CHAPTER 6

The Health Benefits of Calcium Citrate Malate: A Review of the Supporting Science

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Abstract

There has been considerable investigation into the health benefits of calcium citrate malate (CCM) since it was first patented in the late 1980s. This chapter is a comprehensive summary of the supporting science and available evidence on the bioavailability and health benefits of consuming CCM. It highlights the important roles that CCM can play during various life stages. CCM has been shown to facilitate calcium retention and bone accrual in children and adolescents. In adults, it effectively promotes the consolidation and maintenance of bone mass. In conjunction with vitamin D, CCM also decreases bone fracture risk in the elderly, slows the rate of bone loss in old age, and is of benefit to the health and well-being of postmenopausal women. CCM is exceptional in that it confers many unique benefits that go beyond bone health. Unlike other calcium sources that necessitate supplementation be in conjunction with a meal to ensure an appreciable benefit is derived, CCM can be consumed with or without food and delivers a significant nutritional benefit to individuals of all ages. The chemistry of CCM makes it a particularly beneficial calcium source for individuals with hypochlorydia or achlorydia, which generally includes the elderly and those on medications that decrease gastric acid secretion. CCM is also recognized as a calcium source that does not increase the risk of kidney stones, and in fact it protects against stone-forming potential. The versatile nature of CCM makes it a convenient and practical calcium salt for use in moist foods and beverages. The major factor that may preclude selection of CCM as

a preferred calcium source is the higher cost compared to other sources of calcium commonly used for fortification (e.g., calcium carbonate and tricalcium phosphate). However, formation of CCM directly within beverages or other fluid foods and/or preparations, and the addition of a concentrated CCM solution or slurry, are relatively cost-effective methods by which CCM can be incorporated into finished food and beverage products.

I. WHY FOCUS ON CALCIUM CITRATE MALATE?

Regular ingestion of adequate calcium (Ca), particularly by way of consumption of foods naturally high in Ca, requires a deliberate approach to balanced nutrition for a large number of individuals. Everyday exposure to an increasingly wide variety of appealing food and beverage choices can divert the emphasis and preference away from inherently Ca-rich foods that all too often are largely underconsumed by those most in need of them. Under certain circumstances, such as lactose intolerance, allergies, strict vegetarianism, and personal dislikes, dairy foods are not even considered as a viable option or are consumed in insufficient quantity to meet Ca requirements by many subgroups. In situations such as these, Ca supplementation and/or fortification of regularly consumed foods and beverages is an ideal adjunctive approach, conferring benefits to those both interested in reducing the risk for chronic disease and seeking to promote optimal health. Although there are often claims to the contrary, different Ca salts are not, in effect, bioequivalent. While a number of commonly used Ca salts are currently available in the market place, each one is distinct in terms of its chemical composition, the functional and aesthetic properties it imparts to various food matrices, and its efficacy when it comes to delivering health benefits. In many respects, the attributes of calcium citrate malate (CCM), a compound salt of citrate and malate, set it apart from other Ca salt preparations. Other Ca salts do not match CCM's combination of qualities that include: comparatively high aqueous solubility, enhanced absorbability and bioavailability under a wide range of circumstances for all age groups, compositional flexibility (i.e., adjustable molar ratios and mineral content), convenient and practical compatibility with food systems, and an imperceptible presence as a fortificant in foods and beverages at concentrations that make a discernable contribution to health. The choice of a source of Ca is best made from an informed perspective. Recognizing that other Ca salts can also be effectively utilized as fortificants (Rafferty *et al.*, 2007), there has been a wealth of investigation into the bioavailability and health benefits of CCM since it was first patented in the late 1980s. The purpose of this chapter is to present a comprehensive summary of the supporting science and available evidence on the bioavailability and health benefits of consuming CCM.

II. Ca NEEDS AND CURRENT INTAKES

A. The role of Ca in the body

Ca is a mineral essential to the optimal functioning of virtually every cell in the body. It is required by nonskeletal cells for functions such as muscle contraction, neurotransmission, signal transduction, enzyme secretion, vascular function, blood coagulation, and glandular secretion. There is ~1 g of Ca in plasma and extracellular fluids (ECFs), 6–8 g is found in body tissues and within cells, and over 99% of total body Ca is found in bones and teeth, primarily in the form of hydroxyapatite $Ca_{10}(OH)(PO_4)_3$ (Weaver and Heaney, 2006a). The main functional roles played by Ca in the skeleton can be summarized as structural and metabolic. Ca provides the mineral material that principally contributes to growth and development of a dynamic skeletal framework that facilitates stature, posture, is a lever system for locomotion, mechanical strength, provides protection for vital organs, and simultaneously serves as a labile reservoir to enable effective Ca homeostasis in the body.

Ca normally circulates in the bloodstream, within a 2.25–2.50 mmol concentration range, bound to proteins (40–45%), complexed with ions (8–10%), and ionized as Ca^{2+} (45–50%) (Weaver and Heaney, 2006a). Circulating Ca in excess of that required for maintenance of plasma levels is ideally transferred from the blood to be deposited in bone via the bone formation process. The Ca concentration outside of blood vessels in the ECF that bathes cells is tightly regulated close to 1.25 mmol (Weaver and Heaney, 2006a), almost to the point of invariance. It is this ECF Ca pool that cells are immediately reliant upon to sustain vital cellular functions that are imminently critical to the maintenance of life (e.g., cardiac muscle contraction). Circulating Ca is constantly utilized to replenish ECF pools, and when Ca derived from dietary intake is insufficient to replace the amount of Ca used for replenishment, Ca in bone is transferred to the blood via a bone resorption process.

A regular adequate dietary intake of Ca is required to promote the maintenance of Ca balance in tissues and to attenuate undue resorption and subsequent bone loss that can lead to skeletal fragility and fractures. Bidirectional Ca fluxes between the blood and bone are mediated by parathyroid hormone (PTH) and vitamin D ($1,25(OH)_2$ D_3) (Awuney and Bukoski, 2006). Homeostatic regulation of Ca is via an orchestrated interplay among three main organs, the intestines, the kidney, and bones (Fleet, 2006). An increase in PTH from the parathyroid glands stimulates the mobilization of Ca from the skeleton via resorption by osteoclasts, increased renal production of the active form of vitamin D, and through stimulated tubular reabsorption of Ca within the kidneys. Vitamin D stimulates circulating Ca levels by way of increasing intestinal Ca

absorption and renal Ca reabsorption. Decreases in PTH and vitamin D essentially exert the reverse effects.

Bone remodeling, characterized by a spatial and temporal coupling of bone resorptive activities at select loci with subsequent bone formation at the same location, is a natural ongoing process in the skeleton. It facilitates tissue renewal and organ adaptation to stimuli in healthy individuals. High remodeling rates (Dawson-Hughes, 2006a) and/or net remodeling imbalances, caused by bone resorptive activities habitually surpassing those of bone formation, result in bone loss from discrete remodeling packets on the surface of bone matrices. It is indicative of the homeostatic mechanism designed to compensate for hypocalcemia in the event of a plasma Ca insufficiency. Decreases in bone mass subtly but surely weaken the strength and structure of the skeleton over time. Bone fragility is a latent sign of a chronic Ca deficiency which, more often than not, develops imperceptibly in most individuals until it is professionally diagnosed.

In the event of dietary Ca abundance, Ca in excess of adequate circulating concentrations is deposited in the skeleton. This occurs to the extent of the body's ability to store Ca, and any excess beyond this threshold is excreted. Accrual of Ca into bone is governed by such factors as dietary intake (including the absorption, bioavailability, utilization of nutrients and minerals, and other dietary constituents that influence absorption or retention), calciotropic hormones, genetic potential, lifestyle factors, life stage, general health, and the adaptive response to physical/mechanical stimuli within the constraints of metabolic economy.

B. General Ca requirements

Although Ca is required throughout the body to maintain health and reduce chronic disease risk, its retention in the skeleton is used as an indicator of adequate intake (AI) and bone health. Ca intakes that optimize bone health throughout the life span, by promoting attainment of peak bone mass during growth and protecting against subsequent bone loss and fragility, are also considered to provide a buttress against many other chronic disorders. Although the cause of conditions such as hypertension, hypercholesterolemia, colon cancer, premenstrual syndrome, tooth loss, and nephrolithiasis are multifactorial in nature, these disorders may progress without adequate Ca (Bryant *et al.*, 1999). Bone mineral is ~32% Ca. The more bone mass acquired during growth and maintained as we age, the larger is our reservoir of Ca for metabolic and structural needs. The amount of Ca required is varied across age groups and is dependent on the physiological and metabolic needs inherent during different developmental and maturational stages. These include, although are not limited to, periods of rapid growth and age-related changes in absorption and excretion (Bryant *et al.*, 1999).

Recommended intakes of Ca vary widely in different countries around the world. This is because worldwide there are distinct regional food sources, different dietary intakes, as well as varying dietary practices, lifestyles, environmental factors, and unique genetic profiles due to ethnic variability. All of these factors can result in very different exposures and bioavailabilities when it comes to adequate nutrition. Recommended intakes for Ca (mg/day) in the United States rank among the highest in the world (Dawson-Hughes, 2006a) for all life stage categories — with the exception of infants and toddlers for which they are considerably lower in comparison with the broad ranges recommended by a number of other countries (Hicks and Abrams, 2006). AIs have been estimated by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (DRI), the Food and Nutrition Board, and the Institute of Medicine (1997) and herein appear in categorized form in the first two columns on the left side of Table 6.1. The three columns on the right side provide data pertaining to actual Ca intakes reported in National Health and Nutrition Examination Survey (NHANES) conducted during the 1999–2000 period. While the age group categories for actual intakes do not exactly correspond with those defining the groups for AI, they overlap enough to allow one to gauge the general trends of current intakes by age and gender. Diet recall surveys and frequency questionnaires obviously have a number of inherent limitations in terms of supplying exact data on actual intakes; however, they usually provide valuable general information that can be compared against established AIs to determine specific groups at risk, potential health problems if trends persist, and the general level of success pertaining to health interventions.

C. Specific Ca needs

1. Infants

To meet rapid developmental and growth needs during infancy, Ca requirements and Ca absorption are inherently high (Matkovic, 1991). Based on a stable isotope method, mean (±SD) Ca absorption has been observed to be as high as 76.8 ± 14.7% for infants ($n = 18$) fed human breast milk and slightly less for those ($n = 10$) fed formula milks 61.6 ± 14.4% (Hicks and Abrams, 2006). For infants of both sexes born at term, breast milk is the preferred source of nutrition for at least the first 6 months of life. It provides ∼264 mg Ca/liter or ∼210 mg (5.3 mmol) Ca/day based on an average intake of ∼780 ml breast milk/day (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997). Exclusively breast-fed infants are considered to receive ideal nutrition to support optimal growth and rapid development for the first 6 months after birth.

TABLE 6.1 Current dietary reference intake (DRI) established for Ca intake by gender and age group juxtaposed against estimated intakes

Recommended AIs[a]		Data on U.S. Ca intakes (1999–2000)[b]		
Sex and age group	Target intake Ca (mg/d)	Sex and age group[c]	Mean intake Ca (mg/d)	Median intake Ca (mg/d)
Infants (month)				
(♀ & ♂) 0–6	210			
(♀ & ♂) 7–12	270			
Children (year)		Children (year)		
(♀ & ♂) 1–3	500	Girls < 6	785	708
(♀ & ♂) 4–8	800	Boys < 6	916	809
Females (year)		Females (year)		
9–13	1300	6–11	860	812
*14–18	1300	12–19	793	661
*19–30	1000	20–39	797	684
*31–50	1000	40–59	744	621
51–70	1200	≥60	660	563
>70	1200			
Males (year)		Males (year)		
9–13	1300	6–11	915	843
14–18	1300	12–19	1081	956
19–30	1000	20–39	1025	856
31–50	1000	40–59	969	834
51–70	1200	≥60	797	716
>70	1200			

[a] Ca requirements in the United States are currently set as AIs. The recommended AI for Ca is an approximated value estimated to cover the needs of all healthy individuals in the age group based on experimental or observational data that show a mean intake which appears to sustain a desired indicator of health (e.g., desirable Ca retention); however, lack of sufficient evidence precludes specifying with confidence the percentage of individuals covered by this intake (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997).

[b] Estimated from one 24-h dietary recall conducted in the National Health and Nutrition Examination Survey (NHANES, 1999–2000). Data are based on a total of $n = 8640$ individuals (variously spread across gender and age groups) deemed to have complete and reliable recall to include in the analyses (Wright *et al.*, 2003).

[c] Age categories are those recommended in NHANES.

* Pregnancy or lactation does not change the AIs for these age groups.

While one group of investigators observed a positive transient effect of a Ca intake that was in excess of the amount intrinsic to breast milk in infants under 6 months of age (Specker *et al.*, 1997), the improvement in

bone mineral accretion for the formula-fed infants cannot be categorically attributed to the additional Ca. The moderate-mineral infant formula that contained 510 mg Ca/liter would have to have been deemed comparable in terms of nutritional bioavailability, and comprise a nutritional profile that replicates the composition of human milk for all components except the Ca content. Currently there are no convincing data to suggest Ca intake, beyond what is biologically available from breast milk, provides any additional short- or long-term benefit(s) to bone health (Abrams, 2006; Greer *et al.*, 2006).

The concentration of Ca in breast milk appears to be controlled by a mechanism independent of Ca intake. A randomized, double-blind, placebo-controlled trial showed that Gambian women, with chronically low daily Ca intakes (300–400 mg), do not significantly increase the Ca concentration of their milk during lactation when supplemented with an adequate Ca intake by today's standards (i.e., 1500 mg/day) (Jarjou *et al.*, 2006). In fact, maternal supplementation yielded no direct effect on infant birth weight, growth, or bone mineral status during the first year of life. During lactation, physiological mechanisms are suspected to operate to ensure sufficient Ca for breast milk production to meet an infant's natural needs (Prentice *et al.*, 1995). Nevertheless, recommended intakes for early infants in Australia, Belgium, and Ireland are currently as high as 330, 400, and 800 mg Ca/day, respectively (Looker, 2006). It is conceivable that some compensation might be made to adjust for less-efficient absorption of Ca from formula milk, low mineral reserves attributable to a very low birth weight or infant prematurity, or other special circumstances; however, because Ca is considered to be a ''threshold nutrient,'' whereby intakes in excess of an adequate level do not generally provide an added benefit, the rationale for such high intakes in infants remains uncertain.

D. Current intakes

Infant Ca requirements increase slightly from 7 to 12 months of age as body size increases. Breast milk, in combination with the introduction of some solid foods containing Ca, is estimated to provide enough Ca nourishment to sustain normal growth and development. Countries such as Australia, Belgium, and Ireland (with recommended Ca intakes for infants of 550, 600, and 800 mg Ca/day, respectively) exceed the U.S. recommended AI at 270 mg (6.8 mmol) Ca/day for this age group by as much as 49–337%. These recommendations are for higher amounts of Ca than most infants would receive through breast milk. Up until the age of 1 year, the long-term effect(s) of Ca intake levels that greatly exceed those of breast milk remains uncertain. However, infants fed formula may require a higher Ca intake due to reduced absorptive potential from formula milks, although slightly higher Ca retention from alternate sources to

breast milk are reported to compensate for absorption differences (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997). Statistically adjusted data (Nusser *et al.*, 1996) reflecting how well intakes of Ca were met by infants during 1994–1996 was supplied by the US Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals (CSFII) (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997), which indicated median intakes of 457 and 703 mg Ca/day for 0- to 6-month- and 7- to 12-month-old infants, respectively.

1. Children

As is the case for infant groups, gender differences are not a major factor influencing Ca requirements for the 1- to 3-year toddler age group or the 4- to 8-year children's age group. Requirements continue to increase from infancy to the onset of puberty due to continued growth and development. In general, from infancy through childhood Ca needs appear to be approximately matched by AIs based on the 1999–2000 Survey data (Wright *et al.*, 2003) and other data (Storey *et al.*, 2004). Currently, the evidence that prepubertal Ca supplementation with >800 mg/day is of added benefit is largely inconclusive in terms of its meaningful biological significance and long-term influence (Iuliano-Burns *et al.*, 2006). Childhood is an important developmental period in which good dietary habits ought to be established in preparation for the onset of puberty when the Ca accretion rate begins to markedly increase in response to increased needs. Unfortunately, the general trend is for dietary Ca intakes to decline as children get older (Johnson, 2000), with girls generally consuming less Ca than boys (Fioritto *et al.*, 2006; Nicklas, 2003).

2. Preadolescence and adolescence

During adolescence, gender differences in physical development, according to the Tanner scale and Ca needs, emerge. This is due to the timing of the growth spurt during which peak height velocity in girls occurs earlier in Tanner staging (and chronologically) than in boys (i.e., ~11.5 vs ~13.5 years, respectively). Ca accretion rate rapidly peaks during early adolescence and up to 40% of total lifetime bone mass is accumulated by the end of this life stage (Greer *et al.*, 2006). At the completion of growth, boys are generally taller and heavier than girls as a result of undergoing a longer prepubertal growth period, an increased peak height velocity during the growth spurt, and a longer adolescent growth spurt (Anonymous, 2005). Despite these differences, there are no gender-specific AIs for preadolescents (older children 9–13 years) or the adolescent age group (14–18 years). This situation arose as a result of a predominance of Ca studies in girls compared to boys, such that male data were lacking in order to make a case for separate

recommendations in these age groups. Therefore, 1300 mg (32.5 mmol) Ca/day was determined to be adequate for both sexes based on studies fitting with the indicated criteria (Weaver and Heaney, 2006b) (Table 6.2). Intakes in relation to requirements show that >50% of both females and males did not meet the Ca recommendations in preadolescent and adolescent age groups during the 1999–2000 period, with females consuming less Ca than males of the same age (Wright *et al.*, 2003). The ability to respond to exceedingly low Ca intakes is limited and insufficient (Greer *et al.*, 2006).

3. Adults

A lack of regular physical activity, together with a habitually low Ca intake during adolescence, interferes with the attainment of one's genetic potential for peak bone mass during the ages 19 through 30 years.

TABLE 6.2 Criteria upon which Adequate Intake (AI) values were based for calcium by life stage groups[a]

Life stage group[b]	Criterion[c]
Infants (month)	
0–6	Human milk content
6–12	Human milk + solid food
Children (year)	
1–3	Extrapolation of desirable calcium retention from 4 to 8 years
4–8	Calcium accretion/ΔBMC/calcium balance
9–13	Desirable calcium retention/factorial/ΔBMC
14–18	Desirable calcium retention/factorial/ΔBMC
Adults (year)	
31–50	Calcium balance
51–70	Desirable calcium retention/factorial/ΔBMC
>70	Extrapolation of desirable calcium retention from 51 to 70 years/ΔBMD/fracture rate
Pregnancy (year)	
≤18	Bone mineral mass
19–50	Bone mineral mass
Lactation (year)	
≤18	Bone mineral mass
19–50	Bone mineral mass

[a] Table adapted from information published previously (Weaver and Heaney, 2006b)
[b] All groups excluding pregnancy and lactation are males and females.
[c] Criteria are dependent on the data available in the literature.
Desirable calcium retention = the intake at which there is no net loss of calcium.
ΔBMC = change in bone mineral content.
ΔBMD = change in bone mineral density.
Factorial = calcium needs for growth + calcium losses (urine, feces, sweat) adjusted for absorption.

By adulthood, one's final statural height is established; however, gradual consolidation of bone mass is a protracted process that can continue to varying extents at different skeletal sites for up to ~10 years. The 1000 mg Ca/day recommended AI (25 mmol) for this life stage applies to both men and women alike, although the data forming the basis for the recommendations was based predominantly on studies in women. NHANES 1999–2000 findings reveal that women in this age range are, on average, only consuming ~80% of the recommended Ca intake and, disturbingly, the median intake is ~30% less than the AI. Based on average Ca intakes in the same period, men are consuming adequate levels of Ca; however, ≥50% are likely to be anywhere up to ~150 mg/day under the AI recommendations.

4. Middle-aged adults

Based on available Ca balance data, from 31 to 50 years of age a Ca intake of 1000 mg/day is considered to adequately equip both genders to maintain the bone mass attained during young adulthood to the extent that normal physiological processes during this life stage will allow. Age at onset of bone loss is not well defined and appears to vary across anatomical locations (Bainbridge *et al.*, 2002); however, by ~40 years of age general age-related bone loss proceeds at the rate of ~0.5% to 1.0% per year (Bryant *et al.*, 1999). The latter half of this life stage (40–50 years) also coincides with the premenopausal period in women which is associated with a decline in fertility (The Practice Committee of the American Society for Reproductive Medicine, 2006), episodic fluctuations in hormone levels (Santoro *et al.*, 1996), and a fall in Ca absorption (Wishart *et al.*, 2000). There is an approximate 200–250 mg/day shortfall in the mean Ca intake of women included in this age group, and median intakes indicate ≥50% are only consuming just over half of the recommended adequate Ca intake. The average Ca intake of men approximately meets the Ca needs, although median intakes fall short of the AI by just over 100 mg Ca/day.

5. Older adults

Men and women ages 51 through 70 years and beyond require a Ca intake of 1200 mg (30 mmol)/day for maximal Ca retention, which is usually negative during normal physiological functioning during this life stage as a result of the aging process. Averting the exacerbation of involution-induced bone loss is therefore necessary, essentially as a damage control measure when Ca absorptive efficiency, food Ca utilization efficiency, and Ca retention decline during this period (Heaney, 2001a). Moreover, other factors including an overall decrease in physical activity and skeletal muscle mass and a reduction in cutaneous exposure to sunlight (vitamin D), due to more time spent being homebound, all contribute to predisposing

older adults to a lowered bone mass, bone microarchitectural deterioration, compromised bone strength, and increased fracture risk over time (Heaney, 2001a). Compared to the recommend AI in older adults, actual Ca intakes are abysmally low. While the recommended AI for those aged between 51 and 70 years and >70 years old increase by 200 mg above the 1000 mg Ca/day recommended for the prior two decades (31–50 years), actual intakes go in the opposite direction and decrease even further than those reported in the period spanning 40–59 years of age.

Average Ca intakes for women more or less fitting into this older adult age group according to NHANES (1999–2000) are only ~44–56% of the AI (Wright *et al.*, 2003). Furthermore, median intakes show that 50% of women achieve approximately half of the AI for Ca. This is of particular concern during the years of transition to menopause and during early menopause. Natural dysregulation of a female's hypothalamic-pituitary-gonadal axis occurs at this time resulting in the cessation of appreciable ovarian estrogen production. This in turn stimulates an accelerated rate of bone loss which is superimposed on normal age-related bone loss (O'Flaherty, 2000). Estimates of the rate of total bone loss for older women vary, ranging from 3% to 10% per decade at age 50 up to 25–40% per decade by age 60 (O'Flaherty, 2000). After the age of 50, average losses in cortical bone may range from 9% to 12% per decade and in cancellous bone, losses may increase to as high as 13% per decade (O'Flaherty, 2000). The magnitude and consequences of bone loss during this stage of life can be mediated to some extent by entering the menopausal phase with a healthy bone density and by continued adequate Ca intake, without which women are more likely to develop osteoporosis.

As older men age, Ca intakes that were previously comparable to recommended intakes markedly decline and become inadequate. On average, men over the age of 60 have Ca intakes that are ~44% lower than what is recommended, and median intakes are even lower. Inadequate data in men and women >70 years of age preclude determination of an AI based on maximal Ca retention for this age group; therefore, data are currently extrapolated from 51- to 70-year olds (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997).

6. Pregnancy and lactation

Although significant changes in Ca and bone metabolism accompany pregnancy and lactation, the requirements for Ca during these periods have been determined to be identical to those of age-matched nonpregnant and nonlactating women based on current available evidence. During pregnancy, adaptive maternal responses come into effect, peaking by the third trimester of gestation. Intestinal Ca absorption efficiency essentially doubles to meet the ~250–300 mg Ca/day needs of the growing and

developing fetus (Kovacs and Fuleihan, 2006). Pregnant adolescents who are still growing at the time of a pregnancy may require Ca intakes beyond the current AI levels; however, more research needs to be undertaken to substantiate this. Augmentation of Ca absorption is the dominant adaptive mechanism during lactation, although bone is also temporarily borrowed from the skeleton, regardless of Ca intake, to facilitate availability of the ~280–400 mg Ca/day required to breast-feed infants (Kovacs and Fuleihan, 2006). Any acute changes to maternal BMD are typically transient in nature and rapidly reversed after childbirth and postweaning. Furthermore, there are no consistent indications that high parity or extended lactation per se is detrimental in the long term to a woman's BMD or future fracture risk (Karlsson *et al.*, 2001; Streeten *et al.*, 2005).

E. Implications

Adequate amounts of Ca, consumed regularly on a daily basis, are essential throughout the life cycle to promote and protect bone mass and architecture, as well as overall health (Fioritto *et al.*, 2006). Based on recent survey data (Forshee *et al.*, 2006; Wright *et al.*, 2003), the prevalence of Ca inadequacy in the United States beyond infancy still presents a serious public health concern as evidenced by average Ca intakes that fall below recommendations for many groups in the population (Miller *et al.*, 2001). For instance, the probability of Ca adequacy in the American diet has been estimated to be ~46% for adult women and ~59% for adult men. Across an entire lifespan, women can lose up to ~42% of their spinal and ~58% of their femoral peak bone mass (Rosen and KIel, 2006). This is a particularly daunting prospect when one considers that young girls not meeting adequate Ca intakes, of which there are many (Fioritto *et al.*, 2006), may never achieve their genetic potential for peak bone mass to begin with; thus, they are required to draw on an already compromised store of bone Ca later in life.

Calcium has earned a reputation as one of the most at-risk nutrients (Foote *et al.*, 2004) and in 2005 the Dietary Guidelines Advisory Committee classified Ca as a shortfall nutrient because average Ca intake often falls to <60% of the recommended intake in subsets of the population (Kennedy and Meyers, 2005). The pressing need to improve the Ca status of Americans has been highlighted by the Healthy People 2010 Objective, an initiative that endeavors to increase, to at least 75%, the number of US individuals 2 years or older that meet current Ca recommendations (Looker, 2003). The general population requires ongoing education to more fully appreciate the relevance of Ca in relation to current and future health, the imminent risks associated with habitual inadequate intakes, and practical means by which to achieve recommended intakes.

III. DESCRIPTION AND PROPERTIES OF CCM

A. Chemical formula of CCM and its component anions

Citric acid $C_6H_8O_7$
Malic acid $C_4H_6O_5$
Calcium citrate malate $Ca_6(C_6H_5O_7)_2(C_4H_4O_5)_3$
(e.g., hexa-Ca dicitrate trimalate or the anhydrous form of the 6:2:3 molar ratio fully neutralized salt).

CCM is a compound salt of the Ca cation with citrate and malate anions. CCM powder is different from a simple physical blend of Ca citrate and Ca malate powders, as evidenced by a higher aqueous solubility (Fox *et al.*, 1993b) and a unique x-ray powder diffraction pattern (unpublished data). The unique chemical composition of CCM renders it moderately soluble in water, with good compatibility in many food/beverage matrices. CCM is reportedly more bioavailable as a Ca source when added to foods or dietary supplements than other Ca salts currently on the market. CCM does not have a single chemical formula, but rather can be formulated to yield a range of compositions with varying Ca:citrate:malate molar ratios that bracket compositions corresponding to the fully-neutralized salt. CCM molar ratios of 6:2:3, 5:2:2, and 8:2:5 form neutral Ca salts with slightly different solubilities (Fox *et al.*, 1993b). Partial neutralization by Ca and even a slight excess of Ca are also options, such that the molar ratios 4:2:3 and 5:1:1 form slightly acidic and basic CCM salts, respectively. Numerous states of hydration of CCM powder are possible and as few as two to as many as 16–20 H_2O molecules may be present (Fox *et al.*, 1993a). Further details regarding the composition and/or properties of CCM can be obtained from numerous US patents that describe the technology (Andon, 1995; Burkes *et al.*, 1995; Fox *et al.*, 1993a,b; Jacobs, 1994; Nakel *et al.*, 1988; Saltman and Smith, 1993).

B. Ca content

The neutral 6:2:3 molar ratio CCM salt is comprised of 23.7% elemental Ca on a dry weight basis. Various states of hydration of the CCM powder will of course yield slightly lower Ca contents and the preparation of an octahydrate form of CCM powder (6:2:3 molar ratio) that comprises 20.73% Ca by weight has been described previously (Fox *et al.*, 1993b). CCM compositions with a higher proportion of citrate and/or malate moieties (e.g., 4:2:3 molar ratio) will contain a proportionally lower Ca content. Table 6.3 lists the Ca content of various Ca salts.

Dairy products are among the most Ca-dense foods. On average, milk contains 0.12% Ca by weight. Single-strength fruit juice beverages

TABLE 6.3 The Ca content of various Ca salts

Ca salt	Chemical formula (anhydrous salts)	M.W. (g/mol)	% Ca (elemental Ca)
Calcium oxide	CaO	56.08	71.47
Calcium hydroxide	$Ca(OH)_2$	74.10	54.09
Calcium carbonate	$CaCO_3$	100.09	40.04
Tricalcium phosphate	$Ca_3(PO_4)_2$	310.18	39.00
Calcium chloride	$CaCl_2$	110.98	36.11
Calcium oxalate	CaC_2O_4	128.10	31.29
Calcium formate	$Ca(CHO_2)_2$	129.12	31.04
Dicalcium phosphate	$CaHPO_4$	136.06	29.46
Calcium fumarate	$CaC_4H_2O_4$	154.14	26.00
Calcium acetate	$Ca(C_4H_6O_4)$	158.17	25.34
Calcium citrate	$Ca_3(C_6H_5O_7)$	498.44	24.12
Calcium citrate malate	$Ca_6(C_6H_5O_7)_2(C_4H_4O_5)_3$	1014.90	23.70
Calcium malate	$Ca(C_4H_4O_5)$	172.15	23.28
Calcium glycinate	$Ca(C_2H_4NO_2)_2$	188.20	21.30
Calcium lactate	$Ca(C_3H_5O_3)_2$	218.22	18.37
calcium lactate gluconate	$Ca_2(C_{18}H_{32}O_{20})$	648.60	12.36
Calcium lysinate	$CaC_{12}H_{28}N_4O_4$	332.46	12.06
Calcium ascorbate	$Ca(C_{12}H_{14}O_{12})$	390.31	10.27
Calcium gluconate	$Ca(C_6H_{11}O_7)_2$	430.38	9.31

supplemented with CCM can be formulated to include anywhere between 0.05% and about 0.26% by weight (wt) of solubilized Ca, or approximately half to double the level of Ca in milk. CCM-supplemented fruit juice concentrates may comprise between 0.2% and 1.20% by wt. solubilized Ca, which requires a proportionally higher level of added citric and malic acids (Burkes *et al.*, 1995). CCM is currently used to fortify various commercially available beverages such as single-strength orange juice (OJ) at a level of 350 mg Ca/8 fl oz, a fitness water beverage at a level

of 100 mg Ca/8 fl oz, and a 15% juice + 7% skim milk enriched beverage blend at a level of 450 mg Ca/8 fl oz (a level of fortification that is 50% greater than skim milk at 300 mg Ca/8 fl oz).

C. How CCM is made

CCM is formed by neutralization of an alkaline Ca source with citric and malic acids. The alkaline Ca source can be Ca hydroxide ($Ca(OH)_2$), Ca carbonate ($CaCO_3$), or Ca oxide (CaO). The neutralization reactions involved in the formation of the neutral 6:2:3 molar ratio CCM salt from $Ca(OH)_2$ and $CaCO_3$ are as follows:

$$6Ca(OH)_2 + 2(C_6H_8O_7) + 3(C_4H_6O_5) \rightarrow Ca_6(C_6H_5O_7)_2(C_4H_4O_5)_3 + 12H_2O$$

$$6CaCO_3 + 2(C_6H_8O_7) + 3(C_4H_6O_5) \rightarrow Ca_6(C_6H_5O_7)_2(C_4H_4O_5)_3 + 6H_2O + 6CO_2$$

In many cases, $Ca(OH)_2$ is a preferred Ca source for CCM formation because CO_2 is not produced as a byproduct of the neutralization, as it is when $CaCO_3$ is a reactant. If $CaCO_3$ is used as a starting material, the evolution of CO_2 gas that occurs as CCM is formed needs to be considered and controlled.

CCM can be formed by carrying out the neutralization reaction in water to produce a concentrated solution or slurry, which can then be added at low levels to fortify various foods and beverages. Alternatively, the concentrated solution or slurry may be dried to produce a CCM powder that can be used for food and beverage fortification, or as an ingredient in a dietary supplement. In either case, production of the CCM occurs according to the following generalized scheme. Citric acid and malic acid are first dissolved in water with mixing at the desired concentrations according to the target molar ratio of CCM being produced. Complete solubilization of the citric and malic acids together prior to reaction with the alkaline Ca source ensures the simultaneous availability of the two acids during the neutralization reaction. The required amount of an alkaline Ca source ($Ca(OH)_2$, $CaCO_3$, or CaO) is dispersed in a separate quantity of water in another vessel to produce a slurry of the material. The slurry of the Ca source is then added with mixing to the solution of citric and malic acids under a controlled rate of addition. After addition of the slurry, the blend is cooled with mixing and allowed to age for a period of time to allow the neutralization reaction to go to completion. A neutral CCM salt is formed when the weights of alkaline Ca source, citric acid, and malic acid correspond to 6, 2, and 3 moles, respectively. As neutralization proceeds and CCM is formed, the resulting solution eventually becomes saturated and CCM precipitates as a solid. The CCM is recovered by separating it from the supernatant liquid by

means of decantation, filtration, or centrifugation methods. The material is then dried at temperatures ideally not exceeding 100 °C using a conventional drying process to yield CCM powder with moisture content <10% by wt. The resulting dried salt is a stable powder form of CCM that can be milled to the desired mesh size (which typically ranges between 6 and 50 microns) most appropriate for the intended application (Fox *et al.*, 1993b).

Another approach for fortifying liquid foods such as beverages with CCM is to form the CCM complex *in situ* by allowing the neutralization reaction to proceed directly within the product itself. This is accomplished by adding the individual reactants to the beverage in the proper proportion and order of addition (e.g., citric and malic acids, followed by the alkaline Ca source). If the liquid food or beverage inherently contains citric and/or malic acid (e.g., orange and apple juices), the level of acids naturally present should be considered in terms of formulating to yield a given CCM molar ratio in the finished product. The *in situ* approach is the manner in which commercially available CCM-fortified OJ is manufactured, with additional citric and malic acids added to complement the levels naturally occurring in OJ and $Ca(OH)_2$ added as the Ca source.

D. Aqueous solubility

Ca is a comparatively difficult element for the body to absorb and digest. It is essentially only available for consumption associated with various other moieties (e.g., citrate, phosphate, and other anions). Each Ca source has unique physical, structural, and chemical properties such as mass, density, coordination chemistry, and solubility that are largely determined by the anions associated with the Ca^{2+}. Aqueous solubility of various Ca salts can vary markedly and comparisons are frequently made under standardized conditions. The water solubility of CCM is moderate when ranked versus other Ca sources frequently used as dietary supplements and food/beverage fortificants. The solubility of CCM (6:2:3 molar ratio) is 1.10-g salt in 100 ml of H_2O at 25 °C (Fox *et al.*, 1993a). Table 6.4 lists the solubility of various Ca sources in water at specific temperatures, and also includes some information on potential sensory characteristics.

Based on the tabulated values, it can be seen that CCM, with its solubility in water slightly over 1% by weight at 25 °C, is approximately 500 and >150–700 times more soluble than tricalcium phosphate and $CaCO_3$, respectively. As individual Ca salts, Ca malate and Ca citrate have relatively low solubility in water compared to CCM (by approximately three- and tenfold, respectively). Since there are multiple chemical formulas for CCM, solubility profiles will also vary. CCM compositions with less Ca relative to the amount of accompanying organic acids are

TABLE 6.4 Solubility and sensory characteristics of various Ca sources

Ca salt	Potential sensory characteristics	% Solubility Ca salt in H_2O	Temperature (° C)
Calcium chloride·$6H_2O$	Bitter notes, tissue irritant	74	20
Calcium lactate Gluconate	Clean tasting	40	20
Calcium acetate	Vinegary taste	40	0
		30–34	100
Calcium formate	N/A	16	20
Calcium lactate·$5H_2O$	Neutral, bitter at high levels	9	20
Calcium gluconate·H_2O	Neutral taste	3	20
Calcium fumarate·$3H_2O$	Neutral to slight fruity flavor in juice-based products	1.22	20
Calcium citrate malate (6:2:3)	Neutral taste and flavor	1.1	25
Calcium malate·$3H_2O$	Slight sourness	0.31–0.4	25
Calcium citrate	Bitter notes and tangy/sour flavor at high concentrations	0.096	25
Calcium hydroxide	Slight bitter, alkaline taste	0.1	20
Calcium oxide	Alkaline, bitter taste	0.1	25
Dicalcium phosphate	Chalky mouthfeel at neutral pH	0.02	25
Tricalcium phosphate	gritty in liquids	0.002	25
Calcium carbonate	Soapy flavor, lemony taste	0.0014–0.0056	25
Calcium oxalate	N/A	0.00067	20

N/A = not available; RT = room temperature.
Solubility data obtained from material safety data sheets (MSDS), CRC Handbook of Chemistry and Physics (Lide, 2004–2005), and a U.S. patent (Fox *et al.*, 1993a). Sensory descriptions acquired from various sources (Kuntz, 2003; Puspitasari *et al.*, 1991; Quilici-Timmcke, 2002; Tordoff, 2001; Wade, 2004; Yang and Lawless, 2005).

inherently more acidic and, in turn, have higher aqueous solubility (Fox *et al.*, 1993b). For example, a molar ratio of Ca:citrate:malate of 4:2:3 (acidic) versus 6:2:3 (neutral) versus 5:1:1 (alkaline) results in a 100% >91% >45% relative amount of Ca in solution per unit weight of CCM, respectively (Fox *et al.*, 1993a). The high aqueous solubility of CCM compared to other frequently used Ca sources is paramount to its ease of use and functionality when it comes to fortifying foods and beverages.

E. CCM in fortified foods and beverages

In 1994, an NIH consensus statement indicated that a large percentage of Americans fail to meet recommended guidelines for optimal Ca intake (National Institutes of Health, 1994). A decade later the Surgeon General's Report (2004) reiterated this finding by stating that the average American consumes levels of Ca far below the amount recommended for optimal bone health (US Department of Health and Human Services, 2004). To complicate matters, the US population as a whole is living an increasingly sedentary lifestyle compared to past generations, creating a paradoxical situation that essentially limits the amount of food and calories we can and should reasonably consume to ensure we get sufficient nutrition to maintain optimal health.

A large percentage of the American population already consumes excess calories without meeting the recommendations for a number of nutrients and minerals including Ca (e.g., 1000–1500 mg Ca/day is widely recommended for adults). Currently in this country, an estimated 127 million adults are overweight, 60 million are obese, and 9 million are severely obese. Thirty percent of children and adolescents are also overweight, and 15% are presently classified as obese (American Obesity Association, 2006). Therefore, adding more Ca to the diet without introducing additional calories and incidental dietary fat requires improvements in food choices; it calls for the availability and consumption of nutrient-dense foods that include Ca-fortified foods and beverages. For individuals that are lactose maldigesters/intolerant, strict vegetarians, or for those that have allergies to milk proteins, or simply dislike consuming dairy products on a daily basis, Ca fortification offers alternative food sources rich in Ca. If consumed regularly, fortified foods can circumvent what might otherwise likely result in a chronic dietary deficit of Ca.

Three cups of low-fat or fat-free milk per day or an equivalent amount of low-fat yogurt and/or low-fat cheese (1.5 ounces = 1 cup of milk) provide an adequate amount of Ca for most adolescents. Children 2- to 8-years old require two cups of milk per day, or the equivalent in alternative dairy foods. A one cup serving of whole milk supplies ~290 mg Ca. It was recently demonstrated that it was virtually impossible for adolescents not consuming dairy products in their diet, and without an intentional plan for

meeting Ca requirements with dairy substitution, to meet recommended AIs for Ca while also consuming the recommended intake of total calories, fat, and other essential nutrients (Gao *et al.*, 2006). Dairy-free diets in girls and boys result in mean (±SEM) Ca intakes that are only ~40% of the Ca AI (i.e., 498 ± 50.5 and 480 ± 62.4 mg Ca/day, respectively). According to the linear programming performed by Gao *et al.*, the introduction of 0.5–1.5 servings per day of Ca-fortified fruit juice in dairy-free diets with the highest Ca intakes can provide a practical alternative to the inclusion of dairy foods, providing a dietary approach considered to be within established pediatric guidelines. In effect, 1.5 servings of Ca-fortified juice can improve daily Ca intakes for females and males in this category by a corresponding 33% and 29% and supply 1302 and 1640 mg Ca/day, respectively, thus enabling achievement of the desired AI goals.

Supplementation of the diet with CCM-fortified beverages can help avoid problems associated with swallowing pills, which can be a barrier to compliance, especially for children. Swallowing is also problematic for many older persons attempting to consume medications (Griffith, 2005) and/or supplement their diets with additional Ca (Dawson-Hughes *et al.*, 1997). Furthermore, compliance may be improved when consuming whole fortified foods as opposed to supplements because the former involves substituting one food for another that is generally similar in appearance, taste, and availability. This may be particularly true if a conscious decision is made to select nutrient-fortified foods and beverages during the weekly grocery shopping. A weekly habit of purchasing Ca-fortified foods is likely easier to adhere to than a daily supplementation regimen that may require tablets to be taken multiple times per day. Taking supplements on a regular basis is an important determinant of the effectiveness of any oral nutritional intervention (Bruce *et al.*, 2003). Ca-fortified foods have the added advantage over Ca supplements of providing multiple nutrients. Ca fortification of foods began in the late 1980s, although it was not until the late 1990s that the number of fortified foods and consumer awareness appreciably increased (Forshee *et al.*, 2006).

Incorporation of a Ca source into foods and beverages can be a complicated process and it requires careful planning to be successful. Consideration must be given to the type of Ca salt that is best suited for the particular application. Each Ca compound has distinct physicochemical attributes that: (i) influence the convenience of the manufacturing process, (ii) either enhance or diminish the nutritional and organoleptic properties of the end product, and (iii) influence the shelf life and stability. The choice of available FDA-designated GRAS (Generally Recognized as Safe) Ca salts for the purpose of fortification is extensive. However, depending on the food or beverage matrix and the desired level of fortification, certain Ca sources will be better choices for optimizing the organoleptic attributes, stability, and Ca bioavailability.

CCM is a highly absorbable source of Ca that can be used to fortify a wide variety of food and beverage products. It is probably best known as a fortificant of OJ and other fruit juice/drink beverages. The aqueous solubility of CCM ensures that it remains dissolved in the juice, thereby avoiding precipitation that can confer a gritty mouthfeel or unpalatable aftertaste. Less soluble Ca salts may be only partially dissolved, with the insoluble particles either suspended in the beverage along with other juice particulates or sedimented at the bottom of the container. CCM has recently been used to fortify high-temperature, short-time (HTST) pasteurized milk without the need for stabilizers or chelating agents required in many other Ca-fortified protein-containing beverages (Luhadiya *et al.*, 2006). CCM-fortified milk is a stable and hedonically acceptable product. Low concentrations of stabilizers and chelating agents are an available option if an exceptionally high level of Ca fortification is desired. The inherently high Ca concentration of cow's milk, in addition to added Ca provided by CCM, results in a particularly Ca-rich beverage that can be used alone or be incorporated into other beverage/food products. CCM has also been used successfully to fortify plant milks (e.g., soy), milk/juice blends, and fitness waters.

It is important to remember that the effectiveness of any fortified food product fundamentally depends on its palatability. As is the case for any nutrient-fortified product, sensory quality and stability during processing and storage of CCM-fortified foods/beverages need to be confirmed at the desired level of fortification. CCM has been used to fortify beverages at levels sufficient to qualify for an excellent source of Ca nutrient content claim (i.e., $\geq$20% Daily Value/serving), while its effect on taste and appearance is neutral. A number of divalent mineral salts (Ca, Fe, and Zn) are capable of stimulating complex oral and retronasal sensations (Yang and Lawless, 2005). Based on sensory studies, inorganic Ca salts have been associated with bitter tastes and less intense bitter aftertastes (Yang and Lawless, 2005). For example, Ca chloride imparts a disagreeable bitter brackishness at high concentrations and can be a stomach irritant. The bitter taste associated with some divalent salts can often be modified to a certain degree by anions, especially organic anions. Ca lactate gluconate is generally bland tasting, although at 0.10 M it has been identified as bitter by a trained descriptive panel characterizing the oral and sensory properties of divalent salts (Yang and Lawless, 2005). Ca citrate can be very acidic and convey a slight bitter note. Ca acetate can impart a vinegary taste. A soapy flavor may be detected with Ca carbonate, particularly when it is added to a food system with high pH and fat (Wade, 2004). If hydrocolloids are required to maintain less soluble Ca salts in suspension, the texture of the product is usually altered to some extent (van Mossevelde, 1997). Ca can interact with free ionized carboxylic groups on certain nonstarch food polysaccharides, including

alginates, pectins, gellan, and xanthan gums and, hence, influence the textural and rhelogical (i.e., flow) properties. The impact on texture and flow may either be positive or negative depending on the specific product application. Ca can also interact with protein molecules, especially in the presence of heat, leading to poor dispersion stability, sedimentation, flocculation, or gelation. Poor organoleptic properties will prevent even the best fortified product from achieving its prime objective, which ultimately is acceptance and consumption by the target population. Other important factors naturally include price, quality, and perceived value.

Heaney and colleagues conducted an experiment that demonstrated the difference in physical characteristics between Ca sources used to fortify commercially available beverages. The objective was to examine the physical state of the Ca in 14 fortified beverages compared to unfortified fat-free milk (Heaney *et al.*, 2005a). Measurements reflected how well an isotope (i.e., ^{45}Ca) in extrinsically labeled beverages (prepared with 5 μCi ^{45}Ca, agitated and equilibrated overnight) partitioned between the pelleted particulate phase (solid moiety) and the supernatant liquid (soluble moiety) when the beverage was subjected to centrifugation. A Beverage Score was assigned to produce values that increase as both dissolved Ca and specific activity of the Ca in the sedimented pellet increase (Figure 6.1). Determination of the amount of tracer in the pellet (as a percent of predicted) provided an approximate indication of the extent to which unassimilable Ca exchanges with the Ca in solution. A high percentage of total Ca separable by centrifugation, in addition to a poor exchange between the solid and soluble fractions, was surmised to be indicative of relatively poor absorbability and was reflected in a lower Beverage Score. Samples were also ashed and analyzed by atomic absorption spectrophotometry and liquid scintillation spectrometry to compare the measured Ca per serving to the level listed on the Nutrition Facts panel on the package label. In Ca-fortified OJ, CCM was the Ca source that exhibited the lowest amount of separable Ca in the sedimented pellet (8.1% $^{\#5.}$ and 9.7% $^{\#4.}$), the highest percentage of ^{45}Ca exchangeability between the pelleted particulate phase and the supernatant phase, and therefore the highest overall Beverage Scores of the fortified juices (Figure 6.1). CCM-fortified OJ exhibited Beverage Scores comparable to cow's milk, suggesting a potential for excellent bioavailability of Ca from these sources. When used as a sole source of Ca in OJ, tricalcium citrate and tricalcium phosphate yielded the highest level of separable Ca, the lowest level of exchangeable Ca, and consequently the lowest Beverage Scores (#9 and #11 in Figure 6.1, respectively). Overall, the Heaney *et al.* study [155] showed that Ca was more uniformly suspended in OJ beverages than in soy or rice-based beverages.

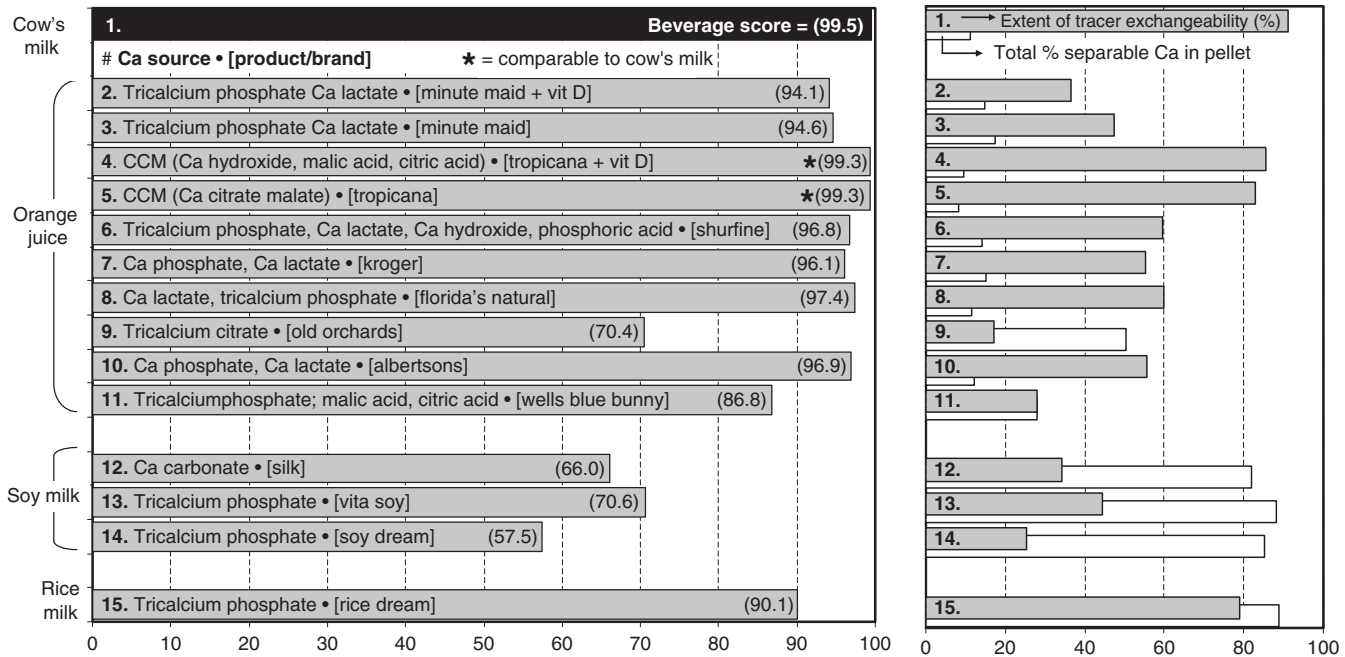

FIGURE 6.1 Graphical illustration of the results of a Ca beverage study (Heaney *et al.*, 2005a). The black bar at the top of the left graph represents the Beverage Score for milk, the traditional Ca-rich referent beverage. In the same graph, the comparative Beverage Score (i.e., dissolved Ca as determined by specific tracer activity as a result of extrinsic labeling) of various Ca sources in other brand name products (#2 through 15) are shown as gray bars with percentage values. The graph on the right depicts gray bars that indicate the corresponding amount of ^{45}Ca exchanged with Ca in solution which likely represents an assimilable source of Ca versus the overlapped white bars that show the percentage of total Ca that remained in the pellet after centrifugation.

F. CCM in dietary supplements

Sales of Ca supplements alone were $875 million in the United States in 2002, and comprised ~60% of all mineral supplement sales (Anonymous, 2004). In 2004, sales of Ca supplements increased by 9.3% (Uhland *et al.*, 2004), possibly to some extent in response to the Surgeon General's report on bone health that was issued that year. More recently in 2006, it was projected that dietary supplement sales in the United States would approach $5 billion (Anonymous, 2006). While Ca derived from a balanced diet is preferable, Ca supplements are a popular noncaloric alternative for increasing daily Ca intake. There are a vast number of oral Ca supplements available in the market place in the form of capsules, tablets, chewable tablets, effervescent tablets, liquids, powders, suspensions, wafers, and granules. However, not all Ca salts are equally soluble or bioavailable and the dose of Ca on the label of a supplement may not necessarily be reflective of the relative amount of available Ca once consumed. Furthermore, the same Ca salt may be more or less bioavailable depending on the production process and materials used to manufacture the supplement.

Many pills and tablets are coated with acid-insoluble coating agents, waxes, or shellac to act as a sealant, mask taste and odor, or ease swallowing. Various methods of preparation may interfere with Ca bioavailability from a supplemental source. Factors such as overcompression of tablets, formulations without the inclusion of starch (a disintegrant), or the inclusion of various other excipients comprising of complex fillers/binders may be a cause of poor disintegration and dissolution of Ca supplements. It is not unusual for chiropractors to visualize undissolved $CaCO_3$ tablets on radiographs in the lower intestine during lumbar spine examinations (Cook, 1994). The variable efficacy of two commercially available $CaCO_3$ tablets, brand-X and brand-Y, was demonstrated by Kobrin *et al.* (1989) in multiple experiments using an official US Pharmacopeia (USP) test method for *in vitro* evaluation and short- and long-term tests to assess *in vivo* effects. The two brands of Ca tablets were comprised of two different oyster shell-derived sources of $CaCO_3$, each containing 500 mg of elemental Ca per tablet. *In vivo* testing involved ingestion of two tablets of a given brand during each meal for 24 h. *In vitro* testing involved tablet dissolution and disintegration tests performed in acetic and hydrochloric acids. The disintegration time of brand-X in vinegar was >15-fold longer than that of brand-Y. The brand-X supplement did not meet USP limits for $CaCO_3$ disintegration and dissolution. *In vitro* assessments may of course be criticized, and with good reason, for not exactly mimicking what occurs *in vivo* in the more complex environment of the gastrointestinal tract. However, given that Ca has a high radiodensity, roentgenographs or x-rays were also used to confirm the presence of undisintegrated $CaCO_3$ tablet remnants in stool samples collected for 48-h postdosing.

In another study, serum phosphorus (P) and Ca levels were assessed in patients with hyperphosphatemia and hypocalcemia, respectively, subsequent to therapeutic supplementation with each brand of $CaCO_3$. Roentgenographs revealed tablet-shaped opacities after supplementation with brand-X, but not after supplementation with brand-Y. Intact tablet remnants of brand-X supplements, which were 99.7% of their original weight, were recovered in the feces of all subjects, although no remnants were recovered for brand-Y. Furthermore, only brand-Y supplements restored serum Ca and P levels to the desired range in patients with medical conditions dependant on Ca bioavailability. Kobrin also cited other investigators that performed *in vitro* studies on 32 brands of commercially available $CaCO_3$ preparations and found that only 7 of them met USP standards for disintegration and dissolution. Intestinal transit time and gastric motility vary within the general population and can influence Ca availability, particularly if tablet disintegration is slow under physiological conditions. A slowly disintegrating Ca salt supplement with inherently poor solubility characteristics, coupled with a fasted state, achlorhydria, or pharmacologically induced achlorhydria, can result in Ca supplements appearing in the rectum of patients that are x-rayed, having bypassed the principal intestinal sites of absorption in the ileum and jejunum. Physical evidence of tablet remnants in stools clearly indicates a Ca supplement has not been available for absorption.

While absorption of CCM is enhanced in the presence of a light meal, it can also be consumed on an empty stomach and still be sufficiently absorbed (Heaney *et al.*, 1989b; Higdon, 2005). The same cannot be said for some other Ca salts and Ca supplements in tablet form (Cook, 1994). Instead, their availability and benefit are contingent upon the presence of sufficient stomach acid and/or the ionic strength of the intestinal contents during a meal. Absorption of Ca from carbonate sources in patients with achlorhydria has been demonstrated to be significantly impaired if supplementation does not coincide with a meal (Recker, 1985).

Ca carbonate is often used in supplement tablets or pills because of the high Ca density and low cost. However, a powdered Ca dietary supplement, intended to be mixed/dissolved into beverages or other fluid foods (e.g., soups and sauces) by the consumer just prior to consumption, is a novel product form applicable only to a soluble Ca salt such as CCM. A powdered Ca supplement would of course avoid the problems some consumers experience with swallowing pills and tablets, as well as eliminate any uncertainty associated with tablet disintegration and dissolution.

Ca excess, or hypercalcemia, from dietary sources of Ca, including fortified foods and supplements is very rare. In relation to daily Ca intakes, hypercalcemia is typically only likely to result in the event of extreme habitual overzealousness, which of course is not recommended. Daily Ca intakes that fall short of systemic needs are infinitely more

prevalent. Excellent traditional food sources of Ca include milk, yogurt, cheese, tofu, and canned fish with soft edible bones, although these foods are not consumed regularly enough by individuals that are unable to meet their Ca needs. Fortification of an expanding number of food and beverage products, including many products that do not supply appreciable levels of Ca in their unfortified state, may prove to be an effective strategy for helping to narrow the gap between current Ca consumption levels and the recommended intakes. We live in an era in which average lifespan and average age of the population are increasing. Basic prophylactic measures such as increased consumption of fortified foods and beverages and mineral supplements comprising highly bioavailable Ca can enhance our protection against chronic debilitating diseases associated with aging, such as osteoporosis.

IV. STUDIES OF Ca BIOAVAILABILITY FROM CCM

In the context of nutrition, bioavailability refers to the difference between the amount of an exogenous nutrient or mineral a person is exposed to, and the amount of that substance that is absorbed and reaches the systemic circulation. Exposure is typically, although not exclusively, via an ingested dose. Bioavailability is considered to reflect accessibility of a substance to a potential site of action (Balant, 1991) for use or storage. Utilization is essentially a separate issue beyond bioavailability. Once absorbed, available nutrients are utilized according to physiological status, metabolic function, and nutritional need (Heaney, 2001b), despite their relative abundance. Movement of a substance across the outer membrane of the gastrointestinal tract represents absorption. Effective absorption of Ca at the intestinal mucosa is at the forefront of the bioavailability process. Absorption is influenced by numerous factors, both intrinsic and extrinsic, that produce effects both singularly and in combination with each other. CCM is often distinguished from other Ca sources on the basis of its ostensibly high absorbability and bioavailability in the face of factors that mediate outcomes in relation to Ca uptake and metabolism. This chapter explains and examines the factors that generally govern and influence Ca absorption; it looks at how agreeably CCM fits with these factors in terms of being able to deliver health benefits based on data available from human and animal studies (summarized in Tables 6.5 and 6.6, respectively).

A. Ca absorption

Intestinal absorption of Ca is via two distinct mechanistic routes which involve the (i) transcellular pathway, a saturable active transfer process that is unidirectional (i.e., mucosal-to-serosal), and (ii) the paracellular

TABLE 6.5 Summary of studies that investigated the absorption/bioavailability of Ca from CCM ingested as a supplement or food fortificant

Author, year	Subjects (n), age, and gender	Treatments Ca loads	Objective and design	Results	Significance
Andon *et al.*, 1996b	$n = 57$ ♀ $\bar{x}$: 57 year	250 mg Ca as CCM via radiolabeled oral dose. Juices were extrinsically labeled with ^{45}Ca. CCM molar ratio in apple juice was 1.0:0.7:1.3 (equivalent to 6:4.2:7.8); CCM molar ratio in OJ was 1.0:1.8:1.5 (equivalent to 6:10.8:9)	Compare Ca bioavailability from CCM in orange (OJ) versus apple juice (AJ) using single oral isotope method; specific activity in serum measured 5-h postdose	% FxAbs of Ca ($\bar{x}$: ±SEM) in serum: CCM-OJ (36 ± 1%) CCM-AJ (42 ± 2%)	CCM-AJ > CCM-OJ $p < .003$
Miller *et al.*, 1989	$n = 6$ children ♂: $n = 3$ ♀: $n = 3$ Age range 11–17 years	250 mg Ca as CCM enriched with tracer in form of chewable tablets	Stable dual isotope tracers used to quantify CaAbs in urine and serum. Comparison of CCM chewable versus CCM swallowable supplements and $CaCO_3$ from data collected previously in same subjects	Based on urinary excretion which correlated with serum data ($r = 0.85$): $\bar{x}$: ±SD% CaAbs from chewable CCM for molar ratio 6:2:3 (41.4 ± 8.2%) > swallowable CCM for molar ratio 5:1:1 (39.5 ± 10.6%) > $CaCO_3$ (26.7 ± 7.8%)	Chewable CCM > $CaCO_3$ ($p = .047$) and swallowable CCM > $CaCO_3$ ($p = .094$)
Miller *et al.*, 1988	$n = 12$ adolescents ♂: $n = 6$ ♀: $n = 6$ Age 10–17 years	Treatments in randomized order with 3-week washout period 1. 250-mg elemental Ca as $CaCO_3$ (enriched with ^{44}Ca)	Compare CaAbs for CCM versus $CaCO_3$ using dual isotope method using a crossover design and measured urinary isotope ratio after 24 h	Mean FxAbs ± SEM results 1. CCM: 36.2 ± 2.7% (range: 27.3–53.3%) 2. CaCO3: 26.4 ± 2.2% (range: 12.8–39.6%)	t-test for FxAbs difference CCM > $CaCO_3$ ($p < .03$)

(*continued*)

TABLE 6.5 (*continued*)

Author, year	Subjects (*n*), age, and gender	Treatments Ca loads	Objective and design	Results	Significance
		2. 250-mg elemental Ca as CCM (enriched with ^{44}Ca) Intrinsically labeled; ^{42}Ca i.v. tracer 30-min after oral Ca load; CCM molar ratio was 5:1:1 (equivalent to 6:1.2:1.2)		Mean difference in FxAbs for CCM versus $CaCO_3$ was 9.7 ± 3.7% or a 37% increase in CaAbs for CCM	
Martini and Wood, 2002	$n = 12$ elderly subjects ♂: $n = 3$, 76 ± 6 year ($\bar{x}$: ±SEM) ♀: $n = 9$, 70 ± 3 year (: ±SEM)	6 weeks on a standardized low-Ca basal diet (300 mg Ca/day) Weeks 2, 4, and 6, one of the following high-Ca treatments (adding 1000 mg Ca/day as a divided oral dose at breakfast and dinner) was randomly added to the basal diet for a 1-week period 1) Milk 2) Ca citrate malate fortified OJ (CCM-OJ) 3) $CaCO_3$ supplement (Os-Cal)	6-week crossover trial in a metabolic unit to compare the relative bioavailability of Ca from three dietary supplemental sources by measuring serum PTH response to Ca intake (an indirect method)	Postprandial suppression of serum PTH not different among the three supplemental sources tested, suggesting Ca bioavailability equivalence among them During 1-week high-Ca diet periods: fasting serum Ca ↑3% ($p < .0001$), serum 1.25-$(OH)_2$ vitamin D ↓20% ($p < .0001$), and bone resorption biomarker (serum NTX) 14% ($p < .02$) compared to low-Ca periods	$p > .05$ for comparison of Ca sources
Brink *et al.*, 2003	$n = 10$ ♀ > 5 year postmenopausal Age 58–65 year	Consumed a western style breakfast with 60.2 mg Ca in food + oral intake of 200 mg of a given Ca salt [CCM, $CaCO_3$,	Assessed true FxAbs of Ca in a 10-week randomized 8-way crossover design study with 7- to 14-day washout periods. Dual	$\bar{x}$: ±SD Ca Abs (%) CCM $n = 8$ 29.6 ± 10.6 $CaCO_3$ $n = 9$ 29.9 ± 12.3 Milk $n = 9$ 29.4 ± 8.8 TCP $n = 9$ 24.7 ± 14.8	CaLG, CaL CCM $CaCO_3$ and milk comparison. ($p > .05$) TCP < CaLG and

		tricalcium phosphate (TCP), Ca lactate gluconate (CaLG), and Ca L-lactate (CaL)]	isotope technique to measure Ca tracer ratios in urine	CaLG $n = 9$ 32.1 ± 7.6 CaL $n = 18$ 31.5 ± 9.3	CaL ($p < .05$) TCP same as CCM, $CaCO_3$, and milk
Smith *et al.*, 1987	$n = 10$ per group for Expt. 1 $n = 12$ per group for Expt. 2 Age range 21–30 years All subjects ♀	250-mg oral Ca load (labeled) for both experiments, and simultaneous i.v. administration of a second tracer, ^{47}Ca Expt. 1—$CaCO_3$ tablets versus Ca citrate malate (CCM) tablets; intrinsically labeled; CCM molar ratio 6:2:3 Expt. 2—milk (2% fat) versus CCM added to OJ; extrinsically labeled; CCM molar ratio 3:3:2 (equivalent to 6:6:4)	Comparison of Ca availability from CCM (tablet form and as a fortificant in juice), $CaCO_3$, and milk Interindividual differences tested, subjects assigned to either treatment alternative in each experiment Double-isotope technique employed to assess serum and urine Ca	Absolute % absorption (±SEM): Expt. 1: CCM (37.3 ± 2.0) vs $CaCO_3$ (29.6 ± 1.7) Expt. 2: CCM-OJ (38.3 ± 1.5) vs Milk (29.4 ± 2.4) Ca availability from CCM was at least as good, if not better than it was in either $CaCO_3$ (in supplement form) or milk	Expt. 1: CCM > $CaCO_3$ ($p < 01$) Expt. 2: CCM-OJ > Milk ($p < .01$)
Jackman *et al.*, 1997	$n = 35$ adolescent girls, postmenarcheal Age range 12–15 years	Mean ± SD Ca content of basal diet provided 799 ± 163 mg/day, and was adjusted for Ca content by adding CCM to dietary beverages so that mean ± SD Ca (mg/day) intake for groups was as follows: *Gp* *n* *Low Ca* *High Ca* A* 10 841 ± 153 1842 ± 153 B* 3 1023 ± 150 2173 ± 149 C* 3 1154 ± 153 1694 ± 142 D* 5 1358 ± 143 2096 ± 153 E 14 1332 ± 102 -	Crossover design* for groups on both high and low intakes Ca balance measured in subjects during two 21-day Ca-balance studies separated by a 4-week washout period;	Ca retention modeled as a nonlinear function of Ca intake Ca intake explained 79% and 6%, respectively of the variation in fecal and urinary Ca excretion. $\bar{x}$ maximal Ca retention was 473 mg/day. 1300 mg Ca/day was the smallest intake that allowed some subjects to achieve 100% of maximal Ca retention (95% CI: 26%, 100%).	$\bar{x}$: maximal Ca retention (95% CI: 245, 701 mg Ca/day)

(*continued*)

TABLE 6.5 (*continued*)

Author, year	Subjects (*n*), age, and gender	Treatments Ca loads	Objective and design	Results	Significance
				Ca retention estimated to plateau at intakes > 2 g/day	
Andon, 2003 Abstr 188.3	n = not specified Group 1: adolescents age range 9–17 years Group 2: ♀ age range 20–30 years Group 3: ♀ age range 40–77 years	All oral Ca test loads (250 mg) intrinsically labeled with Ca tracer and consumed as tablets or in juice	Assessed the impact of molar ratio (MR) on CaAbs in 5 different Ca citrate malate (CCM) compositions comprising a threefold range in MR (data from 154 previous studies).	$\bar{x}$ CaAbs for: 1) tablet (37.7%) vs juice (38.9%) vehicle 2) age group (gp 1: 37.9% vs gp 2: 37.8% vs gp 3: 39.0%) 3) MR (lowest to highest MR yielded % CaAbs of 42.0, 38.8, 36.2, 36.0, 38.3%). CCM indexed to an equimolar Ca dose of milk (Ca index for milk set at 100) consistently exceeded that of milk (138), other dairy products (86–100), Ca salts used as fortificants (86–93), Ca-fortified soy milk (77), and other vegetable sources (18–103).	1) Effect of vehicle Tablet > Juice $p > .05$ 2) Effect of age $p > .05$ 3) Effect of different molar ratios of CCM $p > .05$
Heaney *et al.*, 1989b	n = 47 ♀ used in various comparisons Age range 20–30 years ▪ Observation study: used subgroup of n = 1–3	▪ Observation study. Oral treatments as follows: (1) $CaCO_3$ (2) calf bone substance or CBS	Evaluated the effect of co-ingestion of a light meal with various sources of Ca on CaAbs efficiency. Double-isotope method employed.	▪ Observation study: $\bar{x}$ FxAbs of Ca: without meal for $CaCO_3$ (0.0803), CBS (0.0780), HA (0.077) with meal for milk	▪ Obs: Significance not reported ◆ Expt 1: $p > .05$ $CaCO_3$ w/meal > $CaCO_3$ w/out meal ▲ Expt. 2: $p < .01$

	◆ Expt. 1: $n = 10$ for a 'with-meal' assessment and $n = 26$ for a 'without-meal' assessment ▲ Expt. 2: $n = 10$	(3) synthetic hydroxyapatite or HA (4) milk (5) Ca citrate malate or CCM where: - all Ca loads ~250 mg + labeled with ^{45}Ca and ^{47}Ca i.v. tracer administered to subjects ◆ Expt. 1: 250 mg Ca load (as $CaCO_3$) + ^{45}Ca + ^{47}Ca i.v. tracer: (a) with meal, and (b) without meal. ▲ Expt. 2: 250 mg Ca load (as Ca citrate malate in OJ) + ^{45}Ca + ^{47}Ca i.v. tracer: (a) with meal (b) without meal.	The test Ca source was always consumed at the midpoint of a neutral test meal with no food post-load (4 h). ▪ Observation study: Subjects tested twice under non-meal conditions. ◆ Expt. 1: Not a crossover design ▲ Expt. 2: Subjects crossed over for with- and without-meal comparison	(0.247), and CCM (0.304) ◆ Expt. 1: $\bar{x} \pm$ SEM for FxAbs of Ca after oral intake of $CaCO_3$ with meal = 0.296 ± 0.0170 vs without meal = 0.246 ± 0.0265 (NS) ▲ Expt. 2: $\bar{x} \pm$ SEM for FxAbs of Ca at base-line under fasting conditions for CCM was 0.2847 ± 0.095 vs 0.3743 ± 0.0166 with meal. Difference in FxAbs + 0.0896 ± 0.0246. 90% of subjects exhibited improved FxAbs of Ca when Ca load was ingested with a meal.	CCM w/meal > CCM w/out meal
Heaney *et al.*, 2005b	$n = 25$, premenopausal ♀ Age range 21–43 year, $\bar{x}$: 34.4 year	500 mg Ca as an oral load delivered in OJ as a fortificant with a light meal as follows: 1. Ca citrate malate (CCM) 2. tricalcium phosphate/Ca lactate (TCP/CL)	Pharmacokinetic methods used to assess the bioavailability of two Ca salts used to fortify OJ. Subjects tested 3x's, 2x's with one fortification, and 1x with the other (random sequence); 4-week washout between treatments	$AUC_{9\text{-hr}}$ was 48% greater for CCM vs TCP/CL $\bar{x}$: ±SD (mg) for Ca absorbed was: CCM-OJ (148 ± 9.0) > TCP/CL-OJ (100 ± 8.9)	CCM > TCP/CL ($p < .001$)
Heaney *et al.*, 1990a	Age range 20–40 year adult ♀ $n = 39$ CaC_2O_4 $n = 21$ hydroxyapatite (HA) $n = 10$ $CaCO_3$ w/meal $n = 43$ $CaCO_3$ w/out	All tests under load conditions at intake levels comparable to the Ca content of a typical meal Ca sources in liquid form were extrinsically labeled (i.e., Ca	Compilation of values for Ca FxAbs and approximate solubility of various Ca salts and food sources obtained in 352 studies in human subjects with and without	Data summarized as: () ~ $\bar{x} \pm$ SEM solubility (mM/L) [] [FxAb with *or without a meal*]: CaC_2O_4: (0.04) [0.102	The solubility data is presented as an approximation

(*continued*)

TABLE 6.5 (*continued*)

Author, year	Subjects (*n*), age, and gender	Treatments Ca loads	Objective and design	Results	Significance
	meal $n = 10$ $Ca_3(PO_4)_2$, or TCP $n = 7$ Ca citrate (CC) $n = 20$ CCM $n = 13$ bisglycino-Ca (BS) $n = 34$ spinach $n = 108$ milk w/meal $n = 10$ milk w/out meal $n = 11$ kale $n = 37$ bonemeal (BM)	citrate and milk) or intrinsically labeled during synthesis and ingested as a liquid (i.e., bisglycinocalcium). Solid preparations intrinsically labeled prior to precipitation [i.e., $CaCO_3$, Ca oxalate (CaC_2O_4), TCP, HA, and CCM], spinach and kale grown hydroponically and labeled via a nutrient solution during growth All tests were performed after subjects fasted overnight	the coingestion of food Solubility in this study referred to the amount of the substance that can be dissolved in water at neutral pH Most studies performed using the double-isotope method, some later tests used a 5 h, single isotope method	±.040] w/ HA: (0.08) [0.166 ± .090] *w/out* $CaCO_3$: (0.14) [0.296 ± .054] w/ [*0.235 ±.123*] *w/out* TCP: (0.97) [0.252 ± .130] w/ CC (7.3) [*0.242 ± .049*] w/out CCM: (80) [0.363 ± .076] w/ BS: (1500) [*0.440 ± .104*] w/out Spinach: [0.046 ± .004] w/ Milk: [0.317 ± .023] w/ [*0.267 ±. 025*] *w/out* Kale: [0.409 ± .030] w/ BM: [*0.272 ± .019*] *w/out* The relationship of solubility to absorbability was determined to be tenuous and CaAbs from food sources was considered to be influenced by other food components	
Griffin *et al.*, 2002	$n = 59$ girls at or near menarche Age range: 11.0–13.9 years	Average Ca intake was ~1500 mg/day via consumption of Ca-fortified OJ (i.e., one 8-oz glass AM and PM supplying 700 mg Ca/d as CCM) in	Assessed the effect of oligofructose or inulin + oligofructose vs placebo on Ca Abs in a balanced, randomized, crossover design using dual isotope	Mean (±SD) Ca Abs from CCM due to the placebo treatment alone ranged from 31.8 ± 10.0% to 32.3 ± 9.8%. Ca Abs from CCM increased	Ca Abs from CCM in the presence of inulin + oligofructose > placebo; $p = .01$

		conjunction with the following treatments (3 weeks each) adhered to in randomized order with an intervening 2-week washout period: 1. placebo (sucrose) 2. oligofructose 3. inulin + oligofructose mixture OJ extrinsically labeled with ^{46}Ca as $CaCO_3$.	methodology. Ca Abs measured from the ratio of the fractional excretion of ^{46}Ca:^{42}Ca (the latter delivered intravenously) in a 48-hr urine collection.	significantly in the presence of inulin + oligofructose (38.2 ± 9.8%), although not in response to oligofructose alone (31.8 ± 9.3%).	Ca Abs from CCM in OJ during intake of the placebo or with the non-digestible, fermentable oligosaccharide treatments were high despite the fact that subjects e xceeded the AI recommended for Ca.
Griffin *et al.*, 2003	$n = 54$ girls Mean age: 12.4 ± 1.2 years	Average Ca intake maintained at 1390 ± 453 mg/day via consumption of Ca-fortified OJ (i.e., one 8-oz glass AM and PM supplying 700 mg Ca/d as CCM) in conjunction with the following treatments (3-wk each) adhered to in randomized order with an intervening 2-week washout period: 1. placebo (sucrose) 2. Synergy1 (long-chain inulin enriched with oligofructose) OJ extrinsically labeled with ^{46}Ca as $CaCO_3$.	Assessed benefits due to enhanced Ca absorption resulting from the addition of modest amounts of long-chain inulin enriched with oligofructose in a balanced randomized crossover design. Ca Abs measured using dual-isotope methodology via the ratio of ^{46}Ca with ^{42}Ca (intravenous tracer) as it appeared in urine collected over 48 hr.	Overall, mean (±SD) Ca Abs due to the placebo treatment was 33.1 ± 9.2%. In the presence of Synergy1, Ca Abs significantly increased to 36.1 ± 9.8%. Girls displaying lower Ca absorption during the placebo period benefited most from the addition of non-digestible, fermentable, oligofructose.	Ca Abs from CCM-fortified OJ during intake of the placebo and following consumption of the non-digestible, fermentable, oligofructose treatment was consistently high (Synergy1 > placebo; $p = .027$) despite the subjects exceeding the AI recommended for Ca.

TABLE 6.6 Summary of animal studies that investigated the absorption/retention/bioavailability of Ca from CCM

Author, year	Animals (n)	Treatments Ca loads	Objective and design	Results	Significance
Weaver *et al.*, 2002	Adult male rats $n = 10–15$	Oral gavage with various Ca fortification salts of different solubilities that were intrinsically labeled with ^{44}Ca	Determination of FxAbs of Ca based on the femur uptake model 48-h after feeding	Mean FxAbs of ^{45}Ca (%) ±SEM from the five salts was: Ca fumarate: 30.09 ± 1.02 > Ca malate fumarate: 29.13 ± 1.65 > Ca citrate: 28.69 ±2.25 > CCM: 28.06 ± 1.58 > $CaCO_3$: 27.42 ± 3.09	$p > .05$ for all comparisons
Andon *et al.*, 1996b	Dogs: adult ♀ ($n = 6$)	Animals dosed with ^{47}Ca extrinsically labeled beverages	Whole body ^{47}Ca retention assessed in dogs and rats 72-h postdose	Ca retention for CCM-AJ > CCM-OJ in: Rats 61 ± 2 vs $52 \pm 2\%$ Dogs 29 ± 2 vs $15 \pm 1\%$	CCM-AJ > CCM-OJ Rats: $p < .05$ Dogs $p < .001$
	Rats: young adult ♂ ($n = 6$)	Dogs: 125 mg dose Ca as: (1) CCM-OJ or (2) CCM-AJ Rats: 6 mg dose Ca administered as either (1) 2% fat milk (2) CCM-OJ (3) CCM-AJ	Additional analysis to determine the effect of different carbohydrate and organic acid profiles of juices on CaAbs in rats	Ca from CCM in AJ better retained than that from OJ In rats Ca retention CCM-AJ and CCM-OJ > milk ($42 \pm 2\%$) Higher ratio of fructose: glucose and lower acid content of CCM-AJ improved Ca retention in rats	Comparison of juices $p < .05$ Juices vs milk $p < .05$ Effect of organic acid AJ vs OJ $p = .002$ Effect of sugars AJ vs OJ $p = .0001$

Smith *et al.*, 1987	Expt. 1. $n = 7$/group Expt. 2 $n = 13$/group Rats in Expt. 1 were 30–40% older than those in Expt. 2	Expt. 1. Labeled (^{45}Ca or ^{47}Ca) oral test loads of 6 mg Ca/rat via gastric tubing as CCM or $CaCO_3$ (powder vehicle) Expt. 2 Extrinsically labeled CCM-OJ or milk	Estimated relative Ca retention after 6-days to determine % retention of the oral test load	Mean % ±SEM Ca retention for Expt. 1: CCM powder = 35.1 ±1.6% > $CaCO_3$ powder = 30.8 ± 2.6% Expt. 2 CCM-OJ = 51.1 ± 1.7% > milk = 42.2 ± 1.8%	Expt. 1. CCM > $CaCO_3$; $p > .05$ Expt. 2 CCM-OJ > milk; $p < .001$
Kochanowski, 1990	Young (weanling) growing female rats ($n = 14$/group)	Rats fed either marginal (0.3%) or adequate (0.6%) Ca as CCM or $CaCO_3$ for either 4 or 12 weeks′	Examined bone histomorphometry parameters as an indicator of Ca bioavailability	Rats fed 0.6% CCM were heavier than both groups of $CaCO_3$-fed rats at 4 weeks and 8 weeks Longitudinal bone growth rate (0.3%) CCM > (0.6%) $CaCO_3$ at 4 weeks Trabecular bone 4 weeks: CCM 23–25% > $CaCO_3$ Trabecular bone 12 weeks: CCM 44–47% > $CaCO_3$	Body weight CCM > $CaCO_3$ 4 weeks $p < .05$ CCM > $CaCO_3$ longitudinal growth $p < .05$ CCM > $CaCO_3$ trabecular bone 4- and 12 weeks $p < .05$
Andon *et al.*, 1993	Young adult male rats $n = 6$–7/group	Equimolar amount of intrinsically $_{[int]}$ or extrinsically $_{[ext]}$ labeled Ca as an oral dose of either: CCM $_{[int]}$ CCM $_{[ext]}$ $CaCO_3$ $_{[int]}$	Longitudinal study determined whole body fractional Ca retention at 8, 16, 29, and 32 weeks of age with various Ca salts labeled either	Advancing age decreased Ca retention Rank order relative to CCM $_{[int]}$(100%) > $CaCO_3$ $_{[int]}$ (83±4%) > HAP $_{[int]}$ Ca retention over	Ca retention: young > older age for all Ca salts; $p < .001$. CCM > $CaCO_3$ > HAP at all ages; $p < .001$ Extrinsic > intrinsic labeling values in

(*continued*)

TABLE 6.6 (*continued*)

Author, year	Animals (n)	Treatments Ca loads	Objective and design	Results	Significance
		$CaCO_{3\ [ext]}$ Ca hydroxyapatite $(HAP)_{[int]}$ Ca hydroxyapatite $(HAP)_{[ext]}$	intrinsically or extrinsically	estimated by extrinsic labeling in younger rats	young rats (~20%)
Heaney *et al.*, 1989b	Male rats $n = 6$–24 per group treatment	Oral Ca test load (5.1 mg) by gavage w/ and w/ out a meal Milk or CCM dissolved in a variety of citrus juice beverages	Evaluated the effect of coingestion of $CaCO_3$ vs CCM salts w/ and w/out a meal. Radiolabeled CCM and $CaCO_3$ ingested and activity measured 7 days postdose using whole body counting method to determine Ca retention	Mean Ca retention fraction w/out food was 48% for CCM in juice and 42.7% for milk Mean Ca retention fraction w/ food was 61.2% for CCM in juice and 50.8% for milk	CCM w/ meal > CCM w/out meal $p < .001$ for all juices $CaCO_3$ w/ meal vs $CaCO_3$ w/out meal was $p < .01$ and $p < .001$
Henry and Pesti, 2002	Young boiler chicks $n = 10$ pens of 3–8 chicks per treatment	Expt. 1 CCM vs $CaCO_3$ each at 0.7% and 0.9% Expt. 2. CCM vs Ca CO_3 at 0.50, 0.55, 0.60, 0.65, or 0.70% Ca	Comparison of CCM vs $CaCO_3$ in two feeding studies in terms of bone/body growth and development	Expt. 1. Feed efficiency and growth 0–18 days CCM > $CaCO_3$, no differences in bone development Expt. 2. CCM > $CaCO_3$ for: (1) weight gain (2) dry fat-free tibia weight (3) tibia ash (4) tibia Ca	Expt.1. CCM > $CaCO_3$ for growth $p \leq .05$ Expt. 2. parameters CCM > $CaCO_3$ (1) $p < .022$ (2) $p < .0002$ (3) $p < .023$ (4) $p < .0001$ (5) $p < .047$
Lihono *et al.*, 1997a)	1-day old male broiler chickens	Expt. 1. Animals fed corn/ soybean-meal-based	Feeding study assessed the effects of microbially derived	Adding phytase to CCM in food did not cause changes in weight	Ca bioavailability for CCM w/ or w/ out phytase in

	$n = 20$ birds per treatment	diets, w/ w/out ~0.12% added phytase and either: -Ca from CCM at 0, 0.1, 0.2, and 0.3% -Ca from $CaCO_3$ at 1% Expt. 2. Fed spray-dried soymilk incubated with microbial phytase prior to hydrothermal cooking and either: -Ca from CCM (0.31, 0.46, 0.61%) -Ca from $CaCO_3$ (0.76%).	phytase enzyme treatments on the bioavailability of Ca from soy-based foods over 17 days Examined bone, weight gain and feed intake	gain Adding phytase to $CaCO_3$ in food vs $CaCO_3$ alone improved weight gain	diets was the same $p < .17$ Ca bioavailability for $CaCO_3$ w/ phytase > $CaCO_3$ w/out phytase in diets $p < .05$
(Pointillart and Guéguen, 1993)	Male pigs (2-month old) $n = 9$/group	Fed 0.7% Ca-supplemented diet 0.5% Ca from milk 0.5% Ca from CCM 0.2% Ca from CaCO3 in basal diet Phytate content in diets	Balance study and assessment of bone parameters Pair fed pigs for 10 weeks, 10-day balance trial 2 weeks before euthanization for $n = 6$/group	Milk > CCM for growth rate and feed efficiency Milk = CCM for growth rate and feed efficiency when adjusted for bodyweights Urinary hydroxyproline excretion CCM = Milk Most morphological parameters for bone milk > CCM Bone parameters not related to growth CCM = or > Milk	Before adjustment for bodyweight bone growth milk > CCM $p < .05$ or $p < .03$ after adjustment $p > .05$ Apparent density and stress parameter for metatarsal bone CCM > milk $p < .05$

pathway, a nonsaturable process dependent on diffusion-driven transfer which is potentially bidirectional (i.e., predominantly mucosal-to-serosal, but also potentially serosal-to-mucosal).

Transcellular absorption involves lumenal Ca crossing the brush border of intestinal enterocytes, down its electrochemical potential gradient, and entering the cytosol via Ca channels and membrane-binding transport proteins. The intracellular vitamin D-induced Ca-binding protein calbindin-D_{9K} (rate limiting) and membrane-bound vesicles mediate the translocation of Ca through the cytosol to the basolateral membrane where it is ejected from the enterocyte against its electrochemical potential gradient via transport proteins. When the intracellular concentration of Ca is low, the basolateral membrane Ca-ATPase splits ATP and uses the liberated energy to pump Ca extracellularly. When intracellular concentrations of Ca are high, the Na^+/Ca^{2+} exchanger in the basolateral membrane uses energy derived from the Na^+ gradient to expel intracellular Ca. The membrane-bound vesicles that transport Ca also extrude it from the lateral membrane via exocytosis. The duodenum followed by the jejunum are the intestinal locations that most effectively absorb Ca transcellularly, whereas the ileum is the site of longest residency and, therefore, the region of the intestines of greatest total Ca absorption (Weaver and Heaney, 2006a). Transcellular absorption is regulated by $1,25(OH)_2$ vitamin D_3 and limited by the presence of a finite number of Ca channels and binding sites and therefore functions optimally when Ca intake is relatively low.

A high concentration of Ca in the intestinal lumen relative to the ECF tends to drive Ca absorption via the paracellular route. Water naturally seeps through the ''microspaces'' (Wasserman, 2004), or cellular junctions between adjacent enterocytes, during absorption thus creating a paracellular pathway between which 8–30% of the total Ca absorbed (McCormick, 2002) is entrained as a solute. The transfer of Ca by a solvent drag-induced mechanism is via a passive diffusion process in response to increases in the osmolarity of the lumenal contents. This pathway is not site specific and the opportunity for Ca absorption via this route occurs throughout the entire length of the small intestine (Weaver and Liebman, 2002).

B. Factors that influence Ca absorption

The absorbability and subsequent bioavailability of Ca salts in humans and animals is simultaneously influenced by a number of exogenous and endogenous factors. In summary, these factors include, although are not limited to:

- Ca load (i.e., per day and per dose)
- Measurement methodologies
- Chemistry of the Ca salt
- Food matrix or type of supplement
- The presence or absence of enhancing and/or interfering substances (e.g., inulin-type fructans, fructooligosaccharides, phytates, excipients, medications)
- The composition and/or timing of meals
- Nutritional status (i.e., replete or deficient)
- Health status, family history of osteoporosis
- Lifestyle factors (e.g., physical activity, smoking, alcohol intake)
- Race and genetic factors [e.g., FOK1 gene polymorphism of the vitamin D receptor (VDR)]
- Age, life stage
  - Body size
  - Hormonal status (including seasonal effects)
- Physiological function
  - Gastric acid secretion
  - Endogenous solubility
  - Intestinal motility, mucosal permeability, and mucosal mass
  - Species differences (i.e., endogenous phytase synthesis, coprophagy)

C. How CCM fits with the influencing factors — human studies with CCM

1. Ca intake

The net amount of Ca absorbed increases with increasing intake (Dawson-Hughes, 2006b). Balance and tracer studies have shown that fractional Ca absorption efficiency is generally increased by a low and reduced by a high Ca diet (Heaney *et al.*, 1990b; Norman *et al.*, 1981). Percentage Ca absorption is, for that reason, inversely related to size of the Ca load (Andon *et al.*, 2004; Schulze *et al.*, 2003; Weaver *et al.*, 1996) and usual intake is considered to explain 26% of the interindividual variation in Ca absorption (Barger-Lux *et al.*, 1995). Barger-Lux and Heaney (2005) have described Ca absorption efficiency as more highly variable than Ca intake itself. Dividing up one's daily Ca dose into equal increments that are consumed at regularly spaced intervals over the course of the day is recommended as a useful means by which to increase the absorption efficiency and efficacy of Ca (Blanchard and Aeschlimann, 1989; Heaney, 1991). Ca supplements that include >500 mg Ca per tablet/capsule can quickly overwhelm the active transport mechanism of the intestinal system and passive transport must be more heavily relied upon for absorption of high acute Ca doses (Dawson-Hughes, 2006b).

Ideally, Ca supplements should not be used to replace Ca consumed from natural food sources; although in the event that food choices or caloric intake do not supply adequate levels of Ca, supplements may be of great benefit when used to complement one's dietary intake. However, the reality is that Ca intakes across a number of groups in the population are generally too low (Braun and Weaver, 2006). As a result, larger doses of Ca in the form of supplements are routinely relied upon to supply absorbable Ca and, hence, the various factors that influence absorption become increasingly important.

2. Methodologies

Measurement of Ca absorption and bioavailability is ultimately the means by which the scientific community gauges the effectiveness of a Ca source. Methods used to measure Ca bioavailability have been summarized previously (Heaney, 2001b) and in brief include: (i) balance studies (i.e., INTAKE – EXCRETION = NET ABSORPTION) — which are difficult to perform in a practical sense; (ii) serum concentrations — similar to the pharmacokinetic measurement of the area under the curve (AUC), which has limited sensitivity; (iii) tracer methods — using radioactive or stable isotopes which yield highly sensitive and reproducible results for intrinsically labeled Ca sources; (iv) urinary increment — which is an imprecise method given that only 1–6% of the variation in urinary Ca is explained by Ca intake (Braun *et al.*, 2006; Jackman *et al.*, 1997); (v) target system effects — dependent on the nutritional and physiological homogeneity of populations (e.g., inbred experimental animals); and (vi) *in vitro* methods — that are not entirely relevant to inherently complex *in vivo* environments.

Overall, serum and urinary increment methods have shown that a Ca source (e.g., $CaCO_3$) accompanied by citrate is better absorbed than one that is not (Heaney *et al.*, 1999). Citrate that is absorbed into circulation is inclined to bind Ca ions and thereby artificially elevate incremental data compared to other salts (Heaney, 2001b). A number of studies utilizing the most sensitive isotopic tracer methods have demonstrated that CCM is highly absorbable compared to other Ca sources (Abrams *et al.*, 2003; Heaney *et al.*, 1989b; Miller *et al.*, 1988; Smith *et al.*, 1987).

Direct isotopic labeling of a food source or salt (incorporating an isotope during ingredient synthesis, i.e., intrinsic labeling) is not always possible or practical, and extrinsic labeling (adding an isotopic tracer to the prepared test meal) must be employed instead, as is necessitated when the source cannot be intrinsically labeled such as in the case for mined Ca salts. Most Ca salts can be intrinsically labeled prior to incorporation into the food product being fortified. The accuracy of extrinsic labeling to estimate Ca absorption from a fortified food or beverage is

largely reliant upon a complete exchange between the Ca source in the food or beverage and the added isotope. This exchange should be tested prior to using the extrinsic labeling approach. The high molecular weight (MW) of organic chelates can potentially interfere with exchangeability, and if the Ca source in the fortified food or beverage is partially or completely insoluble (e.g., $CaCO_3$ or tricalcium phosphate), extrinsic labeling is likely to overestimate the true fractional absorption because of incomplete exchange with the added isotope (Andon *et al.*, 1993; Heaney *et al.*, 2005b). This has not been tested for CCM, nevertheless its high solubility suggests that exchange is likely to be complete as long as the isotope form is also soluble. For example, $CaCl_2$ (the common commercial form for radiocalcium tracers) is highly soluble, while $CaCO_3$ (the common commercial form of stable Ca tracers) is not. While not a definitive test, the data from Smith *et al.* (1987) provide some confidence that extrinsic labeling of CCM yields similar results as intrinsic labeling (i.e., similar fractional absorption was measured for intrinsically labeled CCM tablets versus extrinsically labeled CCM OJ; 37.3% vs 38.3%).

3. Supplemental Ca source and form

Supplemental Ca is available from a wide variety of sources and the same type of Ca can be delivered in different forms (e.g., swallowable or chewable tablet, different ratios of components). In terms of substances used to chelate minerals, malate and citrate are considered to be among the best absorbed (Anonymous, 2007). They are easily ionized which enhances the absorption of minerals to which they are bound by increasing the amount of ionized minerals in the intestinal tract. Citrate and malate anions are absorbed in the upper digestive tract (Demigne *et al.*, 2004). Other substances used in this capacity and which facilitate mineral absorption include ethanolamine phosphate, ascorbate, fumarate, succinate, lysinate, glycerate, picolinate, and acetate (Anonymous, 2007). In contrast, carbonate and oxides tend to exert a detrimental effect on mineral absorption (Dawson-Hughes *et al.*, 1986; Prather and Miller, 1992; Seligman *et al.*, 1983). The source and physical form of Ca, whether it be in a supplement or added to foods, has been shown to significantly influence absorption efficiency and bioavailability in both humans and animals.

Miller *et al.* (1989) tested Ca absorption from CCM using single and double stable isotope techniques. Six healthy Caucasian children (three boys and three girls) ranging in age from 11 to 17 years participated in a study to determine whether a single serum sample can provide an accurate estimate of Ca absorption. The stable isotopes ^{44}Ca and ^{42}Ca were quantified in serum and urine. A comparison of the Ca absorption data also was made with a previous study involving the same subjects (Miller *et al.*, 1988). After an overnight fast the children received a standardized breakfast plus three chewable tablets comprising 215 mg Ca from CCM

(6:2:3 molar ratio) that had been enriched via intrinsic labeling with 35 mg ^{44}Ca to provide a total Ca load of 250 mg. Exactly 30 min after the oral dose of Ca, a 10 ml intravenous injection of ^{42}Ca tracer was administered. A 24-h urine sample and 90, 120, 150, 180, and 500-min incremental blood samples were collected for tracer determinations. Average (±SD) Ca absorption estimated from the ratio of urinary tracers was 41.4 ± 8.2%. Serum ^{44}Ca, measured 150 min after the oral dose of Ca, correlated significantly with Ca absorption based on urinary tracer determination ($r = 0.85$, $p < .05$). A paired comparison with the same subjects (in a previous study) showed that mean (±SEM) Ca absorption (%) from a chewable tablet comprising CCM of 6:2:3 molar ratio (41.4 ± 8.2%) was similar to a nonchewable tablet comprising CCM of molar ratio 5:1:1 (39.5 ± 10.6%), and that Ca absorption from a $CaCO_3$ tablet was lower than both CCM tablets (26.7 ± 7.8%) (Figure 6.2). The chewable and nonchewable CCM supplement tablets were better absorbed than the tablets formulated with $CaCO_3$ ($p = .047$ and $p = .094$, respectively).

In another study, the difference in fractional absorption between Ca from CCM and $CaCO_3$ was tested in 12 healthy adolescents (6 males and 6 females) aged 10–17 years using a 2-period crossover design (Miller *et al.*, 1988). The average (±SEM) dietary Ca intake based on a food frequency questionnaire was 600.4 ± 65.7 mg/day. The order of Ca supplementation for groups was randomized and for each treatment two tablets were ingested with a standardized breakfast. Each tablet contained

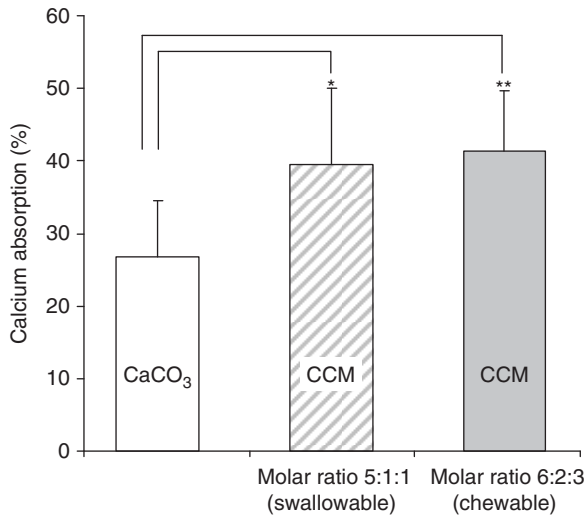

FIGURE 6.2 Data are the mean (±SEM) for absorption of various Ca salts in children ($n = 6$; three males and three females) based on a study by Miller *et al.* (1989). *$p = .094$, **$p = .047$.

114.6 mg elemental Ca (enriched with 10.4 mg ^{44}Ca), either as $CaCO_3$ or 5:1:1 molar ratio CCM (250 mg Ca total dose). Exactly 30 min after tablet ingestion subjects were intravenously injected with 3.6 mg ^{42}Ca. Ca absorption was estimated using high resolution fast-atom-bombardment mass spectrometry to quantify ^{42}Ca and ^{44}Ca relative to ^{40}Ca in urine samples before and 24 h after tracer administration. Fractional absorption of Ca from CCM (mean ± SEM: 36.2 ± 2.7%) was significantly higher ($p < .03$) than from $CaCO_3$ (26.4 ± 2.2%), representing a 37% improvement in Ca absorption from CCM.

Andon (2003) compared five different CCM formulations covering a threefold range of Ca:citrate:malate molar ratios from 154 previous studies in humans. Intrinsically labeled tablets or juices comprising 250 mg Ca as CCM were tested in adolescents and groups of women 20 to 30-years and 40 to 77-years old. A comparison of mean values for age groups, molar ratios, and vehicles revealed no differences. Comparison with reported values in the literature, after adjustment to equalize Ca doses and indexing versus a standard (i.e., milk = 100), revealed Ca absorption from CCM consistently exceeded absorption from other sources including milk, various dairy products, fortified foods, and Ca supplements.

4. Food/beverage matrices

Different foods and beverages vary in their compositional matrices, a factor known to affect the absorption of the same Ca salt from different sources. For instance, CCM added to beverages does not result in an equivalent across-the-board percent absorption of Ca. Rather, Ca absorption from CCM tends to be higher when added to apple juice than when added to OJ (Andon *et al.*, 1996b), and both of these juice vehicles supply a more absorbable source of Ca than can be obtained from fortification of lemon juice with CCM (Mehansho *et al.*, 1989b). Heaney and others maintain that fractional Ca absorption values for most of the Ca salts commonly used to fortify foods or formulated as supplements are similar to absorption values from milk, with the exception of CCM which is slightly higher (Heaney *et al.*, 1990a; Weaver *et al.*, 1999). A comparison of Ca-fortified food sources with the highly ranked natural sources of absorbable Ca found in milk and yogurt (based on a 1 cup serving and an equivalent 300 mg Ca content) exemplifies the relative potential value of CCM-fortified foods in terms of estimated absorption efficiency (%) and absorbability (mg) per serving (Figure 6.3; Braun and Weaver, 2006; Weaver *et al.*, 1999).

In the same food/beverage matrices, the Ca source used for fortification of the system can significantly influence the amount of Ca that is bioavailable. A product's label generally states the amount of Ca added to a fortified product; however, this is not always a good indicator of what the consumer can expect to be absorbed or bioavailable following

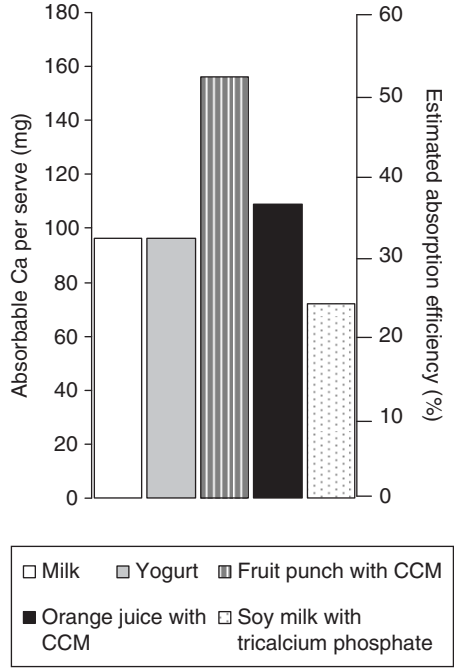

FIGURE 6.3 Graph compares the absorption characteristics of naturally Ca-rich foods with beverages that have been fortified with CCM. The left axis indicates the absorbable Ca per serve and the right axis shows the corresponding absorption efficiency estimated for each product.

consumption. This point was clearly demonstrated in a randomized cross-over pharmacokinetic study of 25 healthy premenopausal women between the ages of 21 and 43 years that consumed a 500 mg Ca load from CCM versus tricalcium phosphate/Ca lactate (TCP/CL) (Heaney *et al.*, 2005b). Each Ca source was delivered in an OJ vehicle at the midpoint of a light breakfast, that itself was low in Ca. The participants were tested three times, twice with one fortification source and once with the other in a random sequence. Care was taken to synchronize test periods with menstrual period stage by testing 29 ± 3 days apart. The AUC of serum Ca plotted against time through 9-h postdosing ($AUC_{0–9\ h}$) was assessed to determine the serum Ca increment above baseline after the test load. Net absorbed Ca, calculated from $AUC_{(9\text{-}h)}$, was 48% greater for CCM versus TCP/CL ($p < .001$) and the mean (±SEM) amount of Ca absorbed was 148 ± 9.0 mg versus 100 ± 8.9 mg, respectively. The less sensitive pharmacokinetic method of measuring Ca bioavailability was used for this study rather than the more sensitive isotopic tracer method, which requires a market-ready product be extrinsically labeled and the

isotopic tracer uniformly equilibrated between phases for accurate results. Tricalcium phosphate is an insoluble Ca source that exists in the juice in particulate form, which impedes uniform exchange of Ca isotope between the solution and dispersed particulate phases and precludes the use of extrinsic labeling for estimating bioavailability. Heaney has advocated that manufacturers be encouraged to disclose bioavailability information to consumers that may naively presume bioequivalence of Ca sources in fortified products (Anonymous, 2002; Heaney, 2001b).

To compare fractional absorption of Ca from two CCM-fortified juices in humans, a study was designed for two groups of $n = 57$ women (mean ages: 56 and 58 years) that were administered 5 μCi ^{45}Ca with 250 mg of ^{40}Ca as CCM in either OJ or apple juice (Andon *et al.*, 1996b). Including both endogenous and added citric and malic acids, the CCM molar ratios were 1.0:0.7:1.3 (equivalent to 6:4.2:7.8) and 1.0:1.8:1.5 (equivalent to 6:10.8:9), respectively. An overnight fast and low-Ca breakfast pretreatment was followed by a 5-h postdose specific activity test of serum. The mean (±SEM) fractional absorption of Ca delivered orally was observed to be significantly higher ($p < .003$) for CCM-AJ (42 ± 2%) versus CCM-OJ (36 ± 1%). This study was not a crossover design whereby uncharacteristic individual differences, if they existed, could be nullified. The method employed is most reliable when used in a crossover design to compare relative bioavailability.

Two recent studies (Griffin *et al.*, 2002, 2003) measured the Ca absorption from fortified OJ in adolescent girls age 10–15 years. For a 3-week period, the girls consumed a daily diet providing ~1500 mg Ca/day, which included two servings per day of CCM-fortified OJ supplying 700 mg Ca/day as CCM (personal communication). At the end of the 3-week adaptation period, fractional Ca absorption was measured utilizing a double-isotope method involving extrinsic labeling of the juice with ^{46}Ca as $CaCO_3$ and a ^{42}Ca intravenous tracer. The labeled juice was consumed with breakfast following an overnight fast and with dinner. Fractional Ca absorption was estimated from the ratio of the two isotopes excreted in a 48-h complete urine collection. Three study cohorts were tested, two in Houston, TX ($n = 30$ and 29, respectively) and one in Omaha, NE ($n = 25$). Ca absorption from the CCM-fortified OJ was consistently high in all three cohorts [mean (±SD): 31.8 ± 10.0%, 32.3 ± 9.8%, and 33.6 ± 9.4%, respectively]. These studies are important to highlight because they demonstrate high Ca bioavailability from CCM even when the daily Ca intake exceeds the current AI for adolescents (1300 mg/day) and, perhaps more importantly, after a 3-week adaptation period. The primary objective of these studies was not to specifically measure bioavailability of Ca from CCM, rather it was to assess the effect of nondigestible, fermentable, oligosaccharides such as inulin and fructooligosaccharide (FOS), on Ca absorption in girls at or near menarche.

On this point, the findings were mixed, as a significant increase in fractional Ca absorption ($p < .01$) resulting from consumption of 8 g/day of a blend of inulin and FOS was measured in the Houston cohort; however, no significant effect was observed in the Omaha cohort. No gastrointestinal issues associated with the use of inulin were reported for these studies.

5. Age and life stage

A host of factors that are age related serve to impair Ca absorption as we get older. Compared to healthy young adults, healthy older men and women absorb Ca less efficiently as they age (Gallagher *et al.*, 1979; Ireland and Fordtran, 1973; Scopacasa *et al.*, 2004). This predisposition in the elderly is thought to be at least partly attributable to Ca absorption being dependent on adequate vitamin D levels to hinder PTH stimulation and hyperparathyroidism. Vitamin D is necessary for the active transport of Ca across the intestinal mucosa (Heaney, 2003c), and Ca absorption efficiency has been shown to improve when the precursor molecule to bioactive vitamin D, 25(OH)D (i.e., calcidiol) is at the higher end of the reference range in postmenopausal women (Heaney *et al.*, 2003). However, no direct relationship between serum 25(OH)D and Ca absorption was observed in young adolescents (Abrams *et al.*, 2005b). Age has been shown to adversely affect $1,25(OH)_2D_3$ serum levels in men (Agnusdei *et al.*, 1998) and intestinal responsiveness to $1,25(OH)_2D_3$ (Ebeling *et al.*, 1992; Pattanaungkul *et al.*, 2000) in women, both of which reduce Ca absorption. Exposure of the skin to sunlight, which is required for *in vivo* vitamin D synthesis, is generally less frequent and efficient in the elderly who are more likely to be housebound or institutionalized (Kinyamu *et al.*, 1997). Although studies focusing on Ca absorption efficiency in relation to CCM and vitamin D are currently lacking, CCM in combination with vitamin D has been shown to be effective in terms of averting the typical seasonal (wintertime) increase in PTH in women residing in northern latitudes. This dietary combination is also linked to a reduction in fracture risk in older men and women.

Longitudinal data on Ca absorption, collected over the course of 17 years in middle-aged women as they transitioned to menopause, reveals the negative correlation that exists between estrogen status and Ca absorption efficiency over time (Heaney *et al.*, 1989a). Heaney and colleagues established that the reduction in estrogen levels after menopause, together with the natural decrease in Ca absorption attributable to aging, accounts for an approximate 20–25% decline in absorptive potential in women from age 40 to 60 years. As previously discussed, CCM has been shown to be a highly absorbable Ca source in postmenopausal women when administered in fruit juices (Andon *et al.*, 1996b).

Martini and Wood (2002) tested the bioavailability of 3 different sources of Ca in 12 healthy elderly subjects (9 women and 3 men of mean ± SEM age: 70 ± 3 and 76 ± 6 years, respectively) in a 6-week crossover trial conducted in a Human Study Unit. Each Ca source supplied 1000 mg Ca/day and was ingested for 1 week with meals (as 500 mg Ca 2x/day), thus contributing to a high-Ca intake (1300 mg Ca/day). A low-Ca intake (300 mg Ca/day strictly from the basal diet) was adhered to for 1 week in-between each treatment. The Ca sources included skim milk, CCM-fortified OJ, and a dietary supplement of $CaCO_3$. Assessment parameters were indirect measures predicted to reflect the relative bioavailability of Ca postprandially via an acute PTH suppression test (hourly for 4 h). Longer-term responses to Ca supplementation were assessed via a number of urinary and serum hormone, mineral, and bone resorption biomarkers (i.e., vitamin D, Ca, phosphorus, and collagen type 1 N-telopeptide cross-links).

The postprandial PTH suppression tests revealed no significant difference among Ca sources or Ca-responsive measures over 1 week in response to the three Ca sources. Conversely, high-Ca versus low-Ca diets were significantly different for a number of parameters, which inexplicably did not include serum Ca. Based on their results, the investigators of this study inferred that the three Ca sources tested were equivalent in terms of Ca bioavailability. These data subsequently aroused some criticism (Heaney, 2003b). It was pointed out that, peculiarly, serum Ca measurements did not distinguish absorptive calcemia in response to the oral Ca loads that contributed to suppression of PTH in serum and the increase in urinary Ca 4-h postprandially. One key factor of many that could obscure a demarcation in response to different Ca sources in the elderly is consumption of the Ca test load in conjunction with a more complex test meal than is typically served under assessment conditions. In general, humans tend to absorb less soluble sources of Ca, such as $CaCO_3$, less efficiently than more highly soluble forms, such as CCM, on an empty stomach (Heaney *et al.*, 1989b) or in the absence of sufficient food. In the presence of adequate or more complex foods, gastric emptying is slowed (Hunt, 1980) and Ca absorption and bioavailability may not be appreciably different among Ca sources due to prolonged gastric emptying (Song *et al.*, 2001) and/or slowing of intestinal motility. Citric acid, a component used in the formulation of CCM, has also been shown to delay gastric emptying in fasted volunteers administered freeze-dried Ca in the form of Ca alginate beads (Stops *et al.*, 2006).

The prevalence of hypo- and achlorhydria is high in the elderly population (Bo-Linn *et al.*, 1984). Furthermore, sufficient food consumption in the elderly is not always possible and in some situations potent gastric antisecretory medications may be prescribed for long-term use in older people (Bo-Linn *et al.*, 1984; Evenepoel, 2001). A source of Ca that is more

likely to be absorbed and made bioavailable under a wider range of conditions may, under such circumstances, be preferable to Ca sources dependent upon food intake and sufficient gastric acid secretions for adequate absorption. CCM delivers highly absorbable Ca in the presence or absence of food and may be among the best choices as a supplemental Ca source, particularly for elderly populations.

6. Intrinsic conditions

Ca salts differ from one another in terms of the anions and molecules that they are associated with. CCM's characteristic aqueous solubility is directly related to the citrate and malate anions. The *in vitro* solubility of any Ca salt is essentially constant under standard conditions (e.g., at neutral pH in water). Once a Ca source is consumed, it encounters a host of variable environmental factors, such as pH changes, interactions with other food components, and the hormonal milieu, that alter its solubility and/or potential for absorption. It is the net impact of these internal factors, together with the combination of introduced variables, such as the food matrix in which Ca is incorporated and the timing of Ca intake, that contribute to determining just how beneficial to health a Ca source will be.

In vitro solubility characteristics of six Ca salts, namely Ca lactate, Ca phosphate (CaP) (monobasic), Ca citrate, Ca gluconate, $CaCO_3$, and CCM, were compared by Roth-Bassell and Clydesdale (1992). Ca salts (10 mM) in a mineral stock solution were tested under conditions designed to simulate the gastric acid environment of the stomach (pH 2.0 using 1.0 N HCl) followed by the neutral environment of the intestines (pH 7.0, using 1.0 N NaOH). Total Ca, total soluble Ca, and ionic Ca were measured. CCM and Ca citrate formed significantly higher levels of a soluble Ca complex. The Ca from Ca lactate and $CaCO_3$ that did solubilize was entirely in the ionic form. CaP and Ca gluconate was predominantly soluble in the ionic form. Ionic Ca at neutral pH is considered to be reactive and capable of forming insoluble complexes with various intestinal food constituents, a propensity that can alter the potential for Ca absorption. Information pertaining to the statistical analysis for this *in vitro* experiment was not, for some undisclosed reason, published in the paper. Bench top simulations designed to mimic physiological conditions provide information that has some theoretical merit, although results acquired this way may not truly represent *in vivo* conditions that are infinitely more complex.

It is somewhat counterintuitive to think that the aqueous solubility of a Ca source has little impact on its capacity to be absorbed at the intestinal mucosal surface. However, contrary to conventional wisdom, Heaney *et al.* (1990a) have demonstrated that solubility plays a limited role with the coingestion of food. Weaver and Liebman (2002) concede that only

salts at the extreme ends of the solubility spectrum have appreciably different Ca absorption efficiencies.

Ca is usually liberated from complex dietary compounds during digestion in the highly acidic milieu of the stomach. Pancreatic and biliary bicarbonate secretion occurs in response to duodenal acidification to neutralize the pH of the stomach chyme entering the intestines. Ca is typically considered to be present in a soluble and/or apparently ionized form (Ca^{2+}) in the small intestine preceding absorption (Guéguen and Pointillart, 2000). Nevertheless, it has been demonstrated *in vivo* that dissociation of a Ca salt, with a low MW, neutral charge, and comparatively lower solubility, is not necessarily a prerequisite for absorption since paracellular diffusion of intact Ca oxalate (MW 128.10) and $CaCO_3$ salts (MW 100.09) can occur (Hanes *et al.*, 1999). Ca chelated to an amino acid (i.e., bisglycinocalcium or Ca bis-glycinate, MW 188.20) has also been shown to be absorbed intact (Heaney *et al.*, 1990a). CCM is a large soluble salt with an MW $>$ 1000 (i.e., MW 1014.90 for the anhydrous form of CCM with a 6:2:3 molar ratio of Ca:citrate:malate), which may be too large to traverse tight paracellular cellular junctions. The overall effect(s) exerted as a consequence of intact Ca salts being absorbed and entering the general circulation or reaching a target organ is uncertain (e.g., Ca oxalate in the kidneys). Citrate and malate anions chelated to Ca in CCM are considered to enhance Ca absorption (Weaver and Liebman, 2002), possibly by forming relatively stable soluble complexes, such that precipitation of Ca by phosphate in the gut is not chemically favored and the likelihood of Ca absorption is improved. Generally, Ca absorption is expected to be enhanced by substances which increase its solubility [e.g., hydrochloric acid (Hardy and Ball, 2005), ascorbic acid, and citric acid].

7. Meal effects

The positive effects of a coingested meal on Ca absorption in humans has been demonstrated in two studies by Heaney *et al.* (1989b). Absorption of Ca (250 mg) both with and without a neutral test meal was assessed in 20- to 30-year-old women. The Ca sources tested were CCM ($n = 10$ subjects involved in a cross-over, within-subject design) and $CaCO_3$ ($n = 26$ subjects without a meal and $n = 10$ with a meal). The double isotope method was employed; ^{45}Ca served as the oral tracer and ^{47}Ca as the intravenous tracer. The meal effect on the mean (±SEM) fractional absorption of labeled Ca test loads in women was significant for CCM ($p < .01$), ranging from 0.2848 (±.0300) without a meal to 0.3743 (±.0166) with a meal. The overall 31% increase in Ca absorption fraction when CCM was coingested with a meal was due to 9 of the 10 subjects demonstrating increased absorption efficiency. Following $CaCO_3$ ingestion, Ca fractional absorption was 0.246 (±.0265) without a meal, and only increased by 20% to 0.296 (±.0170) with a meal ($p > .05$). Variation in the results for $CaCO_3$

under nonmeal conditions was widespread in women, ranging from under ~.05 to >.50. When consumed with a meal, outcomes narrowed substantially from >.20 to <.40. CCM results were significantly less variable than those for $CaCO_3$ ($p < .01$).

The general enhancing effect of a meal on Ca absorption observed by Heaney (Heaney *et al.*, 1989b) was not detected by Brink and coworkers when a Ca L-lactate salt was ingested by healthy postmenopausal women (Brink *et al.*, 2003). In Brink's study, subjects were evaluated to determine true Ca absorption via the dual labeling stable isotope technique. Mean (±SD) Ca absorption was substantially higher in the absence of a meal (45.0 ± 10.2%), rather than after coingestion of the Ca with an Asian or Western style breakfast (29.7 ± 8.6 and 31.5 ± 9.3%, respectively; $p < .0001$). The large negative effect of a meal on Ca absorption was attributed to the high fiber content of the breakfasts. No effects of the different food matrices due to the style of breakfasts were observed. Brink also examined Ca absorption from 6 different intrinsically labeled Ca sources (i.e., 200 mg elemental Ca from milk, $CaCO_3$, CCM, tricalcium phosphate, Ca L-lactate, and Ca lactate/gluconate) in 10 postmenopausal women using a randomized 8-way crossover design with a washout period of 7 or 14 days. True Ca absorption (%) after a single oral test load was similar among all Ca sources consumed with a meal, with the exception of tricalcium phosphate which was significantly lower.

8. Interfering substances

Various constituents in plant foods can impede Ca absorption. Plant-based diets can be high in oxalate and phytate, which are recognized as inhibitors of Ca absorption. In fact, Ca absorption is considered to be inversely proportional to oxalic acid content of the food (Weaver *et al.*, 1999). Phytic acid poses Ca absorption problems for those species unable to endogenously synthesize phytase (e.g., humans, birds, and pigs). The Ca in CCM is chelated with the citrate and malate anions, which may make CCM less reactive than other sources of Ca toward food components known to interact with Ca^{2+} cations. For example, Lihono *et al.* (1997a) reported data suggesting that the Ca in CCM may be less likely to complex with phytates than other Ca salts. On this basis, CCM may be more appropriate for the fortification of soy or other phytic acid-containing products.

Protein has long been classified as a factor that causes Ca to be wastefully excreted from the body. Less is documented in relation to how it affects Ca absorption. Dawson–Hughes has reported that a dietary protein increase of 20% combined with a low Ca intake of ~800 mg/day in elderly men and women lowers the amount of absorbable Ca by 23%. In contrast, a high protein diet (between 18.16% and 29.14% of total dietary energy from protein) in the presence of a high Ca intake

(>1300 mg Ca/day in the form of CCM) plus vitamin D supplementation, increased fractional Ca absorption overall, compensating for the adverse effects of protein on Ca absorption observed in the placebo group consuming inadequate Ca (Dawson-Hughes and Harris, 2002).

9. Various other factors

Body size and statural height have a direct effect on the length of the intestinal tract, intestinal transit time, and mucosal mass, all of which impact Ca absorption because they lengthen exposure to absorptive surfaces. A height advantage of 4 inches in women can result in a 30% increase in Ca absorptive potential (Barger-Lux and Heaney, 2005), while smaller increases in Ca absorption attributable to height in young girls have also been observed (i.e., 3–3.5%) (Abrams *et al.*, 2005c). A larger mucosal mass has been shown to be a direct determinant of Ca absorptive transport capacity in rats (Yeh and Aloia, 1984), and the trend is presumed to be similar in humans (Barger-Lux and Heaney, 2005). The general health of the intestinal mucosa (e.g., the absence of inflammatory bowel conditions such as colitis, Crohn's disease) is also important for maximizing Ca absorption. Certain medications can either directly or indirectly affect Ca absorption, among them corticosteroids and anticonvulsants, respectively. Smoking (Krall and Dawson-Hughes, 1999) and alcohol intake (Wolf *et al.*, 2000), both of which are modifiable behaviors, are also disruptive to Ca absorption efficiency. Fok1 gene polymorphisms of the vitamin D receptor (VDR), which constitutes a genetic rather than a modifiable factor, influences Ca absorption (Abrams *et al.*, 2005a; Ames *et al.*, 1999), as does race, with blacks being better able to absorb Ca as opposed to whites (Abrams *et al.*, 1995). CCM has not been specifically evaluated as the administered Ca source for a number of these additional factors that influence Ca absorption, and more research with CCM is warranted in these areas.

D. Animal studies with CCM

A number of studies designed to test the effectiveness of CCM as a Ca source under various conditions have also been performed in various animal models including rats, chicks, pigs, and dogs (Table 6.6). Based on rodent studies, there is evidence to suggest both positive effects and no particular advantage of CCM in terms of absorption and bioavailability relative to other Ca salts. There was no difference in fractional Ca absorption among CCM and four other Ca salts of varying solubilities [i.e., Ca fumarate (CF), Ca malate fumarate (CMF), Ca citrate, CCM, and $CaCO_3$] in rats administered an intrinsically labeled Ca dose via oral gavage after being fasted (Weaver *et al.*, 2002). Forty-eight hours postdose the rats ($n = 15$/group) were euthanized and the harvested femurs (whole bones)

were dissolved and subjected to scintillation counting to determine tissue uptake of ^{45}Ca for each Ca salt. Mean (±SEM) values for fractional absorption ranged from 27.42 ± 3.09% for $CaCO_3$ to 30.09 ± 1.02% for CF and were not significantly different. Fractional absorption of Ca from CCM was 28.6 ± 1.58%, which is considerably lower than that determined by other investigators using extrinsic labeling. The accuracy of the later method is highly contingent upon a satisfactory exchange of the isotopic label. Weaver's results are essentially in agreement with a human study (Brink *et al.*, 2003) in which true fractional absorption of Ca in postmenopausal women was determined not to be different between intrinsically labeled $CaCO_3$ and CCM under meal conditions (mean ± SD: 29.9 ± 12.3% vs 29.6 ± 10.6%, respectively); however, food is considered to have a potential equalizing effect on the absorption of Ca salts (Heaney *et al.*, 1989b). Smith *et al.* (1987) also determined there was no difference between percent retention of powdered sources of CCM and $CaCO_3$ that were labeled with ^{47}Ca while in solution phase and administered via gastric tubing to older rats (35.1 ± 1.6% vs 30.8 ± 2.6%, respectively; $p > .05$). The lower retention values for this rodent experiment, compared to higher retention values in younger animals, were considered to reflect the Ca absorption decrease expected in older rats due to a larger proportion of the Ca pool being turned over and accounted for by excretion. In contrast to studies suggesting there is no advantage of CCM compared to other less soluble Ca salts in rodents, another five studies indicate that CCM is significantly better absorbed, more bioavailable, clearly influenced by the vehicle in which it is delivered, and/or is associated with health benefits.

In animals, as is the case with humans, absorption and bioavailability is influenced by age. Smith's result of Ca retention equivalence between CCM and $CaCO_3$ in older rats is different to how younger rodents responded (Smith *et al.*, 1987). CCM in OJ was determined to be relatively better absorbed and retained than the Ca in 2% fat milk (mean ± SEM retention rates: 51.1 ± 1.7 vs 42.2 ± 1.8%; $p < .001$), respectively. This result was reproduced when, based on extrinsic labeling, the Ca from CCM in both AJ and OJ was reported to be better absorbed in juvenile rats than was the Ca from milk ($p < .05$) (Andon *et al.*, 1996b).

The efficacy of any Ca source as a food/beverage fortificant is also dependent upon accompanying ingredients and food components. During Andon's investigation of juice vehicles, it was also determined that the mean (±SEM) Ca retention from CCM-AJ was significantly higher than from CCM-OJ in rats (61 ± 2 vs 52 ± 2%, respectively; $p < .05$) (Andon *et al.*, 1996b). This difference was attributed to the intrinsic profile of organic acids present in the juices in which the molar ratios of Ca: citrate:malate were 1.0:1.8:1.5 in CCM-OJ and 1.0:0.7:1.3 in CCM-AJ, while the respective molar ratios for glucose:fructose were 1:1 and 1:2.

These results indicate that the absorption of CCM as a beverage fortificant is significantly influenced by the chemistry and composition of the delivery vehicle, being optimized by a lower organic acid content and a higher fructose to glucose ratio. To systematically determine the contribution to Ca absorption coming from the organic acids versus the carbohydrates in fruit juices, another group of rats was administered mock juices formulated to mimic the citric and malic acid profile of actual juices, except minus the carbohydrate content (Andon *et al.*, 1996b). The result was that the variation in Ca absorbability in rats consuming CCM-fortified OJ versus AJ test solutions was eliminated, whereas adding back carbohydrates resulted in the mean Ca retention of AJ exceeding that of OJ ($p < .002$). A subsequent increase in the level of fructose added to CCM-fortified mock OJ, equal to that present in the AJ, significantly improved Ca retention from the CCM-OJ test solution ($p < .0001$) (Andon *et al.*, 1996b). Taken together, these results showed that the organic acids and carbohydrates in CCM fortified juice were essentially equipotent in terms of their capacity to modify Ca absorption.

A combination of age, type of Ca salt, and the isotopic labeling method used to assess the effects of various Ca salts are all important factors in the determination of Ca absorption and bioavailability. Therefore, in an entirely separate animal study Andon and colleagues investigated the effect of age (at 2, 4, 5, and 8 months), Ca source (CCM, $CaCO_3$, and hydroxyapatite or HA), and radiolabeling method (intrinsic $_{[int]}$ vs extrinsic $_{[ext]}$ for both $CaCO_3$ and HA) on whole body retention (WBR) of ^{47}Ca in a longitudinal study using growing male rats (Andon *et al.*, 1993). Advancing age was found to be associated with a decrease in percent whole body retention of ^{47}Ca for all Ca sources (at a rate of $\sim -3.4\%$/week, $p < .001$), even though gastric acid secretion was determined to be greater in older rats. The relative bioavailability of each Ca salt tested was consistent at all ages ($p < .001$), and the combined mean ($\pm$SEM) percent fractional ^{47}Ca retention values (measured from 3 days postdose/baseline at each time point assessed) during aging were summarized (proportional to CCM) as: $CCM_{[int]}$ (100%) > $CaCO_{3\ [int]}$ ($83 \pm 4\%$) > $HAP_{[int]}$ ($57 \pm 4\%$). As previously mentioned, extrinsic radiolabeling is not always as accurate as intrinsic methods, and in this experiment extrinsic tags yielded retention values that were determined to be overestimated in younger animals ($\sim$20%) compared to intrinsic measures, although differences due to this factor subsided with advancing age. According to Andon, overstated extrinsic data would have erroneously altered the Ca salt rank order to: $CaCO_{3\ [ext]} > CCM_{[ext]} > HA_{[ext]}$ in younger rats. Data from this study indicated that Ca source did in fact influence the extent of Ca absorption, and the results are in agreement with human absorption studies (Miller *et al.*, 1989, 1988). Despite the reasonably good precision of whole body

retention studies, in the end they cannot provide mechanisms of action for the results obtained (Weaver and Heaney, 2006c).

A Ca salt ingested in the presence of food versus the same salt consumed on an empty stomach will usually be better absorbed with food regardless of the salt's solubility index. The solubility properties of a specific Ca salt tend to influence just how big the differential is for absorption between the fed and unfed state. The effects of a coingested meal on Ca absorption in animals were demonstrated in a series of six separate rat experiments (Heaney *et al.*, 1989b). After a 16-h fast, rodents received a CCM-fortified juice beverage or a milk drink, each of which was radiolabeled with ^{47}Ca and administered orally in either the absence or presence of food that was tantamount to a meal. Seven days later, fractional Ca retention was assessed via whole body counting. A significant increase ($p < .001$) in Ca retention attributable to meal effects was evident in all CCM rat experiments using citrus juices as the vehicle, and in one out of two comparisons using milk ($p < .10$ and $p < .001$). On average, Ca retention in the absence of food was 42.7% for milk and 48% for CCM in juice. When consumed with a meal, 50.8% of the Ca in milk versus 61.2% of the Ca from CCM in juice was absorbed. It is expected that any Ca-rich beverage consumed in the presence of solid food will be mixed with the food in the stomach and as a result endure a longer transit time through the gastrointestinal tract giving the Ca an extended opportunity to be absorbed.

Measurements of Ca absorption and bioavailability are undeniably of great importance; however, in actuality they do not in themselves provide us with a direct measure of the health benefit of a Ca source. Not all Ca that is absorbed, determined to be bioavailable, and/or retained in the body can be presumed to provide a discernible physiological benefit. Physical quantitative endpoints, such as a change in bone parameters, represent an integrated measurement of absorption, bioavailability, utilization, storage, and efficacy. Such all-encompassing assessments may serve to elucidate possible differences among various Ca salts in homogeneous populations (e.g., inbred experimental animals) at times when they are not identified by other investigators focusing strictly on conventional bioavailability measures. To assess the physiological value of one Ca salt versus another, Kochanowski examined bone histomorphometry parameters in young (weanling) growing female rats ($n = 14$/group) that were fed either marginal (0.3%) or adequate (0.6%) Ca as CCM or $CaCO_3$ for either 4 or 12 weeks (Kochanowski, 1990). Rats are usually weaned around 30 days of age and are growing rapidly by 12-week old. Rats fed 0.6% CCM were heavier than both groups of $CaCO_3$-fed rats at 4 weeks ($p < .05$) and 8 weeks ($p > .05$). Ca source did not appear to influence tibia and femur bone fat-free dry weight. The longitudinal bone growth rate for

rats fed the lowest level of CCM (0.3%) exceeded that of rats fed high amounts of $CaCO_3$ (0.6%) at 4 weeks (170.6 μm/day vs 148.8 μm/day, respectively); however, there were no significant differences by 12 weeks. Differences detected in trabecular bone volume (TBV) attributable to Ca source persisted throughout the study. TBV in the metaphyses of the tibia was 23–25% higher for CCM-fed rats than for $CaCO_3$-fed rats at 4 weeks ($p < .05$), and 44–47% higher at 12 weeks ($p < .05$). While cortical bone was not affected by either Ca level or source, the effect of Ca source in trabecular bone was independent of Ca level; this was a finding that the investigators presupposed would only be evident at marginal Ca intakes. On the basis of sensitive histomorphometry analysis, CCM was concluded to be more bioavailable than $CaCO_3$. However, in this instance it may be more appropriate to conclude that the relative ''efficacy'' of absorbed Ca from CCM was responsible for the protective effect observed via bone parameters. The anions that accompany the Ca in CCM may, for example, improve Ca efficacy in terms of providing a relative cation excess that can protect bone from a predominantly acidic diet (Heaney, 2001b).

Only one study assessing the Ca absorption capacity for CCM in dogs has been performed (Andon *et al.*, 1996b). Two year old mature female beagle dogs ($n = 6$/group) were administered extrinsically labeled CCM-fortified orange (CCM-OJ) and CCM-fortified apple juice (CCM-AJ). Based on whole body scintillation counting performed 72-h postdose, Ca retention for CCM-AJ was significantly better than that for CCM-OJ in dogs (29.2% vs 15.1%, respectively; $p < .001$). The general outcome for dogs was similar to that for rats, with the exception of a much higher percent Ca retention occurring in rats for both fortified beverages than in dogs. Age and developmental stage, considering the rats were still growing and the dogs were already mature, probably contributes to the higher absorption of Ca seen in the rats.

The efficacy of CCM has been evaluated in pigs by Pointillart and Guéguen (1993). The inherently high requirement of growing pigs for Ca was the impetus for comparing the bioavailability of Ca from a diet containing milk (i.e., skim milk powder) with one containing added CCM in 2-month-old crossbred male pigs (two groups of $n = 9$). The animals were fed a 0.7% Ca-supplemented diet (0.5% Ca from either milk or CCM, and an extra 0.2% Ca from $CaCO_3$ in the basal diet) for a period of 10 weeks which included a 10-day metabolic balance period 2 weeks prior to euthanization. While the diets were matched for energy and protein content, they differed with respect to protein type and in this study the complement of proteins in the milk-based diet appeared to surpass the CCM-based diet as a facilitator of growth rate and feed efficiency. Urinary hydroxyproline, a bone resorption marker, in addition to absorption and retention measures of Ca and phosphorus, were not different between treatments. Bone parameters related to growth

rate (i.e., tibia and metatarsal fresh weight and total ash) were significantly higher in the milk group ($p < .05$ or $p < .03$); however, after adjusting for body weight, the milk- and CCM-diet exerted similar effects on bone ($p > .05$). Bone parameters that were not correlated with growth rate were either similar for milk versus CCM (i.e., bending moment, stress, ash percentage) or significantly higher for the CCM diet [i.e., apparent density (g/cm^3) and stress (N/mm^2) $p < .05$]. It was concluded that Ca absorption from CCM was essentially equivalent to that of milk, with the differences related to growth being attributed to the higher protein efficiency of the milk-based diet.

An important factor appears to have been overlooked in Pointillart's study concerning the different grain sources used for the milk diet (37% barley, 24% corn) versus the CCM diet (29% wheat, 25% corn, 13% soy meal) in the attempt to achieve equivalence in terms of energy, amount of protein, crude fiber, fat, and vitamins. Each of these grains contains a variable concentration of phytic acid/phytates in addition to phosphorus-rich compounds known to inhibit Ca absorption and account for up to 70% of the total phosphorus content in the diet of an animal (Brumm, 2000). However, the phosphate in phytate is largely indigestible and unavailable for the maintenance of optimal bone status. This is a documented problem in swine (Spencer *et al.*, 2000) that unlike rodents (Lihono *et al.*, 1997b; Mason *et al.*, 1993) do not typically exhibit the required intestinal phytase activity to effectively hydrolyze phytate (Veum *et al.*, 2002). Low-phytase cultivars and phytase-treated grain sources can be fed to livestock; however, this issue was not addressed in the results of this study, aside from an acknowledgment that phosphorus levels in the two diets were not equivalent. Interestingly, organic acids such as citric acid and its salt improve phytate-phosphorus utilization in swine (Boling *et al.*, 2000). The presence of CCM in the gut could also be speculated to interfere with phytate-Ca associations. Nevertheless, different grain compositions of diets containing variable phytate concentrations are confounding, with or without being treated for phytase, and for this reason the results of Pointillart's study may have been biased to a certain extent.

CCM is considered to ameliorate the interfering effect of phytates consumed by animals, and as a result enhance Ca absorption and bioavailability. A study in chicks by Lihono *et al.* (1997a) directly investigated this possibility in food derived from soy beans. The effects of microbially derived phytase enzyme treatments on the bioavailability of Ca from soy-based foods fed to young male broiler chickens were examined in two separate experiments. In experiment one, the effect of phytase was tested when day-old chicks were fed corn/soybean-meal-based diets, with or without ~0.12% added phytase, that also included Ca from CCM (at levels 0%, 0.1%, 0.2%, and 0.3% to provide total Ca of 0.45%, 0.55%, 0.65%, and 0.75%, respectively) or Ca from $CaCO_3$ (at the 1% level, which

included Ca from the basal diet) for a total of 17 days. For experiment two, chicks were fed diets that included spray-dried soymilk that had been incubated with microbial phytase prior to hydrothermal cooking. The diets also contained Ca from CCM (at levels 0.31%, 0.46%, 0.61%) or Ca from $CaCO_3$ (at the 0.76% level). Results revealed that phytase had no effect on weight gain, feed intake, tibia/body weight, ash%, and ash Ca% when CCM was added to the corn/soy meal diet or the hydrothermally cooked soymilk diet ($p < .17$). However, the same two diets with $CaCO_3$ as the Ca source, but without phytase, reduced the mean weight gain, feed intake, tibia/body weight, and ash % compared to the $CaCO_3$ supplemented diets with phytase added ($p < .05$). The Ca delivered as CCM appeared to be less prone to Ca:phytate complex formation and was the more effective Ca fortificant when compared to $CaCO_3$.

Young boiler chicks were also the animal model used by Henry and Pesti (2002) in an experiment related to the effect of CCM on bone development versus commercial-grade limestone (i.e., $CaCO_3$). Both Ca sources at 0.7% and 0.9% resulted in no differences for measures of dry fat-free tibia, tibia weight, tibia ash, or tibia Ca content. However, CCM-fed chicks up to the time they were 18 days old gained more weight and had better feed conversion ratios than $CaCO_3$-fed chicks. Diets comprising 0.50%, 0.55%, 0.60%, 0.65%, or 0.70% of Ca as CCM or limestone, each with added dicalcium phosphate (DCP), were also fed to chicks. A positive effect of CCM versus limestone as a Ca source was observed in relation to weight gain during growth ($p < .022$), dry fat-free tibia weight ($p < .0002$), tibia ash ($p < .023$), and tibia Ca ($p < .0001$) in CCM-fed chicks. The level of CCM was only significant for tibia ash ($p < .047$). Furthermore, the incidence of tibia dyschondroplasia (TD) was also reduced with CCM + DCP administration compared to limestone in male chicks. Notwithstanding species differences, Henry's results in this instance are very similar to those of Kochanowski's rodent experiment in which more robust growth was associated with CCM in young growing animals (Kochanowski, 1990). While CCM was considered a good source of Ca for growing chicks, the seemingly positive effect of CCM was attributed more to the inadequacy of limestone as a Ca source rather than remarkable increases in Ca bioavailability from CCM. Nevertheless, it should also be considered that the anions present in CCM may exert positive effects in and of themselves considering they are Krebs cycle intermediates and required by the body for energy production. For example, only relatively small amounts of exogenous malate are required to increase mitochondrial oxidative phosphorylation and adenosine triphosphate (ATP) production (Abraham and Flechas, 1992) to generate increased amounts of potential energy *in vivo*. If this energy is tapped for the purposes of growth and development or tissue repair, it may be a contributing

factor to the improved growth observed in animals following CCM supplementation.

Effective absorption and bioavailability are extremely important if significant health benefits are to be derived from supplemental sources of Ca. Both human studies and animal experiments confirm that CCM performs extremely well in terms of providing Ca that is overall reliably and consistently absorbed, easily administered, and suitable for use under a wide range of conditions. When considering only published data from human studies, CCM has been shown to be highly absorbable when administered to both children and adults, in both tablet and beverage form, in doses ranging from acute (200 mg Ca) to chronic (700 mg Ca/day) and for compositions that cover a broad range of Ca:citrate: malate molar ratios from 5:1:1 (equivalent to 6:1.2:1.2) to 1.0:1.8:1.5 (equivalent to 6:10.8:9). To provide some perspective, the CCM in commercially available CCM-fortified OJ has a molar ratio of ~6:9:5 including both the endogenous and added citric and malic acids (unpublished data), a molar ratio that falls within the aforementioned range of compositions that have been tested and shown to be highly bioavailable.

V. STUDIES OF Ca RETENTION AND BONE BUILDING IN CHILDREN AND ADOLESCENTS

Adequate Ca must be consumed, absorbed, and effectively retained in the skeleton to build strong healthy bones during childhood and adolescence. Achieving the highest percentage of one's genetic potential for bone mass by the end of the skeletal maturation period is considered an important determinant of fracture risk as a result of osteoporosis later in life (Bonjour *et al.*, 1994). The retention of Ca in bone is constantly under strong homeostatic control via regulation by genetics, calciotropic hormones, and weight bearing exercise (Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine, 1997). During youth, an increase in intestinal net Ca absorption efficiency also acts to facilitate the phase of continuous net Ca retention that drives the accrual of bone mineral (Loud and Gordon, 2006; Manz and Schoenau, 2002). Hormone-dependent changes during this time trigger a growth spurt resulting in a drastic increase in bone size and mass which requires a proportional amount of Ca. Regularly drinking milk with meals is currently the exception rather than the norm (Weaver, 2006); over the past few decades, advertising of nondairy Ca-poor beverages and foods has affected mealtime choices (French *et al.*, 2001). This is especially true for American adolescents, a substantial proportion of whom are at risk for low total Ca retention because they habitually replace the consumption of traditional Ca-rich foods with more

visually appealing and psychologically motivated beverage choices. In fact, by the 1990s it was determined that adolescent girls and boys consumed twice as much soft drinks as milk, with intakes reaching 44 gallons of soft drink per capita in 1997 (Lytle and Kubik, 2003). Public policy suggests dairy as the first choice for meeting Ca recommendations because of the package of nutrients contained in milk that support bone growth (DRIs, Surgeon General's Report, and Dietary Guidelines). For those who do not consume adequate dairy products to achieve their recommended intake of Ca, there is a role for the intake of Ca supplements and various Ca-fortified foods and beverages, especially during the period of exponentially high bone mass accretion.

In 1994, Andon reviewed the available evidence pertaining to Ca supplementation with CCM in trials conducted during childhood and adolescence. He made the observation that Ca requirements at the time were based on the premise that Ca absorption is highly efficient in youth. Considering many conditions and factors can negatively affect the skeleton (Loud and Gordon, 2006), Andon contended high absorptive efficiency during youth may not necessarily hold true for all populations studied (Andon *et al.*, 1994). Furthermore, the point was made that recommendations for Ca intake were also based on Ca absorption estimates of 40% in populations in which it was assumed skeletal Ca accretion was optimal with respect to bone mass. While CCM provides Ca that is generally recognized as being well absorbed (Liebman, 1998), it can and has been argued that this is not the case for all Ca sources. Updating Andon's concerns to represent the same issues he raised in the 1990s, according to the current (2007) established AI for children and adolescents between 9 and 18 years, an intake of 1300 mg Ca /day that is 36–40% absorbable would provide ~468–520 mg of available Ca/day at a time when Ca accretion can reach levels as high as ~500 mg/day or more with optimal intakes. A meta-analysis of pooled Ca balance studies during late childhood and adolescence determined mean Ca absorption from various sources did not typically exceed 30% (Matkovic and Heaney, 1992), which only translates to ~390 mg of available Ca/day during this important period of growth and development. Male adolescents (aged 11–14 years) participating in a Spanish study designed to determine dietary mean (±SD) Ca utilization, absorbed up to 31% of dietary Ca (i.e., 271.7 ± 51.7 mg/day) and only retained ~20% (i.e., 170.6 ± 50.9 mg/day) of the total Ca intake which was 881.7 ± 39.9 mg/day (Seiquer *et al.*, 2006). The amount of Ca required for optimal Ca retention and bone mass accumulation during childhood and adolescence may, in part, depend on the Ca source. Ca intake recommendations may need to be revised upwards to account for less absorbable Ca sources in order for young adolescents to reach peak bone mass. CCM as a supplementary Ca source for children and adolescents during this critical window for bone accretion is featured

in the following review of balance studies, and short- and long-term bone density studies that are currently available.

A. Ca balance studies

Ca retention can be determined by measuring the retention of an orally administered isotope (e.g., ^{47}Ca) by means of a whole-body gamma counter (Shipp *et al.*, 1987), although radiation issues may arise when the study population is young. Using excreta-recovery methods, retention is generally calculated as Ca intake − fecal Ca − urinary Ca (Zafar *et al.*, 2004), although sometimes sweat losses are also factored in. Balance studies can also be used to determine Ca requirements based on determination of the maximal Ca intake at which Ca retention improves before a threshold is reached, above which Ca intake is not the limiting factor (Weaver *et al.*, 2004). Data that identifies the optimal Ca intake for maximal Ca retention in adolescent boys was unavailable until 2006 when Braun and colleagues published the results of a rigorous 3-week metabolic balance study with a crossover design in which 31 boys aged 12–15 years were randomly assigned to Ca intakes ranging from 700 to 2100 mg/day (Braun *et al.*, 2006). During the study, the boys resided under observation and strictly controlled conditions in a campus environment and their Ca intake was adjusted at meal times via the inclusion of beverages fortified with various amounts of Ca as CCM. Following a 1-week equilibration period, subjects were randomized to one of five ''low-level'' dietary Ca intakes (ranging between 693 ± 68 and 1081 ± 70 mg Ca/day) which was consumed by each participant for 2 weeks. A washout period ensued (2 weeks), followed by another equilibration period (1 week) before Ca intakes were increased to one of five correspondingly ''higher-levels'' (ranging between 1176 ± 67 and 1986 ± 71 mg Ca/day) for the final 2 weeks. Maximal Ca retention as a function of Ca intake (i.e., based on dietary Ca intake minus Ca excreted via the urine and feces) was determined using a nonlinear regression model and compared against previous data collected from 35 girls of similar sexual maturity that participated in an identically designed study by the same investigators. The lowest mean Ca intake that resulted in the maximal accretion of 628.9 mg Ca/day in boys was 1140 mg Ca/day. Ca retention in boys was 171 ± 38 mg/day higher than that for girls; however, the average Ca intake that maximized Ca retention in the skeletons of girls was not different to that of boys, despite males generally being larger in size and exhibiting a greater skeletal mass. Ca retention curves for boys and girls displayed an approximate parallel trajectory, with boys exceeding girls at all intakes due to more efficient Ca absorption and lower urinary Ca excretion.

The relationship between Ca retention and dietary Ca intake in 21 healthy adolescent girls aged 12–15 years was assessed by Jackman *et al.* (1997). Two 21-day Ca balance studies were carried out while subjects resided in housing at a university campus. Each study comprised a 7-day equilibration phase and a 14-day test period. A crossover design was employed and a 14-day washout period separated the two 21-day periods. Ca intakes were controlled so that they ranged from 841 to 2173 mg/day. This enabled the investigators to determine whether Ca retention actually plateaus due to an upper threshold effect. In order to balance the study, each subject was stratified to a group according to baseline measurements known to impact Ca retention (e.g., BMI, postmenarcheal age). A basal diet containing ~800 mg Ca/day, mostly from dairy products, was prepared; it also included a beverage fortified with various added amounts of Ca in the form of CCM so that one of four ''low,'' or one of four relatively ''high'' amounts of supplemental Ca could be randomly administered to subjects during each test period of the trial. Ca retention was measured by subtracting the total Ca in excreta from total Ca intake. Results from another similar study in which Ca retention in age-matched girls was tested at 1332 mg/day were also added to the data pool during analysis (Weaver *et al.*, 1995). Mean maximal Ca retention was 473 mg/day (95% CI: 245, 701 mg/day), and the minimal Ca intake required to achieve it was 1300 mg/day. For Ca intakes >2 g/day, retention continued to improve. At the 1300 mg Ca/day intake level, maximal Ca retention was observed to decrease with postmenarcheal age. Using a nonlinear regression model, Ca intake explained 79% of the variation in fecal Ca excretion, and only 6% of the variation in urinary Ca excretion as measured by atomic-absorption spectrophotometry.

Kinetic studies provide additional information over balance studies as various parameters of Ca metabolism such as absorption and bone formation and resorption rates can be elucidated concurrently (Weaver *et al.*, 2004). The mechanisms by which a high Ca intake (mean ± SD: 1896 ± 48 mg/day), versus a low Ca intake (848 ± 80 mg/day), increases Ca retention in adolescent girls was investigated in a randomized, crossover study utilizing Ca tracers and kinetic modeling (Wastney *et al.*, 2000). Changes in bone turnover biomarkers were also monitored to provide circumstantial validation of the kinetic changes detected. The subjects included $n = 10$ healthy white adolescent girls ranging in age from 11 to 14 years old (mean ± SD: 12 ± 1 year) and on average >9-month postmenarcheal (range: 12-month premenarcheal to 32-month postmenarcheal) that had been spontaneously consuming ≤800 mg Ca/day. Each study arm comprised a 7-day adaptation period to the designated Ca intake and a 14-day metabolic study period. The latter was initiated by administering an oral dose of ^{44}Ca as $CaCO_3$ to subjects followed 1 h afterward by an intravenous dose of ^{42}Ca as $CaCl_2$; all excreta

(urine and feces) and periodic blood samples were collected thereafter. Each arm of the study was separated by a 1-month washout period. High Ca intakes were attained by supplying CCM-fortified fruit-flavored beverages. Collected samples were analyzed by atomic absorption spectroscopy to determine Ca levels and isotope ratios were ascertained by fast atom bombardment mass spectrophotometry. Tracer data from serum, feces, and urine were fitted to a three-compartmental model.

A high versus a low Ca intake in Wastney's study resulted in a respective increase in the amount of Ca absorbed (mean ± SD: 19.6 ± 7.5 vs 8.0 ± 2.5 mmol/day; $p < .05$), urinary Ca excreted (2.8 ± 1.7 vs 2.1 ± 1.1 mmol/day; $p < .001$), fecal Ca excreted (26.4 ± 12.1 vs 12.6 ± 5.51 mmol/day; $p < .05$), and bone Ca retained (14.5 ± 8.9 vs 3.24 ± 3.59 mmol/day; $p < .001$). Fractional absorption of Ca did not change with higher Ca intakes during the pubertal growth stage of these girls. The constant Ca absorption efficiency suggests that the range of Ca intakes studied were all above the saturation intake for active Ca absorption when passive Ca absorption is dominant. Of the Ca absorbed, the percentage retained on the high intake was 74% compared to 40% on the low intake, and Ca retention was highest in girls within 6 months of menarche and lowest in subjects >10-month premenarcheal and >20-month postmenarcheal. In agreement with the kinetic data, Ca deposition in bone was not determined to be different between the two intakes based on bone formation markers. The changes in urinary bone resorption biomarkers normalized for creatinine (NTX:Cr, PYR:Cr, DPX:Cr) were somewhat variable and ranged from −11% to −23%. A marked reduction in the collagen degradation product hydroxyproline (−23%) was the only biochemical indicator of a significant suppressive effect on bone resorption pertaining to a higher Ca intake ($p < .05$), whereas kinetic data showed that the additional Ca supplied as CCM resulted in a 32% decrease in bone resorption compared to the low Ca intake. The data from this study showed that high Ca intakes due to CCM fortification of beverages can profoundly decrease bone resorption and increase Ca retention in the bones of adolescent girls, particularly around the time of menarche when Ca absorption appears to be more efficient.

B. Bone density studies (2–3 years)

Bone mineral density (BMD) measured using dual x-ray absorptiometry (DEXA) is the current standard method by which to assess BMD in children and adolescents (Loud and Gordon, 2006). It has some limitations in that it only measures bone in two dimensions (g/cm^2) and by utilizing the projected area for areal measurements does not account for bone volume or distance of the subject from the beam [i.e., surrounding tissue mass and (re)positioning]. Moreover, the continuous changes in

bone shape and size commensurate with growth during adolescence serve to complicate the interpretation of BMD (Loud and Gordon, 2006). The gold standard non-invasive three-dimensional (g/cm^3) method by which to evaluate bone is via quantitative computed tomography (QCT); however, this technology is used sparingly in young children due to the associated radiation dose (Loud and Gordon, 2006). To date, more short- rather than longer-term Ca intervention bone density studies have been completed in adolescents.

The most rapid increases in bone mass occur in girls between 11 and 14 years of age. From this time, until approximately age 20, between 40% and 50% of adult bone mass is accrued (Lloyd *et al.*, 1996). Considering the average Ca intake of adolescent girls is typically well below recommended intakes [i.e., not more than 66% of the AI according to 1999–2000 survey data (Wright *et al.*, 2003)], the effect of Ca supplementation on various bone parameters in 112 healthy Caucasian girls (mean $\pm$ SD age: 11.9 $\pm$ 0.5 year) was investigated in a 2-year double-blind, placebo-controlled trial (Lloyd *et al.*, 1996). A stratified randomization was used to balance groups with respect to natural differences in body mass index (BMI) and initial bone density. The girls consumed their normal diet, which provided 960 mg dietary Ca/day. They were assigned to either the supplemented group, that received two 250 mg Ca tablets per day in the form of CCM, or the placebo group that received two inert microcrystalline cellulose tablets. Total body and region-of-interest bone parameters were measured using DEXA at baseline and at 18 and 24 months to determine percent change. Nutrition, anthropometric, and pubertal stage assessments, as well as clinical chemistry measures including urinary Ca, hormone and gonadotropin concentrations were also performed. Over the course of the trial, 21 girls dropped out of the study, although each group was similarly affected in terms of reduced subject numbers ($n = 11$ Ca vs $n = 10$ placebo). With compliance in the Ca group at 71%, the average amount of supplemental Ca ingested daily was 360 mg. At 18 months, the Ca supplemented group compared to the placebo group gained more total body bone mass (TB-BMD: 9.6% vs 8.3%; $p < .05$) and increased lumbar spine bone mass (LS-BMD: 18.7% vs 15.8%; $p < .03$) (Lloyd *et al.*, 1993). Supplementation with CCM made a significant difference; it increased bone gain by 24 g/year which converts to a 1.3% increase in bone mass per annum.

In the same study by Lloyd *et al.* (1996), but after 2 years of CCM supplementation, the Ca group versus the placebo group demonstrated significantly higher BMD (12.2% vs 10.1%; $p = .005$) and bone mineral content increases (BMC: 39.9% vs 35.7%; $p = .01$) for total body, while bone area remained similar between groups ($p = .15$). At the lumbar spine and pelvis, supplemental Ca improved bone accretion compared to placebo by as much as 12–24%. Annualized bone acquisition rate was highest in Ca supplemented subjects with above-median values for

Tanner scores ($p < .006$). Overall, the results were impressive; added Ca from CCM resulted in a 20% average increase in the rate of bone gain, or an additional 61 g of bone mineral, in teenage girls over a 2-year period. The authors estimated that the Ca intake for the supplemented group in this study included substantially more available Ca due to the high absorbability of Ca derived from CCM. A number of human studies substantiate that the percentage of Ca absorbed from CCM is relatively high [e.g., 42% and 36% (Andon *et al.*, 1996b), 41.4% (Miller *et al.*, 1989), 37.43% (Heaney *et al.*, 1989a), 36.2% (Miller *et al.*, 1988)]. Based on an improvement in the rate of bone gain resulting from the level of Ca supplementation in this study, CCM added to the diet of teenage girls provided an adequate daily intake of Ca which could potentially manifest in a 5–6% increase in absolute peak bone mass over a 4-year period — providing the rate of increase observed for the 2 years of this study is sustained. According to epidemiological data (Matkovic *et al.*, 1979; Wasnich and Miller, 2000), a gain of this magnitude may be sufficient to significantly decrease hip fracture risk later in life (Matkovic *et al.*, 1979).

Seventy pairs of identical twins comprising $n = 86$ girls and $n = 54$ boys, of average (±SD) age 10 ± 2 year and ranging from 6 to 14 years old, were enrolled in a 3-year double-blind, placebo-controlled trial designed to investigate the effect of 1000 mg/day of supplemental Ca from CCM versus placebo tablets (Johnston *et al.*, 1992). One twin from each pair was randomized to ingest 2×250 mg CCM tablets in the morning and evening, while the other twin consumed placebo tablets on the same schedule. A co-twin study is superlative in that it automatically controls for a multitude of variables that are usually difficult to control for in non-twin cohorts and thereby enables detection of small differences. Bone density measurements in each region of interest were performed via absorptiometry at baseline. At 6 months, and annually from the first to the third year, the distal and midshaft of the radius were reassessed. In the hip (i.e., at Ward's triangle, the femoral neck, and greater trochanter) and lumbar spine region (L2 to L4), bone mass was reassessed at 3 years. Twenty-five twin pairs dropped out during the course of the study. Spontaneous Ca intake averaged 908 mg/day from the diet in the placebo group, whereas the supplemented twins consumed 984 mg Ca/day from food and, after compliance was factored in, it was estimated they averaged an additional 718 mg Ca/day for a total intake of 1612 mg. Throughout the trial, 22 twins remained prepubertal. It was this specific subgroup in which Ca supplementation with CCM demonstrated a significantly increased rate of bone accrual from 6 month onwards, as evidenced by a BMD gain with 95% confidence intervals that did not include zero. The overall average increase in BMD at all six sites measured was +2.9%, with positive effects occurring at the midshaft (+5.1%), distal radius (+3.8%), and lumbar spine (+2.8%). Gains related to Ca supplementation were not

apparent after puberty. This was possibly due to other factors, including sex steroids, which may potentially have dominated Ca accretion to the point that bone is optimally stimulated. Alternatively, the supplemental Ca effects may have been diminutive in comparison to other rapid physiological changes occurring at this time.

The question of whether bone density gains resulting from increased Ca intakes are sustained after an intervention was subsequently put to the test. As a follow-up to Johnston's 1992 co-twin study, Slemenda and coworkers enrolled 13 of the male and 32 of the female twin pairs (ages 6–14 years) that had already participated in the aforementioned 3-year trial and monitored them for 3 years postsupplementation to examine the effects on skeletal growth of withdrawal of the added 1000 mg Ca/day as CCM (Slemenda *et al.*, 1997). Each twin in a pair had been administered one of the two treatments. Methods included absorptiometry (dual x-ray and dual photon) for bone mass measurements of the radius, lumbar spine, and proximal femur. Assays were performed for serum biochemical markers of bone turnover [i.e., osteocalcin (OC), a measure of bone formation during puberty (Kanbur *et al.*, 2002) and tartrate resistant acid phosphatase (TRAP), a marker reflecting osteoclast resorptive activity], anthropological techniques, and Tanner staging. During growth, lower serum concentrations of bone turnover markers are generally associated with a higher bone mass, and this was the case during CCM supplementation in the study by Johnston *et al.* (1992). A within-pair –15.1% difference in serum osteocalcin of prepubertal subjects was observed after 3 years of Ca supplementation compared to placebo ($p < .05$). Three years post-supplementation the within-pair differences in OC attributed to Ca intake did not persist. An erosion of the significant benefit to bone mass bestowed by Ca to prepubertal subjects during supplementation was also evident at 3 years post-supplementation. Among all subjects, TRAP levels were not significantly different between groups during Ca supplementation or 3 years after supplementation ceased. Supplementation with CCM appeared to slow bone turnover and promoted more bone accrual compared to the placebo, although the residual benefits stemming from the earlier period of supplementation were not sustainable in this study. Considering that during the 3-year follow-up period, spontaneous Ca intake was only 920 mg/day, or ~400 mg/day below what is currently recommended as an adequate amount of daily Ca for adolescents, it is not entirely surprising that bone status would eventually be compromised.

C. Long-term bone density studies (4–7 years)

A double-blind, placebo-controlled trial to determine the effect of 500 mg Ca/day supplementation as CCM on bone gain in 112 adolescent girls ages 12–16 years (Lloyd *et al.*, 1997) represented a continuation of

previously described studies (Lloyd *et al.*, 1993, 1996). After 2 years of Ca or placebo treatment, which includes the 2-year intervention study of Lloyd *et al.* (1996), the subjects were rerandomized into four groups comprising: placebo tablets for 4 years from age 12 to 16 years (PP); placebo tablets for 2 years from age 12 to 14 years and then Ca supplementation for 2 years from age 14 to 16 years (PC); Ca supplementation for 2 years during age 12–14 years and then placebo tablets for 2 years from age 14 to 16 years (CP); Ca supplementation for 4 years from age 12 to 16 years (CC). Throughout the 4-year intervention, spontaneous Ca intakes averaged 983 mg/day among all participants. After compliance was factored in, supplemental Ca intake averaged 350 mg Ca/day, resulting in an average total Ca intake of 1333 mg/day during periods when Ca supplementation occurred. During the initial 2 years of the study, bone gain was significantly higher in the groups that were supplemented with Ca (Lloyd *et al.*, 1996). In terms of ranking the overall effects, the average 5-year gain (4-year intervention plus 1-year post-intervention) for total body BMC and BMC of the lumbar spine region was PP $<$ CP $<$ PC $<$ CC ($p > .05$). Gains for BMD were similarly ranked such that PP $<$ CP $<$ PC $<$ CC ($p > .05$). Bone area was lowest in the PP group compared to the other treatments supplying supplemental Ca; however, intergroup differences were not significant. It is highly possible that a lack of intergroup differences was indicative of a sample size problem resulting from the relatively high dropout rate (26%) which reduced the total number of subjects ($n = 13$–22/group). In addition, the 1-year post-intervention period, during which subjects did not receive any Ca supplementation, likely diminished the intergroup differences that may have existed at the end of treatment. Data at the conclusion of the 4-year treatment period were not reported. One year after completion of the Ca intervention, when the participants were 17 years old, the benefits attributable to previous Ca supplementation (Lloyd *et al.*, 1996) were no longer significant. Lloyd concluded that an assessment of the benefit of Ca to bone in a cohort that is some years away from skeletal maturity may not demonstrate a positive effect. This may be because benefits imparted by Ca in subjects with very high remodeling activity levels may be representative of a very pronounced bone remodeling transient. This is a phenomenon that occurs when a bone-active agent/nutrient initially acts to suppress bone remodeling activation and bone resorption, slowing bone loss due to an interruptive partial closure of the remodeling space. This can transiently alter the remodeling balance and, in the short-term, may provide a nonsustainable gain in bone mass, possibly an increase as high as 30%, until a steady state is re-established (Heaney, 2003a). It is difficult to logically reconcile that the growth and development of adolescent skeletons are virtually unaffected by previous Ca intakes; however, the dynamics of Ca metabolism during growth are complex. A number of

factors aside from those related to nutrition are involved; and overall, mechanisms governing bone metabolism at this time are not as clearly understood as may be expected. On the balance of existing evidence, it seems prudent to maintain an abundant intake of Ca throughout adolescence, via supplementation if need be, to avoid a Ca shortfall.

The pubertal growth spurt is a time of increased bone mass acquisition and when bone modeling markedly alters the size and geometry of bones to support longitudinal bone growth. In late adolescence, bone consolidation mediates endosteal apposition and periosteal expansion. While the short-term benefits attributable to Ca supplementation during growth have been established, little was known about the long-term effects of Ca supplementation on bone mass during the transitional period from childhood to young adulthood. This gap in the knowledge was addressed via a long-term (4 years + additional 3 years non-obligatory extension = total of 7 years), randomized, double-blind, placebo-controlled clinical trial that was organized for young girls. The aim of the trial was to evaluate the effectiveness of Ca supplementation versus a placebo on bone accretion (Matkovic *et al.*, 2005). An observational study was also run in parallel to evaluate the effect of higher intake levels of dairy products (Matkovic *et al.*, 2000, 2004a). Healthy prepubertal (stage 2) Caucasian females of average age (±SD) 10.8 ± 0.8 years with a spontaneous Ca intake <1480 mg/day at baseline (determined by food records) were initially stratified according to baseline total body BMD (TBBMD) and BMI (kg/m^2) measurements to ensure the initial equivalence of groups. Subjects were supplemented with 1000 mg Ca/day as CCM (via a 500-mg dose AM and PM) or else followed the same regimen with placebo tablets. The dairy group was comprised of those participants already consuming >1480 mg/day at baseline.

Data pertaining only to the first 4 year period for the placebo group ($n = 120$) versus the supplemented group ($n = 100$) reflected the effect of an average spontaneous Ca intake of 830 mg/day (from diet) and 1500 mg/day (from diet + CCM supplementation), respectively. Primary outcome measures included TBBMD and BMD of the radius (proximal and distal) via DEXA, and metacarpal cortical area and total area (CA and TA) via radiogrammetry. Based on follow-up univariate analyses, when baseline measurements were used as covariates, additional Ca via supplementation with CCM positively influenced bone acquisition. This occurred at all skeletal regions of interest throughout the bone modeling phase during the pubertal growth spurt compared to the placebo ($p < .05$). However, bone turnover markers, stature, bone width, and bone mineral area turned out not to be significantly different between the supplemented and placebo groups after 4 years. A limitation of the 4 years analyses in this study pertains to the omission of an adjustment to correct for baseline differences.

Subjects evaluated over 7 years included $n = 79$ receiving Ca supplementation, $n = 100$ administered the placebo, and $n = 85$ consuming a dairy-rich diet. Data pertaining to the supplemented versus placebo group at 7 years, by which time the pubertal growth spurt was over, revealed that the significant improvement conferred by Ca supplementation to TBBMD and radial BMD after 4 years was lost. Significant gain in metacarpal CA and CA:TA attributable to Ca supplementation after 4 years was sustained up to the 7-year endpoint. Bone mass acquisition was determined to be more rapid throughout the entire trial in the Ca supplemented group. Although fracture rate was not a primary research outcome, fewer subjects in the Ca group reported a bone fracture due to moderate trauma ($n = 9$) than was the case in the placebo group ($n = 20$). This was also true for forearm fractures, the most common fracture site during adolescence, with 3 reported forearm fractures in the Ca group versus 11 in the placebo group.

Results during the last 3 years of this study also included areal BMD of the hip (femur neck and trochanter) and lumbar spine (L2-L4) as measured by DEXA, as well as a final peripheral quantitative tomography (pQCT) measurement at the proximal radius. In order to account for compliance, which was estimated to average (±SD) ~65 ± 22% with Ca supplementation over 7 years, analysis for these measures was via post-hoc stratification of participants into two subgroups based on total cumulative Ca intake above and below the median intake of 1006 mg/day (excluding baseline). This resulted in an average (±SD) Ca intake of 1494 ± 292 mg/day for the ''high Ca'' group versus 748 ± 161 g/day for the ''low Ca'' intake group. Daily Ca intake among participants in the observational dairy study averaged ~1200 mg/day and this was coupled with a higher protein intake ($p < .001$) than was observed among those in the clinical trial. This nutritional combination commensurate with a high dairy intake conferred benefits in terms of bone growth and periosteal expansion; subjects were taller and cortical bone area in the proximal radius was higher in the dairy groups versus either a high or low intake of Ca among clinical subjects ($p = .0003$). BMD of the hip and forearm was significantly augmented by both Ca supplementation and high dairy consumption. High versus low Ca intake made a significant difference at the proximal radius for volumetric BMD and CA:TA. BMD at the lumbar spine was not affected by Ca supplementation, although in the dairy group a significant BMD increase at L2-L4 was observed. Milk with its combination of minerals and protein appeared to impact bone growth and bone area, while Ca supplementation promoted bone accretion via an improvement in volumetric bone density, particularly less than 1 year before and after menarche.

A ''catch-up'' phenomenon is being hypothesized for female adolescents ingesting less than adequate Ca levels during the pubertal growth

spurt. Bone modeling slows down after menarche, and it has been suggested that Ca absorption, as well as the requirement for Ca or Ca intake threshold, also declines at this time. Under these circumstances, current average Ca intakes may be sufficient enough to enable unimpaired consolidation of bone mass after growth, such that existing bone mineralization deficits may be reversible at this life stage. It has been proposed that an increase in Ca intake during the most crucial years of growth and development may simply accelerate the attainment of peak bone mass (Andon *et al.*, 1994). Alternatively, the Ca intakes classified as ''low'' in most of the adolescent studies comparing a high versus a low Ca intake may not be low enough to significantly or permanently compromise bone accretion at a life stage characterized by dynamic hormonal changes that may do more to dominate bone accretion than a suboptimal Ca intake. Based on Matkovic's 7-year trial, a catch-up in bone acquisition was evident in participants on the placebo diet in terms of BMD measurements for total body and the radius for example, but did not occur at the metacarpals. Furthermore, taller persons with a larger skeleton clearly benefited from Ca supplementation with CCM, resulting in significantly higher BMD at the proximal radius after 7 years ($p < .05$). Moreover, subjects who consumed a high habitual Ca intake over the course of the 7-year study, irrespective of the assigned group (averaging 1353 mg Ca/day), displayed significantly higher BMD at the proximal radius ($p < .05$) that persisted into early adulthood compared to subjects who consumed a low habitual Ca intake (averaging 668 mg Ca/day). This finding provides support for the conclusion that meeting the current Ca DRI of 1300 mg/day throughout adolescence from a combination of diet and supplementation can benefit BMD as a young adult. The results of Matkovic's study are particularly important in that they reflect long-term outcomes rather than short-lived changes consistent with a short-term bone remodeling transient.

In comparison, physical activity may not have the same ''catch-up'' capacity. The case has been made that exercise when young is likely to provide lifelong benefits to bone structure and strength (Warden *et al.*, 2007). It is possible that comparisons of ''catch-up'' with diet and physical activity are not yet possible until we have comparable ranges of deficiency and timing of deficiency prior to a period of adequacy. To generalize from the Matkovic study, dietary Ca intakes in the United States in placebo arms of randomized controlled trials are only moderately deficient compared to the very low Ca intakes of children in certain regions of Oriental (Lee *et al.*, 1993, 1995) and Third World countries (Dibba *et al.*, 2000), for example, who consume little or no milk.

Data from Matkovic's same cohort of young females, that were involved in the 7-year clinical trial that monitored the transitional period from childhood to early adulthood, was also used to assess BMD of the

skull and lower extremities during growth with Ca supplementation versus placebo (Matkovic *et al.*, 2004b). The skull is unusual in that it is a non-weight bearing site compared to bones in the lower limbs. It is comprised of comparatively dense bone, makes a relatively larger contribution to TBBMD during childhood than it does in later years, and it has a high volume to projected area when scanned by DEXA. The effectiveness of Ca supplementation was determined using data only from subjects attending 7 or more of the 15 planned semiannual visits for assessment purposes; however, the statistical analysis was described as being performed on an ''intent-to-treat'' basis. Intent-to-treat implies there is no definitive cut off criterion for compliance — providing at least one post-baseline assessment has been obtained. Unless the arbitrary requirement for seven or more assessment visits to warrant inclusion of a subject in the statistical evaluation was specified apriori, the data from subjects completing anywhere between one and seven visits should be included in an ''intent-to-treat'' analysis. The biological efficacy of Ca treatment in relation to bone accretion was evaluated by way of a subgroup analysis performed using a posthoc stratification procedure that assessed cumulative Ca intake in the lower and upper terciles ($<$824 mg/day and $>$1305 mg/day, respectively). Variables were analyzed relative to menarche, and a linear mixed effect model (i.e., fixed group effects and random subject effects) was employed. BMD of the skull in the Ca group supplemented with CCM reached a higher level more rapidly than it did in the placebo arm ($p < .0001$). Nevertheless, the difference between groups during the bone consolidation period that followed the bone modeling phase of the pubertal growth spurt at the skull was minimal. This suggests the catch-up phenomenon influenced the bone of the skull of subjects administered the placebo. The rate of bone accretion in the lower limbs increased most during the $\pm$2 YSM (Years Since Menarche) period and BMD of the Ca-supplemented group was significantly greater than the placebo group ($p < .0001$). In contrast to the subjects in the lower tercile, those subjects in the upper tercile of Ca intake ($>$1305 mg/day) were able to maintain the additional bone mass acquired. This outcome provides further support that a recommendation to meet the current DRI through a combination of dietary and supplemental Ca is prudent advice to facilitate the building of bone mass during adolescence that persists into young adulthood. Catch-up growth in the lower extremities, a region prone to higher and more dynamic mechanical stresses and a higher rate of bone remodeling, was limited when Ca intake was low. In adolescents, the skeleton appears to be particularly responsive to Ca supplementation before pubertal maturation (Bonjour and Rizzoli, 2002). The Ca salt used to supplement diets may also modulate the nature of the bone response (Bonjour and Rizzoli, 2007).

The studies outlined in this chapter demonstrate the effectiveness of CCM as a Ca source in children and adolescents. Periods of marginal Ca deficiency may be partially or totally corrected by the ''catch-up'' phenomenon depending on the timing and length of the suboptimal period, the skeletal site, and the degree of insufficiency relative to programmed body size.

VI. STUDIES OF BONE MAINTENANCE IN ADULTS

The amount of bone amassed at skeletal maturity (peak bone mass) and the rate of bone loss thereafter determines how dense and fracture resistant bones will be in old age. A lifelong AI of Ca is one of the best defenses against pathological bone loss in later adult life as the body becomes less efficient at balancing Ca needs with advancing age. Ca facilitates the maximal accrual of bone mass and contributes to the maintenance of bone density during aging by slowing the rate at which bone is inevitably lost. Osteoporosis is a latent chronic disease process involving excessive bone loss, as well as microarchitectural and material properties deterioration of the skeleton leading to skeletal fragility. Both men and women are afflicted with osteoporosis, although it is more prevalent in women than men. This is due mainly to the sudden hormonal changes women experience at menopause, compared to the more gradual endocrinological changes men experience, and the longer lifespan and smaller bone mass of women in general. Osteoporosis develops due to a combination of factors that usually always includes a long-term Ca insufficiency and/or hypoestrogenicity. In 2005, 30% of osteoporosis-related fractures occurred in patients 50–64 years of age, which corresponds with the average age of onset of menopause in women and the postmenopausal years, respectively (Burge *et al.*, 2007). By 2025, the burden of osteoporosis in the United States is projected to increase by 50%, the annual number of fractures could exceed three million, and the associated economic cost could be as high as $25.3 billion each year. Up to 70% of the fractures and 80% of the economic burden is likely to be borne by those $\geq$65 years of age (Burge *et al.*, 2007). Vitamin D works in conjunction with Ca to protect the skeleton; it has been shown to be effective in reducing the incidence of muscle atrophy and there is evidence to suggest it reduces the risk of falls in women who have suffered a stroke (Sato *et al.*, 2005). A meta-analysis of randomized clinical trials (Bischoff-Ferrari *et al.*, 2005) demonstrated that oral vitamin D supplementation reduces the risk of fracture (hip and nonvertebral) in the elderly, and an extension of this investigation established that the efficacy of vitamin D (vitD) is dependent on the addition of supplemental Ca (Boonen *et al.*, 2007). The following studies

outline the benefits of supplementation with CCM as the Ca source for postmenopausal women and elderly women and men.

A. Studies in postmenopausal women

The rate of bone loss in response to different levels of supplemental vitamin D (100 or 700 IU/day) was studied in a 2-year double-blind, randomized trial in 247 healthy ambulatory postmenopausal women (Dawson-Hughes *et al.*, 1995). All subjects resided in a northern latitude (42 °N) where exposure of the skin to the sun is limited in the winter time and adequate vitamin D synthesis may be reduced. The usual dietary vitamin D and Ca intakes of the subjects (mean age ± SD: ∼63.3 ± 5.2 years) were in the vicinity of 100 ± 90 IU/day and 450 ± 250 mg/day (mean ± SD), respectively. Added to usual dietary intakes, supplementation essentially raised respective total daily intakes of vitamin D to ∼200 or 800 IU/day. The current recommended AI for vitamin D is 400 IU. Regardless of the dosage of supplemental vitamin D, all participants were administered 500 mg Ca/day in the form of CCM (with a Ca:citrate: malate molar ratio of 6:2:3). Despite the supplemental Ca added to the usual dietary Ca at the time of the study, intakes by current standards were at least ∼200 mg/day short of the AI levels recommended for women of this age (i.e., 1200 mg Ca/day). At 6-month intervals, which coincided with the peak and nadir of seasonal sun exposure and circulating 25OH vitamin D, dual x-ray absorptiometry measures of hip, lumbar spine, and whole body bone density were used to gauge the rate of bone loss in response to treatments. In these women with low dietary Ca intakes to begin with, total intakes of ∼200 and 800 IU vitamin D/day for 2 years had similar effects on lumbar spine and whole body bone loss in the presence of an additional 500 mg/day supplemental Ca supplied by CCM. Overall, 800 IU vitamin D/day with added Ca made a significant impact, reducing the mean (±SEM) percentage of bone lost at the femoral neck (–1.06 ± 0.34%; $p = .003$), compared to 200 IU vitamin D/day + Ca (–2.54 ± 0.37%). The attenuation of bone loss attributable to the higher vitamin D intake mostly pertained to the winter/spring months (70%) rather than the fall/summer months (30%) when exposure of the skin to ultraviolet light is increased. Inadequate vitamin D (∼200 IU/day) resulted in a bone density loss at the femoral neck in postmenopausal women supplemented with levels of Ca that were below the recommended AI, but not when vitamin D levels exceeded recommended intakes. Ca as CCM appears to be more effective in postmenopausal women when dietary vitamin D levels are adequate.

Wintertime presents additional nutritional challenges, particularly to postmenopausal women living in northern regions where exposure to sunlight, which is important for the synthesis of vitamin D, is limited.

The benefit of vitamin D supplementation (400 IU of vitamin D) was evaluated in a 1-year double-blind, placebo-controlled trial involving 249 healthy postmenopausal women (mean ± SEM age: 61.4 to 61.9 ± 0.5 year). They also all received 377 mg/day of supplemental Ca (250 mg/day of elemental Ca as CCM and 127 mg/day of elemental Ca as Ca phosphate). The purpose of this study was to determine whether bone loss varies according to seasonal changes in exposure to sunlight at a latitude of 42°N in the United States when vitamin D is made orally available (Dawson-Hughes *et al.*, 1991). By current standards, the usual baseline vitamin D intakes of 100 IU in these subjects at the time of the trial were at least 300 IU/day less than the intake levels presently recommended. Although it is still considered highly controversial, a number of experts in the field of nutrition contend that 400 IU of vitamin D/day is still largely inadequate (Norman *et al.*, 2007) and levels that exceed the present upper tolerable limits are more appropriate to reduce the likelihood of vitamin D deficiencies (Vieth, 2006). In the months primarily corresponding to summertime and fall (period 1), lumbar spinal bone density (L2-L4) and whole body density increased similarly in both the placebo and vitamin D-supplemented groups of women. During the main months of winter and spring (period 2), bone loss in the spine was reduced by more than half in the vitamin D group compared to the placebo-treated women ($p = .032$), with the overall annual benefit to spinal bone density being significant for vitamin D + Ca ($p = .04$). Ca supplementation alone, which increased mean intakes to ~800 mg Ca/day in both groups, was equally as effective as vitamin D + Ca in terms of changes in whole body BMD throughout winter and summertime periods. It was not as effective in the spine during period 2 or over the course of the entire year. Nevertheless, the supplemental Ca in this trial, comprising 66% CCM, did exert a positive effect by reducing the rate of bone modeling in postmenopausal women. The addition of supplemental vitamin D contributed to a lowering of PTH levels and higher 25OH vitamin D levels during wintertime, which generally serves to protect bone mass.

A 2-year randomized, double-blind, placebo-controlled trial in 301 healthy postmenopausal women demonstrated that by increasing the Ca intake of women previously habituated to inadequate intakes (i.e., extremely low = <400 mg Ca/day or low = >400 to <650 mg Ca/day), the bone loss that characteristically occurs during postmenopause can be attenuated or even halted (Dawson-Hughes *et al.*, 1990). The protective effects of Ca were dependent on the anatomical site, years since menopause, and the source of supplemental Ca used. Early postmenopausal women (≤5 years) generally experience a more accelerated rate of bone loss compared to women ≥6-year postmenopausal, in whom the rapid rate of bone loss finally slows. The subjects were randomized to

receive 500 mg/day of elemental Ca, as either $CaCO_3$ or CCM (with a Ca:citrate:malate molar ratio of 6:2:3) or microcrystalline cellulose placebo tablets. Results were analyzed after separating early and late postmenopausal women, of respective mean (±SD) ages 54.5 ± 3.4 years and 59.9 ± 5.4 years, and women with extremely low basal Ca intakes from those that were slightly higher before supplementation. The 500 mg Ca/day supplementation in this trial significantly increased serum levels of ionized Ca ($p < .05$), although it could not prevent bone loss in the spine associated with the early stage of menopause. In retrospect, the levels of Ca in this 1990 study can hardly be expected to achieve as much considering the current recommended AI levels of 1200 mg Ca/day for 51- to 70-year-old women exceeds the maximum amount of Ca that was consumed by these subjects via diet and supplements (i.e., Ca intakes ranged between 900 and 1150 mg/day). At the time of this study the RDA was a mere 800 mg Ca/day. Among late-stage postmenopausal women, bone loss was less rapid for Ca treatments versus placebo. Based on the mean percentage change in bone density from baseline in women with low basal Ca intakes (<400 mg/day), additional Ca in the form of CCM versus placebo (in that order) attenuated bone loss at the femoral neck (mean ± SE: +0.87 ± 1.01% vs –2.11 ± 0.93%; $p < .05$) and radius (+1.05 ± 0.75% vs –2.33 ± 0.72%; $p < .05$). It also reduced bone loss in the spine (–0.38 ± 0.82% vs –2.85 ± 0.77%; $p < .05$). In comparison to the placebo treatment, $CaCO_3$ maintained baseline bone density at the femoral neck (+0.08 ± 0.98%; $p < .05$) and radius (+0.24 ± 0.70%; $p < .05$), but not in the spine (–2.54 ± 0.85%). Although the $CaCO_3$ treatment exerted positive effects in this category of women, it was at least nine, three, and five times less effective at ameliorating bone loss or maintaining bone density in the femoral neck, radius, and spine of postmenopausal women (respectively), than was CCM. The higher overall Ca intakes from both supplemental sources in later postmenopausal women maintained bone in the femoral neck and radius; however, bone loss in the spine did persist. At the supplementation level in this study over a 2-year period, CCM was more effective than $CaCO_3$ at protecting bone mass in women ≥6-year postmenopausal on extremely low Ca intakes.

Ca not only works in conjunction with vitamin D to enhance bone health, its effects on bone maintenance have been surmised to be enhanced in postmenopausal women by the presence of other minerals. A 2-year double-blind, placebo-controlled trial evaluated the effect of supplementary Ca (1000 mg elemental Ca/day as CCM) on lumbar spine bone loss in the presence and absence of a combination of trace minerals integral to bone maintenance (i.e., copper, 2.5 mg/day; manganese, 5.0 mg/day; zinc, 15.0 mg/day). Participants included 59 healthy postmenopausal women of mean age (±SD) 66 ± 7 years who were on average 18.1 ± 8.9-year postmenopausal (Strause *et al.*, 1994). At baseline, the mean Ca

intake was ~600 mg Ca/day, which is low by current standards. Subjects were randomized to one of four treatments: (i) Ca placebo + trace mineral placebo, (ii) Ca placebo + active trace minerals, (iii) Ca + trace mineral placebo, and (iv) Ca + active trace minerals. CCM was selected as the Ca source on account of its comparatively high bioavailability. It was delivered in a divided dose as 500 mg Ca with the morning meal and 500 mg Ca 2-h postprandial in the evening. The Ca:citrate:malate molar ratio was 6:2:3. Basal dietary Ca intake did not change from baseline among subjects receiving the Ca placebo. After 2 years, the CCM + trace mineral group ($n = 14$) was the only treatment that completely halted bone loss from the spine with a mean (±SEM) percent change in L2-L4 vertebral BMD of +1.48 ± 1.40% ($p = .0099$ vs placebo), while the placebo group ($n = 18$) showed significant bone loss from baseline (–3.5 ± 1.24%; $p = .0061$). BMD changes for the CCM ($n = 13$) and the trace mineral ($n = 14$) groups were intermediate (–1.25 ± 1.46% and –1.89 ± 1.40%, respectively) and not significantly different from any of the other treatments. The interaction between Ca and trace minerals was not significant, yet Ca was significant as a main effect ($p = .045$). Lack of a significant difference between CCM and the placebo treatment was attributed to the high dropout rate of participants. Fifty four of the original 113 subjects dropped out resulting in diminished power to detect differences. Supplementation with various other sources of Ca (e.g., $CaCO_3$) has been observed to cause interactions that interfere with mineral bioavailability, particularly with respect to iron (Cook *et al.*, 1991; Prather and Miller, 1992) and zinc (Wood and Zheng, 1997). In this study though, CCM in combination with trace minerals enhanced the maintenance of spinal BMD in older postmenopausal women.

The years surrounding menopause are typically associated with weight gain, which as a consequence increases risk factors associated with coronary heart disease (Wing *et al.*, 1991). Conversely, weight loss brought about by habitual caloric restriction usually stimulates generalized bone loss (Compston *et al.*, 1992; Jensen *et al.*, 2001; Villareal *et al.*, 2006). During periods of dieting, Ca intake is also frequently restricted to suboptimal levels, a situation that can easily be reversed with supplementary Ca that may serve to protect against diet-induced bone loss. Ricci and coworkers evaluated the effect of supplemental Ca from CCM (1 g/day in a divided dose) on BMD and bone turnover markers in a randomized, double-blind, placebo-controlled trial. The main goal of the trial was to test whether additional Ca reduces the risk of bone dissolution and bone mineral loss during a weight reduction regimen in obese postmenopausal women (Ricci *et al.*, 1998). The weight loss trial was completed by 16 women in the placebo group and 15 women in the Ca-supplemented group. The mean (±SD) age of participants was 58.3 ± 9.1 years. BMIs ranged from 28 to 48 kg/m^2 at baseline, and after 25 weeks of moderate dieting the

Ca and placebo groups [consuming a mean (±SD) total of 1646 ± 182 and 515 ± 105 mg Ca/day, respectively; $p < .05$], on average (±SD) lost 10.0 ± 5.3% of their body weight ($p > .05$). The decline in BMD from baseline was 1.4% higher in the placebo group versus the Ca-supplemented group, although not statistically different ($p < .08$). Urinary pyridinum and deoxypyridinoline cross-links (bone resorption markers), osteocalcin (bone formation marker), and serum PTH were significantly suppressed by Ca supplementation ($p < .05$, $< .01$, $< .05$, respectively). During any period of calorie restriction, it is imperative to continue to meet nutrient needs, especially those for Ca, in order to limit corresponding adverse effects on bone density. Supplementation with CCM did not hinder weight reduction and it ensured that weight loss was less likely to be accompanied by a loss of bone mass.

B. Studies in elderly men and women — including effects on fracture risk and risk of falls

In addition to microarchitectural deterioration and a reduction in the material properties of bone, an age-related decrease in bone mass potentiates fracture risk among the elderly (Heaney, 2001a; Prince *et al.*, 1997). To determine the extent to which supplementation with Ca and vitamin D can lessen fracture risk in men and women 65 years of age, a 3-year, double-blind, placebo-controlled trial was designed to examine changes in BMD, biochemical markers of bone metabolism, and the incidence of nonvertebral fractures every 6 months (Dawson-Hughes *et al.*, 1997). Healthy, ambulatory, home-dwelling subjects (176 men and 213 women with mean dietary intakes at baseline of <800 mg Ca/day and ~200 IU vitamin D/day) received either 500 mg Ca/day as CCM plus 700 IU of vitamin D_3 or placebo. Of the 389 enrolled subjects, only 318 completed the study. During the first year of the study, the Ca + vitamin D group showed mean BMD improvements from baseline at the femoral neck and lumbar spine (L2-L4) compared to the placebo group that demonstrated bone loss at these sites ($p = .05$ and $p < .001$, respectively). At the same time, the loss of total body BMD during the first year was significantly less for the Ca + vitamin D group versus the placebo group ($p < .001$). During the second and third years, the advantages of supplementation at the femoral neck and spine observed during the first year were maintained, although not increased. However, the mean (±SD) annual change in total body BMD was significantly greater in both sexes due to Ca + vitamin D treatment versus placebo during the second and third years (+0.23 ± 0.70% vs −0.14 ± 0.68% change/year, respectively; $p < .001$). Based on these findings, the investigators concluded that CCM + vitamin D supplementation provided a long-term benefit to the skeleton as a whole. Biochemical markers of bone metabolism changed significantly in a

favorable direction for the supplement versus the placebo-treated group, particularly for plasma 25OH vitamin D_3, serum PTH, serum osteocalcin, and 24-h urinary Ca:creatinine ratio ($p < .005$ for all measurements for both men and women). The number of falls reported in the two groups was similar; however, the cumulative incidence (all 389 subjects) of a first fracture (non-vertebral) at 3 years was 5.9% in the Ca + vitamin D group ($n = 11$) and 12.9% ($n = 26$) in the placebo group ($p < .02$). Furthermore, the 3-year cumulative incidence of a first pathological fracture, classified as ''osteoporotic,'' was significantly lower in the Ca + vitamin D versus the placebo group ($p = .01$). While the fracture risk outcomes must be interpreted with care due to the relatively small sample size in this trial by Dawson-Hughes *et al.*, based on these results and the balance of other similar studies with larger sample sizes (reviewed in detail elsewhere, Boonen *et al.*, 2004), there exists a strong case for supplementation with Ca+ vitamin D as a cost-effective prophylactic measure to combat fractures due to osteoporosis. It should also be noted that genetics and physical activity are also integral to the health and fracture resistance of the skeleton. The baseline physical activity scores were similar for men and women participating in the Dawson-Hughes *et al*. study, although genetic differences were not assessed. The type of supplements used, in addition to the characteristics of the subjects and their daily environment (e.g., home-dwelling vs institutionalized), are possible reasons for variations in outcomes among similar trials.

Further analysis of the risk of falling, at least once, during the above 3-year study was evaluated via an intent-to-treat analysis (Bischoff-Ferrari *et al.*, 2006). A total of 231 participants, of which 97 were men (49%) and 134 were women (55%), self-reported at least one fall during the 3-year period, with most falls occurring in the first year. Fifty-four percent of falls were sustained by individuals in the placebo group while 46% of reported falls were in the Ca + vitamin D group ($p > 0.05$). Stratification by activity levels (i.e., more or less active) revealed active individuals were somewhat more prone to falls. While the odds ratio for falls was not affected by supplementation based on the entire population of subjects or among men, a 46% reduction in risk was observed among women supplemented with CCM + vitamin D versus placebo; and that was significant as early as 1 year into the study. Less active women benefited most, particularly those who received ongoing treatment during the follow-up period when it was observed that CCM + vitamin D contributed to a 74% reduction in falls. A limitation noted by the authors of this study is the initial lack of power to detect effect modification, and the interaction terms of sex, activity level, and baseline 25OH vitamin D level not reaching significance. In addition, results pertaining to free-living older persons may not apply to individuals confined to nursing homes or other care centers.

Age-related bone loss occurs in the elderly (Meta *et al.*, 2006), particularly as dietary intake of Ca tends to decrease with age (Heaney *et al.*, 1982; Volkert *et al.*, 2004). Maintaining dietary Ca and serum 25OH vitamin D concentrations at the upper levels of normal ranges in the elderly may contribute toward limiting the loss of BMD in older persons. This theory was tested in 316 women and 122 men (mean ages 73.7 and 75.9 years, respectively) who were enrolled in a 4-year placebo-controlled, double-blind trial and randomized to receive daily supplementation with 750 mg Ca/day from CCM, 15 μg/day of 25OH vitamin D_3, or a placebo treatment as three equivalent divided doses with meals (Peacock *et al.*, 2000). At baseline and 6-month intervals, BMD of the hip region, lumbar spine, and total body was assessed. Bone structure at the hip was evaluated by measuring cortical bone thickness and femoral medulla width at the upper femur from x-ray radiographs taken at baseline and at 12-month intervals. Blood and urinary biochemical tests were also performed, and fracture history and incidence were recorded. Based on an intent-to-treat analysis of the men and women combined, the placebo group lost BMD compared to baseline (-0.0144 g/cm^2; $p = .0001$) at the total hip region at a rate of ~0.5% per year ($p < .001$), whereas the small change in BMD from baseline at the total hip for the Ca supplemented group was not significant (–0.0023 g/cm^2; $p = .45$). The change in BMD for the 25OH vitamin D_3 group (-0.0095 g/cm^2; $p = .002$) was intermediate and not significantly different from either the placebo or CCM group. Overall, changes at the greater trochanter, femoral neck, and Ward's triangle followed a similar pattern to the total hip. Cortical bone thickness of the femoral shaft decreased and medullary width increased significantly more in the placebo group than in the Ca group ($p < .002$) or 25OH vitamin D_3 group ($p < .03$). This indicated increased resorptive activity and age-related expansion of the medullary cavity in the absence of supplementation with Ca or 25OH vitamin D. Based on the bone density and structural data, the investigators concluded that two important components of bone strength at the hip were preserved with CCM supplementation (i.e., BMD and cortical bone thickness). Taken as a whole, Ca as CCM supplied at the upper end of the normal range surpassed the placebo in terms of minimizing bone turnover, supplying mineral for deposition in bone, and reversing the secondary hyperparathyroidism evident at baseline. Benefits beyond the placebo alone were provided by 25OH vitamin D_3, although in terms of ameliorating bone loss and slowing bone turnover these benefits were minimal. Based on results pertaining to subjects in this study, 25OH vitamin D_3 became less important to BMD, serum Ca, and serum PTH in the presence of adequate Ca. While the results of this study can only be generalized to older (60- to 90-year old) Caucasian, community-dwelling persons at risk of Ca and vitamin D insufficiency, it can be concluded that the treatments were safe and

demonstrated the benefits associated with CCM supplementation in the elderly.

An issue sometimes raised in relation to bone maintenance in the elderly concerns the endogenous acid generated by the metabolism of dietary protein, which when regularly excreted in the urine can cause calciuresis via various mechanisms (Dawson-Hughes, 2003a,b). Opinions have varied in terms of the extent to which protein-induced increases in Ca excretion may impact bone mass via bone dissolution and/or resorption (Barzel and Massey, 1998; Heaney, 1998, 2002). Protein intake in conjunction with Ca supplementation has now been positively associated with bone accrual, and only more recently has the notion of Ca economy been considered as a compensatory mechanism in response to an acute increase in the dietary protein load (Bonjour, 2005; Kerstetter *et al.*, 2005).

Dawson-Hughes and Harris investigated associations between protein intake (as a percentage of energy) and rates of bone loss in response to a placebo or supplemental Ca (500 mg/day from CCM + 700 IU/day vitamin D) treatment in elderly men ($n = 161$) and women ($n = 181$) over 65 years of age (Dawson-Hughes and Harris, 2002). Subjects completed a 3-year randomized, placebo-controlled trial during which BMD (total body, femoral neck, and spine) and protein intake were assessed at 6-month intervals and at 18-month intervals, respectively. Participants consumed a relatively high mean (±SD) protein intake of 79.1 ± 25.6 g/day in conjunction with daily Ca intakes of 871 ± 413 mg/day for the placebo group, or 1346 ± 358 mg Ca/day for the CCM + vitamin D-supplemented subjects. A higher protein intake positively increased the percentage change in total body BMD ($p < .042$) and femoral neck BMD ($p > .05$) in the supplemented subjects, although not in the placebo-treated group which lost bone. Supplemental Ca from CCM + vitamin D not only protected the skeleton from bone loss, it also promoted bone gain in the presence of an increased protein intake. Conservation of bone mass may occur via Ca lowering the turnover rate of bone, or via minimization of the adverse effects of mild acidosis on bone resorption (Dawson-Hughes, 2003b). In the placebo group, Ca absorption actually declined with increasing protein intake as a percentage of energy (ANCOVA; $p = .017$), although the absorption method used did not predict true Ca absorption. In the elderly, an increased protein intake in concert with supplemental Ca from CCM + vitamin D at recommended intake levels may promote BMD gain. The chemical nature of CCM is such that it provides 25 mEq of additional alkali, which may have compensated somewhat for the protein-related increase in acid load (Remer, 2001). Since there is an age-related functional decline in the ability to excrete acid, CCM as a Ca source for the elderly may be especially beneficial (Bushinsky, 2001).

Above all, it is important for the elderly population to meet current recommended intakes for Ca and vitamin D (Ca + vitD) on a regular basis for maximum skeletal benefit. Unfortunately, compliance with taking dietary supplements that may have been recommended, prescribed, or co-prescribed in conjunction with standard medical treatments for osteoporosis (e.g., bisphosphonates) is often poor and intermittent, thus limiting their effectiveness (Bayly *et al.*, 2006; Prince *et al.*, 2006; Rossini *et al.*, 2006). To determine whether a sustained benefit related to supplementation exists with Ca + vitamin D on BMD, 295 healthy ambulatory men and women (≥68 years old), who were participants in the previously discussed 3-year randomized, placebo-controlled trial involving simultaneous administration of Ca (500 mg/day as CCM) and vitamin D (700 IU/day), were followed-up for an additional 2 years (Dawson-Hughes *et al.*, 2000). Obligatory supplementation ceased after the 3-year intervention, although subjects were free to take supplements during the follow-up period if they chose to. Postintervention use of all supplements and medications was recorded and found to be comparatively evenly distributed among participants according to prior treatment classifications. Mean baseline BMD at 0 months was considered the reference point for evaluating subsequent changes in each group after the follow-up period. Overall, mean femoral neck, lumbar spine (L2-L4), and total body BMD improvements resulting from the 3 years of CCM + vitamin D supplementation were lost in elderly men and women by the end of the 2-year post-intervention period. The only exception to this was a modest persistent positive effect ($p < .05$) on total body BMD in men, mostly attributed to maintenance of bone mass in the legs. The observed reduction in bone remodeling rate during the supplementation regimen was also diminished during the 2-year follow-up based on serum osteocalcin and intact PTH biochemical measures. In summary, no cumulative skeletal benefit of Ca + vitamin D supplementation was identified for elderly women 2 years beyond the treatment intervention, and only a very modest and limited continuing benefit was observed for elderly men, suggesting that prolonged Ca supplementation in the elderly is advisable to maintain bone health.

VII. OTHER HEALTH BENEFITS

A. Oral health

1. Tooth retention

An AI of Ca and vitamin D is essential for retarding the rate of systemic bone loss that occurs naturally as a consequence of aging and declining hormone levels (e.g., menopause). Declining BMD can lead to osteopenia,

osteoporosis, and an increased susceptibility to pathologic bone fractures. Early data from rodent experiments suggested that oral bone surrounding and mechanically supporting teeth, particularly alveolar bone, is also dependent on an AI of Ca (Erricsson and Ekberg, 1975; Ohya *et al.*, 1992; Shoji, 2000) and vitamin D (Davideau *et al.*, 2004) to provide some protection against bone resorption in this region. Therefore, it is not unexpected that periodontal disease in humans may be more prevalent in osteopenic and osteoporotic subjects than in individuals with higher bone densities (Mohammad *et al.*, 2003; Wactawski-Wende *et al.*, 2005) and that it possibly shares a number of common etiologies (Nishida *et al.*, 2000; Yoshihara *et al.*, 2005).

Periodontal disease is characterized by a sequence of chronic oral inflammation and excessive alveolar bone resorption (i.e., receding alveolar bone) that results in root surface exposure of teeth, increased sensitivity, eventual detachment of the periodontal ligament, and subsequent tooth loss. Alveolar ridge bone exhibits intrinsic porosity, a structural fragility, and a proximity to vasculature that in effect virtually ensures it has the potential to be a vulnerable site in times of rapid bone resorption, much like the trabecular-rich regions in the hip and spine. Subsequent retention of the quantity and quality of bone in edentulous jaws also becomes critically important in terms of being able to provide surface support for dental implants and dentures that are desirable for both functional and cosmetic purposes (Bodic *et al.*, 2005) (Figure 6.4).

The impact of Ca and vitamin D supplementation on tooth loss in 145 healthy elderly subjects ($\geq$65 years of age, and not taking medications or supplements that alter Ca metabolism) was assessed during a 3-year randomized, placebo-controlled trial that was primarily designed to investigate bone loss from the hip (Dawson-Hughes *et al.*, 1997; Krall *et al.*, 2001). Participants randomized to the supplemented group consumed 500 mg/day of elemental Ca as CCM and 700 IU/day of cholecalciferol (vitamin D_3). The placebo group consumed an equivalent number of inert microcrystalline cellulose pills daily. Observations in the same subjects also continued during a 2-year follow-up period that began after cessation of the compulsory supplementation regimen (Dawson-Hughes *et al.*, 2000; Krall *et al.*, 2001). During the second phase, participants were divided into two categories based on either a lower ($<$1000 mg) or higher ($>$1000 mg) daily Ca intake. Questionnaires were used to gather information relevant to tooth loss at 1.5, 3, and 5 years. Actual tooth counts were conducted at 1.5 and 5 years, and a final periodontal exam also was scheduled at 5 years. The rate of compliance was based on pill counts at 6-month intervals and averaged 92% and 93% for the placebo and supplemented groups, respectively. During the randomized trial, the incidence of one or multiple teeth being lost in subjects was 13% in the supplemented group and 27% in the placebo group ($p < .05$). By the end

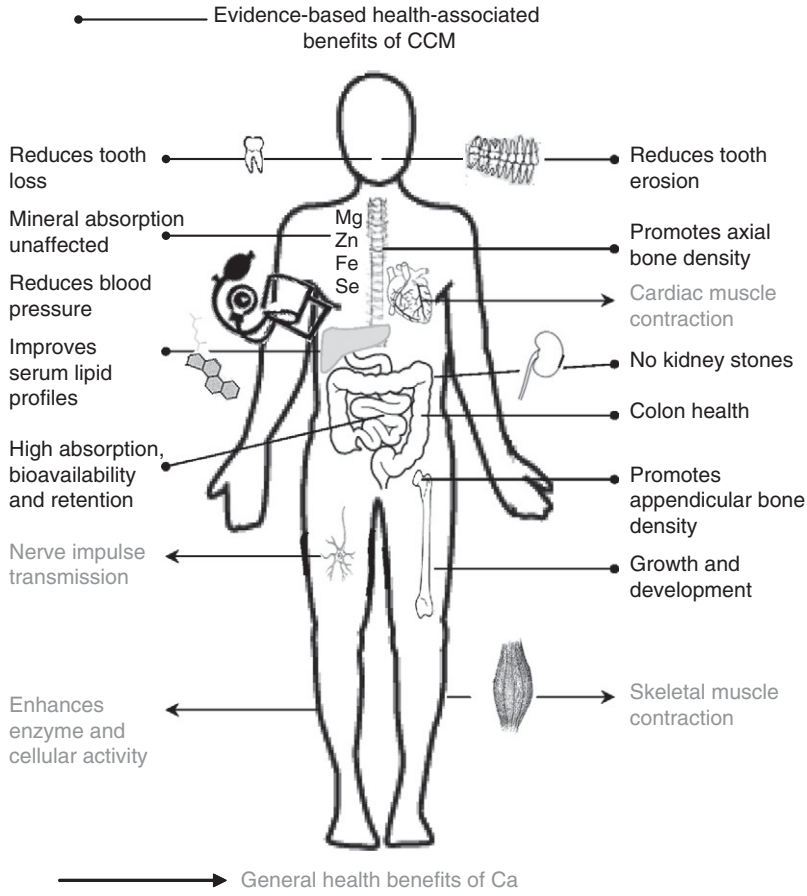

FIGURE 6.4 Illustrated summary of the health benefits pertaining to CCM.

of the follow-up period, 59% of subjects consuming <1000 mg Ca and 40% of subjects regularly consuming higher levels of Ca lost one or more teeth. Based on the odds ratio and the 95% confidence interval for tooth loss, it was determined that supplementation during the randomized clinical trial and the level of Ca intake (but not vitamin D intake) during the follow-up period significantly reduced the risk of tooth loss ($p < .05$ and $p < .03$, respectively). These results suggest that prophylactic CCM and vitamin D supplementation for osteoporosis also exerts a beneficial effect on tooth retention.

A prior study by Krall and colleagues demonstrated an association between dental status and BMD in healthy postmenopausal women, thus lending support to the hypothesis that systemic bone loss may also contribute to tooth loss (Krall *et al.*, 1994). A later prospective study within

a 7-year period assessed the association of tooth loss with systemic bone loss in the whole body, hip, and spine of postmenopausal women via three nutritional interventions (Krall *et al.*, 1996). Subjects randomized to the first arm of the intervention were divided into a group supplemented with 500 mg/day elemental Ca as either CCM or $CaCO_3$, and a second group taking a placebo. At the end of 2 years, more women ($p = .054$) in the placebo group than in the Ca-supplemented group lost teeth (11% compared to 4%, respectively) and the association was stronger ($p = .04$) among nonsmokers in the placebo group (12%) and the supplemented group (3%). The second and third interventions involved randomization of subjects to groups that were all supplemented with Ca, and additionally supplemented with either vitamin D (400 IU/day) or placebo for 1-year (Study 2), or a low (100 IU) or high (700 IU) daily dose of vitamin D for 2 years (Study 3), respectively. Subsequently, vitamin D was not found to be associated with tooth loss. However, compilation of data from all of the intervention groups demonstrated that the rate of change from baseline for BMD of the whole body and femoral neck was positively associated with the likelihood of tooth loss. BMD of the spine was increased in all groups; however, in subjects that lost teeth, the total gain was less at this site. This study showed that mitigation of systemic bone loss may simultaneously lessen oral bone loss and promote tooth retention. Furthermore, it revealed that supplementation with Ca was a nutritional intervention of consequence in terms of slowing the rate of BMD decline in the whole body and hip, as well as increasing tooth retention.

Surgical treatments for oral bone loss alone were estimated to cost in the vicinity of $5–$6 billion per year at least 5 years ago, an amount that does not include the costs associated with impaired dentition due to tooth loss (Hildebolt, 2007). The potential associated psychological, social, and physical harm that may have to be endured was also not considered. Therefore, it seems prudent to ensure that one's dietary intake of bioavailable Ca is adequate to avoid at least one potentially modifiable risk factor for tooth loss.

2. Tooth erosion

The erosive potential of acidic foods and beverages on dental enamel has been well documented (Ganss *et al.*, 2002; Johansson *et al.*, 2002; Yip *et al.*, 2003). Frequent exposure to extrinsic sources of organic acids found in pickled foods, fruits, fruit juices, juice blends, and soft drinks can readily give rise to chemical dissolution of the surface enamel and underlying dentine. Together with the high sugar content in many beverages, a low pH imparts a desirable flavor profile and organoleptic qualities (e.g., bite, tang, freshness, sourness), as well as functionalities including preservation and stabilization (Jandt, 2006). However, a beverage that induces an

oral cavity pH $\leq$ 5.5 is critical in relation to the biological hydroxyapatite [i.e., a nonstoichiometric carbonated Ca hydroxyapatite (Simmer, 1995)] that comprises tooth enamel (Brunton and Hussain, 2001). This is because acid in the beverage rapidly associates with ions that are normally supersaturated in saliva (Ca, phosphate, and hydroxyl ions), thus reducing salvia ion concentration. When the degree of ion saturation with respect to tooth mineral diminishes, the Law of Mass Action takes effect and causes mineral to be leached from teeth, which in effect softens and demineralizes exposed tooth surfaces. Based on this phenomenon, it stands to reason that the addition of Ca ions and/or Ca salts to acidic beverages and foodstuffs should therefore partially protect tooth enamel from the ingested organic acids by slowing the rate of enamel dissolution. In fact, findings from other studies support this notion (Attin *et al.*, 2003; Hughes *et al.*, 1999, 2000; Jensdottir *et al.*, 2005; Lussi *et al.*, 2005; Parry *et al.*, 2001). Moreover, a number of different product modifications have already been the subject of experiments targeting the problem of tooth erosion associated with consumption of acidified beverages (Attin *et al.*, 2005; Barbour *et al.*, 2005; Grenby, 1996).

As discussed in a previous section, adding Ca to beverages can be problematic in terms of solubility and precipitation effects (Parker, 2004). Furthermore, in certain beverages (e.g., cranberry juice) interactions of free Ca with matrix components can cause changes in color and loss of clarity (Klahorst, 2001). Simply adding less acid has been shown to adversely affect the taste and flavor profile of soda beverages (Barbour *et al.*, 2003). Another option is to use a combination of acids, such as citric acid as the primary acidulant together with a weaker acidulant, such as malic acid (Grenby, 1996). In terms of further reducing the erosive potential of acidic beverages, a potentially better strategy may involve the addition of CCM. Adding citric and malic acid in combination with Ca provides a soluble salt complex that has health and dental benefits (Andon *et al.*, 1992; Parker, 2004). Fortification of an orange-flavored beverage with increasing amounts of CCM has been shown to increase the pH of the system and, hence, reduce the erosive potential in a Ca concentration-dependent manner. This has been achieved without influencing the beverage sensory properties or reducing consumer acceptability (Assmann *et al.*, 2003).

To test the hypothesis that the addition of CCM reduces dental erosion, the erosive effects of four different drinks was compared: a citric acid-based orange-flavored soft drink fortified with CCM (pH 4.0, 1344 mg Ca/liter); the same drink without CCM (pH 3.6, 72 mg Ca/liter); and positive and negative controls consisting of a diet phosphoric acid-based cola (pH 3.1, 35 mg Ca/liter) and distilled water, respectively (Rugg-Gunn *et al.*, 1998). In a randomized cross-over design comprised of four 6-day periods, 11 subjects were required to wear a palatal

intra-oral appliance that held sterilized bovine incisor enamel slabs in place. During each of the 6-day periods, participants removed and immersed the slabs in one of the room temperature drinks for 15 min four times daily before replacing it in their mouth. The intra-oral appliances were routinely removed and kept moist during meals. Prolifometry was used to quantify the loss of slab enamel attributable to the exposure to each drink. Results showed a significant effect of the type of drink on enamel loss ($p < .001$), however, only the phosphoric acid-based cola drink was significantly different ($p < .01$) from the rest of the drinks with respect to depth of enamel loss (14.3 μm). There was no statistically significant difference for this parameter in response to distilled water (5.0 μm), or the citric acid-based soft drink with and without CCM (5.2 μm and 6.1 μm, respectively). However, this study appeared to have some limitations. First, bovine and human enamel are not equivalent in a number of chemical and structural respects; for example, bovine and human enamel possess some exclusive and significant chemical elements, bovine enamel is comprised of larger crystal grains and more lattice defects than human enamel, there are response differences in terms of acid-resistance with respect to the development of caries-like lesions, and it has been suggested that enamel etching could, as a result, vary between bovine and human enamel (Abuabara *et al.*, 2004). Consequently, the comparative value of the model needs to be carefully considered. Second, the 15-min period of slab exposure to an unstirred beverage is unlikely to be representative of the *in vivo* dynamics associated with various drinking habits (e.g., fast or slow sipping versus the steady swallowing of larger mouthfuls or guzzling). Third, as noted by the authors, the exposure of bovine enamel to drinks was at room rather than body temperature, a factor known to decrease erosion potential in the presence of acids (Amaechi *et al.*, 1999). It was unexpected that distilled water caused the amount of erosion exhibited and, although the erosion for the orange drinks trended in the expected direction, more subjects are likely required to demonstrate significant differences.

Human studies and rodent experiments demonstrating the potential for CCM to reduce the risk of tooth enamel erosion are included in the patent of Andon *et al.* (1992). One experiment assessed rats provided with soft drink, soft drink with added CCM, or water as their only source of fluids for 21 days. Based on a predefined erosion scale, the average extent of dental erosion compared to the unfortified soft drink was 4.5 and sixfold less in water and soft drink + CCM, respectively. Another study used the Vickers hardness measurement to assess the mean (±SEM) reduction in surface hardness of human enamel specimens ($n = 8$ per group) immersed for 60 min in 15 ml of OJ (–101 ± 8.7), OJ + CCM (0.9 ± 5.8), grapefruit juice (–130 ± 12.7), grapefruit juice + CCM (2.8 ± 6.4), or

water (–0.4 ± 4.3). This later method uses a nonnatural setting, as the buffering effect of saliva is negated *in vitro* and the time of enamel exposure is unusually long. Despite these limitations, the results are remarkable in terms of the magnitude of protection that CCM provided against the erosive potential of fruit juices. Considering fruit juice and soft drink consumption is widespread, particularly among children and adolescents (Lussi *et al.*, 2000; Rampersaud *et al.*, 2003), and bearing in mind that these beverages often replace dairy consumption in the diet (Harnack *et al.*, 1999), adding CCM to juices and other acidified beverages to improve the nutritive value and to lower erosive potential appears to be a practical initiative.

B. Blood pressure reduction

High blood pressure (hypertension) is a condition more frequently associated with adults; however, it can be present at any age (e.g., infancy). In fact, over the past two decades, the incidence of hypertension among children and adolescents has been documented to be on the rise (Sorof *et al.*, 2004). In 2002, among a cohort of 5102 students aged 10–19 years, the prevalence of elevated blood pressure (BP) was estimated to be as high as 4.5%. The cause of hypertension in children is increasingly being linked to excess weight gain, unhealthy eating habits, stress, and insufficient physical activity (Sorof *et al.*, 2004). Conversely, a family history of hypertension can be a predisposing factor, as can an underlying renal or cardiac pathology (i.e., secondary hypertension). Childhood hypertension is considered to be a long-term health risk as it predicts adult hypertension, and even mild-to-moderate hypertension can, over time, result in damage to the heart, kidneys, and blood vessels. The absence of a pathologic etiology for hypertension in children usually indicates a nonpharmacologic approach to BP management involving dietary and lifestyle modifications and can be an effective intervention to restore normotension. Dietary CCM supplementation has already been implicated in a BP lowering effect in children with low baseline Ca intakes (Gillman *et al.*, 1995).

In a randomized, double-blind, placebo-controlled trial, 101 students (50 girls and 51 boys, average age 11 years) were assigned to either (i) an intervention group ($n = 51$) that consumed two servings of 300 mg Ca-fortified fruit juice per day for 12 weeks (with CCM as the daily source of 600 mg supplemental Ca) or (ii) a placebo group ($n = 50$) that consumed two servings of an identical-looking unfortified fruit juice per day for 12 weeks. Subjects included 61 black, 9 Hispanic, 16 Asian, and 15 white students. An automated devise was used to record four BP measurements per subject each sitting. The final three measurements were averaged and recorded at the baseline, study midpoint, during the final stage of the

study, and post-intervention. In summary, the mean overall change in BP and the average difference in BP change between the CCM and placebo group were calculated. Anthropometry measurements were performed at baseline and at 12 weeks. Dietary assessment, medication questionnaires, and compliance monitoring were carried out as planned during the course of the intervention to strengthen confidence in the results.

Diastolic BP (DBP), which measures the pressure in the arteries when the heart is at rest, was largely unaffected by the intervention. Systolic BP (SBP), or the maximum pressure exerted when the heart contracts, did change in response to CCM supplementation in most children. The data specifically showed that over 12 weeks, children in the lowest quartile of baseline daily Ca intake (150– $<$ 347 mg/1000 kcal) were affected most significantly by CCM supplementation in terms of a reduction in systolic BP (effect estimate: –3.5 mm Hg), whereas children in the highest quartile of baseline daily Ca intake (514– $<$ 882 mg/1000 kcal) demonstrated no appreciable reduction in systolic BP due to CCM supplementation. Children in quartiles two and three of the baseline Ca intake benefited from a CCM-induced reduction in SBP with the effect estimated to be –2.8 mm Hg and –1.3 mm Hg, respectively. The overall trend for the estimated effect of Ca intake on BP across quartiles was highly significant ($p = 0.009$).

Covariate adjusted estimates of the mean difference in SBP revealed that the CCM group was 1.1 mm Hg lower than that of the placebo group at the midpoint of the trial (6 weeks). From baseline to the final stage of the study (12 weeks), SBP in the CCM group was 1.8 mm Hg less than that of the placebo group (effect estimate: –1.8 mm Hg; 95% CI: –4.0, 0.3). However, the reduction in SBP promoted by CCM supplementation was essentially lost by 6-weeks post-intervention. Subgroup analyses, which also included adjustments for confounding variables, indicated that the difference in SBP change was –2.0 mm Hg among black children, –1.5 mm Hg among nonblack children, –2.3 mm Hg among boys, and –0.9 mm Hg among girls. It was also generally observed that the placebo group gained slightly more weight (2.9 kg) than the intervention group (2.7 kg) after 12 week and SBP in the placebo group increased during the trial.

Despite the short duration of this study and the low-dose supplementation regimen with CCM, a modest lowering of SBP among subjects was observed. This effect was more prominent among boys and among black children, who may consume lower amounts of dairy products due to a higher incidence of lactose intolerance. The data provide evidence to support the hypothesis that a highly absorbable form of Ca can potentially influence SBP in ''at risk'' younger populations. Even seemingly small reductions in SBP of a large population over time can potentially impact public health by way of reducing the risk of coronary and stroke incidents in the future.

C. No increase in the risk of kidney stones

The incidence of kidney stones in the United States has steadily increased over the previous three decades [National Kidney and Urologic Diseases Information Clearing House (NKUDIC), 2005]. In 2000, more than 600,000 patients went to emergency rooms for problems related to kidney stones [National Kidney and Urologic Diseases Information Clearing House (NKUDIC), 2005], 177,496 patients were hospitalized and the cost of evaluating and treating kidney stones was $2.07 billion dollars (Pearle *et al.*, 2004). It is predicted that 10% of the US population will have a kidney stone at some point in time (Sullivan, 2005). While there are many types of kidney stones, the most common are formed from dissolved urinary Ca and oxalate that forms an insoluble accretion [i.e., Ca oxalate monohydrate crystals (Qiu *et al.*, 2003)] in the kidneys or ureters. Sooner or later, if the crystalline mass becomes large enough ($\geq$6 mm), the stone initiates symptoms of discomfort.

Oxalate is the dissociated form of oxalic acid, either derived from dietary sources (10–20%) (Holmes *et al.*, 2001) or synthesized endogenously in the liver ($\sim$80%) (Gershoff and Faragalla, 1959; Morozumi *et al.*, 2006). Oxalic acid is a ubiquitous substance in animal tissues and occurs naturally in a large number of plants (Monje and Baran, 2002). The main sources of dietary oxalic acid are plant-derived foods with relatively high concentrations (i.e., $\geq$200 ppm), for example, buckwheat, star fruit, black pepper, purslane, poppy seeds, rhubarb, tea, spinach, plantains, cocoa and chocolate, ginger, almonds, cashews, garden sorrel, mustard greens, bell peppers, sweet potatoes, soybeans, tomatillos, beets and beet greens, oats, pumpkin, cabbage, green beans, mango, eggplant, tomatoes, lentils, and parsnips. In human tissues, oxalic acid levels generally range between 0.6 and 4 mg/kg in blood, kidney, liver, muscle, brain and bone, with the highest concentrations occurring in the kidneys (Committee for Veterinary Medicinal Products, 2003).

The presence of Ca in kidney stones and the abnormally high Ca levels in idiopathic (absorptive) hypercalciuric individuals that are inherently more prone to kidney stones, initially led to the belief that dietary Ca may be a cause of renal stone formation (Coe *et al.*, 1992). Recent evidence suggests that, as a therapeutic approach to reducing the risk for kidney stones, Ca-restricted diets may pose a greater risk to normocalciuric individuals prone to kidney stone formation; such an approach may increase urinary oxalate and the likelihood of recurrent stones, as well as promote bone loss (Borghi *et al.*, 2002; Coe *et al.*, 1997; Curhan *et al.*, 1997). The amount of oxalate excreted in urine has been found to be positively associated with Ca oxalate supersaturation and stone formation (Holmes *et al.*, 2001). While free oxalic acid is readily absorbed from the gut lumen (Morozumi *et al.*, 2006), an increased dietary Ca to oxalate

ratio reduces gastrointestinal oxalate absorption and subsequent oxalate excretion (Morozumi *et al.*, 2006; Williams *et al.*, 2001). Citrate, the dissociated form of citric acid and a Krebs cycle intermediate, is also recognized as playing an important role in the reduction of kidney stones when it is excreted in the urine (Qiu *et al.*, 2003). Its chemical composition is such that it can chelate metal ions and impart increased solubility to a salt complex. Moreover, citrate is protective by way of contributing to the control of crystal habit and growth of renal stones via modulation of the morphology and growth kinetics of Ca oxalate crystals (Qiu *et al.*, 2003). Citrate is usually effective in this capacity at very low concentrations (Tiselius, 2005). Malate, the dissociated form of the dicarboxylic acid malic acid, also has been implicated in chelating Ca and forming soluble salts that protect against urinary tract calculi in rodents (Thomas and Thomas, 1977). CCM therefore includes three chemical components considered to be of potential benefit in terms of protecting against kidney stones.

To further evaluate Ca's role in the presence of oxalate, Liebman and Chai examined the effect of oxalate loading (OL) over 24 h on mean oxalate absorption and urinary oxalate excretion in 10 healthy non-kidney stone forming (6 males and 4 females, mean ±SD age: 28 ± 7 years) and 4 kidney stone forming subjects (2 males and 2 females, 35 ± 11 years) in response to concurrent dosing with a 300 mg source of elemental Ca (Liebman and Chai, 1997). Three OL tests were performed in a crossover design with a ≥1-week washout period between each test. Oxalate loading consisted of 180 mg unlabeled + 18 mg labeled 1,2[$^{13}C_2$]oxalic acid and tests took place in the following order: (i) alone (baseline), (ii) with $CaCO_3$ (OL + Ca), and (iii) with CCM (OL + CCM). For all urinary indices tested, stone forming and non-stone forming individuals were similar, and subsequently pooled into one group with each subject serving as his/her own control. Mean 24-h oxalate absorption decreased from 18.3% (baseline) to 8.1% and 7.2% for OL + Ca and OL + CCM, respectively. Mean 24-h exogenous oxalate was significantly reduced from 36.2 mg (baseline) to 16.1 mg (OL + Ca) and 14.3 mg (OL + CCM), whereas endogenous oxalate was unchanged across all treatments. Post-OL urine sampling for Ca showed that, of the two Ca sources, CCM was more bioavailable than $CaCO_3$; however, oxalate absorption did not differ significantly between the two Ca sources.

Six subjects also completed 24-h oxalate excretion and absorption tests so that the effectiveness of various Ca doses (100, 200, and 300 mg Ca) in conjunction with an OL versus baseline (0 mg Ca) could be assessed. Total urinary oxalate excretion was significantly reduced in the presence of Ca at all doses, although endogenous oxalate remained unchanged. Zero mg Ca and 100 mg Ca resulted in higher oxalate absorption (11.3% and 9.1%, respectively) compared to 200 mg Ca (5.9%) and 300 mg Ca (7.6%).

In summary, ≥200 mg elemental Ca administered either as $CaCO_3$ or CCM reduced oxalate absorption and excretion in the event of an oxalic acid challenge.

A randomized study was designed to compare the stone-forming potential of low-fat milk versus CCM-fortified OJ in 12 idiopathic hypercalciuric adults (6 healthy males and 6 females aged 18–30 years) (Coe *et al.*, 1992). After a washout and subsequent baseline period (7 days each), at which times it was confirmed that participants were on basal Ca intakes of <800 mg/day, one of the beverages was added to their daily diet for 28 consecutive days. The beverages provided a total of 600 mg additional Ca/day in divided doses over the course of the day. After a 7-day washout period, testing was repeated with the other beverage. Apart from subjects being asked to avoid excessive dairy consumption and all fluid milk consumption that was not a test beverage, usual dietary habits were encouraged during the study. During the course of the study, 24-h urine samples were collected to evaluate the effect of Ca supplementation on a number of urinary measures, including Ca, citrate, malate, and Ca oxalate levels, pH, and Ca:citrate ratio.

In both sexes, CCM-OJ provided an alkali load that significantly increased urinary pH compared to basal levels and versus milk consumption, and also increased urinary citrate excretion versus basal levels. An elevated urine pH and citrate level are generally considered to reduce Ca oxalate supersaturation and crystallization potential (Odvina, 2006). However, in this study the relative supersaturation measurement for Ca oxalate was not different between the CCM-OJ and milk treatment groups, or between either treatment and the basal levels. Although the alkalizing effect of milk was less than that of CCM-OJ, it also induced a higher urinary pH compared to basal levels ($p < .01$ and $p < .05$ in women and men, respectively).

The biochemical changes in urine associated with both CCM-OJ and milk consumption in this study indicated the two beverages were equally effective at modifying stone formation risk factors in hypercalciuric adults. These findings suggest that normocalciuric individuals are therefore unlikely to be put at increased risk of kidney stones due to consumption of these beverages at the moderate intake levels tested in this study. Furthermore, vegans, and individuals with lactose intolerance and/or a milk protein allergy, should be able to supplement with CCM-OJ with minimal risk for stone formation.

D. No effect on the status of other minerals (Fe, Zn, Se, and Mg)

The chemistry of metal ions and their interactions with other molecules or ions in food/beverage matrices, in dietary supplements, and in the body itself, has great biological relevance. For decades now, Ca has been

heavily promoted by health professionals as one of the most important minerals to regularly consume in adequate amounts to reach one's potential peak bone mass and to protect against bone loss. As is expected in complex systems, a high Ca intake naturally alters the body's mineral status, which conceivably might exert an interactive effect in terms of altering the bioavailability of other minerals, such as magnesium (Mg), iron (Fe), zinc (Zn), and selenium (Se). That possibility has been a concern which has been investigated in both human studies and animal experiments over the years. It has already been established that the potential exists for various forms and sources of Ca to interact with and interfere with micro- and macromineral bioavailability (Cook *et al.*, 1991; Dawson-Hughes *et al.*, 1986; Deehr *et al.*, 1990; Smith, 1988).

Based on an overview of numerous studies, the extent to which Ca and trace mineral interactions occur appears to be related to such factors as the source of Ca, the ratio of Ca in relation to other minerals, the timing of Ca and trace mineral intake, meal interactions, food formulations, and natural food chemical compositions (Smith, 1988). As described in the following sections, CCM has been evaluated for its impact on the absorption of other minerals and, based on the results of these studies, appears to provide a unique delivery system for dietary Ca that does not appreciably affect the availability or status of other minerals.

1. Iron

Iron deficiency is considered to be the most prevalent nutrient deficiency worldwide (Ilich-Ernst *et al.*, 1998; Turnlund *et al.*, 1990). Depletion of iron in the blood as a result of low dietary iron intake, inadequate intestinal absorption, or increased iron losses can lead to iron-deficient anemia (Haas and Brownlie, 2001). Iron exists in two forms, heme and nonheme. Heme iron (Fe^{2+}) is the central pigmented oxygen-carrying portion of hemoglobin and myoglobin protein molecules in animal tissues. Nonheme iron (Fe^{3+}) is derived from plant tissues and animal tissues other than hemoglobin and myoglobin. Heme iron is better absorbed than nonheme iron because the former binds fewer of the luminal iron chelators that more readily bind inorganic iron (Conrad and Umbreit, 2002). The availability of iron is affected by dietary components known to inhibit iron absorption in a dose-dependent manner (Hallberg, 1998). Inhibitors of iron bioavailability generally include, although are not limited to: phytates (Hurrell *et al.*, 1992), fibers (Hallberg, 1987), Ca (Hallberg, 1998), phosphate (Monsen and Cook, 1976), ethylenediaminetetraacetic acid (EDTA) (Cook and Monsen, 1976), tannic acids (Brune *et al.*, 1989), and other polyphenols (Cook *et al.*, 1995). Chronic exposure to elevated levels of supplemental Ca has also been implicated in reducing hemoglobin concentrations in both animals (Smith, 1988) and humans (Hallberg, 1998).

Hemoglobin is the iron-containing oxygen-transport metalloprotein in red blood cells (RBCs) of the blood in humans and animals.

Heme and non-heme iron each enter intestinal mucosal cells via different specific receptors on the luminal mucosal surface (i.e., Fe^{2+} via divalent metal transporter-1 and Fe^{3+} via mobilferrin-integrin) (Roughead *et al.*, 2002). Since the absorption of both types of iron can be affected by Ca, it is hypothesized that a disruption to iron absorption, caused for example by competitive binding (Hallberg, 1998), may occur at some point of transfer within the mucosal cells or at the site of release from the intestinal cell into the circulation (Perales *et al.*, 2006; Smith, 1988). The potential for Ca to interfere with the bioavailability of Fe can also be related to numerous other factors including the presence of Fe chelating agents, such as various organic acids, that form acid–Fe complexes of variable solubilities (Salovaara *et al.*, 2002).

The effect of the source of Ca on the magnitude of Ca–Fe interactions *in vivo* was assessed in rodents (Smith, 1988), using a whole body radioisotopic retention test as an endpoint to determine true iron bioavailability (i.e., Fe that is absorbed and utilized). A single 50 μg liquid dose of ^{59}Fe-labeled $FeCl_3$ was administered by oral gavage to rats at a Ca:Fe ratio of 60:1 and 120:1 to replicate a human iron intake of ~15 mg/day and a Ca intake of 800 mg/day or 1600 mg/day, respectively. Ca sources included $CaCO_3$, Ca Phosphate (CaP), bone meal, and Ca hydroxyapatite (CaHA), while the control dose contained no Ca and was normalized to represent 100% Fe retention for comparison purposes. Isotope counts were performed immediately after dosing (to measure 100% retention) and subsequent counts over 6 days were divided by the 100% count to estimate Fe retention. For $CaCO_3$, Fe retention was 68% at a Ca:Fe ratio of 60:1, and only declined a further 2% when the ratio was increased to 120:1. Fe retention values for other forms of Ca at a 60:1 Ca:Fe ratio were as follows: 77% for bone meal, 89% for CaP, and 99% for CaHA. Fe retention decreased in response to the higher Ca:Fe ratio of 120:1 (i.e., Fe retention in the presence of bone meal, CaHA, and CaP was 49%, 72%, and 78%, respectively). This is indicative of a dose-response effect of Ca on Fe retention. This study also underscored the importance of the source of Ca in relation to Fe retention.

Possibly due to the organic anions that accompany the Ca in CCM (Deehr *et al.*, 1990) and/or the components of the food matrix to which CCM is added, the Ca–Fe interactions that typically interfere with Fe bioavailability seem not to be of significant nutritional detriment (Mehansho *et al.*, 1989a). The positive nutritional effects of the fruit beverage components citric acid and ascorbic acid were demonstrated in a rat experiment ($n = 6$/group) that evaluated Ca and Fe bioavailability following consumption of OJ with added CCM versus an aqueous control, that is, CCM in deionized water (H_2O) (Mehansho *et al.*, 1989a).

Whole-body isotope retention experiments were performed (via the method described above in the Smith experiment and with confirmed corrections for isotope decay rates), using extrinsically labeled ferric chloride hexahydrate and Ca from CCM (i.e., [^{59}Fe]$FeCl_3$ and [^{47}Ca]$CaCl_2$) to determine the bioavailability of these minerals at an Fe:Ca ratio of 1:167 mol/mol. CCM solubility was also assessed via a filtration method.

CCM solubility was 73.3% in H_2O compared to 100% in OJ. To put these solubility values in perspective, $CaCO_3$ in water was tested using the same method and its solubility was only 5%. Subsequent whole-body isotopic counting revealed that Ca in H_2O significantly reduced Fe retention from 43.1% for Fe + H_2O to 12.3% for Ca + Fe + H_2O. Compared to water (control), OJ significantly ($p < .05$) enhanced the bioavailability of Ca ([^{47}Ca] retention was 42.8% vs 33.0%, respectively) and Fe ([^{59}Fe] retention was 38.0% vs 8.0–12.3%, respectively). Various components of OJ, including citric acid, ascorbic acid, fructose, and sucrose, were then methodically added back to the aqueous control in the presence of Fe and Ca to establish which of these components, alone or in combination, were contributing to the improved Ca and Fe retention associated with the OJ vehicle.

The addition of citric acid to water, at a level equivalent to the amount present in OJ (41.6 mM, pH 3.9), lowered the pH of water from 6.5 to 4.0 and completely solubilized added CCM. Nonetheless, the extent to which *in vitro* solubility is relevant to final absorption is an issue that remains controversial (Heaney, 2001b). In fact, added citric acid did not appear to alter Ca absorption from the water vehicle; however, citric acid significantly increased Fe retention when added to water containing CCM (8.0% vs 23.7%). Fe retention was further increased (23.7–37.6%) by the addition of ascorbic acid (i.e., Fe + Ca + cit + AA + H_2O). Conversely, ascorbic acid added to H_2O + Fe + Ca in the absence of citric acid did not alleviate the significant reduction (30.8%) in Fe retention attributable to Ca–Fe interactions. In OJ, Ca–Fe interactions were not significant in terms of interfering with Fe retention. In water, added citric and ascorbic acid mitigated the negative effect of Ca on Fe absorption and, as a result, Fe retention was comparable to OJ. Addition of fructose and/or sucrose, with or without added citric or ascorbic acid, did not significantly influence ^{59}Fe retention. Citric acid was determined to be the most effective component in OJ contributing to high Fe retention in the presence of CCM.

In the Mehansho *et al.* experiment, the potential for ascorbic acid to solubilize Fe was reported to be limited to low pH environments. Other investigators have reported that ascorbic acid facilitates iron absorption by forming a chelate with ferric iron at an acidic pH that remains soluble at the alkaline pH of the duodenum (Lynch and Cook, 1980). Salovaara

and coworkers observed a 70-fold increase in Fe^{3+} uptake resulting from 80 µmol/L AA at a low acid concentration in the CaCo-2 cell line (Salovaara *et al.*, 2002). The presence of both citric and ascorbic acids in a beverage that also delivers a soluble source of Ca therefore appears to be most desirable for maximizing Fe bioavailability.

The value of CCM as a Ca source that does not antagonize Fe absorption was confirmed among a cohort of 19 healthy postmenopausal women (mean ± SD: 63 ± 6 years) using a single-blind, placebo-controlled, randomized crossover design (Deehr *et al.*, 1990). Whole-body retention of ^{59}Fe was tested at baseline and immediately after the ingestion of an extrinsically labeled test meal containing the radiolabel ^{59}Fe at 1.85×10^5 Bq as $FeSO_4$. Immediately after the test meal, each subject received either placebo tablets with 450 ml water or 500 mg elemental Ca in the form of (i) 450 ml whole milk, (ii) 450 ml OJ + CCM (comprising a 6:2:3 molar ratio of Ca:citrate:malate), or (iii) two CCM tablets with 450 ml water. Precisely 30 min after the start of the meal a second whole-body count was performed and subjects were subsequently fasted for 4 h, with the exception of drinking 250 ml distilled water. A steady state condition for ^{59}Fe in humans is typically reached between 10 and 14 days (Wienk *et al.*, 1999). Participants returned every 2 weeks for an additional whole body scan before repeating the same procedure with the next treatment. Serum concentrations of ferritin, which have a direct correlation with the total amount of Fe stored in the body (Jacobs and Worwood, 1975), were also measured via radioimmunoassay (RIA).

In comparison to the placebo, Fe absorption from milk and CCM in H_2O was reduced by 60% and 30%, respectively ($p < .05$). A relatively small reduction in Fe retention (11%) in response to CCM in OJ was not significantly different from placebo. In humans, the Ca in milk has been reported to be as inhibitory to Fe absorption as Ca in the form of $CaCO_3$ and HA (Dawson-Hughes *et al.*, 1986). The lack of antagonism to Fe absorption associated with CCM in OJ is believed to be attributable to the presence of citric acid and ascorbic acid in the OJ and the organic anions in CCM. Cow's milk also contains organic acids, although at much lower levels. Cow's milk contains ~150 mg citrate/100 ml (Linzell *et al.*, 1976) and 2 mg ascorbic acid/100 ml (Platt and Moncrieff, 1947), whereas OJ contains between 838 and 2539 mg citric acid/100 ml as free and combined citrate (Walton *et al.*, 1945) and ~30–40 mg ascorbic acid/100 ml (Lee and Coates, 1997). Smith and Rotruck (1988) previously suggested that Ca and Fe do not compete for the same ligands in OJ. However, citrate is a polyvalent anion with binding affinity for both trivalent (Fe^{3+}) and divalent cations (Ca^{2+}) (Pierre and Gautier-Luneau, 2000). It therefore appears that OJ has an ample supply of organic anions (e.g., citrate, malate, and ascorbate) to effectively chelate, solubilize, and enhance the bioavailability of both mineral cations.

Ilich-Ernst *et al*. (1998) have confirmed the lack of effect of Ca from CCM on Fe status over 4 years in a randomized, double-blind, placebo-controlled intervention trial involving 354 healthy Caucasian girls (mean ± SD: 10.8 ± 0.8 years). The participants were initially at Tanner stage 2 of puberty and subsequently became menarcheal. Girls at this stage of development are considered to have high Ca and Fe requirements (i.e., 1300 mg Ca/day and 8–15 mg Fe/day according to current nutritional guidelines). Subjects consumed either placebo tablets or 1000 mg elemental Ca as CCM in tablet form. After assessment of dietary records and adjustments for treatment compliance, Ca supplemented subjects during the course of the study had a total Ca intake of ~1500 mg Ca/day, of which ~700 mg was CCM. Participants in the placebo group averaged ~829 mg Ca/day from dietary sources. Serum ferritin and RBC indexes were evaluated annually and at 4 years, respectively. Serum ferritin concentrations were not significantly different between girls in the CCM or placebo group at baseline or at any annual assessment point over 4 years. The lack of antagonism of CCM on Fe status was corroborated by RBC indicators (i.e., hemoglobin, hemocrit, and corpuscular indexes) that were also similar at 4 years between both groups. In summary, results from both rodent and human studies indicate that OJ is an excellent vehicle for the Ca source CCM in terms of ensuring high bioavailability of both Ca and Fe.

2. Zinc

The potential for interaction between supplemental Ca and the trace element Zn, resulting in impairment of Zn utilization, is of particular concern now that recommended optimal intakes of Ca are as high as 1500 mg/day (NIH Consensus Development Panel on Optimal Calcium Intake, 1994). Ca antagonism of Zn absorption or utilization has been reported in some studies, but not in others. Wood and colleagues demonstrated that milk and lactose-free milk significantly reduced Zn absorption in both lactose-tolerant and intolerant postmenopausal women (Wood and Hanssen, 1988). Bertolo *et al*. (2001) reported that an amount of Ca similar to that present in infant formulas results in a 42% reduction in Zn uptake in piglets. Various other animal studies have also indicated that Ca interferes with Zn status (Dursun and Aydogan, 1994; Hoekstra *et al*., 1967; Smith, 1988). Zinc is an essential trace element required to support normal growth and development (Lind *et al*., 2004). It is also integral to a healthy immune system (Frassinetti *et al*., 2006), DNA synthesis (Wu and Wu, 1987), and serves as a cofactor to a large number of enzymes and transcription factors in the body (Chimienti *et al*., 2003). The requirement for zinc is especially important in rapidly growing children, developing adolescents, and pregnant women because of the

increased need to endogenously synthesize numerous Zn-containing proteins during these life stages.

A number of researchers have suggested that antagonism among ingested minerals at the site of uptake in the intestines represents the underlying mechanism of action for Ca interference with Zn absorption, since divalent minerals appear to compete with each other for brush border membrane transport mechanisms (Gunshin *et al.*, 1991; Roth-Bassell and Clydesdale, 1991). Although this is a viable hypothesis, it does not account for the lack of effect of Ca on Zn bioavailability reported in certain studies (Dawson-Hughes *et al.*, 1986; Lonnerdal *et al.*, 1984; Spencer *et al.*, 1984; Yan *et al.*, 1996). It is likely that interactions between ingested Ca and Zn are complex, involving a combination of various factors that may influence bioavailability. These include the following: Ca:Zn ratios, the timing of ingestion of each mineral (e.g., simultaneously or separately; with a meal or between meals), competitive interactions between Ca and Zn for binding sites of ligands present in food or beverages [e.g., phytates, hemicelluloses (Walsh *et al.*, 1994)], the solubility of Ca and Zn in relation to their own concentration and the concentration of other minerals present, their solubility in the presence of certain food components (Corneau *et al.*, 1996) [e.g., formation of poorly soluble, digested, and absorbed Ca-phytate-Zn or phytate-Zn-Ca-amino acid side-chain complexes (Erdman and Fordyce, 1989)], and possibly the Ca source that is ingested.

The source of Ca as a factor integral to Zn bioavailability was investigated by Smith via whole-body retention studies in rats that were administered a single-dose of ^{65}Zn-labeled $ZnSO_4$ and supplemented with either $CaCO_3$, CaP, bone meal, or CaHA at Ca:Zn ratios of 60:1 and 120:1 (Smith, 1988). The 120:1 Ca:Zn ratio best approximates the daily recommended intakes of Ca and Zn for U.S. women aged between 19 and 50 years (i.e., 1000 mg Ca/day and 8 mg Zn/day or Ca:Zn of 125:1). Similar dosing and retention assessment protocols were used as described previously for ^{59}Fe retention in rats (Smith, 1988). Compared to the placebo group receiving no Ca, rats supplemented with $CaCO_3$ demonstrated a 25% decrease in Zn retention at the 120:1 ratio, while CaP, bone meal, and CaHA depressed Zn retention as much as 50–60%. Although these animal data suggest that Ca source can significantly impact Zn retention, Dawson-Hughes *et al.* (1986) did not detect an effect on Zn bioavailability from dosing postmenopausal women with either $CaCO_3$ or CaHA based on whole-body extrinsically labeled ^{65}Zn retention.

For reasons pertaining to poor patterns of food selection and low energy intakes, many adolescent girls in industrialized countries do not consume diets containing the recommended amount of Zn to meet their physiological requirements (DRI for adolescent girls in the US ranges from 8 to 9 mg/day) (Gibson *et al.*, 2002). Antagonism of Zn utilization

from a high-Ca diet could potentially exacerbate a suboptimal Zn intake. A metabolic study was designed to determine the effect of long-term Ca supplementation on Zn utilization in 26 healthy Caucasian adolescent girls (mean ± SD: 11.3 ± 0.5 years), all of which were within one SD for various anthropometry and bone mass measurements (McKenna *et al.*, 1997). Subjects were randomized to receive either 1000 mg Ca/day as CCM ($n = 13$) or placebo tablets ($n = 13$). To facilitate adaptation of the girls to higher Ca intakes, supplementation with CCM began 15 weeks prior to a 14-day balance study. The initial 7 days of the balance study involved equilibration and adaptation to a controlled basal dietary intake of 722 mg Ca/day and 6.3 mg Zn/day, in addition to the assigned treatment. All biological samples (feces and urine) were collected within discrete 24-h periods during days 8–14 and identical meal samples were chemically analyzed for Ca and Zn content by standard laboratory procedures.

According to results of the McKenna *et al.* study, a high-Ca intake (1000 mg/day as CCM + 667 mg Ca/day on average from the diet) did not antagonize Zn absorption ($p > .05$) when 5.5 mg Zn was in the diet (~46% of the RDA). During the 15 week lead up to the balance study, the intake of Zn among participants, based on dietary records, was estimated to be 67% of the RDA. Five girls (four in the placebo group and one in the CCM group) were determined to be in negative Zn balance. Ideally, for a balance study it is preferable to employ a crossover design in the same subjects to minimize any confounding effects that are constant within an individual (Weaver and Heaney, 2006c). Overall, the high Ca level in this study did exceed the optimal recommended intake for adolescent girls and Zn intake was marginal, yet supplementary CCM did not appear to exert an adverse effect on Zn absorption.

3. Selenium

Selenium (Se) is a non-metal element predicted to interact predominantly with nutrients that have an effect on the pro-oxidant/antioxidant balance of cells (Dodig and Cepelak, 2004). As an essential trace mineral, Se shares many chemical properties with sulfur (Burk, 1983). It is an integral cofactor of the Se-dependent glutathione peroxidase system (GSH-Px), which functions as a group of water-soluble enzymes to catalyze destruction or neutralization of water-soluble and membrane-bound hydroperoxides that are capable of causing free radical damage due to reactive oxygen species (Bettger, 1993). Se is also essential for normal functioning of the thyroid gland and immune system and it has a protective, as well as therapeutic, role in different diseases (Dodig and Cepelak, 2004; Greger *et al.*, 1981).

The amount of Se derived from both vegetable and animal products in the diet is dependent on the Se content of the soil in the geographical area

in which animals are raised and produce is grown. Therefore, determining the propensity for dietary Ca and Se interactions has been of particular interest to those raising livestock in Se-poor regions. Animals in these regions generally have a compromised Se status, so that any additional interference to the utilization of ingested Se by high Ca intakes is obviously undesirable. In swine, an increase in dietary Ca enhanced Se retention, whereas low levels of dietary Ca appeared to interfere with Se absorption (Buescher *et al.*, 1961; Lowry *et al.*, 1985). In dairy calves, a wide range of dietary Ca intakes had no significant effect on ^{75}Se absorption (Alfaro *et al.*, 1987). Alternatively, others showed that dietary Ca derived from limestone (comprised primarily of $CaCO_3$) and the Ca naturally present in hay quantitatively affected apparent Se absorption in a curvilinear manner in non-lactating cows (Harrison and Conrad, 1984). Interactions between dietary Ca and Se that significantly influence overall Se retention do not appear, thus far, to have been reported in humans (Greger *et al.*, 1981).

A couple of theories have been proposed to explain how dietary Ca might possibly affect Se utilization. It has been suggested that Se availability may be directly influenced by intestinal interactions involving Ca or minerals linked to Ca utilization (e.g., phosphorus) (Lowry *et al.*, 1985). Indirect effects on the capacity of a target tissue to respond to Se are also considered possible means by which bioavailability or retention might be influenced (Parizek, 1978). It has also been conjectured (Hill and Matrone, 1970; Howell and Hill, 1978) that elements with valence shell electronic structures most similar to Se (i.e., Se^{2-}, Se^{4+}, and Se^{6+}) are most likely to act antagonistically. Based on this criterion, Ca^{2+} does not fit the profile of a probable Se antagonist.

To address the question of whether Ca intake influences Se utilization, the impact of Ca supplementation during puberty on Se parameters in adolescent girls was investigated (Holben *et al.*, 2002). The objective was to test their hypothesis of no interactive effect of Ca in the form of CCM on Se status. Subjects included 16 healthy Caucasian adolescent girls in Tanner pubertal stage 2 that ranged in age from 11 to 14 years during the 3 year intervention. Annual 2-week balance studies were conducted under strictly controlled conditions in a metabolic ward, during which data were collected to estimate Se absorption, retention, and blood status (i.e., erythrocyte Se and GSH-Px and serum GSH-Px). The effect of supplementation with 1000 mg Ca/day as CCM tablets ($n = 7$) versus methylcellulose placebo tablets ($n = 9$) was evaluated, while dietary Se was maintained at a level of ~100 μg/day and Ca from food was the same for each group. Self-selected diets prior to and between each balance study were assessed for Se and Ca intakes via dietary records. Girls were adapted to the metabolic diet during week 1 of the balance study and during week 2 total daily excreta were collected for chemical analysis by

standard methods. Se retention (μg/day), calculated as Se intake − [fecal Se + urinary Se], and apparent Se absorption (%), calculated as 100x [(Se intake − fecal Se)/(Se intake)], were subsequently measured annually for each group and statistically compared.

A lack of difference in all Se parameters, except for higher fecal Se excretion during the second balance study in the Ca-supplemented group (by ∼5 μg Se/day), persuaded the investigators to pool the data and not stratify results by Ca treatment rather than by years. By the investigator's own admission, the power to detect treatment differences may have been limited by the small sample size. The overall conclusion was that Se status in the adolescent girls was not adversely affected by long-term daily consumption of 1775 mg Ca/day, of which 1000 mg Ca/day came from CCM. Moreover, the apparent percent absorption of Se in this cohort actually exceeded that of adults based on the results reported in previous Se balance studies (Holben *et al.*, 2002).

4. Magnesium

Magnesium (Mg) is a divalent element required for energy metabolism, muscle contraction and relaxation, nerve impulse transmission, bone mineralization, enzyme function, and numerous other physiological functions (Dominguez *et al.*, 2006; Rude, 1998). A chronic deficiency of Mg may influence cardiac and vascular diseases, renal failure, hypoparathyroidism, and bone health. Dietary Ca and Mg reportedly interact via numerous dynamic and complex mechanisms that are not fully understood. Recommendations for higher daily Ca intake raise the question of whether Mg status might be negatively influenced, particularly when Mg intake is marginal. To date, results of studies that assessed the effect of high-Ca intake on Mg status in adults are inconsistent, although according to Sojka *et al.* (1997) the majority of investigators believe that Ca does not interfere with Mg balance. Other reports concerning the effect of high Ca intake as CCM on Mg status in adolescent girls also exist (Andon *et al.*, 1996a; Sojka *et al.*, 1997).

The effect of a high-Ca intake on Mg balance in 26 healthy, Caucasian, adolescent females (mean age: 11.3 years) was investigated in a 14-day double-blind study (Andon *et al.*, 1996a). The subjects comprised a subset of girls from a larger ongoing placebo-controlled Ca trial, selected based on the closeness of their anthropometric and bone measurements (all within one SD). Girls were assigned to either a low-Ca group ($n = 13$) that consumed placebo tablets, or a high-Ca group ($n = 13$) that consumed 1000 mg elemental Ca/day as CCM. Treatments began 15 weeks prior to the balance study to facilitate adaptation to the higher Ca intake. Food intake during this period was self-selected and monitored by dietary records.

The 14-day balance study was conducted in a metabolic unit and the first week served as an adaptation period. During week 2, all excreta and duplicate food samples were collected for analysis by standard analytical methods. The basal diet for all subjects was rigorously controlled at the metabolic facility. The mean daily intake of Mg among the girls in this study was 176 mg/day, which is less than the current DRI of 240 mg/day for females aged 9–13 years. Daily Ca intake from food sources was 667 mg/day or approximately half the current RDA. Even though girls in the CCM-supplemented group consumed a significantly higher amount of Ca (1667 mg/day) than the placebo group (667 mg/day) ($p = 0.0001$) for an extended period of time, Mg utilization was not different ($p > 0.05$) between treatments in terms of urinary, fecal, and total Mg excretion (mg/day), or Mg balance (mg/day). Furthermore, Mg absorption as a percentage of intake was not significantly different (50% vs 55% for the low and high-Ca intake groups, respectively). Based on these results, it was concluded that a high-Ca intake comprising 1000 mg Ca/day as CCM in addition to 667 mg/day dietary Ca, does not put adolescent girls at risk of compromising the utilization of dietary Mg.

A randomized cross-over design was employed to assess the effect of high versus low Ca intake on Mg metabolism (Sojka *et al.*, 1997). Five healthy adolescent girls (mean ± SD: 12.8 ± 0.8 years) participated in two 21-day balance studies separated by a 5-week washout period. The control (low-Ca) diet contained 800 mg Ca/day from dietary sources, whereas the high-Ca diet included an additional 1000 mg Ca/day as CCM from four servings of a fortified fruit drink (total intake of 1800 mg Ca/day). Both diets included 300 mg Mg/day. Subjects adapted to the study diet for 7 days prior to Mg tracer administration. An oral dose of 40 mg ^{26}Mg 1 h after breakfast (measures dietary Mg), was followed by an intravenous injection of 20 mg ^{25}Mg 1 h later (measures Mg removal from the blood). Relative to the time of injection, blood samples were drawn at 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hr. Complete urine collections were performed at 8, 8–16, and 16–24 h, as well as every 24 h thereafter through 14 days. Complete fecal collections were also made through 14 days. Excreta and diet samples were chemically analyzed for Mg by atomic absorption spectroscopy and stable isotope enrichment was measured using thermal ionization mass spectroscopy. Tracer data were fitted on the basis that Mg was in a steady state and analysis was via an eight-compartment model using the SAAM (Simulation Application for the Analysis of Models) kinetic modeling program.

The results showed that a low- versus a high-Ca diet did not significantly ($p > 0.05$) influence the Mg kinetics or balance based on fecal and urinary excretion (mg/day) and absorption parameters (% and mg/day). The low-Ca intake resulted in a mean Mg absorption of 44%, whereas absorption averaged 39% on the high-Ca diet. The urinary Mg excretion

data in this study has been criticized (Sabatier *et al.*, 2003) for being underestimated based on a divergence of the kinetic model-generated curve and observed values in the early part of the curve (see Fig. 2c in the following reference, Sojka *et al.*, 1997).

A kinetic modeling approach has the advantage of providing an understanding of pool sizes of Mg in relation to compartments and the rate of Mg transfer between them, as opposed to balance studies in which the measured balance (positive or negative) may reflect changes in body pool sizes rather than in total body stores (Sojka *et al.*, 1997). Utilization of a crossover design in Andon's study (Andon *et al.*, 1996a) would have strengthened the conclusions, although this may not have been possible with the subset of participants being selected from another larger trial. However, in general the Andon *et al.* and Sojka *et al.* studies are in agreement with regard to the conclusion that CCM does not negatively influence Mg status in adolescent girls consuming in excess of the RDA for Ca.

Based on the balance of the evidence to date, it appears that CCM does not significantly interfere with the status of other minerals including Fe, Zn, Se, and Mg. It is widely acknowledged that Ca does have the potential to interact with other elements based on its chemical properties in certain environments. In many instances, accompanying food components or ingredients can change the dynamics of these mineral interactions by either facilitating or interfering with mineral solubility, absorption, and utilization (i.e., bioavailability). In the case of CCM, more often than not its chemical composition in complex systems or as a dietary supplement is advantageous or neutral, rather than inhibitory, in relation to the utilization of other minerals.

E. Effect on serum lipids

Over time, hypercholesterolemia, or high blood cholesterol, can accelerate the onset of atherosclerosis and increase the risk of cardiovascular events such as stroke, myocardial infarction, and transient ischemic attacks. Excess saturated fatty acids (SFA) in the diet have the most dramatic effect in terms of elevating serum cholesterol level, particularly the low density lipoprotein (LDL) fraction that is implicated in exacerbating cardiovascular disease risk. There is increasing evidence suggesting that the absorption of saturated fatty acids can be reduced by the presence of high levels of Ca in the diet (Denke *et al.*, 1993; Reid *et al.*, 2002; Shahkhalili *et al.*, 2001; Welberg *et al.*, 1994). The mechanism involves formation of insoluble Ca-SFA soaps in the gastrointestinal tract (Teegarden, 2006) that precipitate out of solution and are excreted in the feces rather than incorporated into biliary micelles. The tendency for significant fecal elimination of Ca soaps is dependent upon the amount and nature of the Ca

compounds and fats present, in addition to the acidity of the intestinal contents (Boyd *et al.*, 1931).

$CaCO_3$ has been the subject of most of the Ca-SFA interaction investigations thus far. Bell and coworkers reported an LDL cholesterol (LDL-C) reduction of 4.4% ($p = .001$) and a high density lipoprotein cholesterol (HDL-C) increase of 4.1% ($p = .031$) in hypercholesterolemic patients administered 1200 mg $CaCO_3$/day for 6 weeks in a placebo controlled crossover study (Bell *et al.*, 1992). The HDL-C fraction of serum lipids is associated with protective effects in relation to cardiovascular health. Bostick and colleagues on the other hand found no significant effect of supplementation with 1000 or 2000 mg $CaCO_3$/day over 4 months on serum total cholesterol or HDL-C concentrations in 193 men and women (Bostick *et al.*, 2000). No effect of 1000 mg $CaCO_3$/day on LDL-C was detected in hypotensive and normotensive outpatients during 8-week test periods in a double-blind, placebo-controlled crossover study (Karanja *et al.*, 1987). A similar study design tested the effect of 1000 mg supplemental Ca/day as $CaCO_3$ during two 10-week periods on serum cholesterol concentrations in 50 children with familial hypercholesterolemia (type II-A; age range 4–19 years) (Groot *et al.*, 1980). LDL-C in the children decreased by 4% ($p < .05$) and apolipoprotein A-1 (or Apo A-1, which improves the cholesterol clearing capacity of HDL-C) increased by 4% ($p < .05$). Children in the Groot *et al.* study were on low cholesterol high polyunsaturated fat diets that have not been shown to result in significantly increased fecal fatty acid excretion. A short-term, randomized, double-blind, crossover study examined the effect of $CaCO_3$ supplementation (900 mg Ca/day) on digestibility of saturated fat in chocolate containing high levels of cocoa butter (Shahkhalili *et al.*, 2001). In this study, Ca supplementation resulted in a substantial LDL-C reduction of 15% ($p < .02$). A 1-year randomized study assessed the effects of 1000 mg Ca/day as Ca citrate on the serum lipid profile of 223 healthy postmenopausal women (Reid *et al.*, 2002). A nonsignificant ($p > .05$) 6% reduction in LDL-C, a 7% increase in HDL-C ($p = .01$) was observed along with a significantly improved HDL-C:LDL-C ratio ($p = .001$) for Ca citrate versus placebo. The LDL-C lowering effect of supplemental Ca appears to be quite variable.

Only one study thus far has evaluated the effect of CCM fortification of the diet on serum lipid profiles. It was a randomized, single-blind, crossover metabolic diet study with two 10-day periods separated by a 10-day washout (Denke *et al.*, 1993). The subjects included 13 healthy men (mean ± SD: 43 ± 4 years) classified as moderately hypercholesterolemic (mean ± SD: 6.19 ± 0.37 mmol serum cholesterol at baseline) and with a low baseline Ca intake (mean ± SD: 466 ± 199 mg Ca/day). The low-Ca basal diet contained 34% energy from fat (primarily as beef tallow), 13% from saturated fat, 240 mg cholesterol/day, and 410 mg Ca/day. The high-Ca diet was similar in composition except that CCM

supplementation brought the total Ca intake to 2200 mg/day. CCM was added to the daily diet in the form of fortified OJ (550 mg Ca) and muffins (750 mg Ca), as well as two CCM tablets (500 mg Ca). Baseline serum cholesterol concentrations were measured and during the last 3 days of each dietary period, subjects were monitored in a metabolic ward and fecal, urinary, and blood samples were collected. It was assumed, based on the results of other studies cited by the authors, that by this time 90% of the dietary induced effects on LDL-C would have reached a steady state.

Results of the Denke *et al.* study demonstrated that fecal SFA excretion was increased by 7% with the addition of high levels of Ca as CCM. Relative to the low-Ca diet, the high-Ca diet also resulted in an 11% reduction of LDL-C, a 6% reduction in total cholesterol, and a 7% decrease in apolipoprotein B (or Apo B, which is a primary component of LDL-C). The changes in the serum lipid profile were statistically significant ($p < .05$). HDL-C and Apo A-1 were not significantly different between low- and high-Ca intake, nor was there a Ca effect on bile acid excretion. Further analysis of the data revealed that the increase in fecal fat excretion did not entirely account for the higher than expected reduction in serum cholesterol level, suggesting that the hypocholesterolemic effect of CCM may involve other mechanisms that are not yet understood.

Diets high in SFA have been shown to be the most responsive to the cholesterol lowering effect of Ca supplementation. Although only evaluated in a single study, CCM appears comparable to other Ca sources in terms of its cholesterol lowering potential. Higher intakes of Ca, up to 2 g/day, generally appear to be most effective at reducing LDL-C and increasing fecal fat excretion. Supplementation of Ca at high doses may raise concerns about predisposing certain individuals to kidney stone formation. However, as previously discussed, this is less of an issue with CCM as a Ca source considering its citrate and malate content, its excellent assimilation as a fortificant in OJ, and the reduced lithogenic (i.e., calculi formation) potential (Harvey *et al.*, 1985).

F. Colon health

Colon cancer is currently the third leading cause of cancer deaths for men and women in the United States (American Cancer Society, 2006). The American Cancer Society predicted that during 2006 the incidence of new cases of colon and rectal cancer among Americans could be as high as 106,680 and 41,930, respectively (Jemal *et al.*, 2006). During that same period, as many as 55,000 deaths were expected to be attributable to colorectal cancers (Jemal *et al.*, 2006). The etiology of colorectal cancer is complex and multifactorial; it is governed by dynamics such as genetic predisposition, family history, and exposure to infectious agents. In addition, modifiable factors include obesity, physical activity, alcohol and

tobacco intake (Kamangar *et al.*, 2006; Kushi *et al.*, 2006), and habitual consumption of a Western-diet high in fat and phosphate and low in Ca and vitamin D (Newmark *et al.*, 1990; Richter *et al.*, 1995).

While early epidemiological studies examining the association between Ca intake and colorectal cancer largely yielded inconsistent results (Chia, 2004; Martinez and Willett, 1998), evidence from more recent prospective trials suggest a modest and more consistent inverse association between Ca intake and incidence of colorectal cancer (Schatzkin and Peters, 2004). It has been hypothesized that Ca counteracts the potential irritancy of secondary bile salts, essentially cytotoxic surfactants (Govers *et al.*, 1996) produced during fat digestion, via the formation of poorly absorbable/insoluble soaps (Flood *et al.*, 2005). The Ca fatty acid soap complexes formed in the lumen of the colon have a neutralizing, rather than an aggravating, effect on the colonic mucosa. Therefore, excessive proliferation of the mucosal cells is hindered (Flood *et al.*, 2005). Ca also may exert direct protective effects on the proliferative rate of the colonic mucosa via other mechanisms independent of soap complex formation (Chakrabarty *et al.*, 2005; Martinez and Willett, 1998).

To test the effect of nutrition on murine colonic mucosa, a nutrient poor rodent diet (i.e., a stress diet) was formulated to mimic a Western-diet that is high in fat (40% corn oil) and phosphate (0.8 mg P/kcal), and low in Ca (0.11 mg Ca/kcal) and vitamin D (0.11 IU/kcal) (Richter *et al.*, 1995). The stress diet was fed to 74 6-week-old mice for 8 weeks. Subgroups of the mice were then either sacrificed or randomized to remain on the stress diet or were switched to one of two Ca-enriched rescue diets. The tricalcium phosphate (TCP) rescue diet was equivalent to the stress diet with the exception of substituting the low Ca content with 0.9 mg Ca/kcal as TCP. The CCM rescue diet was comprised of the stress diet with 0.9 mg Ca/kcal as CCM. After another 6 weeks of feeding the designated diets (i.e., 14 weeks into the study), a subgroup of mice from each treatment leg was sacrificed for histological analysis. The remaining mice continued in their respective groups for an additional 6 weeks, at which time they were also sacrificed (20 weeks into the study). A nutrient adequate control diet (AIN-76) was fed to a fourth group of control mice throughout the experiment.

After as few as 8 weeks, significant adverse mucosal alterations were induced in the sigmoid colon of mice fed the stress diet compared to control mice. Abnormal cell multiplication (i.e., hyperplasia) and a high rate of cell division (i.e., hyperproliferation) were observed. After 20 weeks, mice on the stress diet exhibited mucosal morphological abnormalities similar to those expected in animals administered carcinogens for the purpose of tumor induction (i.e., hyperplasia characterized by elongation of occasional colonic crypts in addition to enlargement and elongations of nuclei at the base of the crypts). Conversely, 6 weeks after

switching to the Ca rescue diets, [^{3}H] thymidine labeling of colonic epithelial cells was not different in both rescue diets when compared to control levels. Epithelial cell numbers of mice on rescue diets also demonstrated no differences compared to control mice 6 weeks after switching to the TCP rescue diet and 12 weeks after switching to the CCM rescue diet.

Despite the continuing risk to colon health from a stress diet high in fat and low in vitamin D, the addition of adequate Ca (as either TCP or CCM) to the diet reversed the induced hyperplasia and hyperproliferation. TCP is predicted to be more reactive in the lower gastrointestinal tract due to a lower solubility (0.002 g TCP solubilizes in 100 ml water under standard conditions) (Richter *et al.*, 1995). The protective effect of CCM, a moderately soluble Ca salt [1.10 g of 6:2:3 ratio CCM/100 ml water under standard conditions (Fox *et al.*, 1993a)] with potentially higher than average bioavailability, is presumed to be related to its reactivity in the upper gastrointestinal tract (Richter *et al.*, 1995). Considering Ca is associated with numerous health benefits, maintaining an adequate Ca intake via diet and supplementation to also potentially increase protection against colorectal cancer seems like a reasonable recommendation.

VIII. LIMITATIONS OF CCM

The benefits of CCM, which are supported by scientific research, have been well described in this chapter; however, potential limitations also need to be discussed. While the Ca content of anhydrous CCM (23.7% for the 6:2:3 molar ratio formulation) is higher than certain other soluble Ca salts (e.g., Ca lactate, Ca gluconate; see Table 6.3), it is well recognized that the Ca content of CCM is lower than that of insoluble Ca sources often used for food fortification. For example, $CaCO_3$ and TCP are ~40% and 39% elemental Ca by weight, respectively. Thus, fortification of a food or beverage to a given Ca level will require approximately half as much added $CaCO_3$ or TCP as would be required for CCM. In certain non-fluid or low-moisture foods, a lower level of addition of the more Ca dense sources may translate into a sensory or processing advantage.

Fortification of food and beverage products with premade, dried, CCM powder will cost more than fortification with $CaCO_3$ or TCP to an equivalent Ca level. However, the cost of fortifying with CCM powder is likely to be comparable to fortification with other soluble Ca salts. For beverages and certain fluid foods, fortification can often be accomplished using an *in situ* approach that is a relatively cost-effective strategy for incorporating CCM into finished food and beverage products. *In situ* fortification refers to the formation of the CCM complex directly within the product of interest via addition of an alkaline Ca source (e.g., $Ca(OH)_2$ or $CaCO_3$) that reacts with the endogenous and/or added citric and malic

acids in the proper proportion to form CCM. This is the manner in which commercial CCM-fortified 100% juice, such as not-from-concentrate OJ, is produced. Another potentially cost-effective approach for CCM fortification is to produce a separate concentrated solution or slurry of CCM, starting with the alkaline Ca source and the citric and malic acids in the correct molar ratio. The concentrated CCM solution or slurry may then be added at a low level to the finished food or beverage product of interest to yield the desired level of fortification (Luhadiya *et al.*, 2006).

With respect to dietary supplements, $CaCO_3$ is often favored because of the high Ca density and low cost. The number of tablets (or the size of the tablets) that one is required to consume to achieve a desired level of Ca supplementation is smaller for $CaCO_3$ than for CCM or other soluble Ca salt of relatively low Ca density. However, a powdered dietary supplement, intended to be mixed/dissolved into beverages or other fluid foods (e.g., soups, sauces) by the consumer just prior to consumption, is a novel product form applicable only to a soluble Ca salt such as CCM.

CCM is moderately soluble in water and has higher aqueous solubility compared to a number of other Ca sources often used for food fortification and in dietary supplements. However, the Ca lactate and gluconate salts have higher solubility that conceivably may be advantageous in unique applications when a very high concentration of the Ca salt is required (e.g., concentrated syrups and/or liquid nutritional supplements).

It is well known that soluble Ca salts can potentially destabilize food systems if the conditions under which they are added are not carefully controlled (Vyas and Tong, 2004) or an incompatible Ca salt is used. CCM has been shown to be compatible with protein-rich beverages (e.g., milk-based, soy-based) that are heat pasteurized with a high-temperature short-time (HTST) process (Luhadiya *et al.*, 2006). However, fortification of ultra-high temperature (UHT) treated milk products with high levels of soluble Ca may lead to excessive flocculation, thickening and/or sedimentation resulting from Ca-protein interactions. Addition of stabilizers and/or ingredients to reduce the Ca-protein complexation may help to minimize these undesired textural changes.

While CCM is exceptionally well suited to fortification of juice beverages because of the compatibility of its organic anions with those naturally present in fruit, it does not provide a desirable taste profile in cola flavored beverages (Chang *et al.*, 2002). Table 6.7 presents a comparison between CCM and $CaCO_3$.

IX. CONCLUSION

Ca consumption habitually falls short of the recommended AI for many subgroups in the population. These shortfalls represent a major health problem, one that is getting progressively worse over time as evidenced

by the increasing incidence of osteopenia, osteoporosis, and pathologic bone fractures in the United States and other industrialized nations. Currently, dietary Ca insufficiency appears to be related to food choice, based on the fact that calorie intake per person reached an all time high in the United States at the beginning of the twenty-first century (United States Department of Agriculture, 2003). Continual public health recommendations to consume adequate low-fat dairy products go unheeded by consumers. In the advent of a dietary Ca deficit, supplementation and fortification of foods and beverages with Ca can easily provide an additional amount of Ca without the addition of extra calories. The cornerstone to enhancing bone health depends largely on consumers being aware of the Ca-bone health connection and acknowledging the long-term implications nutrition can have on the quality of their lives. Making a conscious effort to discriminate between products and purchase Ca-fortified foods and beverages, or to regularly supplement, depends on a perception that adequate nutrition confers protective and beneficial health effects. To encourage consumption, Ca-fortified foods must be palatable to the extent that their taste and texture are equally or more preferred to the non-fortified products on the market.

Ideal Ca fortificants are inconspicuous in food formulations and are adequately assimilable or bioavailable from the products in which they are incorporated. This is where CCM has an important and distinct advantage over other Ca sources that may be prone to precipitating out of solution, impart an undesirable taste or texture, and/or are not as bioavailable. CCM has been shown to be highly absorbable in numerous studies and its unique chemical composition lends itself to inclusion in a variety of food and beverage products that are consumed every day. CCM can be incorporated into many popular foods and beverages in amounts per serving that make an appreciable nutritional contribution, without significantly affecting taste and texture.

On the balance of the available evidence from human and animal studies presented in this chapter, it appears that CCM is well absorbed across a wide range of compositions and circumstances. For example, studies using isotopic and pharmacokinetic methods have shown that CCM is highly absorbable by both children and adults, in both tablet and beverage form, when consumed at levels ranging from an acute dose to chronic consumption (i.e., 200 mg Ca to 700 mg Ca/day, respectively), and for compositions covering a broad range of Ca:citrate:malate molar ratios that bracket the 6:2:3 neutral salt (i.e., molar ratios from 5:1:1 to 1.0:1.8:1.5 or the equivalent 6:10.8:9).

This chapter has highlighted the current body of scientific evidence that demonstrates CCM plays an important role in terms of facilitating Ca retention and bone accrual in children and adolescents. CCM effectively enables adults to consolidate and maintain bone mass, it works in

TABLE 6.7 Comparison of CCM with Ca carbonate

	CCM	$CaCO_3$
Absorbability	Organic form of Ca in a soluble ionized state — the presence of stomach acid is less essential for adequate Ca absorption	Inorganic form of Ca, negligible aqueous solubility, requires the presence of food to stimulate gastric acid secretion for adequate absorption
Ca density	Intermediate (23.7%)	High (40%)
Cost	More expensive on an equivalent Ca basis	Less expensive (raw material abundant and low cost)
Ease of use in a dietary supplement	Lower Ca density translates to more pills/tablets (or larger pills/tablets) to deliver a given Ca dose	Higher Ca density allows for fewer (or smaller) pills/tablets
	Dissolution rate can be tailored which, along with the moderate aqueous solubility, allows for a powdered dietary supplement that can be mixed/dissolved into beverages or other fluid foods (e.g., soups, sauces) by the consumer just prior to consumption	Not applicable to a powdered dietary supplement form
Optimal application as a food fortificant	Compatible in neutral and acid systems. Optimal in beverages and fluid food matrices	Optimal in non-fluid or low-moisture products, in which the higher Ca density may be advantageous (e.g., dry cereal or food bars)
Stability	Does not precipitate in beverages at high concentrations.	Greatly increases the heat stability of skim milk powders. Potential for

(*continued*)

TABLE 6.7 (*continued*)

	CCM	$CaCO_3$
	Compatible with protein-rich beverages (e.g., milk-based; soy-based) treated with high-temperature short-time (HTST) pasteurization. However, may be issues with flocculation, thickening, or sedimentation due to Ca-protein interactions when subjected to ultra-high temperature (UHT) heat treatment	gritty texture and tendency to sediment in liquid products
Taste profile	Generally neutral, no noticeable off-flavor	Sometimes associated with a soapy off-flavor or chalky mouthfeel

conjunction with vitamin D to decrease fracture risk from falls in the elderly, slows the rate of bone loss in old age, and in this respect and others is also of immense benefit to the health and well-being of postmenopausal women. CCM is exceptional in that it confers a number of unique health benefits that go beyond Ca retention and maintenance of bone mass in the appendicular and axial skeleton. Unlike other Ca sources that necessitate supplementation be in conjunction with a meal to ensure that the optimal benefit is derived, CCM can be consumed with or without food and delivers a significant nutritional benefit to individuals of all ages. The unique chemistry of CCM makes it an especially beneficial Ca source for individuals with hypochlorydia or achlorydia, which generally includes the elderly and those on various long-term prescription medications that impair gastric acid secretion. CCM is also recognized as a Ca source that does not increase the risk of kidney stones, and in fact it protects against stone-forming potential. CCM has been shown to be effective in promoting oral health, that is, tooth retention is enhanced

and tooth erosion minimized. Other benefits include BP reduction, improved serum lipid profiles, colon health, and evidence of no interfering effect on the status of other minerals essential to the body.

From a food technology perspective, CCM is a relatively adaptable and ''user-friendly'' Ca salt, particularly in moist foods and beverages. The major factor that may preclude selection of CCM as a preferred Ca source is the higher cost compared to other sources of Ca commonly used for fortification (e.g., $CaCO_3$; TCP). However, *in situ* formation of CCM directly within beverages or other fluid foods and/or preparation and addition of a concentrated CCM solution or slurry, are relatively cost-effective strategies for incorporating CCM into finished food and beverage products. Furthermore, from a societal perspective, the cost of habitually purchasing good quality Ca-fortified foods and supplements is negligible compared to the exorbitant costs associated with substandard nutrition and the resulting pathologies.

REFERENCES

Abraham, G. E., and Flechas, J. D. (1992). Management of fibromyalgia: Rationale for the use of magnesium and malic acid. *J. Nutr. Med.* **3**, 49–59.

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., O'Brien, K. O., Liang, L. K., and Stuff, J. E. (1995). Differences in calcium absorption and kinetics between black and white girls aged 5–16 years. *JBMR* **10**, 829–833.

Abrams, S. A., Griffin, I. J., Davila, P. M., and Hawthorne, K. M. (2003). Calcium absorption is similar from milk and two types of fortified orange juice at high intake levels. *JBMR* **18**, Abst. S104.

Abrams, S. A., Griffin, I. J., Hawthorne, K. M., Chen, Z., Gunn, S. K., Wilde, M., Darlington, G., Shypailo, R. J., and Ellis, K. J. (2005a). Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. *JBMR* **20**, 945–953.

Abrams, S. A., Griffin, I. J., Hawthorne, K. M., Gunn, S. K., Gundberg, C. M., and Carpenter, T. O. (2005b). Relationships among Vitamin D Levels, parathyroid hormone, and calcium absorption in young adolescents. *J. Clin. Endocrinol. Metab.* **90**, 5576–5581.

Abrams, S. A., Griffin, I. J., Hawthorne, K. M., and Liang, L. (2005c). Height and height Z-score are related to calcium absorption in five- to fifteen-year-old girls. *J. Clin. Endocrinol. Metab.* **90**, 5077–5081.

Abuabara, A., dos Santos, A. J. S., Aguiar, F. H. B., and Lovadino, J. R. (2004). Evaluation of microleakage in human, bovine, and swine enamels. *Braz. Oral. Res.* **18**, 312–316.

Agnusdei, D., Civitelli, R., Camporeale, A., Parisi, G., Gennari, L., Nardi, P., and Gennari, C. (1998). Age-related decline of bone mass and intestinal calcium absorption in normal males. *Calcif. Tissue Int.* **63**, 197–201.

Alfaro, E., Neathery, M. L., Miller, W. J., Gentry, R. P., Crowe, C. T., Fielding, A. S., Etheridge, R. E., Pugh, D. G., and Blackmon, D. M. (1987). Effects of varying the amounts of dietary calcium on selenium metabolism in dairy calves. *J. Dairy Sci.* **70**, 831–836.

Amaechi, B. T., Higham, S. M., and Edgar, W. M. (1999). Factors influencing the development of dental erosion *in vitro*: Enamel type, temperature and exposure time. *J. Oral. Rehab.* **26**, 624–630.

American Cancer Society (2006). Cancer facts and figures 2006. Available from: http://www.cancer.org/downloads/STT/CAFF2006PWSecured.pdf [Accessed October 2006].

American Obesity Association (2006). Updated statistics on overweight and obesity in the U.S.

Ames, S., Ellis, K., Gunn, S., Copeland, K., and Abrams, S. (1999). Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *JBMR* **14**, 740–746.

Andon, M. B. (1995). Concentrated bioavailable calcium source U.S. Patent No. 5,468,506. The Procter and Gamble Company.

Andon, M. B. (2003). Effect of citrate: malate molar ratio on calcium absorption from calcium citrate malate. *In* ''FASEB Conference, Abst. 188.3,'' Experimental Biology, San Diego, California.

Andon, M. B., Kanerva, R. L., Rotruck, J. T., and Smith, K. T. (1992). Method of preventing tooth enamel erosion utilizing an acidic beverage containing calcium. U.S. Patent No. 5,108,761.

Andon, M. B., Kanerva, R. L., Schulte, M. C., and Smith, K. T. (1993). Effect of age, calcium source, and radiolabeling method on whole body ^{47}Ca retention in the rat. *Am. J. Physiol. Endocrinol. Metab.* **265**, E554–E558.

Andon, M. B., Lloyd, T. A., and Matkovic, V. (1994). Supplementation trials with calcium citrate malate: Evidence in favor of increasing the calcium RDA during childhood and adolescence. *J. Nutr.* **124**, 1412S–1417S.

Andon, M. B., Ilich, J. Z., Tzagournis, M. A., and Matkovic, V. (1996a). Magnesium balance in adolescent females consuming a low- or high-calcium diet. *Am. J. Clin. Nutr.* **63**, 950–953.

Andon, M. B., Peacock, M., Kanerva, R. L., and DeCastro, J. A. S. (1996b). Calcium absorption from apple and orange juice fortified with calcium citrate malate (CCM). *J. Am. Coll. Nutr.* **15**, 313–316.

Andon, M. B., Griffin, I. J., Hicks, P. D., and Gunn, S. K. (2004). Pubertal girls only partially adapt to low dietary calcium intakes. *JBMR* **19**, 759–763.

Anonymous (2002). Calcium in orange juice not always well absorbed. Available at: http://www.iconocast.com/News_Files/HNewsF3_XX_XX/News7.htm [Accessed July 2007].

Anonymous (2004). Role of calcium in bone building less than exercise. Available from: http://www.foodnavigator-usa.com/news/news-ng.asp?id=52720-role-of-calcium [Accessed November 2007].

Anonymous (2005). The merk manuals online medical dictionary: Physical growth. Available from: http://www.merck.com/mmpe/sec19/ch269/ch269b.html [Accessed January 2007].

Anonymous (2006). Packaged facts: Nutritional supplements in the U.S. Available from: http://www.gii.co.jp/sample/catalog/e_FD.pdf [Accessed December 2006].

Anonymous (2007). Vitamin B12. Available from: http://www.gastricbypassfamily.com/B12.html [Accessed January 2007].

Assmann, S., Medeiros, D. M., and Chambers, E. (2003). Fortification with calcium citrate malate may not influence the sensory properties of an orange flavored beverage. *J. Food Qual.* **26**, 395–407.

Attin, T., Meyer, K., Hellwig, E., Buchalla, W., and Lennon, A. M. (2003). Effect of mineral supplements to citric acid on enamel erosion. *Arch. Oral Biol.* **48**, 753–759.

Attin, T., Weiss, K., Becker, K., Buchalla, W., and Wiegand, A. (2005). Impact of modified acidic soft drinks on enamel erosion. *Oral Dis.* **11**, 7–12.

Awuney, E. M., and 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 Inc., Totowa, NJ.

Bainbridge, K. E., Sowers, M. F., Crutchfield, M., Lin, X., Jannausch, M., and Harlow, S. D. (2002). Natural history of bone loss over 6 years among premenopausal and early postmenopausal women. *Am. J. Epidemiol.* **156**, 410–417.

Balant, L. (1991). Is there a need for more precise definitions of bioavailability? *Eur. J. Clin. Pharmacol.* **40**, 123–126.

Barbour, M. E., Parker, D. M., Allen, G. C., and Jandt, K. D. (2003). Human enamel dissolution in citric acid as a function of pH in the range $2.3 \leq \text{pH} \leq 6.30$ - a nanoindentation study. *Eur. J. Oral Sci.* **111**, 258–262.

Barbour, M. E., Shellis, R. P., Parker, D. M., Allen, G. C., and Addy, M. (2005). An investigation of some food-approved polymers as agents to inhibit hydroxyapatite dissolution. *Eur. J. Oral Sci.* **113**, 457–461.

Barger-Lux, M., and Heaney, R. P. (2005). Calcium absorptive efficiency is positively related to body size. *J. Clin. Endocrinol. Metab.* **90**, 5118–5120.

Barger-Lux, M. J., Heaney, R. P., Lanspa, S. J., Healy, J. C., and DeLuca, H. F. (1995). An investigation of sources of variation in calcium absorption efficiency. *J. Clin. Endocrinol. Metab.* **80**, 406–411.

Barzel, U. S., and Massey, L. K. (1998). Excess dietary protein can adversely affect bone. *J. Nutr.* **128**, 1051–1053.

Bayly, J. R., Hollands, R. D., Riordan-Jones, S. E., Yemm, S. J., Brough-Williams, I., Thatcher, M., Woodman, N. M., and Dixon, T. (2006). Prescribed vitamin D and calcium preparations in patients treated with bone remodelling agents in primary care: A report of a pilot study. *Curr. Med. Res. Opin.* **22**, 131–137.

Bell, L., Halstenson, C. E., Halstenson, C. J., Macres, M., and Keane, W. F. (1992). Cholesterol-lowering effects of calcium carbonate in patients with mild to moderate hypercholesterolemia. *Arch. Intern. Med.* **152**, 2441–2444.

Bertolo, R. F. P., Bettger, W. J., and Atkinson, S. A. (2001). Divalent metals inhibit and lactose stimulates zinc transport across brush border membrane vesicles from piglets. *J. Nutr. Biochem.* **12**, 73–90.

Bettger, W. J. (1993). Zinc and selenium, site-specific versus general antioxidation. *Can. J. Phys. Pharmacol.* **71**, 721–724.

Bischoff-Ferrari, H. A., Willet, W. C., Wong, J. B., Giovannucci, E., Dietrich, T., and Dawson-Hughes, B. (2005). Fracture prevention with vitamin D supplementation: A meta-analysis of randomized controlled trials. *JAMA* **293**, 2257–2264.

Bischoff-Ferrari, H. A., Orav, E. J., and Dawson-Hughes, B. (2006). Effect of cholecalciferol plus calcium on falling in ambulatory older men and women. *Arch. Intern. Med.* **166**, 424–430.

Blanchard, J., and Aeschlimann, J. M. (1989). Calcium absorption in man: Some dosing recommendations. *J. Pharmacokin. Pharmacodyn.* **17**, 631–644.

Bo-Linn, G. W., Davis, G. R., Buddrus, D. J., Morawsji, S. G., Ana, C. S., and Fordtran, J. S. (1984). An evaluation of the importance of gastric acid secretion in the absorption of dietary calcium. *J. Clin. Invest.* **73**, 640–647.

Bodic, F., Hamel, L., Lerouxel, E., Basle, M. F., and Chappard, D. (2005). Bone loss and teeth. *Joint Bone Spine* **72**, 215–221.

Boling, S. D., Webel, D. M., Mavromichalis, I., Parsons, C. M., and Baker, D. H. (2000). The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *J. Anim. Sci.* **78**, 682–689.

Bonjour, J. P. (2005). Dietary protein: An essential nutrient for bone health. *J. Am. Coll. Nutr.* **24**, 526S–536S.

Bonjour, J. P., and Rizzoli, R. (2002). Bone mass acquisition. *In* ''Osteoporosis: Pathophysiology and Clinical Management'' (E. S. Orwoll and M. Bliziotes, eds.), pp. 69–82. Humana Press, New Jersey.

Bonjour, J. P., and Rizzoli, R. Peak bone mass and prevention of osteoporosis. Available from: http://www.nnia.co.za/CPD/articles/peakbonemass.pdf [Accessed February 2007].

Bonjour, J. P., Theintz, G., Law, F., Slosman, D., and Rizzoli, R. (1994). Peak bone mass. *Osteoporos. Int.* **4**, 7–13.

Boonen, S., Lips, P., Bouillon, R., Bischoff-Ferrari, H. A., Vanderschueren, D., and Haentjens, P. (2007). Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: Evidence from a comparative meta-analysis of randomized controlled trials. *J. Clin. Endocrinol. Metab.* **92**, 1415–1423.

Boonen, S., Rizzoli, R., Meunier, P. J., Stone, M., Nuki, G., Syversen, U., Lehtonen-Veromaa, M., Lips, P., Johnell, O., and Reginster, J. Y. (2004). The need for clinical guidance in the use of calcium and vitamin D in the management of osteoporosis: A consensus report. *Osteoporos. Int.* **15**, 511–519.

Borghi, L., Schianchi, T., Meschi, T., Guerra, A., Allegri, F., Maggiore, U., and Novarini, A. (2002). Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N. Eng. J. Med.* **346**, 77–84.

Bostick, R. M., Fosdick, L., Grandits, G. A., Grambsch, P., Gross, M., and Louis, T. A. (2000). Effect of calcium supplementation on serum cholesterol and blood pressure: A randomized, double-blind, placebo-controlled, clinical trial. *Arch. Fam. Med.* **9**, 31–38.

Boyd, O., Crum, C. L., and Lyman, J. F. (1931). The absorption of calcium soaps and the relation of dietary fat to calcium utilization in the white rat. *J. Biol. Chem.* **95**, 29–41.

Braun, M., and Weaver, C. M. (2006). A call to evaluate the impact of calcium-fortified foods and beverages. *Nutr. Today* **41**, 40–47.

Braun, M., Martin, B. R., Kern, M., McCabe, G. P., Peacock, M., Jiang, Z., and Weaver, C. M. (2006). Calcium retention in adolescent boys on a range of controlled calcium intakes. *Am. J. Clin. Nutr.* **84**, 414–418.

Brink, E. J., van den Heuvel, E. G. H. M., and Muijs, T. (2003). Comparison of six different calcium sources and meal type on true fractional calcium absorption in postmenopausal women. *Curr. Top. Nutraceut Res.* **1**, 161–168.

Bruce, D., Laurance, I., McGuiness, M., Ridley, M., and Goldswain, P. (2003). Nutritional supplements after hip fracture: Poor compliance limits effectiveness. *Clin. Nutr.* **22**, 497–500.

Brumm, M. C. (2000). Reducing phosphorus content of swine manure. *Manure Matters* **6**, 1–2.

Brune, M., Rossander, L., and Hallberg, L. (1989). Iron absorption and phenolic compounds: Importance of different phenolic structures. *Eur. J. Clin. Nutr.* **43**, 547–557.

Brunton, P. A., and Hussain, A. (2001). The erosive effect of herbal tea on dental enamel. *J. Dent.* **29**, 517–520.

Bryant, R. J., Cadogan, J., and Weaver, C. M. (1999). The new dietary reference intakes for calcium: Implications for osteoporosis. *J. Am. Coll. Nutr.* **18**, 406S–412S.

Buescher, R. G., Bell, M. C., and Berry, R. K. (1961). The effect of excessive calcium on selenium-75 in swine. *J. Anim. Sci.* **20**, 368–372.

Burge, R., Dawson-Hughes, B., Solomon, D. H., Wong, J. B., King, A., and Tosteson, A. (2007). Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. *JBMR* **22**, 465–475.

Burk, R. F. (1983). Biological activity of selenium. *Ann. Rev. Nutr.* **3**, 53–70.

Burkes, A. L., Butterbaugh, J. L., FIeler, G. M., Gore, W. J., and Zuniga, M. E. (1995). Storage stable calcium-supplemented beverage concentrates. U.S. Patent No. 5,422,128.

Bushinsky, D. A. (2001). Acid-base imbalance and the skeleton. *Eur. J. Nutr.* **40**, 238–244.

Chakrabarty, S., Wang, H., Canaff, L., Hendy, G. N., Appelman, H., and Varani, J. (2005). Calcium sensing receptor in human colon carcinoma: Interaction with Ca^{2+} and 1,25-dihydroxyvitamin D_3. *Cancer Res.* **65**, 493–498.

Chang, P., Lee, T., Olszewski, J., Edgar, B. G., Orr, R., and Curtiss, H. (2002). Calcium supplemented beverages and methods of making that same. PepsiCo, Inc., U.S. Patent No. 20,020,102,331.

Chia, V. (2004). Brief critical reviews—calcium and colorectal cancer: Some questions remain. *Nutr. Rev.* **62**, 115–120.

Chimienti, F., Aouffen, M., Favier, A., and Seve, M. (2003). Zinc homeostasis-regulating proteins: New drug targets for triggering cell fate. *Curr. Drug Targets* **4**, 323–338.

Coe, F. L., Parks, J. H., and Webb, D. R. (1992). Stone-forming potential of milk or calcium-fortified orange juice in idiopathic hypercalciuric adults. *Kidney Int.* **41**, 139–142.

Coe, F. L., Parks, J. H., and Favus, M. J. (1997). Diet and calcium: The end of an era. *Ann. Intern. Med.* **126**, 553–555.

Committee for Veterinary Medicinal Products (2003). Oxalic acid: Summary report. Available from: http://www.emea.eu.int/pdfs/vet/mrls/089103en.pdf [Accessed November 2007].

Compston, J. E., Laskey, M. A., Croucher, P. I., Coxon, A., and Kreitzman, S. (1992). Effect of diet-induced weight loss on total body bone mass. *Clin. Sci. (Lond.)* **82**, 429–432.

Conrad, M. E., and Umbreit, J. N. (2002). Pathways of iron absorption. *Blood Cell Mol. Dis.* **29**, 336–355.

Cook, A. (1994). Osteoporosis—do I eat TUMs? Available from: http://www.chiroweb.com/archives/12/21/01.html [Accessed November 2007]

Cook, J. D., and Monsen, E. R. (1976). Food iron absorption in man II. The effect of EDTA on absorption of dietary non-heme iron. *Am. J. Clin. Nutr.* **29**, 614–620.

Cook, J. D., Dassenko, S. A., and Whittaker, P. (1991). Calcium supplementation: Effect on iron absorption. *Am. J. Clin. Nutr.* **53**, 106–111.

Cook, J. D., Reddy, M. B., and Hurrell, R. F. (1995). The effect of red and white wines on non-heme iron absorption in humans. *Am. J. Clin. Nutr.* **61**, 800–804.

Corneau, L., Lavigne, C., and Zee, J. A. (1996). Effect of calcium and zinc concentrations and calcium source on *in vitro* calcium and zinc solubility in a fiber-fortified enteral formula. *Nutr. Res.* **16**, 1659–1669.

Curhan, G. C., Willett, W. C., Speizer, F. E., Spiegelman, D., and 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.

Davideau, J. L., Lezot, F., Kato, S., Bailleul-Forestier, I., and Berdal, A. (2004). Dental alveolar bone defects related to vitamin D and calcium status. *J. Steroid Biochem. Mol. Biol.* **89–90**, 615–618.

Dawson-Hughes, B. (2003a). Calcium and protein in bone health. *Proc. Nutr. Soc.* **62**, 505–509.

Dawson-Hughes, B. (2003b). Interaction of dietary calcium and protein in bone health in humans. *J. Nutr.* **133**, 852S–854S.

Dawson-Hughes, B. (2006a). Calcium and vitamin D. *In* ''Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism'' (M. J. Favus, ed.), pp. 257–259. The American Society for Bone and Mineral Research, Washington DC.

Dawson-Hughes, B. (2006b). 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., and Harris, S. S. (2002). Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am. J. Clin. Nutr.* **75**, 773–779.

Dawson-Hughes, B., Seligson, F. H., and Hughes, V. A. (1986). Effects of calcium carbonate and hydroxyapatite on zinc and iron retention in postmenopausal women. *Am. J. Clin. Nutr.* **44**, 83–88.

Dawson-Hughes, B., Dallal, G. E., Krall, E. A., Sadowski, L., Sahyoun, N., and Tannenbaum, S. (1990). A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N. Engl. J. Med.* **323**, 878–883.

Dawson-Hughes, B., Dallal, G. E., Krall, E. A., Harris, S. S., Sokoll, L. J., and Falconer, G. (1991). Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann. Intern. Med.* **115**, 505–512.

Dawson-Hughes, B., Harris, S. S., Krall, E. A., Dallal, G. E., and Falconer, G. (1995). Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. *Am. J. Clin. Nutr.* **61**, 1140–1145.

Dawson-Hughes, B., Harris, S. S., Krall, E. A., and 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., and 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.

Deehr, M. S., Dallal, G. E., Smith, K. T., Taulbee, J. D., and Dawson-Hughes, B. (1990). Effects of different calcium sources on iron absorption in postmenopausal women. *Am. J. Clin. Nutr.* **51**, 95–99.

Demigne, C., Sabboh, H., Puel, C., Remsey, C., and Coxam, V. (2004). Organic anions and potassium salts in nutrition and metabolism. *Nutr. Res. Rev.* **17**, 249–258.

Denke, M. A., Fox, M. M., and Schulte, M. C. (1993). Short-term dietary calcium fortification increases fecal saturated fat-content and reduces serum-lipids in men. *J. Nutr.* **123**, 1047–1053.

Dibba, B., Prentice, A., Ceesay, M., Stirling, D. M., Cole, T. J., and Poskitt, E. M. E. (2000). Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. *Am. J. Clin. Nutr.* **71**, 544–549.

Dodig, S., and Cepelak, I. (2004). The facts and controversies about selenium. *Acta Pharm.* **54**, 261–276.

Dominguez, L. J., Barbagallo, M., Lauretani, F., Bandinelli, S., Bos, A., Corsi, A. M., Simonsick, E. M., and Ferrucci, L. (2006). Magnesium and muscle performance in older persons: The INCHIANTI study. *Am. J. Clin. Nutr.* **84**, 419–426.

Dursun, N., and Aydogan, S. (1994). Comparative effects of calcium deficiency and supplements on the intestinal absorption of zinc in rats. *Jpn. J. Physiol.* **44**, 157–166.

Ebeling, P. R., Sandgren, M. E., DiMagno, E. P., Lane, A. W., DeLuca, H. F., and Riggs, B. L. (1992). Evidence of an age-related decrease in intestinal responsiveness to vitamin D: Relationship between serum 1,25-dihydroxy D3 and intestinal vitamin D receptor concentrations in normal women. *J. Clin. Endocrinol. Metab.* **75**, 176–182.

Erdman, J. W., Jr., and Fordyce, E. J. (1989). Soy products and the human diet. *Am. J. Clin. Nutr.* **49**, 725–737.

Erricsson, Y., and Ekberg, O. (1975). Dietetically provoked general and alveolar osteopenia in rats and its prevention or cure by calcium and fluoride. *J. Periodont. Res.* **10**, 256–269.

Evenepoel, P. (2001). Alteration in digestion and absorption of nutrients during profound acid suppression. *Best Pract. Res. Clin. Gastroenterol.* **15**, 539–551.

Fioritto, L. M., Mitchell, D. C., Smiciklas-Wright, H., and Birch, L. L. (2006). Girl's calcium intake is associated with bone mineral density during middle childhood. *J. Nutr.* **136**, 1281–1286.

Fleet, J. C. (2006). Molecular regulation of calcium metabolism. *In* ''Calcium in Human Health'' (C. M. Weaver and R. P. Heaney, eds.), pp. 163–189. Humana Press, Inc., Totowa, NJ.

Flood, A., Peters, U., Chatterjee, N., Lacey, J. V., Jr., Schairer, C., and Schatzkin, A. (2005). Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. *Cancer Epidemiol. Biomarkers Prev.* **14**, 126–132.

Foote, J. A., Murphy, S. P., Wilkens, L. R., Basiotis, P. P., and Carlson, A. (2004). Dietary variety increases the probability of nutrient adequacy among adults. *J. Nutr.* **134**, 1779–1785.

Forshee, R. A., Anderson, P. A., and Storey, M. L. (2006). Changes in calcium intake and association with beverage consumption and demographics: Comparing data from CSFII 1994–1996, 1998 and NHANES 1999–2002. *J. Am. Coll. Nutr.* **25**, 108–116.

Fox, M. M., Heckert, D. C., and Luhrsen, K. R. (1993a). Calcium citrate malate composition. U.S. Patent No. 5,186,965.

Fox, M. M., Luhrsen, K. R., and Burkes, A. L. (1993b). Calcium fortified salad dressing product. The Procter and Gamble Company, U.S. Patent No. 5,215,769.

Frassinetti, S., Bronzetti, G., Caltavuturo, L., Cini, M., and Della Croce, C. (2006). The role of zinc in life: A review. *J. Environ. Pathol. Toxicol. Oncol.* **25**, 597–610.

French, S. A., Story, M., and Jeffery, R. W. (2001). Environmental influences on eating and physical activity. *Annu. Rev. Public Health* **22**, 309–335.

Gallagher, J. C., Riggs, B. L., Eisman, J., Hamstra, A., Arnaud, S. B., and DeLuca, H. F. (1979). Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. *J. Clin. Invest.* **64**, 729–736.

Ganss, C., Klimek, J., and Borkowski, N. (2002). Characteristics of tooth wear in relation to different nutritional patterns including contemporary and medievil subjects. *Eur. J. Oral. Sci.* **110**, 54–60.

Gao, X., Wilde, P. E., Lichtenstein, A. H., and Tucker, K. L. (2006). Meeting adequate intake for dietary calcium without dairy foods in adolescents aged 9 to 18 years (National Health and Nutrition Examination Survey 2001–2002). *J. Am. Diet. Assoc.* **106**, 1759.

Gershoff, S. N., and Faragalla, F. F. (1959). Endogenous oxalate synthesis and glycine, serine, deoxypyridoxine interrelationships in vitamin B6-deficient rats. *J. Biol. Chem.* **234**, 2391–2393.

Gibson, R. S., Health, A. L. M., and Ferguson, E. L. (2002). Risk of suboptimal iron and zinc nutriture among adolescent girls in Australia and New Zealand: Causes, consequences, and solutions. *Asia Pac. J. Clin. Nutr.* **11**, 543–552.

Gillman, M. W., Hood, M. Y., Moore, L. L., Nguyen, U. S. D. T., Singer, M. R., and Andon, M. B. (1995). Effect of calcium supplementation on blood pressure in children. *J. Pediatr.* **127**, 186.

Govers, M. J., Termont, D. S., Lapre, J. A., Kleibeuker, J. H., Vonk, R. J., and van der Meer, R. (1996). Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. *Cancer Res.* **56**, 3270–3275.

Greer, F. R., Krebs, N. F., and Committee on, N. (2006). Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics* **117**, 578–585.

Greger, J. L., Smith, S. A., and Snedeker, S. M. (1981). Effect of dietary calcium and phosphorus levels on the utilization of calcium, phosphorus, magnesium, manganese, and selenium by adult males. *Nutr. Res.* **1**, 315–325.

Grenby, T. H. (1996). Lessening dental erosive potential by product modification. *Eur. J. Oral Sci.* **104**, 221–228.

Griffin, I. J., Davila, P. M., and Abrams, S. A. (2002). Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. *Br. J. Nutr.* **87**, S187–S191.

Griffin, I. J., Hicks, P. M. D., Heaney, R. H., and Abrams, S. A. (2003). Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. *Nutr. Res.* **23**, 901–909.

Griffith, R. (2005). Managing difficulties in swallowing solid medication: The need for caution. *Nurse Prescr.* **3**, 201–203.

Groot, P. H. E., Grose, W. F. A., Dijkhuis-Stofflesma, R., Fernandes, J., and Ambagtsheer, J. J. (1980). The effect of oral calcium carbonate administration on serum lipoproteins of children with familial hypercholesterolaemia (Type II-A). *Eur. J. Pediatr.* **135**, 81–84.

Guéguen, L., and Pointillart, A. (2000). The bioavailability of dietary calcium. *J. Am. Coll. Nutr.* **19**, 119S–136S.

Gunshin, H., Noguchi, T., and Naito, H. (1991). Effect of calcium on the zinc uptake by brush border membrane vesicles isolated from the rat small intestine. *Agric. Biol. Chem.* **55**, 2813–2816.

Haas, J. D., and Brownlie, T. I. V. (2001). Iron deficiency and reduced work capacity: A critical review of the research to determine a causal relationship. *J. Nutr.* **131**, 676S–690S.

Hallberg, L. (1987). Wheat fiber, phytates and iron absorption. *Scand. J. Gastroenterol. Suppl.* **129**, 73–79.

Hallberg, L. (1998). Does calcium interfere with iron absorption? *Am. J. Clin. Nutr.* **68**, 3–4.

Hanes, D. A., Weaver, C. M., Heaney, R. P., and Wastney, M. (1999). Absorption of calcium oxalate does not require dissociation in rats. *J. Nutr.* **129**, 170–173.

Hardy, G., and Ball, P. (2005). Clogbusting: Time for a concerted approach to catheter occlusions? *Curr. Opin. Clin. Nutr. Metab. Care* **8**, 277–283.

Harnack, L., Stang, J., and Story, M. (1999). Soft drink consumption among US children and adolescents: Nutritional consequences. *J. Am. Diet. Assoc.* **99**, 436–441.

Harrison, J. H., and Conrad, H. R. (1984). Effect of dietary calcium on selenium absorption by the non-lactating dairy cow. *J. Dairy Sci.* **67**, 1860–1864.

Harvey, J. A., Zobitz, M. M., and Pak, C. Y. (1985). Calcium citrate: Reduced propensity for the crystallization of calcium oxalate in urine resulting from induced hypercalciuria of calcium supplementation. *J. Clin. Endocrinol. Metab.* **61**, 1223–1225.

Heaney, R. P. (1991). Calcium supplements: Practical considerations. *Osteoporos. Int.* **1**, 1433–2965.

Heaney, R. P. (1998). Excess dietary protein may not adversely affect bone. *J. Nutr.* **128**, 1054–1057.

Heaney, R. P. (2001a). Calcium needs of the elderly to reduce fracture risk. *J. Am. Coll. Nutr.* **20**, 192S–197S.

Heaney, R. P. (2001b). Factors influencing the measurement of bioavailability, taking calcium as a model. *J. Nutr.* **131**, 1344S–1348S.

Heaney, R. P. (2002). Protein and calcium: Antagonists or synergists? *Am. J. Clin. Nutr.* **75**, 609–610.

Heaney, R. P. (2003a). Advances in therapy for osteoporosis. *Clin. Med. Res.* **1**, 93–99.

Heaney, R. P. (2003b). Sensitivity of parathyroid hormone response to calcium intake. *Am. J. Clin. Nutr.* **78**, 493–497.

Heaney, R. P. (2003c). Vitamin D depletion and effective calcium absorption. *JBMR* **18**, 1342.

Heaney, R. P., Gallagher, J. C., Johnston, C. C., Neer, R., Parfitt, A. M., and Whedon, G. D. (1982). Calcium nutrition and bone health in the elderly. *Am. J. Clin. Nutr.* **36**, 986–1013.

Heaney, R. P., Recker, R. R., Stegman, M. R., and Moy, A. J. (1989a). Calcium absorption in women: Relationships to calcium intake, estrogen status, and age. *JBMR* **4**, 469–475.

Heaney, R. P., Smith, K. T., Recker, R. R., and Hinders, S. M. (1989b). Meal effects on calcium absorption. *Am. J. Clin. Nutr.* **49**, 372–376.

Heaney, R. P., Recker, R. R., and Weaver, C. M. (1990a). Absorbability of calcium sources: The limited role of solubility. *Calcif. Tissue Int.* **46**, 300–304.

Heaney, R. P., Weaver, C. M., and Fitzsimmons, M. L. (1990b). Influence of calcium load on absorption fraction. *JBMR* **5**, 1135–1138.

Heaney, R. P., Dowell, R., and Barger-Lux, M. J. (1999). Absorption of calcium as carbonate and citrate salts with some observations on method. *Osteoporos. Int.* **9**, 19–23.

Heaney, R. P., Dowell, M. S., Hale, C. A., and Bendich, A. (2003). Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J. Am. Coll. Nutr.* **22**, 142–146.

Heaney, R. P., Rafferty, K., and Bierman, J. (2005a). Not all calcium-fortified beverages are equal. *Nutr. Today* **40**, 39–44.

Heaney, R. P., Rafferty, K., Dowell, M. S., and Bierman, J. (2005b). Calcium fortification systems differ in bioavailability. *J. Am. Diet. Assoc.* **105**, 807–809.

Henry, M. H., and Pesti, G. M. (2002). An investigation of calcium citrate malate as a calcium source for young broiler chicks. *Poult. Sci.* **81**, 1149–1155.
Hicks, P. D., and Abrams, S. A. (2006). Formula-fed full term infants have lower fractional absorption but greater total calcium absorption than human milk-fed infants. *FASEB J.* **20**, A991.
Higdon, J. (2005). Preventing osteoporosis through diet and lifelstyle. Linus Pauling Institute Spring/Summer 2005 Research Report.
Hildebolt, C. F. Oral bone loss assessment and repair. Available from: http://research.medicine.wustl.edu/ocfr/Research.nsf/s/B4883B6A205D60BA8625677D00592BBC [Accessed March 2007].
Hill, C. H., and Matrone, G. (1970). Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. *Fed. Proc.* **29**, 1474–1481.
Hoekstra, W. G., Faltin, E. C., Lin, C. W., Roberts, H. F., and Grummer, R. H. (1967). Zinc deficiency in reproducing gilts fed a diet high in calcium and its effect on tissue zinc and blood serum alkaline phosphatase. *J. Anim. Sci.* **26**, 1348–1357.
Holben, D. H., Smith, A. M., Ilich, J. Z., Landoll, J. D., Holcomb, J. P., and Matkovic, V. (2002). Selenium intakes, absorption, retention, and status in adolescent girls. *J. Am. Diet. Assoc.* **102**, 1082–1087.
Holmes, R. P., Goodman, H. O., and Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int.* **59**, 270–276.
Howell, G. O., and Hill, C. H. (1978). Biological interaction of selenium with other trace elements in chicks. *Environ. Health Perspect.* **25**, 147–150.
Hughes, J. A., West, N. X., Parker, D. M., Newcombe, R. G., and Addy, M. (1999). Development and evaluation of a low erosive blackcurrant juice drink. Final drink and concentrate, formulae comparison in situ and overview of the concept. *J. Dent.* **27**, 345–350.
Hughes, J. A., West, N. X., Parker, D. M., van den Braak, M. H., and Addy, M. (2000). Effects of pH and concentration of citric, malic, and lactic acids on enamel, *in vitro*. *J. Dent.* **28**, 147–152.
Hunt, J. N. (1980). A possible relation between the regulation of gastric emptying and food intake. *Am. J. Physiol. Gastrointest. Liver Physiol.* **239**, G1–G4.
Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S. R., Dassenko, S. A., and Cook, J. D. (1992). Soy protein, phytate, and iron absorption in humans. *Am. J. Clin. Nutr.* **56**, 573–578.
Ilich-Ernst, J. Z., McKenna, A. A., Badenhop, N. E., Clairmont, A. C., Andon, M. B., Nahhas, R. W., Goel, P., and Matkovic, V. (1998). Iron status, menarche, and calcium supplementation in adolescent girls. *Am. J. Clin. Nutr.* **68**, 880–887.
Ireland, P., and Fordtran, J. S. (1973). Effect of dietary calcium and age on jejunal calcium absorption in humans studied by intestinal perfusion. *J. Clin. Invest.* **52**, 2672–2681.
Iuliano-Burns, S., Wang, X. F., Evans, A., Bonjour, J. P., and Seeman, E. (2006). Skeletal benefits from calcium supplementation are limited in children with calcium intakes near 800 mg daily. *Osteoporos. Int.* **17**, 1794–1800.
Jackman, L. A., Millane, S. S., Martin, B. R., Wood, O. B., McCabe, G. P., Peacock, M., and Weaver, C. M. (1997). Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females. *Am. J. Clin. Nutr.* **66**, 327–333.
Jacobs, A., and Worwood, M. (1975). Ferritin in serum: Clinical and biological implications. *N. Eng. J. Med.* **292**, 951–956.
Jacobs, S. A. (1994). Calcium supplements. U.S. Patent No. 5,314,919.
Jandt, K. D. (2006). Probing the future in functional soft drinks on the nanometre scale—towards tooth friendly soft drinks. *Trends Food Sci. Technol.* **17**, 263–271.
Jarjou, L. M. A., Prentice, A., Sawo, Y., Laskey, M. A., Bennett, J., Goldberg, G. R., and Cole, T. J. (2006). Randomized, placebo-controlled, calcium supplementation study in pregnant Gambian women: Effects on breast-milk calcium concentrations and infant

birth weight, growth, and bone mineral accretion in the first year of life. *Am. J. Clin. Nutr.* **83**, 657–666.
Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., Smigal, C., and Thun, M. J. (2006). Cancer Statistics, 2006. *CA Cancer J. Clin.* **56**, 106–130.
Jensdottir, T., Bardow, A., and Holbrook, P. (2005). Properties and modification of soft drinks in relation to their erosive potential *in vitro*. *J. Dent.* **33**, 569–575.
Jensen, L. B., Kollerup, G., Quaade, F., and Sorensen, O. H. (2001). Bone mineral changes in obese women during a moderate weight loss with and without calcium supplementation. *JBMR* **16**, 141–147.
Johansson, A. K., Lingstrom, P., and Birkhed, D. (2002). Comparison of factors potentially related to the occurrence of dental erosion in high- and low-erosion groups. *Eur. J. Oral Sci.* **110**, 204–211.
Johnson, R. K. (2000). Changing eating and physical activity patterns of US children. *Proc. Nutr. Soc.* **59**, 295–301.
Johnston, C. C., Miller, J. Z., Slemenda, C. W., Reister, T. K., Hui, S., Christian, J. C., and Peacock, M. (1992). Calcium supplementation and increases in bone mineral density in children. *N. Engl. J. Med.* **327**, 82–87.
Kamangar, F., Dores, G. M., and Anderson, W. F. (2006). Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. *J. Clin. Oncol.* **24**, 2137–2150.
Kanbur, N. O., Derman, O., and Kinik, E. (2002). Osteocalcin. A biochemical marker of bone turnover during puberty. *Int. J. Adolesc. Med. Health* **14**, 235–244.
Karanja, N., Morris, C., Illingworth, D. R., and McCarron, D. A. (1987). Plasma lipids and hypertension: Response to calcium supplementation. *Am. J. Nutr.* **45**, 60–65.
Karlsson, C., Obrant, K. J., and Karlsson, M. (2001). Pregnancy and lactation confer reversible bone loss in humans. *Osteoporos. Int.* **12**, 828–834.
Kennedy, E., and Meyers, L. (2005). Dietary reference intakes: Development and uses for assessment of micronutrient status of women—a global perspective. *Am. J. Clin. Nutr.* **81**, 1194S–1197S.
Kerstetter, J. E., O'Brien, K. O., Caseria, D. M., Wall, D. E., and Insogna, K. L. (2005). The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J. Clin. Endocrinol. Metab.* **90**, 26–31.
Kinyamu, H. K., Gallagher, J. C., Balhorn, K. E., Petranick, K. M., and Rafferty, K. A. (1997). Serum vitamin D metabolites and calcium absorption in normal young and elderly free-living women and women living in nursing homes. *Am. J. Clin. Nutr.* **65**, 790–797.
Klahorst, S. (2001). Design elements: Calcium's role. Available from: http://www.foodproductdesign.com/articles/463/463_0101de.html [Accessed March 2007].
Kobrin, A. M., Goldstein, S. J., Shangraw, R. F., and Raja, R. M. (1989). Variable efficacy of calcium carbonate tablets. *Am. J. Kidney Dis.* **XIV**, 461–465.
Kochanowski, B. A. (1990). Effect of calcium citrate-malate on skeletal development in young, growing rats. *J. Nutr.* **120**, 876–881.
Kovacs, C. S., and Fuleihan, G. E. (2006). Calcium and bone disorders during pregnancy and lactation. *Endocrinol. Metab. Clin. North Am.* **35**, 21–51.
Krall, E. A., and Dawson-Hughes, B. (1999). Smoking increases bone loss and decreases intestinal calcium absorption. *JBMR* **14**, 215–220.
Krall, E. A., Dawson-Hughes, B., Papas, A., and Garcia, R. I. (1994). Tooth loss and skeletal bone density in healthy postmenopausal women. *Osteoporos. Int.* **2**, 104–109.
Krall, E. A., Garcia, R. I., and Dawson-Hughes, B. (1996). Increased risk of tooth loss is related to bone loss at the whole body, hip, and spine. *Calcif. Tissue Int.* **59**, 433–437.
Krall, E. A., Wehler, C., Garcia, R. I., Harris, S. S., and Dawson-Hughes, B. (2001). Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am. J. Med.* **111**, 452–456.

Kuntz, L. A. (2003). Elemental calcium facts. Available from: http://www.foodproductdesign.com/archive/2003/0203INI.html [Accessed March 2007].

Kushi, L. H., Byers, T., Doyle, C., Bandera, E. V., McCullough, M., Gansler, T., Andrews, K. S., and Thun, M. J. (2006). American Cancer Society guidelines on nutrition and physical activity for cancer prevention: Reducing the risk of cancer with healthy food choices and physical activity. *CA Cancer J. Clin.* **56**, 254–281.

Lee, H. S., and Coates, G. A. (1997). Vitamin C contents in processed Florida citrus juice products from 1986–1995 survey. *J. Agric. Food Chem.* **45**, 2550–2555.

Lee, W. T. K., Leung, S. S. F., Ng, M. Y., Wang, S. F., Xu, Y. C., Zeng, W. P., and Lau, J. (1993). Bone mineral content of two populations of Chinese children with different calcium intakes. *Bone Miner.* **23**, 195–206.

Lee, W. T. K., Leung, S. S. F., Leung, D. M. Y., Tsang, H. S. Y., Lau, J., and Cheng, J. C. Y. (1995). A randomized double-blind controlled calcium supplementation trial, and bone and height acquisition in children. *Br. J. Nutr.* **74**, 125–139.

Lide David, R. (ed.) (2004–2005) *In* ''CRC Handbook of Chemistry and Physics'' 85th Ed. CRC Press, Boca Raton, Florida.

Liebman, B. (1998). Calcium supplements: The way to go? Available from: http://www.encyclopedia.com/doc/1G1-20467468.html [Accessed March 2007].

Liebman, M., and Chai, W. (1997). Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *Am. J. Clin. Nutr.* **65**, 1453–1459.

Lihono, M. A., Serfass, R. E., Sell, J. L., and Palo, P. E. (1997a). Bioavailability of calcium citrate malate added to microbial phytase-treated, hydrothermally cooked soymilk. *J. Food Sci.* **62**, 1226–1230.

Lihono, M. A., Serfass, R. E., Sell, J. L., and Palo, P. E. (1997b). Bioavailability of calcium citrate malate added to microbial phytase-treated, hydrothermally cooked soymilk. *J. Food Sci.* **62**, 1226–1230.

Lind, T., Lonnerdal, B., Stenlund, H., Gamayanti, I. L., Ismail, D., Seswandhana, R., and Persson, L. A. (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: Effects on growth and development. *Am. J. Clin. Nutr.* **80**, 729–736.

Linzell, J. L., Mepham, T. B., and Peaker, M. (1976). The secretion of citrate into milk. *J. Physiol.* **260**, 739–750.

Lloyd, T., Andon, M. B., Rollings, N., Martel, J. K., Landis, J. R., Demers, L. M., Eggli, D. F., Kieselhorst, K., and Kulin, H. E. (1993). Calcium supplementation and bone mineral density in adolescent girls. *JAMA* **270**, 841–844.

Lloyd, T. A., Martel, J. K., Rollings, N. J., Andon, M. B., Kulin, H., Demers, L. M., Eggli, D. F., Kieselhorst, K., and Chinchilli, V. M. (1996). The effect of calcium supplementation and Tanner stage on bone density, content, and area in teenage women. *Osteoporos. Int.* **6**, 276–283.

Lloyd, T. A., Rollings, N. J., and Chinchilli, V. M. (1997). The effect of enhanced bone gain achieved with calcium supplementation during ages 12 to 16 does not persist in late adolescence. *In* ''Third International Symposium on Nutritional Aspects of Osteoporosis'' (P. Burckhardt, B. Dawson-Hughes, and R. P. Heaney, eds.), Springer-Verlag, London.

Lonnerdal, B., Cederblad, A., Davidsson, L., and Sandstrom, B. (1984). The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. *Am. J. Clin. Nutr.* **40**, 1064–1070.

Looker, A. C. (2003). Interaction of science, consumer practices and policy: Calcium and bone health as a case study. *J. Nutr.* **133**, 1987S–1991S.

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 Inc., Totowa, NJ.

Loud, K. J., and Gordon, C. M. (2006). Adolescent bone health. *Arch. Pediatr. Adolesc. Med.* **160**, 1026–1032.

Lowry, K. R., Mahan, D. C., and Corley, J. R. (1985). Effect of dietary calcium on selenium retention in postweaning swine. *J. Anim. Sci.* **60**, 1429–1437.

Luhadiya, A. P. C., Yang, D. K., and Heisey, M. T. (2006). Calcium fortified beverages. The Procter and Gamble Company, U.S. Patent No. 6,994,877.

Lussi, A., Jaeggi, T., and Zero, D. T. (2005). Impact of modified acidic soft drink on enamel erosion. *Oral Dis.* **11**, 7–12.

Lussi, A., Kohler, N., Zero, D. T., Schaffner, M., and Megert, B. (2000). A comparison of the erosive potential of different beverages in primary and permanent teeth using an *in vitro* model. *Eur. J. Oral Sci.* **108**, 110–114.

Lynch, S. R., and Cook, J. D. (1980). Interaction of vitamin C and iron. *Ann. NY Acad. Sci.* **355**, 32–44.

Lytle, L. A., and Kubik, M. Y. (2003). Nutritional issues for adolescents. *Best Pract. Res. Clin. Endocrinol. Metab.* **17**, 177.

Manz, F., and Schoenau, E. (2002). Calcium requirements. *Ann. Nestle* **60**, 117–128.

Martinez, M. E., and Willett, W. C. (1998). Calcium, vitamin D, and colorectal cancer: A review of the epidemiologic evidence. *Cancer Epidemiol. Biomarkers Prev.* **7**, 163–168.

Martini, L., and Wood, R. J. (2002). Relative bioavailability of calcium-rich dietary resources in the elderly. *Am. J. Clin. Nutr.* **76**, 1345–1350.

Mason, A. C., Weaver, C. M., Kimmel, S., and Brown, R. K. (1993). Effect of soybean phytate content on calcium bioavailability in mature and immature rats. *J. Agric. Food Chem.* **41**, 246–249.

Matkovic, V. (1991). Calcium metabolism and calcium requirements during skeletal modeling and consolidation of bone mass. *Am. J. Clin. Nutr.* **54**, 245S–260S.

Matkovic, V., and Heaney, R. P. (1992). Calcium balance during human growth: Evidence for threshold behavior. *Am. J. Clin. Nutr.* **55**, 992–996.

Matkovic, V., Kostial, K., Simonovic, I., Buzina, R., Brodarec, A., and Nordin, B. E. (1979). Bone status and fracture rates in two regions of Yugoslavia. *Am. J. Clin. Nutr.* **32**, 540–549.

Matkovic, V., Badenhop-Stevens, N. E., Landoll, J. D., Goel, P., and Li, B. (2000). Long-term effect of calcium supplementation and dairy products on bone mass of young females. *JBMR* **17**, S172.

Matkovic, V., Landoll, J. D., Badenhop-Stevens, N. E., Ha, E. Y., Crncevic-Orlic, Z., Li, B., and Goel, P. (2004a). Nutrition influences skeletal development from childhood to adulthood: A study of hip, spine, and forearm in adolescent females. *J. Nutr.* **134**, 701S–705S.

Matkovic, V., Landoll, J. D., Goel, P., Badenhop-Stevens, N., Ha, E. J., Li, B., and Crncevic-Orlic, Z. (2004b). Bone mineral density of skull and lower extremities during growth and calcium supplementation. *In* ''Nutritional Aspects of Osteoporosis,'' (P. Burckhardt, B. Dawson-Hughes, and R. P. Heaney, eds.), pp. 3–15. Elsevier Academic Press, Burlington, MA.

Matkovic, V., Goel, P. K., Badenhop-Stevens, N. E., Landoll, J. D., Li, B., Ilich, J. Z., Skugor, M., Nagode, L. A., Mobley, S. L., Ha, E. J., Hangartner, T. N., and Clairmont, A. (2005). Calcium supplementation and bone mineral density in females from childhood to young adulthood: A randomized controlled trial. *Am. J. Clin. Nutr.* **81**, 175–188.

McCormick, C. C. (2002). Passive diffusion does not play a major role in the absorption of dietary calcium in normal adults. *J. Nutr.* **132**, 3428–3430.

McKenna, A. A., Ilich, J. Z., Andon, M. B., Wang, C., and Matkovic, V. (1997). Zinc balance in adolescent females consuming a low- or high-calcium diet. *Am. J. Clin. Nutr.* **65**, 1460–1464.

Mehansho, H., Kanerva, R. L., Hudepohl, G. R., and Smith, K. T. (1989a). Calcium bioavailability and iron-calcium interaction in orange juice. *J. Am. Coll. Nutr.* **8**, 61–68.

Mehansho, R., Hudepohl, G. R., Kanerva, R. L., Luhrsen, K. R., and Smith, K. T. (1989b). Calcium bioavailability in fruit juices. *FASEB J.* **3**, A771.

Meta, M., Lu, Y., Keyak, J. H., and Lang, T. (2006). Young-elderly differences in bone density, geometry and strength indices depend on proximal femur sub-region: A cross sectional study in Caucasian-American women. *Bone* **39**, 152–158.

Miller, G. D., Jarvis, J. K., and McBean, L. D. (2001). The importance of meeting calcium needs with foods. *J. Am. Coll. Nutr.* **20**, 168S–185S.

Miller, J. Z., Smith, D. L., Flora, L., Slemenda, C., Jiang, X. Y., and Johnston, C. C. J. (1988). Calcium absorption from calcium carbonate and a new form of calcium (CCM) in healthy male and female adolescents. *Am. J. Clin. Nutr.* **48**, 1291–1294.

Miller, J. Z., Smith, D. L., Flora, L., Peacock, M., and Johnston, C. C. J. (1989). Calcium absorption in children estimated from single and double stable calcium isotope techniques. *Clin. Chim. Acta* **183**, 107–114.

Mohammad, A. R., Hooper, D. A., Vermilyea, S. G., and Mariotti, A. (2003). An investigation of the relationship between systemic bone density and clinical periodontal status in postmenopausal Asian-American women. *Int. Dent. J.* **53**, 121–125.

Monje, P. V., and Baran, E. J. (2002). Characterization of calcium oxalates generated as biominerals in cacti. *Plant Physiol.* **128**, 707–713.

Monsen, E. R., and Cook, J. D. (1976). Food iron absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. *Am. J. Clin. Nutr.* **29**, 1142–1148.

Morozumi, M., Hossain, R. Z., Yamakawa, K., Hokama, S., Nishijima, S., Oshiro, Y., Uchida, A., Sugaya, K., and Ogawa, Y. (2006). Gastrointestinal oxalic acid absorption in calcium-treated rats. *Urol. Res.* **34**, 168–172.

Nakel, G. M., Russell, W. E., Dake, T. W., and Heckert, D. C. (1988). Beverages and beverage concentrates nutritionally supplemented with calcium. The Procter and Gamble Company, U.S. Patent No. 4,737,375.

National Institutes of Health (1994). National Institutes of Health Consensus Development Conference Statement: Optimal calcium intake. Available from: http://consensus.nih.gov/1994/1994OptimalCalcium097html.htm [Accessed November 2007].

National Kidney and Urologic Diseases Information Clearing House (NKUDIC) (2005). What you should know about kidney stones. *In* ''Urinary Tract and Kidney Diseases and disorders Sourcebook'' I. L. Alexander (ed.), (2005). pp. 348–360. Omnigraphics Inc., Detroit, MI.

Newmark, H. L., Lipkin, M., and Maheshwari, N. (1990). Colonic hyperplasia and hyperproliferation induced by a nutritional stress diet with four components of Western-style diet. *J. Natl. Cancer Inst.* **82**, 491–496.

Nicklas, T. (2003). Calcium intake trends and health consequences from childhood through adulthood. *J. Am. Coll. Nutr.* **22**, 340–356.

NIH Consensus Development Panel on Optimal Calcium Intake (1994). NIH Consensus conference. Optimal calcium intake. *JAMA* **272**, 1942–1948.

Nishida, M., Grossi, S., Dunford, R. G., Ho, A. W., TRevisan, M., and Genco, R. J. (2000). Calcium and the risk for periodontal disease. *J. Periodont.* **71**, 1057–1066.

Norman, A. W., Bouillon, R., Whiting, S. J., Vieth, R., and Lips, P. (2007). 13th Workshop consensus for vitamin D nutritional guidelines. *J. Steroid Biochem. Mol. Biol.* **103**, 204–205.

Norman, D. A., Fordtran, J. S., Brinkley, L. J., Zerwekh, J. E., Nicar, M. J., Strowig, S. M., and Pak, C. Y. (1981). Jejunal and ileal adaptation to alterations in dietary calcium: Changes in calcium and magnesium absorption and pathogenetic role of parathyroid hormone and 1,25-dihydroxyvitamin D. *J. Clin. Invest.* **67**, 1599–1603.

Nusser, S. M., Carriquiry, A. L., Dodd, K. W., and Full, W. A. (1996). A semi-parametric transformation approach to estimating usual daily intake distributions. *J. Am. Stat. Assoc.* **91**, 1440–1449.

O'Flaherty, E. J. (2000). Modeling normal aging bone loss, with consideration of bone loss in osteoporosis. *Toxicol. Sci.* **55**, 171–188.

Odvina, C. V. (2006). Comparative value of orange juice versus lemonade in reducing stone-forming risk. *Clin. J. Am. Soc. Nephrol.* **1**, 1269–1274.

Ohya, K., Amano, H., and Ogura, H. (1992). Alveolar bone resorption process in the molar tooth region in calcium-deficient rats. *Kokubyo Gakkai Zasshi* **59**, 585–593.

Parizek, J. (1978). Interactions between selenium compounds and those of mercury or cadmium. *Environ. Health Perspect.* **25**, 53–55.

Parker, D. M. (2004). Liquid oral compositions comprising calcium compound and an acidulant. U.S. Patent No. 6,719,963 B2.

Parry, J., Shaw, L., Arnaud, M. J., and Smith, A. J. (2001). Investigation of mineral waters and soft drinks in relation to dental erosion. *J. Oral Rehab.* **28**, 766–772.

Pattanaungkul, S., Riggs, B. L., Yergey, A. L., Vieira, N. E., O'Fallon, W. M., and Khosla, S. (2000). Relationship of intestinal calcium absorption to 1,25-dihydroxyvitamin D [1,25 $(OH)_2D$] levels in young versus elderly women: Evidence for age-related intestinal resistance to 1,25$(OH)_2D$ action. *J. Clin. Endocrinol. Metab.* **85**, 4023–4027.

Peacock, M., Liu, G., Carey, M., McClintock, R., Ambrosius, W., Hui, S., and Johnston, C. C. (2000). Effect of calcium or 25OH vitamin D_3 dietary supplementation on bone loss at the hip in men and women over the age of 60. *J. Clin. Endocrinol. Metab.* **85**, 3011–3019.

Pearle, M. S., Calhoun, E. A., and Curhan, G. C. (2004). Urolithiasis. *In* ''Urologic Diseases in America. DHHS, Public Health Service (PHS), NIH, NIDDK'' (M. S. Litwin and C. S. Saigal, eds.), pp. 3–39. Government Publishing Office (GPO), 2004; NIH Publication No. 04–5512, Washington, DC.

Perales, S., Barbera, R., Lagarda, M. J., and Farre, R. (2006). Fortification of milk with calcium: Effect on calcium bioavailability and interactions with iron and zinc. *J. Agric. Food Chem.* **54**, 4901–4906.

Pierre, J. L., and Gautier-Luneau, I. (2000). Iron and citric acid: A fuzzy chemistry of ubiquitous biological relevance. *Biometals* **13**, 91–96.

Platt, B. S., and Moncrieff, A. (1947). Nutritional comparison of human and cow's milk for infant feeding. *Br. Med. Bull.* **5**, 177–180.

Pointillart, A., and Guéguen, L. (1993). Calcium bioavailability in pig fed a dry milk-based diet versus a standard diet containing Ca citrate malate. *In* ''Bioavailability '93: Nutritional, Chemical and Food Processing Implications of Nutrient Availability''. (U. Schlemmer, ed.), pp. 1–5. Ettlingen, May 9–12th [Proceedings].

Prather, T. A., and Miller, D. D. (1992). Calcium carbonate depresses iron bioavailability in rats more than calcium sulphate or sodium carbonate. *J. Nutr.* **122**, 327–332.

Prentice, A., Jarjou, L. M., Cole, T. J., Stirling, D. M., Dibba, B., and Fairweather-Tait, S. (1995). Calcium requirements of lactating Gambian mothers: Effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. *Am. J. Clin. Nutr.* **62**, 58–67.

Prince, R. L., Dick, I. M., Lemmon, J., and Randell, D. (1997). The pathogenesis of age-related osteoporotic fracture: Effects of dietary calcium deprivation. *J. Clin. Endocrinol. Metab.* **82**, 260–264.

Prince, R. L., Devine, A., Dhaliwal, S. S., and Dick, I. M. (2006). Effects of calcium supplementation on clinical fracture and bone structure—results of a 5-year, double-blind, placebo-controlled trial in elderly women. *Arch. Intern. Med.* **166**, 869–875.

Puspitasari, N. L., Lee, K., and Greger, J. L. (1991). Calcium fortification of cottage cheese with hydrocolloid control of bitter flavor defects. *J. Dairy Sci.* **74**, 1–7.

Qiu, A. R., Wierzbicki, J. A., Orme, C. A., Cody, A. M., Hoyer, J. R., Nancollas, G. H., Zepeda, S., and De Yoreao, J. J. (2003). Molecular modulation of calcium oxalate crystallization by osteopontin and citrate. *PNAS* **101**, 1811–1815.

Quilici-Timmcke, J. (2002). Opportunities expanding for calcium-fortified foods. Available from: http://www.ffnmag.com/asp/articleDisplay.asp?strArticleId=104&strSite=FFNSite [Accessed April 2007].

Rafferty, K., Walters, G., and Heaney, R. P. (2007). Calcium fortificants: Overview and strategies for improving calcium nutriture of the U.S. population. *J. Food Sci.* **72**, R152–R158.

Rampersaud, G. C., Bailey, L. B., and Kauwell, G. P. (2003). National survey beverage consumption data for children and adolescents indicate the need to encourage a shift toward more nutritive beverages. *J. Am. Diet. Assoc.* **103**, 97–100.

Recker, R. R. (1985). Calcium absorption and achlorhydria. *N. Engl. J. Med.* **313**, 70–73.

Reid, I. R., Mason, B., Horne, A., Ames, R., Clearwater, J., Bava, U., Orr-Walker, B., Wu, F., Evans, M. C., and Gamble, G. D. (2002). Effects of calcium supplementation on serum lipid concentrations in normal older women: A randomized controlled trial. *Am. J. Med.* **112**, 343.

Remer, T. (2001). Influence of nutrition on acid-base balance—metabolic aspects. *Eur. J. Nutr.* **40**, 214–220.

Ricci, T. A., Chowdhury, H. A., Heymsfield, S. B., Stahl, T., Pierson, R. N., and Shapses, S. A. (1998). Calcium supplementation suppresses bone turnover during weight reduction in postmenopausal women. *JBMR* **13**, 1045–1050.

Richter, F., Newmark, H. L., Richter, A., Leung, D., and Lipkin, M. (1995). Inhibition of Western-diet induced hyperproliferation and hyperplasia in mouse colon by two sources of calcium. *Carcinogenesis* **16**, 2685–2689.

Rosen, C. J., and KIel, D. P. (2006). Age-related osteoporosis. *In* ''Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism'' (M. J. Favus, ed.), American Society for Bone and Mineral Research, Washington, DC.

Rossini, M., Bianchi, G., Di Munno, O., Giannini, S., Minisola, S., Sinigaglia, L., and Adami, S. (2006). Determinants of adherence to osteoporosis treatment in clinical practice. *Osteoporos. Int.* **17**, 914–921.

Roth-Bassell, H. A., and Clydesdale, F. M. (1991). The influence of zinc, magnesium, and iron on calcium uptake in brush border membrane vesicles. *J. Am. Coll. Nutr.* **10**, 44–49.

Roth-Bassell, H. A., and Clydesdale, F. M. (1992). *In vitro* solubility characteristics of six calcium salts. *J. Food Prot.* **55**, 1003–1005.

Roughead, Z. K., Zito, C. A., and Hunt, J. R. (2002). Initial uptake and absorption of nonheme iron and absorption of heme iron in humans are unaffected by the addition of calcium as cheese to a meal with high iron bioavailability. *Am. J. Clin. Nutr.* **76**, 419–425.

Rude, R. K. (1998). Magnesium deficiency: A cause of heterogenous disease in humans. *J. Bone Miner. Res.* **13**, 749–758.

Rugg-Gunn, A. J., Maguire, A., Gordon, P. H., McCabe, J. F., and Stephenson, G. (1998). Comparison of erosion of dental enamel by four drinks using an intra-oral device. *Caries Res.* **32**, 337–343.

Sabatier, M., Pont, F., Arnaud, M. J., and Turnlund, J. R. (2003). A compartmental model of magnesium metabolism in healthy men based on two stable isotope tracers. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R656–R663.

Salovaara, S., Sandberg, A., and Andlid, T. (2002). Organic acids influence iron uptake in the human epitheleal cell line Caco-2. *J. Agric. Food Chem.* **50**, 6233–6238.

Saltman, P. D., and Smith, K. T. (1993). Calcium and trace mineral supplements comprising estrogen. U.S. Patent No. 5,232,709.

Santoro, N., Brown, J. R., Adel, T., and Skurnick, J. H. (1996). Characterization of reproductive hormonal dynamics in the perimenopause. *J. Clin. Endocrinol. Metab.* **81**, 1495–1501.

Sato, Y., Iwamoto, J., Kanoko, T., and Satoh, K. (2005). Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: A randomized controlled trial. *Cerebrovasc. Dis.* **20**, 187–192.

Schatzkin, A., and Peters, U. (2004). Advancing the calcium-colorectal cancer hypothesis. *J. Natl. Cancer Inst.* **96**, 893–894.
Schulze, K. J., O'Brien, K. O., Germain-Lee, E. L., Baer, D. J., Leonard, A., and Rosenstein, B. J. (2003). Efficiency of calcium absorption is not compromised in clinically stable prepubertal and pubertal girls with cystic fibrosis. *Am. J. Clin. Nutr.* **78**, 110–116.
Scopacasa, F., Wishart, J. M., Horowitz, M., Morris, H. A., and Need, A. G. (2004). Relation between calcium absorption and serum calcitriol in normal men: Evidence for age-related intestinal resistance to calcitriol. *Eur. J. Clin. Nutr.* **58**, 264–269.
Seiquer, I., Lopez-Frias, M., Munoz-Hoyos, A., Galdo, G., Delqado-Andrade, C., Mesias, M., and Navarro, M. P. (2006). Dietary calcium utilization among a group of Spanish boys aged 11–14 years on their ususal diets. *J. Physiol. Biochem.* **62**, 9–16.
Seligman, P. A., Caskey, J. H., Frazier, J. L., Zucker, P. M., Podell, E. R., and Allen, R. H. (1983). Measurements of iron absorption from prenatal multivitamin-mineral supplements. *Obstet. Gynecol.* **61**, 356–362.
Shahkhalili, Y., Murset, C., Meirim, I., Duruz, E., Guinchard, S., and Cavadini, C. (2001). Calcium supplementation of chocolate: Effect on cocoa butter digestibility and blood lipids in humans. *Am. J. Clin. Nutr.* **73**, 246–252.
Shipp, C. C., Maletskos, C. J., and Dawson-Hughes, B. (1987). Measurement of 47 calcium retention with a whole-body counter. *Calcif. Tissue Int.* **41**, 307–312.
Shoji, K. (2000). Bone mineral density of alveolar bone in rats during pregnancy and lactation. *J. Periodont.* **71**, 1073–1078.
Simmer, J. P. (1995). Molecular mechanisms of dental enamel formation. *Crit. Rev. Oral Biol. Med.* **6**, 84–108.
Slemenda, C. W., Peacock, M., Hui, S., Zhou, L., and Johnston, C. C. (1997). Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. *JBMR* **12**, 676–682.
Smith, K. T. (1988). Calcium and trace mineral interactions. *Cereal Foods World* **33**, 776–782.
Smith, K. T., and Rotruck, J. T. (1988). Trace mineral and calcium interactions. *In* ''Trace Elements in Man and Animals 6'' (L. S. Hurley, C. L. Keen, B. Lonnerdal, and R. B. Rucker, eds.), pp. 221–228. Plenum Publishing Corporation, NY.
Smith, K. T., Heaney, R. P., FLora, L., and Hinders, S. M. (1987). Calcium absorption from a new calcium delivery system (CCM). *Calcif. Tissue Int.* **41**, 351–352.
Sojka, J., Wastney, M., Abrams, S., Lewis, S. F., Martin, B., Weaver, C., and Peacock, M. (1997). Magnesium kinetics in adolescent girls determined using stable isotopes: Effects of high and low calcium intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **273**, R710–R715.
Song, Y. M., Sheu, W. H. H., and Lee, W. J. (2001). Acute biochemical variations induced by calcium citrate and calcium carbonate in Type 2 diabetic patients: Impaired calcium absorption in Type 2 diabetic patients with prolonged gastric emptying time. *J. Diabet. Complic.* **15**, 97–102.
Sorof, J. M., Lai, D., Turner, J., Poffenbarger, T., and Portman, R. J. (2004). Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics* **113**, 475–482.
Specker, B. L., Beck, A., Kalkwarf, H., and Ho, M. (1997). Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. Available from: http://pediatrics.aappublications.org/cgi/content/abstract/99/6/e12 [Accessed July 2007].
Spencer, H., Kramer, L., Norris, C., and Osis, D. (1984). Effect of calcium and phosphorus on zinc metabolism in man. *Am. J. Clin. Nutr.* **40**, 1213–1218.
Spencer, J. D., Allee, G. L., and Sauber, T. E. (2000). Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *J. Anim. Sci.* **78**, 675–681.

Standing Committee of the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine (1997). *In* ''Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride.'' National Academy Press, Washington, DC.

Stops, F., Fell, J. T., Collett, J. H., Martini, L. G., Sharma, H. L., and Smith, A. M. (2006). The use of citric acid to prolong the *in vivo* gastro-retention of a floating dosage form in the fasted state. *Int. J. Pharm.* **308**, 8–13.

Storey, M. L., Forshee, R. A., and Anderson, P. A. (2004). Associations of adequate intake of calcium with diet, beverage consumption, and demographic characteristics among children and adolescents. *J. Am. Coll. Nutr.* **23**, 18–33.

Strause, L., Saltman, P., Smith, K. T., Bracker, M., and Andon, M. B. (1994). Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. *J. Nutr.* **124**, 1060–1064.

Streeten, E. A., Ryan, K. A., McBride, D. J., Pollin, T. I., Shuldiner, A. R., and Mitchell, B. D. (2005). The relationship between parity and bone mineral density in women characterized by a homogeneous lifestyle and high parity. *J. Clin. Endocrinol. Metab.* **90**, 4536–4541.

Sullivan, M. (2005). Shattering myths about kidney stones. *In* ''Urinary Tract and Kidney Diseases and Disorders Sourcebook'' (I. L. Alexander, ed.), pp. 361–365. Omnigraphics Inc., Detroit, MI.

Teegarden, D. (2006). Dietary calcium and obesity. *In* ''Calcium in Human Health'' (A. Beddich, ed.), pp. 327–339. Humana Press, Totowa, NJ.

The Practice Committee of the American Society for Reproductive Medicine (2006). The menopausal transition. *Fertil. Steril.* **86**, S253–S256.

Thomas, J., and Thomas, E. (1977). Experimental lithiasis in the rat. Urinary level of malic acid and oxaloacetic acid increased by administration of succinic acid, malic acid and oxaloacetic acid. *Ann. Urol.* **11**, 89–94.

Tiselius, H. G. (2005). Aetiological factors in stone formation. *In* ''Oxford Textbook of Clinical Nephrology'' (A. M. A. Davidson, J. S. Cameron, J.-P. Grunfeld, C. Ponticelli, E. Ritz, C. G. Winearls, and C. van Ypersele, eds.), Oxford University Press, USA.

Tordoff, M. G. (2001). Calcium: Taste, intake, and appetite. *Physiol. Rev.* **81**, 1567–1597.

Turnlund, J. R., Smith, R. G., Kretsch, M. J., Keyes, W. R., and Shah, A. G. (1990). Milk's effect on the bioavailability of iron from cereal-based diets in young women by use of *in vitro* and *in vivo* methods. *Am. J. Clin. Nutr.* **52**, 373–378.

U.S. Department of Health and Human Services (2004). *In* ''Bone Health and Osteoporosis: A Report of the Surgeon General.'' U.S. Department of Health and Human Services, Office of the Surgeon General, Rockville, MD.

Uhland, V., Lewis, K., and Spehar, C. (2004). American Top 25: Supplements climbing the charts. Available from: http://www.naturalfoodsmerchandiser.com/ASP/articleDisplay.asp?strArticleId=1046&strSite=NFMSITE&Screen=CURRENTISSUE [Accessed December 2006].

United States Department of Agriculture (2003). Agriculture Fact Book 2001–2002. Available from: http://www.usda.gov/factbook/2002factbook.pdf [Accessed March 2007].

van Mossevelde, B. (1997). Culinary cure: Calcium fortification. Available from: http://www.foodproductdesign.com/archive/1997/0997CSP.html [Accessed March 2007].

Veum, T. L., Ledoux, D. R., Bollinger, D. W., Raboy, V., and Cook, A. (2002). Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs. *J. Anim. Sci.* **80**, 2663–2670.

Vieth, R. (2006). Critique of the considerations for establishing the tolerable upper intake level for vitamin D: Critical need for revision upwards. *J. Nutr.* **136**, 1117–1122.

Villareal, D. T., Fontana, L., Weiss, E. P., Racette, S. B., Steger-May, K., Schechtman, K. B., Klein, S., and Holloszy, J. O. (2006). Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: A randomized controlled trial. *Arch. Intern. Med.* **166**, 2502–2510.

Volkert, D., Kreuel, K., Heseker, H., and Stehle, P. (2004). Energy and nutrient intake of young-old, old-old and very-old elderly in Germany. *Eur. J. Clin. Nutr.* **58**, 1190–1200.

Vyas, H. K., and Tong, P. S. (2004). Impact of source and level of calcium fortification on the heat stability of reconstituted skim milk powder. *J. Dairy Sci.* **87**, 1177–1180.

Wactawski-Wende, J., Hausmann, E., Hovey, K., Trevisan, M., Grossi, S., and Genco, R. J. (2005). The association between osteoporosis and alveolar crestal height in postmenopausal women. *J. Periodont.* **76**, 2116–2124.

Wade, M. A. (2004). Calcium: The chosen form: Fortifying products with calcium requires an understanding of the benefits of various forms and their interactions. *Prepared Foods* **175**, 54–58.

Walsh, C. T., Sandstead, H. H., Prasad, A. S., Newberne, P. M., and Fraker, P. J. (1994). Zinc: Health effects and research priorities for the 1990's. *Environ. Health Perspect.* **102**, 5–46.

Walton, B., Sinclair, E., Bartholomew, E. T., and Ramsey, R. C. (1945). Analysis of the organic acids of orange juice. *Plant Physiol.* **20**, 3–18.

Warden, S. J., Fuchs, R. K., Castillo, A. B., Nelson, I. R., and Turner, C. H. (2007). Exercise when young provides lifelong benefits to bone structure and strength. *JBMR* **22**, 251–259.

Wasnich, R. D., and Miller, P. D. (2000). Antifracture efficacy of antiresorptive agents are related to changes in bone mineral density. *J. Clin. Endocrinol.* **85**, 231–236.

Wasserman, R. H. (2004). Vitamin D and the dual processes of intestinal calcium absorption. *J. Nutr.* **134**, 3137–3139.

Wastney, M. E., Martin, B. R., Peacock, M., Smith, D., Jiang, X. Y., Jackman, L. A., and Weaver, C. M. (2000). Changes in calcium kinetics in adolescent girls induced by high calcium intake. *J. Clin. Endocrinol. Metab.* **85**, 4470–4475.

Weaver, C. M. (2006). Back to basics: Have milk with meals. *J. Am. Diet. Assoc.* **106**, 1756–1758.

Weaver, C. M., and Heaney, R. P. (2006a). Calcium. *In* ''Modern Nutrition in Health and Disease'' (M. E. Shils, M. Shike, A. L. Ross, R. J. Cabollero, and R. J. Cousins, eds.), pp. 194–210. Lippincott Williams and Wilkins, Philadelphia.

Weaver, C. M., and Heaney, R. P. (2006b). Appendix 1. *In* ''Calcium in Human Health'' (A. Bendich, ed.), p. 433. Humana Press, Totowa, NJ.

Weaver, C. M., and Heaney, R. P. (2006c). Clinical approaches for studying calcium metabolism and its relationship to disease. *In* ''Calcium in Human Health'' (A. Bendich, ed.), . 433. Humana Press, Totowa, NJ.

Weaver, C. M., and Liebman, M. (2002). Biomarkers of bone health appropriate for evaluating functional foods designed to reduce risk of osteoporosis. *Br. J. Nutr.* **88**, S225–S232.

Weaver, C. M., Martin, B. R., Plawecki, K. L., Peacock, M., Wood, O. B., Smith, D. L., and Wastney, M. E. (1995). Differences in calcium metabolism between adolescent and adult females. *Am. J. Clin. Nutr.* **61**, 577–581.

Weaver, C. M., Heaney, R. P., Teegarden, D., and Hinders, S. M. (1996). Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *J. Nutr.* **126**, 303–307.

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

Weaver, C. M., Martin, B., Costa, M. B., Saleeb, F. Z., and Huth, P. J. (2002). Absorption of calcium fumarate is equivalent to other calcium salts when measured in the rat model. *J. Agric. Food Chem.* **50**, 4974–4975.

Weaver, C. M., Wastney, M. E., and Spence, L. A. (2004). Quantitative clinical nutrition approach to the study of calcium and bone metabolism. *In* ''Nutrition and Bone Health'' (M. Holick and B. Dawson-Hughes, eds.), pp. 307–326. Humana Press, New Jersey.

Welberg, J. W., Monkelbaan, J. F., de Vries, E. G., Muskiet, F. A., Cats, A., Oremus, E. T., Boersma-van, E. W., van Rijsbergen, H., van der Meer, R., and Mulder, N. H. (1994). Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. *Ann. Nutr. Metab.* **38**, 185–191.

Wienk, K. J. H., Marx, J. J. M., and Beynen, A. C. (1999). The concept of iron bioavailability and its assessment. *Eur. J. Nutr.* **38**, 51–75.

Williams, C. P., Child, D. F., Hudson, P. R., Davies, G. K., Davies, M. G., John, R., Anandaram, P. S., and De Bolla, A. R. (2001). Why oral calcium supplements may reduce renal stone disease: Report of a clinical pilot study. *J. Clin. Pathol.* **54**, 54–62.

Wing, R. R., Matthews, K. A., Kuller, L. H., Meilahn, E. N., and Plantinga, P. L. (1991). Weight gain at the time of menopause. *Arch. Intern. Med.* **151**, 97–102.

Wishart, J. M., Scopacasa, F., Horowitz, M., Morris, H. A., Need, A. G., Cliftin, P. M., and Nordin, B. E. (2000). Effect of perimenopause on calcium absorption: A longitudinal study. *Climacteric* **3**, 102–108.

Wolf, R. L., Cauley, J. A., Baker, C. E., Ferrell, R. E., Charron, M., Caggiula, A. W., Salamone, L. M., Heaney, R. P., and Kuller, L. H. (2000). Factors associated with calcium absorption efficiency in pre- and perimenopausal women. *Am. J. Clin. Nutr.* **72**, 466–471.

Wood, R. J., and Hanssen, D. A. (1988). Effect of milk and lactose on zinc absorption in lactose-intolerant postmenopausal women. *J. Nutr.* **118**, 982–986.

Wood, R. J., and Zheng, J. J. (1997). High dietary calcium intakes reduce zinc absorption and balance in humans. *Am. J. Clin. Nutr.* **65**, 1803–1809.

Wright, J. D., Wang, C. Y., Kennedy-Stephenson, J., and Ervin, R. B. (2003). Dietary intake of ten key nutrients for public health, United States: 1990–2000. *Advanced data from vital and health statistics*. **no. 334**. Hyattsville, Maryland: National Center for Health Statistics, .

Wu, F. Y. H., and Wu, C. W. (1987). Zinc in DNA replication and transcription. *Ann. Rev. Nutr.* **7**, 251–272.

Yan, L., Prentice, A., Dibba, B., Jarjou, L. M., Stirling, D. M., and Fairweather-Tait, S. (1996). The effect of long-term calcium supplementation on indices of iron, zinc and magnesium status in lactating Gambian women. *Br. J. Nutr.* **76**, 821–831.

Yang, H. H. L., and Lawless, H. T. (2005). Descriptive analysis of divalent salts. *J. Sens. Stud.* **20**, 97–113.

Yeh, J. K., and Aloia, J. F. (1984). Effect of hypophysectomy and 1,25-dihydroxyvitamin D on duodenal calcium absorption. *Endocrinology* **114**, 1711–1717.

Yip, K. H. K., Smales, R. J., and Kaidonis, J. A. (2003). The diagnosis and control of extrinsic acid erosion of tooth substance. *Gen. Dent.* **51**, 350–353.

Yoshihara, A., Seida, Y., Hanada, N., Nakashima, K., and Miyazaki, H. (2005). The relationship between bone mineral density and the number of remaining teeth in community dwelling older adults. *J. Oral Rehab.* **32**, 735–740.

Zafar, T. A., Weaver, C. M., Zhao, Y., Martin, B. R., and Wastney, M. E. (2004). Nondigestible oligosaccharides increase calcium absorption and suppress bone resorption in ovariectomized rats. *J. Nutr.* **134**, 399–402.