10

Nutritional Aspects of Minerals in Bovine and Human Milks

C.D. Hunt and F.H. Nielsen

10.1. Introduction

This review summarizes the nutritional aspects of the 21 mineral elements present in bovine and human milks and considered essential or beneficial for human health (Table 10.1). This includes discussion of their respective physiological roles, signs of deficiency and toxicity, current recommended intakes, chemical presence as compounds that affect bioavailability and utilization and known enhancers and/or inhibitors of their absorption. The term "mineral" is not an accurate descriptor for the chemical nature of some of these elements but is a widely accepted terminology in the field of nutrition. The mineral elements occur in the body in one or more chemical forms, including inorganic ions and salts, complexes or constituents of organic molecules.

Fourteen of the minerals present in bovine and human milks (calcium, chloride, cobalt, copper, iodine, iron, magnesium, manganese, molybdenum, sodium, phosphorus, potassium, selenium and zinc) have well-established essential physiological functions that range from structural components of body tissues to essential components of many enzymes and other biologically important molecules. Another seven minerals (arsenic, boron, chromium, fluorine [as fluoride], nickel, silicon and vanadium) are not considered essential but may be beneficial, based on the evidence that they have a role in some physiological processes in one or more mammalian species.

C.D. Hunt and F.H. Nielsen • US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, P.O. Box 9034, Grand Forks, ND, USA, 58202-9034.

Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents.
Edited by P.L.H. McSweeney and P.F. Fox, DOI 10.1007/978-0-387-84865-5_10,

Table 10.1. Mean and/or range of concentration (per L) of minerals in mature human milk, bovine milk and humanized infant formulae

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
Arsenic (μg)	0.2–0.6	(Anke, 1986)	20–60	(Anke, 1986)		
Boron (μg)	28–30	(Hunt *et al.*, 2004)	280	(Hunt and Meacham, 2001)	120[a]	(Hunt and Meacham, 2001)
	30–50	(Hunt *et al.*, 2005)				
Calcium (mg)	279	(Fransson and Lonnerdal, 1983)	1060	(Pennington *et al.*, 1987)	560[a]	(Hunt and Meacham, 2001)
	294	(Butte *et al.*, 1987)	1130	(Hunt and Meacham, 2001)	510[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	250	(Yamawaki *et al.*, 2005)	1043–1283	(Gaucheron, 2005)		
	268–281	(Hunt *et al.*, 2005)				
	250	(Mastroeni *et al.*, 2006)				
Chloride (mg)	359	(Yamawaki *et al.*, 2005)	772–1207	(Gaucheron, 2005)		
Chromium (ng)	250	(Casey *et al.*, 1985)	<500	(Anderson *et al.*, 1992)		
Cobalt (μg)	0–6	(Friel *et al.*, 1999)				

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
Copper (μg)	443	(Alkanani *et al.*, 1994)	100	(Fransson and Lonnerdal, 1983)	620[a]	(Hunt and Meacham, 2001)
	400–710	(Al-Awadi and Srikumar, 2000)	90	(Pennington *et al.*, 1987)	500	(US Department of Agriculture Agricultural Research Service, 2007)
	360–650	(Hunt *et al.*, 2004)	90	(Hunt and Meacham, 2001)		
	120–160	(Domellof *et al.*, 2004)				
	350	(Yamawaki *et al.*, 2005)				
Fluoride (μg)	19	(Koparal *et al.*, 2000)	22	(Koparal *et al.*, 2000)	76–1053	(Buzalaf *et al.*, 2004)
	17	(Chuckpaiwong *et al.*, 2000)	80	(Atac *et al.*, 2001)	21–118	(Koparal *et al.*, 2000)
					101	(Atac *et al.*, 2001)
Iodine (μg)	142; 21–281	(Bruhn and Franke, 1983)	178; 48–661	(Bader *et al.*, 2005)		
	50–60	(Parr *et al.*, 1991)	140–195	(Li *et al.*, 2006)		
	94	(Bazrafshan *et al.*, 2005)				

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
	22	(Skeaff *et al.*, 2005)				
	169; 33–348	(Bader *et al.*, 2005)				
	155 (median); 27–1968	(Pearce *et al.*, 2007)				
Iron (μg)	308; 242–160	(Butte *et al.*, 1987)	290	(Fransson and Lonnerdal, 1983)	11700[a]	(Hunt and Meacham, 2001)
	270–430	(Al-Awadi and Srikumar, 2000)	700	(Pennington *et al.*, 1987)	4300[b]	(Hunt and Meacham, 2001)
	225–355	(Hunt *et al.*, 2004)	200	(Hunt and Meacham, 2001)	11800[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	210–290	(Domellof *et al.*, 2004)				
	900	(Mastroeni *et al.*, 2006)				
Manganese (μg)	4–6	(Al-Awadi and Srikumar, 2000)	40	(Pennington *et al.*, 1987)	70[a]	(Hunt and Meacham, 2001)
	6		20		100[c]	

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
		(Alkanani *et al.*, 1994)		(Hunt and Meacham, 2001)		(US Department of Agriculture Agricultural Research Service, 2007)
	11	(Yamawaki *et al.*, 2005)				
Magnesium (mg)	35	(Fransson and Lonnerdal, 1983)	117	(Fransson and Lonnerdal, 1983)	50[a]	(Hunt and Meacham, 2001)
	30	(Butte *et al.*, 1987)	98	(Pennington *et al.*, 1987)	50[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	27	(Yamawaki *et al.*, 2005)	97–146	(Gaucheron, 2005)		
	29–33	(Hunt *et al.*, 2005)				
	30	(Mastroeni *et al.*, 2006)				
Molybdenum (μg)	5	(Alkanani *et al.*, 1994)	97	(Hunt and Meacham, 2001)	55[a]	(Hunt and Meacham, 2001)
	0–4	(Friel *et al.*, 1999)				
Nickel (μg)	16	(Alkanani *et al.*, 1994)				

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
	0–28	(Friel *et al.*, 1999)				
	11–16	(Parr *et al.*, 1991)				
Phosphorus (mg)	153	(Butte *et al.*, 1984)	830	(Pennington *et al.*, 1987)	440[a]	(Hunt and Meacham, 2001)
	150	(Yamawaki *et al.*, 2005)	880	(Hunt and Meacham, 2001)	350[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	137	(Mastroeni *et al.*, 2006)	930–992	(Gaucheron, 2005)		
Potassium (mg)	430–527	(Dewey and Lonnerdal, 1983)	1340	(Pennington *et al.*, 1987)	820[a]	(Hunt and Meacham, 2001)
	443	(Butte *et al.*, 1987)	1480	(Hunt and Meacham, 2001)	710[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	470	(Yamawaki *et al.*, 2005)				
	462	(Mastroeni *et al.*, 2006)				
Selenium (μg)	12–20	(Arnaud *et al.*, 1993)	17–23	(al-Saleh *et al.*, 1997)	18[c]	(US Department of Agriculture Agricultural Research Service, 2007)

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
	112 (seleniferous region)	(Bratter *et al.*, 1997)	22	(Muniz-Naveiro *et al.*, 2005)		
	14	(Bianchi *et al.*, 1999)	19	(Juniper *et al.*, 2006)		
	19–27	(Hunt *et al.*, 2004)				
	17	(Yamawaki *et al.*, 2005)				
Sodium (mg)	134–264	(Dewey and Lonnerdal, 1983)	420	(Pennington *et al.*, 1987)	170[a]	(Hunt and Meacham, 2001)
	168	(Butte *et al.*, 1984)	350	(Hunt and Meacham, 2001)	200[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	112	(Butte *et al.*, 1987)	391–644	(Gaucheron, 2005)		
	135	(Yamawaki *et al.*, 2005)				
	205	(Mastroeni *et al.*, 2006)				
Zinc (mg)	0.46–0.70	(Domellof *et al.*, 2004)	3.50	(Fransson and Lonnerdal, 1983)	5.70[a]	(Hunt and Meacham, 2001)

(*Continued*)

Table 10.1. (*Continued*)

Mineral	Mature human milk, term pregnancy; ≤12 wk postpartum	Reference	Bovine milk	Reference	Humanized infant formula	Reference
	1.19–4.06	(Hunt *et al.*, 2004)	3.70	(Pennington *et al.*, 1987)	6.6[c]	(US Department of Agriculture Agricultural Research Service, 2007)
	1.45	(Yamawaki *et al.*, 2005)	3.90	(Hunt and Meacham, 2001)		
	1.00–2.30	(Hunt *et al.*, 2005)				
	1.50	(Mastroeni *et al.*, 2006)				

[a] Infant formula, Gerber, with iron, ready-to-feed
[b] Infant formula, Gerber, iron low, ready-to-feed
[c] Infant formula, Mead Johnson, Enfamil Lipil, with iron, ready-to-feed, arachidonic acid (ARA) and docosahexaenoic (DHA) acid.

Several other mineral elements (aluminum, bromine, cadmium, cesium, lead, lithium, strontium, mercury, silver, rubidium and tin) are present normally in very low amounts in bovine and human milks. They are considered important because of their high orders of toxicity but are not reviewed here because of the lack of compelling evidence for their beneficial functions. All essential or beneficial minerals are toxic when ingested in excess. Therefore, an upper limit (UL) of intake has been established for each (Table 10.2). Because individual ULs are generally well above the total amounts supplied by typical diets, the toxicological aspects of each mineral are discussed only when they are considered relevant.

Human milk and bovine milk are markedly different in several aspects, including mineral content (Table 10.1). Furthermore, the bioavailability of most minerals in human milk is much higher than from bovine milk or infant formula (Emmett and Rogers, 1997). Frequent revision of humanized infant formulae to match the nutritive value of human milk complicates comparisons and notable current differences in content or bioavailability are identified in the discussion. This discussion focuses on mature human milk which has a mineral content considerably different from that of early and transitional human milks. In human milk, the concentrations of most minerals remain fairly constant throughout the course of lactation. Notable exceptions are zinc, copper and iron, but even these elements have predictable patterns of change over time, falling for several months after parturition (Emmett and Rogers, 1997).

Mineral concentrations in human milk are resistant to changes in maternal dietary mineral intakes (except for selenium; Kumpulainen *et al*., 1985), age, parity or lactational history (Butte *et al*., 1987). Differences in mineral composition of milk between term and preterm mothers are not universal and the lack of agreement among investigators may be due to differences in sample collection, a wide range of gestational ages and greater individual variation in the composition of milk from mothers of premature infants (Friel *et al*., 1999). Reported values for the concentration of a given mineral in milk among laboratories are remarkably consistent (Table 10.1). Although each mineral is discussed separately, interactions of minerals with each other, with other constituents of milk and with other food constituents are considered to be of paramount importance.

10.2. Sodium, Chloride and Potassium

The cation sodium and the anion chloride are required to maintain extracellular volume and plasma osmolarity. Human populations have the capacity to survive at extremes of sodium intake from less than 0.2 g (10 mmol)/d by the

Table 10.2. Dietary reference intake values by life-stage group for the United States and Canada for arsenic, boron, calcium, chloride, chromium, cobalt, copper, fluoride, iodine, iron, magnesium, manganese, molybdenum, nickel, sodium, phosphorus, potassium, selenium, vanadium and zinc

Age/sex	As (mg/d)	B (mg/d)	Ca (mg/d)	Cl^- (g/d)	Cr (μg/d)	Cu (μg/d)	F^- (mg/d)	I (μg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Mo (μg/d)	Ni (mg/d)	Na (g/d)	P (mg/d)	K (g/d)	Se (μg/d)	V (mg/d)	Zn (mg/d)
Recommended dietary allowance (RDA)																			
0–6 month	ND[1]	ND	210, AI[2]	0.18, AI	0.2, AI	200, AI	0.01, AI	110, AI	0.27, AI	30, AI	0.003, AI	2, AI	ND	0.12, AI	100, AI	0.4, AI	15, AI	ND	2, AI
7–12 month	ND	ND	270, AI	0.57, AI	5.5, AI	220, AI	0.5, AI	130, AI	11	75, AI	0.6, AI	3, AI	ND	0.37, AI	275, AI	0.7, AI	20, AI	ND	3
1–3 yr	ND	ND	500, AI	1.5, AI	11, AI	340	0.7, AI	90	7	80	1.2, AI	17	ND	1.0, AI	460	3.0, AI	20	ND	3
4–8 yr	ND	ND	800, AI	1.9, AI	15, AI	440	1, AI	90	10	130	1.5, AI	22	ND	1.2, AI	500	3.8, AI	30	ND	5
9–13 yr; M	ND	ND	1300, AI	2.3, AI	25, AI	700	2, AI	120	8	240	1.9, AI	34	ND	1.5, AI	1250	4.5, AI	40	ND	8
9–13 yr; F	ND	ND	1300, AI	2.3, AI	21, AI	700	2, AI	120	8	240	1.6, AI	34	ND	1.5, AI	1250	4.5, AI	40	ND	8
14–18 yr; M	ND	ND	1300, AI	2.3, AI	35, AI	890	3, AI	150	11	410	2.2, AI	43	ND	1.5, AI	1250	4.7, AI	55	ND	11
14–18 yr; F	ND	ND	1300, AI	2.3, AI	24, AI	890	3, AI	150	15	360	1.6, AI	43	ND	1.5, AI	1250	4.7, AI	55	ND	9
19–30 yr; M	ND	ND	1000, AI	2.3, AI	35, AI	900	4, AI	150	8	400	2.3, AI	45	ND	1.5, AI	700	4.7, AI	55	ND	11
19–30 yr; F	ND	ND	1000, AI	2.3, AI	25, AI	900	3, AI	150	18	310	1.8, AI	45	ND	1.5, AI	700	4.7, AI	55	ND	8
31–50 yr; M	ND	ND	1000, AI	2.3, AI	35, AI	900	4, AI	150	8	420	2.3, AI	45	ND	1.5, AI	700	4.7, AI	55	ND	11
31–50 yr; F	ND	ND	1000, AI	2.3, AI	25, AI	900	3, AI	150	18	320	1.8, AI	45	ND	1.5, AI	700	4.7, AI	55	ND	8
51–70 yr; M	ND	ND	1200, AI	2.0, AI	30, AI	900	4, AI	150	8	420	2.3, AI	45	ND	1.3, AI	700	4.7, AI	55	ND	11

(*Continued*)

Table 10.2. (*Continued*)

Age/sex	As (mg/d)	B (mg/d)	Ca (mg/d)	Cl^- (g/d)	Cr (μg/d)	Cu (μg/d)	F^- (mg/d)	I (μg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Mo (μg/d)	Ni (mg/d)	Na (g/d)	P (mg/d)	K (g/d)	Se (μg/d)	V (mg/d)	Zn (mg/d)
51–70 yr; F	ND	ND	1200, AI	2.0, AI	20, AI	900	3, AI	150	8	320	1.8, AI	45	ND	1.3, AI	700	4.7, AI	55	ND	8
>70 yr; M	ND	ND	1200, AI	1.8, AI	30, AI	900	4, AI	150	8	420	2.3, AI	45	ND	1.2, AI	700	4.7, AI	55	ND	11
>70 yr; M	ND	ND	1200, AI	1.8, AI	20, AI	900	3, AI	150	8	320	1.8, AI	45	ND	1.2, AI	700	4.7, AI	55	ND	8
Pregnancy																			
14–18 yr	ND	ND	1300, AI	2.3, AI	29, AI	1000	3, AI	220	27	400	2.0, AI	50	ND	1.5, AI	1250	4.7, AI	60	ND	13
19–30 yr	ND	ND	1000, AI	2.3, AI	30, AI	1000	3, AI	220	27	350	2.0, AI	50	ND	1.5, AI	700	4.7, AI	60	ND	11
31–50 yr	ND	ND	1000, AI	2.3, AI	30, AI	1000	3, AI	220	27	360	2.0, AI	50	ND	1.5, AI	700	4.7, AI	60	ND	11
Lactation																			
14–18 yr	ND	ND	1300, AI	2.3, AI	44, AI	1300	3, AI	290	10	360	2.6, AI	50	ND	1.5, AI	1250	5.1, AI	70	ND	14
19–30 yr	ND	ND	1000, AI	2.3, AI	45, AI	1300	3, AI	290	9	310	2.6, AI	50	ND	1.5, AI	700	5.1, AI	70	ND	12
31–50 yr	ND	ND	1000, AI	2.3, AI	45, AI	1300	3, AI	290	9	320	2.6, AI	50	ND	1.5, AI	700	5.1, AI	70	ND	12
Upper limit (UL)																			
0–6 month	ND	ND	ND	ND	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	ND	ND	45	ND	4
7–12 month	ND	ND	ND	ND	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	ND	ND	60	ND	5
1–3 yr	ND	3	2.5	2.3	ND	1000	1.3	200	40	65	2	300	0.2	1.5	3000	ND	90	ND	7
4–8 yr	ND	6	2.5	2.9	ND	3000	2.2	300	40	110	3	600	0.3	1.9	3000	ND	150	ND	12
9–13 yr	ND	11	2.5	3.4	ND	5000	10	600	40	350	6	1100	0.6	2.2	4000	ND	280	ND	23
14–18 yr	ND	17	2.5	3.6	ND	8000	10	900	45	350	9	1700	1.0	2.3	4000	ND	400	ND	34
19–70 yr	ND	20	2.5	3.6	ND	10000	10	1100	45	350	11	2000	1.0	2.3	4000	ND	400	1.8	40
>70 yr	ND	20	2.5	3.6	ND	10000	10	1100	45	350	11	2000	1.0	2.3	3000	ND	400	1.8	40

(*Continued*)

Table 10.2. (*Continued*)

Age/sex	As (mg/d)	B (mg/d)	Ca (mg/d)	Cl^- (g/d)	Cr (μg/d)	Cu (μg/d)	F^- (mg/d)	I (μg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Mo (μg/d)	Ni (mg/d)	Na (g/d)	P (mg/d)	K (g/d)	Se (μg/d)	V (mg/d)	Zn (mg/d)
Pregnancy																			
14–18 yr	ND	17	2.5	3.6	ND	8000	10	900	45	350	9	1700	1.0	2.3	3500	ND	400	ND	34
19–50 yr	ND	20	2.5	3.6	ND	10000	10	1100	45	350	11	2000	1.0	2.3	3500	ND	400	ND	40
Lactation																			
14–18 yr	ND	17	2.5	3.6	ND	8000	10	900	45	350	9	1700	1.0	2.3	4000	ND	400	ND	34
19–50 yr	ND	20	2.5	3.6	ND	10000	10	1100	45	350	11	2000	1.0	2.3	4000	ND	400	ND	40

[1] ND, not determinable because of lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

[2] AI, adequate intake was established because of insufficient data to establish an RDA.

Yanomamo Indians of Brazil to over 10.3 g (450 mmol)/d in Northern Japan (Food and Nutrition Board: Institute of Medicine, 2005). Under normal circumstances, dietary deficiency of sodium or chloride does not occur, but the body can be depleted of sodium and chloride under extreme conditions, e.g., heavy perspiration, chronic diarrhea or renal disease. Chlorine deficiency in breast-fed infants is rare, and it is generally accepted that the chlorine level of breast milk is not affected by maternal diet (Lonnerdal, 1986). The ability to survive at extremely low levels of sodium intake reflects the capacity of the normal human body to conserve sodium by markedly reducing losses of sodium in the urine and sweat. Under conditions of maximal adaptation and without sweating, the minimal amount of sodium required to replace losses is around 0.18 g (8 mmol)/d (Food and Nutrition Board: Institute of Medicine, 2005). Still, it is unlikely that a diet providing this level of sodium is sufficient to meet dietary requirements for other nutrients.

Because of insufficient data from dose–response trials, an Estimated Average Requirement (EAR) for sodium could not be established by the U.S. Food and Nutrition Board (FNB). Thus, a Recommended Dietary Allowance (RDA) could not be derived for any sex–age group. Hence, only Adequate Intakes (AIs) are provided (Table 10.2). For example, the AI for sodium for infants aged 0–6 months is based on the average amount of sodium in human milk. For young adults, it is the amount that ensures that the overall diet provides an adequate intake of other important nutrients and to cover sweat losses of sodium in unacclimatized individuals who are exposed to high temperatures or who become physically active. This AI does not apply to individuals who lose large volumes of sodium in sweat. The AI for chloride is set at a level equivalent to that of sodium on a molar basis.

Bovine milk contributes only 7% of dietary sodium in the United Kingdom, but some dairy products, such as cheese and butter, contain added salt and can be significant sources of sodium in some countries (e.g., about 13% of total sodium intake in the United Kingdom; Hazell, 1985). It has been estimated that milk and dairy products provide 20% of total sodium in the diet in Ireland and the United Kingdom (Flynn *et al.*, 1990). Mature human milk contains considerably less sodium and chloride than bovine milk (Table 10.1). No relationship has been demonstrated between maternal dietary sodium or chloride intakes and the concentrations of these electrolytes in milk (Ereman *et al.*, 1987). The chloride concentration in bovine milk increases sharply toward the end of lactation and is independent of dietary intake. Sodium and chloride are believed to be present in milk almost entirely as free ions (Holt, 1993). Almost all sodium and chloride in milk are absorbed in the gastrointestinal tract, although much of what is absorbed is not retained.

In the young infant, clinical problems, including dehydration, may arise if there are excessive intakes of sodium and chloride. In these individuals, the

capacity to concentrate solids is limited and the renal solute load exerts a major effect on water balance. Renal solute load is determined mainly by sodium, chloride, potassium, phosphorus and protein. Bovine milk has a much higher potential renal solute load (~300 mOsmol/l) than human milk (~93 mOsmol/l) (Ziegler and Fomon, 1989). The high renal solute load resulting from the ingestion of bovine milk may be of relatively little significance in healthy growing infants without increased evaporative water losses, because the kidney excretes a more concentrated urine. However, this reduces the margin of safety against dehydration that can occur in conditions of diarrhea, fever or low water intake. For this reason, it is recommended that the upper limit of potential renal solute load in formulae for young infants should be about 220 mOsmol/l (Ziegler and Fomon, 1989).

Potassium plays many roles in the body (Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005; Preuss, 2006), including acid–base balance, maintenance of osmotic pressure and blood pressure, cellular uptake of amino acids and as a co-factor or activator in many enzyme systems. Potassium has a critical role in membrane transport and carbohydrate and energy metabolism. Cellular membrane polarization depends upon the internal and external concentrations of potassium. As a result, the major clinical disturbances of severe abnormal potassium status usually are associated with an altered membrane function, especially in neuromuscular and cardiac conduction systems. Both deficient (hypokalemia) and excess (hyperkalemia) circulating potassium results in disorders in cardiac, muscle and neurological function (Preuss, 2006). Adverse effects of hypokalemia include cardiac arrhythmias, muscle weakness and glucose intolerance. Adverse effects of moderate potassium deficiency without hypokalemia include increased blood pressure, salt sensitivity and bone turnover. Cardiac arrest caused by abnormal electrical conduction is the most serious clinical manifestation of hyperkalemia. Neuromuscular symptoms of potassium excess include tingling, paresthesia, weakness and flaccid paralysis.

The FNB determined that an EAR could not be established for potassium, so a RDA could not be derived (Food and Nutrition Board: Institute of Medicine, 2001). The FNB also stated that the health effects of potassium intake in infants and children are uncertain. Thus, only an AI was set that reflected a calculated mean potassium intake through human milk (age 0–6 months) or a combination of human milk and complementary foods (age 7–12 months). The AI for children was derived by extrapolating from the adult AI of 4.7 g (120 mmol) potassium/d (Table 10.2).

The adult human body contains about 45 mmol K/kg body weight or about 3150 mmol (1230 g) for a 70 kg adult (Preuss, 2006). Intracellular potassium accounts for 98% of the potassium and for 75% of total intracellular cations. Because extracellular fluid contains only 2% of the potassium in the

body, plasma potassium is a poor indicator of tissue concentrations. The average concentration of potassium in mature human milk is 0.5 g/l, which is considerably less than that of bovine milk (Food and Nutrition Board: Institute of Medicine, 2001). Lactating dairy cows have a very high dietary potassium requirement (10 g/kg dry matter), and potassium is the mineral element found in the highest concentration in milk (1.5 g/l) (Hunt and Meacham, 2001; Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005). Potassium concentration in bovine colostrum is higher than that in mature milk (Ontsouka *et al.*, 2003). No relationship between maternal dietary potassium and its concentration in milk has been demonstrated. Almost all potassium in milk is absorbed from the digestive tract (Flynn, 1992).

Bovine milk and dairy products can be major contributors to the total dietary intake of potassium (24–29% in Ireland and the United Kingdom; Flynn *et al.*, 1990), because the richest sources, vegetables and fruits, often are not consumed in recommended amounts. Potassium deficiency is unlikely unless excessive alimentary (e.g., diarrhea) or renal (e.g., diuretic use) losses occur because many foods contain significant amounts of potassium (Preuss, 2006). The dietary intake of potassium in the United States and Canada, however, is considerably lower than the AI, which was based on the need to blunt the severe salt sensitivity prevalent in African-American men, and decreasing the risk of kidney stones (Food and Nutrition Board: Institute of Medicine, 2001). A diet rich in fruits, vegetables and milk would assure a healthy intake of potassium.

10.3. Calcium

Calcium is the most abundant mineral in the body (~1000 g adults) (Favus *et al.*, 2006). Approximately 1% of total body calcium is found in extracellular fluids, intracellular structures and cell membranes where it serves as a second messenger, coupling intracellular responses to extracellular signals (Awumey and Bukoski, 2006; Kirchhoff and Geibel, 2006). As such, calcium mediates muscle contraction, nerve transmission and glandular secretion. The remaining 99% of total body calcium is found in bones and teeth where its chemical properties are indispensable for skeletal function and dental structure and function.

Bone crystals (an analogue of the geological mineral, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]) have a significant (~39%) calcium content. These crystals, with the ability to resist compression, are arrayed in a protein matrix that has the ability to withstand tensile loads. Changes in either the inorganic (e.g., hydroxyapatite) or the organic (e.g., collagen) matrix components can alter

bone strength (Rubin and Rubin, 2006). The bone calcium deposition rate *in vivo* (V_o+) during early prepuberty (8.3 yr) was estimated at 1504 mg/d; puberty (10.2 yr), 1952 mg/d (Abrams *et al*., 2000); menarche, 3000 mg/d; and postmenarche (60 months), 1000 mg/d (Abrams *et al*., 1996). Accordingly, the rate of calcium deposition is much higher than either calcium consumption (~1200 mg/d) or the gut absorption (~400 mg/d) observed in human studies (Bronner and Abrams, 1998; Abrams *et al*., 2000). Skeletal tissue is replaced every 10–12 yr, on average (Heaney, 2006), because the skeleton is a metabolically active organ and must undergo continuous remodeling throughout life to adapt its internal microstructure to changes in the mechanical and physiological environment. Furthermore, bone is renewed continuously to repair micro-damage to minimize the risk of fracture. If the bone repairing function is slower than micro-damage accumulation, a "stress fracture" (very small, incomplete fracture) finally occurs (Garcia-Aznar *et al*., 2005).

Factors that contribute to skeletal fragility include suboptimal calcium intake, genetics, lifestyle, smoking, decrease in sex hormone production and certain medications. Cases of rickets caused by calcium deficiency have been reported in developed (Davidovits *et al*., 2006) and developing (Thacher *et al*., 2006) countries. Age-related osteoporosis is a major metabolic bone disease of unknown but apparent multifactorial etiology with nutritional, lifestyle, genetic and endocrine components. It is characterized by compromised bone strength that predisposes a person to increased fracture risk. A diagnosis of osteoporosis requires both low bone mass and a preexisting loss of bone tissue (Harvey *et al*., 2006). A distinguishing characteristic of this disease, compared to osteomalacia, is a normal mineral/collagen ratio (Looker *et al*., 1993). One in two women and one in five men who are 50 yr of age will have an osteoporotic fracture in their remaining lifetime (Harvey *et al*., 2006).

Calcium intake recommendations vary widely worldwide, with that of the US National Academy of Sciences (NAS) among the highest. The NAS established AIs, rather than EARs or RDAs, for Ca (Table 10.2). This decision was based on several concerns, including uncertainties in the precision and significance of balance studies needed to determine a desirable retention model and lack of concordance between mean Ca intake and experimentally derived values predicted to achieve a desirable level of Ca retention (Food and Nutrition Board: Institute of Medicine, 1997). NAS recommendations for pregnancy and lactation are specific for the age of the mother. The dietary reference nutrient intake values for calcium in the United Kingdom are lower than in the United States: ages 0–12 months, 525 mg (17.1 mmol); 1–3 yr, 350 mg (11.4 mmol); 4–6 yr, 450 mg (14.6 mmol); 7–10 yr, 550 mg (17.9 mmol); 11–18 yr (male), 1000 mg (32.5 mmol);

11–18 yr (female), 800 mg (26.0 mmol); and 19+ yr, 700 mg (22.8 mmol)/d (Francis, 2008). Dietary allowance of calcium recommended by the Indian Council of Medical Research is 400 mg/d for adults (Harinarayan *et al*., 2007). A recent analysis of primary calcium balance data from a group of separate, but tightly controlled metabolic feeding studies, indicates a balance point of 741 mg/d and a presumptive RDA of 1035 mg/d for calcium for men and women (Hunt and Johnson, 2007). Several countries and organizations, including the United States and the European Community, have identified an upper limit for calcium (2500 mg/d) (Looker, 2006).

Calcium intakes vary considerable among countries. In the United States, mean usual calcium intakes from food calculated for males from NHANES 2001–2002 data (Moshfegh *et al*., 2005) were as follows: ages 1–3 yr, 972 mg; 4–8 yr, 960 mg; 9–13 yr, 1139 mg; 19–30 yr, 1098 mg; 31–50 yr, 1021 mg; 51–70 yr, 874; and 51+ yr, 817 mg/d. For females: ages 1–3 yr, 972 mg; 4–8 yr, 960 mg; 9–13 yr, 865 mg; 19–30 yr, 784 mg; 31–50 yr, 755 mg; 51–70 yr, 701; and 51+ yr, 666 mg/d. Calcium intake data from the UK Women's Cohort Study (Cade *et al*., 2004) with 35,372 women, aged 35–69 yr, indicate that British women consume a mean of 1141 mg calcium per day. Average calcium intake was 558 mg/d in 647 subjects from Reus, Spain (Garcia-Lorda *et al*., 2007). Calcium intake data (four standardized 24-h dietary recalls collected 3–6 weeks apart) for 4680 men and women, aged 40–59 yr, in Japan, China, the United Kingdom and the United States indicated mean daily calcium intakes of 605, 356, 1013 and 882 mg in those countries, respectively (Zhou *et al*., 2003). Healthy urban and rural men of Tirupati, southern Andhra Pradesh, India, consumed on average 323 and 271 mg calcium per day, respectively, similar to women living in the same urban and rural areas (306 and 262 mg/d, respectively) (Harinarayan *et al*., 2007).

In the United States, the current calcium AI for infants 0–6 months of age is based on the reported intake of milk and on the reported average calcium concentration in human milk after 1 month of lactation. For infants aged 7–12 months, the AI is based on the average calcium intake from human milk and solid foods. For females aged 1–8 yr, calcium accretion in the range of 60–200 mg/d has been predicted. Therefore, the AI for children aged 4–8 yr reflects data from balance studies with girls (and findings extrapolated to boys) that indicate that a calcium intake of 800–900 mg/d would result in mean calcium retention of up to 174 mg/d. These data were extrapolated to set the AI for younger children aged 1–3 yr.

Adequate dietary calcium intake is required to achieve full accretion of bone mass prescribed by genetic potential. On the other hand, the efficacy of calcium supplementation in healthy children as a public health intervention remains highly controversial. Catch-up mineralization later in puberty

appears likely if calcium intake is consistent with usual average intakes in the United States (Abrams, 2005). For example, a recent meta-analysis of 19 randomized controlled trials involving 2859 children (aged 3–18 yr) examined the effects of the addition of 300–1200 mg calcium per day from supplementation or dairy products (Winzenberg *et al*., 2006). This analysis concluded that there was no significant persistent effect of calcium supplementation on femoral neck or lumbar spine bone mineral density (BMD). A small long-term effect of supplementation on BMD in the upper limb seemed unlikely to reduce the risk of fracture, either in childhood or in later life, to a degree of major public health importance. During sexual maturation (~9–18 yr), calcium retention increases to a peak and then declines, a physiological phenomenon that complicates derivation of estimates of calcium needs. The AI for this age group has been estimated from three major lines of evidence: (1) a factorial approach (summation of calcium needs for growth plus calcium losses with adjustments for absorption), (2) calcium retention to meet peak bone mineral accretion and (3) clinical trials in which bone mineral content was measured in response to variable calcium intakes. There is no consensus as to when peak bone mass occurs (Nilas, 1993) and there may be an overall increase, not decrease, in total skeletal mass of about 4% between 18 and 50 yr (Matkovic *et al*., 1994). The AI for calcium for men and women aged 19–50 yr is based on balance studies and bone mineral density data.

It is well recognized that calcium has a limited role in maintaining bone health because calcium adequacy alone does not provide full protection against bone loss (Shapiro and Heaney, 2003) associated with age and menopause (Heaney, 2006). In perimenopausal women, significant metacarpal and lumbar bone loss occurs even with supplements of 1000 or 2000 mg calcium per day (Elders *et al*., 1991). Spinal BMD lost at menopause can be attributed completely to estrogen deprivation, and femoral neck losses are a composite of estrogen deprivation and age-related loss (Recker *et al*., 2000). The AI for calcium for men and women aged $\geq$51 is based on the assumption that individuals in this age range would have calcium needs somewhat higher than that of the 19- through 30-yr age group (Food and Nutrition Board: Institute of Medicine, 1997). However, among the several studies of calcium intake and fracture risk, no consistent association has been demonstrated between reported calcium intake over periods of up to 10 yr and fractures (Cumming and Nevitt, 1997; Dawson-Hughes, 2006; Jackson *et al*., 2006), the only sequela of importance in osteoporosis. Also, most (Kochersberger *et al*., 1990; Chapuy *et al*., 1992; Chevalley *et al*., 1994; Dawson-Hughes *et al*., 1997; 2000; Hitz *et al*., 2007), but not all (Phang *et al*., 1969), studies on adults indicating a positive influence of high dietary Ca in reducing the rate of bone remodeling were confounded by the presence of vitamin D as an experimental co-variable.

Evidence to support a role for dietary calcium in the prevention of a number of diseases other than osteoporosis (Heaney, 1996; Food and Nutrition Board: Institute of Medicine, 1997; Weaver, 2006) remains controversial. Higher calcium intake (>1250 mg/d versus ≤500 mg/d) is associated with a reduced risk of distal, but not proximal, colon cancer, but calcium intakes beyond moderate levels were not associated with a further risk reduction (Wu *et al*., 2002). Calcium supplementation (1200 mg/d) was associated with a significant, though moderate, reduction (31 versus 38%) in the risk of recurrent colorectal adenomas (Baron *et al*., 1999). On the other hand, elevated calcium intake (> 2000 mg/d) was related positively to advanced prostate cancer (Giovannucci *et al*., 1998), a finding not in agreement with a similar prospective study (Schuurman *et al*., 1999) that found no positive association between calcium intake and prostate cancer risk. Dietary calcium was associated with reduced incidence of kidney stones in an observational study on men (Curhan *et al*., 1993). However, in a separate study on women, moderate intake of supplemental calcium appeared to increase risk for symptomatic kidney stones whereas high intake of dietary calcium appeared to reduce that risk (Curhan *et al*., 1997). In a separate study with healthy postmenopausal women supplemented with 1000 mg calcium per day and 400 IU vitamin D per day, the subjects had increased risk of kidney stones and did not have significantly reduced hip fracture (Jackson *et al*., 2006). A meta-analysis of randomized, controlled calcium supplementation trials indicated that calcium supplementation leads to a small reduction in systolic, but not diastolic, blood pressure (Bucher *et al*., 1996). Even so, increasing attention has been given to the influence of dietary patterns rather than individual nutrients on disease prevention (Appel *et al*., 1997).

Milk products, the most calcium-dense foods in Western diets, contain about 300 mg (7.5 mmol) calcium per serving (244 g of milk or yogurt or 42.5 g of Cheddar cheese) (Food and Nutrition Board: Institute of Medicine, 1997). Bovine milk and other dairy products, such as cheese and yoghurt, provided 84% of the dietary calcium in the United States in 1989–1993. However, milk is being replaced by sweetened soft drinks and juices such that Americans drank nearly 2.5 times more soda than milk in 2001 (Weaver and Heaney, 2006). Grains are not particularly rich in calcium, but because they may be consumed in large quantities, they can account for a substantial proportion of dietary calcium. Among Mexican American adults, corn tortillas are the second most important food source of calcium, after milk (Looker *et al*., 1993).

The concentration of calcium in mature human milk is 250–300 mg/l (Butte *et al*., 1984; Hunt, 1988; Lonnerdal, 1997), and findings from several longitudinal studies indicate that the calcium concentration in breast milk is

stable (Kirksey *et al.*, 1979; Lonnerdal, 1997), exhibits a transient decrease (Friel *et al.*, 1999), or decreases slightly but significantly (Hunt *et al.*, 2005) during the first 4 months of lactation. Generally, the maternal diet is not believed to affect the concentration of calcium in breast milk (Lonnerdal, 1986). However, there is some evidence that calcium levels in breast milk are lower than normal during extended lactation (Laskey *et al.*, 1990) and that breast milk of lactating young teenage mothers may have lower calcium concentrations than that of older women (Lipsman *et al.*, 1985). The calcium content of human milk is considerably lower than that of bovine milk (~1100 mg/l) (Table 10.1) and also that of milk-based (~500 mg/l) (Hunt and Meacham, 2001) or soy-based (~600 mg/l) formulae. Because formula-fed infants usually consume higher volumes than breast-fed infants, they receive at least twice the amount of calcium that breast-fed infants ingest (Lonnerdal, 1997). The fundamental concept that the body composition of exclusively breast-fed infants in the first 6 months of life is the ideal standard for all full-term infants is now in conflict with the reality that it is technologically possible with infant formulae to increase calcium absorption and bone calcium accretion to levels above those achieved by human milk-fed, full-term infants. However, there are no data to support such a goal or suggest that it is beneficial for short- or long-term bone health (Abrams, 2006). Regardless, in the United States, the mandate of the Infant Formula Act and certain policy statements have led to the marketing of infant formulae with much higher concentrations of calcium than is present in human milk (Abrams, 2006).

Calcium sources should be evaluated on the basis of both content and bioavailability of calcium (Weaver *et al.*, 1999). About 60–70% of calcium is absorbed from human milk, leading to a net retention of about 90–100 mg calcium per day during the first 6 months for babies fed exclusively human milk (Abrams, 2006). Calcium absorption from bovine milk (~32%) is similar to that from other dairy products even though the lactose content and the chemical form of calcium in cheese or yogurt are altered during processing (Weaver *et al.*, 1999). Fractional calcium absorption from infant formulae tends to range between 40 and 60% in most studies (Abrams, 2006). Calcium salts, regardless of solubility, have fractional calcium absorption values similar to that for bovine milk, with the exception of calcium citrate malate, from which absorption is slightly higher (Weaver *et al.*, 1999). Fractional absorption of calcium from low-oxalate vegetables, i.e., broccoli (61%), bok choy (54%) or kale (49%) is actually higher than that from bovine milk (Weaver *et al.*, 1999). Calcium bioavailability is typically greatly reduced in foods containing high level of oxalate and/or phytate (e.g., common dried beans) but foods produced from soybeans, rich in both oxalate and phytate, have relatively high calcium bioavailability. However, increasing dietary

protein from omnivorous sources increases intestinal calcium absorption and substitution of soy protein for meat protein causes an acute decline in dietary calcium bioavailability (Kerstetter *et al.*, 2006). Regardless of the source of calcium, calcium absorption efficiency decreases with increasing intake but total calcium absorbed continues to increase with load (Weaver and Heaney, 2006). Earlier concern that purified proteins in the diet decrease calcium balance (Weaver *et al.*, 1999) was muted by the finding that calcium retention was not reduced when subjects consumed a high-protein diet from common dietary sources such as meat (Roughead *et al.*, 2003). Calcium absorption efficiency is upregulated during puberty and during the third trimester of pregnancy. On the other hand, calcium absorption efficiency declines with age (Weaver and Heaney, 2006). Active calcium absorption is compromised by hyperparathyroidism and diseases of the kidney (Weaver and Heaney, 2006).

In bovine milk, 99% of the calcium is in the skim milk fraction, whereas, in human milk, 16% of calcium is present in the lipid fraction (bound to the fat globule membrane), 47% is protein bound (6% to casein and the remainder to whey proteins), about 38% is soluble (mainly as calcium ions) (Fransson and Lonnerdal, 1983) and 1% is bound to α-lactalbumin (Lonnerdal and Glazier, 1985). Casein micelles are colloidal protein–calcium-transport complexes with the colloidal calcium phosphate present in nanoclusters with a diameter of $\sim$2.5 nm (Marchin *et al.*, 2007). Therefore, calcium levels are higher in milks rich in caseins (Gaucheron, 2005). Micellar calcium is not exclusively associated with the colloidal inorganic phosphate; part is bound directly to the phosphoserine residues of casein, which are organic phosphate. Thus, the colloidal calcium in milk is a mixture of calcium caseinate (containing organic phosphate) and calcium phosphate (an inorganic phosphate) (Gaucheron, 2005). In bovine milk, two-thirds and one-third of the total calcium are in the micellar and soluble forms, respectively (Gaucheron, 2005). Micellar calcium phosphate is exchangeable with the diffusible fraction, and the calcium bound to phosphoserine residues is more exchangeable than that associated with colloidal phosphate (Gaucheron, 2005). Ionized calcium in the soluble phase accounts for about 10% of the total calcium, with the remaining soluble portion as calcium citrate. Micellar calcium phosphate plays a key role in the maintenance of the structure of the casein micelle, and the physico-chemical properties are exploited for the manufacture of dairy products (Gaucheron, 2005). Casein micelles of human and bovine milk differ in composition such that when clotted by proteolytic enzymes under conditions simulating those found in the infant stomach, either no clot or an almost undetectable very fine curd forms as opposed to the large curds in bovine milk. The small clots may be involved in the proper absorption of the milk constituents into the body for optimal utilization (Sood *et al.*, 1997).

10.4. Phosphorus

Phosphorus is an essential nutrient for humans, and the adult human body contains about 850 g of elemental phosphorus with about 85% in the skeleton; 14% in the soft tissues; and 1% in the extracellular fluids, intracellular structures and cell membranes (Anderson *et al*., 2006). Food phosphorus is a mixture of inorganic phosphate (P_i) and various organic phosphates. Most phosphorus absorption occurs as P_i because intestinal phosphatases hydrolyze the organic forms in foods (Food and Nutrition Board: Institute of Medicine, 1997) and the predominant form of inorganic phosphate in all biological fluids and tissues is the hydrogen phosphate ion (HPO_4^{2-}), a divalent anion. The dietary phosphorus present as phytate, the storage form of phosphorus in food plant seeds (beans, cereals, nuts, peas), is not available directly. Phosphorus bioavailability from these foods depends on the hydrolysis of phytate by phytase produced by yeasts and colonic bacteria and the natural phytase content of all foods. Leavening breads with yeasts that produce phytase improves phosphorus bioavailability from those foods (Anderson *et al*., 2006). Because there is a multitude of factors that influence the presence of phytase, there is considerable flux in phosphorus bioavailability from food phytate in the gut over a given period of time (Food and Nutrition Board: Institute of Medicine, 1997).

The organic phosphates are major components of phospholipids, nucleotides and nucleic acids. Also, the hydroxyapatite-like bone crystals contain a constant ratio of calcium-to-phosphate of approximately 2:1. On the other hand, the whole body P_i compartment comprises a minute fraction of total body phosphorus and is located mainly in the blood and extracellular fluid (ECF). However, the P_i compartment is a critical pool because it accepts phosphate absorbed from the diet and the phosphorus resorbed from bone and is the source of most bone fluid phosphorus and most urinary phosphorus. The normal very high fluxes of P_i between bone and the bone fluid compartment each day (5000 mg) occur by ionic exchange and active bone resorption (Anderson *et al*., 2006). Bone turnover rates are relatively slow, so that dynamic ionic exchange is critical for maintenance of blood P_i concentration.

Phosphorus is found in nearly all foods, with most food sources having high phosphorus bioavailability (55–70%) (Food and Nutrition Board: Institute of Medicine, 1997). The phosphorus content of the US food supply is increasing as phosphate salts are added to processed foods for non-nutrient functions such as moisture retention, smoothness, and binding. As a result, near total starvation is required to produce dietary phosphorus deficiency which manifests as hypophosphatemia (Food and

Nutrition Board: Institute of Medicine, 1997). However, there are several situations where hypophosphatemia may be induced and becomes life-threatening. In intensive care units, hypophosphatemia may be expected in the presence of risk factors including alcohol withdrawal, parenteral nutrition and/or glucose loading, insulin infusion during ketoacidosis treatment and sepsis, dialysis and treatment with antiacids, diuretics and dialysis (Palmese *et al.*, 2005). When ECF P_i levels are low, cellular dysfunction follows. Thus, the effects of hypophosphatemia include anorexia, anemia, muscle weakness, bone pain, rickets and osteomalacia, general debility, increased susceptibility to infection, paresthesias, ataxia, confusion and even death (Lotz *et al.*, 1968). In growing skeletal tissue, the supply of P_i from dietary sources becomes potentially limiting but the typical abundance of phosphorus in the diet minimizes this rate-limiting risk (Anderson *et al.*, 2006).

Excess phosphorus intake from any source is expressed as hyperphosphatemia, an abnormally high level of plasma P_i (Food and Nutrition Board: Institute of Medicine, 1997). There are several potential problems of P_i metabolism related to excessive phosphorus consumption. Most clinical studies of acute and longer exposure to phosphorus loading show an increase in PTH levels. Thus, a chronic high phosphorus intake may impair the adaptive mechanism needed for adequate calcium absorption and optimal bone accretion (Anderson *et al.*, 2006). Even so, the FNB concluded that current phosphorus intakes thought to be experienced by the US population are unlikely to adversely affect bone health (Food and Nutrition Board: Institute of Medicine, 1997). Calcification of non-skeletal tissues (metastatic calcification) is a serious and harmful effect of hyperphosphatemia, a situation that occurs when calcium and phosphorus concentrations of the extracellular fluid exceed limits of calcium phosphate solubility. Metastatic calcification of the kidney is not known to occur from dietary sources alone in persons with adequate renal function but hyperphosphatemia is an almost universal finding in patients with end-stage renal disease and is associated with increased all-cause mortality, cardiovascular mortality and vascular calcification (Kooienga, 2007).

Dietary phosphorus is derived mainly from foods high in protein or from foods where phosphate salts are added to improve non-nutritive properties including moisture retention, smoothness, or binding. In adults, total dietary phosphorus intake varies more with total food intake than with food composition. Even so, increased consumption of soft drinks that use phosphoric acid as the acidulant or dairy products will increase phosphorus density values; the phosphorus densities of either cola or bovine milk are higher than those of most other foods in a typical diet (Food and Nutrition

Board: Institute of Medicine, 1997). In the United States, median phosphorus intakes exceed DRIs for both genders by 300–600 mg/d. Furthermore, it is not difficult for adults aged 19–70 yr to exceed the UL for phosphorus (4000 mg/d). For example, consumption of certain high-energy bars and shakes or creatinine monophosphate supplements at the manufacturer's recommended daily dose alone will provide up to 3000 mg/d of phosphorus (Anderson *et al.*, 2006). In the United States, intakes of calcium are consistently lower than those of phosphorus in the absence of calcium supplementation, the significance of which remains controversial.

Infants, toddlers and adolescents derive 32–48% of their dietary phosphorus from milk and milk products, whereas dairy foods provide only 20–30% of phosphorus for most adult age and sex groups (Anderson *et al.*, 2006). Particular attention must be paid to increased risk of hyperphosphatemia in neonates because renal handling of phosphorus is developmentally immature in this age group. Persistent hyperphosphatemia during early infancy may cause parathyroid hyperplasia, ectopic calcifications, low serum calcitriol, and hypocalcaemia severe enough to induce neonatal tetany. This condition is more prevalent in artificially fed than in breast-fed infants (Food and Nutrition Board: Institute of Medicine, 1997) because bovine milk or formulae may contain, respectively, six or three times as much phosphorus as human milk (see Table 10.1). The phosphorylation level of caseins is believed to have major implications for the formation of micelles that are involved in delivering valuable calcium phosphate and other minerals to the newborn (Kjeldsen *et al.*, 2007). Even so, human milk with its low phosphorus content is both safer and better suited to the growth needs of the infant than bovine milk. Casein micelle structure is not characterized fully, but in all compositional models, micellar calcium phosphate is an integral part of the casein micelle and is responsible for the structure and stability of these particles. In bovine milk, about 54% of P_i is soluble and about 46% is associated with casein micelles as calcium phosphate, probably by binding mainly to casein phosphoserine residues (organic phosphate) and glutamate and aspartate residues (Gaucheron, 2005). In human milk, about 15% of phosphorus occurs in an inorganic form, 23% is protein bound and about 62% is present with lipids (Renner, 1983).

10.5. Magnesium

Magnesium is the most prevalent intracellular divalent cation and is needed for enzymatic reactions vital to every metabolic pathway (Food and Nutrition Board: Institute of Medicine, 1997; Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005; Volpe, 2006). These

reactions involve synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), protein and, adenylate cyclase glycolysis, cellular energy production and storage and preservation of cellular electrolyte composition. Magnesium regulates intracellular calcium and potassium at the cell membrane level, and thus is a controlling factor in nerve transmission, skeletal and smooth muscle contraction, cardiac excitability, vasomotor tone, blood pressure and bone turnover.

Severe magnesium deficiency, usually the result of dysfunctional states causing malabsorption or excessive excretion, results in numerous signs and symptoms including loss of appetite, nausea, vomiting, fatigue and weakness. As the deficiency becomes more severe, numbness, tingling, muscle contractions and cramps, seizures, personality changes and coronary spasms (angina pectoris) occur. A chronic low intake of magnesium may result in cardiovascular dysfunctions such as hypertension and dysrhythmias, bone loss leading osteoporosis, and insulin resistance and impaired insulin secretion leading to diabetes mellitus. The major effect of excessive magnesium intake is diarrhea (Food and Nutrition Board: Institute of Medicine, 1997; Volpe, 2006), but nausea and abdominal cramping may also occur. High serum magnesium (hypermagnesmia) causing more severe magnesium toxicity signs is most commonly associated with the combination of impaired renal function and high intakes of non-food sources of magnesium such as magnesium-containing laxatives and antacids. Signs of hypermagnesmia include muscle weakness, extremely low blood pressure, difficulty in breathing, irregular heartbeat and change in mental status.

The FNB (Food and Nutrition Board: Institute of Medicine, 1997) found no functional criteria on which to base an RDA for infants. Thus, they set an AI for magnesium that reflects the mean intake of infants fed principally with human milk (Table 10.2). An RDA for magnesium was set for children aged 1–3 yr by interpolating data from other age groups. Data from the US National Health and Nutrition Examination Survey (NHANES) 2001–2002 indicated that the majority of people consumed less than the EAR for magnesium (Moshfegh *et al.*, 2005). For example, 64% of women aged 51–70 yr did not attain the magnesium EAR. A recent analysis of primary magnesium balance data from tightly controlled metabolic feeding studies indicates a presumptive EAR of 165 mg/d and a presumptive RDA of 237 mg/d for magnesium for men and women (Hunt and Johnson, 2006).

The adult human body contains about 25 g (1028 mmol) of magnesium, which is about equally divided between bone and soft tissue (Volpe, 2006). Less than 1% of the total body magnesium is in blood. Approximately one-third of skeletal magnesium is exchangeable, and acts as a pool for maintaining normal concentrations of extracellular magnesium. Thus, extracellular magnesium stays relatively constant, even if skeletal and intracellular levels

are decreasing. This results in plasma or serum magnesium being a poor indicator of tissue magnesium status. The concentration of magnesium in human colostrum is about 30% higher than in mature human milk (Rajalakshmi and Srikantia, 1980), which is about 34 mg (1.4 mmol)/l (Food and Nutrition Board: Institute of Medicine, 1997; Hunt *et al*., 2005). Mature bovine milk contains about 100 mg (4.1 mmol)/l (Hunt and Meacham, 2001; Volpe, 2006). Magnesium concentration in bovine colostrum is two to three times that in mature milk, but decreases to the mature milk value within the first 1–3 d of lactation (Hidiroglou and Proulx, 1982). The concentration of magnesium in human milk increases slightly between months 1 and 4 (Hunt *et al*., 2005) but remains relatively constant in bovine mature milk (Hidiroglou and Proulx, 1982) over the first year of lactation. Milk magnesium concentration is unaffected by dietary magnesium intake. Because bovine milk contains over three times as much magnesium as human milk, commercial formulae that are based on bovine milks are generally higher in magnesium concentration (40–50 mg or 1.7–2.1 mmol/l) than human milk (Food and Nutrition Board: Institute of Medicine, 1997).

In human milk, 2% of magnesium is in the fat fraction, 6% in casein, 36% associated with whey proteins and 58% as low-molecular-weight forms (Fransson and Lonnerdal, 1983). In bovine milk, 98–100% of the magnesium is in the skim milk phase (Fransson and Lonnerdal, 1983), with 65% in a soluble form (40% as magnesium citrate, 7% as magnesium phosphate, and 16% as free magnesium ions). The other 35% of magnesium is colloidal and associated with casein micelles (Holt, 1985). Premature and full-term infants absorb 45% of the magnesium in human milk (Food and Nutrition Board: Institute of Medicine, 1997) and absorption varies from 40 to 71% in bovine milk-based infant formulae (Food and Nutrition Board: Institute of Medicine, 1997).

Green leafy vegetables, whole grains and nuts are the richest sources of magnesium (Volpe, 2006). However, because milk and milk products are moderate sources of magnesium, increasing their intake would be a reasonable recommendation for assuring a healthy intake of this element.

10.6. Iron

Dietary iron is required for a wide variety of biochemical processes. Biological systems utilize the chemical reactivity of iron and interconvert iron oxidation states. This interconversion allows iron to participate in electron transfer as well as the reversible binding to ligands (Food and Nutrition Board: Institute of Medicine, 2001). Iron is suited to participate in oxygen transport and storage, electron transfer and substrate oxidation–reduction.

For example, one of the main functions of iron is the movement of oxygen from the environment to body tissues (Beard, 2006). As a result, approximately 66% of iron in the body is found in hemoglobin of circulating erythrocytes. Another 25% is in the readily mobilizable iron store and the remaining 15% is contained in the myoglobin of muscle tissue and a variety of enzymes required for oxidative metabolism and other functions in cells. In humans, there are four major classes of iron-containing proteins: heme proteins (hemoglobin, myoglobin, cytochromes), iron–sulfur enzymes (flavoproteins, heme-flavoproteins), iron transport and storage proteins (lactoferrin, transferrin, ferritin, hemosiderin) and other iron-containing or activated enzymes (sulfur, non-heme enzymes). Iron is critical for the regulation of genes, cell growth and differentiation, hematopoiesis and cognitive development during infancy (Kelleher and Lonnerdal, 2005).

Iron-deficiency anemia is the late stage of negative iron balance and is diagnosed as low serum transferrin saturation (<15%), a low serum ferritin concentration (<12 μg/l) and an elevated soluble transferrin receptor (>6 mg/dL) in a background of microcytic anemia. The physical symptoms of iron-deficiency anemia are tiredness, listlessness, apathy and general feelings of lack of energy. Endurance exercise is markedly impaired. These symptoms represent true reductions in muscle functioning but may also reflect pathological changes in central nervous system functioning (Beard, 2001). Iron-deficiency anemia affects an estimated 20–25% of infants worldwide and appears to cause irreversible developmental delays (Lozoff *et al.*, 2003). Other clinical signs of iron deficiency include inflammation or infection of the tongue (glossitis), fissuring in the corners of the lips (angular stomatitis), spoon nails (koilonchyia), blue sclera, esophageal webbing and behavior disturbances (pica, the abnormal consumption of non-food items) (Beard, 2006).

Ferritin and hemosiderin (a water-insoluble degradation product of ferritin) comprise 95 and 5%, respectively, of the storage forms of iron in the liver (Beard, 2006). Because both the human host and most pathogens require iron, the host must meet cellular demands while simultaneously preventing excess accumulation. Iron overload can be induced by several factors including blood transfusions, hereditary hemochromatosis or particular forms of food and their preparation (i.e., consuming beer cooked in iron pots). In iron overload, there is gross cellular accumulation of both ferritin and hemosiderin in several tissues but particularly in the liver where the rate of increase of hemosiderin is 10 times that of ferritin (Miyazaki *et al.*, 2002).

Iron requirements are highest during pregnancy (Table 10.2). On the other hand, the requirement for exogenous iron is virtually zero for the normal full-term infant at birth because of the normally very high

hemoglobin concentration and high iron stores in the newborn (Food and Nutrition Board: Institute of Medicine, 2001). Maternal iron deficiency is not associated with neonatal iron-deficiency anemia but neonatal iron stores are reduced (Kelleher and Lonnerdal, 2005), which puts the newborn at increased risk for iron deficiency if they do not consume adequate amounts of iron-rich complementary foods after 4–6 months of age (Domellof *et al*., 2002). Regulation of iron absorption changes between 6 and 9 months of age that apparently enhances the ability of the infant to adapt to a low-iron diet. That is, fractional iron absorption from breast milk can double between 6 and 9 months of age in infants not supplemented with iron. Thus, some infants can avoid iron deficiency despite low-iron intake in late infancy (Domellof *et al*., 2002). Men have higher iron stores than women and the requirement for iron is much higher for reproductive-age females than any other sex–age group. This is a reflection of the additional iron requirement associated with menstrual blood loss (Food and Nutrition Board: Institute of Medicine, 2001). The iron requirement increases considerably for lactoovovegetarians compared to non-vegetarians because non-heme iron absorption is considerably lower in the former (Hunt and Roughead, 1999).

Heme iron is highly bioavailable, is soluble in the alkaline luminal environment (such that binding proteins are not necessary for luminal absorption) and enters the intestinal cell intact. On the other hand, non-heme iron absorption depends on the solubilization of predominately ferric food iron in the acid milieu of the stomach and subsequent reduction to the ferrous form (Beard, 2006). This reduction is dependent upon interaction with endogenous compounds such as a ferrireductase (present at the mucosal surface of duodenal enterocytes) (Han *et al*., 1995) and dietary ascorbic acid (Food and Nutrition Board: Institute of Medicine, 2001). Because mammals lack a regulated pathway for iron excretion, iron homeostasis is regulated primarily by iron absorption by duodenal mucosal cells. The hepatic antimicrobial peptide, hepcidin, plays a key role in the negative feedback regulation of intestinal iron absorption (Ward and Conneely, 2004).

The iron concentration in breast milk is probably under homeostatic control because anemic women as well as women taking iron supplements have levels of iron in their milk that are similar to those of non-anemic women (Lonnerdal, 1997). Prematurity does not affect human milk iron concentrations (Hunt *et al*., 2004). Maternal dietary iron supplementation with up to 30 mg of iron per day does not affect its concentration in milk (Siimes *et al*., 1984). Apparent homeostatic control of iron in breast milk is probably achieved by up- and down-regulation of transferrin receptors in the mammary gland such that in a situation of maternal iron deficiency, a higher number of mammary transferrin receptors facilitates the uptake of iron into the gland and ensures a normal iron level in the milk (Lonnerdal, 1997).

Debate continues as to whether the iron content of human milk is sufficient for the breast-fed infant and whether these infants should be supplemented with iron. The concentration of iron in human milk is normally very low and decreases significantly further during the first 12 weeks of lactation (Butte *et al.*, 1987; Davidsson *et al.*, 1994; Hunt *et al.*, 2004; Kelleher and Lonnerdal, 2005). Even so, several studies have demonstrated that the iron status of exclusively breast-fed infants is satisfactory up to 6 months of age and, in some studies, up to 9 or 12 months of age (Lonnerdal, 1997). A panel of experts (American Academy of Pediatrics, 2005) concluded that exclusive breast-feeding is sufficient to support optimal growth and development for approximately the first 6 months of life but complementary foods rich in iron should be introduced gradually beginning around 6 months of age. Preterm and low-birth-weight infants have high iron requirements (Griffin and Abrams, 2001). More recently, a report suggested that iron supplementation from 1 to 6 months of age results in higher hemoglobin and mean corpuscular volume at 6 months of age and significantly higher visual acuity and psychomotor developmental indices at 13 months of age (Friel *et al.*, 2003). On the other hand, new evidence (Sazawal *et al.*, 2006) suggests that current guidelines for universal supplementation with iron and folic acid in areas of high malaria transmission should be revised. Children in those areas who received iron and folic acid were more likely to die or need treatment in hospital for an adverse event. In general, iron-deficient and anemic children can benefit from supplementation but supplementation of those who are not iron deficient might be harmful.

Earlier estimates (Lonnerdal, 1989) of typical iron absorption from human milk (50%) are considerably higher than newer estimates (~20%) obtained with stable isotope technology (Fomon *et al.*, 1993; Lonnerdal, 1997). Also, formula-fed infants absorb considerably less iron (~7%) than breast-fed infants (Fomon *et al.*, 1993). Reasons for the higher bioavailability of iron in human milk are not understood. Lactoferrin is a highly efficient mammalian iron-scavenging defense protein (~80 kDa) (Weinberg, 2007), a glycoprotein that has a very high association constant for iron (K_a, 1.0×10^{30} M^{-1}) (Lonnerdal, 1997) and is found at high concentrations in all human milk, including colostrum (~9.8 g/l) and mature breast milk (~2.4 g/l) (Velona *et al.*, 1999). Despite a very high binding affinity, there is still no good evidence that lactoferrin plays any role as an iron transporter or is involved in "mainstream" iron metabolism (Brock, 2002). In fact, in the murine model, lactoferrin ablation did not result in iron-deficiency anemia (Ward and Conneely, 2004) and, in infants older than 4 months of age, iron absorption was found to be slightly higher from lactoferrin-free milk than from intact breast milk (Lonnerdal, 1997).

Human milk, as well as bovine milk and milk products, are poor sources of iron (Table 10.1) (Pennington *et al.*, 1987). To prevent iron deficiency and

anemia by 6–9 months of age, most infant formulae are supplemented with iron but at various concentrations (Lonnerdal, 1997). In the United States, infants fed typical iron-fortified formula (11.8 mg/l) (Hunt and Meacham, 2001) in typical quantities (0.78 l/d) may have an iron intake of 9.2 mg/d, a level of iron that corresponds to as much as 34 times more iron per day than that consumed by breast-fed infants (0.27 mg Fe/d). Because most grain products are fortified with non-heme iron, approximately 50% of ingested iron comes from bread and other grain products, including cereals and breakfast bars. Heme iron represents only 7–10% of dietary iron for girls and women and 8–12% for boys and men.

Distribution of iron among compartments in human milk is complex and distribution within a compartment differs with species (Fransson and Lonnerdal, 1983). In bovine and human milks, the lipid fraction contains ~14 and ~33% of total milk iron, respectively. Within the lipid fraction of human milk, iron is thought to bind to xanthine oxidoreductase (an enzyme located in the fat globule membrane), one molecule of which contains eight atoms of iron. In both bovine and human milks, whey proteins bind similar amounts of iron (~26 to ~26%) but lactoferrin, a whey protein, has a much more prominent role in binding iron in human milk than in bovine milk. Another ~24% of total bovine milk iron is present in casein, considerably more than the amount of total iron present in human casein (~9%). Finally, in both bone and human milks, ~32% of total iron is associated with low-molecular-weight compounds, most likely citrate.

10.7. Zinc

Because of its stability and coordination flexibility, zinc performs catalytic, structural and regulatory functions in protein, nucleic acid, carbohydrate and lipid metabolism (Food and Nutrition Board: Institute of Medicine, 2001; Cousins, 2006). Approximately 300 enzymes contain zinc. Zinc has a catalytic role in enzymes of all six classes. Zinc also has a structural role in some enzymes and in zinc finger proteins of transcription factors. Through binding to trans-acting factors, zinc regulates the expression of some genes. Mild signs of zinc deficiency described for infants and young children include suboptimal growth, poor appetite and impaired taste acuity. Zinc is a relatively non-toxic element, but chronic zinc supplementation greater than 100 mg/d may result in impaired immune function, decreased HDL cholesterol and gastric distress (Food and Nutrition Board: Institute of Medicine, 2001; Cousins, 2006).

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) found no functional criteria on which to base a zinc RDA for early infancy. Thus, they set an AI of 2.0 mg/d for infants aged 0–6 months based on the

mean intake exclusively from human milk. RDAs for zinc for all other sex–age groups are listed in Table 10.2.

The adult human body contains between 1.5 and 2.5 g of zinc (Cousins, 2006), 85% is in skeletal muscle and bone (Food and Nutrition Board: Institute of Medicine, 2001). About 95% of body zinc is intracellular, with 40% found in the nucleus. Plasma contains about 0.1% of the body's zinc, which responds markedly to external stimuli including zinc intake, fasting and acute stressors such as infection (Cousins, 2006). Thus, using plasma zinc to asses zinc status should be done with caution. In humans, plasma zinc is reduced in severe zinc deficiency, but not with moderate zinc deprivation (Food and Nutrition Board: Institute of Medicine, 2001). Zinc concentrations in human milk decline rapidly during the first 6 months of lactation (Food and Nutrition Board: Institute of Medicine, 2001; Hunt *et al*., 2005). They decline from approximately 4 mg/l at 2 weeks to 3 mg/l at 1 month, 2 mg/l at 2 months, 1.5 mg/l at 3 months and 1.2 mg/l at 6 months postpartum. No significant correlation was observed between dietary zinc intake and zinc concentration in human milk, and zinc supplementation does not appreciably affect its zinc concentration (Lonnerdal, 1997). In contrast to human milk, the zinc concentration in mature bovine milk changes little after the first 3 d of lactation when the concentration decreases by 50% in colostrum (de Maria, 1978). Mature bovine milk contains between 3 and 5 mg zinc/l (Lonnerdal *et al*., 1981; Hunt and Meacham, 2001).

The reported mean distribution of zinc in human milk is 29% in the lipid fraction (bound to the fat globule membrane), 14% associated with casein, 28% associated with whey proteins and 29% in the form of a low-molecular-weight compound (probably as citrate) (Lonnerdal *et al*., 1982; Fransson and Lonnerdal, 1983). The distribution of zinc in bovine milk is significantly different; most is found in the skim milk fraction, with only 1–3% in the lipid fraction (Fransson and Lonnerdal, 1983). Approximately 32% of the zinc is bound to casein and most of the remaining zinc is bound to colloidal calcium phosphate; only a small amount (~5%) is associated with a low-molecular-weight compound, which was identified as citrate (Blakeborough *et al*., 1983; Singh *et al*., 1989). Proteins in human milk, which bind most of the zinc, are believed to be more easily digestible than casein, the major protein in bovine milk. This difference may explain the higher zinc bioavailability from human milk (Cousins, 2006). Pasteurization of human milk decreases zinc bioavailability to the point of affecting zinc balance in preterm infants, a situation probably caused by redistribution and alterations in the zinc-binding pattern (Goes *et al*., 2002).

Because increasing zinc concentration from 1.8 to 5.8 mg/l in infant formulae resulted in increased growth in male infants (Walravens and Hambidge, 1976), zinc supplementation of formulae is practiced widely.

The zinc concentration of bovine milk-based infant formulae in the United States is in the range of 4.0–7.4 mg/l (Hamill *et al.*, 1989; Hunt and Meacham, 2001). Dairy products such as milk, cheese and yoghurt are moderate sources of zinc for adults. It has been estimated that milk and dairy products contribute between 19 and 31% of the total zinc intake in Western countries (Hazell, 1985; Renner *et al.*, 1989).

10.8. Copper

Copper functions in a metal center of (co-factor) about 10 enzymes that bind oxygen and produce water, superoxide or hydrogen peroxide in addition to producing molecules involved in energy production, iron absorption and utilization, extracellular matrix maturation, neuropeptide activation and neurotransmitter synthesis (Food and Nutrition Board: Institute of Medicine, 2001; Prohaska, 2006). Clinically evident (Frank) copper deficiency has been reported for infants fed milk formulae and bovine milk, malnutrition associated with chronic diarrhea and patients nourished with prolonged total parenteral nutrition. Signs of copper deficiency include normocytic hypochromic anemia, leukopenia, neutropenia and osteoporosis. Severe copper deficiency caused by the genetic disorder, Menke's syndrome, and animal deprivation studies suggest that a chronic low intake of copper may have pathological consequences for cardiovascular health, immune response and neuronal function.

Acute excessive copper intake (e.g., >3–4 mg/l drinking water) induces gastrointestinal distress including abdominal pain, cramps, nausea, diarrhea and vomiting (Food and Nutrition Board: Institute of Medicine, 2001; Prohaska, 2006). Chronic copper toxicity is rare unless some hereditary defect in copper homeostasis is present (Food and Nutrition Board: Institute of Medicine, 2001). Liver damage in childhood cirrhosis in India has been associated with the use of copper-contaminated water to prepare infant formula and the consumption of other foods that were stored or prepared in brass and/or copper vessels (Tanner, 1998).

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) found no functional criteria of copper status to use for establishing a copper RDA for infants. Thus, they set AIs that reflected the mean intake of infants principally fed with human milk; these AIs were 200 μg/d for age 0–6 months and 220 μg/d for age 7–12 months. Extrapolation from the RDA for adults was the basis for setting RDAs for children (Table 10.2)

The human body contains about 1 mg copper/kg body weight (Prohaska, 2006). While liver, brain and kidney have the highest concentrations, muscle and bone, because of their overall greater mass, account for 50–70 % of total body copper (Harris, 1997). Low serum copper can indicate copper deficiency

(Food and Nutrition Board: Institute of Medicine, 2001). However, using serum copper to assess copper status requires caution because concentrations increase during pregnancy and with a number of diseases. The concentration of copper in human milk is highest during early lactation and declines with time (Food and Nutrition Board: Institute of Medicine, 2001; Hunt *et al.*, 2004). The mean copper concentration in human milk is about 250 μg/l during the first 6 months of lactation and declines to between 100 and 200 μg/l between 7 and 12 months postpartum (Food and Nutrition Board: Institute of Medicine, 2001). No correlation was found between dietary copper intake and human milk copper concentrations. The mean concentration of copper is higher in human milk than in bovine milk (60–90 μg/l) (Fransson and Lonnerdal, 1983; Hunt and Meacham, 2001). The concentration of copper in bovine milk decreases by up to 50% during the first 3 d of lactation (de Maria, 1978) but can be increased by dietary copper supplementation (Murthy and Thomas, 1974) or by contact with copper-containing containers and processing equipment (Roh *et al.*, 1976). The copper content of commercial infant formulae is often similar to that in human milk (Table 10.1).

In human milk, 9–15% of copper is in the fat fraction, 39–56% bound to whey proteins (mainly the albumin fraction of milk), 7–28% bound to casein and 21–24% in a low-molecular-weight form (bound mainly to citrate) (Lonnerdal *et al.*, 1982; Lonnerdal, 1985). In bovine milk, 2% of copper is in the fat fraction, 8% bound to whey proteins, 44% bound to casein and 47% is in a low-molecular-weight fraction (Lonnerdal, 1985). It has been reported that infants absorb much more copper from human milk than from a bovine milk-based formula (Food and Nutrition Board: Institute of Medicine, 2001). However, suckling rats absorbed about 80% of copper from human or bovine milks (Lonnerdal, 1985).

Milk and milk products are considered poor sources of copper (Pennington *et al.*, 1987; Renner *et al.*, 1989; Hunt and Meacham, 2001). Bovine milk contributes very little to the total dietary intake of copper.

10.9. Manganese

Manganese functions as an enzyme activator and is a constituent of several metalloenzymes (Leach and Harris, 1997; Food and Nutrition Board: Institute of Medicine, 2001; Nielsen, 2006). The numerous enzymes that can be activated by manganese include oxidoreductases, lyases, ligases, hydrolases, kinases, decarboxylases and transferases. Most enzymes activated by manganese in higher animals and humans can also be activated by other metals, especially magnesium; exceptions are the manganese-specific activation of glycosyltransferases, glutamine synthetase, farnesyl pyrophosphate synthetase and

phosphoenolpyruvate carboxykinase. The few manganese enzymes in higher animals and humans include arginase, pyruvate carboxylase and manganese superoxide dismutase. Manganese deficiency has been difficult to induce or identify in humans, and thus is generally not considered to be of much nutritional concern. The most likely case of manganese deficiency is that of a child on long-term parenteral nutrition who exhibited diffuse bone demineralization and poor growth that were corrected by manganese supplementation. Other probable signs of manganese deficiency are a finely scaling, minimally erythematous rash, decreased plasma cholesterol concentration and increased serum alkaline phosphatase activity. Low dietary manganese or low blood and tissue manganese have been associated with osteoporosis, diabetes, epilepsy, atherosclerosis, impaired wound healing and cataracts (Wedler, 1994). Findings from animal experiments have provided some support for these associations. In the past, manganese was considered to be one of the least toxic of the essential mineral elements (Food and Nutrition Board: Institute of Medicine, 2001; Nielsen, 2006). Recently, however, magnetic resonance imaging has shown that signals for manganese in brain are strongly associated with neurological symptoms (e.g., sleep disturbances) exhibited by patients with chronic liver disease. Such findings suggest that high intakes of manganese are ill-advised because of potential neurotoxicological effects, especially in people with compromised homeostatic mechanisms or infants whose homeostatic control of manganese is not fully developed.

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) found no functional criteria of manganese status to use for establishing an RDA for manganese for infants. Thus, they set AIs for manganese that reflected the mean intake of infants fed principally human milk; these AIs were 0.003 mg/d for age 0–6 months and 0.6 mg/d for age 6–12 months. Intake data were used to determine AIs for other age groups (Table 10.2).

Manganese is fairly uniformly distributed in soft tissues at concentrations generally less than 3 μg/g (Leach and Harris, 1997; Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005). Bone contains a substantial amount of manganese but should not be considered a reservoir. Serum or plasma manganese concentrations apparently are somewhat sensitive to large variations in manganese intake, with normal values near 1 μg/l (Food and Nutrition Board: Institute of Medicine, 2001). Mean concentrations of manganese in human milk were found to be highest during the first few days postpartum (6–12 μg/l); at 1 month it was found to be approximately 4.0 μg/l, and apparently declined to near 2.0 μg/l by 3 months postpartum (Food and Nutrition Board: Institute of Medicine, 2001). A correlation between dietary intake and human milk concentration has been reported (Vuori *et al.*, 1980). The mean concentration of manganese in mature bovine milk is 30 μg/l (Lonnerdal *et al.*, 1981). The concentration in

bovine colostrum is 100–160 μg/l but declines by over 50% during the first 3 d of lactation (de Maria, 1978). Oral supplementation of large doses of manganese for an extended time can increase the manganese content of milk from bovine milk (Archibald, 1958).

In human milk, 67% of manganese is bound to lactoferrin, 11% to casein, 18% to the fat globule membrane, and 4% in a low-molecular-weight form (Lonnerdal *et al*., 1985). In bovine milk, 67% of manganese is bound to casein, 1% in the fat globule membrane, 14% to whey proteins and 18% in a low-molecular-weight form (Lonnerdal *et al*., 1985). Very little is known about the availability of manganese in milk to infants. Manganese absorption by human adults was higher (8.4%) from human milk than from bovine milk (2.4%) or bovine milk-based infant formula (3.1%) (Davidsson *et al*., 1989). However, suckling rats absorbed about the same percentage (between 80 and 89%) of manganese from human milk, bovine milk and bovine milk-based formula (Keen *et al*., 1986); this finding suggests that infants absorb a greater percentage of manganese from milk than adults do.

Based on the fact that infants do not have fully developed homeostatic control of manganese, its content in formulae has raised concerns. In the early 1980s, supplementation of formulae resulted in some having 100–1000 times higher amounts of manganese than in human milk (Lonnerdal *et al*., 1983; Stastny *et al*., 1984). Even without manganese supplementation, milk-based infant formulae contain more manganese than human milk (2–4 μg/l) because of the higher concentration of this element in bovine milk (30 μg/l). Manganese supplementation of formulae has now ceased, so they now contain manganese in the range of 34–300 μg/l (Lonnerdal, 1985; Johnson *et al*., 1998), which is lower than the recommended UL of 335–600 μg/l in infant formula (Lonnerdal, 1985; Hambidge and Krebs, 1989). The FNB did not set a manganese UL for infants, but set 2 mg/d as an UL for children aged 1–3 yr (Food and Nutrition Board: Institute of Medicine, 2001).

For adults, milk and milk products are considered poor sources of manganese. Bovine milk has been estimated to contribute 1–3% of the dietary intake of manganese in Western countries (Hazell, 1985).

10.10. Selenium

Selenium is involved in many biological functions, including thyroid, neural and immune function and protection against oxidative stress and gastrointestinal disease (Food and Nutrition Board: Institute of Medicine, 2000; Burk and Hill, 2005; Esworthy *et al*., 2005; Sunde, 2006). Research with Coxsackievirus indicates that oxidative stress in a selenium-deficient host leads to genomic changes of an RNA virus that can increase virulence (Beck *et al*.,

2003). This finding has proven important for understanding Keshan disease, an endemic cardiomyopathy that appears to involve a viral co-factor along with severe selenium deficiency (Beck *et al*., 2003).

Most selenium in animal tissues is present as selenomethionine or selenocysteine. In plants, selenium is metabolized as though it was sulfur to form the amino acid selenomethionine, which is incorporated randomly in place of methionine into proteins. Selenomethionine cannot be synthesized by humans, and in plants and animals, this compound does not have a physiological function separate from that of methionine. The metabolite selenide ($H_2Se/HSe^-/Se^{2-}$) is derived from reductive metabolism of inorganic selenium (selenite or selenate) or from catabolism of selenocysteine derived from food selenoproteins or trans-selenation of food selenomethionine. As such, selenide occupies a central role in selenium metabolism; it is the obligate selenium donor in the co-translational synthesis of selenocysteine, in selenoproteins, accounting for the nutritional activity of the element.

Selenoproteins have been identified in all phylogenetic kingdoms, and, with one known exception (a structural role in a sperm selenoprotein), selenium serves as a catalytic component in selenoproteins (Sunde, 2006). Selenium is best known for its role as an antioxidant, especially as a component of the glutathione peroxidases. Extracellular glutathione peroxidase (GPX1) was the first of the 25 selenoproteins discovered to date. Even so, GPX1 *per se* does not appear to have a critical role under normal conditions and has been postulated to function as a biological selenium buffer than can be used to expand and store selenium before excretion mechanisms are activated (Sunde, 2006). However, knockout of GPX1 renders experimental animals susceptible to severe acute oxidative stress and overexpression confers extra protection against the insult (Lei *et al*., 2007).

Implementation of dietary selenium supplementation in the human population must be done with caution because selenium has a narrow therapeutic range and may be toxic (Sunde, 2006). For example, indiscriminate supplementation of AIDS patients with selenium may aid the human skin poxvirus, *Molluscum contagiosum*, during infection. This poxvirus has acquired in its genome a cDNA sequence for GPX1. Excess selenium for this pox GPX, with antioxidant activity, may block full activation of antivirus mechanisms, which include peroxidative stimulation of programmed cell death (Sunde, 2006). In addition, selenium and iodine deficiencies exacerbate each other to contribute to the etiology of endemic myxedematous cretinism. Administration of selenium alone apparently aggravates this disease by restoring selenium-dependent deiodinase activity, which in turn exacerbates iodine deficiency through increased use of iodine (Vanderpas *et al*., 1993). Finally, supranutritional selenium supplementation (200 μg/d) increased the incidence of type 2 diabetes (Stranges *et al*., 2007) in a

randomized trial with patients with a history of skin cancer. In a separate study, a probability sample of the US population also indicated that high serum selenium levels (>130 ng/ml) were positively associated with the prevalence of diabetes (Bleys *et al*., 2007). The NHANES III survey found that the mean serum selenium level for all Americans aged >4 yr is 125 ng/ml (Food and Nutrition Board: Institute of Medicine, 2000). Increased risk for diabetes suggests asymptomatic changes related to chronic selenium toxicity that could take place at intake levels lower than the currently defined UL (400 μg/d for adults). There are some contrary data; a substudy of the Health Professionals Follow-up Study found an inverse association between toenail selenium levels and the prevalence of diabetes at baseline (Rajpathak *et al*., 2005).

Additional findings from the supranutritional selenium supplementation study discussed above (Stranges *et al*., 2007) indicated that selenium supplementation (200 μg/d) increased the risk of squamous cell carcinoma (25%) and total non-melanoma skin cancer (17%) (Duffield-Lillico *et al*., 2003) despite reductions in total cancer incidence and mortality (Duffield-Lillico *et al*., 2002). Also, participants with baseline plasma selenium concentrations in the lowest two tertiles (<121.6 ng/ml) experienced reductions in total cancer incidence, whereas those in the highest tertile (>121.6 ng/ml) showed a possible small increase in total cancer risk.

The FNB found no functional criteria on which to base an RDA for selenium for infants. Thus, they set AIs for selenium (Table 10.2) that reflected the mean intake of infants fed principally human milk. In the age group 7–12 months, an amount was added for the contribution obtained from weaning foods. For children and adolescents aged 1–18 yr and all adults aged 19–50 yr, the selenium EARs were based on selenium intakes expected to maximize plasma extracellular glutathione peroxidase (GSHPx-3) activity. Because the aging process does not appear to impair selenium absorption or utilization, selenium EARs for adults aged >50 yr are also based on maximal GSHPx-3 activity. Data from the available human studies are generally compatible with the possibility that intakes of selenium above those needed to maximize selenoproteins have an anticancer effect in humans. However, confounding factors, small sample sizes and the potential for excess selenium supplementation to raise cancer risk collectively do not allow use of these studies as the basis for determining dietary selenium requirements at this time (Food and Nutrition Board: Institute of Medicine, 2000).

Dietary selenium is consumed principally in the form of selenomethionine (plant, animal and supplements sources), selenocysteine (mainly animal sources) or selenate and selenite (from supplements). Significant sources of dietary selenium vary widely because the wide range in soil selenium content is reflected in the selenium content in foods of plant origin as well as that of

grazing livestock. Accordingly, handbook values for selenium in foods should be considered unreliable unless confirmed by actual analysis. Drinking water usually has negligible selenium content (Sunde, 2006). In the United Kingdom, meat, poultry and fish make up the largest contribution to selenium intake. In North America, wheat-derived foods make a substantial contribution to selenium intake (Rayman, 2000). Average selenium intake ranges from $\leq$11 μg/d (in areas of endemic Keshan disease in China), to 25 μg/d (in New Zealand and Finland with no reported occurrence of Keshan disease), to 79–104 μg/d (across the United States), to 113–220 μg/d (across Canada)

Selenomethionine is a less available metabolic source of selenium than selenite or selenate but is more effective at increasing apparent selenium status because of its non-specific incorporation and retention in body proteins in place of methionine, a characteristic that increases its toxicity during long-term consumption. More than 90% of selenomethionine, the major dietary form of the element, is absorbed. Selenate and selenite are inorganic forms of selenium commonly used to fortify foods. Selenate is absorbed almost completely but a significant fraction is lost in the urine before tissue incorporation. Selenite has a more variable absorption (generally $>$50%) but once absorbed is retained better than selenate (Food and Nutrition Board: Institute of Medicine, 2000).

Human milk selenium apparently is not under homeostatic control because the concentration of selenium in breast milk is strongly dependent on the maternal intake of selenium (McGuire *et al.*, 1993; Bratter *et al.*, 1997; Lonnerdal, 1997). For example, the mean concentration of selenium in breast milk varies from ~10 μg/l (collected in Finland with low soil selenium) (Kumpulainen *et al.*, 1985), to ~22 μg/l (collected in St John's, Canada) (Hunt *et al.*, 2004), to 112 μg/l (collected in a seleniferous region in Venezuela; Bratter *et al.*, 1997). Selenium concentration in human milk typically ranges from ~12 to 30 μg/l (Arnaud *et al.*, 1993; al-Saleh *et al.*, 1997; Bianchi *et al.*, 1999; Hunt *et al.*, 2004; Yamawaki *et al.*, 2005). Ready-to-feed infant formulae manufactured by American companies have imputed selenium concentrations that range from 13 to 18 μg/l (US Department of Agriculture Agricultural Research Service, 2006). Compared to infants fed humanized formulae not supplemented with selenium, infants fed human milk or humanized formula supplemented with selenium have increased plasma and serum selenium concentrations and higher plasma glutathione peroxidase activity (Carver, 2003). The concentration of selenium in human milk decreases during the first 12 weeks of lactation (Levander *et al.*, 1987; Hunt *et al.*, 2004). Selenium speciation studies suggest that there is a minimum of five selenium-binding compounds in human milk (Bratter *et al.*, 1998). In bovine milk whey, most selenium is

incorporated non-specifically as selenomethionine into the two major whey proteins, β-lactoglobulin and α-lactalbumin. Another approximately 25–33% of bovine whey selenium is found in the low-molecular fraction (Hoac *et al.*, 2007). There are also distinctive selenium isotope distribution profiles in human milk whey. For example, there is considerably more ^{78}Se than either ^{82}Se or ^{77}Se in human milk whey (Bratter *et al.*, 1998).

10.11. Iodine

Iodine is an essential nutrient but has only one clearly established function, a component of the thyroid hormones. The thyroid gland selectively concentrates iodine and stores 70–80% of total body iodine (15–20 mg). In the thyroid gland, iodine is attached to the glycoprotein thyroglobulin to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT), the precursors of thyroid hormone. Through complex biochemical reactions, linkage of two DIT molecules produces thyroxine (T_4; tetraiodothyronine) and linkage of a MIT and DIT produces triiodothyronine (T_3). Enzymatic degradation of thyroglobulin releases T_4 and T_3 into circulation. In target tissues, T_4 is deiodinated to T_3, the main physiologically active form of thyroid hormone (Zimmermann, 2006). Other than the lactating breast, the significance of iodine concentration in extra-thyroidal tissues, such as the gastric mucosa, is less well understood (Josefsson *et al.*, 2002).

The thyroid hormones are important in regulating the basal metabolic rate and brain development. Iodine deficiency disorders (IDD) are characterized by loss of energy, mental retardation, hypothyroidism, goiter, cretinism and varying degrees of other growth and developmental abnormalities. The most serious adverse effect of iodine deficiency is damage to reproduction. For example, thyroid hormones are required for neuronal cell migration and myelination of the central nervous system. Maternal thyroid is the only source of T_4 and T_3 for the brain of the fetus because its thyroid gland does not start contributing to fetal requirements until mid-gestation in humans. Therefore, severe iodine deficiency during pregnancy increases the incidence of stillbirths, abortions and congenital abnormalities. Cretinism, the most severe form of neurological damage from foetal hypothyroidism, is characterized by gross mental retardation along with various degrees of deaf mutism and spasticity (Auso *et al.*, 2004; Perez-Lopez, 2007).

Many geographical regions inhabited by significant human populations have iodine-deficient soils as a result of soil leaching which depletes surface soils of iodide. Areas of frequent flooding and erosion (especially in south and southeast Asia), high alpine areas (e.g., the Alps, Andes, Atlas and Himalaya ranges) and several inland areas (including central Asia and Africa and central

and eastern Europe) are typically iodine deficient (Zimmermann, 2006). In goitrous children, iodine deficiency is exacerbated by inadequate intakes of selenium, iron or vitamin A, or by consumption of goitrogens. Some goitrogens are chemical agents (e.g., polychlorinated biphenyls, organophosphate pesticides and dioxin) that can permanently alter the pituitary–thyroid axis if exposure occurs during the perinatal period. Thyroid disruptor properties have also been attributed to several plant-derived substances. Examples are flavonoids, C-glycosylflavones (in millet), linamarin (from insufficiently processed cassava leaves) and thioglucosides (from cabbage, Brussel sprouts, broccoli and sorghum) (Fountoulakis *et al*., 2007).

The newest estimates of the iodine requirements for several age–sex groups have been revised upward. For example, the iodine requirement for infants aged 0–12 months are based on the current mean iodine intake of American infants exclusively fed human milk (Table 10.2). The AI iodine values established in 2001 for infants aged 0–6 months (110 μg/l) are much higher than the RDA values established earlier for the same age group (40 μg/d) (Food and Nutrition Board: Institute of Medicine, 2001). Likewise, the iodine AI for infants aged 7–12 months was increased also (130 μg versus 50 μg/d). The estimate average requirement for iodine during lactation is based on the average requirement of adolescent girls and non-pregnant women (95 μg/d) plus the average daily loss of iodine in human milk (114 μg/d). The RDA for iodine during lactation is 290 μg/d (Food and Nutrition Board: Institute of Medicine, 2001). Data appear insufficient to assess whether providing preterm infants with supplemental iodine (to match fetal accretion rates) prevents morbidity and mortality in preterm infants (Ibrahim *et al*., 2006).

Since 1990, the widespread introduction of iodized salt has greatly reduced the global prevalence of iodine deficiency. However, the World Health Organization recently estimated the worldwide prevalence of iodine deficiency at nearly 2 billion individuals, of whom 285 million are school-aged children (Zimmermann, 2006). Furthermore, the median iodine urine concentration (a standard indicator of iodine nutrition) has fallen in the United States from 321 μg/l in the 1970s to 145 μg/l currently (Zimmermann, 2006). The current average urine iodine concentration indicates adequate iodine nutriture but a reminder of the importance of regular monitoring. Socioeconomic status is not necessarily a predictor of iodine sufficiency for the general population. Despite the generally high standard of living in New Zealand, the mean iodine concentration in breast milk of mothers living in the South Island as late as 1998 and 1999 was 22 μg/l, a value only 15% of the average US value (Skeaff *et al*., 2005). The prevalence of iodine deficiency is the lowest (10.1%) in North and South Americas where the proportion of households consuming iodized salt is highest (90%). Europe has the highest

prevalence of iodine deficiency (59.9%), and the proportion of households consuming iodized salt is the lowest (27%). Most Europeans countries have weak or non-existent national programmes for iodine supplementation (Zimmermann, 2006). In Spain, almost half of pregnant women may have iodine deficiency (Perez-Lopez, 2007). In some countries, "silent iodine prophylaxis" occurs as improvements made in socioeconomic status allowed easier access to imported food products richer in iodine. Ironically, this approach sometimes has resulted in change of phenotypic expression of thyroid disease from endemic goiter to goiter associated with autoimmune thyroiditis from excessive iodine intakes (Fountoulakis *et al*., 2007).

Dietary iodine in the United States has generally been adequate since the 1920s but median urinary iodine level, a biomarker for dietary iodine, has decreased by over 50% from 1971 to 1994 among American women of childbearing age. However, The NHANES 2001–2002 data confirm the current stability of the US iodine intake and continued adequate iodine nutrition for the country (Caldwell *et al*., 2005).

Evidence supporting the protective effect of breast-feeding on the infant's thyroid metabolism is overwhelming, and breast milk is the only source of organic I, mostly thyroid hormones (T-4, T-3 and metabolites), in early infancy (Dorea, 2007). The iodine concentration in both human and bovine milks reflects maternal intake and thus are highly variable (see Table 10.1). In the United States, average breast milk iodine concentration of mothers is 146 μg/l. In countries with endemic IDD, breast milk iodine concentration is typically lower than 50 μg/l. Bovine milk is an important source of dietary iodine. The iodine content in bovine milk varies linearly with intake in the normal dietary range if other conditions are equal (Miller *et al*., 1975). Iodine content also varies with the season (Dahl *et al*., 2003) to the point that the incidence of thyrotoxicosis in Britain may be causally related to the high milk iodine levels in winter–spring (Phillips *et al*., 1988). In the United States, supplementation of animal feed with organic iodine has increased the amount of iodine in bovine milk by 300–500% between 1980 and 1986 (Food and Nutrition Board: Institute of Medicine, 2001). On the other hand, replacement of iodophors by other sanitizers in the dairy industry is associated with a decrease in iodine content of bovine milk and a re-emergence of iodine deficiency (Li *et al*., 2006).

Iodine enters bovine milk primarily as inorganic iodide, and iodine in bovine milk, as naturally secreted, is only about 10% bound. About 16% of total bovine milk iodine is in the cream but nearly all of the iodine in cream is in the non-fat (serum) portion (Miller *et al*., 1975). Cream separation, pasteurization and spray-drying of milk appear not to affect the concentration on a dry weight basis of either natural or iodophor-derived iodine in bovine milk. Boiling apparently does not induce iodine volatilization significantly

(0.02%) (Wheeler *et al*., 1983). The concentration of iodine is higher in whey cheeses than in casein cheeses (Dahl *et al*., 2003).

10.12. Molybdenum

Molybdenum is essential for the synthesis of a molybdenum co-factor containing a pterin nucleus that is required for the activity of sulfite oxidase, xanthine dehydrogenase and aldehyde oxidase in higher animals and humans (Nielsen, 2006). These enzymes catalyze the conversion of sulfite to sulfate, the transformation of hypoxanthine to xanthine and the oxidation and detoxification of various pyrimidines, purines and pteridines. Nutritional molybdenum deficiency has not been identified unequivocally in humans other than in one individual nourished by total parenteral nutrition (Abumrad *et al*., 1981). Thus, molybdenum generally is considered to be of no practical nutritional concern for humans. The individual fed by parenteral nutrition and individuals with genetic molybdoenzyme deficiencies (Johnson, 1997) have enabled the description of signs and symptoms of molybdenum deficiency; these include hypermethioninemia, hypouricemia, hyperoxypurinemia, hypouricosuria, low sulfate excretion and mental disturbances. Inadequate data exist to identify any adverse health outcomes caused by excessive molybdenum intake by normal, apparently healthy individuals.

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) found no functional criteria on which to base a RDA for infants. Thus, they set AIs for molybdenum that reflected the mean intake of infants fed principally human milk; these AIs were 2 μg/d for age 0–6 months and 3 μg/d for age 7–12 months. Extrapolation from the RDA for adults was the basis for setting RDAs for children (Table 10.2).

Concentrations of molybdenum in tissues, blood and milk vary with molybdenum intake (Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005). Highest concentrations of molybdenum are found in liver, kidney and bone (normally >1 mg/kg dry weight) (Johnson, 1997). The concentration of molybdenum in other tissues usually is between 0.14 and 0.20 mg/kg dry weight (Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005). Molybdenum concentration decreases rapidly in human milk with stage of lactation; for example, in a study of full-term infants, the median concentration of molybdenum in mothers' milk fell from 4 μg/l at 1 week postpartum to nearly undectable levels by 12 weeks postpartum (Friel *et al*., 1999). Another study found postpartum concentrations (μg/l) of 15 on day 1, 2.8 at 7–10 d and 2.6 by

1 month (Bougle *et al*., 1988). In addition, a study found that human milk contained 1.42 μg/l at 42–60 d postpartum and 1.78 μg/l at 293 d (Rossipal and Krachler, 1998). The FNB (Food and Nutrition Board: Institute of Medicine, 2001) used an average value of 2 μg molybdenum/l of human milk to calculate the AI for infants aged 0–6 months. The concentration of molybdenum is much higher in bovine milk and humanized infant formulae, which reports indicate ranges from 50 to 100 μg/l (Archibald, 1958; Tsongas *et al*., 1980; Hunt and Meacham, 2001). The concentration in milk can be increased substantially by ammonium molybdate supplementation (Archibald, 1951). Much of the molybdenum in human (Zeise and Zikakis, 1987) and bovine (Hart *et al*., 1967) milks is associated with xanthine oxidase. Xanthine oxidase activity of human colostrum is about 10% that of bovine milk (Oliver *et al*., 1971). Milk and milk products are considered rich sources of molybdenum for humans (Nielsen, 2006).

10.13. Cobalt

The only known function of cobalt in humans relates to its role in the structure of the cobalamins, a group of cobalt-containing compounds (corrinoids) (Stabler, 2006). The cobolamins are required as a co-factor for only two, but very important, enzymes in humans: L-methylmalonyl CoA mutase and methionine synthase. Only microorganisms retain the ability to synthesize cobalamins such that the source of cobalamins in all higher animals is the product of microbial synthesis. Characteristic of these compounds, cobalt is the central metal ion with four of the six coordination sites provided by a corrin ring (similar to the porphyrin ring found in heme). The fifth cobalt coordination site is through 5,6-dimethylbenzimidazole but the sixth coordination site, the center of reactivity, is variable: cyano, hydroxyl, glutathione or coenzyme forms (methyl or adenosyl). Thus, cyanocobalamin (the scientific term for "vitamin B_{12}") can be converted to either of the two cobalamin coenzymes that are active in human metabolism: methylcobalamin and 5-deoxyadenosylcobalamin. Ironically, cyanocobalamin is an artifact formed as a result of the use of cyanide in the purification procedures. Furthermore, cyanocobalamin, compared to hydroxocobalamin, binds to serum proteins less well and is excreted more rapidly (Tudhope *et al*., 1967).

Unless cobalt is absorbed as an integral part of vitamin B_{12}, the amount of cobalt in milk or other foods is not relevant *per se*. However, B_{12} deficiency is a serious problem in human populations and is caused either by an inadequate dietary intake of vitamin B_{12} or by malabsorption (e.g., lack of intrinsic

factor critical for B_{12} internalization and absorption in the ileal mucosa). Untreated vitamin B_{12} deficiency results in potentially irreversible neurological damage and life-threatening anemia. Infants fed predominantly human milk usually demonstrate clinical signs of B_{12} deficiency if the mother has been a strict vegetarian for at least 3 yr or has untreated pernicious anemia (Food and Nutrition Board: Institute of Medicine, 1998). Humans ordinarily obtain vitamin B_{12} from animal foods, mainly meat, fish and poultry. Milk and milk drinks are the second category contributing the most B_{12} to the diets of women. However, there is some evidence that boiling milk for 10 min reduces B_{12} content by $\sim$50%. This practice may of particular concern for certain lactovegetarians. For example, boiling milk was described as a common cooking practice among Hindu women in the United Kingdom (Stewart *et al.*, 1970).

10.14. Fluoride

Unequivocal or specific signs of fluorine deficiency have not been described for higher animals or humans (Nielsen, 2006). Fluoride, the ionic form of fluorine, has a well-established beneficial function in humans; it protects against pathological demineralization of calcified tissues as a pharmacological agent (Food and Nutrition Board: Institute of Medicine, 1997). Fluoride inhibits tooth enamel degradation by two separate mechanisms. Uptake of fluoride by tooth enamel crystallites and the formation of fluorhydroxyapatite during pre-eruptive tooth development reduce the risk of dental caries; the fluorhydroxyapatite is less acid-soluble than hydroxyapatite. After eruption, fluoride protects tooth enamel by reducing acid production by plaque bacteria and increasing the rate of enamel re-mineralization during an acidogenic challenge (Sieck *et al.*, 1990). Sodium fluoride (NaF) has been shown repeatedly and reproducibly to increase spinal bone mass in a dose-dependent manner. Yet, despite numerous studies, NaF has never been convincingly demonstrated to reduce the vertebral fracture rate in established spinal osteoporosis (Kleerekoper and Mendlovic, 1993).

Although fluoride intakes by fully breast-fed infants is low, fluoride intakes by partially breast-fed infants and by formula-fed infants is highly variable, depending primarily on the fluoride content of the water used to dilute concentrated liquid or powdered infant formula products. Prolonged exposure to high intakes of fluoride during infancy is much more common now than in the past because of a trend toward more extended feeding of formula (Fomon and Ekstrand, 1999). Infants fed human milk consume about 0.01 mg/d fluoride but those fed a formula reconstituted with fluoridated water may receive as much as 1.0 mg/d. For infants aged 0–6 months,

the current recommendation for adequate intake of fluoride is based on fluoride normally received from human milk, 0.01 mg/d, an amount 10–50 times less than the estimated safe and adequate intake established in 1989 (0.1–0.5 mg/d) (Food and Nutrition Board: Institute of Medicine, 1997).

Excessive intake of fluoride causes fluorosis, characterized by mottling of teeth (with hypomineralized and porous enamel), and sometimes the more severe skeletal fluorosis with lower extremity pain and microfracture (Hallanger Johnson *et al.*, 2007). For infants, the lowest-observed-adverse-effect level is 0.10 mg/kg/d for moderate enamel fluorosis. On this basis, the upper limit of fluorine is 0.7 and 0.9 mg fluoride/d for infants aged 0–6 months and 7–12 months, respectively.

The mean concentration of fluoride in mature human milk is approximately 18 μg/l (Table 10.1). The fluoride content of bovine milk is variable (Table 10.1). About 46–64% of the fluoride in bovine milk occurs as free fluoride ions with the remainder bound to proteins (Esala *et al.*, 1982). Essentially 100% of fluoride ingested in the fasted state as fluoridated water and 50–80% of fluoride ingested with food is absorbed from the gastrointestinal tract (Nielsen, 2006).

10.15. Boron

Boron is a bioactive element of low molecular weight (atomic weight = 10.81 $g \cdot mol^{-1}$) that is essential for all vascular plants (Loomis and Durst, 1992). There are four lines of evidence, derived in large part from research in animal models, that dietary boron can have beneficial effects on humans: (1) In amounts typically found in human and animal diets, boron improved bone health (independent of vitamin D status) by increasing bone development in frogs (Fort *et al.*, 2000), bone breaking strength in pigs (Armstrong *et al.*, 2000), broilers (Rossi *et al.*, 1993) and growing pullets (Wilson and Ruzler, 1997) and bone calcium concentration in chicks (Hunt *et al.*, 1994). (2) Boron interacts with specific steroid hormones; it counteracts the deleterious effects of dietary vitamin D deficiency on body growth in chicks (Bai and Hunt, 1996) and growth plate morphology in embryonic (King *et al.*, 1991) or hatched chicks (Hunt, 1989; Hunt *et al.*, 1994). In addition, boron increased the circulating concentration of 17β-estradiol in humans (Nielsen *et al.*, 1992; Naghii and Samman, 1997) and, together with injections of 17β-estradiol, increased trabecular bone surfaces in ovariectomized rats (Sheng *et al.*, 2001). (3) Physiologic amounts of boron apparently reduce the amount of insulin required to maintain plasma glucose in rats (Bakken and Hunt, 2003). (4) Borate or borate analogs can inhibit the *in vitro* activity of several enzymes in the eicosanoid pathway related to inflammation and

immune function (Spielberg *et al.*, 1979; Belver and Donaire, 1983; Rajendran *et al.*, 1994).

Evidence suggests that boron may be under metabolic control, possibly through the function of a boron transporter localized recently in tissues with excretory functions (kidney, parotid gland, submandibular gland, pancreas and liver) (Park *et al.*, 2004). Human blood boron concentrations are insensitive to changes in dietary boron intake (Hunt *et al.*, 1997), and the concentration of boron in milk from mothers of full-term healthy infants is highly conserved across time despite the fact that dietary intake of boron typically varies widely with food intake patterns and drinking water sources (Hunt *et al.*, 2004).

Because no biological function has been identified for boron in humans, neither an EAR nor an RDA nor an AI has been established for the element. The DRI UL for boron is 20 mg/d for adults; boron is considered to have a low order of toxicity (Table 10.2).

Boron is present naturally in all foods (Hunt and Meacham, 2001). Recent findings indicate that the mean concentration of boron in human milk from full-term mothers over the first 12 weeks of lactation (28 μg/l) is much less than that present in ready-to-eat infant formulae (~120 μg B/l) or in bovine whole milk (280 μg B/l; fluid, 3.3% milkfat) (Hunt and Meacham, 2001). Assuming that breast-fed full-term healthy infants consume milk at a daily rate of ~0.74 l during the first 4 months of lactation (Butte *et al.*, 1987), their total daily boron intake during the first 12 weeks of lactation is estimated at 0.022 mg/d. Even so, at a slightly older age (6–11 months), American infants are estimated to consume 0.55 mg B/d from all dietary sources (Hunt and Meacham, 2001). For males aged 51–70 yr, boron intake was estimated at 1.34 mg/d (1st percentile, 0.39; 99th percentile, 3.34 mg/d); for lactating females, 1.39 mg/d (1st percentile, 0.38; 99th percentile, 3.49 mg/d) (Food and Nutrition Board: Institute of Medicine, 2001). In postmenopausal women, diets that provide 0.36 mg B/2000 kcal (and otherwise nutritionally replete with minor supplements) result in negative boron balance (Hunt *et al.*, 1997).

The bioavailability of boron in human milk is likely to be high. It is known that low amounts of naturally occurring dietary boron (0.36 mg B/d) as well as supplemental inorganic forms (2.87 mg B/d; as orthoboric acid) are absorbed almost completely and excreted in the urine (Hunt *et al.*, 1997) of post-menopausal women. Little is known about the speciation of boron in natural foodstuffs. However, boron transport molecules in breast milk are probably associated with the soluble, instead of fat, fraction because the boron content of bovine whole milk (3.3% milkfat) and skim milk (0.08% milkfat) are not significantly different: 280 and 310 μg/l, respectively (Hunt and Meacham, 2001).

10.16. Chromium

Trivalent chromium was described as the glucose tolerance factor that alleviated impaired glucose tolerance in rats fed torula yeast-sucrose diets by Schwarz and Mertz (1959). Shortly thereafter, the glucose tolerance factor, or GTF, evolved into a speculated organic form of chromium. In 1977, it was reported that chromium supplementation overcame what was considered to be signs of chromium deficiency in a patient receiving total parenteral nutrition (Jeejeebhoy *et al*., 1977). This report occurred during the time when a mineral element often was accepted as essential based simply on evidence that dietary deprivation consistently induced a change in a biological function that was preventable or reversible by physiological amounts of the element. As a result, chromium was widely regarded as essential with a role in glucose metabolism. Thus, an estimated safe and adequate daily dietary intake was established for chromium by the US Food and Nutrition Board in 1980 (National Research Council, 1980). However, doubts about the nutritional essentiality of chromium arose after it was found that chromium analyses before 1980 were not valid and repeated efforts to characterize definitively a chromium-containing GTF were not successful. Additionally, studies on chromium essentiality subsequent to 1985 were not successful in showing that chromium deprivation consistently impaired a biological function that was prevented by physiological or nutritional amounts of chromium. Furthermore, early studies of chromium essentiality provided supplements to controls that resulted in chromium intakes over 100 times that of normal nutritional intakes. Thus, the early reports of chromium essentiality may have been describing pharmacologic or supranutritional actions of chromium.

Although chromium apparently is losing its designation as an established essential nutrient, there is much evidence showing that chromium is a bioactive and beneficial element for higher animals and humans. Numerous studies show that chromium beneficially affects circulating glucose, insulin and lipids in humans and a variety of animal species (Stoecker, 2006). A study that has received much attention found that 1000 μg chromium per day as chromium picolinate for 4 months markedly reduced blood glucose and glycated hemoglobin in diabetic Chinese subjects (Anderson *et al*., 1997b). The basis for this finding may be chromodulin, a naturally occurring oligopeptide composed of glycine, cysteine, aspartate and glutamate that tightly binds four chromium ions (Vincent and Bennett, 2007). Chromodulin apparently amplifies the tyrosine kinase activity of insulin-activated insulin receptor; the amplification is directly dependent upon the chromium content of chromodulin (Vincent and Bennett, 2007).

Methods of administration have resulted in controversy about the toxicity of chromium. Rats intravenously injected daily with 5 μg of chromium as a picolinate complex for 60 d exhibited signs of oxidative stress and DNA damage (Hepburn *et al*., 2003). In contrast, mice fed 5000 μg Cr/l in drinking water as chromium acetate for 17 months (Schroeder *et al*., 1963), and rats fed 100,000 μg Cr/kg diet as chromium picolinate for 24 weeks (Anderson *et al*., 1997a), exhibited no apparent signs of toxicity. However, a single acute oral dose of 895,000 μg of chromium as aqueous $CrCl_3$ induced increases in several markers of oxidative stress (Bagchi *et al*., 2002). Ingested trivalent chromium probably has a low order of toxicity because its complexes with oxygen-based ligands are usually electrochemically inactive and have poor ability to cross cell membranes.

The FNB did not find sufficient evidence to set EARs, so AIs were set for chromium-based estimated mean intakes (Food and Nutrition Board: Institute of Medicine, 2001). An AI of 0.2 μg/d for infants aged 0–6 months was based on a mean chromium intake principally from human milk. Intake data were used to determine AIs for other age groups (Table 10.2), including 5.5 μg/d for infants aged 7–12 months. An UL was not established for chromium because few serious adverse effects have been associated with excess intake of chromium.

Chromium is present in biological tissues at very low concentrations with picomolar concentrations occurring in liver, kidney, testis, bone and spleen (Stoecker, 2006). The average concentration of chromium in human milk has been estimated to be 0.25 μg/l (Casey *et al*., 1985). Bovine milk contains very little chromium. One reliable analysis indicated that chromium concentration in whole or skim milk was less than 0.5 μg/l (Anderson *et al*., 1992). Thus, milk and milk products are poor dietary sources of chromium. The chemical form of chromium in milk is unknown, but chromium in foods is generally in the trivalent state.

10.17. Arsenic

Arsenic can be toxic and carcinogenic, but some evidence also suggests that it may have beneficial actions in low amounts. Arsenic affects the utilization of labile methyl groups arising from methionine in higher animals. Thus, arsenic may beneficially affect the methylation of metabolically or genetically important molecules such as DNA and *S*-adenosylmethionine, the functions of which are dependent on or influenced by methyl incorporation. Most adverse effects associated with arsenic occur upon ingestion of inorganic arsenic present at high amounts in drinking water (Nielsen, 2006). The classical symptoms of arsenic toxicosis include numbness; tingling and paresthesia in

the extremities; decreased touch, pain and temperature sensation; and muscular tenderness. Chronic consumption of high amounts of inorganic arsenic in drinking water results in hyperkeratosis of the hands and feet, symmetrical pigmentation, conjunctivitis, tracheitis, acrocyanosis and polyneuritis and skin cancer.

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) set no dietary reference intakes (DRI) for arsenic. Animal data would suggest that intakes of 12–25 μg/d may be beneficial (Nielsen, 2006). Recent surveys indicate that arsenic intakes from food are less than this; the median intakes of adult men and women in the United States are approximately 2.0–2.9 and 1.7–2.1 μg/d, respectively (Food and Nutrition Board: Institute of Medicine, 2001). Earlier surveys indicated higher mean intakes of arsenic ranging from 23 to 72 μg/d (Food and Nutrition Board: Institute of Medicine, 2001).

The mean arsenic concentration in healthy adult human tissues was reported to be highly variable and between 40 and 90 μg/kg dry weight (Anke *et al*., 1997). Human milk was found to contain 0.2–6 μg arsenic/l, with no differences between colostrum and mature milk (Anke *et al*., 1997). Normal bovine milk was found to contain 15–60 μg arsenic/l (Anke *et al*., 1997). Increasing dietary arsenic did not increase the arsenic concentration in bovine milk. Reported arsenic concentrations in milk indicate that dairy products can contribute a substantial portion of the total daily dietary intake of arsenic.

10.18. Nickel

Nickel is not generally regarded as an essential nutrient for higher animals and humans, apparently because of the lack of a clearly defined specific biochemical function (Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005; Nielsen, 2006). However, nickel has been identified as an essential component of seven different enzymes involved in hydrolysis and redox reactions in some lower forms of life (plants and some bacteria). Interestingly, in microbes, the substrates or metabolites of the enzymatic reactions are dissolved gases of hydrogen, carbon monoxide, carbon dioxide, methane, oxygen and ammonia. Additionally, nickel deprivation studies show that it has beneficial actions in higher animals (Nielsen, 2006). Nickel deprivation detrimentally affects reproductive function and bone strength. Nickel deprivation also has been shown to increase sensitivity to salt, increase triacylglycerol levels in serum and liver and reduce the activity of enzymes that degrade glucose. Nickel might have a function that is associated with vitamin B_{12}, because lack of this vitamin inhibits the response to

nickel supplementation when dietary nickel is low, and nickel can alleviate vitamin B_{12} deficiency in higher animals. No chronic nickel toxicosis signs caused by oral intake have been reported for humans (Food and Nutrition Board: Institute of Medicine, 2001; Nielsen, 2006). Toxicosis through oral intake is limited to a few case reports of acute affects resulting from the ingestion of high doses of soluble nickel salts; this resulted in nausea, abdominal pain, diarrhea, vomiting and shortness in breath. Nickel-sensitive individuals may show a contact dermatitis-like reaction after a high intake (i.e., 0.6 mg) of soluble nickel after fasting.

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) set no RDA or AI for nickel, or an UL for infants; however, an UL of 0.2 mg/d was set for children aged 1–3 yr. Animal data suggest that intakes near 100 μg nickel per day may be beneficial (Nielsen, 2006). Typical daily dietary intakes for nickel are 70–400 μg/d (Food and Nutrition Board: Institute of Medicine, 2001).

Nickel is widely distributed in tissues at concentrations generally between 0.01 and 0.2 mg/kg wet weight (Eder and Kirchgessner, 1997; Nielsen, 2006). At 38 d postpartum, the mean nickel concentration in human milk was reported to be 1.2 μg/l (Casey and Neville, 1987). However, another report indicated that human milk contained a much higher concentration of nickel of 41 μg/l (Anke *et al.*, 1993). Mature bovine milk was found to contain 10–30 μg nickel/l (Casey, 1977; Anke *et al.*, 1993). Thus, dairy products could supply a significant proportion of the daily intake of nickel. This suggestion is supported by the finding that Canadian infants (age 0–12 months) had a nickel intake of 38 μg/d supplied by both human milk and formula consumption (Dabeka, 1989).

10.19. Silicon

Although silicon deprivation has been reported to produce aberrant metabolism of connective tissue and impaired immune function, silicon is still not generally accepted as an essential nutrient for higher animals. Recently, however, dietary silicon correlated positively and significantly with bone mineral density at all hip sites in men and pre-menopausal women in a large cross-sectional, population-based study (Jugdaohsingh *et al.*, 2004). No acute oral silicon toxicity signs have been identified for humans. Extremely high amounts of silicon are needed to have just relatively minor effects on growth in experimental animals (Committee on Minerals and Toxic Substances in Diets and Water for Animals, 2005). Silica stones have been found in people on long-term antacid therapy with magnesium trisilicate (Food and Nutrition Board: Institute of Medicine, 2001). The FNB did not set an UL for silicon

because of the lack of data showing adverse effects of silicon (Food and Nutrition Board: Institute of Medicine, 2001).

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) set no RDA or AI for silicon. Based on the US Total Diet Study (1991–1997), the median intake of silicon was 14 and 21 mg/d for women and men, respectively (Food and Nutrition Board: Institute of Medicine, 2001). The range of intakes (1st and 99th percentiles for all individuals) was 3.5–80 mg/d.

Animal studies indicate that the silicon content of connective tissue (e.g., aorta and tendon) is four to five times richer in silicon than soft tissues (e.g., liver, heart and muscle), which contain 2–10 μg/g dry weight (Carlisle, 1997). The mean concentration of silicon in human milk has been reported to be 0.47 mg/l up to 5 months postpartum (Anderson, 1992). Bovine milk apparently contains a similar amount of silicon (Jugdaohsingh *et al*., 2002). Thus, dairy products contribute very little to the dietary intake of silicon.

10.20. Vanadium

Vanadium is not generally accepted as an essential nutrient; however, it can be bioactive. Its ability to inhibit selectively protein tyrosine phosphatases at submicromolar concentrations probably explains a broad range of effects found for vanadium, most notably insulin-mimetic action. Additionally, limited animal deprivation studies suggested that vanadium deprivation alters thyroid hormone metabolism, impairs reproduction and induces bone and joint abnormalities. In lower forms of life, some haloperoxidases require vanadium. Vanadium is a relatively toxic element for humans (Nielsen, 1997; Food and Nutrition Board: Institute of Medicine, 2001). The threshold for toxicity through ingestion apparently is between 10 and 20 mg/d. Signs of toxicity include abdominal pain, anorexia, nausea and diarrhea.

The FNB (Food and Nutrition Board: Institute of Medicine, 2001) set no RDA or AI for vanadium, or an UL for infants. An UL of 1.8 mg/d was set for adults older than 18 yr. Typical intakes of vanadium are 6–18 μg/d for adults (Pennington and Jones, 1987).

Vanadium concentrations in tissues normally are <10 ng/g fresh weight (Nielsen, 1997). Bone apparently is a major sink for excessive retained vanadium. Human colostrum, transitional and mature milk were found to generally contain <1 ng vanadium/g dry weight (Kosta *et al*., 1983). Bovine milk may be a significant dietary source of vanadium because it reportedly contains about 3 ng vanadium/g (Myron *et al*., 1977). However, a fivefold variation in the vanadium content of milk, depending upon geographic location, has been described (Soremark, 1967). Apparently, the vanadium in milk

is water soluble, because it is relatively more abundant in skim milk than in butter (Myron *et al*., 1977).

10.21. Summary and Conclusions

Defining the roles of dietary minerals in human health has advanced remarkably in recent years. This new knowledge has led to significant revision of dietary recommendations and guidelines worldwide. Even so, there are still considerable deficits in our understanding of mineral nutrition. A primary challenge for current human mineral nutrition research is more complete characterizations of interactions among mineral salts and with other nutrients and environmental (e.g., infectious agents and exercise) and genetic factors to maintain health.

Progress in analytical methodologies has greatly improved the confidence in reported macromineral and trace element content in human and bovine milks and humanized infant formulae. With few exceptions (e.g., iodine and selenium), the values for human and bovine milks are remarkably similar across a wide range of geographical locations. Milk and milk products are significant dietary sources of arsenic, boron, calcium, cobalt (as vitamin B_{12}), iodine, molybdenum, nickel, phosphorus, potassium, sodium and zinc.

The mineral content of human breast milk remains the "gold standard" for establishing dietary mineral requirements of infants for the first 6 months of life. The concentrations of minerals in human milk are generally lower than those in bovine or humanized infant formulae. Thus, approaches for infant nutrition other than exclusive breast feeding should be taken with caution. Attempts to provide universal iron and folic acid supplementation in areas of high malaria transmission have caused increased morbidity and mortality. The high phosphorus content of bovine milk compared to human milk increases the risk of hyperphosphatemia in neonates fed bovine milk-derived formulae. Infants fed formulae may consume amounts of fluoride above the lowest-observed level known to induce moderate enamel fluorosis, a level nearly 100 times that consumed by exclusively breast-fed infants.

Recommendations for mineral nutrition pertinent to the consumption of milk and milk products must remain vigilant of changes in marketplace activities and changes in food choices that affect mineral intakes and bioavailability. Increased consumption of sweetened soft drinks and juices reduces milk consumption, a shift in dietary practice that tends to reduce calcium intake. Increased use of phosphate salts in processed foods for nonnutrient functions and increased consumption of those products increase the phosphorus content of the US food supply. Replacement of iodophors by other sanitizers in the dairy industry has contributed to the re-emergence of

iodine deficiency in some countries while increases in "silent iodine prophylaxis" has increased the incidence of iodine toxicity in other countries. Because zinc and iron requirements are not met by exclusive breast-feeding after approximately 6 months of age, proper selection of complementary foods with low phytate-to-mineral ratios becomes critical for normal growth and development.

References

Abrams, S.A. 2005. Calcium supplementation during childhood: long-term effects on bone mineralization. *Nutr. Rev.* **63**, 251–255.

Abrams, S.A. 2006. Building bones in babies: can and should we exceed the human milk-fed infant's rate of bone calcium accretion? *Nutr. Rev.* **64**, 487–494.

Abrams, S.A., Copeland, K.C., Gunn, S.K., Gundberg, C.M., Klein, K.O., Ellis, K.J. 2000. Calcium absorption, bone mass accumulation, and kinetics increase during early pubertal development in girls. *J. Clin. Endocrinol. Metab.* **85**, 1805–1809.

Abrams, S.A., O'Grien, K.O., Stuff, J.E. 1996. Changes in calcium kinetics associated with menarche. *J. Clin. Endocrinol. Metab.* **81**, 2017–2020.

Abumrad, N.N., Schneider, A.J., Steel, D., Rogers, L.S. 1981. Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. *Am. J. Clin. Nutr.* **34**, 2551–2559.

Al-Awadi, F.M., Srikumar, T.S. 2000. Trace-element status in milk and plasma of Kuwaiti and non-Kuwaiti lactating mothers. *Nutrition* **16**, 1069–1073.

al-Saleh, I., al-Doush, I., Faris, R. 1997. Selenium levels in breast milk and cow's milk: a preliminary report from Saudi Arabia. *J. Environ. Pathol. Toxicol. Oncol.* **16**, 41–46.

Alkanani, T., Friel, J.K., Jackson, S.E., Longerich, H.P. 1994. Comparison between digestion procedures for the multielemental analysis of milk by inductively coupled plasma mass spectrometry. *J. Agric. Food Chem.* **42**, 1965–1970.

American Academy of Pediatrics 2005. Breastfeeding and the use of human milk. *Pediatrics* **115**, 496–506.

Anderson, R.R. 1992. Comparison of trace elements in milk of four species. *J. Dairy Sci.* **75**, 3050–3055.

Anderson, R.A., Bryden, N.A., Polansky, M.M. 1992. Dietary chromium intake. Freely chosen diets, institutional diet, and individual foods. *Biol. Trace Elem. Res.* **32**, 117–121.

Anderson, R.A., Bryden, N.A., Polansky, M.M. 1997a. Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Am. Coll. Nutr.* **16**, 273–279.

Anderson, R.A., Cheng, N., Bryden, N.A., Polansky, M.M., Chi, J., Feng, J. 1997b. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes* **46**, 1786–1791.

Anderson, J.J.B., Klemmer, P.J., Watts, M.L.S., Garner, S.C., Calvo, M.S. 2006. Phosphorus. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 383–399, International Life Sciences Institute, Washington, DC.

Anke, M. 1986. Arsenic. In: *Trace Elements in Human and Animal Nutrition*, 5th edn. (W. Mertz, ed.), pp. 347–372, Academic Press, Orlando, Florida.

Anke, M., Angelow, M., Müller, M., Glei, M. 1993. Dietary trace element intake and excretion of man. In: *Trace Elements in Man and Animals – TEMA 8* (M. Anke, D. Meissner and C.F. Mills, eds.), pp. 180–188, Verlag Media Touristik, Gersdorf.

Anke, M., Glei, M., Arnhold, C., Drobner, C., Seifert, M.F. 1997. Arsenic. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 631–639, Marcel Dekker, New York.

Appel, L.J., Moore, T.J., Obarzanek, E., Vollmer, W.M., Svetkey, L.P., Sacks, F.M., Bray, G.A., Vogt, T.M., Cutler, J.A., Windhauser, M.M., Lin, P.H., Karanja, N. 1997. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N. Engl. J. Med.* **336**, 1117–1124.

Archibald, J.G. 1951. Molybdenum in bovine milk. *J. Dairy Sci.* **34**, 1026–1029.

Archibald, J.G. 1958. Trace elements in milk: a review – part II. *Dairy Sci. Abstr.* **20**, 799–812.

Armstrong, T.A., Spears, J.W., Crenshaw, T.D., Nielsen, F.H. 2000. Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites. *J. Nutr.* **130**, 2575–2581.

Arnaud, J., Prual, A., Preziosi, P., Favier, A., Hercberg, S. 1993. Selenium determination in human milk in Niger: influence of maternal status. *J. Trace Elem. Electrolytes Health Dis.* **7**, 199–204.

Atac, A., Altay, N., Olmez, S. 2001. Fluoride content of infant formulas and market milk in Turkey. *Turk. J. Pediatr.* **43**, 102–104.

Auso, E., Lavado-Autric, R., Cuevas, E., Del Rey, F.E., Morreale De Escobar, G., Berbel, P. 2004. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. *Endocrinology* **145**, 4037–4047.

Awumey, E.M., Bukoski, R.D. 2006. Cellular functions and fluxes of calcium. In: *Calcium in Human Health* (C.M. Weaver and R.P. Heaney, eds.), pp. 13–35, Humana Press, Totowa, NJ.

Bader, N., Moller, U., Leiterer, M., Franke, K., Jahreis, G. 2005. Pilot study: tendency of increasing iodine content in human milk and cow's milk. *Exp. Clin. Endocrinol. Diabetes* **113**, 8–12.

Bagchi, D., Stohs, S.J., Downs, B.W., Bagchi, M., Preuss, H.G. 2002. Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology* **180**, 5–22.

Bai, Y., Hunt, C.D. 1996. Dietary boron enhances efficacy of cholecalciferol in broiler chicks. *J. Trace Elem. Exp. Med.* **9**, 117–132.

Bakken, N.A., Hunt, C.D. 2003. Dietary boron decreases peak pancreatic *in situ* insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *J. Nutr.* **133**, 3516–3522.

Baron, J.A., Beach, M., Mandel, J.S., van Stolk, R.U., Haile, R.W., Sandler, R.S., Rothstein, R., Summers, R.W., Snover, D.C., Beck, G.J., Bond, J.H., Greenberg, E.R. 1999. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N. Engl. J. Med.* **340**, 101–107.

Bazrafshan, H.R., Mohammadian, S., Ordookhani, A., Abedini, A., Davoudy, R., Pearce, E.N., Hedayati, M., Azizi, F., Braverman, L.E. 2005. An assessment of urinary and breast milk iodine concentrations in lactating mothers from Gorgan, Iran, 2003. *Thyroid* **15**, 1165–1168.

Beard, J. 2006. Iron. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 430–444, International Life Sciences Institute, Washington, DC.

Beard, J.L. 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. *J. Nutr.* **131**, 568S–580S.

Beck, M.A., Levander, O.A., Handy, J. 2003. Selenium deficiency and viral infection. *J. Nutr.* **133**, 1463S–1467S.

Belver, A., Donaire, J.P. 1983. Partial purification of soluble lipoxygenase of sunflower cotyledons: action of boron on the enzyme and lipid constituents. *Z. Pflanzenphysiol.* **109**, 309–317.

Bianchi, M.L., Cruz, A., Zanetti, M.A., Dorea, J.G. 1999. Dietary intake of selenium and its concentration in breast milk. *Biol. Trace Elem. Res.* **70**, 273–277.

Blakeborough, P., Salter, D.N., Gurr, M.I. 1983. Zinc binding in cow's milk and human milk. *Biochem. J.* **209**, 505–512.

Bleys, J., Navas-Acien, A., Guallar, E. 2007. Serum selenium and diabetes in U.S. adults. *Diabetes Care* **30**, 829–834.

Bougle, D., Bureau, F., Foucault, P., Duhamel, J.F., Muller, G., Drosdowsky, M. 1988. Molybdenum content of term and preterm human milk during the first 2 months of lactation. *Am. J. Clin. Nutr.* **48**, 652–654.

Bratter, P., Blasco, I.N., Negretti de Bratter, V.E., Raab, A. 1998. Speciation as an analytical aid in trace element research in infant nutrition. *Analyst* **123**, 821–826.

Bratter, P., Bratter, V.E., Recknagel, S., Brunetto, R. 1997. Maternal selenium status influences the concentration and binding pattern of zinc in human milk. *J. Trace Elem. Med. Biol.* **11**, 203–209.

Brock, J.H. 2002. The physiology of lactoferrin. *Biochem. Cell Biol.* **80**, 1–6.

Bronner, F., Abrams, S.A. 1998. Development and regulation of calcium metabolism in healthy girls. *J. Nutr.* **128**, 1474–1480.

Bruhn, J.C., Franke, A.A. 1983. Iodine in human milk. *J. Dairy Sci.* **66**, 1396–1398.

Bucher, H.C., Cook, R.J., Guyatt, G.H., Lang, J.D., Cook, D.J., Hatala, R., Hunt, D.L. 1996. Effects of dietary calcium supplementation on blood pressure. A meta-analysis of randomized controlled trials. *J. Am. Med. Assoc.* **275**, 1016–1022.

Burk, R.F., Hill, K.E. 2005. Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annu. Rev. Nutr.* **25**, 215–235.

Butte, N.F., Garza, C., Johnson, C.A., Smith, E.O., Nichols, B.L. 1984. Longitudinal changes in milk composition of mothers delivering preterm and term infants. *Early Hum. Dev.* **9**, 153–162.

Butte, N.F., Garza, C., Smith, E.O., Wills, C., Nichols, B.L. 1987. Macro- and trace-mineral intakes of exclusively breast-fed infants. *Am. J. Clin. Nutr.* **45**, 42–48.

Buzalaf, M.A., de Almeida, B.S., Cardoso, V.E., Olympio, K.P., Furlani Tde, A. 2004. Total and acid-soluble fluoride content of infant cereals, beverages and biscuits from Brazil. *Food Addit. Contam.* **21**, 210–215.

Cade, J.E., Burley, V.J., Greenwood, D.C. 2004. The UK Women's Cohort Study: comparison of vegetarians, fish-eaters and meat-eaters. *Public Health Nutr.* **7**, 871–878.

Caldwell, K.L., Jones, R., Hollowell, J.G. 2005. Urinary iodine concentration: United States National Health and Nutrition Examination Survey 2001–2002. *Thyroid* **15**, 692–699.

Carlisle, E.M. 1997. Silicon. In: Handbook of Nutritionally Essential Mineral Elements (B.L. O'Dell and R.A. Sunde, eds.), pp. 603–618, Marcel Dekker, New York.

Carver, J.D. 2003. Advances in nutritional modifications of infant formulas. *Am. J. Clin. Nutr.* **77**, 1550S–1554S.

Casey, C.E. 1977. The content of some trace elements in infant milk foods and supplements available in New Zealand. *NZ Med. J.* **85**, 275–278.

Casey, C.E., Hambidge, K.M., Neville, M.C. 1985. Studies in human lactation: zinc, copper, manganese and chromium in human milk in the first month of lactation. *Am. J. Clin. Nutr.* **41**, 1193–1200.

Casey, C.E., Neville, M.C. 1987. Studies in human lactation 3: molybdenum and nickel in human milk during the first month of lactation. *Am. J. Clin. Nutr.* **45**, 921–926.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D., Meunier, P.J. 1992. Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N. Engl. J. Med.* **327**, 1637–1642.

Chevalley, T., Rizzoli, R., Nydegger, V., Slosman, D., Rapin, C.H., Michel, J.P., Vasey, H., Bonjour, J.P. 1994. Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin-D-replete elderly patients. *Osteoporos. Int.* **4**, 245–252.

Chuckpaiwong, S., Nakornchai, S., Surarit, R., Soo-ampon, S. 2000. Fluoride analysis of human milk in remote areas of Thailand. *Southeast Asian J. Trop. Med. Public Health* **31**, 583–586.

Committee on Minerals and Toxic Substances in Diets and Water for Animals (2005). *Mineral Tolerance of Animals*. National Academies Press, Washington, DC.

Cousins, R.J. 2006. Zinc. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 445–457, International Life Sciences Institute, Washington, DC.

Cumming, R.G., Nevitt, M.C. 1997. Calcium for prevention of osteoporotic fractures in post-menopausal women. *J. Bone Miner. Res.* **12**, 1321–1329.

Curhan, G.C., Willett, W.C., Rimm, E.B., Stampfer, M.J. 1993. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N. Engl. J. Med.* **328**, 833–838.

Curhan, G.C., Willett, W.C., Speizer, F.E., Spiegelman, D., Stampfer, M.J. 1997. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Ann. Intern. Med.* **126**, 497–504.

Dabeka, R.W. 1989. Survey of lead, cadmium, cobalt and nickel in infant formulas and evaporated milks and estimation of dietary intakes of the elements by infants 0-12 months old. *Sci. Total Environ.* **89**, 279–289.

Dahl, L., Opsahl, J.A., Meltzer, H.M., Julshamn, K. 2003. Iodine concentration in Norwegian milk and dairy products. *Br. J. Nutr.* **90**, 679–685.

Davidovits, M., Levy, Y., Avramovitz, T., Eisenstein, B. 2006. Calcium-deficiency rickets in a four-year-old boy with milk allergy. *J. Pediatr.* **122**, 249–251.

Davidsson, L., Cederblad, A., Lonnerdal, B., Sandstrom, B. 1989. Manganese absorption from human milk, cow's milk, and infant formulas in humans. *Am. J. Dis. Child.* **143**, 823–827.

Davidsson, L., Kastenmayer, P., Yuen, M., Lonnerdal, B., Hurrell, R.F. 1994. Influence of lactoferrin on iron absorption from human milk in infants. *Pediatr. Res.* **35**, 117–124.

Dawson-Hughes, B. 2006. Calcium throughout the life cycle. In: *Calcium in Human Health* (C.M. Weaver and R.P. Heaney, eds.), pp. 371–385, Humana Press, Totowa, NJ.

Dawson-Hughes, B., Harris, S.S., Krall, E.A., Dallal, G.E. 1997. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N. Engl. J. Med.* **337**, 670–676.

Dawson-Hughes, B., Harris, S.S., Krall, E.A., Dallal, G.E. 2000. Effect of withdrawal of calcium and vitamin D supplements on bone mass in elderly men and women. *Am. J. Clin. Nutr.* **72**, 745–750.

de Maria, C.G. 1978. Trace element content in colostrum of different ruminant species at various post-partum intervals. *Ann. Rech. Vet.* **9**, 277–280.

Dewey, K.G., Lonnerdal, B. 1983. Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. *J. Pediatr. Gastroenterol. Nutr.* **2**, 497–506.

Domellof, M., Lonnerdal, B., Abrams, S.A., Hernell, O. 2002. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. *Am. J. Clin. Nutr.* **76**, 198–204.

Domellof, M., Lonnerdal, B., Dewey, K.G., Cohen, R.J., Hernell, O. 2004. Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *Am. J. Clin. Nutr.* **79**, 111–115.

Dorea, J.G. 2007. Maternal smoking and infant feeding: breastfeeding is better and safer. *Matern. Child Health J.* **11**, 287–291.

Duffield-Lillico, A.J., Reid, M.E., Turnbull, B.W., Combs, G.F., Jr., Slate, E.H., Fischbach, L. A., Marshall, J.R., Clark, L.C. 2002. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiol. Biomarkers Prev.* **11**, 630–639.

Duffield-Lillico, A.J., Slate, E.H., Reid, M.E., Turnbull, B.W., Wilkins, P.A., Combs, G.F., Jr., Park, H.K., Gross, E.G., Graham, G.F., Stratton, M.S., Marshall, J.R., Clark, L.C. 2003. Selenium supplementation and secondary prevention of nonmelanoma skin cancer in a randomized trial. *J. Natl. Cancer Inst.* **95**, 1477–1481.

Eder, K., Kirchgessner, M. 1997. Nickel. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 439–451, Marcel Dekker, New York.

Elders, P.J., Netelenbos, J.C., Lips, P., van Ginkel, F.C., Khoe, E., Leeuwenkamp, O.R., Hackeng, W.H., van der Stelt, P.F. 1991. Calcium supplementation reduces vertebral bone loss in perimenopausal women: a controlled trial in 248 women between 46 and 55 years of age. *J. Clin. Endocrinol. Metab.* **73**, 533–540.

Emmett, P.M., Rogers, I.S. 1997. Properties of human milk and their relationship with maternal nutrition. *Early. Hum. Dev.* **49** Suppl, S7–28.

Ereman, R.R., Lonnerdal, B., Dewey, K.G. 1987. Maternal sodium intake does not affect postprandial sodium concentrations in human milk. *J. Nutr.* **117**, 1154–1157.

Esala, S., Vuori, E., Helle, A. 1982. Effect of maternal fluorine intake on breast milk fluorine content. *Br. J. Nutr.* **48**, 201–204.

Esworthy, R.S., Yang, L., Frankel, P.H., Chu, F.F. 2005. Epithelium-specific glutathione peroxidase, Gpx2, is involved in the prevention of intestinal inflammation in selenium-deficient mice. *J. Nutr.* **135**, 740–745.

Favus, M.J., Bushinsky, D.A., Lemann, J.J. 2006. Regulation of calcuim, magnesium, and phosphate metabolism. In: *Rimer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th edn. (1 M.J. Favus, ed.), pp. 76–83, American Society for Bone and Mineral Research, Washington, DC.

Flynn, A. 1992. Minerals and trace elements in milk. *Adv. Food Nutre. Res.* **36**, 209–252

Flynn, A., Shortt, C., Morrissey, P.A. 1990. Sodium and potassium intakes in Ireland. *Proc. Nutr. Soc.* **49**, 323–332.

Fomon, S.J., Ekstrand, J. 1999. Fluoride intake by infants. *J. Public Health Dent.* 59, 229–234.

Fomon, S.J., Ziegler, E.E., Nelson, S.E. 1993. Erythrocyte incorporation of ingested ^{58}Fe by 56-day-old breast-fed and formula-fed infants. *Pediatr. Res.* **33**, 573–576.

Food and Nutrition Board: Institute of Medicine 1997, *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy Press, Washington, DC.

Food and Nutrition Board: Institute of Medicine 1998, *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline*. National Academy Press, Washington, DC.

Food and Nutrition Board: Institute of Medicine 2000, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. National Academy Press, Washington, DC.

Food and Nutrition Board: Institute of Medicine 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press, Washington, D.C.

Food and Nutrition Board: Institute of Medicine 2005. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. National Academy Press, Washington, DC.

Fort, D.J., Stover, E.L., Rogers, R.L., Copley, H.F., Morgan, L.A., Foster, E.R. 2000. Chronic boron or copper deficiency induces limb teratogenesis in *Xenopus*. *Biol. Trace Elem. Res.* **77**, 173–187.

Fountoulakis, S., Philippou, G., Tsatsoulis, A. 2007. The role of iodine in the evolution of thyroid disease in Greece: from endemic goiter to thyroid autoimmunity. *Hormones (Athens)* **6**, 25–35.

Francis, R.M. 2008. What do we currently know about nutrition and bone health in relation to United Kingdom public health policy with particular reference to calcium and vitamin D? *Br. J. Nutr.* **99**, 155–159.

Fransson, G.B., Lonnerdal, B. 1983. Distribution of trace elements and minerals in human and cow's milk. *Pediatr. Res.* **17**, 912–915.

Friel, J.K., Andrews, W.L., Jackson, S.E., Longerich, H.P., Mercer, C., McDonald, A., Dawson, B., Sutradhar, B. 1999. Elemental composition of human milk from mothers of premature and full-term infants during the first 3 months of lactation. *Biol. Trace Elem. Res.* **67**, 225–247.

Friel, J.K., Aziz, K., Andrews, W.L., Harding, S.V., Courage, M.L., Adams, R.J. 2003. A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J. Pediatr.* **143**, 582–586.

Garcia-Aznar, J.M., Rueberg, T., Doblare, M. 2005. A bone remodelling model coupling micro-damage growth and repair by 3D BMU-activity. *Biomech. Model Mechanobiol.* **4**, 147–167.

Garcia-Lorda, P., Salas-Salvado, J., Fernandez Ballart, J., Murphy, M.M., Bullo, M., Arija, V. 2007. Dietary calcium and body mass index in a Mediterranean population. *Int. J. Vitam. Nutr. Res.* **77**, 34–40.

Gaucheron, F. 2005. The minerals of milk. *Reprod. Nutr. Dev.* **45**, 473–483.

Giovannucci, E., Rimm, E.B., Wolk, A., Ascherio, A., Stampfer, M.J., Colditz, G.A., Willett, W.C. 1998. Calcium and fructose intake in relation to risk of prostate cancer. *Cancer Res.* **58**, 442–447.

Goes, H.C., Torres, A.G., Donangelo, C.M., Trugo, N.M. 2002. Nutrient composition of banked human milk in Brazil and influence of processing on zinc distribution in milk fractions. *Nutrition* **18**, 590–594.

Griffin, I.J., Abrams, S.A. 2001. Iron and breastfeeding. *Pediatr. Clin. North Am.* **48**, 401–413.

Hallanger Johnson, J.E., Kearns, A.E., Doran, P.M., Khoo, T.K., Wermers, R.A. 2007. Fluoride-related bone disease associated with habitual tea consumption. *Mayo Clin. Proc.* **82**, 719–724.

Hambidge, K.M., Krebs, N.F. 1989. Upper limits of zinc, copper and manganese in infant formulas. *J. Nutr.* **119**, 1861–1864.

Hamill, T.W., Young, E.R., Eitenmiller, R.R., Hogarty, C.D., Soliman, A.M. 1989. Ca, P, Mg, Cu, Mn, Na, K and Cl contents of infant formulas manufactured in the United States. *J. Food Compos. Anal.* **2**, 132–139.

Han, O., Failla, M.L., Hill, A.D., Morris, E.R., Smith, J.C., Jr. 1995. Reduction of Fe(III) is required for uptake of nonheme iron by Caco-2 cells. *J. Nutr.* **125**, 1291–1299.

Harinarayan, C.V., Ramalakshmi, T., Prasad, U.V., Sudhakar, D., Srinivasarao, P.V., Sarma, K.V., Kumar, E.G. 2007. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. *Am. J. Clin. Nutr.* **85**, 1062–1067.

Harris, E.D. 1997. Copper. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 231–273, Marcel Dekker, New York.

Hart, L.I., Owen, E.C., Proudfoot, R. 1967. The influence of dietary molybdenum on the xanthine oxidase activity of the milk of ruminants. *Br. J. Nutr.* **21**, 617–630.

Harvey, N., Earl, S., Cooper, C. 2006. Epidemiology of osteoporotic fracgtures. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* (M.J. Favus, ed.), pp. 244–248, American Society for Bone and Mineral Research, Washington, DC.

Hazell, T. 1985. Minerals in foods: dietary sources, chemical forms, interactions, bioavailability. *World Rev. Nutr. Diet.* **46**, 1–123.

Heaney, R.P. 1996. Calcium. In: *Principles of Bone Biology* (J.P. Bilezikian, L.G. Raisz and G.A. Rodan, eds.), pp. 1007–1018, Academic Press, San Diego.

Heaney, R.P. 2006. Bone as the calcium nutrient reserve. In: *Calcium in Human Health* (C.M. Weaver and R.P. Heaney, eds.), pp. 7–12, Humana Press, Totowa, NJ.

Hepburn, D.D.D., Burney, J.M., Woski, S.A., Vincent, J.B. 2003. The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids *in vivo*. *Polyhedron* **22**, 455–463.

Hidiroglou, M., Proulx, J.G. 1982. Factors affecting the calcium, magnesium and phosphorus content of beef cow milk. *Can. J. Comp. Med.* **46**, 212–214.

Hitz, M.F., Jensen, J.E., Eskildsen, P.C. 2007. Bone mineral density and bone markers in patients with a recent low-energy fracture: effect of 1 y of treatment with calcium and vitamin D. *Am. J. Clin. Nutr.* **86**, 251–259.

Hoac, T., Lundh, T., Purup, S., Onning, G., Sejrsen, K., Akesson, B. 2007. Separation of selenium, zinc, and copper compounds in bovine whey using size exclusion chromatography linked to inductively coupled plasma mass spectrometry. *J. Agric. Food Chem.* **55**, 4237–4243.

Holt, C. 1985. The milk salts: their secretion, concentrations and physical chemistry. In: *Developments of Diary Chemistry–3: Lactose and Minor Constituents* (P.F. Fox, ed.), pp. 143–181, Elsevier Applied Science Publishers, London.

Holt, C. 1993. Interrelationships of the concentrations of some ionic constituents of human milk and comparison with cow and goat milks. *Comp. Biochem. Physiol. Comp. Physiol.* **104**, 35–41.

Hunt, C.D. 1988. Boron homeostasis in the cholecalciferol-deficient chick. *Proc. N. Dakota Acad. Sci.* **42**, 60.

Hunt, C.D. 1989. Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. *Biol. Trace Elem. Res.* **22**, 201–220.

Hunt, C.D., Butte, N.F., Johnson, L.K. 2005. Boron concentrations in milk from mothers of exclusively breast-fed healthy full-term infants are stable during the first four months of lactation. *J. Nutr.* **135**, 2383–2386.

Hunt, C.D., Friel, J.K., Johnson, L.K. 2004. Boron concentrations in milk from mothers of full-term and premature infants. *Am. J. Clin. Nutr.* **80**, 1327–1333.

Hunt, C.D., Herbel, J.L., Idso, J.P. 1994. Dietary boron modifies the effects of vitamin D_3 nutriture on indices of energy substrate utilization and mineral metabolism in the chick. *J. Bone Miner. Res.* **9**, 171–181.

Hunt, C.D., Herbel, J.L., Nielsen, F.H. 1997. Metabolic response of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium, and magnesium absorption and retention and blood mineral concentrations. *Am. J. Clin. Nutr.* **65**, 803–813.

Hunt, C.D., Johnson, L.K. 2006. Magnesium requirements: new estimations for men and women by cross-sectional statistical analyses of metabolic magnesium balance data. *Am. J. Clin. Nutr.* **84**, 843–852.

Hunt, C.D., Johnson, L.K. 2007. Calcium requirement: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. *Am. J. Clin. Nutr.* **86**, 1054–1063.

Hunt, C.D., Meacham, S.L. 2001. Aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc: concentrations in common Western foods and estimated daily intakes by infants, toddlers, and male and female adolescents, adults, and seniors in the United States. *J. Am. Diet. Assoc.* **101**, 1058–1060; Table 1 available at www.eatright.com/images/journal/0901/hunt_tables.pdf.

Hunt, J.R., Roughead, Z.K. 1999. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. *Am. J. Clin. Nutr.* **69**, 944–952.

Ibrahim, M., Sinn, J., McGuire, W. 2006. Iodine supplementation for the prevention of mortality and adverse neurodevelopmental outcomes in preterm infants. *Cochrane Database Syst. Rev.*, CD005253.

Jackson, R.D., LaCroix, A.Z., Gass, M., Wallace, R.B., Robbins, J., Lewis, C.E., Bassford, T., Beresford, S.A., Black, H.R., Blanchette, P., Bonds, D.E., Brunner, R.L., Brzyski, R.G., Caan, B., Cauley, J.A., Chlebowski, R.T., Cummings, S.R., Granek, I., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Johnson, K.C., Judd, H., Kotchen, J.M., Kuller, L.H., Langer, R.D., Lasser, N.L., Limacher, M.C., Ludlam, S., Manson, J.E., Margolis, K.L., McGowan, J., Ockene, J.K., O'Sullivan, M.J., Phillips, L., Prentice, R.L., Sarto, G.E., Stefanick, M.L., Van Horn, L., Wactawski-Wende, J., Whitlock, E., Anderson, G.L., Assaf, A.R., Barad, D. 2006. Calcium plus vitamin D supplementation and the risk of fractures. *N. Engl. J. Med.* **354**, 669–683.

Jeejeebhoy, K.N., Chu, R.C., Marliss, E.B., Greenberg, G.R., Bruce-Robertson, A. 1977. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr.* **30**, 531–538.

Johnson, J.L. 1997. Molybdenum. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 413–438, Marcel Dekker, New York.

Johnson, M.A., Smith, M.M., Edmonds, J.T. 1998. Copper, iron, zinc, and manganese in dietary supplements, infant formulas, and ready-to-eat breakfast cereals. *Am. J. Clin. Nutr.* **67**, 1035S–1040S.

Josefsson, M., Grunditz, T., Ohlsson, T., Ekblad, E. 2002. Sodium/iodide-symporter: distribution in different mammals and role in entero-thyroid circulation of iodide. *Acta Physiol. Scand.* **175**, 129–137.

Jugdaohsingh, R., Anderson, S.H., Tucker, K.L., Elliott, H., Kiel, D.P., Thompson, R.P., Powell, J.J. 2002. Dietary silicon intake and absorption. *Am. J. Clin. Nutr.* **75**, 887–893.

Jugdaohsingh, R., Tucker, K.L., Qiao, N., Cupples, L.A., Kiel, D.P., Powell, J.J. 2004. Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the Framingham offspring cohort. *J. Bone Miner. Res.* **19**, 297–307.

Juniper, D.T., Phipps, R.H., Jones, A.K., Bertin, G. 2006. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine, and feces. *J. Dairy Sci.* **89**, 3544–3551.

Keen, C.L., Bell, J.G., Lonnerdal, B. 1986. The effect of age on manganese uptake and retention from milk and infant formulas in rats. *J. Nutr.* **116**, 395–402.

Kelleher, S.L., Lonnerdal, B. 2005. Molecular regulation of milk trace mineral homeostasis. *Mol. Aspects Med.* **26**, 328–339.

Kerstetter, J.E., Wall, D.E., O'Brien, K.O., Caseria, D.M., Insogna, K.L. 2006. Meat and soy protein affect calcium homeostasis in healthy women. *J. Nutr.* **136**, 1890–1895.

King, N., Odom, T.W., Sampson, H.W., Yersin, A.G. 1991. The effect of *in ovo* boron supplementation on bone mineralization of the vitamin D-deficient chicken embryo. *Biol. Trace Elem. Res.* **31**, 223–233.

Kirchhoff, P., Geibel, J.P. 2006. Role of calcium and other trace elements in the gastrointestinal physiology. *World J. Gastroenterol.* **12**, 3229–3236.

Kirksey, A., Ernst, J.A., Roepke, J.L., Tsai, T.L. 1979. Influence of mineral intake and use of oral contraceptives before pregnancy on the mineral content of human colostrum and of more mature milk. *Am. J. Clin. Nutr.* **32**, 30–39.

Kjeldsen, F., Savitski, M.M., Nielsen, M.L., Shi, L., Zubarev, R.A. 2007. On studying protein phosphorylation patterns using bottom-up LC-MS/MS: the case of human alpha-casein. *Analyst* **132**, 768–776.

Kleerekoper, M., Mendlovic, D.B. 1993. Sodium fluoride therapy of postmenopausal osteoporosis. *Endocr. Rev.* **14**, 312–323.

Kochersberger, G., Bales, C., Lobaugh, B., Lyles, K.W. 1990. Calcium supplementation lowers serum parathyroid hormone levels in elderly subjects. *J. Gerontol.* **45**, M159–M162.

Kooienga, L. 2007. Phosphorus balance with daily dialysis. *Semin. Dial.* **20**, 342–345.
Koparal, E., Ertugrul, F., Oztekin, K. 2000. Fluoride levels in breast milk and infant foods. *J. Clin. Pediatr. Dent.* **24**, 299–302.
Kosta, L., Byrne, A.R., Dermelj, M. 1983. Trace elements in some human milk samples by radiochemical neutron activation analysis. *Sci. Total Environ.* **29**, 261–268.
Kumpulainen, J., Salmenpera, L., Siimes, M.A., Koivistoinen, P., Perheentupa, J. 1985. Selenium status of exclusively breast-fed infants as influenced by maternal organic or inorganic selenium supplementation. *Am. J. Clin. Nutr.* **42**, 829–835.
Laskey, M.A., Prentice, A., Shaw, J., Zachou, T., Ceesay, S.M., Vasquez-Velasquez, L., Fraser, D.R. 1990. Breast-milk calcium concentrations during prolonged lactation in British and rural Gambian mothers. *Acta Paediatr. Scand.* **79**, 507–512.
Leach, R.M., Harris, E.D. 1997. Manganese. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 335–355, Marcel Dekker, New York.
Lei, X.G., Cheng, W.H., McClung, J.P. 2007. Metabolic regulation and function of glutathione peroxidase-1. *Annu. Rev. Nutr.* **27**, 41–61.
Levander, O.A., Moser, P.B., Morris, V.C. 1987. Dietary selenium intake and selenium concentrations of plasma, erythrocytes, and breast milk in pregnant and postpartum lactating and nonlactating women. *Am. J. Clin. Nutr.* **46**, 694–698.
Li, M., Waite, K.V., Ma, G., Eastman, C.J. 2006. Declining iodine content of milk and re-emergence of iodine deficiency in Australia. *Med. J. Aust.* **184**, 307.
Lipsman, S., Dewey, K.G., Lonnerdal, B. 1985. Breast-feeding among teenage mothers: milk composition, infant growth, and maternal dietary intake. *J. Pediatr. Gastroenterol. Nutr.* **4**, 426–434.
Lonnerdal, B. 1985. Bioavailability of trace elements from human milk, bovine milk and infant formulas. In: *Composition and Physiological Properties of Human Milk*, (J. Schaub, ed.), pp. 3–16, Elsevier Science Publishers, New York.
Lonnerdal, B. 1986. Effects of maternal dietary intake on human milk composition. *J. Nutr.* **116**, 499–513.
Lonnerdal, B. 1989. Trace element absorption in infants as a foundation to setting upper limits for trace elements in infant formulas. *J. Nutr.* **119**, 1839–1845.
Lonnerdal, B. 1997. Effects of milk and milk components on calcium, magnesium, and trace element absorption during infancy. *Physiol. Rev.* **77**, 643–669.
Lonnerdal, B., Glazier, C. 1985. Calcium binding by alpha-lactalbumin in human milk and bovine milk. *J. Nutr.* **115**, 1209–1216.
Lonnerdal, B., Hoffman, B., Hurley, L.S. 1982. Zinc and copper binding proteins in human milk. *Am. J. Clin. Nutr.* **36**, 1170–1176.
Lonnerdal, B., Keen, C.L., Hurley, L.S. 1981. Iron, copper, zinc, and manganese in milk. *Annu. Rev. Nutr.* **1**, 149–174.
Lonnerdal, B., Keen, C.L., Hurley, L.S. 1985. Manganese binding proteins in human and cow's milk. *Am. J. Clin. Nutr.* **41**, 550–559.
Lonnerdal, B., Keen, C.L., Ohtake, M., Tamura, T. 1983. Iron, zinc, copper, and manganese in infant formulas. *Am. J. Dis. Child.* **137**, 433–437.
Looker, A.C. 2006. Dietary calcium. Recommendations and intakes around the world. In: *Calcium in Human Health* (C.M. Weaver and R.P. Heaney, eds.), pp. 105–127, Humana Press, Totowa, NJ.
Looker, A.C., Loria, C.M., Carroll, M.D., McDowell, M.A., Johnson, C.L. 1993. Calcium intakes or Mexican Americans, Cubans, Puerto Ricans, non-Hispanic whites, and non-Hispanic blacks in the United States. *J. Am. Diet. Assoc*. **93**, 1274–1279.
Loomis, W.D., Durst, R.W. 1992. Chemistry and biology of boron. *BioFactors* **3**, 229–239.
Lotz, M., Zisman, E., Bartter, F.C. 1968. Evidence for a phosphorus-depletion syndrome in man. *N. Engl. J. Med.* **278**, 409–415.

Lozoff, B., De Andraca, I., Castillo, M., Smith, J.B., Walter, T., Pino, P. 2003. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. *Pediatrics* **112**, 846–854.

Marchin, S., Putaux, J.L., Pignon, F., Leonil, J. 2007. Effects of the environmental factors on the casein micelle structure studied by cryo transmission electron microscopy and small-angle x-ray scattering/ultrasmall-angle x-ray scattering. *J. Chem. Phys.* **126**, 045101.

Mastroeni, S.S., Okada, I.A., Rondo, P.H., Duran, M.C., Paiva, A.A., Neto, J.M. 2006. Concentrations of Fe, K, Na, Ca, P, Zn and Mg in maternal colostrum and mature milk. *J. Trop. Pediatr.* **52**, 272–275.

Matkovic, V., Jelic, T., Wardlaw, G.M., Ilich, J.Z., Goel, P.K., Wright, J.K., Andon, M.B., Smith, K.T., Heaney, R.P. 1994. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. *J. Clin. Invest.* **93**, 799–808.

McGuire, M.K., Burgert, S.L., Milner, J.A., Glass, L., Kummer, R., Deering, R., Boucek, R., Picciano, M.F. 1993. Selenium status of infants is influenced by supplementation of formula or maternal diets. *Am. J. Clin. Nutr.* **58**, 643–648.

Miller, J.K., Swanson, E.W., Spalding, G.E. 1975. Iodine absorption, excretion, recycling, and tissue distribution in the dairy cow. *J. Dairy Sci.* **58**, 1578–1593.

Miyazaki, E., Kato, J., Kobune, M., Okumura, K., Sasaki, K., Shintani, N., Arosio, P., Niitsu, Y. 2002. Denatured H-ferritin subunit is a major constituent of haemosiderin in the liver of patients with iron overload. *Gut* **50**, 413–419.

Moshfegh, A., Goldman, J., Cleveland, L., 2005. What we eat in America, NHANES 2001-2002: Usual nutrient intakes from food compared to dietary reference intakes. www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/usualintaketables2001-02.pdf, accessed 30 September 2005.

Muniz-Naveiro, O., Dominguez-Gonzalez, R., Bermejo-Barrera, A., Cocho de Juan, J.A., Fraga Bermudez, J.M., Goris Pereiras, A., Lopez Santamarina, A., Martinez Lede, I., Valledor Puente, J., Fernandez-Couto Gomez, L., Bermejo-Barrera, P. 2005. Selenium content and distribution in cow's milk supplemented with two dietary selenium sources. *J. Agric. Food Chem.* **53**, 9817–9822.

Murthy, G.K., Thomas, J.W. 1974. Trace elements in milk. *Crit. Rev. Environ. Control* **4**, 1–37.

Myron, D.R., Givand, S.H., Nielsen, F.H. 1977. Vanadium content of selected foods as determined by flameless atomic absorption spectroscopy. *J. Agric. Food Chem.* **25**, 297–300.

Naghii, M.R., Samman, S. 1997. The effect of boron supplementation on its urinary excretion and selected cardiovascular risk factors in healthy male subjects. *Biol. Trace Elem. Res.* **56**, 273–286.

National Research Council 1980. *Recommended Dietary Allowances*. National Academy of Sciences, Washington, DC.

Nielsen, F.H. 1997. Vanadium. In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R.A. Sunde, eds.), pp. 619–630, Marcel Dekker, New York.

Nielsen, F.H. 2006. Boron, manganese, molybdenum, and other trace elements. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 506–526, International Life Sciences Institute, Washington, DC.

Nielsen, F.H., Gallagher, S.K., Johnson, L.K., Nielsen, E.J. 1992. Boron enhances and mimics some effects of estrogen therapy in postmenopausal women. *J. Trace Elem. Exp. Med.* **5**, 237–246.

Nilas, L. 1993. Calcium intake and osteoporosis. *World Rev. Nutr. Diet.* **73**, 1–26.

Oliver, I., Sperling, O., Liberman, U.A., Frank, M., De Vries, A. 1971. Deficiency of xanthine oxidase activity in colostrum of a xanthinuric female. *Biochem. Med.* **5**, 279–280.

Ontsouka, C.E., Bruckmaier, R.M., Blum, J.W. 2003. Fractionized milk composition during removal of colostrum and mature milk. *J. Dairy Sci.* **86**, 2005–2011.

Palmese, S., Pezza, M., De Robertis, E. 2005. Hypophosphatemia and metabolic acidosis. *Minerva Anestesiol.* **71**, 237–242.

Park, M., Li, Q., Shcheynikov, N., Zeng, W., Muallem, S. 2004. NaBC1 is a ubiquitous electrogenic Na(+)-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. *Mol. Cell* **16**, 331–341.

Parr, R.M., DeMaeyer, E.M., Iyengar, V.G., Byrne, A.R., Kirkbright, G.F., Schoch, G., Niinisto, L., Pineda, O., Vis, H.L., Hofvander, Y., Omololu, A. 1991. Minor and trace elements in human milk from Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire. Results from a WHO/IAEA joint project. *Biol. Trace Elem. Res.* **29**, 51–75.

Pearce, E.N., Leung, A.M., Blount, B.C., Bazrafshan, H.R., He, X., Pino, S., Valentin-Blasini, L., Braverman, L.E. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J. Clin. Endocrinol. Metab.* **92**, 1673–1677.

Pennington, J.A., Wilson, D.B., Young, B.E., Johnson, R.D., Vanderveen, J.E. 1987. Mineral content of market samples of fluid whole milk. *J. Am. Diet. Assoc.* **87**, 1036–1042.

Pennington, J.A.T., Jones, J.W. 1987. Molybdenum, nickel, cobalt, vanadium, and strontium in total diets. *J. Am. Diet. Assoc.* **87**, 1644–1650.

Perez-Lopez, F.R. 2007. Iodine and thyroid hormones during pregnancy and postpartum. *Gynecol. Endocrinol.* **23**, 414–428.

Phang, J.M., Berman, M., Finerman, G.A., Neer, R.M., Rosenberg, L.E., Hahn, T.J. 1969. Dietary perturbation of calcium metabolism in normal man: compartmental analysis. *J. Clin. Invest.* **48**, 67–77.

Phillips, D.I., Nelson, M., Barker, D.J., Morris, J.A., Wood, T.J. 1988. Iodine in milk and the incidence of thyrotoxicosis in England. *Clin. Endocrinol. (Oxf)* **28**, 61–66.

Preuss, H.G. 2006. Electrolytes: sodium, chloride, and potassium. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 409–421, International Life Sciences Institute, Washington, DC.

Prohaska, J.R. 2006, Copper. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 458–470, International Life Sciences Institute, Washington, DC.

Rajalakshmi, K., Srikantia, S.G. 1980, Copper, zinc, and magnesium content of breast milk of Indian women. *Am. J. Clin. Nutr.* **33**, 664–669.

Rajendran, K.G., Burnham, B.S., Sood, C.A., Spielvogel, B.F., Shaw, B.R., Hall, I.H. 1994, Anti-inflammatory and anti-osteoporotic activities of base-boronated nucleosides and phosphate-boronated nucleotides in rodents. *J. Pharm. Sci.* **83**, 1391–1395.

Rajpathak, S., Rimm, E., Morris, J.S., Hu, F. 2005, Toenail selenium and cardiovascular disease in men with diabetes. *J. Am. Coll. Nutr.* **24**, 250–256.

Rayman, M.P. 2000. The importance of selenium to human health. *Lancet* **356**, 233–241.

Recker, R., Lappe, J., Davies, K., Heaney, R. 2000. Characterization of perimenopausal bone loss: a prospective study. *J. Bone Miner. Res.* **15**, 1965–1973.

Renner, E. 1983. *Milk and Dairy Products in Human Nutrition*. Volkswirtschaftlicher Verlag, Munich.

Renner, E., Schaafsma, G., Scott, K.J. 1989. Micronutrients in milk. In: *Micronutrients in Milk and Milk-Based Food Products* (E. Renner, ed.), pp. 1–70, Elsevier Applied Science, London.

Roh, J.K., Bradley, R.L., Richardson, T., Weckel, K.G. 1976. Removal of copper from milk. *J. Dairy Res.* **59**, 382–385.

Rossi, A.F., Miles, R.D., Damron, B.L., Flunker, L.K. 1993. Effects of dietary boron supplementation on broilers. *Poul. Sci.* **72**, 2124–2130.

Rossipal, E., Krachler, M. 1998. Pattern of trace elements in human milk during the course of lactation. *Nutr. Res.* **18**, 11–24.

Roughead, Z.K., Johnson, L.K., Lykken, G.I., Hunt, J.R. 2003. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *J. Nutr.* **133**, 1020–1026.

Rubin, C., Rubin, J. 2006. Biomechanics and mechanobiology of bone. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th edn. (M.J. Favus, ed.), pp. 36–42, American Society for Bone and Mineral Research, Washington, D.C.

Sazawal, S., Black, R.E., Ramsan, M., Chwaya, H.M., Stoltzfus, R.J., Dutta, A., Dhingra, U., Kabole, I., Deb, S., Othman, M.K., Kabole, F.M. 2006. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* **367**, 133–143.

Schroeder, H.A., Vinton, W.H., Jr., Balassa, J.J. 1963. Effect of chromim, cadmium and other trace metals on the growth and survival of mice. *J. Nutr.* **80**, 39–47.

Schuurman, A.G., van den Brandt, P.A., Dorant, E., Goldbohm, R.A. 1999. Animal products, calcium and protein and prostate cancer risk in The Netherlands Cohort Study. *Br. J. Cancer* **80**, 1107–1113.

Schwarz, K., Mertz, W. 1959. Chromium(III) and the glucose tolerance factor. *Arch. Biochem. Biophys.* **85**, 292–295.

Shapiro, R., Heaney, R.P. 2003. Co-dependence of calcium and phosphorus for growth and bone development under conditions of varying deficiency. *Bone* **32**, 532–540.

Sheng, M.H.-C., Taper, L.J., Veit, H., Thomas, E.A., Ritchey, S.J., Lau, K.-H.W. 2001. Dietary boron supplementation enhances the effects of estrogen on bone mineral balance in ovariectomized rats. *Biol. Trace Elem. Res.* **81**, 29–45.

Sieck, B., Takagi, S., Chow, L.C. 1990. Assessment of loosely-bound and firmly-bound fluoride uptake by tooth enamel from topically applied fluoride treatments. *J. Dent. Res.* **69**, 1261–1265.

Siimes, M.A., Salmenpera, L., Perheentupa, J. 1984. Exclusive breast-feeding for 9 months: risk of iron deficiency. *J. Pediatr.* **104**, 196–199.

Singh, H., Flynn, A., Fox, P.F. 1989. Zinc binding in bovine milk. *J. Dairy Res.* **56**, 249–263.

Skeaff, S.A., Ferguson, E.L., McKenzie, J.E., Valeix, P., Gibson, R.S., Thomson, C.D. 2005. Are breast-fed infants and toddlers in New Zealand at risk of iodine deficiency? *Nutrition* **21**, 325–331.

Sood, S.M., Herbert, P.J., Slattery, C.W. 1997. Structural studies on casein micelles of human milk: dissociation of beta-casein of different phosphorylation levels induced by cooling and ethylenediaminetetraacetate. *J. Dairy Sci.* **80**, 628–633.

Soremark, R. 1967. Vanadium in some biological specimens. *J. Nutr.* **92**, 183–190.

Spielberg, S.P., Butler, J.D., MacDermot, K., Schulman, J.D. 1979. Treatment of glutathione synthetase deficient fibroblasts by inhibiting gamma-glutamyl transpeptidase activity with serine and borate. *Biochem. Biophys. Res. Commun.* **89**, 504–511.

Stabler, S.P. 2006. Vitamin B_{12}. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 302–313, International Life Sciences Institute, Washington, DC.

Stastny, D., Vogel, R.S., Picciano, M.F. 1984. Manganese intake and serum manganese concentration of human milk-fed and formula-fed infants. *Am. J. Clin. Nutr.* **39**, 872–878.

Stewart, J.S., Roberts, P.D., Hoffbrand, A.V. 1970. Response of dietary vitamin-B12 deficiency to physiological oral doses of cyanocobalamin. *Lancet* **2**, 542–545.

Stoecker, B.J. 2006. Chromium. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 498–505, International Life Sciences Institute, Washington, DC.

Stranges, S., Marshall, J.R., Natarajan, R., Donahue, R.P., Trevisan, M., Combs, G.F., Cappuccio, F.P., Ceriello, A., Reid, M.E. 2007. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann. Intern. Med.* **147**, 217–223.

Sunde, R.A. 2006. Selenium. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 480–497, International Life Sciences Institute, Washington, DC.

Tanner, M.S. 1998. Role of cupper in Indian childhood cirrhosis. *Am. J. Clin. Nutr.* **67**, 1074S–1081S.

Thacher, T.D., Fischer, P.R., Strand, M.A., Pettifor, J.M. 2006. Nutritional rickets around the world: causes and future directions. *Ann. Trop. Paediatr.* **26**, 1–16.

Tsongas, T.A., Meglen, R.R., Walravens, P.A., Chappell, W.R. 1980. Molybdenum in the diet: an estimate of average daily intake in the United States. *Am. J. Clin. Nutr.* **33**, 1103–1107.

Tudhope, G.R., Swan, H.T., Spray, G.H. 1967. Patient variation in pernicious anaemia, as shown in a clinical trial of cyanocobalamin, hydroxocobalamin and cyanocobalamin–zinc tannate. *Br. J. Haematol.* **13**, 216–228.

US Department of Agriculture Agricultural Research Service, 2006. USDA Nutrient Database for Standard Reference, Release 19. Available at: www.nal.usda.gov/fnic/foodcomp., accessed 28 August, 2007.

US Department of Agriculture Agricultural Research Service, 2007. USDA National Nutrient Database for Standard Reference, Release 20. Nutrient Data Laboratory Home Page. www.ars.usda.gov/ba/bhnrc/ndl., accessed 29 December, 2007.

Vanderpas, J.B., Contempre, B., Duale, N.L., Deckx, H., Bebe, N., Longombe, A.O., Thilly, C.H., Diplock, A.T., Dumont, J.E. 1993. Selenium deficiency mitigates hypothyroxinemia in iodine-deficient subjects. *Am. J. Clin. Nutr.* **57**, 271S–275S.

Velona, T., Abbiati, L., Beretta, B., Gaiaschi, A., Flauto, U., Tagliabue, P., Galli, C.L., Restani, P. 1999. Protein profiles in breast milk from mothers delivering term and preterm babies. *Pediatr. Res.* **45**, 658–663.

Vincent, J.B., Bennett, R. 2007. Potential and purported roles for chromium in insulin signaling: the search for the holy grail. In: *The Nutritional Biochemistry of Chromium (III)* (J.B. Vincent, ed.), pp. 139–160, Elsevier, Amsterdam.

Volpe, S.L. 2006. Magnesium. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 400–408, International Life Sciences Institute, Washington, DC.

Vuori, E., Makinen, S.M., Kara, R., Kuitunen, P. 1980. The effects of the dietary intakes of copper, iron, manganese, and zinc on the trace element content of human milk. *Am. J. Clin. Nutr.* **33**, 227–231.

Walravens, P.A., Hambidge, K.M. 1976. Growth of infants fed a zinc supplemented formula. *Am. J. Clin. Nutr.* **29**, 1114–1121.

Ward, P.P., Conneely, O.M. 2004. Lactoferrin: role in iron homeostasis and host defense against microbial infection. *Biometals* **17**, 203–208.

Weaver, C. 2006. Calcium. In: *Present Knowledge in Nutrition* (B.A. Bowman and R.M. Russell, eds.), pp. 373–382, International Life Sciences Institute, Washington, DC.

Weaver, C.M., Heaney, R.P. 2006. Food sources, supplements, and bioavailability. In: *Calcium in Human Health* (C.M. Weaver and R.P. Heaney, eds.), pp. 129–142, Humana Press, Totowa, NJ.

Weaver, C.M., Proulx, W.R., Heaney, R. 1999. Choices for achieving adequate dietary calcium with a vegetarian diet. *Am. J. Clin. Nutr.* **70**, 543S–548S.

Wedler, F.C. 1994. Biochemical and nutritional role of manganese: an overview. In: *Manganese in Health and Disease*, pp. 1–35, CRC, Boca Raton.

Weinberg, E.D. 2007. Antibiotic properties and applications of lactoferrin. *Curr. Pharm. Des.* **13**, 801–811.

Wheeler, S.M., Fleet, G.H., Ashley, R.J. 1983. Effect of processing upon concentration and distribution of natural and iodophor-derived iodine in milk. *J. Dairy Sci.* **66**, 187–195.

Wilson, J.H., Ruzler, P.L. 1997. Effects of boron on growing pullets. *Biol. Trace Elem. Res.* **56**, 287–294.

Winzenberg, T., Shaw, K., Fryer, J., Jones, G. 2006. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. *Bmj* **333**, 775.

Wu, K., Willett, W.C., Fuchs, C.S., Colditz, G.A., Giovannucci, E.L. 2002. Calcium intake and risk of colon cancer in women and men. *J. Natl. Cancer Inst.* **94**, 437–446.

Yamawaki, N., Yamada, M., Kan-no, T., Kojima, T., Kaneko, T., Yonekubo, A. 2005. Macronutrient, mineral and trace element composition of breast milk from Japanese women. *J. Trace Elem. Med. Biol.* **19**, 171–181.

Zeise, L., Zikakis, J.P. 1987. Characterization of human colostral xanthine oxidase. *J. Agric. Food Chem.* **35**, 942–947.

Zhou, B.F., Stamler, J., Dennis, B., Moag-Stahlberg, A., Okuda, N., Robertson, C., Zhao, L., Chan, Q., Elliott, P. 2003. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. *J. Hum. Hypertens.* **17**, 623–630.

Ziegler, E.E., Fomon, S.J. 1989. Potential renal solute load of infant formulas. *J. Nutr.* **119**, 1785–1788.

Zimmermann, M.B. 2006. Iodine and the iodine deficiency disorders. In: *Present Knowledge in Nutrition*, (B.A. Bowman and R.M. Russell, eds.), pp. 471–479, International Life Sciences Institute, Washington, DC.