium contamination) plus a limited ability to replicate

normal living conditions. In order to circumvent these problems, Charles et al. (1983) used ^{47}Ca and kinetic modelling to measure dermal losses of calcium in a study of calcium metabolism in patients with different calcium metabolic disorders. As part of this study 15 healthy adults were given an intravenous injection of ^{47}Ca and a daily retention curve was generated over 10 days by measuring ^{47}Ca excretion in stools and urine. This was compared with retention measured by whole body counting, and the difference assumed to be dermal calcium loss. In the absence of exercise and with minimal sweating the median dermal loss of calcium was 55 mg/day (range 50–94 mg/day). Charles et al. (1983) concluded that body size may be responsible for some of the inter-individual variation as there was a correlation between dermal calcium loss and body surface area. In this study dermal losses from the whole body were determined and the average loss during a 7-day-period was calculated. However, there may be an error in count rate introduced by ^{47}Ca redistribution within the body, which leads to an overestimation of dermal losses; the authors calculated that this error could lead to a maximum overestimation of dermal calcium loss of 35 %. Charles et al. (1991) reviewed the literature on dermal calcium loss and although the loss of calcium through the skin is difficult to assess a minimum obligatory dermal loss of 32–40 mg/day was proposed.

The Panel notes that dermal losses are difficult to measure accurately and are very variable. There are no data on dermal losses in children but in adults there is a significant correlation between dermal calcium loss and body surface area (Charles et al., 1983). Therefore, the Panel considers that dermal losses in infants and children can be estimated by interpolation from the adult value using the mean body surface area for each age group. The data from a radio-isotope study (Charles et al., 1991), where the mean dermal loss was 55 mg/day, is considered to be the most reliable, but may be an overestimate, and when the maximum potential error is taken into account, the dermal calcium loss falls to 36 mg/day. The Panel considers that a value of 40 mg/day represents dermal losses in adults.

2.3.6.4. Breast milk

Breast milk calcium concentrations are homeostatically regulated and are not influenced by the mother's intake of calcium (Olausson et al., 2012). There are compensatory physiological changes to maintain the calcium supply to the infant, including increased maternal efficiency of absorption in the later stages of lactation, enhanced renal reabsorption, and reduced BMD; the magnitude of bone loss is directly related to feeding practices, but there are no long-term effects on bone that can be attributed to lactation (Olausson et al., 2012). Calcium in breast milk (post-colostrum) is relatively constant for the first three months of lactation, with a concentration of 200–300 mg/L (5.0–7.5 mmol/L), and from then on it progressively declines (Atkinson et al., 1995). The concentration is independent of the volume of milk produced but there are large inter-individual variations in the calcium content of breast milk (Jarjou et al., 2012). The reasons for the differences are uncertain although, as calcium is associated with the casein, phosphate and citrate fractions of milk, factors that regulate the concentration of these fractions will, by default, affect calcium concentration; genotype may also play a role (Olausson et al., 2012). The Panel considers that the calcium concentration of breast milk over the first three months of lactation is 200–300 mg/L.

2.3.7. Interaction with other nutrients

There is an interaction between vitamin D and calcium that affects vitamin D economy. High calcium intakes increase the half-life of 25(OH)D (Lips, 2012), which may be one of the reasons why clinical trials in which combined vitamin D and calcium supplements are given to decrease fracture incidence generally show more positive results than trials using vitamin D or calcium supplements alone (Lips, 2012).

Calcium and phosphorus are both required for bone mineral deposition and maintenance throughout life. Outside the skeleton, their essential but distinct physiological functions are controlled by specific transporters and hormonal systems, which also serve to secure the appropriate supply for bone health. Several interactions between phosphorus and calcium have been documented at both the intestinal and renal levels. Phosphate decreases urinary calcium excretion and increases calcium balance (Fenton et al., 2009). The consumption of a high phosphorus/low calcium diet and, inversely, of a high

calcium/low phosphorus diet can result in reduced absorption of the lower dose mineral which can lead to disturbances in calcium or phosphorus homeostasis, with possible detrimental consequences on bone health.

Increasing the intake of sodium results in a higher urinary calcium excretion (Zarkadas et al., 1989) and this may affect bone calcium balance. In a cross-over study in postmenopausal women, comprised of four successive five-week periods of controlled dietary intervention, each separated by a minimum four-week washout, the effects of moderately low and high calcium intakes (518 versus 1 284 mg/day) and salt (3.9 versus 11.2 g/day) in a Western-style diet were compared (Teucher et al., 2008). Stable isotope labelling techniques were used to measure calcium absorption and excretion, compartmental modelling (with bone as one of five body compartments) was undertaken to estimate bone calcium balance, and biomarkers of bone formation and resorption were measured in blood and urine. The high salt intake elicited a significant increase in urinary calcium excretion ($P = 0.0008$); with the low calcium diet the 24-hour mean calcium excretion increased from 123 to 141 mg/day, and with the high calcium diet the 24-hour mean calcium excretion increased from 159 to 192 mg/day. With a high salt diet, there was no effect on bone calcium balance when intakes of calcium were high, but with a low calcium intake, the balance became negative irrespective of salt intake. The Panel notes that high intakes of sodium appear to have a detrimental effect on bone calcium balance when intakes of calcium are low.

The positive association between fruits and vegetables and bone health has been suggested to partly result from their relatively high potassium content since potassium bicarbonate supplements have been shown to be hypocalciuric (Sebastian et al., 1994). However, data from balance studies show that potassium intake is inversely associated with both urinary calcium excretion and intestinal calcium absorption (possibly through changes in renal phosphate retention which then affect $1,25(OH)_2D_3$ synthesis), resulting in no net change in calcium balance, suggesting that the effect observed in the supplement studies is due to bicarbonate, not potassium (Rafferty and Heaney, 2008), and indicating that there may be other components of fruits and vegetables that have a beneficial effect on bone health. In a retrospective analysis of data from California and North East Scotland, in which postmenopausal women were enrolled in long-term randomised, placebo-controlled studies on the effects of low- or high-dose dietary potassium supplements on bone turnover, there was no effect of treatment on BMD change or bone resorption (Frassetto et al., 2012).

In a study undertaken in 37 healthy women comparing the effect of sulphate-rich mineral water and milk on calcium balance there was a significantly lower calcium balance during the period when the sulphate-rich water was consumed, which was due to a higher urinary calcium excretion (Brandolini et al., 2005). The authors suggest that the acidogenic action of sulphate may have been responsible for the increased calciuria.

2.4. Biomarkers

2.4.1. Biomarkers of intake

In order to circumvent the problems encountered when measuring dietary intake, entailing the collection of calcium intake data from all sources (food, drinks and supplements), availability of comprehensive up-to-date food composition data, and information on the calcium content of water and other drinks, the use of an independent surrogate biomarker of intake has some advantages. Firstly, changes in habitual dietary patterns which are frequently associated with prospective dietary assessment are not an issue. Secondly, the biomarker can reflect total calcium intake more accurately as it does not rely on dietary recall (memory) or the collection of complete dietary records. Since urinary calcium excretion depends on calcium intake, it has been proposed as a surrogate biomarker of calcium intake. Some epidemiological studies have reported a linear relationship between dietary and urinary calcium (Kesteloot and Joossens, 1990). However, in both cross-sectional (Charlton et al., 2005; Toren and Norman, 2005) and long-term intervention (Zhu et al., 2011) studies there is no clear

relationship between dietary calcium intake and 24-hour urinary excretion. The Panel concludes that there are no reliable biomarkers of calcium intake.

2.4.2. Biomarkers of status

Serum calcium concentrations are maintained within a narrow range from the large calcium bone reservoir, irrespective of dietary calcium intake or whole body calcium content/status. Serum ionised calcium concentration can be used to identify disturbances in calcium metabolism but are not useful for assessing status in healthy humans (Gibson, 2005).

BMD and/or BMC can be used to assess the response to changes in intake over a relatively long period of time (> 1 year) (Gibson, 2005), but not to measure calcium status *per se*. Serum markers of bone formation (osteocalcin and bone-specific alkaline phosphatase) and urinary markers of bone resorption (pyridinoline and deoxypyridinoline) reflect changes more rapidly and have been measured in shorter-term interventions (Seamans et al., 2011). The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine suggested that serum procollagen type 1 amino-terminal propeptide and serum cross-linked C-terminal telopeptide of type 1 collagen could be used as reference bone turnover markers but require international reference standards (Vasikaran et al., 2011), although the Panel notes that results of a recent systematic review suggest that bone turnover biomarkers have a very low diagnostic value for osteoporosis (Biver et al., 2012). These markers are influenced by a number of environmental and lifestyle factors, and change in relation to circadian rhythm (Chubb, 2012) and the length of the bone modelling transient (Aloia et al., 2008). The measurements are also assay-specific (Eastell et al., 2012), and further work is required to develop reference ranges and the standardisation of methods for bone turnover markers to be a useful adjunct in the assessment of status in different population groups.

The Panel concludes that there are no suitable biomarkers of calcium status.

2.5. Influence of genotype

BMD is highly heritable, but there are age- and site-related differences. For example, using a classical twin design model it was shown that the genetic proportion of total variance for spine BMD was 88 % in premenopausal women and 77 % in postmenopausal women (Hunter et al., 2001). A study was carried out to examine the relationship between polymorphisms of the vitamin D receptor (VDR) gene and BMD (Stathopoulou et al., 2011). In a group of 578 Greek menopausal women genotyping was performed for the BsmI, TaqI and Cdx-2 polymorphisms of the VDR gene. These polymorphisms were not associated with BMD, osteoporosis or osteoporotic fractures, but when stratified by calcium intake in the low calcium group (< 680 mg/day) all polymorphisms were associated with the BMD of the lumbar spine ($P < 0.05$). After adjustment for potential covariates, BsmI and TaqI polymorphisms were associated with osteoporosis ($P < 0.05$), while the presence of the minor A allele of Cdx-2 polymorphism was associated with a lower spine BMD ($P = 0.025$). In the higher calcium intake group (> 680 mg/day), no significant differences were observed within the genotypes for all polymorphisms. It appears that the VDR gene only affects BMD in women with a low calcium intake. In addition to the proposed effects of target genes there are well-described ethnic differences in BMD. For example, despite lower dietary calcium intake and serum $1,25(OH)_2D_3$ concentrations, African Americans have a higher BMD and develop osteoporosis less frequently than European Americans (Freedman and Register, 2012).

3. Dietary sources and intake data

3.1. Dietary sources

Rich food sources of calcium include dairy products, selected vegetables (such as spinach, purslane, chard, endive, broccoli), legumes, nuts, fish with soft bones (e.g. tinned sardines), and calcium-fortified foods.

Currently, calcium carbonate, calcium chloride, calcium salts of citric acid, calcium gluconate, calcium glycerophosphate, calcium lactate, calcium salts of orthophosphoric acid, calcium hydroxide, calcium oxide, and calcium sulphate may be added to both foods[6] and food supplements.[7] The calcium content of infant and follow-on formulae[8] and processed cereal-based foods and baby foods for infants and young children[9] is regulated.

The calcium content of tap water varies widely. In tap water collected from 492 Spanish towns the calcium concentration ranged from 0–337 mg/L and in 182 bottled waters commercially available in Europe the concentration varied from 0.5–672 mg/L with 16 % having a concentration > 100 mg/L and two > 300 mg/L (Martinez-Ferrer et al., 2008).

The main dietary sources of calcium in different European countries differ, although dairy products are generally the most important food group (Welch et al., 2009); water may also contribute significantly to the daily intake in hard water areas. In Belgium, cow's milk, sweetened milk drinks and cheese are the main sources of calcium intakes (26, 25 and 11 %, respectively) in Flemish pre-school children (Huybrechts et al., 2011), and cow's milk and dairy products contributed 48 % of the daily calcium intake of men and women in the Republic of Ireland (Burke et al., 2005), 59 % in Italy (Lombardi-Boccia et al., 2003), and they are the main source of calcium in Croatia (Mandic-Puljek et al., 2005). Young Swedish vegans obtained approximately 30 % of their calcium from supplements followed by vegetables, potatoes and legumes, whereas animal products were the main source of calcium for omnivores (Larsson and Johansson, 2005).

3.2. Dietary intake

EFSA estimated dietary intakes of calcium from food consumption data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011) combined with data on the calcium content of foods from the EFSA nutrient composition database (Roe et al., 2013). Data of 13 dietary surveys from nine countries, i.e. Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK were included in the assessment after consistency checks. Food composition information of Finland, France, Germany, Italy, theNetherlands, Sweden and the UK were used to calculate calcium intakes in these countries, assuming that the best intake estimate would be obtained when both the consumption data and the composition data are from the same country. For nutrient intake estimates of Ireland and Latvia, food composition data from the UK and Germany, respectively, were used, because no specific composition data from these countries were available. The amount of borrowed calcium values in the seven composition databases used varied between 15 and 78 %. The data covered all age groups from infants to adults aged 75 years and older (Appendix A). Estimates were based on food consumption only (i.e. without dietary supplements). Nutrient intake calculations were performed only on subjects with at least two reporting days. Data on infants were available from Finland, Germany, the UK, and Italy. The contribution of human milk was taken into account if the amounts of human milk consumed (Italian INRAN SCAI survey and the UK DNSIYC survey) or the number of breast milk consumption events (German VELS study) were reported. In case of the Italian INRAN SCAI survey, human milk consumption had been estimated based on the number of eating occasions using standard portions per eating occasion. In the Finnish DIPP study only the information "breast fed infants" was available, but without any indication about the number of breast milk consumption events during one day or the amount of breast milk consumed per event. For the German VELS study, the total amount of breast milk was calculated based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion at different ages, i.e. the amount of

[6] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26

[7] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51

[8] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1.

[9] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children, OJ L 339, 6.12.2006, p. 16.

breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants aged 6–7 months and to 100 g/eating occasion for infants aged 8–12 months.

Average calcium intakes ranged between 307 and 584 mg/day (135–179 mg/MJ) in infants (aged between 1 and 11 months, four surveys), between 533 and 838 mg/day (125–192 mg/MJ) in children aged 1–< 3 years (five surveys), between 589 and 986 mg/day (97–178 mg/MJ) in children aged 3–< 10 years (seven surveys), between 675 and 1 273 mg/day (88–156 mg/MJ) in adolescents (10–< 18 years) (six surveys), and between 690 and 1 122 mg/day (87–143 mg/MJ) in adults (≥ 18 years) (eight surveys). Average daily intakes were in most cases slightly higher in males (Appendix B) compared to females (Appendix C) mainly due to larger quantities of food consumed per day.

The main food group contributing to calcium intakes was milk and dairy products. While liquid milk products (not including food products for the young population, such as infant formula) were the most important contributors to calcium intakes in infants, young and older children, cheese was the main source of calcium in the older age groups. Grains and grain-based products also contributed significantly to calcium intakes, probably at least party due to milk-based ingredients in the products. Differences in main contributors to calcium intakes between sexes were minor (Appendix D and E).

EFSA's calcium intake estimates in mg/day were compared with published intake values from the same survey and dataset and the same age class using the German EsKiMo and VELS surveys in children (Kersting and Clausen, 2003; Mensink et al., 2007), the DIPP study in Finnish children (Kyttälä et al., 2008; Kyttälä et al., 2010), the study in Finnish adolescents (Hoppu et al., 2010), the French national INCA2 survey (Afssa, 2009), the Irish NANS Survey (IUNA, 2011), the FINDIET 2012 Survey (Helldán et al., 2013), the Italian INRAN-SCAI Survey (Sette et al., 2011), the Dutch National Dietary Survey (van Rossum et al., 2011), the Swedish national survey Riksmaten (Amcoff et al., 2012), the DNSIYC-2011 Study in UK infants and toddlers (Lennox et al., 2013) and the UK NDNS Survey (Bates et al., 2012) (Table 3).

Table 3: EFSA's average daily calcium intake estimates, expressed as percentages of intakes reported in the literature

Country	% of published intake (% range over different age classes in a specific survey)
Finland	89 (DIPP young children, 1–< 3 years), 98 (DIPP children, 3–< 10 years), 100–101 (Finnish adolescents), 89–91 (FINDIET 2012)
France	92–96 (INCA2)
Germany	80–82 (VELS infants), 92–98 (VELS children), 84–95 (EsKiMo)
Ireland	105–114 (NANS)
Italy	94–100 (INRAN-SCAI)
NL	94–97 (Dutch National Dietary Survey)
Sweden	109–112 (Riksmaten)
UK	96 (DNSIYC), 94–99 (NDNS–Rolling Programme, Years 1–3, adolescents), 101-108 (NDNS–Rolling Programme, Years 1–3, other age groups)

When the EFSA intake estimates were compared with published intake estimates from the same survey and age range, the EFSA estimates differed up to around 10 % from the published values in all countries and surveys, except for the Irish and Swedish national surveys, where up to 12–14 % overestimation was seen, and for German VELS infants and EsKiMo children, where values were underestimates of up to 18–20 %. For young children of the DIPP and for children in the EsKiMo study the underestimation can partly be explained by the fact that both the DIPP and the EsKiMo study included calcium supplement consumption in their data. The contribution of the supplements has, however, been reported to be minor compared to the calcium intake from foods (Mensink et al., 2007; Kyttälä et al., 2008). Overall, several sources of uncertainties may contribute to these differences, including inaccuracies in mapping food consumption data according to food classifications and in nutrient content estimates available from the food composition tables, the use of borrowed calcium values from other countries in the food composition database, and replacing missing

calcium values by values of similar foods or food groups in the calcium intake estimation process. As the intake calculations rely heavily on estimates of both food composition and food consumption, it is not possible to conclude which of these intake estimates would be closer to the actual calcium intakes.

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The German-speaking countries (D-A-CH, 2013) considered results of a pooled analysis of calcium balance studies with 82 men and 73 women (Hunt and Johnson, 2007) and assumed that the calcium intake associated with null balance in that study is equivalent to the AR. For deriving the PRI 30 % were added to the AR of 741 mg/day to take into account the variation in calcium requirement in the population. The PRI of 1 000 mg/day was set for all adults, as there was no clear evidence that higher calcium intakes may lead to a lower reduction in bone density in postmenopausal women or a lower fracture risk in adults over 65 years of age.

For adults aged 19–50 years, the US Institute of Medicine (IOM, 2011) set an Estimated Average Requirement (EAR) of 800 mg/day and a Recommended Dietary Allowance (RDA) of 1 000 mg/day, based on calcium balance data (Hunt and Johnson, 2007) showing null calcium balance at an intake of 741 mg/day (rounded up to obtain the EAR), with the upper limit of the 95 % confidence interval (CI) of 1 035 mg/day (rounded to obtain the RDA). For adults aged 51–70 years, the main indicator for the setting of the RDA was the degree of bone loss. For men, IOM considered the data of Hunt and Johnson (2007), although only two men over 50 years of age were included: there was no evidence of changes in skeletal maintenance in men of that age, hence no reason was seen to have a different RDA than in younger adults. For women aged 51–70 years, the data of Hunt and Johnson (2007) were considered as well, though there was no stratification on the basis of menopausal status while about half of the included women were over 50 years of age. Data on BMD (Jackson et al., 2006; Tang et al., 2007) judged as reliable predictor of fracture risk later in life were also taken into account, while data on fracture risk in this population group were not considered relevant. The earlier bone loss in women compared to men, due to the onset of menopause, was taken into account and the considerable variability in the age of onset of menopause. An EAR of 1 000 mg and an RDA of 1 200 mg/day was derived. For adults beyond 70 years of age, the lack of calcium balance data was stressed and data on fracture risk taken into account (Peacock et al., 2000; Grant et al., 2005; Prince et al., 2006; Tang et al., 2007), although it was noted that the results were inconsistent, there was limited evidence of a dose-response relationship and a lack of information on background calcium intake. IOM concluded that bone loss was similar in both sexes at this age. An EAR of 1 000 mg/day and an RDA of 1 200 mg/day were set for both sexes.

The World Health Organization (WHO/FAO, 2004) used data from 210 calcium balance experiments (n = 81 subjects; duration between 6 and 480 days, mean of 90 days) (Steggerda and Mitchell, 1939; Owen et al., 1940; Steggerda and Mitchell, 1941, 1946; Johnston et al., 1952; Bogdonoff et al., 1953; Malm, 1958; Clarkson et al., 1970) to derive regression curves on a) calcium output on calcium intake, insensible calcium losses (skin, hair, nails) and urinary calcium, and b) net absorbed calcium according to intake and urinary calcium excretion according to intake. Both approaches yielded a mean apparent calcium requirement of about 520 mg/day. After adding to this value the insensible calcium losses (60 mg), the intercept between the curve of net absorbed calcium and the regression line of urinary calcium increased to 840 mg/day. Thus, the recommended intake for premenopausal women and men until 65 years of age was set at 1 000 mg/day. Menopause was considered to raise urinary calcium by about 30 mg/day (Nordin and Polley, 1987; Prince et al., 1995; Nordin et al., 1999), but not to increase calcium absorption (Heaney et al., 1989; Nordin, 1997). WHO/FAO reported on 20 prospective trials in 857 postmenopausal women and 625 controls showing a suppression of bone loss after calcium supplementation (Nordin, 1997), as well as a meta-analysis showing that calcium supplementation significantly enhanced the anabolic effect of oestrogen on bone (Nieves et al., 1998). For postmenopausal women, the AR was set at 1 100 mg/day and the recommended intake at 1 300 mg/day. Calcium absorption was considered to decrease with age in

both sexes (Morris et al., 1991; Ebeling et al., 1994; Need et al., 1998). Despite the existence of stronger evidence for an increased calcium requirement in postmenopausal women compared to men (Owen et al., 1940; Bogdonoff et al., 1953), as a precautionary measure, the same recommended intake as for postmenopausal women was set for men aged 65 years and older.

The Nordic countries (NNR, 2004) set the recommended intake at 800 mg/day, for both sexes, based on studies indicating that men with an intake of about 800 mg/day had a lower incidence of hip fracture than men with about half that intake (Matkovic et al., 1979; Cooper et al., 1988; Holbrook et al., 1988), that bone density of the lumbar vertebrae and upper femur was correlated to calcium intake in men (Kelly et al., 1990) and that a high supplemental intake of calcium may reduce fracture incidence in men (Horowitz et al., 1994). For postmenopausal women, it was noted that long-term balance studies had not been performed, that supplementation with calcium in osteoporotic patients had resulted in some reduction in bone loss in late menopausal women (Reid et al., 1993, 1995), but that the oestrogen deficiency-related bone loss observed early after menopause was not appreciably altered by calcium supplementation. For NNR 2012, the recommended intake of 800 mg/day from NNR 2004 was maintained for adults above 20 years since no strong evidence has emerged to justify a change (Nordic Council of Ministers, 2014). The recommended intake of adolescents of 900 mg/day was extended to young adults noting that some bone mass is still accreted beyond 17 years of age and that the increased demand for calcium is also reflected in a higher absorption efficiency up to the age of 24 years.

The French Food Safety Agency (Afssa, 2001) applied the factorial method and considered daily losses in urine (130 mg), faeces (110 mg) and sweat (20 mg) (Spencer et al., 1986; Charles et al., 1991; Lemann, 1993; Heaney and Recker, 1994). The minimum maintenance requirement was estimated to be 260 mg/day for adults, 280 mg/day for women beyond 55 years and men beyond 65 years of age. Calcium absorption was assumed to be 35–40 % in younger adults, taking into account calcium absorption from diets with almost no dairy products and providing about 500 mg/day of calcium, and 30 % for women beyond 55 years and men beyond 65 years of age (Weaver, 1994). Afssa noted that the average calcium intake yielding a positive or null balance in 50 % of subjects was shown to be below 650 mg/day in one balance study (Marxhall et al., 1976) and set an AR of 690 mg/day and a PRI of 900 mg/day for women until 55 years and men until 65 years of age. For women beyond 55 years and men beyond 65 years of age, the AR was set at 930 mg/day and the PRI was calculated as 1.3 (CV = 15 %) times the AR, i.e. 1 200 mg/day.

For adults aged 19–30 years, the Health Council of the Netherlands (2000) used the factorial method and estimated faecal calcium losses to be 110 mg/day (Heaney and Recker, 1982; Spencer et al., 1984), urinary losses to be 140 mg/day (Melvin et al., 1970; Marxhall et al., 1976; Matkovic, 1991), skin losses to be 30 mg/day (Allen et al., 1979; Charles et al., 1983; Peacock, 1991), and the average total loss to be 280 mg/day based on studies in which the average calcium intake was about 500 mg/day. The Council noted that already 92–95 % of peak bone mass is achieved at age 18-20 years and 100 % ten years later (Recker et al., 1992; Matkovic et al., 1994; Teegarden et al., 1995), and estimated calcium retention to be 10 mg/day (American Academy of Pediatrics. Committee on Nutrition, 1978). Assuming calcium absorption to be 30–40 % a value of 730–970 mg/day was derived. The Council considered that the results of the balance and observational studies (Matkovic and Heaney, 1992) supported the results from the factorial method, and concluded on an AI of 1 000 mg/day. No reason was identified to expect different calcium losses and absorption in adults aged 31–50 years, for which balance studies showed an equilibrium at an intake of 1 000 mg/day (Heaney et al., 1975; Heaney et al., 1977, 1978a, 1978b). The Council considered that calcium absorption is reduced with age and after menopause (Avioli et al., 1965; Ireland and Fordtran, 1973; Recker et al., 1988; Heaney et al., 1989; Ebeling et al., 1994; Heaney, 1995; Kinyamu et al., 1997; Ensrud et al., 2000), that balance studies supported an AI of 1 200 mg/day for adults aged 51–70 years, and that intervention and observational studies in relation to bone mass, bone loss or fracture risk supported an AI of 1 000–1 200 mg/day for this age range. Hence, an AI of 1 100 mg/day was set for adults aged 51–70 years. For adults aged 71 years and over, the Council considered that the factorial estimate would be higher and set an AI of 1 200 mg/day.

The Scientific Committee for Food (SCF, 1993) and the UK COMA (DH, 1991) derived a PRI (or Reference Nutrient Intake, RNI) of 700 mg/day for adults including older adults. Using the factorial approach, calcium losses via urine, sweat, faeces, hair and nails (160 mg/day) and a calcium absorption of 30 % were used to set the AR, to which twice its standard deviation (SD) was added. The Lower Threshold Intake (or Lower RNI) was set at 400 mg/day.

Table 4: Overview of Dietary Reference Values for calcium for adults

	Nordic Council (2014)	D-A-CH (2013)	IOM (2011)	WHO/FAO (2004)	Afssa (2001)	NL (2000)[a]	SCF (1993)	DH 1991)
Age (years)	18–20	≥ 19	19–50	19–65 (m) 19–menopause (f)	20–65 (m) 20–55 (f)	19–50	≥ 18	≥ 19
PRI								
Men (mg/day)	900	1 000	1 000	1 000	900	1 000	700	700
Women (mg/day)	900	1 000	1 000	1 000	900	1 000	700	700
Age (years)	≥ 21		51–70	≥ 65 (m) postmenopausal (f)	≥ 66 (m) ≥ 56 (f)	51–70		
PRI								
Men (mg/day)	800		1 000	1 300	1 200	1 100		
Women (mg/day)	800		1 200	1 300	1 200	1 100		
Age (years)			≥ 70			≥ 70		
PRI								
Men (mg/day)			1 200			1 200		
Women (mg/day)			1 200			1 200		

NL, Health Council of the Netherlands; m, males; f, females; PRI, Population Reference Intake.
(a): Adequate Intake.

4.2. Children and adolescents

For infants from 4–< 12 months, D-A-CH (2013) estimated a calcium intake of 188.5 mg/day from 650 mL of breast milk and an amount of 140 mg/day via complementary foods (IOM, 2011). Thus, after rounding, an AI of 330 mg/day was set. Calcium requirements of children were estimated factorially, assuming a calcium retention of 140 mg/day for children aged 1–< 4 years (Lynch et al., 2007), 120 mg/day for children aged 4–< 7 years (Ames et al., 1999), and 150 mg/day for those aged 7–< 10 years (Ellis et al., 1996; Abrams et al., 1999; IOM, 2011). Urinary calcium losses were assumed to amount to 37 mg/day, 45 mg/day and 55 mg/day for the respective age groups (Weaver, 1994), and endogenous faecal losses were estimated as 37 mg/day, 40 mg/day and 50 mg/day, respectively (Abrams et al., 1991; Weaver, 1994). No sweat calcium losses were assumed for children aged 1–< 4 years, whereas those aged 4–< 7 years and 7–< 10 years were estimated to have sweat calcium losses of 30 and 40 mg/day, respectively (Weaver, 1994). Summing up losses and the requirement for calcium retention, ARs were derived by assuming a calcium absorption of 45.6 % for children aged 1–< 4 years (Lynch et al., 2007), and 38 % for those aged 4–< 7 years and 7–< 10 years, respectively (Wastney et al., 1996). The factorial approach was also used for older children and adolescents, assuming calcium retention based on the findings by (Vatanparast et al., 2010). Urinary calcium losses (Abrams et al., 1997a), endogenous faecal losses (Abrams et al., 1991; Weaver, 1994; Abrams et al., 1997a), and sweat losses (Weaver, 1994; Palacios et al., 2003) were also taken into account. Due to differences in the timing of the pubertal growth spurt, a calcium absorption of 38 % was assumed for boys aged 10–< 13 years and girls aged 13–< 19 years (Wastney et al., 1996), and of 42 % for girls aged 10–< 13 years and boys aged 13–< 19 years (Jackman et al., 1997; Braun et al., 2006). For all children, PRIs were derived by adding 20 % to the ARs.

The IOM (2011) set an AI for infants aged 7–12 months based on the assumption that the calcium requirement of infants is met by human milk. Taking into account data on mean intake of human milk (0.6 L/day during the second six months of life) (Dewey et al., 1984), mean calcium content of breast milk (about 200 mg/L during this stage of lactation) (Atkinson et al., 1995), calcium absorption (60 %) and retention (about 100 mg/day during the first year of life), and the additional intake of calcium from complementary foods (140 mg/day in formula-fed infants, assumed to be similar in breast-fed

infants at that age), the AI was set at 260 mg/day. For children, IOM followed the factorial method. For children aged 1–3 years, an EAR of 500 mg/day (after rounding) and an RDA of 700 mg/day were set, based on average calcium retention (142 mg/day), urinary losses (34 mg/day), faecal losses (40 mg/day) and a calcium absorption of 46 % in this population (Lynch et al., 2007). For children aged 4–8 years, the EAR was set at 800 mg/day and the RDA at 1 000 mg/day, based on an increased calcium retention due to pre-puberty (140–160 mg/day), urinary losses (40 mg/day), faecal losses (50 mg/day), and a calcium absorption of 30 % (Abrams et al., 1999; Ames et al., 1999). For children aged 9–18 years, IOM used data on average calcium retention (92–210 mg/day according to age and sex), urinary losses (106 mg/day in girls, 127 mg/day in boys), faecal losses (112 mg/day in girls, 105–108 mg/day in boys according to the age considered), sweat losses (55 mg/day) and a calcium absorption of 38 % (Vatanparast et al., 2010). The variability in the onset of puberty and the pubertal growth spurt was considered as small. The EAR was set at 1 100 mg/day based on interpolation of the calcium intakes to achieve the average calcium retention estimated for girls and boys aged 9–18 years (Vatanparast et al., 2010), and an RDA of 1 300 mg/day was set for both sexes.

WHO/FAO (2004) estimated calcium retention for infants aged 7–12 months to be about 100 mg/day, urinary calcium excretion to be about 10 mg/day (Widdowson et al., 1963; Widdowson, 1965; Hanna et al., 1970; Williams et al., 1970; Shaw, 1976) and insensible losses to be about 10 mg/day. Thus, the required quantity of absorbed calcium was assumed to be 120 mg/day. Calcium absorption from cow's milk was considered to be lower than that from human milk, and about 0.5 SD above the normal adult slope of calcium absorption according to intake (see Section 4.1.). From these curves and the value of 120 mg/day, WHO/FAO derived an AR of about 300 mg/day and a recommended intake of 400 mg/day for infants aged 7–12 months. For children aged 2–9 years, calcium retention was considered to be about 120 mg/day based on data on total body dual-energy X-ray absorptiometry (DXA) and calculations from growth analyses (Leitch and Aitken, 1959). To this value, average daily urinary calcium losses of 60 mg (Matkovic, 1991) and dermal losses of 40 mg were added, resulting in an average required quantity of absorbed calcium of 220 mg/day. Considering a net absorption of calcium by children of 1 SD above that of adults (see Section 4.1.), the AR was considered to be 440 mg/day and the recommended intake to be 600 mg/day in children aged 4–6 years, somewhat lower in young children (500 mg/day, 1–3 years) and somewhat higher in children aged 7–9 years 700 mg/day). For adolescents, considering the increased calcium retention (300 mg/day) (Leitch and Aitken, 1959), and urinary (100 mg/day) (Matkovic, 1991), and dermal calcium losses (40 mg/day), the required quantity of absorbed calcium during at least part of adolescence was set at 440 mg/day. A higher absorption (+2 SD above that of adults) was taken into consideration, thus the AR was set at 1 040 mg/day and the recommended intake at 1 300 mg/day for both sexes during the peak growth phase.

The Nordic Countries (NNR, 2004) recommended a calcium intake of 600 mg/day for children aged 1–5 years, which was assumed to ensure a calcium retention of about 60–200 mg/day observed between in children aged 1–8 years based on DXA estimation of BMC. For puberty, calcium retention was considered to be much higher. Calcium supplementation was reported to be associated with increased bone density up to puberty. Adaptation to an increased calcium requirement (Weaver et al., 1995; O'Brien et al., 1996) and the efficient calcium absorption were noted and a calcium intake of 900 mg/day recommended for children aged 10–17 years. The possible inhibitory effect on iron absorption of higher calcium intakes was mentioned (Cook et al., 1991; Hallberg et al., 1991). The recommended intakes for infants and children of all ages remained unchanged for NNR 2012 (Nordic Council of Ministers, 2014).

The French Food Safety Agency (Afssa, 2001) followed the factorial method. The minimum maintenance requirement was considered to be the same in adolescents aged 15–18 years as in adults (i.e. 260 mg/day). It was considered to vary according to body weight, and thus to be 50 mg/day in children aged 1–3 years and 100 mg/day in those aged 4–9 years (Abrams et al., 1991; Matkovic and Ilich, 1993). The requirement for growth according to age was estimated to be 90 mg/day (1–3 years), 140 mg/day (4–9 years), 250 mg/day (10-14 years), and 100 mg/day (15–18 years) (Comar and Bronner, 1964; Peacock, 1991; Fomon and Nelson, 1993; Chan et al., 1995; Ruiz et al., 1995; Bonjour

et al., 1997). Absorption was assumed to be 40 % in children aged 1–9 and 15–18 years, and 45 % in children aged 10–14 years. Hence, the ARs were set at 350 mg/day (1–3 years), 600 mg/day (4–9 years), 930 mg/day (10–14 years) and 920 mg/day (15–18 years), and the PRIs were calculated from the ARs considering twice a CV of 15 %.

Using the factorial method, the Health Council of the Netherlands (2000) estimated calcium losses to be 60 mg/day and calcium retention to be 100 mg/day for infants aged 6–11 months. An AI of 450 mg/day was derived based on a calcium absorption of about 50 % and adding to the requirement of 320 mg/day two SD. For children aged 1–3 years, losses were estimated to be 80 mg/day, retention to be 100 mg/day (Fomon et al., 1982; Matkovic, 1991) and the AI to be 500 mg/day (Matkovic, 1991; Matkovic and Heaney, 1992). For children aged 4–8 years, losses were considered to be 130 mg/day and retention to be 100 mg/day. Assuming a calcium absorption of 50 %, an intake of 460 mg/day was considered necessary. Taking into account data from balance studies and intervention studies with a sufficiently long duration, the Council set an AI of 700 mg/day. For children aged 9-18 years, calcium losses were considered to be about 220–230 mg/day (Greger et al., 1978; Matkovic, 1991; Weaver et al., 1995; O'Brien et al., 1996; Wastney et al., 1996; Abrams et al., 1997a) and calcium retention to be about 160–210 mg/day (Mazess, 1973; American Academy of Pediatrics. Committee on Nutrition, 1978; Fomon et al., 1982). Considering calcium absorption to be about 35–50 %, the Council set an AI of 1 200 mg/day for boys and of 1 100 mg/day for girls aged 9–18 years.

For infants aged 6–11 months, due to lack of data, the SCF (1993) proposed the PRI for children aged 1–3 years, i. e. 400 mg/day. The UK COMA (DH, 1991) considered for infants from 0–12 months a calcium requirement for retention of 160 mg/day, an absorption efficiency of 40 % from infant formula and consequently an EAR and an RNI of 400 mg/day and 525 mg/day, respectively. For children between one and ten years of age, the SCF (1993) and the UK COMA (DH, 1991) used the factorial approach and an estimated calcium retention of 70–150 mg/day (Leitch and Aitken, 1959), a net absorption of 35 %, and two SD to cover individual variation. For adolescents, a mean retention of 250 mg/day (girls) and 300 mg/day (boys) and a net absorption of 40 % were assumed, and adding 30 % for individual variation, the PRIs (or RNIs) for girls and boys aged 11–17 (or 18) years were set at 800 mg/day and 1 000 mg/day, respectively.

Table 5: Overview of Dietary Reference Values for calcium for children

	Nordic Council (2014)	D-A-CH (2013)	IOM (2011)	WHO/ FAO (2004)	Afssa (2001)	NL (2000) [a]	SCF (1993)	DH (1991)
Age (months)	6–11	4–<12	6–12	7–12		6–11	6–11	0–12
PRI (mg/day)	540	330	260 [a]	400		450	400	525
Age (years)	1–5	1–<4	1–3	1–3	1–3	1–3	1–3	1–3
PRI (mg/day)	600	600	700	500	500	500	400	350
Age (years)	6–9	4–<7	4–8	4–6	4–6	4–8	4–6	4–6
PRI (mg/day)	700	750	1 000	600	700	700	450	450
Age (years)	10–17	7–<10	9–18	7–9	7–9	9–18	7–10	7–10
PRI (mg/day)	900	900	1 300	700	900	1 200 (m) 1 100 (f)	550	550
Age (years)		10–<13		10–18	10–19		11–17	11–18
PRI (mg/day)		1 100		1 300	1 200		1 000 (m) 800 (f)	1 000 (m) 800 (f)
Age (years)		13–<19						
PRI (mg/day)		1 200						

NL, Health Council of the Netherlands; PRI, Population Reference Intake; m, males; f, females.
(a): Adequate Intake

4.3. Pregnancy and lactation

D-A-CH (2013) considered that pregnancy is associated with a doubling of calcium absorption, an increase in urinary calcium excretion and some bone resorption, but that these physiological adaptations are transient. In addition, it was stated that interventions with calcium have not shown a benefit of calcium supplementation during pregnancy (Koo et al., 1999). The German-speaking countries considered that a higher calcium intake during lactation does not prevent the loss of calcium from bone nor does it influence the calcium concentration of human milk. The recommended intake for pregnant and lactating women was therefore the same as for non-pregnant non-lactating women, i.e. 1 000 mg/day for adults and 1 200 mg/day for adolescents.

For pregnant women and adolescents, IOM (2011) used the same EARs and RDAs as for non-pregnant women and adolescents, as randomised controlled trials did not show that calcium supplementation (beyond non-pregnant requirements) during pregnancy would be beneficial to the mother or fetus (Koo et al., 1999; Jarjou et al., 2010). It was also stated that parity may be associated with a neutral or even protective effect on maternal BMD or fracture risk according to observational studies (Sowers, 1996; Kovacs and Kronenberg, 1997; O'Brien et al., 2003; Chantry et al., 2004), and that fractional calcium absorption doubles during pregnancy and compensates for the increased calcium transferred to the fetus (200–250 mg/day). For lactating adults and adolescents, the EARs and RDAs of non-lactating women and adolescents were also considered appropriate. This was based on evidence that the calcium content of human milk is not affected by intake (Kalkwarf et al., 1997; Jackson et al., 2006), that the transient maternal bone resorption observed in lactating women (Kalkwarf et al., 1997; Specker et al., 1997; Kalkwarf, 1999) is not suppressed by an increased calcium intake (Cross et al., 1995; Fairweather-Tait et al., 1995; Prentice et al., 1995; Kalkwarf et al., 1997; Laskey et al., 1998; Polatti et al., 1999), that maternal bones are restored after lactation without additional calcium intake (Cross et al., 1995; Prentice et al., 1995) and that there is no evidence suggesting that lactation impairs achievement of peak bone mass in adolescents (Chantry et al., 2004).

WHO/FAO (2004) reported the calcium content of the newborn infant to be about 24 g, most of which is laid down in the last trimester of pregnancy during which the fetus retains about 240 mg/day (American Academy of Pediatrics. Committee on Nutrition, 1978). Using the factorial approach, WHO/FAO considered an increased calcium absorption during pregnancy (Heaney and Skillman,

1971; Kumar et al., 1979; Kent et al., 1991), maternal urinary calcium losses of 120 mg/day and dermal losses of 60 mg/day, summing up to a requirement for absorbed calcium of 420 mg/day. Considering an absorption of + 2 SD above that of non-pregnant, non-lactating adults, the corresponding AR was set at 940 mg/day, and the recommended intake at 1 200 mg/day. For lactating women, WHO/FAO considered daily calcium losses via milk of about 280 mg based on a calcium content in human milk of 360 mg/L (Nordin, 1976) and a secreted amount of about 0.75 L/day. Maternal urinary calcium excretion was considered to be 100 mg/day, and maternal skin losses to be 60 mg/day, summing up to losses of 440 mg/day. WHO/FAO stated that calcium absorption does not increase and possibly even decreases during lactation and that lactational bone loss is not affected by calcium intake (Sowers et al., 1996). Thus, no extra calcium allowance was set for lactating women.

The Nordic countries (NNR, 2004) recommended the same calcium intake of 900 mg/day for pregnant and lactating women as for non-pregnant non lactating women. It was noted that calcium absorption increases during pregnancy (Shenolikar, 1970; Heaney and Skillman, 1971), that calcium supplementation does not influence calcium retention (Ashe et al., 1979), and that dietary calcium intakes in the Nordic countries are already about 800–1 000 mg/day. It was also noted that calcium supplementation does not alter % absorption (Fairweather-Tait et al., 1995; Kalkwarf et al., 1997), that bone resorption increases during lactation (Affinito et al., 1996), that there is renal conservation of calcium (Specker et al., 1994), that these adaptive changes are not influenced by calcium intake, and that bone loss is regained when ovarian function and menstruation resume. This recommendation was maintained in NNR 2012 since no strong evidence has emerged to justify a change (Nordic Council of Ministers, 2014).

The French Food Safety Agency (Afssa, 2001) followed the factorial approach. For pregnant women, the minimum maintenance requirement was assumed to be lower than for non-pregnant women, i.e. 200 mg/day, due to a higher intestinal absorption of endogenous calcium. The fetus was considered to retain about 20 g of calcium during the last trimester of pregnancy, i.e. on average 220 mg/day. Based on a calcium absorption of 55 % for pregnant women (Kent et al., 1991), the AR was calculated as 760 mg/day and the PRI set at 1.3 (CV = 15 %) times the estimated AR, i.e. 1 000 mg/day, for pregnant women in the third trimester. A calcium content in human milk of 320 mg/L and a daily volume of 0.8 L were taken into account to estimate calcium losses of 250 mg/day during lactation (Lönnerdal, 1997). For lactating women, the minimum maintenance requirement was assumed to be lower than for non-pregnant women, i.e. 200 mg/day, due to the reduction in urinary calcium excretion. Based on a calcium absorption of 45 % (Kent et al., 1991; Kalkwarf et al., 1996), the AR was calculated as 1 000 mg/day, which was also the value chosen as PRI, considering that losses of bone mass during breastfeeding would be later compensated by an increased bone retention (Drinkwater and Chesnut, 1991; Specker et al., 1991; Sowers et al., 1993; Prentice, 1994; Cross et al., 1995; Laskey et al., 1998; Ritchie et al., 1998). AFSSA also derived a PRI for women after the breastfeeding period; considering a calcium retention of 200 mg/day to restore bone calcium content and a calcium absorption of 50 %, an AR of 800 mg/day was derived and the PRI set at 1.3 (CV = 15 %) times the estimated AR, i.e. 1 000 mg/day to be applied for the same number of months as those of breastfeeding.

The Health Council of the Netherlands (2000) considered that pregnant women do not need to increase their calcium intake (Allen, 1982; Schaafsma, 1992; IOM, 1997). It was reported that the number of pregnancies was either not correlated with maternal bone density or fracture risk later in life (Cumming et al., 1997; IOM, 1997) or even associated with a higher bone density (Aloia et al., 1983) and a lower fracture risk (Hoffman et al., 1993). The same calcium intake as for non-pregnant women was also proposed for lactating women, as there was no clear indication that a higher intake would be beneficial (Prentice, 2000).

For pregnant women, the SCF (1993) and the UK COMA (DH, 1991) considered that the calcium required for fetal growth is provided through an increased absorption and the mobilisation of calcium from maternal bone (Purdie, 1989), and set the same PRI as for non-pregnant women. For lactating women, the SCF (1993) proposed an additional calcium intake of 500 mg/day for the calcium required

in milk, assuming an absorption of 40 % and adding 2 SD. The additional calcium intake proposed by the UK COMA (DH, 1991) was estimated by taking into account an amount of calcium secreted with breast milk of 300 mg/day, assuming an absorption of 40 % and also considering that the EAR of lactating women is lower than that of non-lactating adults (400 mg/day instead of 525 mg/day).

Table 6: Overview of Dietary Reference Values for calcium for pregnant and lactating women

	Nordic Council (2014)	D-A-CH (2013)	IOM (2011)	WHO/FAO (2004)	Afssa (2001)	NL (2000)	SCF (1993)	DH (1991)
Pregnancy								
PRI (mg/day)	900	As for non-pregnant women	As for non-pregnant women	1 200	1 000 (3rd trim)	As for non-pregnant women	As for non-pregnant women	As for non-pregnant women
Lactation								
PRI (mg/day)	900	As for non-pregnant women	As for non-pregnant women	As for non-pregnant women	1 000	As for non-pregnant women	≥ 500, i.e. 1 200	+ 550
After lactation								
PRI (mg/day)					1 000 (a)			

NL, Health Council of the Netherlands; PRI, Population Reference Intake; Trim, trimester.
(a): for the same number of months as those of breastfeeding.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of calcium requirement

Although there are no direct biomarkers of calcium status (see Section 2.4.2), the role that calcium plays in skeletal health provides a basis for deriving DRVs. The quantity of dietary calcium that is sufficient for bone growth and turnover and to replace obligatory body losses in 50 % of the population is the criterion upon which the AR is calculated. For extraskeletal outcomes (see below) the evidence is inconsistent and causality is inconclusive so these cannot be used for deriving DRVs.

5.2. Calcium balance in adults

Balance studies are based on the assumption that a healthy subject on an adequate diet maintains an equilibrium or a null balance between nutrient intakes and nutrient losses: at this null balance, the intake matches the requirement determined by the given physiological state of the individual. When intakes exceed losses (positive balance), there is nutrient accretion that may be attributable to growth or to weight gain, anabolism or repletion of stores; when losses exceed intakes (negative balance), nutrient stores are progressively depleted resulting, in the long term, in clinical symptoms of deficiency. When performed at different levels of intakes, balance studies enable the quantification of obligatory losses by regression to zero. In addition to numerous methodological concerns about the accuracy and precision in the determination of intakes and losses (Baer et al., 1999), the validity of balance studies for addressing requirements has been questioned: they might possibly reflect only adaptive changes before reaching a new steady-state (Young, 1986) or only the conditions for maintenance of nutrient stores in the context of a given diet, the relevance of the pool size for health remaining to be established for each nutrient (Mertz, 1987).

There is a positive correlation between calcium balance and intake at lower levels of intake which reaches a plateau at higher levels of intake (Matkovic and Heaney, 1992). Once requirements for bone growth and turnover are satisfied, any additional absorbed calcium will be excreted in the urine. The value at which the plateau occurs depends on age because of differences in calcium requirements for bone growth (the effect of sex is unknown because data from males and females from birth to 30 years of age were combined for the regression analysis). Ascertaining values for the threshold value in different population groups was attempted by Matkovic and Heaney (1992), but small sample size, high inter-individual variation and the inherent imprecision in balance data made it impossible to derive accurate values.

In order to provide figures that could be used to establish calcium requirements for the North American Dietary Reference Intakes (DRIs), balance data from well-controlled metabolic studies, collected in 155 adults (73 women and 82 men) aged 19–75 years with different levels of calcium intake (ranging from 415–1 740 mg/day) and intakes of sodium and protein typical for diets consumed in industrialised countries were collated and analysed (IOM, 2011). Only studies with balance periods of ≥ 18 days (following a minimum equilibration period of seven days) were included in order to allow sufficient time for physiological adaptation to take place according to the level of intake, and calcium intake and excretion during the final 6–12 days of each metabolic balance period was measured accurately by chemical analysis. The participants were apparently healthy people, living in North America, and with no evidence of osteomalacia. The data were combined and the relationship between intake and excretion examined by fitting random coefficient models. The models predict a null calcium balance at intakes of 741 mg/day, irrespective of age or sex (Hunt and Johnson, 2007).

The same balance data from the studies which were used to derive DRIs for North American adults, were further analysed by EFSA (see Appendix F), with some important differences. Firstly, data from additional studies in which calcium supplements were given (not included in the analysis by Hunt and Johnson (2007)) were added to the database, which resulted in data from a total of 27 studies being analysed. Secondly, individual data from younger adults (< 25 years) were excluded as there is evidence that additional calcium continues to be deposited in bones after they have ceased growing (Teegarden et al., 1995; Ohlsson et al., 2011; Darelid et al., 2012), which is dependent on bone site (Recker et al., 1992; Hui et al., 1999). The Panel notes that calcium metabolism cannot be considered to be in a steady state until the age of 25 years (see Section 2.3.4).

EFSA applied a mixed linear model (Brown and Prescott, 1999) to establish the dietary calcium intake able to predict a null balance for half the population (Appendix F). It was assumed that in order to be representative of a healthy population, the range of average individual values for calcium balance in any one study should include zero. After excluding data from studies that did not meet this criterion, a total of 170 individuals (females and males) and 378 observations were considered in the final analysis. Outliers (6 extreme observations) were removed, leaving 169 subjects (110 women aged 25–81 years, 59 men aged 25–65 years) and 372 observations in total (229 for females and 143 for males). The effect of age, sex and body weight were not significant, so they were removed from the final model which only contained calcium intake as the explanatory variable. The mean intake of calcium where intake equals excretion (null balance) was 715 mg/day. The PRI is defined as the level of intake that is adequate for 97.5 % of subjects in a population group. This parameter is estimated via the upper bound of the marginal prediction interval at the level corresponding to a null balance for the population mean. The 95 % marginal prediction interval is the estimate of the individual values in a population provided by the model with 95 % confidence. Its upper bound represents the 97.5 percentile of the distribution of the individual predictions for each level of the predictor (dietary calcium intake) at the population average random effects. This prediction interval upper bound at the level of calcium null balance for the population mean is equal to 904 mg/day (lower bound at 525 mg/day). The Panel considers that calcium excretion used in the model is an underestimate due to the fact that dermal calcium losses were not measured in the metabolic studies. The extent of underestimation would depend on the type and extent of physical activity by the subjects during the study periods, which varied considerably as indicated in the publications of the individual studies, and no information on this was provided to EFSA. The Panel considers that the range of values for the dietary calcium intake and excretion reflects the situation in the EU. The Panel also considers that it is not appropriate to conclude on the representativeness of dietary consumption patterns, age and sex composition, due to the lack of data and the relatively small sample size.

5.3. Calcium balance in infants and children

There are very few published data on calcium balance in infants and children. A stable isotope study in 19 breast-fed infants aged 8–10 weeks (Hicks et al., 2012) reported a mean calcium intake of 246 ± 20 mg/day and a fractional calcium absorption of 76.0 ± 2.9 %. The total absorbed calcium was calculated to be 187 ± 16 mg/day. In comparison, in a group of 30 infants of the same age calcium

intake from cow's milk formula was 557 ± 16 mg/day, fractional calcium absorption was 59.2 ± 2.3 %, and total calcium absorbed was 328 ± 13 mg/day. The Panel notes that this study was designed to measure calcium absorption, not retention. Butte et al. (2000) undertook repeated anthropometric and body composition measurements in infants from birth until 24 months of age. Exclusive breastfeeding for at least four months (n = 40) resulted in lower BMC than in formula-fed infants (n = 36) at 12 months but the difference disappeared by 24 months. Specker et al. (1997) reported that during the first six months of life both breast milk and low-mineral (439 mg/L of calcium and 240 mg/L of phosphorus) formula were associated with lower bone mass accretion than high-mineral (1 350 mg/L of calcium and 900 mg/L of phosphorus) formula, but by 12 months of age there were no differences in bone mass between the groups.

Lynch et al. (2007) measured calcium absorption in 28 children aged 15–48 months using a dual-tracer stable-isotope technique; endogenous faecal excretion was measured in a subset of eight children, and net calcium balance was calculated. Mean calcium intake was 551 mg/day (range 124–983 mg/day), and mean (± SEM) calcium retention was 161 ± 17 mg/day. Both linear and nonlinear modelling of balance data showed that a calcium intake of approximately 470 mg/day led to a calcium retention of 140 mg/day.

Matkovic & Heaney (1992) pooled balance data from a number of published articles in order to examine the relationship between calcium intake and balance. At high intakes, balance tended to flatten and become constant whereas at lower intakes balance was highly correlated with intake. The Panel notes that during periods of growth, a positive balance is required in order for calcium to be supplied to the developing bones, and therefore balance data can only be used for deriving calcium requirements in infants and children when combined with bone accretion data.

5.4. Calcium requirements in pregnancy and lactation

In pregnancy, there are additional demands for calcium to meet the requirements of the developing fetal skeleton. The accretion of calcium takes place mainly in the second half of pregnancy with the estimated rate of 50 mg/day at 20 weeks gestation increasing to 330 mg/day at 35 weeks (Forbes, 1976). During lactation there is an additional requirement for calcium for the mammary gland. The average secretion of calcium in breast milk is 200 mg/day but it can be as high as 400 mg/day (Prentice, 2003) (see Section 2.3.6.4).

Calcium absorption increases during pregnancy and early lactation (Heaney and Skillman, 1971; Kent et al., 1991). Urinary calcium excretion is also raised, but this may be a consequence of increased absorption, and calcium balances are generally positive (King et al., 1992). There are conflicting reports on bone changes during pregnancy, but the majority of studies demonstrate maternal bone mobilisation from some sites, but this has been shown to be unrelated to dietary calcium intake (reviewed by Prentice (1994)).

Olausson et al. (2012) reviewed the literature on calcium requirements during pregnancy and lactation. They concluded that in both of these population groups changes are induced in calcium and bone metabolism to support the transfer of calcium from the mother to the child. These are generally independent of maternal calcium intake in populations where dietary intakes are close to current recommendations.

The Panel acknowledges the existence of physiological adaptive processes that ensure sufficient calcium for fetal growth and breast milk production. These may obviate the need for additional calcium in the diet, provided intakes are close to the DRV for adults.

5.5. Calcium intake and health consequences

A systematic review of the literature pertaining to calcium and vitamin D and health outcomes was published in 2009 (Chung et al., 2009). The studies included primary intervention or observation studies that reported outcomes in human subjects in relation to vitamin D and/or calcium intake/status,

as well as systematic reviews that met the inclusion and exclusion criteria. Cross-sectional and retrospective case-control studies were excluded. Outcomes of relevance to calcium where evidence was found included bone and skeletal health, cancer, cardiovascular disease, and hypertension. The review was not specifically targeted at life stages, except for pregnant and postmenopausal women, and there was a large variation in the methodological quality of the studies examined which limited the possibilities for meta-analysis. In 165 primary studies and 11 systematic reviews (which included > 200 primary studies), the available evidence focused mainly on bone health, cardiovascular diseases and cancer. The authors concluded that the majority of the findings concerning vitamin D, calcium, or a combination of both nutrients on the different health outcomes were inconsistent, and because the literature was so heterogeneous it was not possible to derive a dose–response relationship between intakes of either vitamin D, calcium, or both nutrients and health outcomes. One of the key challenges was the difficulty in separating the effects of calcium and vitamin D in many studies due to their close interrelationship. Furthermore, there were very few randomised controlled trials or clinical trials that focussed on extraskeletal outcomes as the primary endpoint.

A recent systematic review undertaken to inform the NNR5 project on calcium requirements and upper intake levels (Uusi-Rasi et al., 2013) reported on the effects of calcium intake for a number of health outcomes. The time frame for the search was January 2000 until December 2011. Life stages covered were infants, children, adolescents, adults, elderly, and pregnancy and lactation, and the population groups considered were primarily Caucasians. Outcome measures included pregnancy outcomes and growth, bone health (fractures, BMD, osteoporosis, bone mass, bone quality), muscle strength, all cancers (and breast, colorectal, and prostate cancer), autoimmune diseases, type 2 diabetes, obesity/weight control, total mortality, and cardiovascular disease clinical outcomes. The main limitations of this review were that most were calcium supplementation studies and did not report total calcium intake, there was high heterogeneity amongst study protocols (widely varying intakes of calcium, different study duration), and dose–response studies were not reported.

5.5.1. Bone health

The NNR review (Uusi-Rasi et al., 2013) was not able to draw any conclusions on the effects of calcium intake on measures of bone health (skeletal growth, BMD and fractures) in any population group. The greatest limitations when evaluating the effect of calcium on bone health are methodological (differences in the measurement of BMD or BMC, lack of RCTs due to the need for an intervention lasting for at least one year to attain measureable differences in BMD/BMC, and few data for some population groups, such as premenopausal women and men. There was high heterogeneity in protocols amongst the studies.

There was insufficient evidence on maternal calcium intake and fetal growth to draw any conclusions (Uusi-Rasi et al., 2013).

The Panel considers that measures of bone health cannot be used to derive DRVs for calcium.

5.5.2. Cardiovascular disease-related outcomes

The NNR review (Uusi-Rasi et al., 2013) identified 13 studies (seven systematic reviews, three RCTs, three cohort studies) that addressed the effects of calcium on different cardiovascular outcomes, but there was no consistent evidence of any association between calcium intake and cardiovascular outcomes apart from systolic blood pressure. However, as these were calcium supplementation studies, with no information on total calcium intake, the Panel considers that the results could not be used for deriving DRVs.

The Panel considers that evidence related to cardiovascular outcomescannot be used to derive DRVs for calcium.

5.5.3. Cancer

Results of a meta-analysis (Chen et al., 2010) reported a 19 % (RR 0.81, 95% CI 0.72–0.90) decrease in breast cancer risk for women with the highest quantile of calcium intake compared to the lowest quantile, but there was significant heterogeneity among the studies and evidence of publication bias. Chung et al (2009) reviewed primary studies that evaluated associations between calcium intake and incidence and mortality of prostate cancer. Twelve studies reported data on subjects with a mean age ranged from 53–67 years. Seven studies did not find an association between calcium intake and the risk of prostate cancer. Five studies found that the risk was higher in the groups that took more calcium (diet plus supplements) compared to the groups that took lower amount (adjusted OR 1.2–2.2). The higher amount ranged from 921 to at least 2 000 mg/day of calcium; the lower amount ranged from 455 to 1 000 mg/day. Three studies also reported on the association between calcium intake and mortality from prostate cancer. Two studies found no association, and one study found an increased risk comparing the group that took at least 2 000 mg/day of calcium with the group that took 500 to 749 mg/day (adjusted RR 2.02, 95 % CI 1.14–3.58). Results from the US Prostate Cancer Prevention Trial (Kristal et al., 2010) found a positive association between dietary calcium intake (quartile 4 (> 1 165 mg/day) versus quartile 1 (< 598 mg/day)) and low-grade cancer (OR 1.27, 95 % CI 1.02–1.57) but an inverse association with high-grade cancer (OR 0.43, 95 % CI 0.21–0.89).

The NNR review (Uusi-Rasi et al., 2013) included nine studies (five systematic reviews, one meta-analysis, three cohort studies) with cancer as an outcome. There was no consistent relationship between the level of calcium intake and different types of cancers; some showed a protective effect whilst in others calcium increased the risk. The Panel notes that, due to the nature of the health outcome, an evaluation of the effect of calcium intake on cancer risk needs an exposure of several years, making intervention studies impossible, and restricting studies to observational studies, in which intakes of calcium need to be assessed and monitored accurately, a situation which is rarely achieved.

The Panel considers that evidence related to cancer cannot be used to derive DRVs for calcium.

6. Data on which to base Dietary Reference Values

In the absence of suitable biomarkers of status or function and of suitable data on calcium intake and health outcomes, the Panel decided to derive DRVs for calcium using a factorial approach for children and balance data for adults. The data required to derive ARs in different population groups are the calcium intakes that are needed to replace endogenous losses, and hence achieve null calcium balance, plus the quantities needed for growth and lactation, where appropriate.

6.1. Infants aged 7–11 months

Infants are growing and need to be in positive calcium balance. If a factorial approach is used to derive the physiological requirement, the quantity of calcium required for bone accretion must be added to the endogenous losses. However, factorial estimates of calcium requirements are difficult to calculate accurately in infants due to limited data. In exclusively breast-fed infants calcium retention is estimated to be 100 mg/day, most of which is used for bone growth and hence broadly equivalent to bone calcium accretion (Section 2.2.4). Endogenous losses have been reported to range from 2–5 mg/kg body weight per day (Abrams et al., 1999) in infants from 7–11 months. Assuming the lowest endogenous losses (2 mg/kg per day) and 60 % absorption (Section 2.3.1), the intake to balance losses and enable adequate calcium accretion into bones is calculated as 196 mg/day, and using the highest endogenous losses (5 mg/kg per day), the intake needed is 241 mg/day.

The Panel notes the wide range and resultant uncertainty in factorial estimates for infants aged 7–11 months.

Although it is possible for formula-fed infants to increase calcium absorption and bone calcium accretion to levels above those achieved in breast-fed infants, this does not result in differences in BMC at 12 months (Specker et al., 1997). Therefore, the Panel decided to estimate the quantity of

calcium absorbed by exclusively breast-fed infants and to extrapolate these values to older infants, taking into account body weight changes. The calcium concentration of breast milk over the first three months of lactation is 200–300 mg/L (Olausson et al., 2012). Assuming a mean concentration over the first six months of lactation of 250 mg/L, an average breast milk consumption of infants aged 0–6 months of 0.8 L/day (Butte and King, 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009) and an absorption efficiency of 60 % (see Section 2.3.1), the amount of absorbed calcium will be 120 mg/day. The AI for infants over six months of age can be derived by extrapolation from this figure, using isometric scaling (linear with body weight) and assuming an absorption of 60 % (Abrams et al., 1997a; Abrams et al., 1997b; Abrams, 2010b, 2010a). The median body weight-for-age of infants aged 9 months and 3 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006) served as reference body weights. For infants aged 7–11 months, the AI is estimated to be 280 mg/day. This is close to the value derived from the highest estimated endogenous losses using the factorial approach (241 mg/day).

6.2. Children

The AR is derived using the factorial approach. The total quantity of calcium required for bone accretion (Section 2.3.4) and replacement of endogenous losses (Section 2.3.6) are adjusted to account for % absorption (Section 2.3.1). The estimates used in the factorial approach to derive the AR for calcium for children are given in Table 6 (see also Appendix G for mode of calculation).

Table 7: Estimates used in the factorial approach to calculate dietary requirements for calcium for children

Age	Reference weight (kg)	Calcium losses (mg/day) [a]			Requirement for bone calcium accretion (mg/day) [b]	Physiological requirement (mg/day) [c]	% Absorption [d]	Dietary requirement (mg/day) [e]
		Urinary	Faecal	Dermal				
1–3 years	11.9 [f]	24	18	13	120	174	45	388
4–6 years	19.0 [g]	38	28	18	120	204	30	681
7–10 years	28.8 [h]	58	43	24	111	235	35	672
11–14 years	44.8 [i]	89	67	32	189	378	40	944
15–17 years	59.8 [j]	120	90	39	143	391	45 (m), 35 (f)	965

m, males; f, females. Calculations were done with the unrounded figures, whereas figures in the tables are given without decimals.
(a): see Sections 2.3.6.1, 2.3.6.2 and 2.3.6.3. In the absence of data on dermal calcium losses in children, these were extrapolated from adult losses of 40 mg/day using body weight to the power of 0.67 as a proxy for body surface area.
(b): see Section 2.3.4.
(c): Sum of losses and requirement for bone calcium accretion
(d): see Section 2.3.1
(e): Dietary requirement = [(urinary losses + faecal losses + dermal losses) + calcium accretion in bone]/% absorption
Example calculation for boys aged 2 years:
Dietary requirement = [(1.5 mg/kg per day * 12.2 kg) + (2 mg/kg per day * 12.2 kg) + 13 mg/day + 120 mg/day]/0.45 = 390 mg/day
(f): Mean of body weight-for-age at 50th percentile of boys and girls aged 1, 2 (WHO Multicentre Growth Reference Study Group, 2006) and 3 years (van Buuren et al., 2012)
(g): Mean of body weight at 50th percentile of boys and girls aged 4, 5, and 6 years (van Buuren et al., 2012)
(h): Mean of body weight at 50th percentile of boys aged 7, 8, 9, and 10 years (van Buuren et al., 2012)
(i): Mean of body weight at 50th percentile of girls aged 11, 12, 13 and 14 years (van Buuren et al., 2012)
(j): Mean of body weight at 50th percentile of boys and girls aged 15, 16, and 17 years (van Buuren et al., 2012)

For children aged 1–3 years, the requirement for bone calcium accretion is 120 mg/day, endogenous faecal calcium loss is 1.5 mg/kg body weight per day, urinary calcium loss is 2 mg/kg body weight per day, and dermal losses are 13 mg/day, extrapolated by allometric scaling (body weight$^{0.67}$) from the value for adults (40 mg/day, Section 2.3.6.2) and averaged over the three years. Using median body weights of boys and girls aged 1, 2, (WHO Multicentre Growth Reference Study Group, 2006) and 3 years (van Buuren et al., 2012), physiological requirements were calculated for both sexes combined and per year. These were averaged and the dietary requirement was derived, assuming a % calcium absorption of 45 % (see Section 2.3.1). A dietary requirement of 388 mg/day was calculated, and the Panel derived an AR of 390 mg/day.

In children aged 4–6 years, the Panel assumed a similar calcium requirement for bone calcium accretion (120 mg/day) and endogenous faecal calcium losses of 1.5 mg/kg body weight per day. Urinary losses were assumed to be 2 mg/kg body weight per day. Dermal losses were extrapolated by allometric scaling (body weight$^{0.67}$) from the value for adults (40 mg/day, Section 2.3.6.2) and averaged over the three years. Using median body weights of boys and girls aged 4, 5, and 6 years (van Buuren et al., 2012), physiological requirements were calculated for the combined sexes at each year of age. These were averaged and the dietary requirement of 681 mg/day was derived, assuming a % calcium absorption of 30 %.

In children aged 7–10 years, a similar approach was used to calculate endogenous faecal (43 mg/day), urinary (58 mg/day) and dermal (24 mg/day) losses. The requirement for bone calcium accretion was assumed to be 120 mg/day in children aged 7 and 8 years and as estimated by Vatanparast et al. (2010) for ages 9 and 10 years. Physiological requirements were calculated for the combined sexes at each year of age and thereafter averaged. Assuming 35 % calcium absorption, a dietary requirement of 672 mg/day was calculated. As the dietary requirement of children aged 4–6 and 7–10 years is similar, the Panel decided to derive an AR of 680 mg/day for children aged 4–10 years.

In older children aged 11–17 years, additional calcium is required for accelerated bone growth associated with puberty. From the height-for-age data of children in EU countries, the growth velocity appears to be highest at ages 14–17 years in boys and 12–15 years in girls (van Buuren et al., 2012). The Panel decided to use calcium bone accretion data from a longitudinal study (Vatanparast et al., 2010) (Section 2.3.4). Combining the bone accretion data and growth velocity charts for European children, the Panel decided to derive combined DRVs for boys and girls, for ages 11–14 and 15–17 years. Endogenous faecal losses (1.5 mg/kg body weight per day) observed in children aged 11–14 years (Section 2.3.6.2) were calculated based on median body weights at ages 11, 12, 13, and 14 years (van Buuren et al., 2012). Urinary losses were assumed to be 2 mg/kg body weight per day, and dermal losses were extrapolated by allometric scaling (body weight$^{0.67}$) from the values for adults (40 mg/day, see Section 2.3.6.2). Daily requirements for bone calcium accretion were based on data by Vatanparast et al. (2010). Physiological requirements were calculated for each sex and per year. These were then averaged and a dietary requirement of 944 mg/day was derived, assuming a % calcium absorption of 40 % (see Section 2.3.1). For children aged 15–17 years, the same approach and database was used as in children aged 11–14 years. A dietary requirement of 965 mg/day was calculated, assuming 35 % absorption in girls and 45 % in boys (due to the different pubertal status and hence bone calcium accretion). As the dietary requirement of children aged 11–14 and 15–17 years is similar, the Panel decided to derive an AR of 960 mg/day for children aged 11–17 years.

In the absence of knowledge about the variation in requirement, PRIs for children of the various age groups were estimated based on a CV of 10 %, and rounded down to the nearest 50 (see Table 8).

6.3. Adults

6.3.1. Young adults (18–24 years)

The accretion of calcium in bone continues for a few years after growth has stopped; therefore, there is an additional requirement for calcium in young adults, aged 18–24 years (Section 2.3.4).

As this additional requirement for calcium in young adults is unknown, the AR is derived as the intermediate value between the AR for children aged 11–17 years and that for adults ≥ 25 years, and is 860 mg/day. In the absence of knowledge about the variation in requirements, the PRI was estimated based on a CV of 10 %, and rounded down to the nearest 50.

6.3.2. Adults (25 years and upwards)

The Panel has analysed balance data obtained from North American adults (Section 5.2). The mean intake of calcium where intake equals excretion was 715 mg/day. The calcium excretion data used to compute calcium balance do not include dermal losses. Hunt and Johnson (2007) assumed that dermal losses in adults are negligible, but the Panel has decided to add a value of 40 mg/day to the estimated mean and upper limit of the mean calcium intake with which null calcium balance was achieved in North American adults to make an allowance for dermal losses (Section 2.3.6.3) and derived an AR of 750 mg/day.

The 95 % marginal prediction interval is the estimate of the individual values in a population provided by the model with 95 % confidence. Its upper bound represents the 97.5 percentile of the distribution of the individual predictions for each level of the predictor (dietary calcium intake) at the population average random effects. This prediction interval upper bound at the level of calcium null balance for the population mean is equal to 904 mg/day. Adding to this value dermal losses of 40 mg/day and rounding up to the nearest 50, a PRI of 950 mg/day is derived for men and women aged 25 years and above. Using the "classical" approach (EFSA NDA Panel, 2010) of deriving the PRI from the AR of 750 mg/day by assuming a CV of 10 % would result in a value of 900 mg/day.

6.4. Pregnancy

The adaptive physiological changes that occur during pregnancy (e.g. enhanced efficiency of absorption) are largely independent of maternal calcium intake, unless intakes are very low (reviewed by Olausson (2012)) (see Section 5.4). Therefore, the Panel concludes that additional calcium is not required for pregnant women.

6.5. Lactation

The adaptive physiological changes that occur during lactation (e.g. enhanced efficiency of absorption, loss of calcium from bone) are largely independent of maternal calcium intake, unless intakes are very low (reviewed by Olausson (2012). In two randomised, placebo-controlled trials Kalkwarf et al. (1997) found no effect of calcium supplementation (1 000 mg/day) on bone density in the forearm or on the calcium concentration in breast milk, demonstrating that bone loss cannot be prevented with higher intakes of calcium. The Panel concludes that additional calcium is not required during lactation.

CONCLUSIONS

The Panel concludes that ARs and PRIs for calcium can be derived for adults based on calcium balance data from North America. Adding to the mean value where calcium intake equals excretion (null balance) an allowance for dermal losses of calcium an AR is derived for adults ≥ 25 years. Adding dermal losses to the upper bound 95 % confidence interval at the level corresponding to null balance for the population mean allowed estimation of the PRI. The PRI for young adults (18–24 years), who are still accumulating calcium in bones, is derived as the intermediate value between adolescents aged 15–17 years and adults ≥ 25 years. For infants (7–11 months) an AI was derived by extrapolating the average amount of calcium absorbed by exclusively breast-fed infants using isometric scaling and taking into account % calcium absorption. For children, ARs were estimated based on factorial calculation of losses and considering the need for calcium accretion in bone, and taking into account % calcium absorption at various ages. In the absence of knowledge about the variation in requirement, PRIs for children and young adults were estimated based on a CV of 10 %. Taking into consideration adaptive changes in calcium metabolism that occur during pregnancy and lactation, the AR for adult women aged 18–24 years and ≥ 25 years, respectively, also applies to pregnant and lactating women.

Table 8: Summary of DRVs for calcium for infants, children and adults

Age	AI (mg/day)	Average Requirement (mg/day)	Population Reference Intake (mg/day)
7–11 months	280		
1–3 years		390	450
4–10 years		680	800
11–17 years		960	1 150
Adults 18–24 years [(a)]		860	1 000
Adults ≥ 25 years [(a)]		750	950

(a): including pregnancy and lactation

RECOMMENDATIONS FOR RESEARCH

The Panel recommends that studies be undertaken to generate data required for deriving calcium requirements in young children using the factorial approach (measurements of obligatory losses and bone accretion/calcium retention).

The Panel recommends research that will provide more accurate values for dermal calcium losses to be undertaken.

The Panel recommends research on the effects of very old age on calcium requirements (measurements of efficiency of absorption, obligatory losses and changes in bone calcium content).

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APPENDICES

A. DIETARY SURVEYS IN THE UPDATED EFSA COMPREHENSIVE EUROPEAN FOOD CONSUMPTION DATABASE INCLUDED IN THE NUTRIENT INTAKE CALCULATION AND NUMBER OF SUBJECTS IN THE DIFFERENT AGE CLASSES

Country	Dietary survey (year)	Year	Method	Days	Age (years)	Number of subjects [b]						
						Infants 1-11 mo	Children 1–< 3 y	Children 3–< 10 y	Adolescents 10–< 18 y	Adults 18–< 65 y	Adults 65–< 75 y	Adults ≥ 75 y
Finland/1	DIPP	2000–2010	Dietary record	3	0.5–6	499	500	750				
Finland/2	NWSSP	2007–2008	48-hour dietary recall [a]	2x2 [a]	13–15				306			
Finland/3	FINDIET2012	2012	48-hour dietary recall [a]	2 [a]	25–74					1 295	413	
France	INCA2	2006–2007	Dietary record	7	3–79			482	973	2 276	264	84
Germany/1	EsKiMo	2006	Dietary record	3	6–11			835	393			
Germany/2	VELS	2001–2002	Dietary record	6	<1–4	158	347	299				
Ireland	NANS	2008–2010	Dietary record	4	18–90					1 274	149	77
Italy	INRAN-SCAI 2005-06	2005–2006	Dietary record	3	<1–98	16 [b]	36 [b]	193	247	2 313	290	228
Latvia	FC_PREGNANTWOMEN 2011	2011	24-hour dietary recall	2	15–45				12 [b]	991 [c]		
Netherlands	DNFCS	2007–2010	24-hour dietary recall	2	7–69			447	1 142	2 057	173	
Sweden	RISKMATEN	2010–2011	Dietary records (Web)	4	18–80					1 430	295	72
UK/1	DNSIYC	2011	Dietary record	4	0.3–1.5	1 369	1 314					
UK/2	NDNS-Rolling Programme (1–3 y)	2008–2011	Dietary record	4	1-94		185	651	666	1 266	166	139

mo, months; y, years; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): A 48-hour dietary recall comprises two consecutive days.

(b): 5^{th} or 95^{th} percentile intakes calculated over a number of subjects lower than 60 require cautious interpretations as the results may not be statistically robust (EFSA, 2011) and therefore for these dietary surveys/age classes the 5^{th}, 95^{th} percentile estimates will not be presented in the intake results.

(c): One subject with only one 24-hour dietary recall day was excluded from the dataset, i.e. the final n = 990.

2326 **B. CALCIUM INTAKES IN MALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY**

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			N [(a)]	Average	Median	P5	P95	N	Average	Median	P5	P95
Infants	Finland	DIPP_2001_2009	247	312	293	13	665	245	136	148	35	216
	Germany	VELS	84	440	431	230	703	84	137	134	69	214
	Italy	INRAN_SCAI_2005_06	9	502	476	[(b)]	[(b)]	9	165	162	[(b)]	[(b)]
	United Kingdom	DNSIYC_2011	699	584	576	347	832	699	174	176	108	225
1 to < 3	Finland	DIPP_2001_2009	245	671	640	202	1193	245	180	175	97	287
	Germany	VELS	174	591	568	285	964	174	128	120	67	208
	Italy	INRAN_SCAI_2005_06	20	729	711	[(b)]	[(b)]	20	151	130	[(b)]	[(b)]
	United Kingdom	DNSIYC_2011	663	784	767	395	1204	663	188	183	113	279
	United Kingdom	NDNS-RollingProgrammeYears1-3	107	838	824	406	1310	107	170	167	99	250
3 to < 10	Finland	DIPP_2001_2009	381	986	1001	461	1468	381	168	170	81	245
	France	INCA2	239	808	793	439	1289	239	132	125	69	217
	Germany	EsKiMo	426	757	743	380	1172	426	99	97	56	142
	Germany	VELS	146	617	584	325	1041	146	110	106	64	182
	Italy	INRAN_SCAI_2005_06	94	743	731	435	1162	94	103	99	57	162
	Netherlands	DNFCS 2007-2010	231	854	804	366	1499	231	100	99	44	164
	United Kingdom	NDNS-RollingProgrammeYears1-3	326	799	766	411	1280	326	128	124	71	199
10 to < 18	Finland	NWSSP07_08	136	1273	1203	539	2258	136	156	146	73	253
	France	INCA2	449	846	834	397	1387	449	108	107	59	168
	Germany	EsKiMo	197	809	775	430	1318	197	100	97	57	161
	Italy	INRAN_SCAI_2005_06	108	863	812	363	1486	108	88	87	44	139
	Netherlands	DNFCS 2007-2010	566	976	910	375	1753	566	93	88	37	164
	United Kingdom	NDNS-RollingProgrammeYears1-3	340	822	781	407	1355	340	101	96	56	156
18 to < 65	Finland	FINDIET2012	585	1121	1026	399	2188	585	121	117	52	208
	France	INCA2	936	913	876	401	1521	936	105	101	59	164
	Ireland	NANS_2012	634	1089	1037	519	1836	634	109	104	63	168
	Italy	INRAN_SCAI_2005_06	1068	793	758	326	1390	1068	87	84	43	141
	Netherlands	DNFCS 2007-2010	1023	1122	1054	447	2042	1023	102	95	42	181
	Sweden	Riksmaten 2010	623	1058	983	444	1817	623	108	104	59	172
	United Kingdom	NDNS-RollingProgrammeYears1-3	560	943	908	439	1605	560	108	105	59	167

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			N [(a)]	Average	Median	P5	P95	N	Average	Median	P5	P95
65 to < 75	Finland	FINDIET2012	210	945	899	353	1814	210	115	110	55	194
	France	INCA2	111	893	849	466	1393	111	105	99	66	154
	Ireland	NANS_2012	72	993	948	370	1591	72	112	109	72	157
	Italy	INRAN_SCAI_2005_06	133	764	710	374	1273	133	89	85	47	144
	Netherlands	DNFCS 2007-2010	91	980	918	330	1564	91	107	106	48	167
	Sweden	Riksmaten 2010	127	997	1009	474	1602	127	116	110	71	170
	United Kingdom	NDNS-RollingProgrammeYears1-3	75	1017	1017	489	1747	75	123	115	78	196
≥ 75	France	INCA2	40	836	743	(b)	(b)	40	109	100	(b)	(b)
	Ireland	NANS_2012	34	969	913	(b)	(b)	34	125	123	(b)	(b)
	Italy	INRAN_SCAI_2005_06	69	859	818	346	1426	69	98	100	52	143
	Sweden	Riksmaten 2010	42	987	964	(b)	(b)	42	117	116	(b)	(b)
	United Kingdom	NDNS-RollingProgrammeYears1-3	56	879	840	(b)	(b)	56	122	116	(b)	(b)

P5, 5th percentile; P95, 95th percentile; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): 5th or 95th percentile intakes calculated over a number of subjects lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011) and therefore for these dietary surveys/age classes the 5th and 95th percentile estimates will not be presented in the intake results.

C. CALCIUM INTAKES IN FEMALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY

Ageclass	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			N [(a)]	Average	Median	P5	P95	N	Average	Median	P5	P95
Infants	Finland	DIPP_2001_2009	253	307	308	15	697	251	147	155	44	231
	Germany	VELS	75	392	377	211	658	75	135	133	77	207
	Italy	INRAN_SCAI_2005_06	7	522	529	[(b)]	[(b)]	7	179	185	[(b)]	[(b)]
	United Kingdom	DNSIYC_2011	670	528	511	298	815	670	173	175	102	227
1 to < 3	Finland	DIPP_2001_2009	255	672	652	160	1171	255	192	187	61	308
	Germany	VELS	174	533	502	288	915	174	125	121	68	199
	Italy	INRAN_SCAI_2005_06	16	685	652	[(b)]	[(b)]	16	151	159	[(b)]	[(b)]
	United Kingdom	DNSIYC_2011	651	734	710	361	1144	651	186	184	111	270
	United Kingdom	NDNS-RollingProgrammeYears1-3	78	703	685	339	1083	78	157	156	83	242
3 to < 10	Finland	DIPP_2001_2009	369	935	938	474	1361	369	178	176	101	260
	France	INCA2	243	724	710	440	1073	243	132	127	80	209
	Germany	EsKiMo	409	709	681	347	1146	409	105	101	58	163
	Germany	VELS	147	589	561	332	978	147	114	106	71	176
	Italy	INRAN_SCAI_2005_06	99	697	675	368	1099	99	97	92	58	156
	Netherlands	DNFCS 2007-2010	216	819	775	323	1624	216	101	99	39	181
	United Kingdom	NDNS-RollingProgrammeYears1-3	325	733	716	362	1137	325	124	121	70	182
10 to < 18	Finland	NWSSP07_08	170	1020	1007	464	1762	170	154	157	82	238
	France	INCA2	524	707	702	306	1160	524	112	110	61	169
	Germany	EsKiMo	196	767	751	352	1218	196	104	99	51	166
	Italy	INRAN_SCAI_2005_06	139	732	688	417	1255	139	92	86	52	142
	Latvia	FC_PREGNANTWOMEN_2011	12	1058	955	[(b)]	[(b)]	12	102	99	[(b)]	[(b)]
	Netherlands	DNFCS 2007-2010	576	867	836	329	1534	576	100	96	41	178
	United Kingdom	NDNS-RollingProgrammeYears1-3	326	675	636	318	1136	326	100	94	56	165
18 to < 65	Finland	FINDIET2012	710	980	908	432	1762	710	137	131	68	224
	France	INCA2	1340	813	786	390	1312	1340	128	121	72	211
	Ireland	NANS_2012	640	856	816	421	1385	640	117	113	72	180
	Italy	INRAN_SCAI_2005_06	1245	730	702	337	1193	1245	101	96	54	161
	Latvia	FC_PREGNANTWOMEN_2011	990	801	750	380	1383	990	95	90	47	160
	Netherlands	DNFCS 2007-2010	1034	951	893	396	1692	1034	117	109	54	203
	Sweden	Riksmaten 2010	807	885	856	412	1441	807	125	113	64	185
	United Kingdom	NDNS-RollingProgrammeYears1-3	706	788	749	378	1280	706	120	113	67	194

Ageclass	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			N [(a)]	Average	Median	P5	P95	N	Average	Median	P5	P95
65 to < 75	Finland	FINDIET2012	203	828	770	322	1392	203	133	130	68	213
	France	INCA2	153	776	761	376	1202	153	127	117	69	215
	Ireland	NANS_2012	77	936	801	492	1659	77	137	131	88	213
	Italy	INRAN_SCAI_2005_06	157	690	680	322	1151	157	101	97	48	171
	Netherlands	DNFCS 2007-2010	82	896	880	445	1394	82	126	117	68	209
	Sweden	Riksmaten 2010	168	900	870	434	1470	168	129	126	76	198
	United Kingdom	NDNS-RollingProgrammeYears1-3	91	820	793	458	1310	91	137	129	87	225
≥ 75	France	INCA2	44	806	766	(b)	(b)	44	135	128	(b)	(b)
	Ireland	NANS_2012	43	865	903	(b)	(b)	43	139	136	(b)	(b)
	Italy	INRAN_SCAI_2005_06	159	735	754	336	1157	159	112	105	60	189
	Sweden	Riksmaten 2010	30	985	1024	(b)	(b)	30	139	140	(b)	(b)
	United Kingdom	NDNS-RollingProgrammeYears1-3	83	864	816	484	1278	83	143	143	90	208

P5, 5th percentile; P95, 95th percentile; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): 5th or 95th percentile intakes calculated over a number of subjects lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011) and therefore for these dietary surveys/age classes the 5th and 95th percentile estimates will not be presented in the intake results.

(c): Pregnant women only.

D. MINIMUM AND MAXIMUM % CONTRIBUTION OF DIFFERENT FOOD GROUPS TO CALCIUM INTAKES IN MALES

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives, flavours, baking and processing aids	<1	<1	0	0	0	0	0
Alcoholic beverages	<1	<1	<1	<1	1 – 3	1 – 2	1 – 2
Animal and vegetable fats and oils	<1	<1	<1	<1	<1	<1 – 1	<1 – 1
Coffee, cocoa, tea and infusions	<1	<1 – 1	<1 – 2	<1 – 3	1 – 11	1 – 10	<1 – 10
Composite dishes	<1 – 2	<1 – 5	<1 – 7	<1 – 12	<1 – 10	1 – 9	<1 – 8
Eggs and egg products	<1	<1 – 1	<1 – 1	<1 – 1	<1 – 1	<1 – 2	<1 – 1
Fish, seafood, amphibians, reptiles and invertebrates	<1	<1 – 1	<1 – 3	<1 – 3	<1 – 3	<1 – 4	1 – 2
Food products for young population	30 – 60	3 – 21	<1 – 1	<1	<1	–	–
Fruit and fruit products	<1 – 4	1 – 2	1 – 2	1 – 2	1 – 3	1 – 5	1 – 3
Fruit and vegetable juices and nectars	<1	<1 – 2	1 – 2	1 – 2	<1 – 2	<1 – 2	<1 – 1
Grains and grain-based products	<1 – 6	3 – 12	2 – 19	2 – 22	7 – 27	7 – 33	6 – 35
Human milk	<1 – 24	<1 – 1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	<1 – 1	<1 – 2	<1 – 2	<1 – 2	1 – 2	1 – 2	<1 – 1
Meat and meat products	<1	<1 – 1	1 – 2	1 – 2	1 – 2	1 – 2	1 – 2
Milk and dairy products	21 – 30	62 – 74	55 – 84	43 – 83	38 – 69	39 – 67	39 – 62
Products for non-standard diets, food imitates and food supplements or fortifying agents	<1	0 – 1	<1 – 1	<1 – 1	<1 – 2	<1	<1 – 1
Seasoning, sauces and condiments	<1	<1 – 1	<1 – 1	<1 – 1	<1 – 2	<1 – 2	<1 – 2
Starchy roots or tubers and products thereof, sugar plants	<1 – 1	<1 – 1	<1 – 1	1 – 2	1 – 2	1 – 2	1 – 2
Sugar, confectionery and water-based sweet desserts	<1	<1 – 4	1 – 7	1 – 7	<1 – 2	<1 – 1	<1 – 1
Vegetables and vegetable products	<1 – 3	1 – 3	2 – 5	2 – 6	1 – 9	2 – 11	2 – 8
Water and water-based beverages	1 – 17	2 – 9	1 – 13	2 – 15	3 – 16	2 – 15	2 – 13

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

E. MINIMUM AND MAXIMUM % CONTRIBUTION OF DIFFERENT FOOD GROUPS TO CALCIUM INTAKES IN FEMALES

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives, flavours, baking and processing aids	<1	0	0	0	0	0	0
Alcoholic beverages	0	<1	<1	<1	<1 – 1	<1 – 2	<1 – 1
Animal and vegetable fats and oils	<1	<1	<1	<1	<1	<1 – 1	<1 – 1
Coffee, cocoa, tea and infusions	<1	<1 – 1	<1 – 2	<1 – 3	1 – 11	1 – 11	1 – 11
Composite dishes	<1 – 2	<1 – 5	<1 – 7	<1 – 13	1 – 10	<1 – 8	<1 – 9
Eggs and egg products	<1	<1 – 1	<1 – 2	<1 – 1	<1 – 1	<1 – 1	<1 – 1
Fish, seafood, amphibians, reptiles and invertebrates	0	<1 – 1	<1 – 2	<1 – 4	<1 – 3	1 – 2	1 – 2
Food products for young population	31 – 63	4 – 16	<1 – 2	<1 – 1	<1	–	<1
Fruit and fruit products	1 – 4	1 – 2	1 – 2	1 – 4	1 – 5	2 – 7	1 – 4
Fruit and vegetable juices and nectars	<1	<1 – 2	1 – 2	1 – 2	<1 – 1	<1 – 1	<1 – 1
Grains and grain-based products	1 – 6	2 – 14	2 – 19	3 – 21	7 – 26	6 – 28	6 – 28
Human milk	<1 – 12	1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	<1 – 1	<1 – 2	<1 – 2	<1 – 2	1 – 2	1 – 2	1
Meat and meat products	<1	<1 – 1	1 – 2	1 – 2	1 – 2	1	1
Milk and dairy products	12 – 41	60 – 73	54 – 85	40 – 78	39 – 67	43 – 65	45 – 60
Products for non-standard diets, food imitates and food supplements or fortifying agents	<1	<1 – 1	0 – 1	<1 – 2	<1 – 3	<1 – 2	<1 – 3
Seasoning, sauces and condiments	<1	<1 – 1	<1 – 1	<1 – 1	<1 – 2	<1 – 1	<1 – 2
Starchy roots or tubers and products thereof, sugar plants	<1 – 1	1	1	1 – 2	<1 – 2	1	1
Sugar, confectionery and water-based sweet desserts	<1 – 1	<1 – 3	1 – 7	1 – 7	1 – 3	<1 – 1	<1 – 1
Vegetables and vegetable products	1 – 3	1 – 3	2 – 5	2 – 6	2 – 9	2 – 10	2 – 8
Water and water-based beverages	2 – 12	2 – 11	1 – 13	2 – 15	4 – 18	3 – 16	3 – 16

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

F. ANALYSIS OF CALCIUM BALANCE DATA FOR ADULTS

Specific objectives

The specific objectives of the analysis were to estimate the level of calcium intake that corresponds to a null balance in the healthy adult population based on experimental data. The estimated mean value leading to null balance in the sampled population is assumed to correspond to the Average Requirement (AR), the level of intake that is adequate for half of the people in a population group. Traditionally, a Population Reference Intake (PRI), i.e. the level of intake that is adequate for 97.5 % of people in a population group, is derived from the AR by adding two times the standard deviation of the requirement in the population (EFSA NDA Panel, 2010).

In contrast to the methodology commonly adopted to derive a PRI, a new approach was taken in this work following Hunt and Johnson (2007). A model was set up in order to establish the dietary calcium intake level able to predict a null balance for half of the population (mean predicted value, assuming a normal distribution). The PRI was estimated as the value corresponding to the 97.5th percentile of the population derived from the same model (upper level of the marginal prediction interval at the level corresponding to a null balance for the estimated population mean). For estimating model parameters, metabolic data collected by the US Department of Agriculture, Agricultural Research Service were used. Part of these data were previously analysed by Hunt and Johnson (2007) in their work.

Methodological difference with analysis performed by Hunt and Johnson (2007)

A similar work was performed by Hunt and Johnson (2007). An average value of dietary calcium intake corresponding to a null balance (excretion equal to intake) was established as 741 (when expressed in mg/day), 9.39 (when expressed in mg/kg body weight per day) and 0.279 (when expressed in mg/kcal per day). These values were assumed by the authors as ARs.

The motivation for performing a further analysis on the same set of data was given by the decision to:

1. Consider different eligibility criteria for the study selection such as:
   - Exclusion of subjects younger than 25 years;
   - Inclusion of studies with calcium supplementation;
2. Use a different structure of the variance/covariance matrix of the explanatory model in terms of
   - Random component (‘study’ instead of ‘individual’);
   - covariance structure considered in the error component (correlation among multiple replicates on the same subject).
3. Use a different approach for the derivation of the PRI:
   - A calibration methodology has been used by Johnson et al. for the derivation of the intake requirement corresponding to the calcium excretion at null balance (Oman, 1998)
   - The upper limit of the prediction interval for the population calcium excretion at the null balance has been adopted for the current estimate.

The above-mentioned methodological differences can eventually justify differences in the results between the publication by Hunt and Johnson (2007) and results presented in this Opinion.

Sources of information

Hunt and Johnson (2007) used experimental data collected from metabolic studies in humans, including measures of dietary calcium intake and the corresponding excretion in urine and faeces. The list of 19 studies considered by the authors as well as their main characteristics is provided in Table 1 of Hunt and Johnson (2007). Based on a request for data, EFSA received a set of individual data belonging to 27 studies (eight of those not included in the list of Table 1 in Hunt and Johnson (2007)).

All studies were carried out at the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Centre between 1976 and 1995. These experiments were designed to meet various objectives and various target populations corresponding to a wide range of individual characteristics (e.g. obese women, young men carrying out very intense physical activity).

Each study was run over subsequent dietary periods whose number ranged from 1 to 6. Therefore, replicated observations over time were available for each subject in most of the studies. The minimum length of any dietary period was 18 days.

The provision of data was limited to the subset of variables considered by Hunt and Johnson (2007). They included age, sex and body weight of the subjects as well as measures of dietary calcium intake, excretion and balance, all of them expressed in mg/day, mg/kg body weight per day, mg/kcal of dietary intake per day.

Calcium content of the diet as well as urinary and faecal calcium excretion were determined analytically in all studies. However, no data were available in the metabolic studies provided to EFSA on the amount of calcium eliminated via sweat loss. Consequently, the latter was not accounted for in the current analysis. The lack of consideration of the sweat loss represents a source of bias (potential underestimation of calcium excretion) that needs to be considered while drawing conclusions.

The individual data are property of the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Centre. Therefore, they cannot be disclosed by EFSA.

Summary statistics of the characteristics of the subjects included in the studies provided to EFSA are reported in Table 9. A total of 247 subjects were considered for a total of 566 observations (part of which are correlated since measurements were replicated in the same subject over different periods of time). Data on 144 females (306 observations in total) and 103 males (260 observations in total) were available.

Table 9: Sex, number of subjects and observations (not all independent) by study

Study	Sex	Sample size (n. subjects)	Total n. observations	Study	Sex	sample size (n. subjects)	Total n. observations
1	M	13	57	**15**	F	14	27
2	M	9	15	**16**	F	12	14
3	M	2	4	**17***	F	6	6
4	M	4	7	**18**	M	14	42
5	M	10	17	**19**	F	8	8
6	M	6	11	**20**	M	11	22
7	M	9	16	**21**	M	3	3
8	M	8	30	**22**	F	3	3
9	M	7	19	**23**	F	14	42
10	F	7	42	**24**	F	13	51
11	F	7	9	**25**	F	14	27
12	F	5	20	**26**	F	13	14
13*	F	14	14	**27**	F	14	29
14	M	7	17				

*Studies 13 and 17 were weight loss studies on obese women. Only maintenance diet data were extracted for these studies

The distribution by age classes of the subjects in the sample provided to EFSA was quite uneven by sex, with the majority of women being older than 50, while men over 50 were highly underrepresented (Table 10).

2425 Table 10: Population included in the studies by sex and age classes

Sex	Age class	N. subjects
F	< 25 years	12
F	25–50	42
F	≥ 50 years	90
M	< 25 years	34
M	25–50	64
M	≥ 50 years	5
Total		247

2426

2427 Main summary statistics for the 247 subjects in the dataset are provided in Table 11. These statistics
2428 were computed after averaging over the various replicates for each subject. Calcium excretion and
2429 intake have similar ranges and main statistics (mean and median). The variability tends to be slightly
2430 larger for the calcium output. The mean and median positive values for the balance could be an
2431 indicator of a slight underestimating in the excretion measurements. This could be due either to the
2432 lack of measurements carried out for calcium sweat losses or to a partial loss of faecal/urine material
2433 during the collection. This potential source of bias should be taken into consideration while
2434 interpreting results.

2435 Table 11: All studies and subjects: summary statistics of the main variables

Variables	n. of subjects	Min	Max	Median	Mean	Std dev
Calcium intake (mg/day)	247	556.58	1501.67	788.96	852.98	200.00
Calcium output (mg/day)	247	333.33	1507.67	781.00	802.42	218.41
Balance* (mg/day)	247	-222.14	696.50	18.00	50.56	121.61
Calcium intake (mg/kg)	247	6.04	21.96	11.40	11.84	2.97
Calcium output (mg/kg)	247	3.90	21.97	10.86	11.11	3.08
Balance* (mg/kg)	247	-3.75	10.26	0.25	0.73	1.74
Calcium intake (mg/kcal)	247	0.19	0.55	0.35	0.35	0.07
Calcium output (mg/kcal)	247	0.11	0.55	0.32	0.32	0.08
Balance* (mg/kcal)	247	-0.1	0.21	0.007	0.02	0.05
Body weight (kg)	247	45.93	133.19	71.50	73.79	15.19

2436 *Balance computed as difference between dietary calcium intake and the excretion

2437 Boxplots of dietary calcium intake, excretion and balance expressed as mg/day are provided in Figures
2438 1–3. Again for each individual a single value was obtained averaging over the various replicates (from
2439 1 to 6 measures depending on the study). The boxplot highlights the distribution mean (diamond
2440 symbol), median (horizontal line) and quartiles (interior and extremes of the box), minimum and
2441 maximum in a range of 1.5-fold the 25th and 75th percentiles (extreme of the whiskers) and potential
2442 outliers defined as values above 1.5-fold the 25th and 75th percentile (dots).

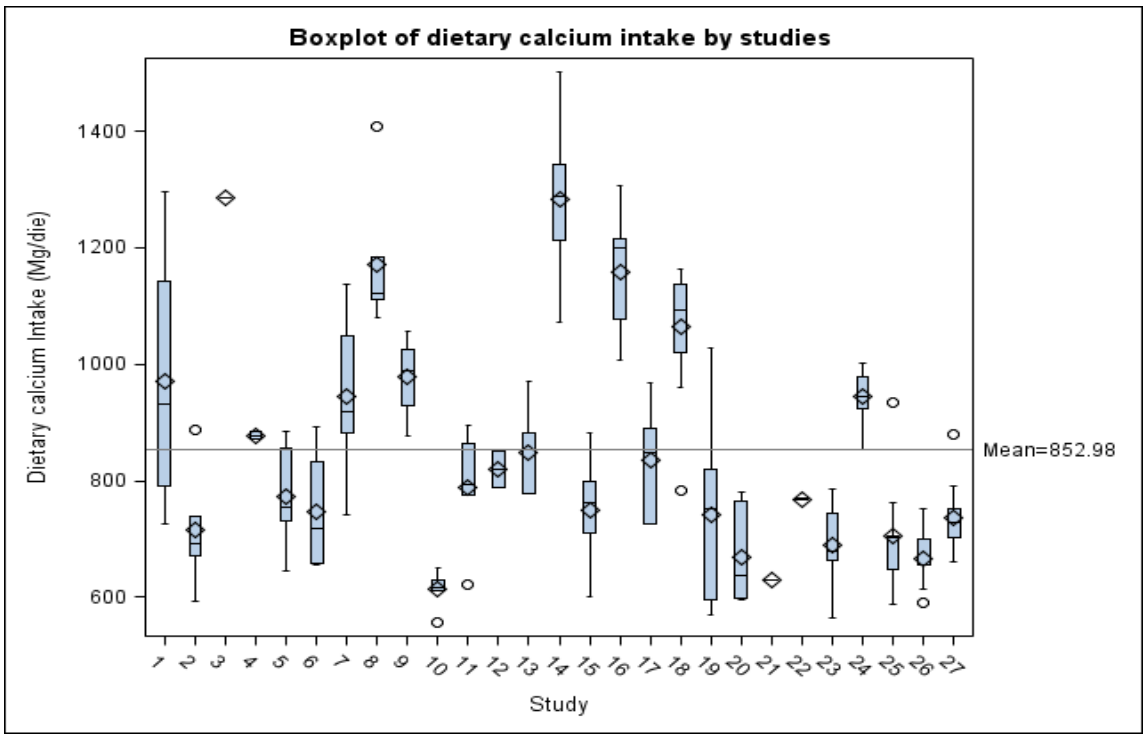


Figure 1: Boxplot of dietary calcium intake by study

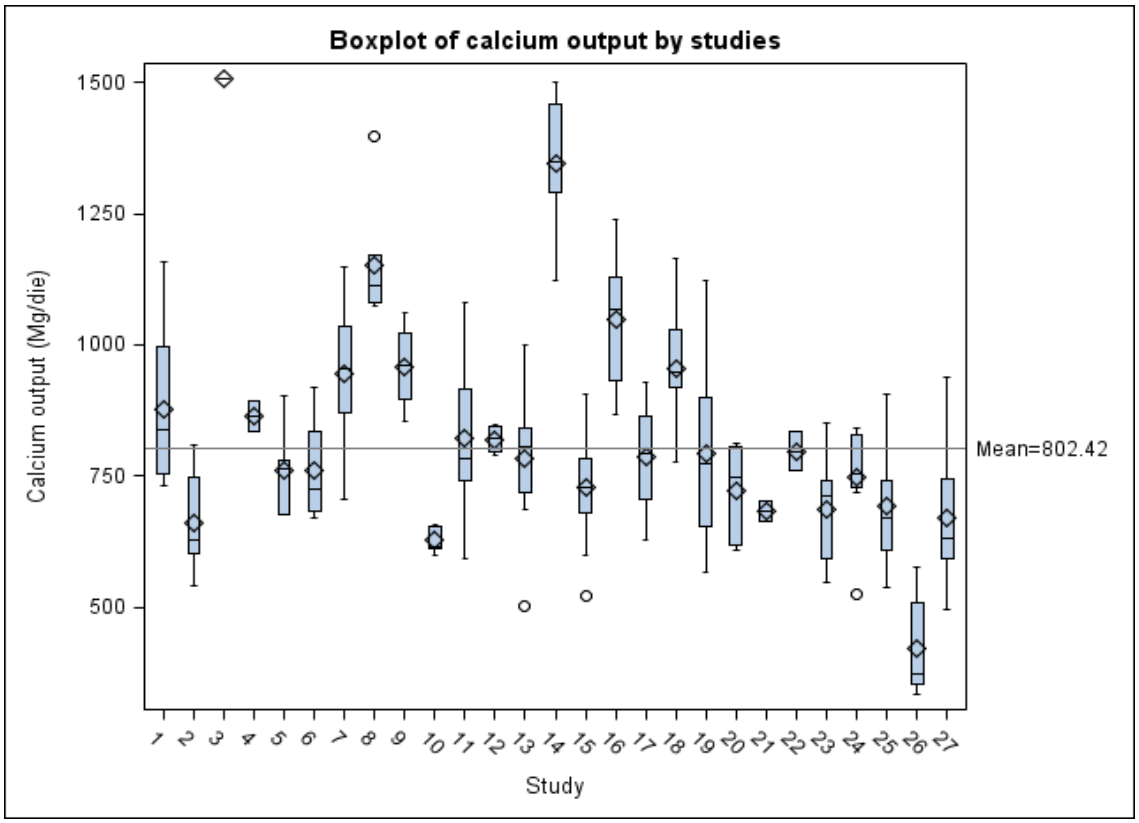


Figure 2: Boxplot of dietary calcium output by study

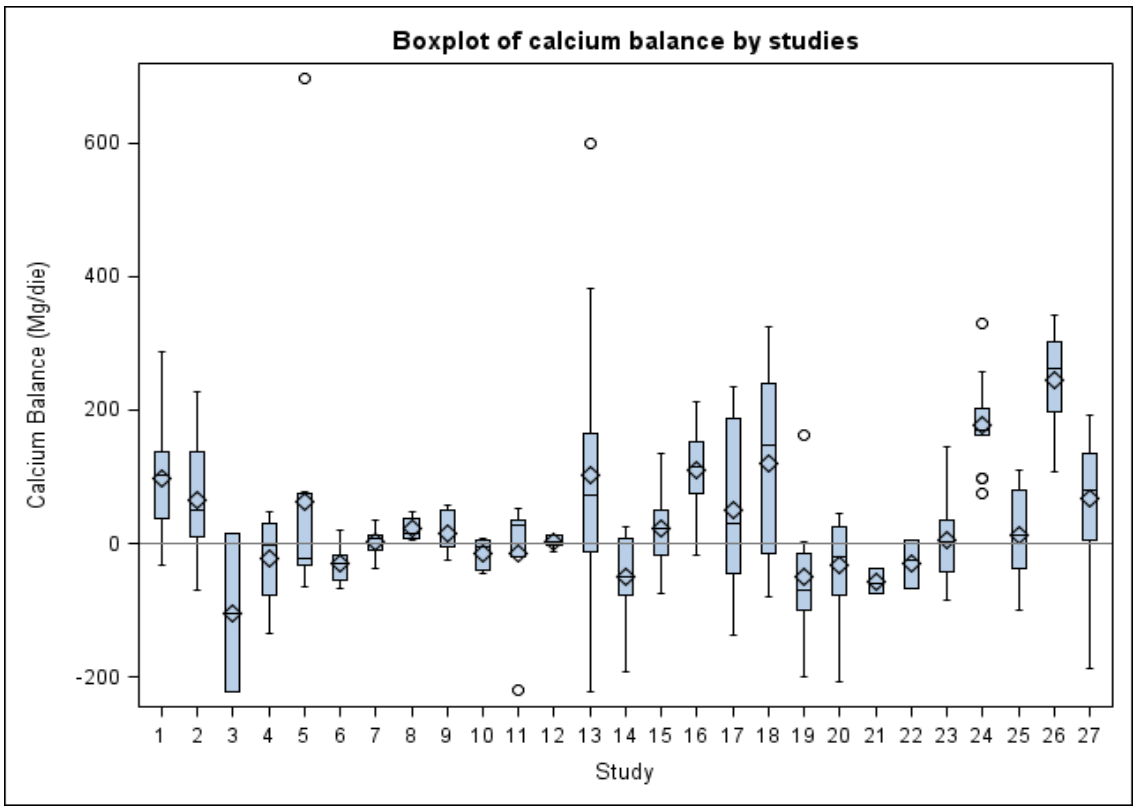


Figure 3: Boxplot of calcium balance by study

Eligibility criteria

Eligibility criteria were established in order to select studies and subjects within studies to include in the analysis to get representative results. The criteria reflect the relevance of the studies and subjects for the objective of the assessment.

It was deemed appropriate to exclude from the analysis:

- people younger than 25 years (people of 25 years and above are included);
- studies with a range of values for the average calcium balance (intake minus excretion) at the individual level not including the null value.

The exclusion of younger adults from the sample was motivated by the assumption that calcium is still being deposited in the bones after their growth has ceased; calcium accretion has been reported to continue until around 25 years in young men and women (Teegarden et al., 1995; Ohlsson et al., 2011; Darelid et al., 2012) or even later, depending on the bone site (Recker et al., 1992; Hui et al., 1999). Therefore, it was assumed that their calcium metabolism cannot be considered in a steady state whereas this was deemed to be the case for older adults (the sample includes individuals up to the age 81 years).

It was also assumed that in order to be representative of a population in a healthy status as for calcium metabolism, the range of the average individual values for calcium balance in a study should include zero (ideally the distribution of the calcium balance should be concentrated around a zero value).

Studies involving calcium supplementation (numbered 20 to 27) and excluded in the paper by Hunt and Johnson (2007) were considered in the analysis, provided that they fulfilled the previous criteria, despite the fact that no information was provided about the proportion of supplemental to total calcium intake. It was assumed that calcium metabolism (i.e. efficiency of absorption) is unaffected by the source of intake.

Both sexes were considered in order to evaluate whether the relationship between intake and excretion is sex-dependent.

Selection by age led to the exclusion of 46 individuals (12 females and 34 males). Therefore, the remaining sample was composed of 201 subjects in total (132 females and 69 males).

After the exclusion of people younger than 25 years, the distribution of the calcium balance (input minus output) in studies n. 3, 8, 21, 24 and 26 did not include the null value (see boxplot in Figure 4). Studies 24 and 26 also have median and mean values quite far from the null (around 200 mg/day) meaning that excretion was systematically below intake for the subjects involved. In both studies supplement use was allowed. Consistent with the pre-established eligibility criteria, the five studies (31 subjects in total, of which 21 females) were not included in the analysis on the assumption that they could not be considered representative of a population in a steady state for calcium metabolism. A total of 170 individuals (females and males) and 378 observations were considered for the final analysis.

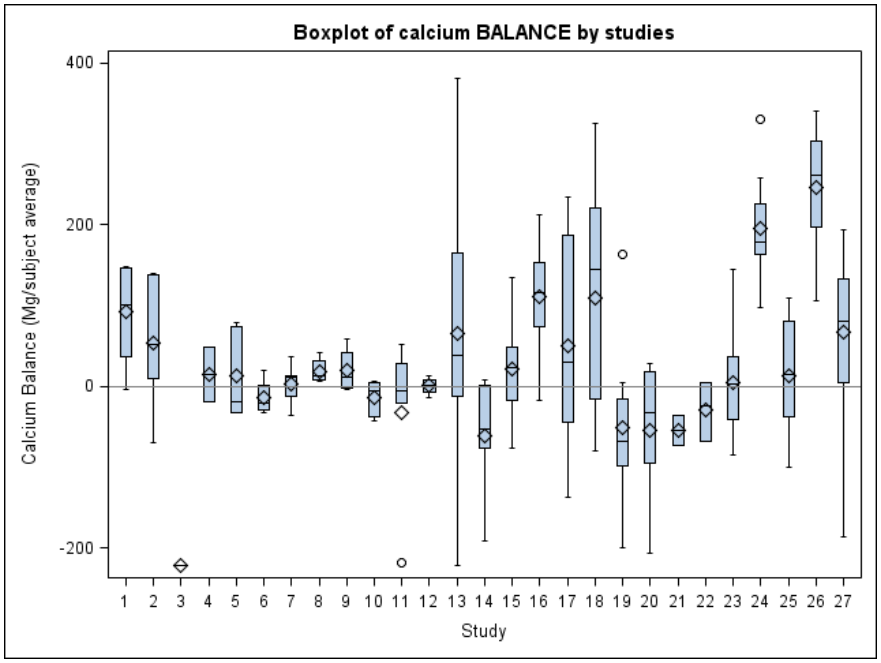


Figure 4: Boxplot of calcium balance by study after exclusion of younger subjects

Summary statistics of the final sample are reported in Table 12. For all the variables and measurements the mean is larger than the median indicating a positive skewness (i.e. the tendency of the distribution to deviating from the symmetry of a normal distribution, exhibiting with larger frequency values lower than the mean). The age range for the selected subjects is between 25 and 65 years for men and 25 and 81 years for women.

Table 12: Subjects younger than 25 years and studies 3, 8, 21, 24, 26 excluded: summary statistics of the main variables

Variables	n. of subjects	Min	Max	Median	Mean	Std dev
Calcium intake (mg/day)	170	556.5833	1501.667	777.857	835.7503	193.402
Calcium output (mg/day)	170	494.25	1500	781.4583	806.9923	191.8591
Balance* (mg/day)	170	-222.143	380.7143	11.8333	28.75797	96.88093
Calcium intake (mg/kg)	170	6.042383	21.95609	10.9200	11.43211	2.821512
Calcium output (mg/kg)	170	5.147166	19.72182	10.5818	11.05357	2.793955
Balance* (mg/kg)	170	-3.75036	5.090926	0.14978	0.37854	1.32219
Calcium intake (mg/kcal)	170	0.193282	0.550093	0.342957	0.344946	0.073938
Calcium output (mg/kcal)	170	0.167381	0.55037	0.323345	0.333778	0.075626
Balance* (mg/kcal)	170	-0.1	0.126905	0.00468	0.011169	0.038984
Body weight (kg)	170	45.925	133.1929	72.9208	74.8215	15.02533

*Balance computed as difference between the dietary calcium intake and the excretion

Data quality

Information about setting of the studies and methodology used to collect data (including laboratory techniques) can be found in the references provided by Hunt and Johnson for each individual study in their paper (2007). A description of the studies with calcium supplementation is provided in Table 12.

Table 13: Studies with calcium supplementation

Study n.	Study description	Reference
20	Copper intake: copper balance, absorption, and indicators of status	Milne (1990)
21	Zinc intake: whole-body surface loss of zinc	Canfield (1982)
22	Marginal zinc intakes: ethanol metabolism	Milne et al. (1987)
23	Aluminum, boron, and magnesium intakes: boron, calcium, and magnesium absorption and retention	Hunt et al. (1997)
24	Calcium and manganese intakes: menstrual cycle symptoms	Penland and Johnson (1993)
25	Boron and magnesium intakes: central nervous system activity	Nielsen (2004)
26	Magnesium intakes: magnesium status indicators	Nielsen (1990)
27	Magnesium intakes: neuronal function	No publication

One of the major strengths of the data is represented by the controlled setting in which individuals resided during the study period which reduced the confounding factors. As reported in the paper "the subjects consumed only and all foods, beverages (including water), and vitamin, mineral, or other supplements provided by the centre". On the other hand, since the study requirements for compliance were quite demanding (e.g. people had to stay most of their time in a confined environment for some months, consume only and all food provided by the centre and perform prescribed physical activity), individuals were selected on a voluntary basis. This could have introduced a bias in the sample selection in terms, for instance, of dietary consumption habits and life style before entering the study. Information on these aspects is missing in the dataset.

Similar considerations apply to the subjects and/or observations on the same subject that were considered not eligible by Hunt and Johnson (2007) excluded from the sample and not provided to EFSA. Although a rationale is provided by the authors to justify their choice, it was not possible to perform an independent evaluation of the opportunity to exclude subjects/observations and not even to assess the impact of the exclusion on the final estimates since a list of these subjects/observations was

not provided. They state that "data from a specific dietary period for an individual are excluded when intakes of magnesium, copper, iron, phosphorus or zinc fell below the respective EAR or exceeded the respective 99th percentiles of usual intakes from the 1994 Continuing Survey of Food Intakes by Individuals…. to avoid confounding the results with concurrent nutritional stress. To maximize the consistency in the data across individuals, balance periods < 6 or > 12 days in length were eliminated. To meet the design criteria suggested by the Food and Nutrition Board, the minimum dietary adaptation period was 12 days (median: 31 days, maximum: 109 days)".

Methods of analysis

Data processing

From a preliminary analysis of the data it appeared that seven subjects participated in two studies. Their mean calcium intake, excretion and balance were evaluated (Table 14) in order to decide which strategy to adopt to treat them (i.e. use as independent subjects, put their replicates together as coming from a single study, deleting replicates related to one of the two studies). Eventually it was decided to treat these subjects as if they were independent observations given the substantial differences observed on their calcium metabolism in the couple of studies they took part in. No formal tests were performed to compare measures obtained on subjects included in pairs of studies because of the limited number of observations available.

Table 14: Mean values of calcium intake, excretion and balance for subjects included in more than one study

Subject code	Study	Calcium intake (mg/day)	Calcium output (mg/day)	Calcium balance (mg/day)
210	5	855	781	74
210	7	883.25	871.25	12
545	2	680	670	10
545	4	872	891.75	-19.75
661	1	1143	997	146
661	2	671	629	42
705	4	883.75	835.5	48.25
705	20	596.5	691.75	-95.25
714	1	1296.83	1159.33	137.5
714	2	886.67	746.33	140.33
786	1	921.83	838.5	83.33
786	2	693	626.5	66.5
952	6	655	671.5	-16.5
952	7	742	706	36

Model formulation

A mixed linear model (Brown and Prescott, 1999) was used in order to investigate the association of calcium excretion to dietary calcium intake. Sex and body weight were considered as potential covariates that might have an effect on the output. Therefore, they were included in the model as well as the intake and tested for significance.

The same model was fitted to calcium intake and excretion expressed, respectively, as mg/day, mg/kg per day and mg/kcal per day.

Since the studies included in the analysis exhibited a level of heterogeneity in terms of experimental setting conditions, a graphical exploratory analysis was performed in order to evaluate the opportunity to incorporate a random factor explaining the variability component due to experimental design. Although regression lines over most of the studies overlapped, some of them showed deviations from

the overall trend (Figure 5). Therefore, it was decided to include this factor as a random component in the model and to evaluate formally whether its contribution to the variance explanation is statistically significant.

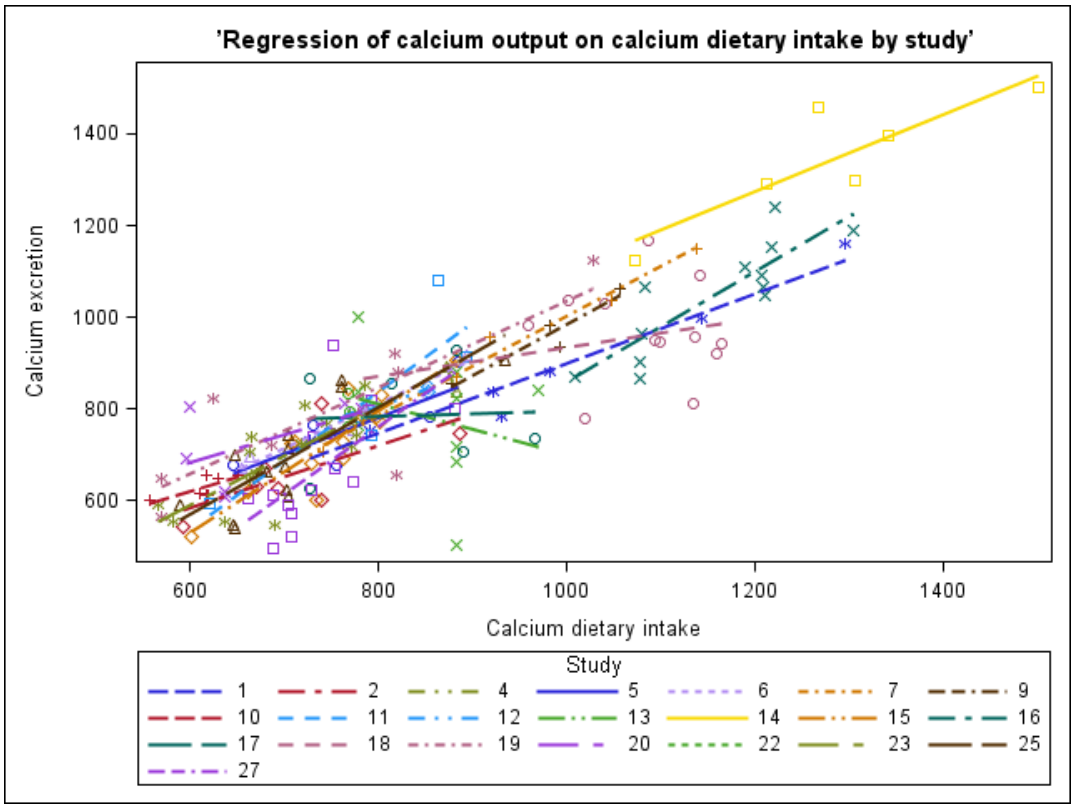


Figure 5: Regression of calcium output (mg/day) on dietary intake (mg/day) by studies

The form of the model is given in equation [1]

$$Y_{ij} = X_{ij}\beta + Z_{ij}\gamma + \varepsilon_{ij} \qquad [1]$$

Where

X_{ij} and Z_{ij} are design matrices for fixed and random factors on replicate j-th on individual i-th

β is the vector of fixed effects

γ is the vector of random effects with $\gamma \propto N(0,G)$

ε_i is the random error term on individual i-th with $\varepsilon \propto N(0,R)$ and $\text{cov}(\varepsilon,\gamma) = 0$

In addition the following assumptions hold for the components of the model

- $E(Y) = X\beta \qquad Var(Y) = ZGZ^t + R$
- G includes a covariance component to account for the correlation between subjects belonging to the same study
- R includes a covariance component to account for the correlation between observations taken on the same subject at different times.

The response variable is represented by calcium excretion (expressed as mg/day, mg/kg, mg/kcal). The fixed components, tested for inclusion in the model, include: dietary calcium intake (expressed as mg/day, mg/kg, mg/kcal), sex, age classes (between 25 and 50 years, above 50 years) and weight (in kg).

The random component of the model is represented by the study. Both the random factor and the error component include a covariance structure to account for the correlation between the couple of individuals participating in the same study, and the couple of observations taken on the same individual at different times.

Different covariance structures were investigated.

Various models have been tested in order to evaluate whether:

- The factors, sex, age classes and body weight, have to be included among fixed effects
- the inclusion of the random component (study) improves the fitting to the data (residual log-likelihood, the Akaike (AIC) and Bayesian (BIC) information criteria were used to compare different models);
- which structure of the covariance matrix has to be considered for the error component reflecting the correlation among replicates (unstructured [UN], compound symmetry [CS] and autocorrelation of the 1st order [AR(1)] were considered);
- which structure of the covariance matrix has to be considered for the random component (study) reflecting the correlation among individuals in the same study (unstructured [UN], and compound symmetry [CS] were considered)

The three possible structures of the error and random component are made explicit in the following:

$$UN = \begin{bmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{13} & \sigma_{13} & \sigma_{15} & \sigma_{16} \\ \sigma_{12} & \sigma_2^2 & \sigma_{23} & \dots & \dots & \dots \\ \sigma_{13} & \sigma_{23} & \sigma_i^2 & \dots & \dots & \dots \\ \sigma_{14} & \dots & \dots & \dots. & \dots & \dots \\ \sigma_{15} & \dots & \dots & \dots & \dots & \dots \\ \sigma_{16} & \dots & \dots & \dots & \dots & \sigma_n^2 \end{bmatrix}$$

$$AR(1) = \sigma^2 \begin{bmatrix} 1 & \rho & \rho^2 & \rho^3 & & \rho^5 \\ \rho & 1 & \rho & \rho^2 & & \rho^4 \\ \rho^2 & \rho & 1 & \rho & \rho^2 & \\ & \rho^2 & \rho & 1 & \rho & \rho^2 \\ & & \rho^2 & \rho & 1 & \rho \\ & & \rho^3 & \rho^2 & \rho & 1 \end{bmatrix}$$

$$CS = \begin{bmatrix} \sigma^2 + \sigma_1 & \sigma_1 & \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma^2 + \sigma_1 & \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma_1 & \sigma^2 + \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma_1 & \sigma_1 & \sigma^2 + \sigma_1 \end{bmatrix}$$

The most parsimonious structures in terms of the number of parameters to be estimated are the AR(1) and the CS. They request, though, stronger assumptions to be done with respect to the unstructured version of the matrix where no assumptions are needed. The ARIMA of the first order assumes that the correlation between a couple of replicated observations on the same subjects decreases with time. The compound symmetry structure requires the covariance between couple of replicates/individuals in the same study being the same irrespective of the time of observation/study membership.

Software

The SAS software version 9.3 for Windows 7 was used to process and analyse data. The output of the procedure MIXED was further processed modifying the code of Kunthel By (2005) for the estimation of Prediction Intervals. The detailed code is provided in the internal report provided by EFSA's Assessment and Methodological Support Unit (AMU).

Results

Calcium expressed as mg/day

Among those models for which convergence was met, the indicators for the fitting process are reported in Table 15.

Table 15: Model fit indicators

Model	Random component	Cov structure	-2 log	AIC	BIC
1	Random study int	Unstructured	4515.6	4559.6	4515.6
	Replicates	Unstructured			
2	Random study int	Unstructured	4560.4	4566.4	4560.4
	Replicates	Compound symmetry			
3	Random study int	Compound symmetry	4560.4	4568.4	4560.4
	Replicates	Compound symmetry			
4	Random study slope	Unstructured	4553.7	4559.7	4553.7
	Replicates	Compound symmetry			

AIC, Akaike information criterion; BIC, Bayesian information criterion

Model selection was performed aiming at a parsimonious (minimum parameters) well-fitting models (smallest values for fit indicators) for the response being measured. Therefore the model 4 requesting less number of parameters to be estimated was chosen, although its goodness of fit was slightly lower with respect to model 1.

Based on the statistical analysis, age, sex and body weight came out to be not relevant factors in explaining the variability of the calcium excretion once dietary intake is considered (results presented only for the selected model, see Table 16). Therefore, they were removed from the final model that contained ultimately only the dietary intake as explanatory variable.

Table 16: Fixed parameter estimates

TYPE	PARMS	STDERR	T	PVALUE	L95B	U95B
Intercept	156.28	50.8426	3.07	0.0025	55.8981	256.65
Calcium input	0.7469	0.05141	14.53	<.0001	0.6455	0.8482
Sex (F)	-33.7790	26.2713	-1.29	0.2003	-85.6457	18.0877
Age (2)	-2.3737	20.6812	-0.11	0.9088	-43.2040	38.4566
Weight	0.7076	0.5436	1.30	0.1945	-0.3642	1.7793

All the components of the variance-covariance matrix ended up to be statistically significant, confirming the need to keep them in the model (Table 17).

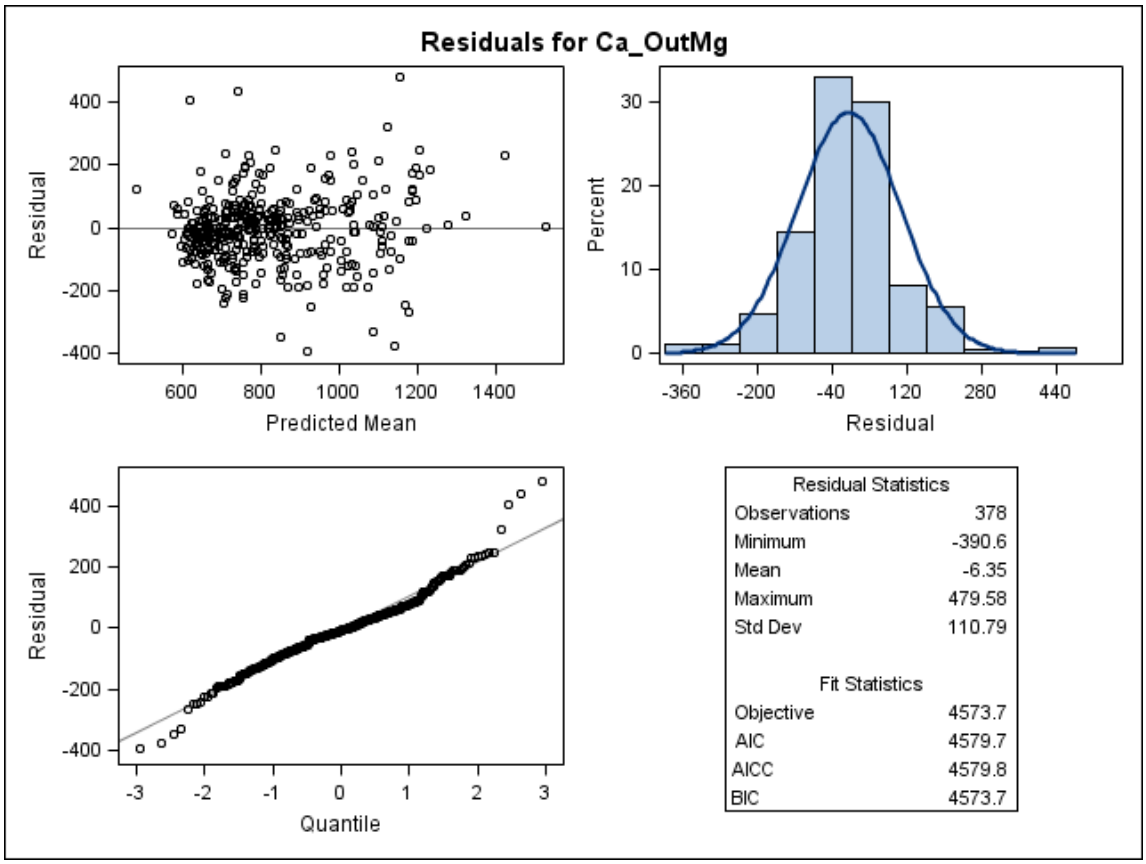
2627 Table 17: Variance/covariance estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
UN(1,1)	Study	0.002857	0.001376	2.08	0.0189
CS	Subject	2138.92	796.19	2.69	0.0072
Residual		8168.93	787.28	10.38	<.0001

2628 **Diagnostic analysis – outlier detection and test for normality and homoschedasticity**

2629 Prior to further statistical analysis, the data were culled for outliers and influential points defined by
2630 Externally Studentized Residual greater than 3 in absolute value. The identified points are those that
2631 are not well fitted by the selected model.

2632 The diagnostic tests performed on the data (including graphical check for normality and
2633 homoschedasticity) are presented in Figure 6.



2634

2635 **Figure 6:** Diagnostic plot for assessing normality and homoschedasticity

2636 Six outliers were identified and eventually removed from the analysis (Table 18). For these replicated
2637 observations the balance values were not corresponding to the expected null balance and quite extreme
2638 with respect to the overall distribution (365 mg/day on a replicate or greater in absolute value). The
2639 final sample included one subject less than the original dataset (169 of which 110 women and 59 men)
2640 and 372 observations in total (229 for females and 143 for males).

Table 18: Outliers and their characteristics

Study	Subject	Repl	Sex	Age	weight_m	CAL_IN_m	CAL_OUT_m	CAL_diff_m
13	791	1	F	38	97.55714	882.8571	502.1429	380.7143
14	523	1	M	27	79.21667	1266.667	1631.667	-365
18	529	4	M	25	64.25833	967.5	525.8333	441.6667
18	762	3	M	27	69.4	1251.667	765	486.6667
20	779	2	M	25	72.675	586.5	1022.5	-436
27	279	2	F	57	67.85	742	1175	-433

Model outcomes

After removal of the outliers the final fit of the model and estimation of the parameters was performed. Results are shown in Table 19.

Table 19: Fixed parameter estimates

TYPE	PARMS	STDERR	T	PVALUE	L95B	U95B
Intercept	140.41	33.3707	4.21	<.0001	74.5337	206.29
Calcium input	0.8036	0.04142	19.40	<.0001	0.7219	0.8852

Again all the components of the variance-covariance matrix ended up to be significant (as reported in Table 20), confirming the need to keep them into the model.

Table 20: Random component estimates

Cov Parm	Subject	Estimate	Standard Error	Z Value	Pr Z
UN(1,1)	Study	0.002090	0.000940	2.22	0.0131
CS	CODE	1517.28	618.85	2.45	0.0142
Residual		6598.99	643.95	10.25	<.0001

The fit of the model is further improved as indicated by the goodness of fit indicators (Table 21) and the overall null model likelihood ratio test (Table 22).

Table 21: Goodness of fit

Model	Random component	Cov structure	-2 log	AIC	BIC
1	Random study int	Unstructured	4414.2	4420.2	4414.2
	Replicates	Compound Symmetry			

AIC, Akaike information criterion; BIC, Bayesian information criterion

Table 22: Null model likelihood ratio test

DF	Chi_square	Pr
2	52.10	<.0001

Computation of the AR and PRI

The AR represents the level of intake that is adequate for half of the people in a population group. The purpose of this work is to estimate the AR for dietary calcium intake to which a null balance is expected at the population level. Therefore, it is straightforward to estimate it as the mean value estimated by the model at the level where calcium intake and excretion equal. A mean value of 715 mg/day was estimated (Table 23).

The PRI is defined as the level of intake that is adequate for 97.5 % of people in a population group. This parameter is naturally estimated via the upper bound of the Prediction interval at the level corresponding to a null balance for the population mean. The 95 % marginal prediction interval is the estimated range of the individual values in a population provided by the model with 95 % confidence (blue dotted lines in Figure 7) at the population average random effects. Its upper bound represents the 97.5 percentile of the distribution of the individual predictions for each level of the predictor (dietary calcium intake). As indicated in Figure 7 this prediction interval upper bound at the level of calcium null balance for the population mean is equal to 904 mg/day.

Table 23: Estimated calcium Average Requirement

Estimated mean at null balance (mg/day)	Lower bound of prediction interval of estimated mean at null balance (mg/day)	Upper bound of prediction interval of estimated mean at null balance (mg/day)
715	525	904

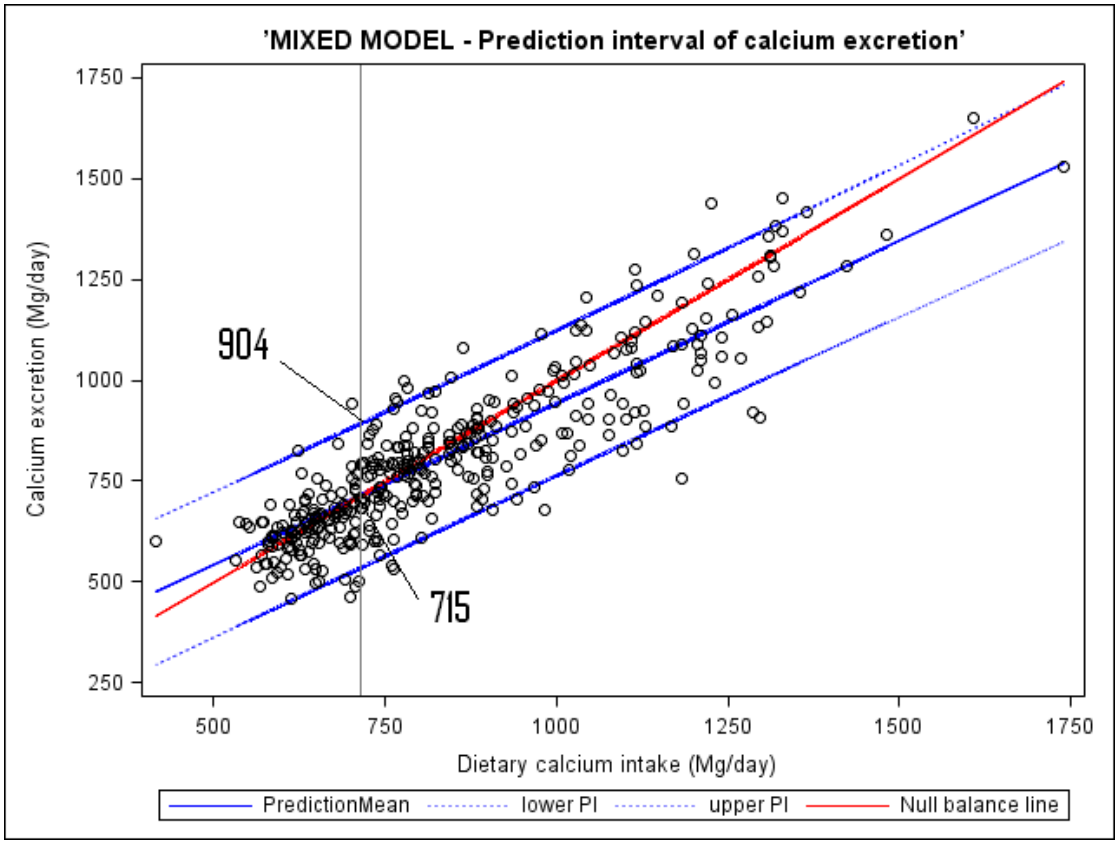


Figure 7: Individual prediction interval for the calcium excretion model

It is worth noting that the estimated relationship between dietary calcium intake and excretion provides predicted values for the calcium output that are systematically above the intake when the intake is low and vice-versa. This trend of the model implies a prediction of a negative balance when the calcium intake is low and a positive one when the calcium intake is higher. As regards the biological plausibility of this pattern the NDA Panel concluded that when intakes are very low or high, there are homeostatic adaptations (changes in absorption and in losses). Therefore, although the model predicts this, the data are not taken from extremely low or high calcium intakes, and consequently the adaptation cannot be incorporated into the model.

2679 **Sources of uncertainty and their potential impact on the final estimates**

2680 The model used to set up the AR and PRI relies on some assumptions about the structure of the model
2681 in terms of the types of factors to be included (fixed and random), and structure of the
2682 variance/covariance matrix. The structure of the variance/covariance model represents a way to
2683 account for the variability in the phenomenon. Nonetheless they are also sources of uncertainty that
2684 can influence the final results. Indeed the structure of the model determines the size of the estimated
2685 interval estimates and consequently their upper bounds. Different choices could lead to different
2686 results. If the model had not random error, the prediction interval would simply account for the natural
2687 variability existing in the reference population among individuals. Similar considerations also apply to
2688 the factors included in the model (Table 24).

2689 Table 24: Sources of uncertainty and their effect on the outcome

Outcome	**Source of uncertainty**	**Direction of the effect on the outcome**
Estimates of the dietary calcium intake and calcium excretion	Lack of information about: • exclusion of some replicates/subjects from the dataset; • contribution of supplemental calcium to the total intake not given in calcium supplement studies It is assumed that dietary calcium intake and calcium supplements are metabolised similarly	It is difficult to evaluate the impact of this on the estimate of dietary calcium intake and excretion. Nonetheless the explanations provided by the authors for exclusion indicate that these subjects had extreme intakes for minerals raising doubts about their representativeness of a healthy adult population. It is difficult to predict what could be the impact of this exclusion on the AR and PRI since being extreme does not necessary implies being outliers. If assumption on the supplemented calcium metabolism is not correct results could be not representative of calcium dietary intake
Representativeness of the healthy European adult population	Individuals were volunteers and involved in studies with varying objectives, not studying calcium balance *per se*. In addition the studies date back to the 1980s. The representativeness of the sample in terms of aspects that might impact on calcium metabolism other than dietary calcium intake was not assessed	The range of values for the dietary calcium intake and excretion was considered by the WG representing well the situation in EU. No conclusions have been drawn with regard to the representativeness of dietary consumption pattern, age and gender composition. Due to the lack of information it is difficult to predict what could be the direction of these sources of uncertainty on the finale estimates.
Estimate of excretion	No measurements were made of sweat losses in the metabolic studies. The type and amount of physical exercise, considerably varied between individuals, and was not included in the information provided to EFSA	The calcium excretion used in the model is predicted to be underestimated. This underestimate would depend on the activity done by the subjects during the study period. However Hunt & Johnson refer to unpublished data estimating the calcium excretion via sources other than faces and urines and evaluate the collective level of excretion from these sources as negligible.
Estimate of AR and PRI	Use of a point estimate resulting	The use of a point value makes the

Outcome	Source of uncertainty	Direction of the effect on the outcome
	from the intercept of the line of null balance with the predicted mean and the upper bound of the prediction interval.	results sensitive to any change in the parameters estimate (intercept and slope) and their variability in the sample. Inclusion/exclusion of some replicates/units could in principle also lead to different estimates for AR and PRI. It is difficult to predict in which direction this uncertainty could affect the final results. It is true though that in a healthy population it is expected that the relationship between dietary calcium intake and excretion should be close to 1. The more the slope of the model goes to 1 the larger the upper bound of the prediction interval becomes. In principle the effect of the uncertainty could be a slight underestimation of the dietary intake corresponding to null balance. It is re-assuring though that the estimate of the slope is already not far from 1 and the fitness of the model quite good. There is a need to cumulate more data of this kind in the future in order to make predictions at the individual level more robust.

2690

2691 **ABBREVIATIONS**

Afssa	Agence française de sécurité sanitaire des aliments
AI	Adequate Intake
AR	Average Requirement
BMC	Bone mineral content
BMD	Bone mineral density
CaBP	Calcium binding protein, calbindin
CaSR	Calcium-sensing receptor
CI	Confidence interval
COMA	Committee on Medical Aspects of Food Policy
CV	Coefficient of variation
D-A-CH	Deutschland-Austria-Confoederatio Helvetica
DoH	Department of Health
DBP	Diastolic blood pressure
DRV	Dietary Reference Value
DXA	Dual-energy X-ray absorptiometry
EAR	Estimated Average Requirement
EU	European Union
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
IOM	U.S. Institute of Medicine of the National Academy of Sciences
NNR	Nordic Nutrition Recommendations
PBM	Peak bone mass
PRI	Population Reference Intake
PTH	Parathyroid hormone
RDA	Recommended Dietary Allowance
RNI	Reference Nutrient Intake
SBP	Systolic blood pressure

SCF	Scientific Committee for Food
SD	Standard deviation
UNU	United Nations University
VDR	Vitamin D receptor
WHO	World Health Organization

2692