Introduction

Dairy Food Consumption and Health: State of the Science on Current Topics

Helen Bishop-MacDonald, MSc, RD, FDC

Dairy Farmers of Canada, Montreal, Quebec, CANADA

The International Dairy Federation (IDF) is a coalition of milk producers and processors from around the world. Its members are concerned with issues that vary from cow comfort to dairy science and technology to, of course, the nutritional value of dairy foods. Nutritionists who specialize in the role of milk in the diet make up one of IDF's standing committees—the Standing Committee on Nutrition and Health—and have been instrumental in bringing together the papers presented in this supplement.

Many mainstream health and nutrition organizations worldwide recommend daily consumption of dairy products for optimal health. Nevertheless, the last decade or so has seen an increase in the number and variety of claims made against the inclusion of milk and/or its products in the diet. A single supplement cannot address all such matters, but the purpose of this supplement is to address in a scientific and objective manner the validity of some of these concerns. Specialists in several key areas of dairy and health were invited to submit manuscripts for publication in this supplement so that health professionals, and other interested parties, would have a comprehensive overview to which to refer when confronted with conflicting viewpoints.

As the year 2005 draws to a close, the International Dairy Federation's Standing Committee on Nutrition and Health is pleased to bring to light the views of some of the world's top nutrition scientists on this food that has served mankind for over 10,000 years. To have people question its consumption on the basis of flawed and faulty science is to no one's benefit . . . neither does it serve to have unwarranted claims disseminated. Milk is not a one-nutrient food, nor is its impact restricted to one condition such as osteoporosis. Its many bioactive components are only just beginning to be defined and explained, and it is hoped that this supplement will support, in a meaningful and practical way, a greater understanding of its contribution to the human condition.

Journal of the American College of Nutrition, Vol. 24, No. 6, 525S (2005)
Published by the American College of Nutrition

Review

Dietary Protein: An Essential Nutrient For Bone Health

Jean-Philippe Bonjour, MD

Service of Bone Diseases, Faculty of Medicine, University of Geneva, SWITZERLAND*

Key words: animal proteins, vegetal proteins, acid-base, bone mineral, potassium, calcium metabolism, bone mass, osteoporosis, fracture

Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings. In addition to calcium in the presence of an adequate vitamin D supply, proteins represent a key nutrient for bone health, and thereby in the prevention of osteoporosis. In sharp opposition to experimental and clinical evidence, it has been alleged that proteins, particularly those from animal sources, might be deleterious for bone health by inducing chronic metabolic acidosis which in turn would be responsible for increased calciuria and accelerated mineral dissolution. This claim is based on an hypothesis that artificially assembles various notions, including in vitro observations on the physical-chemical property of apatite crystal, short term human studies on the calciuric response to increased protein intakes, as well as retrospective inter-ethnic comparisons on the prevalence of hip fractures. The main purpose of this review is to analyze the evidence that refutes a relation of causality between the elements of this putative patho-physiological "cascade" that purports that animal proteins are causally associated with an increased incidence of osteoporotic fractures. In contrast, many experimental and clinical published data concur to indicate that low protein intake negatively affects bone health. Thus, selective deficiency in dietary proteins causes marked deterioration in bone mass, micro architecture and strength, the hallmark of osteoporosis. In the elderly, low protein intakes are often observed in patients with hip fracture. In these patients intervention study after orthopedic management demonstrates that protein supplementation as given in the form of casein, attenuates post-fracture bone loss, increases muscles strength, reduces medical complications and hospital stay. In agreement with both experimental and clinical intervention studies, large prospective epidemiologic observations indicate that relatively high protein intakes, including those from animal sources are associated with increased bone mineral mass and reduced incidence of osteoporotic fractures. As to the increased calciuria that can be observed in response to an augmentation in either animal or vegetal proteins it can be explained by a stimulation of the intestinal calcium absorption. Dietary proteins also enhance IGF-1, a factor that exerts positive activity on skeletal development and bone formation. Consequently, dietary proteins are as essential as calcium and vitamin D for bone health and osteoporosis prevention. Furthermore, there is no consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and risk reduction of fragility fractures.

Key teaching points:

- Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings.
- In addition to calcium in the presence of an adequate vitamin D supply, proteins represent a key nutrient for bone health, and thereby in the prevention of osteoporosis.
- Experimentally selective deficiency in dietary proteins causes marked deterioration in bone mass, micro-architecture and strength, the hallmark of the osteoporosis disease.
- Clinically large prospective epidemiologic studies indicate that relatively high protein intake is associated with increased bone mineral mass and reduced incidence of osteoporotic fracture.
- Low protein intake is often observed in patients with hip fracture and intervention study demonstrates that following orthopedic management, protein supplementation attenuates post-fracture bone loss, increases muscles strength, reduces medical complications and hospital stay.
- There is no consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and risk reduction of fragility fractures.

Journal of the American College of Nutrition, Vol. 24, No. 6, 526S–536S (2005)
Published by the American College of Nutrition

INTRODUCTION

Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings. In addition to dietary calcium, and an adequate vitamin D supply, dietary protein represents a key nutrient for bone health. Well controlled experiments demonstrate that a selective deficiency in dietary proteins, i.e. without any associated insufficiency in other macronutrients, total energy, calcium and vitamin D, causes a rapid and marked alteration in bone mass, microarchitecture and strength. These alterations are the hallmark of the disease osteoporosis. Despite this, it is still repeatedly claimed that dietary proteins, particularly those from animal sources, can be a risk factor for osteoporosis. This claim is based on one hypothesis that artificially assembles various notions, including in vitro observations on the physico-chemical property of apatite crystal, short term human studies on the calciuric response to protein intake, as well as retrospective inter-ethnic comparisons on the prevalence of hip fractures. According to this questionable theory, it is alleged that the consumption of animal proteins would result in a substantial metabolic acid load which in turn would cause the dissolution of bone mineral. This hypothetical connection would explain the increased calciuria, as observed in short term studies testing the effect of high protein intakes on the calcium economy. In turn, it is purported that the hypercalciuria would result in an accelerated loss of bone mineral mass, thereby increasing (in the long term) the risk of osteoporotic fracture in a population consuming a relatively high amount of animal proteins, including those from dairy sources.

The main purpose of this review is to analyse the evidence that refutes a relation of causality between the elements of this putative pathophysiological "cascade", that purports that animal proteins are causally associated with an increased incidence of osteoporotic fractures.

CLAIM 1. DIETARY PROTEINS WOULD INDUCE SYSTEMIC ACIDOSIS AND THEREBY WOULD PROMOTE BONE MINERAL DISSOLUTION

This hypothesis was first built up by analogy to a well established physico-chemical phenomenon indicating that in vitro the solubility of calcium phosphate salt including hydroxyapatite ($3Ca_3(PO4)_2(OH)_2$), which is the most common crystal form found in bone, increases when the environmental pH falls [1]. Based on experiments in rats made severely acidotic by chronic NH_4Cl loading, the observed decrease in skeletal mass was ascribed to the physical-chemical release of alkali from bone mineral [2]. This physico-chemical theory was then applied to the pathophysiology of acidosis-induced osteodystrophy [3, 4] and osteoporosis [5]. Eventually, it provided putative mechanistic support to the hypothesis contending that a high protein diet would negatively affect bone integrity [6]. Thus, this physico-chemical theory considered bone mineral as a vast ion-exchange system that would be in direct contact with the systemic extracellular fluid [5]. This theory did not take into account some fundamental concepts concerning the physico-chemistry of bone mineral.

It should be re-emphasized that bone mineral is not in direct contact with the systemic circulation [1]. A very tight cellular barrier separates the systemic extracellular fluid from the internal bone mineral compartment. As demonstrated by William and Margaret Neuman in their classical reference book on the chemical dynamics of bone mineral: *"The interstitial fluid of bone cannot be equivalent to the extracellular fluid in ionic composition"* [1]. Assuming that the release of bone mineral alkali does occur in acidotic conditions, it could not occur without an alteration in cellular mediated bone turnover. In fact animal studies indicated the possible involvement of osteoclasts in the increased resorption observed in severe metabolic acidosis [7]. In vitro experiments with rat osteoclasts sustained this notion [8]. Further in vitro studies with various osteoclast-like cells cultured on ivory discs indicated that pH variations of the extracellular medium from 7.4 to as low as 6.8 increased cellular resorbing activity, as assessed by monitoring the number of resorption pits formed [9]. This marked decrease in pH corresponds to a four-fold increase in H^+ concentration from about 40 to 160 nMoles/Liter [10]. These in vitro observations help us to understand osteoclast and osteoblast responses to severe acidotic conditions [8, 9, 11, 12]. However, they cannot be extrapolated to the physiological situation prevailing under relatively high protein intake, where there is no evidence that bone buffer release, even in very small amounts, would take place. Indeed, the hypothesis implying that dietary protein-induced bone loss through release of alkali components of hydroxyapatite crystal - whether by a direct physicochemical action or indirectly through the activation of osteoclastic resorption - does not take into account the very high extra-skeletal capacity of an array of biochemical and physiological functions that are involved in the maintainance of the proton concentration in the body fluid compartments [13–15].

The hydrogen ion concentration of the extracellular fluid is closely regulated. The vast majority of hydrogen ions, as generated by cellular metabolism, are bound (buffered) by other

Address reprint requests to: Professor Jean-Philippe Bonjour, Service of Bone Diseases, University Hospital, Rue Micheli-Du-Crest, 1211 Geneva, SWITZERLAND. E-mail: Jean-Philippe.Bonjour@medecine.unige.ch

*WHO Collaborating Center For Osteoporosis Prevention

ions in the extra- and intracellular compartments. The metabolically produced carbon dioxide is the main source of hydrogen ions. It is eliminated by the lungs as rapidly as it is produced by the tissues. The kidney ultimately eliminates excess hydrogen ions, but it is buffering which minimizes changes in hydrogen ion concentration in the extra- and intracellular fluid compartments. In the absence of renal failure, the capacity of the kidney to modify hydrogen ion excretion is very high. The renal tubule adequately responds to large variations in the ingestion of acid yielding organic nutrients, as well as to marked fluctuations in the metabolic production of hydrogen ions. Therefore, in healthy conditions the blood pH is tightly maintained within extremely narrow limits, as a result of the very efficient chemical buffering capacity of the body fluid compartements and the function of both the lungs and kidney in eliminating carbon dioxide and hydrogen ions. Consequently, an increased animal protein intake with its associated load in sulfur-containing amino acids would not lead to such a metabolic acidosis that would require the mobilisation of proton buffer equivalents, carbonate and/or phosphate ions, from the mineralized phase of bony tissue.

Potent inhibition of bone resorption with pharmacological agents such as bisphosphonates does not impair the extrarenal buffering capacity in response to acid loading, unless renal function is abolished [16]. Even in chronic metabolic acidosis, which imposes a higher buffer demand than would a high protein diet, the irrelevance of bone buffering has been well argued, in both qualitative and quantitative terms [14,15]. Theoretically, an increased bone crystal dissolution might contribute to neutralize the increment in acid production resulting from high protein diet by both liberating alkali and changing phosphate ion from the trivalent state (PO_4^{-3}) present in bone crystal to a mixture of divalent and monovalent (HPO_4^{-2}/HPO_4^{-1}) ions [1]. If this response were substantial, one would expect that at similar protein intake, differences in bone resorption rate would result in detectable variations in blood pH and urinary acid excretion. None of the long term and large scale clinical trials carried out in postmenopausal women investigating the effect of bisphosphonates, the most potent inhibitors of bone resorption so far tested, have reported differences in acid-base balance between and the placebo groups [17, 18]. This absence of evidence for a link between bone resorption rate and acid-base balance in human studies is in agreement with experimental investigations mentioned above [16].

The kidney, together with the respiratory system, is the pivotal player in the regulation of the extracellular hydrogen ion concentration. Thus, the difference in renal acid excretion observed in response to variations in protein intake represents a normal homeostatic response. This homeostatic response contributes to the observed maintenance in blood pH in the face of increases in dietary protein intake [19]. Of note, in young healthy adult females, omnivores had a slightly but not significantly higher blood pH than age-matched vegetarians with a lower protein intake [20]. The slightly greater urinary titrable acid output found in the omnivores as compared to the vegetarian group [20], further documents the key role of the kidney in the regulation of acid-base balance in response to variations in nutrient intakes. The renal tubule is extraordinarily well equipped in terms of both bicarbonate reclamation and proton secretion machinery to deal adequately with diets supplying various amounts of alkali and acid [13–15].

Bicarbonate, Potassium, Calcium and Bone Metabolism

An indirect argument put forward in favor of the acid-induced bone dissolution that a protein rich diet might cause, is the reduction in urinary calcium excretion observed under potassium bicarbonate ($KHCO_3$) administration [21, 22]. In postmenopausal women the decreased calciuria associated with short term (18 days) of $KHCO_3$ ingestion was ascribed to an inhibition of bone resorption, evidenced by a 10 percent decrease in urinary hydroxyproline excretion [21]. However, the reported study design did not include the measurement of intestinal calcium absorption, so that the actual effect of $KHCO_3$ on calcium balance remained uncertain [21]. Likewise, this key physiological variable in the calcium economy was not assessed in a recent long term (36 months) study that tested the same kind of intervention in postmenopausal women [23]. In this latter study, no information was provided as to the possible effects on bone mineral density (BMD) or content (BMC) of $KHCO_3$ administered at three dose levels versus placebo in postmenopausal women during 36 months [23]. Initially, and taking into account the acid theory of bone mineral dissolution, the hypocalciuric influence of potassium bicarbonate was ascribed to its alkalinization effect that would counter the "ordinary diet"-related endogenous hydrogen ion production [21]. Nevertheless, this interpretation was not in keeping with the observation that potassium but not sodium bicarbonate reduces urinary calcium excretion in healthy men [24]. Hence, the alternative hypothesis implying potassium per se as the ion responsible for the hypocalciuric effect of $KHCO_3$, through a putative effect on either renal calcium reabsorption or bone mineral dissolution, or both. This apparent beneficial effect of potassium on the calcium economy was taken as one possible mechanistic explanation, along with the estimated reduction in net endogenous acid production, of the positive association found between consumption of fruit and vegetable rich diets and bone mineral density [25, 26]. Note that besides fruits and vegetables, milk and meat also contribute important amounts of potassium to the diet. One liter of milk and 400 g of beef meat each contain about 1400 mg of potassium; this amount is found in approximatively 500 g of fruits and vegetables.

An important caveat regarding the putative positive influence of potassium per se on the calcium economy comes from a recent study in a cohort of about 650 pre- and postmenopausal women with a mean age of 50.2 years [27]. The main findings indicated that dietary K was negatively associated, not only

with urinary calcium, but also with intestinal calcium absorption [27]. Thus, potassium did not exert any beneficial effect on calcium balance since the reduced calciuria was offset by the reduction in intestinal calcium absorption [27]. The role, if any, of potassium per se in the calcium economy and bone health is still more difficult to delineate by considering its relation with acid-base balance in classical pathophysiological situations. Indeed, a potassium deficit generates alkalosis, whereas its excess causes acidosis [10]. Finally, there is no robust evidence supporting the notion that any positive effect of fruits and vegetables on bone health [25, 28, 29] would be mediated by their alkalinizing power and/or their potassium content. There is, rather, negative evidence, since experimental inhibition of bone resorption in vivo as achieved with various vegetable extracts is independent of their base excess and/or potassium content [30]. Therefore, the nutrient(s) that may be associated with a beneficial effect of fruits and vegetables on bone health, remain(s) to be identified.

CLAIM 2. ANIMAL PROTEINS WOULD GENERATE MORE ACID AND BE MORE CALCIURIC THAN VEGETAL PROTEINS

This claim implies that vegetal proteins might be bone protective whereas animal proteins would be harmful for the acquisition and the maintenance of the bone mineral mass. Purportedly, the higher content of sulfur-containing amino acids in animal proteins would lead to increased urinary excretion of calcium and, in the long run, to exacerbation of age-related bone loss.

It should be noted that an increased calciuria does not necessarily equate to a calcium "loss" that would be associated with a negative calcium balance. At steady state it only means that the net input of calcium into the extracellular compartment from either the intestine or bone, or from both sources, is increased. The renal tubular reabsorption of calcium is the key flux in the regulation of the extracellular concentration of calcium [31]. Physiological studies indicate that this regulation takes place mainly in the distal nephron. The main hormonal modulator is parathyroid hormone (PTH) which stimulates the calcium reabsorptive flux [32]. Other influencing factors relevant to this discussion are sulfate anions and the degree of acidification. Increased intraluminal concentration along the distal tubule of sulfate anions or hydrogen ions tend to decrease the tubular reabsorption of calcium [33, 34]. In sheep, feeding a high mineral content diet containing calcium sulfate as compared to calcium carbonate increased at steady state the urinary excretion of calcium without altering the intestinal calcium absorption [35]. At the skeletal level this response was associated with a greater decline in calcium deposition into bone than calcium release from bone [35]. More recent data obtained in healthy young women indicated that a supplement of calcium provided by a sulphate-rich mineral water was associated with a greater urinary calcium excretion than an equivalent amount of calcium supplied by milk [36]. This result corroborates the negative influence of sulfate on the calcium economy as mentioned above. As a complementary but not exclusive interpretation of this study [36], it may also suggest that milk proteins with their sulfur content are less calciuric than sulfate salt contained in mineral water.

Without any scientific evidence it has been often assumed, if not strongly contended, that the sulfur content of animal proteins is greater than that of vegetal proteins. Hence the production of sulfuric acid from the metabolism of sulfur-containing amino acids would be greater with the consumption of animal proteins. This argument does not hold when considering straightforward chemical analysis of the sulfur content of different proteins. Thus, in milk proteins the sulfur content is only half that determined in most cereal proteins [37]. The potential acid as sulfate in sulfur-containing amino acids was calculated [38] from the amino acid composition of various vegetal and animal proteins [39]. It was found to be 82, 69, and 68 mEq/100g protein for oatmeal, whole wheat and white rice, respectively; whereas it was 73, 59 and 55 mEq/100g protein in pork meat, beef meat and milk, respectively [38]. From these data, it can be predicted that the effect of purified proteins on urinary acid and calcium excretion will not be less when isolated from vegetable as compared to animal foods. In agreement with this notion is the finding that a diet containing equal amounts of plant as compared to beef proteins was not associated with a lower urinary excretion of calcium [40]. A very recent controlled feeding study in postmenopausal women indicates that substitution of soy for meat protein did not reduce urinary calcium excretion [41]. This substition neither improved calcium retention, nor modified blood biochemical markers of bone remodeling [41]. Of note, no correlation was detected between urinary acid and calcium excretion [41]. As discussed later, changes in the rate of intestinal calcium absorption appears to be a much stronger determinant of urine calcium excretion than other bone or renal tubular fluxes in response to variations in the protein intake, whether provided from plant or animal food sources.

It is also noteworthy that sulfur-containing amino acids are required in the synthesis of glutathione, and thereby in the capability to confer peroxidative protection, and withstand stresses and environmental challenges such as infections, malnutrition, heart disease or cancer [42–45]. Therefore, the negative view regarding sulfur-containing amino acids is not only unjustified in relation to the calcium economy and bone metabolism (see below), but also when taking into account their essential positive function in both general health and several pathological conditions.

CLAIM 3. THE DIETARY PROTEIN-INDUCED INCREASE IN URINARY CALCIUM EXCRETION WOULD BE DUE TO ENHANCED BONE RESORPTION

The widespread notion that a high protein diet might be harmful for bone health was chiefly based on the hypothesis that the associated increase in calciuria would be the result of an enhanced bone calcium mobilization [46, 47]. Several years later, it was realized that the main source of the increased calciuria was the intestine [48]. Indeed, in young women a relatively low protein intake (0.7 vs 2.1 g/kg b.w.) led to a reduction in intestinal calcium absorption that was associated with an increase in the circulating level of PTH [48, 49]. Therefore, the initial interpretation suggesting that the increased calciuria under a high protein diet reflected bone loss [47] was revisited. This reassessment led to the opposite conclusion: low, rather than high, protein intake is detrimental for bone health [50, 51]. Note that early literature, which remains relevant today, indicated that amino acids such as arginine and lysine are potent stimulators of intestinal calcium absorption [52]. In two recent studies, one in postmenopausal women aged 50–75 years [53] and the other in healthy men and women aged 50 years and over [54], the effect on calcium and bone metabolism of increasing the protein intakes by varying meat consumption from 0.94 to 1.62 and from 0.78 to 1.55 g/kg per day, respectively, was assessed after 5 to 9 weeks. The results of these two trials were very consistent indicating that high protein intakes were associated neither with an increased calciuria, nor with a decrease in calcium retention [53, 54]. Furthermore, the initially higher renal acid excretion in subjects consuming the high as compared to the low protein diet declined significantly with time [53]. Biochemical indicators of bone metabolism were not affected in one study [53], whereas a significant reduction in the urinary exretion of N-telopeptide, a marker of bone resorption, was observed in the other trial [54]. An elevation in the circulating level of the bone growth factor IGF-1 was observed [54]. This finding was in keeping with several human studies indicating a positive relationship between protein intake, from either animal (meat, milk) or plant foods and the production of IGF-1 [55–59]. Taken together, these former and recent observations combining reliable assessments of intestinal absorption and whole body retention of calcium, as well as determinations of biochemical markers of bone metabolism and osteotropic hormones including PTH and IGF-1 [48–59], do not support the claim implying that the protein induced increase in calciuria would reflect an acceleration of bone resorption, and thereby would lead to net calcium "loss" and eventually to osteoporosis. The possibility of a positive influence of increased protein intake on bone mineral mass and its relation with dietary calcium is discussed below.

CLAIM 4. AN INCREASE IN DIETARY PROTEIN INTAKE WOULD EXERT A NEGATIVE EFFECT ON BONE MINERAL MASS

The putative detrimental intake of a high protein diet on bone mineral mass has been often considered as a notion that would have been established according to the stringent criteria of "evidence based medicine". One publication has been frequently cited in support of this putative detrimental effect of high protein diet. This article described a cross-sectional study carried out in 38 young adult women (age range: 24–28 years) [60]. A negative association was found between protein intake, as estimated with a semiquantitative food frequency questionnaire, and areal bone mineral density (aBMD in g/cm^2) measured in the forearm by single photon absorptiometry. However, the negative correlation was only found at one of the two radial sites studied [60]. This observation was interpreted as evidence that relatively high protein intake would exert an adverse effect on bone mineral mass throughout life [61]. However, in several reports such a negative relationship was not observed [62–66]. Furthermore, in a large number of studies a positive relationship between the spontaneous protein intake and bone mineral mass has been found [67–80]. This positive relationship was observed in both women and men. In the Framingham Osteoporosis Study carried out in a large cohort of elderly women and men prospectively followed over 4 years, increased protein intake was protective against spinal and femoral bone loss in both genders [78]. Thus, in contrast to the widely held belief evoked above, high intake of proteins, including those from animal sources, did not adversely affect the skeleton even in the elderly population. In a survey carried out in hospitalized elderly patients, low protein intake was associated with reduced femoral neck aBMD and poor physical performance [72]. The group with a higher protein intake had a greater aBMD, particularly at the femoral neck level, and also had a better improvement of bicipital and quadricipital muscle strength and performance, as indicated by the increased capacity to walk and climb stairs, after four weeks of hospitalization [72]. In hip fracture patients, bone mass was directly proportional to serum albumin, a marker of nutritional status [81]. Altogether, these results indicate that a sufficient protein intake is mandatory for bone health [54, 80, 82–85]. Thus, whereas a gradual decline in caloric intakes with age can be considered as an adequate adjustment to the progressive reduction in energy expenditure, the parallel reduction in protein intakes is certainly detrimental for maintaining the integrity and function of several organs or systems, including skeletal muscles and bone.

There is some evidence that the favorable effect of increasing the protein intake on bone mineral mass is better expressed when the supply of both calcium and vitamin D are adequate [83, 84, 86–88]. Reciprocally, it has been reported that in postmenopausal women with low calcium intake (600 vs 1500 mg/day), a relatively high protein intake (20 vs 10% of energy)

enhanced calcium retention [89]. Further investigation is needed in order to clarify the interaction between protein and calcium intakes on postmenopausal and age-related bone loss. The same holds true for such interaction during skeletal development until the attainment of peak bone mass. Prospective observational studies suggest that both calcium and protein intakes are independent variables of bone mineral mass acquisition, particularly before the onset of pubertal maturation [90, 91]. Indeed, a recent study also suggests that protein intake modulates the effect of calcium supplementation on bone mineral mass gain in prepubertal boys [92]. Therefore it is possible that both protein and calcium played a role in the greater gain of total body aBMD/BMC that has been observed in milk supplemented adolescent girls [93].

CLAIM 5. DIETARY PROTEIN WOULD BE POSITIVELY RELATED TO THE PREVALENCE OR INCIDENCE OF OSTEOPOROTIC FRACTURE

An indirect argument has been put forward for suggesting that high animal protein intakes exert deleterious effects on bone health. This hypothesis was based on a retrospective analysis presenting an increased incidence rate of hip fracture in women older than 50 years of age, living in countries with high protein intake of animal origin [94, 95]. This approach raises two main comments. First, as expected, countries with the highest incidence of hip fracture are those with the longest life expectancy, an important determinant of the risk of osteoporotic fracture. Age adjustment to the 1977 [96] or 1987 [94, 95] distribution of women in the United States does not correct for marked differences in life expectancy between populations with various socio-economic conditions. Second, in this calculated cross-cultural association between animal protein and hip fracture [94, 95], the daily intake was an estimate of the total amount of animal proteins available for the whole population, i.e. the amount produced plus the amount imported minus the amount exported by a given country (data from the Food and Agriculture Organization, FAO, of the United Nations), divided by the number of inhabitants. This estimate does not take into account that in industrialized countries with high incidence of hip fracture, the protein consumption is lower in the elderly than in the young adult population, particularly among patients experiencing fragility fracture of the proximal femur (see for review: [97]).

Other epidemiological data have been obtained in several geographical regions of the world. In the Nurses' Health Study carried out in the United States and which included a large number of subjects followed over 12 years, a trend for hip fracture incidence inversely related to protein intake has been found [98]. In the same study, however, forearm fracture incidence increased in subjects with high protein intake of animal origin [98]. This opposite association might be related to some difference in physical activity and mode of falling between these two types of fracture, of which the maximal incidence occurs at an earlier age in the forearm than in the proximal femur [99, 100]. In a retrospective Norwegian survey an elevated risk of hip fracture was associated with high non-dairy protein intake only when calcium intake was low [101]. In a prospective study (Iowa Women's Health Study) carried out in about 32,000 women aged 55–69, the risk of hip fracture was negatively associated with total protein intake [102]. Thus, the age-adjusted relative risk reduction in hip fracture incidence was 67 and 79% for the highest vs the lowest quartile in total and animal protein intake, respectively [102]. The trend for risk reduction remains significant after further adjustment for body mass index, parity, smoking, alcohol intake, estrogen use, and physical activity [102]. In a case-control study conducted in Utah, the association between the odds ratio of hip fracture decreased across increasing quartiles of total protein intake in participants 50–69 years of age [103]. In this case-control study, such an association was not found in older participants 70–89 years of age [103]. It is unlikely that the positive influence of protein intake would be attenuated from age 70 years and over. Indeed, intervention trials in which protein supplements were demonstrated to exert a beneficial effect on bone mass and remodeling were carried out in patients older than 70 years [55, 104]. As discussed by the authors of the Utah case-control study [103], as well as commented on in a related editorial review [85], the inability to detect a protective effect of protein consumption in the older group might be due to some selection bias, including mostly the "healthiest" hip fracture cases, i.e. those patients able to complete the interview and to provide reliable information on their dietary intakes.

Other studies sustain the notion that under-nutrition with respect to protein intake is a important risk factor for hip fracture. Thus, in the NHANES I Study, hip fracture was higher with low energy intake, low serum albumin levels and low muscle strength [105]. Similarly, low BMI was a significant risk factor for hip fracture in both genders [106, 107]. A low plasma albumin level, which can reflect low nutritional intakes, has been repeatedly found in patients with hip fracture as compared to age-matched healthy subjects or patients with osteoarthritis [81, 108–110]. Dietary proteins positively influence the production and action of the bone anabolic agent, insulin-like growth factor-1 (IGF-1) in both animal and human studies. The "Dietary protein -> IGF-1 -> Bone Health" axis plays a key role in the prevention of osteoporosis. See for review [82]. Preclinical studies in adult animals have documented that an isocaloric low protein diet reduces IGF-1, induces negative bone balance with both decreased formation and increased resorption, thereby leading to a decline in bone strength [111–113]. All these negative effects can be reversed by amino acids administered in the same proportion as in casein [114]. In human studies the risk of spinal and hip fractures was

associated with low plasma levels of IGF-I [115, 116]. Furthermore, muscle mass and strength are important determinants not only of the maintenance of bone quality, but also of the risk and consequences of falling. In the elderly at risk of osteoporotic fractures, marginal dietary protein intake results in losses of muscle mass which is associated with a reduction in the level of IGF-1 [117]. Finally, randomized clinical trials in patients with hip fracture have documented the beneficial effects of correcting the spontaneously low protein intake by giving a casein supplement on the clinical outcome following the acute orthopedic management [55, 110, 118].

CLAIM 6. VEGETAL BUT NOT ANIMAL PROTEINS WOULD REDUCE OSTEOPOROSIS INDUCED BONE FRAGILITY

Several recent human studies do not support the notion that the protective effect of protein on either bone loss or osteoporotic fracture is due to vegetal rather than animal proteins [55, 78, 79, 88, 101–103]. In apparently sharp contrast with these very consistent results, an epidemiological study reported that individuals consuming diets with high ratios of animal to vegetal protein lost bone more rapidly than did those with lower ratios and had a greater risk of hip fracture [119]. The physiological meaning, particularly in terms of impact on calcium-phosphate and bone metabolism, of animal to vegetal protein ratio remains mechanistically quite obscure. Indeed, variations in this calculated ratio can result from differences in the absolute intake of either animal or vegetal proteins. More importantly, however, in this study [119] the statistically negative relationship between the animal to vegetal protein ratio and bone loss was obtained only after multiple adjustments, not only for age but also for energy intake, total calcium intake (dietary plus supplements), total protein intake, weight, current estrogen use, physical activity, smoking status and alcohol intake [119]. In sharp contrast, a positive relationship between the animal to vegetal protein ratio and baseline BMD was found when the statistical model was only adjusted for age [119]. This inconsistency according to the way this set of data was analyzed makes the generalization of these findings, in terms of nutritional recommendations for bone health and osteoporosis prevention, difficult [83].

CONCLUSIONS

The putative beneficial effect of vegetal as compared to the putative detrimental influence of animal protein on bone health has been promulgated over several decades. In the previous sections of this review, the lack of consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and osteoporotic fracture risk reduction has been presented. Both protein sources appear to be important for bone health. Besides their protein content, both plant and animal foods provide other nutrients that can exert positive influences on bone health. Even in groups or among individuals who are favorable to consuming foods from animal sources, whether for economic or palatability reasons, it is generally agreed that a well balanced, nutritionally sound diet includes the regular consumption of fruits and vegetables. In contrast, in some vegetarian circles, there is a certain proselytism against milk and/or meat products. An important aspect of this is the emotional opposition to the consumption of animal foods. As developed above, this rather strong antagonism is in part based on the putative negative influence of animal proteins on bone health. Scientific evidence does not support this negative view, as analysed in detail in the different sections of this review. The opposition to the consumption of animal proteins goes much beyond the legitimate choice of any adult individual to determine what she/he wants to eat and does not want to eat. Fortunately, there is no negative position in scientific or paramedical circles that would dogmatically recommend avoidance of the consumption of fruits and vegetables, among those who consider that animal foods, including meat, fish and dairy products provide useful nutrients for bone health. Proteins from various dietary sources contribute to maintain bone integrity, from early childhood to old age. Along with calcium and vitamin D, an adequate intake of proteins should be recommended in the prevention and treatment of postmenopausal and age-dependent osteoporosis.

REFERENCES

1. Neuman WF, Neuman MW: "The Chemical Dynamics Of Bone Mineral." Chicago: University of Chicago Press, 1958.
2. Barzel US, Jowsey J: The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin Sci 36:517–524, 1969.
3. Green J, Kleeman CR: Role of bone in regulation of systemic acid-base balance. Kidney Int 39:9–26, 1991.
4. Green J: The physicochemical structure of bone: cellular and noncellular elements. Miner Electrolyte Metab 20:7–15, 1994.
5. Barzel US: The skeleton as an ion exchange system: implications for the role of acid-base imbalance in the genesis of osteoporosis. J Bone Miner Res 10:1431–1436, 1995.
6. Barzel US, Massey LK: Excess dietary protein can adversely affect bone. J Nutr 128:1051–1053, 1998.
7. Chan YL, Savdie E, Mason RS, Posen S: The effect of metabolic acidosis on vitamin D metabolites and bone histology in uremic rats. Calcif Tissue Int 37:158–164, 1985.
8. Arnett TR, Dempster DW: Effect of pH on bone resorption by rat osteoclasts in vitro. Endocrinology 119:119–124, 1986.
9. Arnett T: Regulation of bone cell function by acid-base balance. Proc Nutr Soc 62:511–520, 2003.

10. Valtin H: Disorders of h^+ balance: useful tools. In "Renal Dysfunction: Mechanisms Involved in Fluid and Solute Imbalance." Boston: Little, Brown and Company, 1979.
11. Frick KK, Bushinsky DA: Metabolic acidosis stimulates RANKL RNA expression in bone through a cyclo-oxygenase-dependent mechanism. J Bone Miner Res 18:1317– 1325, 2003.
12. Krieger NS, Frick KK, Bushinsky DA: Mechanism of acid-induced bone resorption. Curr Opin Nephrol Hypertens 13:423–436, 2004.
13. Pitts RF: Buffer Mechanisms of Tissues and Body FLuids. In "Physiology of the Kidney and Body Fluids," 2nd ed. Chicago: Year Book Medical Publishers Incorporated, 1970.
14. Oh MS: Irrelevance of bone buffering to acid-base homeostasis in chronic metabolic acidosis. Nephron 59:7–10, 1991.
15. Oh MS: New perspectives on acid-base balance. Semin Dial 13:212–219, 2000.
16. Freudiger H, Bonjour JP: Bisphosphonates and extrarenal acid buffering capacity. Calcif Tissue Int 44:3–10, 1989.
17. Kherani RB, Papaioannou A, Adachi JD: Long-term tolerability of the bisphosphonates in postmenopausal osteoporosis: a comparative review. Drug Saf 25:781–790, 2002.
18. Cranney A, Tugwell P, Adachi J, Weaver B, Zytaruk N, Papaioannou A, Robinson V, Shea B, Wells G, Guyatt G: Meta-analyses of therapies for postmenopausal osteoporosis. III. Meta-analysis of risedronate for the treatment of postmenopausal osteoporosis. Endocr Rev 23:517–523, 2002.
19. Lutz J: Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. Am J Clin Nutr 39:281–288, 1984.
20. Ball D, Maughan RJ: Blood and urine acid-base status of premenopausal omnivorous and vegetarian women. Br J Nutr 78: 683–693, 1997.
21. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris RC: Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med 330:1776–1781, 1994.
22. New SA: Nutrition Society Medal lecture. The role of the skeleton in acid-base homeostasis. Proc Nutr Soc 61:151–164, 2002.
23. Frassetto L, Morris RC, Sebastian A: Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. J Clin Endocrinol Metab 90:831–834, 2005.
24. Lemann J, Gray RW, Pleuss JA: Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney Int 35:688–695, 1989.
25. New SA: Intake of fruit and vegetables: implications for bone health. Proc Nutr Soc 62:889–899, 2003.
26. Macdonald HM, New SA, Fraser WD, Campbell MK, Reid DM: Low dietary potassium intakes and high dietary estimates of net endogenous acid production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. Am J Clin Nutr 81:923–933, 2005.
27. Rafferty K, Davies KM, Heaney RP: Potassium intake and the calcium economy. J Am Coll Nutr 24:99–106, 2005.
28. Lin PH, Ginty F, Appel LJ, Aickin M, Bohannon A, Garnero P, Barclay D, Svetkey LP: The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults. J Nutr 133:3130–3136, 2003.
29. New SA: Do vegetarians have a normal bone mass? Osteoporos Int 15:679–688, 2004.
30. Muhlbauer RC, Lozano A, Reinli A: Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanism independent of their base excess. J Bone Miner Res 17:1230–1236, 2002.
31. Rizzoli R, Bonjour JP: Physiology of calcium and phosphate homeostasis. In Seibel MJ, Robins SP, Bilezikian JP (eds): "Dynamicy of Bone and Mineral Metabolism". San Diego, CA: Academic Press, pp 247–260, 1999.
32. Hugi K, Bonjour JP, Fleisch H: Renal handling of calcium: influence of parathyroid hormone and 1,25-dihydroxyvitamin D3. Am J Physiol 236:F349–356, 1979.
33. Quamme GA: Effects of intraluminal sulfate on electrolyte transfers along the perfused rat nephron. Can J Physiol Pharmacol 59:122–130, 1981.
34. Seldin DW: Renal handling of calcium. Nephron 81 (Suppl 1):2–7, 1999.
35. Gueguen L, Besancon P: Effect of sulfates on phosphorus and calcium metabolis. I. Utilization of calcium sulfate by sheep. Ann Biol Anim Biochim Biophys 12:589–598, 1972.
36. Brandolini M, Gueguen L, Boirie Y, Rousset P, Bertiere MC, Beaufrere B: Higher calcium urinary loss induced by a calcium sulphate-rich mineral water intake than by milk in young women. Br J Nutr 93:225–231, 2005.
37. Anonymous: "Geigy Scientific Tables," Vol.1, 8th ed. Basel, Switzerland: CIBA-GEIGY, 1981.
38. Massey LK: Dietary animal and plant protein and human bone health: a whole foods approach. J Nutr 133:862S–865S, 2003.
39. Hands ES: "Nutrient in Food." Baltimore: Lippencott/Williams & Wilkins, 2000.
40. Massey LK, Kynast-Gales SA: Diets with either beef or plant proteins reduce risk of calcium oxalate precipitation in patients with a history of calcium kidney stones. J Am Diet Assoc 101: 326–331, 2001.
41. Roughead ZK, Hunt JR, Johnson LK, Badger TM, Lykken GI: Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. J Clin Endocrinol Metab 90:181–189, 2005.
42. Fukagawa NK, Yu YM, Young VR: Methionine and cysteine kinetics at different intakes of methionine and cysteine in elderly men and women. Am J Clin Nutr 68:380–388, 1998.
43. Reeds PJ: Dispensable and indispensable amino acids for humans. J Nutr 130:1835S– 1840S, 2000.
44. Young VR, Borgonha S: Nitrogen and amino acid requirements:: the Massachusetts Institute of Technology amino acid requirement pattern. J Nutr 130:1841S–1849S, 2000.
45. Jackson AA, Gibson NR, Lu Y, Jahoor F: Synthesis of erythrocyte glutathione in healthy adults consuming the safe amount of dietary protein. Am J Clin Nutr 80:101– 107, 2004.
46. Heaney RP, Recker RR: Effects of nitrogen, phosphorus, and caffeine on calcium balance in women. J Lab Clin Med 99:46–55, 1982.
47. Kerstetter JE, Allen LH: Dietary protein increases urinary calcium. J Nutr 120:134– 136, 1990.

48. Kerstetter JE, O'Brien KO, Insogna KL: Dietary protein affects intestinal calcium absorption. Am J Clin Nutr 68:859–865, 1998.
49. Kerstetter JE, Caseria DM, Mitnick ME, Ellison AF, Gay LF, Liskov TA, Carpenter TO, Insogna KL: Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. Am J Clin Nutr 66:1188–1196, 1997.
50. Ilich JZ, Kerstetter JE: Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr 19:715–737, 2000.
51. Kerstetter JE, O'Brien KO, Insogna KL: Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am J Clin Nutr 78:584S–592S, 2003.
52. Comar CL, Nold MM, Wasserman RH: The influence of amino acids and other organic compounds on the gastrointestinal absorption of calcium 45 and strontium 89 in the rat. J Nutr 59:371–383, 1956.
53. Roughead ZK, Johnson LK, Lykken GI, Hunt JR: Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. J Nutr 133:1020–1026, 2003.
54. Dawson-Hughes B, Harris SS, Rasmussen H, Song L, Dallal GE: Effect of dietary protein supplements on calcium excretion in healthy older men and women. J Clin Endocrinol Metab 89:1169–1173, 2004.
55. Schurch MA, Rizzoli R, Slosman D, Vadas L, Vergnaud P, Bonjour JP: Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 128:801–809, 1998.
56. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI, Rosen CJ: Dietary changes favorably affect bone remodeling in older adults. J Am Diet Assoc 99:1228–1233, 1999.
57. Khalil DA, Lucas EA, Juma S, Smith BJ, Payton ME, Arjmandi BH: Soy protein supplementation increases serum insulin-like growth factor-I in young and old men but does not affect markers of bone metabolism. J Nutr 132:2605–2608, 2002.
58. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, Wild RA: Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. J Clin Endocrinol Metab 88:1048–1054, 2003.
59. Arjmandi BH, Lucas EA, Khalil DA, Devareddy L, Smith BJ, McDonald J, Arquitt AB, Payton ME, Mason C: One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutr J 4:8, 2005.
60. Metz JA, Anderson JJ, Gallagher PN: Intakes of calcium, phosphorus, and protein, and physical-activity level are related to radial bone mass in young adult women. Am J Clin Nutr 58:537–542, 1993.
61. Anderson JJ, Metz JA: Adverse association of high protein intake to bone density. Challenges of Modern Medicine 7:407–412, 1995.
62. Mazess RB, Barden HS: Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. Am J Clin Nutr 53:132–142, 1991.
63. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB: Bone gain in young adult women. JAMA 268:2403–2408, 1992.
64. Reid IR, Ames RW, Evans MC, Sharpe SJ, Gamble GD: Determinants of the rate of bone loss in normal postmenopausal women. J Clin Endocrinol Metab 79:950–954, 1994.
65. Nieves JW, Golden AL, Siris E, Kelsey JL, Lindsay R: Teenage and current calcium intake are related to bone mineral density of the hip and forearm in women aged 30–39 years. Am J Epidemiol 141:342–351, 1995.
66. Wang MC, Luz Villa M, Marcus R, Kelsey JL: Associations of vitamin C, calcium and protein with bone mass in postmenopausal Mexican American women. Osteoporos Int 7:533–538, 1997.
67. Freudenheim JL, Johnson NE, Smith EL: Relationships between usual nutrient intake and bone-mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. Am J Clin Nutr 44:863–876, 1986.
68. Tylavsky FA, Anderson JJ: Dietary factors in bone health of elderly lactoovovegetarian and omnivorous women. Am J Clin Nutr 48:842–849, 1988.
69. Lacey JM, Anderson JJ, Fujita T, Yoshimoto Y, Fukase M, Tsuchie S, Koch GG: Correlates of cortical bone mass among premenopausal and postmenopausal Japanese women. J Bone Miner Res 6:651–659, 1991.
70. Orwoll ES: The effects of dietary protein insufficiency and excess on skeletal health. Bone 13:343–350, 1992.
71. Hirota T, Nara M, Ohguri M, Manago E, Hirota K: Effect of diet and lifestyle on bone mass in Asian young women. Am J Clin Nutr 55:1168–1173, 1992.
72. Geinoz G, Rapin CH, Rizzoli R, Kraemer R, Buchs B, Slosman D, Michel JP, Bonjour JP: Relationship between bone mineral density and dietary intakes in the elderly. Osteoporos Int 3:242–248, 1993.
73. Michaelsson K, Holmberg L, Mallmin H, Wolk A, Bergstrom R, Ljunghall S: Diet, bone mass, and osteocalcin: a cross-sectional study. Calcif Tissue Int 57:86–93, 1995.
74. Cooper C, Atkinson EJ, Hensrud DD, Wahner HW, O'Fallon WM, Riggs BL, Melton LJ: Dietary protein intake and bone mass in women. Calcif Tissue Int 58:320–325, 1996.
75. Lau EM, Kwok T, Woo J, Ho SC: Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. Eur J Clin Nutr 52:60–64, 1998.
76. Teegarden D, Lyle RM, McCabe GP, McCabe LD, Proulx WR, Michon K, Knight AP, Johnston CC, Weaver CM: Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. Am J Clin Nutr 68:749–754, 1998.
77. Calvo MS, Barton CN, Park YK: Bone mass and high dietary intake of meat and protein: analyses of data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–94). Bone 23 (Suppl):S290, 1998.
78. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP: Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res 15:2504–2512, 2000.
79. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E: Protein consumption and bone mineral density in the elderly : the Rancho Bernardo Study. Am J Epidemiol 155:636–644, 2002.

80. Whiting SJ, Boyle JL, Thompson A, Mirwald RL, Faulkner RA: Dietary protein, phosphorus and potassium are beneficial to bone mineral density in adult men consuming adequate dietary calcium. J Am Coll Nutr 21:402–409, 2002.
81. Thiebaud D, Burckhardt P, Costanza M, Sloutskis D, Gilliard D, Quinodoz F, Jacquet AF, Burnand B: Importance of albumin, 25(OH)-vitamin D and IGFBP-3 as risk factors in elderly women and men with hip fracture. Osteoporos Int 7:457–462, 1997.
82. Bonjour JP, Schurch MA, Chevalley T, Ammann P, Rizzoli R: Protein intake, IGF-1 and osteoporosis. Osteoporos Int 7 (Suppl 3):S36–42, 1997.
83. Heaney RP: Protein intake and bone health: the influence of belief systems on the conduct of nutritional science. Am J Clin Nutr 73:5–6, 2001.
84. Bell J, Whiting SJ: Elderly women need dietary protein to maintain bone mass. Nutr Rev 60:337–341, 2002.
85. Rizzoli R, Bonjour JP: Dietary protein and bone health. J Bone Miner Res 19:527–531, 2004.
86. Heaney RP: Calcium, dairy products and osteoporosis. J Am Coll Nutr 19:83S–99S, 2000.
87. Heaney RP: Protein and calcium: antagonists or synergists? Am J Clin Nutr 75:609– 610, 2002.
88. Dawson-Hughes B, Harris SS: 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, 2002.
89. Roughead ZK: The effects of interaction of dietary protein and calcium on calcium retention: a controlled feeding study. J Bone Miner Res 19 (Suppl 1):S302, 2004.
90. Bonjour JP, Ammann P, Chevalley T, Rizzoli R: Protein intake and bone growth. Can J Appl Physiol 26 (Suppl):S153–166, 2001.
91. Bonjour JP, Ammann P, Chevalley T, Ferrari S, Rizzoli R: Nutritional aspects of bone growth: an overview. In New S., Bonjour JP (Eds): "Nutritional Aspects of Bone Health." Cambridge: The Royal Society of Chemistry, pp 111–127, 2003.
92. Chevalley T, Ferrari S, Hans D, Slosman D, Fueg M, Bonjour JP, Rizzoli R: Protein intake modulates the effet of calcium supplementation on bone mass gain in prepubertal boys. J. Bone Miner Res 17 (Suppl 1): S172, 2002.
93. Cadogan J, Eastell R, Jones N, Barker ME: Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ 315:1255–1260, 1997.
94. Abelow BJ, Holford TR, Insogna KL: Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. Calcif Tissue Int 50:14–18, 1992.
95. Frassetto LA, Todd KM, Morris RC, Sebastian A: Worldwide incidence of hip fracture in elderly women: relation to consumption of animal and vegetable foods. J Gerontol A Biol Sci Med Sci 55:M585–592, 2000.
96. Lewinnek GE, Kelsey J, White AA, Kreiger NJ: The significance and a comparative analysis of the epidemiology of hip fractures. Clin Orthop Relat Res:35–43, 1980.
97. Bonjour JP, Schurch MA, Rizzoli R: Nutritional aspects of hip fractures. Bone 18:139S–144S, 1996.
98. Feskanich D, Willett WC, Stampfer MJ, Colditz GA: Protein consumption and bone fractures in women. Am J Epidemiol 143:472–479, 1996.
99. Cooper C, Melton LJ: Epidemiology of osteoporosis. Trends Endocrinol Metab 3:224– 229, 1992.
100. Cummings SR, Melton LJ: Epidemiology and outcomes of osteoporotic fractures. Lancet 359:1761–1767, 2002.
101. Meyer HE, Pedersen JI, Loken EB, Tverdal A: Dietary factors and the incidence of hip fracture in middle-aged Norwegians. A prospective study. Am J Epidemiol 145:117–123, 1997.
102. Munger RG, Cerhan JR, Chiu BC: Prospective study of dietary protein intake and risk of hip fracture in postmenopausal women. Am J Clin Nutr 69:147–152, 1999.
103. Wengreen HJ, Munger RG, West NA, Cutler DR, Corcoran CD, Zhang J, Sassano NE: Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. J Bone Miner Res 19:537–545, 2004.
104. Hampson G, Martin FC, Moffat K, Vaja S, Sankaralingam S, Cheung J, Blake GM, Fogelman I: Effects of dietary improvement on bone metabolism in elderly underweight women with osteoporosis: a randomised controlled trial. Osteoporos Int 14: 750–756, 2003.
105. Huang Z, Himes JH, McGovern PG: Nutrition and subsequent hip fracture risk among a national cohort of white women. Am J Epidemiol 144:124–134, 1996.
106. Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, Dilsen G, Gennari C, Lopes Vaz A, Lyritis G: Risk factors for hip fracture in European women: the MEDOS Study. Mediterranean Osteoporosis Study. J Bone Miner Res 10:1802– 1815, 1995.
107. Kanis J, Johnell O, Gullberg B, Allander E, Elffors L, Ranstam J, Dequeker J, Dilsen G, Gennari C, Vaz AL, Lyritis G, Mazzuoli G, Miravet L, Passeri M, Perez Cano R, Rapado A, Ribot C: Risk factors for hip fracture in men from southern Europe: the MEDOS study. Mediterranean Osteoporosis Study. Osteoporos Int 9:45–54, 1999.
108. Rapin CH, Lagier R, Boivin G, Jung A, Mac Gee W: Biochemical findings in blood of aged patients with femoral neck fractures: a contribution to the detection of occult osteomalacia. Calcif Tissue Int 34:465–469, 1982.
109. Patterson BM, Cornell CN, Carbone B, Levine B, Chapman D: Protein depletion and metabolic stress in elderly patients who have a fracture of the hip. J Bone Joint Surg Am 74:251–260, 1992.
110. Delmi M, Rapin CH, Bengoa JM, Delmas PD, Vasey H, Bonjour JP: Dietary supplementation in elderly patients with fractured neck of the femur. Lancet 335:1013–1016, 1990.
111. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R: Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. J Bone Miner Res 15:683–690, 2000.
112. Bourrin S, Ammann P, Bonjour JP, Rizzoli R: Dietary protein restriction lowers plasma insulin-like growth factor I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats. Endocrinology 141:3149–3155, 2000.
113. Bourrin S, Toromanoff A, Ammann P, Bonjour JP, Rizzoli R: Dietary protein deficiency induces osteoporosis in aged male rats. J Bone Miner Res 15:1555–1563, 2000.
114. Ammann P, Laib A, Bonjour JP, Meyer JM, Ruegsegger P,

Rizzoli R: Dietary essential amino acid supplements increase bone strength by influencing bone mass and bone microarchitecture in ovariectomized adult rats fed an isocaloric low-protein diet. J Bone Miner Res 17:1264–1272, 2002.

115. Sugimoto T, Nishiyama K, Kuribayashi F, Chihara K: Serum levels of insulin-like growth factor (IGF) I, IGF-binding protein (IGFBP)-2, and IGFBP-3 in osteoporotic patients with and without spinal fractures. J Bone Miner Res 12:1272–1279, 1997.
116. Gamero P, Sornay-Rendu E, Delmas PD: Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women. Lancet 355:898–899, 2000.
117. Castaneda C, Gordon PL, Fielding RA, Evans WJ, Crim MC: Marginal protein intake results in reduced plasma IGF-I levels and skeletal muscle fiber atrophy in elderly women. J Nutr Health Aging 4:85–90, 2000.
118. Tkatch L, Rapin CH, Rizzoli R, Slosman D, Nydegger V, Vasey H, Bonjour JP: Benefits of oral protein supplementation in elderly patients with fracture of the proximal femur. J Am Coll Nutr 11:519–525, 1992.
119. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR: A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. Am J Clin Nutr 73:118–122, 2001.

Received September 9, 2005

Review

The Role of Dairy Foods in Weight Management

Michael B. Zemel, PhD, FACN

The University of Tennessee, Knoxville, Tennessee

Key words: calcium, dairy, whey, obesity, adipocyte, ACE inhibition, branched chain amino acids

Dietary calcium appears to play a pivotal role in the regulation of energy metabolism and obesity risk. High calcium diets attenuate body fat accumulation and weight gain during periods of over-consumption of an energy-dense diet and to increase fat breakdown and preserve metabolism during caloric restriction, thereby markedly accelerating weight and fat loss. This effect is mediated primarily by circulating calcitriol, which regulates adipocyte intracellular Ca^{2+}. Studies of human adipocyte metabolism demonstrate a key role for intracellular Ca^{2+} in regulating lipid metabolism and triglyceride storage, with increased intracellular Ca^{2+} resulting in stimulation of lipogenic gene expression and lipogenesis and suppression of lipolysis, resulting in adipocyte lipid filling and increased adiposity. Moreover, the increased calcitriol produced in response to low calcium diets stimulates adipocyte Ca^{2+} influx and, consequently, promotes adiposity, while higher calcium diets inhibit lipogenesis, promote lipolysis, lipid oxidation and thermogenesis and inhibit diet-induced obesity in mice. Notably, dairy sources of calcium exert markedly greater effects in attenuating weight and fat gain and accelerating fat loss. This augmented effect of dairy products versus supplemental calcium has been localized, in part, to the whey fraction of dairy and is likely due to additional bioactive compounds, such as angiotensin converting enzyme (ACE) inhibitors in dairy, as well as the rich concentration of branched chain amino acids, which act synergistically with calcium to attenuate adiposity; however, these compounds do not fully account for the observed effects, as whey has significantly greater bioactivity than found in these compounds. These concepts are confirmed by epidemiological data as well as recent clinical trials which demonstrate that diets which include at least three daily servings of dairy products result in significant reductions in body fat mass in obese humans in the absence of caloric restriction and markedly accelerates the weight and body fat loss secondary to caloric restriction compared to low dairy diets. These data indicate an important role for dairy products in both the ability to maintain a healthy weight and the management of overweight and obesity.

Key teaching points:

- Dietary calcium modulates circulating calcitriol (1,25-dihydroxyvitamin D) levels that in turn regulate intracellular calcium which affects fat metabolism in human adipocytes.
- Reducing calcitriol levels by increasing dietary calcium results in reduction of body fat in the absence of caloric restriction, substantially increases body weight and fat loss during caloric restriction and reduces weight and fat regain following successful weight loss.
- Dairy sources of calcium are markedly (50–100%) more effective than supplemental calcium in reducing body weight and body fat during caloric restriction. A portion of this additional anti-obesity bioactivity is attributable to the ACE-inhibitory activity of dairy and to the rich concentration of branched chain amino acids.
- This anti-obesity effect of dietary calcium/dairy is supported by cellular mechanistic studies, animal studies human epidemiological studies and clinical trials.
- Incorporating dairy into weight management regimens is associated with significant preservation of lean body mass during caloric restriction.

Address reprint requests to: Michael B. Zemel, PhD, FACN, The University of Tennessee, 1215 W. Cumberland Ave, Room 229, Knoxville, TN 37996-1920. E-mail: mzemel@utk.edu

Journal of the American College of Nutrition, Vol. 24, No. 6, 537S–546S (2005)
Published by the American College of Nutrition

INTRODUCTION

A substantial body of data has emerged over the last five years to indicate that dietary calcium and dairy foods modulate adipocyte lipid metabolism and energy partitioning between adipose tissue and lean body mass, resulting in a significant "anti-obesity" effect. This effect is supported by a clear mechanistic framework, prospective and retrospective epidemiological reports and observational studies, secondary analyses of past clinical trials originally conducted with other primary endpoints (i.e., skeletal, cardiovascular) and prospective clinical trials. Further, these findings are evident in populations of multiple ages and ethnicities, suggestive of a generally robust effect, as discussed in this review.

MECHANISMS

A compelling mechanism for the anti-obesity effect of dietary calcium was provided by our studies of the mechanism of action of the *agouti* gene in regulating murine and human adipocyte metabolism [1–21]. These studies demonstrated a key role for intracellular Ca^{2+} in the regulation of adipocyte metabolism, resulting in modulation of adipocyte triglyceride stores; intracellular Ca^{2+} is regulated by calcitrophic hormones, and this provides the primary mechanistic basis for the anti-obesity effect of dietary calcium.

This regulation of adipocyte lipid metabolism by intracellular Ca^{2+} provides the key framework for dietary calcium modulation of adiposity. We have found both parathyroid hormone [4] and 1,25-$(OH)_2$-D [22,23] stimulate rapid increases in human adipocyte intracellular Ca^{2+}; accordingly, suppression of these hormones by increasing dietary calcium facilitates re-partitioning of dietary energy from lipid storage to lipid oxidation and thermogenesis. Although both parathyroid hormone and 1,25-$(OH)_2$-D both modulate adipocyte intracellular Ca^{2+}, a growing body of evidence indicates that 1,25-$(OH)_2$-D plays a pivotal role in modulation of lipid metabolism, although an additional possible role for parathyroid hormone has not been excluded. Human adipocytes possess membrane (non-genomic) vitamin D receptors which transduce a rapid intracellular Ca^{2+} response to 1,25-$(OH)_2$-D_3 [23,24]; consequently, 1,25-$(OH)_2$-D_3 treatment of human adipocytes results in coordinated activation of fatty acid synthase expression and activity and suppression of lipolysis, leading to an expansion of adipocyte lipid storage [22,24,25]. However, it should be noted that while these data provide a plausible mechanism of action based on *in vitro* studies in human adipocytes, the direct effect of calcitrophic hormones on human adipocyte metabolism has not yet been assessed utilizing *in vivo* techniques, such as microdialysis. Nonetheless, a potential role of 1α,25-$(OH)_2$-D_3 in human obesity is suggested by other data. Polymorphisms in the nuclear vitamin D receptor (nVDR) gene are associated with susceptibility to obesity in humans [26,27], and several lines of evidence demonstrate an alteration of the vitamin D-endocrine system in obese humans, with an increase in circulating 1α,25-$(OH)_2$-D_3 level [28,29]. These observations, coupled with the direct effects of 1α,25-$(OH)_2$-D_3 on adipocyte lipid metabolism, strongly implicate the increase in 1α,25-$(OH)_2$-D_3 found on low calcium diets as a contributory factor to excess adiposity.

In addition to regulating adipocyte metabolism via a non-genomic membrane receptor (the membrane-associated rapid response to steroid, or MARRS protein) [23,30,31], 1α,25-$(OH)_2$-D_3 also acts via the "classical" nuclear vitamin D receptor in adipocytes to inhibit the expression of uncoupling protein2 (UCP2) [32]; further, suppression of 1,25-$(OH)_2$-D_3 levels by feeding high calcium diets to mice results in increased adipose tissue UCP2 expression and attenuation of the decline in thermogenesis which otherwise occurs with energy restriction [25], suggesting that high calcium diets may also affect energy partitioning by suppressing 1,25-$(OH)_2$-D_3-mediated inhibition of adipocyte UCP2 expression. However, the role of UCP2 in thermogenesis is not clear, and the observed thermogenic effect may be mediated by other, as of yet unidentified mechanisms. Moreover, thermogenic effects of dietary calcium and/or dairy products have not yet been demonstrated in humans. Nonetheless, in addition to inducing a mitochondrial proton leak, UCP2 serves to mediate mitochondrial fatty acid transport and oxidation, suggesting that 1,25-$(OH)_2$-D_3 suppression of UCP2 expression may still contribute to decreased fat oxidation and increased lipid accumulation on low calcium diets [32].

Recent data demonstrate that 1,25-$(OH)_2$-D_3 may also modulate adiposity by inhibiting adipocyte apoptosis [33]. This effect is mediated, in part, via inhibition of UCP2 expression and a consequent increase in mitochondrial potential, a key regulator of apoptosis, and in part via 1,25-$(OH)_2$-D_3 regulation of cytosolic Ca^{2+} and of Ca^{2+} flux between endoplasmic reticulum and mitochondria [33 and unpublished data]. Consequently, adipocyte apoptosis is significantly impaired in association with increased 1,25-$(OH)_2$-D_3 levels in mice fed low calcium diets, while there is a marked increase in adipocyte apoptosis in mice fed high calcium and/or high dairy diets [33]. An integrated summary of these mechanisms is shown in Fig. 1.

Increasing dietary calcium may also result in increased fecal fatty acid excretion and, accordingly, it is possible that the resultant increase in fecal energy loss could contribute to the anti-obesity effects of dietary calcium. In support of this concept, Papakonstantinou et al [34] demonstrated that a high calcium diet produced a substantial increase in fecal fat and energy excretion, and attributed the observed reduction in adiposity to fecal energy loss, although a marked decrease in circulating 1,25-$(OH)_2$-D_3 was found as well. More recently, Jacobsen et al [35] reported that a short-term increase in calcium intake from 500 to 1800 mg/day increased fecal fat excretion ~2.5-fold, from 5.9 to 14.2 g/day. However, while such an increase in fecal fat loss will clearly contribute to a

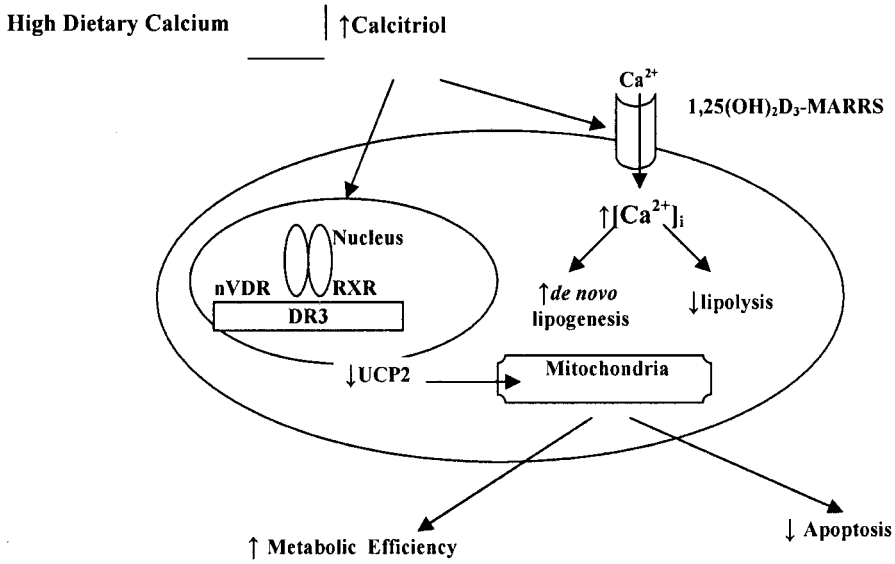

Fig. 1. An integrated summary of mechanisms.

reduction in energy balance, it required a larger level of calcium (1800 mg vs. 1200 mg used in clinical trials of calcium and obesity) to produce a quantitatively small effect (8.3 g additional fecal fat, representing a 75 kcal/day loss) which is insufficient to explain the magnitude of the effects observed in clinical trials (discussed later in this review). Previous studies also demonstrated that large increases in dietary calcium (2–4 g/day) result in statistically significant, but modest, increases in fecal fat loss [36–38]. For example, a supplement of 2 g calcium increased fecal fat excretion from 6.8% to 7.4% of total fat intake [37]. In contrast, in order to achieve a clinically meaningful (albeit modest) contribution to weight loss, the pancreatic lipase inhibitor orlistat must produce approximately a 30% inhibition of total dietary fat absorption, versus the approximately 1–2% found with dietary calcium. Thus, while calcium-inhibition of fat absorption may contribute to an anti-obesity effect, this effect is too small to explain the observed effects. Instead, the primary effect appears to be inhibition of calcitrophic hormone effects on adipocyte energy storage and utilization.

Other Dairy Components

Although dietary calcium appears to inhibit adiposity via the aforementioned 1,25-$(OH)_2$-D_3 mechanisms, data from clinical trials, rodent studies and population studies all indicate a substantially (~two-fold) greater effect of dairy versus supplemental sources of calcium in attenuating adiposity. Accordingly, it is important to identify the additional component(s) of dairy that may be responsible for this augmentation. Our preliminary studies in mice isolate a portion of this additional dairy-derived bioactivity to the whey fraction [39]. Likely candidates for this additional bioactivity include the branched chain amino acid content of dairy protein and specific bioactive whey-derived peptides.

Dairy contains a number of bioactive compounds, which may act either independently or synergistically with calcium to affect lipogenesis, lipolysis, lipid oxidation and/or energy partitioning. Among these, the significant angiotensin converting enzyme (ACE) inhibitory activity contained in whey protein may be relevant to adipocyte lipid metabolism. Angiotensin II upregulates adipocyte fatty acid synthase expression [reviewed in 40], and ACE inhibition mildly attenuates obesity in both mice and in hypertensive patients. Consequently, since adipose tissue has an autocrine renin-angiotensin system, it is possible that a whey-derived ACE inhibitor may contribute to the anti-obesity effects of dairy.

In support of these concepts, a whey-derived ACE inhibitor significantly augmented the effects of dietary calcium on weight and fat loss in energy-restricted mice [39]. However, the combination of the calcium and ACE inhibitor was markedly less potent than either milk or whey in reducing body fat; moreover, milk and whey both substantially preserved skeletal muscle mass during energy restriction while calcium and the calcium/ACE inhibitor combination were without effect. Consequently, although calcium plays a significant role in weight management, and this effect is enhanced by whey-derived ACE-inhibition, a significant portion of the dairy effect remains unexplained. While it is likely that the protective effects of dairy on muscle mass may be attributable to the branched chain amino acid content of whey protein (discussed below), this is unlikely to explain the additional effects of whey on adiposity. An evaluation of whey-derived mineral mix versus calcium carbonate indicates that the other minerals contained in whey do not contribute to the anti-obesity effects of whey [39, and unpublished data]. Present studies in progress are directed

towards identification of the additional components which contribute to the additional anti-obesity bioactivity of dairy.

Although it may be tempting to speculate that the protein content of dairy may play a role in mediating the anti-obesity effect, studies demonstrating an anti-obesity effect of dairy products in both rodents and humans have maintained constant levels of protein intake. Accordingly, the protein content of dairy and whey *per se* cannot be responsible for the additional bioactivity. However, the amino acid composition of dairy protein may play a role. Dairy proteins have a high protein quality score and contain a high proportion (~26%) of branched chain amino acids (BCAA) [41,42]. In addition to supporting protein synthesis, the BCAA (leucine, isoleucine and valine) play specific metabolic roles as energy substrates and in the regulation of muscle protein synthesis, and their potential to participate in these additional metabolic processes are limited by their availability, with first priority provided to new protein synthesis [recently reviewed by Layman, 41]. Accordingly, only diets which provide leucine at levels which exceed requirements for protein synthesis can fully support the intracellular leucine levels required to support additional signaling pathways [41]. Consequently, the abundance of leucine in both casein and whey is of particular interest, as it plays a distinct role in protein metabolism and a pivotal role in translation initiation of protein synthesis [43]. Accordingly, the high concentration of BCAA, and leucine in particular, in dairy products may be an important factor in the re-partitioning of dietary energy from adipose tissue to skeletal muscle [44–46]. This suggests an interaction between the high levels of calcium in dairy in combination with the BCAA content of dairy protein, possibly in concert with other dairy-derived bioactive compounds may work in synergy to minimize adiposity and maximize lean mass.

Modulation of Central Adiposity

Both rodent and human studies demonstrate a shift in the distribution of body fat loss on high versus low calcium diets during energy restriction. In rodents, high calcium and high dairy diets produce a preferential loss of visceral adipose tissue [22,25], while clinical trials demonstrate a preferential loss of fat from the trunk region (i.e. an increase in trunk fat loss as a percentage of total fat loss) [47–50]. Recent studies describing the role of autocrine production of cortisol by adipose tissue provide a plausible and likely mechanism for this effect as well.

Human adipose tissue expresses significant 11 β-hydroxysteroid dehydrogenase-1 (11 β-HSD-1), which can generate active cortisol from cortisone, and visceral adipose tissue exhibits greater 11 β-HSD-1 expression than does subcutaneous adipose tissue [51,52]. Further, selective overexpression of 11-β-HSD-1 in white adipose tissue of mice results in central obesity [53,54], while homozygous 11 β-HSD-1 knockout mice exhibit protection from features of the metabolic syndrome [55]. We have recently found 1,25-$(OH)_2$-D_3 to exert both short-term and long-term regulation of 11 β-HSD-1 in human adipocytes, resulting in ~2-fold increases in 11β-HSD-1 expression and up to 6-fold increases in net cortisol production (56). Thus, the increase in 1,25-$(OH)_2$-D_3 found on low calcium diets is likely to cause selective expansion of visceral adipose tissue, while the observed selective loss of central adiposity on high calcium and high dairy diets appears to be attributable to a reduction in cortisol production by visceral adipocytes [56].

ANIMAL STUDIES

We have confirmed the anti-obesity effect of dietary calcium and dairy products in a series of studies conducted in transgenic mice which express the *agouti* gene in adipose tissue under the control of the aP2 promoter, similar to the human pattern of expression of *agouti* and other obesity-associated genes [22,25,57–60]. These mice are not obese when fed standard chow diets but are susceptible to adult-onset diet-induced obesity. They respond to low calcium diets with accelerated weight gain and fat accretion, while high calcium diets markedly inhibit lipogenesis, accelerate lipolysis, increase thermogenesis and suppress fat accretion and weight gain in animals maintained at identical caloric intakes [22]. Further, low calcium diets impede body fat loss while high calcium diets markedly accelerate weight and fat loss in transgenic mice subjected to identical levels of caloric restriction [25,57–60]. However, there is one report indicating lack of effect of increasing calcium intake on body weight and body fat in rats and mice [61]. The reason for this difference is not apparent, but may be related to the use of older animals with more fully established obesity, as well as the lack of an energy restriction protocol. However, studies in other animal models (Zucker lean and obese rats, Wistar rats and Spontaneously Hypertensive rats) confirm the observation that increased calcium intake lowers body weight and fat content [34,62,63].

Dietary calcium and dairy also alter the partitioning of dietary energy during re-feeding following weight loss in aP2-agouti transgenic mouse model [64]. Although post-obese mice fed a low calcium diet rapidly regained all of the weight and fat that had been lost, re-feeding high calcium diets prevented the suppression of adipose tissue lipolysis and fat oxidation that otherwise accompanies post-dieting repletion and markedly upregulated indices of skeletal muscle fat oxidation [64]. Consequently, although animals re-fed low calcium diets rapidly regained all of the weight and fat that had been lost, animals fed high calcium diets exhibited a 50–85% reduction in weight and fat gain; moreover, dairy exerted markedly greater effects than supplemental calcium on fat oxidation and fat gain [64]. These data are supported by both clinical trials and observational data, as described in the next sections.

CLINICAL STUDIES

The original concept of calcium and dairy modulation of body composition and weight management emerged from data from a hypertension clinical trial, with subsequent corroboration via secondary analysis of other clinical trials originally conducted with skeletal outcomes and finally prospective clinical trials to evaluate the effects of calcium and dairy on adiposity. In the hypertension study, dietary calcium was increased from ~400 to ~1,000 mg/day in obese African Americans without altering dietary energy or macronutrient content. Although body weight did not change, there was a 4.9 kg reduction in body fat [22], which led to the subsequent mechanistic investigations already described. Heaney and colleagues subsequently re-analyzed a series of calcium intervention studies originally designed with primary skeletal endpoints that support a calcium-body weight linkage [65–67]. In an analysis of nine studies, including three controlled trials and six observational studies, a significant negative association between calcium intake and body weight was noted for all age groups studied (third, fifth and eight decades of life). The odds ratio for being overweight was 2.25 for young women below the median calcium intake compared to those above median calcium intake [65], and the controlled trials supported this relationship [65–67]. Overall, increased calcium intake was consistently associated with reduced indices of adiposity (body weight, body fat and/or weight gain); the aggregate effect was each 300 mg increase in daily calcium intake was associated with a 3 kg lower weight in adults and a 1 kg decrease in body fat in children.

Randomized Clinical Trials

Several clinical trials have been conducted to evaluate the effects of dietary calcium and/or dairy on adiposity; to date, all available randomized clinical trial data available are from adults. In the first trial [47], 32 obese adults were maintained on balanced caloric-deficit diets (500 kcal/day deficit) and randomized to control (0–1 serving/day and 400 to 500 mg Ca/day supplemented with placebo), high calcium (control diet supplemented with 800 mg Ca/day), or high dairy (3–4 servings of milk, yogurt and/or cheese/day, total Ca intake of 1200–1300 mg/day). Control subjects lost 5.4% of their body weight over a 24-week study, and this loss was increased to 8.6% on the high calcium diet and to 10.9% on the high dairy diet ($p < 0.01$). Fat loss (via DEXA) followed a similar trend, with the high calcium and high dairy diets augmenting the fat loss found on the low calcium diet by 38 and 64%, respectively ($p < 0.01$). This was accompanied by a marked change in the distribution of body fat loss [47], as fat loss from the trunk region represented 19% of the total fat lost on the low calcium diet, and this was increased to 50% of the fat lost on the high calcium diet and 66% on the high dairy diet; this effect has now been explained via calcium/1,25-$(OH)_2$-D modulation of adipose tissue cortisol production [56], as discussed in a preceding section. These findings demonstrate that increasing dietary calcium from suboptimal to adequate levels can enhance the efficacy of an energy-restricted diet in weight and fat loss, while a markedly greater enhancement is found when dairy foods are used compared to calcium supplements [47].

The effects of dairy in augmenting weight and fat loss secondary to caloric restriction have been confirmed in additional clinical trials. A recent follow-up clinical trial of 34 obese subjects consuming a diet supplemented with three servings of yogurt (total calcium intake of ~1,100 mg/day) compared to a placebo control group (calcium intake of 400–500 mg/day) on a balanced calorie-deficit (−500 kcal/day) for 12 weeks supports these findings [48]. Both groups lost weight, but the yogurt group lost 61% more fat (4.43 vs. 2.75 kg) and 81% more trunk fat (3.16 vs. 1.74 kg) than the control group ($p < 0.001$). Similar to the first clinical trial, the fraction of fat lost from the trunk was markedly higher on the yogurt diet vs. control (60.0 vs. 26.4%). Moreover, there was a significant 31% reduction in the loss of lean tissue mass during energy restriction in the yogurt group compared to the control group. No adverse effects were observed on any serum lipid fraction in either of these trials, and there was an improvement in insulin sensitivity, glucose tolerance and blood pressure in the dairy groups in both trials [47,48]. These findings have been extended in a multi-center trial of 105 overweight and obese adults conducted at The University of Tennessee, Purdue University, USDA, ARS, Western Human Nutrition Research Center at the University of California-Davis, and The Ohio State University [50]. The design was similar to the first clinical trial, with subjects randomized to low calcium, high calcium and high dairy groups on balanced deficit (−500 kcal/day) diets for 12-weeks. Although the calcium supplement exerted little effect, the high dairy diet resulted in significant, marked (~2-fold) increases in fat loss and trunk fat loss, similar to that seen in the first trial [48]. However, in contrast to the first clinical trial [47], the calcium supplement was without significant effect.

These findings have also been replicated in a six-month clinical trial in obese African Americans [49], with essentially similar results. Inclusion of three daily servings of dairy into a balanced deficit diet with no alterations in dietary macronutrients results in ~two-fold increase in weight, fat and trunk fat loss versus those maintained on a low dairy diet. These findings were extended to a six-month study of obese African-American adults in the absence of energy deficit [49]. Isocaloric substitution of three daily servings of dairy products into the diets of obese African-American adults maintained on eucaloric diets for six months results in a 5.4% reduction in total body fat and a 4.6% decrease in trunk fat ($p < 0.01$ for both) in the absence of any change in body weight while the control group maintained on a low calcium/low dairy diet with identical macronutrient composition exhibited no significant changes in total body fat or trunk fat [49]. Bowen et al [68] recently reported

that dairy failed to enhance weight loss during 12 weeks of energy restriction in subjects on high protein diets. However, that work utilized a much higher level of protein intake than that used in the aforementioned trials (34% of energy versus 18%), making a direct comparison difficult, as higher protein intakes have been shown in some studies to be associated with greater weight loss. Indeed, the weight loss found by Bowen et al was approximately twice as high 9.7 vs. 4.99 kg) as that found in the control group in the preceding 12-week study (48). At this higher rate of weight loss (0.8 kg/week), a maximal rate of fat mobilization may already be approached, making additional increments due to dairy (or other factors) unlikely. Moreover, the baseline calcium intakes in the Bowen study were considerably higher (899 and 787 mg/day for men and women, respectively, assigned to the dairy protein diet, and 935 and 737 mg/day for those assigned to the mixed protein diet) than in the aforementioned clinical trials [47,48], in which baseline calcium intakes were $<$600 mg/day. This was considered critical in order to ensure that the effects of correcting suboptimal intakes were studied, rather than the effects of supplementing near-adequate intakes.

Finally, preliminary data demonstrate that a eucaloric high dairy diet markedly attenuates regain of body weight following successful weight loss compared to a low dairy diet (3.03 vs. 1.02 kg weight regain on low vs. high dairy diet, $p < 0.05$) [69]. Similarly, the high dairy diet attenuated regain of body fat (1.959 vs. 0.773 kg on low vs. high dairy diet, $p < 0.01$), and trunk fat (1.546 vs. 0.218 kg on low vs. high dairy diet, $p < 0.01$), indicating that dairy-rich diets attenuate short-term (12-week) weight, fat and trunk fat regain following weight loss. However, longer term assessments are needed to fully evaluate this phenomenon, and are presently in process.

To date, two short-term clinical trials have been conducted to evaluate the mechanisms of the anti-obesity effects of dairy. Both were randomized crossover design studies conducted to evaluate the effects of one-week on each diet and utilized whole-room calorimeters. In the first, level of calcium intake was without effect on 24-hour energy expenditure or fat oxidation, but significantly increased fecal fat and energy excretion [35], as previously discussed. The second study was based upon an observational study in which calcium intake was positively correlated with whole-body fat oxidation in a whole-room calorimeter, with measured calcium intake explaining $\sim$10% of the variance in 24-hour fat oxidation [70]. In the follow-up study, consumption of a high dairy (3–4 servings/day) significantly increased 24-hour fat oxidation by 30 g/day [71]; however, this effect was only significant under conditions of energy deficit ($-$600 kcal/day) produced by a combination of caloric restriction and physical activity. The high dairy diet also resulted in a decreased respiratory quotient during periods of heightened metabolic activity [71]. Thus, the discrepancy between these findings and those of the previous study may be accounted for by the positive energy balance experienced by subjects in the first study [35], while the increased fat oxidation was only significant in the second study during negative energy balance [71].

OBSERVATIONAL AND EPIDEMIOLOGICAL STUDIES

Although there have been a limited number of clinical trials to date, these clinical data are supported by multiple lines of evidence, including observational data noting an inverse relationship between dietary calcium and/or dairy and body weight and/or body fat in children and adolescents [72–76], younger and older women [77–79], African-American women [78], as well as by epidemiological data from NHANES I [79], NHANES III [22], NHANES 1999–2000 [79], the Continuing Study of Food Intake of Individuals [80], the HERITAGE study [81], the Quebec Family Study [82], the CARDIA study [83] and the Tehran Lipid and Glucose study [84].

In a retrospective analysis of a two-year prospective study of 54 normal-weight Caucasian women participating in an exercise intervention, the dietary calcium : energy ratio and the dairy calcium : energy ratio were significant negative predictors of changes in both body weight and body fat [77]. There was a notable interaction between dietary calcium and energy intake in predicting changes in body fat, as calcium, but not energy, intake predicted changes in body weight and body fat for women below the median energy intake (1,876 kcal/day), while energy intake alone predicted changes in weight and fat in women at higher levels of energy intake. Further, the reported effects of calcium appeared to be specific to dairy sources, as dairy calcium predicted changes in body weight and fat, while non-dairy calcium did not [77]. An inverse relationship between energy-adjusted dietary calcium intake and body mass index was also reported in lactose tolerant, but not lactose intolerant, African-American women [78]. Although the reason for the lack of effect in the lactose intolerant group cannot be definitively inferred from this cross-sectional study, the lactose-intolerant group exhibited a uniformly low calcium intake, presumably due to aversion to dairy products, and the lack of women with adequate calcium intakes in this group therefore precluded a clear relationship emerging as it did for the lactose tolerant women.

While most studies reporting the relationship between dietary calcium and/or dairy and indices of adiposity are in adults, there have been a few studies in children and adolescents [72–76,85,86]. Although one study recently reported no relationship between dietary calcium or dairy consumption in a longitudinal assessment of adolescent females [85], the authors noted that dairy consumption was significantly higher for their study cohort compared to that reported by CSFII for a nationally representative survey of the same age group (428 vs. 269 g/day of milk and milk products). Moreover, overall reported median dairy intake was 2.9 servings of dairy and 827 mg of dairy-derived calcium per day. Accordingly, it is possible that

this cohort represented a relatively high dairy consuming population and therefore was sufficiently above a yet-to-be determined threshold of dairy intake to observe an effect on indices of adiposity. In contrast, several other studies of children and adolescents suggest a protective effect of dairy [73–76,86].

A significant inverse relationship between dietary calcium and body fat was reported in a five-year longitudinal study of preschool children studied from two months of age ($R^2 = 0.51$) [72]. The group subsequently extended these longitudinal findings to eight years of age [73]. Overall, in predictive equations that explain 26–34% of the variability in body fat, variations in dietary calcium explained 7–9% of the variability in adiposity [73]. Notably, these longitudinal data strongly suggest that dairy and calcium intake within the first year of life are significant inverse determinants of body fat levels at age 8 [72,73]. Consistent with these findings, longitudinal data from the Framingham Children's Study indicate that higher intakes of calcium early in life (ages 3–5) were associated with decreased gain of body fat over time (early adolescence), with dairy servings being more strongly correlated to reduced body fat than dietary calcium *per se*.

The associations between dairy intake and incidence of the major components of the insulin resistance syndrome (IRS), including obesity, was evaluated in a 10-year population based prospective study of 3,157 black and white adults [83]. Overweight individuals who consumed the most dairy products had a 72% lower incidence of IRS compared to those with the lowest dairy intakes. Moreover, the cumulative incidence of obesity in those who started the study in the overweight category was significantly reduced from 64.8% in those consuming the least amount of dairy foods to 45.1% in the highest dairy food consuming group. Notably, the inverse relationship between dietary calcium and either IRS or obesity incidence in the CARDIA study was explained solely by dairy intake and was not altered by adjustment for dietary calcium, indicating the presence of an additional effect of dairy beyond the mechanisms already cited for dietary calcium in modulating adiposity and obesity risk; this is consistent with both the experimental animal and clinical trial data which also suggest that other dairy components, in addition to calcium, contribute to an anti-obesity effect.

SUMMARY AND CONCLUSIONS

An anti-obesity effect of dietary calcium and dairy foods is now evident from animal studies, observational and population studies and clinical trials. It is important, however, to interpret these findings within the context of overall energy balance. For example, Berkey et al [87] recently reported that adolescents who consume excess calories from milk exhibit higher gains in body mass index than those who do not; however, when adjusted for energy intake, this effect was not evident. Consistent with this, the reported effects of calcium and dairy on body weight and body composition demonstrate accelerated weight and fat loss on energy restricted diets and improvements in body composition with isocaloric substitution of dairy for other components of the diet. Accordingly, these data should not be interpreted to suggest that increasing dairy intake exerts an anti-obesity effect independent of energy balance.

It is also important to interpret these findings to place these findings within the context of optimal calcium and dairy intake. It appears that the effects of calcium on healthy weight management result from correcting suboptimal intakes and thereby preventing the endocrine response (PTH $-$ 1α,25-$(OH)_2$-D_3 axis) which favors adipocyte energy storage. Accordingly, once adequate dietary calcium levels are achieved, minimal responses would be anticipated from further increases in calcium intake, and the available data support this concept. Similarly, the available data indicate that substantial improvements in adiposity are unlikely to result from increasing dairy intake beyond an optimal range (approximately three daily servings).

While there is a strong theoretical framework in place to explain the effects of dietary calcium on energy metabolism, the precise mechanisms whereby dairy products exert substantially greater effects than equivalent amounts of calcium are not yet clear. However, the additional dairy effect appears to be mediated, in part, by several bioactive compounds, including angiotensin converting enzyme inhibitors, the high concentration of branched chain amino acids in dairy protein and other components which have not yet been identified. These data provide the framework for the development of strategies to utilize dairy products and dairy ingredients for the prevention of overweight and obesity and, in conjunction with controlling energy balance, for effective weight management.

REFERENCES

1. Jones BH, Kim JH, Zemel MB, Woychik RP, Michaud EJ, Wilkison WO, Moustaid N: Upregulation of adipocyte metabolism by *agouti* protein: Possible paracrine actions in yellow mouse obesity. Am J Physiol 20:E192–E196, 1996.
2. Zemel MB, Kim JH, Woychik RP, Michaud EJ, Kadwell SH, Patel IR, Wilkison WO: *Agouti* regulation of intracellular calcium: Role in the insulin resistance of viable yellow mice. Proc Nat Acad Sci USA 92:4733–4737, 1995.
3. Xue B, Moustaid-Moussa N, Wilkison WO, Zemel MB: The *agouti* gene product inhibits lipolysis in human adipocytes via a Ca^{2+} dependent mechanism. FASEB J 12:1391–1396, 1998.
4. Xue B, Greenberg AG, Kraemer FB, Zemel MB: Mechanism of intracellular calcium inhibition of lipolysis in human adipocytes. FASEB J 15:2527–2529, 2001.
5. Kim JH, Mynatt RL, Moore JW, Woychik RP, Moustaid N, Wilkison WO, Zemel MB: The effects of calcium channel blockade on *agouti*-induced obesity. FASEB J 10:1646–1652, 1996.
6. Claycombe KH, Wang Y, Jones BH, Kim S, Wilkison WO, Zemel MB, Chun J, Moustaid-Moussa N: Transcriptional regulation of the adipocyte fatty acid synthase gene by the *agouti* gene product: Interaction with insulin. Physiological Genomics 3:157–162, 2000.

7. Shi H, Moustaid-Moussa N, Wilkison WO, Zemel MB: Role of the sulfonylurea receptor in regulating human adipocyte metabolism. FASEB J 13:1833–1838, 1999.
8. Xue B, Zemel MB: Relationship between human adipose tissue agouti and fatty acid synthase (FAS). J Nutr 130:2478–2481, 2000.
9. Voisey J, Imbeault P, Hutley L, Prins JB, van Daal A: Body mass index-related human adipocyte *agouti* expression is sex-specific but not depot-specific. Obes Res 10:447–452, 2002.
10. Zemel MB, Xue B: Agouti/melanocortin interactions with leptin pathways in obesity. Nutr Rev 1998; 56:271–274, 1998.
11. Tebar F, Soley M, Ramirez I: The antilipoytic effects of insulin and epidermal growth factor in rat adipocytes are mediated by different mechanisms. Endocrinology 137:4181–4188, 1996.
12. Xue B, Wilkison WO, Mynatt RL, Moustaid N, Goldman M, Zemel MB: The *agouti* gene product stimulates pancreatic β cell signaling and insulin release. Physiol Genomics 1:11–19, 1999.
13. Mynatt RL, Miltenberger RJ, Klebig ML, Zemel MB, Wilkinson JE, Wilkison WO, Woychik RP: Combined effects of insulin treatment and adipose tissue-specific agouti expression on the development of obesity. Proc Natl Acad Sci USA 94, 919–922, 1997.
14. Mynatt RL, Truett GE: Influence of agouti protein on gene expression in mouse adipose tissue [Abstract]. FASEB J 14:A733, 2000.
15. Zemel MB, Mynatt RL, Dibling D: Synergism between diet-induced hyperinsulinemia and adipocyte-specific agouti expression [Abstract]. FASEB J 13:660.3, 1999.
16. Kwon HY, Bultman SJ, Loffler C, Chen W, Furdon PJ, Powell JG, Usala AL, Wilkison W, Hansmann I, Woychik RP: Molecular structure and chromosomal mapping of the human homolog of the agouti gene. Proc Natl Acad Sci USA 91:9760–9764, 1994.
17. Kim JH, Mynatt RL, Moore JW, Woychik RP, Moustaid N, Wilkison WO, Zemel MB: The effects of calcium channel blockade on *agouti*-induced obesity. FASEB J 10:1646–1652, 1996.
18. Standridge M, Alemzadeh R, Zemel M, Koontz J, Moustaid-Moussa N: Diazoxide down-regulates leptin and lipid metabolizing enzymes in adipose tissue of Zucker rats. FASEB J 14:455–460, 2000.
19. Alemzadeh R, Slonim AE, Zdanowicz MM, Maturo J: Modification of insulin resistance by diazoxide in obese Zucker rats. Endocrinology 133:705–712, 1993.
20. Alemzadeh R, Jacobs AW, Pitukcheewnont P: Antiobesity effect of diazoxide in obese Zucker rats. Metabolism 45:334–341, 1996.
21. Alemzadeh R, Langley G, Upchurch L, Smith P, Slonim AE: Beneficial effect of diazoxide in obese hyperinsulinemic adults. J Clin Endocr Metab 1998; 83:1911–1915.
22. Zemel MB, Shi H, Greer B, DiRienzo D, Zemel PC: Regulation of adiposity by dietary calcium. FASEB J 14:1132–1138, 2000.
23. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB: 1α, 25-dihyroxyvitamin D3 modulates human adipocyte metabolism via non-genomic action. FASEB J 14:2751–2753, 2001.
24. Zemel MB: Role of calcium and dairy products in energy partitioning and weight management. Am J Clin Nutr 79:907S–912S, 2004.
25. Shi H, DiRienzo D, Zemel MB: Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. FASEB J 15:291–293, 2001.
26. Ye WZ, Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G: Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. Eur J Endocrinol 145:181–186, 2001.
27. Barger-Lux MJ, Heaney RP, Hayes HF, DeLuca HF, Johnson ML, Gong G: Vitamin D receptor gene polymorphism, bone mass, body size, and vitamin D receptor density. Calcif Tissue Int 57:161–162, 1995.
28. Andersen T, McNair P, Hyldstrup L, Fogh AN, Nielsen TT, Astrup A, Transbol I: Secondary hyperparathyroidism of morbid obesity regresses during weight reduction. Metabolism 37:425–428, 1988.
29. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S: Evidence for alteration of the vitamin D-endocrine system in obese subjects. J Clin Invest 76:370–373, 1985.
30. Nemere I, Safford SE, Rohe B, DeSouza MM, Farach-Carson MC: Identification and characterization of 1,25-$(OH)_2$-D_3 membrane-associated rapid response, steroid ($1,25D_3$-MARRS) binding protein. J Steroid Biochem Mol Biol 89–90:281–285, 2004.
31. Nemere I, Farach-Carson MC, Rohe B, Sterling TM, Norman AW, Boyan BD, Safford SE: Ribozyme knockdown functionally links a $1,25(OH)_2D_3$ membrane binding protein ($1,25D_3$-MARRS) and phosphate uptake in intestinal cells. Proc Nat Acad Sci USA 101:7392–7397, 2004.
32. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB: 1,25-dihydroxyvitamin D_3 inhibits uncoupling protein 2 expression in human adipocytes. FASEB J 16:1808–1810, 2002.
33. Sun X, Zemel MB: Mechanisms of dual effects of 1α, 25-dihydroxyvitamin D_3 on adipocyte apoptosis. FASEB J 18:1430–1432, 2004.
34. Papakonstantinou E, Flatt WP, Huth PJ, Harris RBS: High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. Obesity Res 11:387–394, 2003.
35. Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen K, Astrup A: Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. Int J Obesity 29:292–301, 2005.
36. Denke MA, Fox MM, Schulte MC: Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J Nutr 123:1047–1053, 1993.
37. Welberg JW, Monkelbaan JF, de Vries EG, Muskiet FA, Cats A, Oremus ET, Boersma-van Ek W, van Rijsbergen H, van der Meer R, Mulder NH, et al: Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. Ann Nutr Metabo 38:185–191, 1994.
38. Shahkhalili Y, Murset C, Meirim I, Duruz E, Guinchard S, Caradini C, Acheson K: Calcium supplementation of chocolate: Effect on cocoa butter digestibility and blood lipids in humans. Am J Clin Nutr 73:246–252, 2001.
39. Causey KR, Zemel MB: Dairy augmentation of the anti-obesity effect of Ca in aP2-agouti transgenic mice [Abstract]. FASEB J A746, 2003.
40. Zemel MB: Role of dietary calcium and dairy products in modulating adiposity. Lipids 2003;38:139–146.
41. Bos C, Gaudichon C, Tome D: Nutritional and physiological criteria in the assessment of milk protein quality for humans. J Am Coll Nutr 19:191S–205S, 2000.
42. Layman DK: The role of leucine in weight loss diets and glucose homeostasis. J Nutr 133:261S–267S, 2003.

43. Anthony JC, Anthony TG, Kimball SR, Jefferson LS: Signaling pathways involved in translational control of protein synthesis in skeletal muscle by protein. J Nutr 131:856S–860S, 2000.
44. Garlick PJ, Grant I: Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Biochem J 254:579–584, 1988.
45. Ha E, Zemel MB: Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. J Nutr Biochem 14:251–258, 2003.
46. Fouillet H, Mariotti F, Gaudichon C, Bos C, Tome D: Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. J Nutr 132:125–133, 2002.
47. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P: Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. Obes Res 12:582–590, 2004.
48. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E: Dairy augmentation of total and central fat loss in obese subjects. Int J Obesity 29:391–397, 2005.
49. Zemel MB, Milstead A, Richards J, Campbell P: Effects of calcium and dairy on body composition and weight loss in African American adults. Obes Res 13:1218–1225, 2005.
50. Zemel MB, Teegarden D, Van Loan M, Schoeller DA, Matkovic V, Lyle M, Craig BA: Role of dairy products in modulating weight and fat loss: A multi-center trial [Abstract]. FASEB J 18:566.5, 2004.
51. Seckl JR, Walker BR: 11 β-hydroxysteroid dehydrogenase Type 1—a tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376, 2001.
52. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, Walker BR: Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metabol 86:1418–1421, 2001.
53. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. Science 294:2166–2170, 2001.
54. Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, Shinyama H, Shart MGF, Fleming S, Mullins JJ, Seckl JR, Flier JS: Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. J Clin Invest 112:83–90, 2003.
55. Kotelevstev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ: 11β-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia in obesity or stress. Proc Natl Acad Sci USA 94:14924–14929, 1997.
56. Morris KL, Zemel MB: 1,25-dihydroxyvitamin D_3 modulation of adipocyte glucocorticoid function. Obesity Res 2005 (in press).
57. Zemel MB, Sun X, Geng X: Effects of a calcium-fortified breakfast cereal on adiposity in a transgenic mouse model of obesity [Abstract]. FASEB J 15: A598, 480.7, 2001.
58. Zemel MB, Geng X: Dietary calcium and yogurt accelerate body fat loss secondary to caloric restriction in aP2-agouti transgenic mice. Obesity Res 9:146S, 2001.
59. Zemel MB, Morgan K: Interaction between calcium, dairy and dietary macronutrients in modulating body composition in obese mice. FASEB J 16:A369, 2002.
60. Causey KR, Zemel MB: Dairy augmentation of the anti-obesity effect of Ca in aP2-agouti transgenic mice. FASEB J A746:453.7, 2003.
61. Zhang Q, Tordoff MG: No effect of dietary calcium on body weight of lean and obese mice and rats. Am J Physiol R669–677, 2004.
62. Bursey RD, Sharkey T, Miller GD: High calcium intake lowers weight in lean and fatty Zucker rats. FASEB J 3:A265, 1989.
63. Metz JA, Karanja N, Torok J, McCarron DA: Modification of total body fat in Spontaneously Hypertensive rats and Wistar-Kyoto rats. Am J Hypertension 1:58–60, 1988.
64. Sun X, Zemel MB: Calcium and dairy products inhibit weight and fat regaind during ad libitum consumption following energy restriction in aP2-agouti transgenic mice. J Nutr 3054–3060, 2004.
65. Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, Hinders S: Calcium intake and body weight. J Clin Endocrinol Metab 85:4635–4638, 2000.
66. Heaney RP, Davies KM, Bargar-Lux MJ: Calcium and weight: Clinical studies. J Am Coll Nutr 21:152S–155S, 2002.
67. Heaney RP: Normalizing calcium intake: Projected population effects for body weight. J Nutr 133:268S–270S, 2003.
68. Bowen J, Noakes M, Clifton PM: Effect of calcium and dairy foods in high protein, energy-restricted diets on weight loss and metabolic parameters in overweight adults. In J Obesity advance online publication; doi:10,1038.sj.ijo.802895, 2005.
69. Zemel MB: Role of dairy products in the prevention of weight regain following weight loss [Abstract]. FASEB J 19, 2005.
70. Melanson EL, Shart TA, Schneider J, Donahoo WT, Grunwald GK, Hill JO: Relation between calcium intake and fat oxidation in adult humans. Int J Obesity 27:196–203, 2003.
71. Melanson EL, Donahoo WT, Dang F, Ida T, Zemel MB: Effect of low- and high-calcium dairy based diets on macronutrient oxidation in humans. Obes Res, in press, 2005.
72. Carruth BR, Skinner JD: The role of dietary calcium and other nutrients in moderating body fat in preschool children. Int J Obesity 25:559–566, 2001.
73. Skinner JD, Bounds W, Carruth BR, Ziegler P: Longitudinal calcium intake is negatively related to children's body fat indexes. JADA 103:1626–1631, 2003.
74. Barba G, Troiano E, Russo P, Venezia A, Siani A: Inverse association between body mass and frequency of milk consumption in children. Brit J Nutr 93:15–19, 2005.
75. Tanasescu M, Ferris AM, Himmelgreen DA, Rodriguez N, Perez-Escamilla R: Biobehavioral factors are associated with obesity in Puerto Rican children. J Nutr 130:1734–1742, 2000.
76. Novotny R, Daida YG, Acharya S, Grove JS, Vogt TM: Dairy intake is associated with lower body fat and soda intake with greater weight in adolescent girls. J Nutr 134:1905–1909, 2004.
77. Lin Y-C, Lyle RM, McCabe LB, McCabe GB, Weaver CM, Teegarden D: Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. J Am Coll Nutr 19:754–760, 2000.
78. Buchowski MS, Semenya J, Johnson AO: Dietary calcium intake in lactose maldigesting intolerant and tolerant African-American women. J Am Coll Nutr 21:47–54, 2002.
79. McCarron DA: Calcium and magnesium nutrition in human hypertension. Ann Intern Med 98:800–805, 1983.

80. Albertson AM, Good CK, Holschuh NM, Eldridge EL: The relationship between dietary calcium intake and body mass index in adult women from three National dietary intake databases [Abstract]. FASEB J 18:6259, 2004.
81. Loos R, Rankinen T, Leon A, Skinner JS, Wilmore JH, Rao DC, Bouchard C: Calcium intake is associated with adiposity in black and white men and white women of the HERITAGE family study. J Nutr 134:1772–1778, 2004.
82. Jaqmain M, Doucet E, Despres J-P, Bouchard C, Tremblay A: Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. Am J Clin Nutr 77:1448–1452, 2003.
83. Pereira MA, Jacobs DR, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS: Dairy consumption, obesity, and the insulin resistance syndrome in young adults. The CARDIA study. JAMA 287:2081–2089, 2002.
84. Mirmiran P, Esmaillzadeh A, Azizi F: Dairy consumption and body mass index. An inverse relationship. Int J Obesity 29:115–121, 2005.
85. Phillips SM, Bandini LG, Cyr H, Colclough-Douglas S, Naumova E, Must A: Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period. Int J Obesity 27:1106–1113, 2003.
86. Moore LL, Singer MR, Bradlee ML, Ellison RC: Dietary predictors of excess body fat acquisition during childhood [Abstract]. Presented at 44th American Heart Association Annual Conference on Cardiovascular Disease Epidemiology and Prevention, 2004.
87. Berkey CS, Helain RS, Willett WC, Colditz GA: Milk, dairy fat, dietary calcium and weight gain" A longitudinal study of adolescents. Arch Pediatr Adolesc Med 159:543–550, 2005.

Received September 9, 2005.

Review

Milk Consumption Does Not Lead to Mucus Production or Occurrence of Asthma

Brunello Wüthrich, MD, Alexandra Schmid, Barbara Walther, PhD, Robert Sieber, PhD

Allergy Unit, Department of Dermatology, University Hospital (B.W.), Zurich, Agroscope Liebefeld-Posieux, Swiss Federal Research Station for Animal Production and Dairy Products (ALP) (A.S., B.W., R.S.), Berne, SWITZERLAND

Key words: milk, dairy products, mucus formation, asthma

There is a belief among some members of the public that the consumption of milk and dairy products increases the production of mucus in the respiratory system. Therefore, some who believe in this effect renounce drinking milk. According to Australian studies, subjects perceived some parameters of mucus production to change after consumption of milk and soy-based beverages, but these effects were not specific to cows' milk because the soy-based milk drink with similar sensory characteristics produced the same changes. In individuals inoculated with the common cold virus, milk intake was not associated with increased nasal secretions, symptoms of cough, nose symptoms or congestion. Nevertheless, individuals who believe in the mucus and milk theory report more respiratory symptoms after drinking milk. In some types of alternative medicine, people with bronchial asthma, a chronic inflammatory disease of the lower respiratory tract, are advised not to eat so-called mucus-forming foods, especially all kinds of dairy products. According to different investigations the consumption of milk does not seem to exacerbate the symptoms of asthma and a relationship between milk consumption and the occurrence of asthma cannot be established. However, there are a few cases documented in which people with a cow's milk allergy presented with asthma-like symptoms.

Key teaching points:

- In alternative medicine, a popular belief is that the consumption of milk and dairy products leads to mucus in upper and lower respiratory tracts.
- Sensations associated with increased mucus production are not specific to cow's milk, but are more likely due to physical characteristics of some beverages.
- In rare cases asthma can occur in patients with confirmed food allergy against cow's milk proteins.
- People with asthma are sometimes advised to abstain from the consumption of dairy products, but research shows that consumption of milk does not significantly change various lung function parameters. In addition, limiting dairy food consumption can lead to low intake of many nutrients, including calcium.

INTRODUCTION

Mucus is a film covering the surface of the mucous membrane of the alimentary and respiratory tracts and protects the organism against a variety of mechanical, thermic and chemical irritations. It is a product of secretory epithelial cells and consists of water, mucins, a mixture of fucose-rich glucosaminoglycans (mucopolysaccharides) and sialic acid-rich glycoproteins, lysozyme, immunoglobulins, different inorganic salts, leucocytes and scaled epithelial cells [1–3]. There is a belief among some members of the public that the consumption of milk and dairy products increases the production of mucus in the upper and lower respiratory tracts - and that, therefore, these foods should be removed from the diet. There is no precise explanation for the mechanism behind this recommendation [4, 5]. The belief can be followed back to the Jewish physician Moses Maimonides, living in the 12th century [6]. Traditional Chinese medicine attributes a humidifying effect in humans to an exaggerated consumption of dairy products - with the exception of butter - as well as chocolate, honey and all other natural sweeteners. It is believed this humidity

Address reprint requests to: Dr. Robert Sieber, Agroscope Liebefeld-Posieux, Swiss Federal Research Station for Animal Production and Dairy Products (ALP), Liebefeld, CH-3003 Berne, SWITZERLAND. E-mail: robert.sieber@alp.admin.ch

Journal of the American College of Nutrition, Vol. 24, No. 6, 547S–555S (2005)
Published by the American College of Nutrition

will thicken to mucus with time [7]. Since an excessive mucus production has been documented in people with asthma, it is not surprising that in alternative medicine these patients are advised not to eat so-called mucus-forming foods, especially all kinds of dairy products (milk, cheese, cream, butter) [8]. But individuals excluding milk products from their daily diet lose an important calcium source and a lack of this mineral may lead to nutritional deficiency and to various health disturbances [9].

The aim of this review is to examine the available evidence regarding the question of whether milk consumption leads to increased mucus formation and whether milk is related to the occurrence of asthma.

MUCUS PRODUCTION

Surveys of Dairy Consumption and Mucus

According to some Australian investigations the belief that milk consumption stimulates mucus production is held by approximately 30% of the population and is accordingly associated with a 38% reduction in their liquid milk intake [10, 11]. The authors identified a milk mucus belief [12].

One study was conducted among 345 randomly-selected Australian shoppers. They were asked about general health perceptions of milk and knowledge about the association between milk and disease. Concerning the question of whether consumption of whole, reduced fat and soy beverage increases mucus, 46% of 111 whole milk drinkers, 25% of 121 reduced fat milk drinkers and 11% of 113 soy milk drinkers agreed [13]. In another study conducted in a pediatric pulmonology office, 330 parents received a 9-question anonymous questionnaire regarding the relationship between milk and mucus. Among these parents 58.5% believed and 21.8% did not believe drinking milk increases mucus, and 19.7% were uncertain. Of the 193 believers 58 parents got their information that milk increases mucus from family members, 19 parents heard it from pediatricians, 36 parents had it from other physicians and 5 parents from other healthcare professionals [14].

In another Australian study a questionnaire was sent to people who were convinced that a relationship exists between milk consumption and mucus formation (n = 70, called believers below) and to others who were not convinced of it (n = 99, non-believers). Respondents were recruited from urban areas and from university and hospital campuses. In the first part of this study, the authors used unstructured questions. The subjects were asked to describe exactly what they felt or what happened when they drank milk. The believers mentioned that the most common site where the sensory perception appeared after drinking milk was the throat (94.3%), followed by back of throat (41.4%), nose (37.1%) and mouth (31.4%). The most common symptoms mentioned were clearing of the throat (52.8%), cough (50.0%), swallow (21.4%), spit (21.4%) and catarrh (10.0%). The terms used by the believers to describe this sensory perception were: thick (35.7%), blocked (20.0%), clogged (12.8%), sticky, coating, choked, heavy (each 10.0%) [12].

In another part of the survey, prompted questions were used. Respondents were asked about specific respiratory and gastrointestinal symptoms experienced after drinking milk. Believers and non-believers differed distinctly in the occurrence of symptoms reported. The believers reported more respiratory symptoms such as throat clearing, moist cough, post-nasal drip, blocked nose and other symptoms (Table 1). The majority of believers (63.2%) needed one glass of milk or less to experience the symptoms and most were certain that whole milk (78.6%) and low fat milk (52.9%) caused the effect. The effect among the believers lasted either a few minutes (12.9%), less than an hour (31.4%) or several hours (24.3%). In an additional trial conducted as part of this study 130 individuals completed a "health" questionnaire. The believers (n = 45) reported more respiratory symptoms related to hay fever, bronchitis or asthma than the non-believers (n = 85) [12].

Experimental Studies on Dairy Consumption and Mucus

Pinnock and Arney [15] conducted a randomised, double-blind trial to investigate the relationship between cow's milk consumption and mucus formation, the so called "milk mucus" effect. They divided 125 subjects into a milk (n = 60) or placebo group (n = 65), of which 43 and 29, respectively, believed that cow's milk consumption produces mucus. These subjects received 300 mL of cow's milk or 300 mL of a soy-based drink (placebo). Both drinks were ultra-heat treated and a cocoa-peppermint flavour-combination was found to be

Table 1. Structured Interview: Percentages of Believers and Non-Believers Experiencing Symptoms after Drinking Milk [12]

Symptom	Believers (n = 70)	Non-believers (n = 99)	Significance
Throat clearing	84.3	20.2	**
Moist cough	34.3	4.0	**
Post-nasal drip	32.7	1.2	**
Blocked nose	30.0	1.0	**
Difficulty swallowing	22.9	6.1	**
Runny nose	22.9	0	**
Other	21.4	5.1	**
Difficulty breathing	20.0	1.0	**
Sneezing	12.9	1.0	**
Dry cough	12.9	1.0	**
Watery eyes	11.4	1.0	**
Headache	4.3	0	*
Diarrhoea	4.3	0	ns
Stomach cramps	2.9	0	ns

**= significant at $p < 0.01$

*= significant at $p < 0.05$

ns = non-significant

most effective in disguising both the mouth-feel of milk and the after-taste of the soy drink and were used for a randomized, double-blind trial. The subjects answered a questionnaire before they received a chilled test drink, and repeated the questionnaire five minutes after, four hours after and the following morning. In both groups three out of 14 indicators of a milk and mucus effect (coating over mouth, back of throat; need to swallow a lot; saliva thicker, harder to swallow) showed statistically significant increases, but only immediately following the test drink in both milk and placebo groups (Table 2). These three indicators were analysed with reference to a belief in a relationship between milk drinking and mucus formation as well as to the assumption by the subjects that they were drinking cow's milk. Subjects who believed in a "milk mucus" effect or thought the drink was milk tended to show larger, though not significant, increases in these three indicators: increases in "coating over mouth", "swallow a lot" and "saliva thicker". The authors concluded that it was possible to detect an increase in three "milk mucus" sensations by the believers after drinking both beverages. The effect which was measured was thus not specific to cow's milk and was also produced by the soy-based drink.

In an earlier study by the same researcher, 60 volunteers aged 18 to 35 were inoculated with the common cold virus (rhinovirus-2). Daily respiratory symptoms and milk intake were recorded over a 10-day period. Fifty one people, who had a cold and from whom satisfactory records of milk intake were received, recorded nasal secretion weights and respiratory symptoms (510 person-days of observation). Symptoms of congestion (nasal discharge, blocked nose, loose cough, post-nasal drip) occurred on 245 person-days. Mean weight of nasal secretion did not increase with increasing milk intake (0–1.9, 2–3.9, >4 glasses). Milk intake was not associated with symptoms of cough, nose symptoms or congestion after infection with the rhinovirus (Table 3). Considering the symptoms by belief, "milk mucus" believers were more likely to report symptoms. For example, believers reported dry cough on 22% of observation days but non-believers on only 12% of observation days. This observation was not accompanied by a parallel increase in the more objective measure of mucus weights. The authors summarized that in healthy adult volunteers challenged with the common cold virus, milk intake was not associated with an increase in symptoms of congestion or nasal secretion weight [10].

Earlier, Blumberger *et al.* [16] showed that drinking hot and cold milk or hot and cold water increased the speed of saliva

Table 2. Mean Milk-Mucus Indicator Scores[1,2] (Upper Part) and Significant Increases of These Scores (Lower Part) in Milk and Placebo Groups at Baseline and after Test Drink [15]

Indicator/Symptom	Milk group (n = 60)				Placebo group (n = 65)			
Time[3]	0	1	2	3	0	1	2	3
Feeling in general	78	74	79	70	83	77	80	76
Coating over mouth	32	43	18	27	28	43	20	22
Mucousy/claggy back of throat	35	38	26	27	34	42	24	29
Cough	25	25	16	17	19	21	16	16
Clear throat	31	38	25	29	30	38	26	25
Swallow a lot	30	45	23	21	30	43	22	25
Mucus dropping down throat	22	25	20	19	23	25	16	20
Saliva thicker	13	31	12	14	11	30	13	16
Spit phlegm	22	23	17	20	15	22	17	21
Chest heavy	10	11	8	11	8	10	9	12
Nose breathing difficult	11	10	17	19	15	16	16	18
Mouth breathing difficult	4	5	4	4	5	9	6	5
Coating over mouth, back of throat		++	−	−		++	−	−
Need to swallow a lot		++	−	−		++	−	−
Saliva thicker, harder to swallow		++	−	−		++	−	−
Want to cough/spit up phlegm/mucus*		−	−	−		+	−	+
Mouth breathing difficult		−	−	−		+	−	−
Need to clear throat		−	−	−		+	−	−
Mucousy/claggy at back of throat		−	−	−		+	−	−
Nose breathing difficult*		+	−	−		−	−	−

[1]not all indicators are shown

[2]for the milk-mucus score a hedonic scaling method was used: 0 = not at all, 100 = very much

[3]time 0, 1, 2, 3: the first questionnaire was completed for baseline measurement before milk consumption (time 0), the second after 5 min (time 1), the third after 4 h (time 2) and the fourth before breakfast on the following day (time 3)

++= significant at $p < 0.01$

+= significant at $p < 0.05$

−= non significant

*= Difference between milk and placebo groups significant at $p < 0.05$

Table 3. Mean Nasal Secretion Weight and Percentages of Symptoms of Cough, Nose or Congestion by Milk Intake [10]

Milk intake glasses	Mucus weight[1] g	Loose cough %	Loose cough/ Total cough	Nose[2] %	congested[3] %
0–1.9	1.32	15.5	0.58	36.9	46.0
2–3.9	0.86	18.6	0.63	37.6	52.4
> 4	1.15	15.0	0.74	37.2	43.4
Significance	ns	ns	ns	ns	ns

[1]Nasal secretion weight
[2]runny/stopped-up nose
[3]one or more of runny, blocked nose, postnasal drip, or loose cough
ns = non significant

secretion by as much as twice the initial value. However, the concentration of neuraminic acid and hexosamine, and therefore also the concentration of the mucopolysaccharides responsible for the viscosity, decreased during drinking. In no case could they show a clear increase in the mucus content of the saliva after milk consumption.

The possibility that milk consumption increases the viscosity or "thickness" of mucus could be explained by the fact that consumption of an emulsion such as milk can lead to droplet floculation after mixing with saliva. This aggregation affects the mouth feel and other sensory aspects [17] and the sensation may be mistaken for mucus.

ASTHMA

Bronchial asthma is a chronic inflammatory disease of the lower respiratory tract (bronchi) and includes swelling, bronchoconstriction, and excess mucus production. For a long time, the consumption of milk and dairy products has been implicated in the exacerbation of asthma. The origin of this view dates back to at least the twelfth century [18, 19]. An explanation for this could be the assumption that the consumption of milk stimulates mucus production in the respiratory tract and that increased mucus formation can result in increased airway resistance, which in turn aggravates asthma symptoms [19]. An association between aspiration of milk into the respiratory tract and exacerbation and/or development of asthma has been suggested [20] and in a murine model recurrent milk aspiration leads to alterations in airway function, lung eosinophilia, and goblet cell hyperplasia [21]. Also in a world-famous book about baby and child care it is suggested that children should avoid milk during respiratory illness [22]. There is a widespread view that people with asthma should limit the intake of milk and dairy products [23, 24]. However, scientific evidence does not support an association between asthma and dairy consumption.

Food Allergy and Asthma

Food allergy is due to immune mechanisms specific to the food in question. In the best-established mechanism in food allergy, food allergies are due to the presence of IgE antibodies against the offending food, respectively to the responsible epitope(s). Food allergens are defined as the antigenic molecules giving rise to the immunological response. Non-IgE-mediated food allergy involves food-IgG-immune complexes or T cell-mediated reactions.

In the fourth quarter of the last century, the prevalence of asthma worldwide increased dramatically [25]. Although there are documented cases of asthma-like symptoms resulting from consumption of or exposure to dairy foods in the literature [26–32], such cases are rare. For example Bernaola *et al.* [28] reported a chocolate confectionery worker who had occupational asthma with lactalbumin as the pathogenic agent. A 24 year-old man who had suffered from severe asthma, urticaria and generalized pruritus since the age of 14 after eating milk and dairy products, presented 15 minutes after consumption of feta cheese with conjunctivitis and a running nose, followed by edema and a severe asthma attack [29]. Blötzer and Wüthrich [33] found among 87 patients with confirmed food allergy one male adolescent with perennial asthma, who was sensitized in the skin and RAST (IgE) test to casein, milk protein (alpha-lactalbumin and beta-lactoglobulin) and various sorts of cheese. A case report describes a 16-year-old boy who showed a moderate degree of bronchial hyperreactivity (cough, bronchial obstruction) two to three minutes after a drop of whey from a sandwich containing fresh cheese fell onto his skin [34]. Among 34 previous non atopic adult patients (aged from 16 to 56 years; 31 females) having an IgE-mediated cow's milk allergy (main allergens were caseins followed by whey proteins), an asthma attack was observed in two patients, one after inhalation of baby powder containing hydrolyzed casein and one after inhalation of cow's milk protein-containing vapors during cooking [35]. In a cross-sectional epidemiologic study, 4 of 1141 randomly selected young adults had a positive skin prick test to cow's milk. One subject showed a probable IgE-mediated food allergy to milk, but a relationship to current asthma, asthma and doctor-diagnosed asthma was not detected [36].

In a study with 19 asthma sufferers and 38 control children (average age: 9.4 years, range 1.8–16 years), poorly controlled asthma and food allergy was found to be significant risk factors for life-threatening asthma. Ten of the cases had a food allergy whereof one was to milk. It was suggested that food allergy

Table 4. Relationship Between Consumption of Dairy Products and Prevalence of Asthma and Wheeze in Pre-School Children (Adjusted Model) [43]

	"Ever asthma" (n = 195)	Recent asthma (n = 145)	Recent wheeze (n = 442)
Full cream milk daily	0.54[a]	0.53[a]	0.81
Full cream milk regularly	0.83	0.73	0.87
Butter daily	0.42	0.25[a]	0.49[a]
Butter regularly	0.97	0.73	1.12
Milk products daily	0.74	0.82	0.68[b]
Semi-skimmed milk daily	0.83	0.75	0.99
Semi-skimmed milk regularly	1.07	0.72	1.05
Margarine daily	0.94	0.82	0.96
Margarine regularly	1.03	0.87	0.96
Breast-fed > 8 weeks	0.69[a]	0.63[a]	0.62[b]

Values are presented as odds ratio
[a]$p < 0.05$ [b]$p < 0.01$

might be a marker for severe asthma. Since most allergies, particularly to egg and milk, are outgrown before the age of 5, the persistence of food allergy suggests an increased atopic state [37]. In a community-based cross-sectional study, 1601 young adults with and without asthma were interviewed and tested. Of the 47 analyzed foods, whole milk was negatively ($p < 0.05$) associated with current asthma, doctor-diagnosed asthma and bronchial hyperreactivity, and butter was negatively associated with doctor-diagnosed asthma and bronchial hyperreactivity. However, ricotta, low-fat cheese and soy beverage showed a partially increased risk of current asthma, doctor-diagnosed asthma and bronchial hyperreactivity. The authors stress that their results do not indicate cause and effect [38]. The occurrence of food allergy-induced asthma reaction was established in a further double-blind study. Of 300 patients with asthma, one patient had a positive response to the milk challenge, but developed no asthma symptoms [39].

The findings above show that cases of asthma from dairy are relatively rare.

Survey on Dairy Consumption and Asthma

Based on the belief that mucus formation aggravates asthma symptoms, and milk consumption increases mucus production, asthma patients are commonly advised to reduce milk consumption. However, because the data do not support this recommendation many people may be limiting their dairy food intake unnecessarily, putting themselves at risk for shortages of calcium and other essential nutrients. In a survey of 135 adult asthma patients, 12% indicated that they avoid consumption of dairy products, 16% had renounced them in the past and 36% blamed the consumption of dairy products for having induced asthma symptoms. Among these 135 patients answering a "food and asthma" questionnaire, 54% declared that they received dietary restriction advice from a "Doctor/Specialist" and 21% from a "Doctor/Specialist and a Dietetian". The most common restriction was dairy foods [24]. It has been shown that calcium deficiency can occur in children who have limited their intake of foods containing calcium because of suspected food allergy [40–42].

In the above-mentioned study among 345 Australian shoppers, 20% of whole milk drinkers, 8% of reduced fat milk drinkers and 5% of soy milk drinkers indicated that consumption of the whole, reduced fat and soy beverage caused asthma whereas 20, 26 and 18% respectively gave the answer "don't know" [13]. In a prospective birth cohort study (natural history study in which no intervention took place; the so-called PIAMA [Prevention and Incidence of Asthma and Mite Allergy] study), 2978 children (age: 3 years) showed a lower prevalence of recent asthma symptoms when they consumed full cream milk and butter daily at the age of 3 than those who did not. The results of this study are summarized in Table 4 [43]. In Saudi Arabia, children (age: 12 years) with a history of asthma and wheezing consumed significantly less milk than controls [44]. In addition, there are some indications that milk drinking may possibly protect the respiratory epithelium [45].

Experimental Studies on Dairy Consumption and Asthma

In 1991 Haas *et al.* [19] could not find any indication in the scientific literature that milk consumption aggravated the symptoms of patients with asthma. Hence, they gave 11 asthmatic subjects (23 to 58 years) and 11 non-asthmatic subjects (22 to 50 years) each approximately 450 mL of whole milk, skim milk or water. The forced expiratory volume in 1 second (FEV_1)* and the

*Different parameters of the lung function are measured with a spirometer: vital capacity = maximum volume expelled after maximum inspiration. Forced expiratory volume in 1 second (FEV_1) = volume of air that can be forced out in one second after taking a deep breath, also given as percentage of forced vital capacity. Forced vital capacity (FVC) = maximum volume of air which can be expired as quickly and forcibly as possible after maximum inspiration.

Table 5. Baseline Values and Mean Changes in Forced Expiratory Volume in 1 Second (FEV_1) and FEV_1/Forced Vital Capacity (FVC) in a Double-Blind, Placebo-Controlled Study of Reaction to Cow's Milk in Non-Cow's-Milk-Sensitive Asthmatic Patients [46]

Challenge type		0 h x	sx	30 min.	%	1 h	%	7 h	%
FEV_1	L								
Cow's milk		2.86	0.71	−0.09	3.3*	−0.05	1.8	0.04	1.8
Placebo		2.85	0.69	−0.02	0.8	−0.07	2.8	−0.01	0.6
FEV_1/FVC	%								
Cow's milk		81.4	6.6	−2.32	2.7*	−0.68	0.7	0.12	0.3
Placebo		81.8	7.0	−1.44	1.7	−1.44	1.7	−0.68	0.7

* = statistically significant in comparison to baseline value (0 h)

On each challenge day, spirometry was done at baseline (0 h) (effective values), 30 min, 1 h and 7 h after challenge (indicated as effective and percent changes against the initial values)

airflow at 50% of vital capacity were not significantly changed in either group after consumption of whole milk, skim milk and water. However, in the asthmatic group, the pulmonary diffusing capacity was reduced by 21.0 ± 3.2% three hours after consumption of whole milk whereas a statistically non-significant reduction of 9.6 ± 2.4% was reached after skim milk consumption and of 10.0 ± 4.0% after water intake. In non-asthmatic subjects the maximum reductions amounted to 9.0 ± 2.7 (whole milk), 8.9 ± 5.3 (skim milk) and 6.6 ± 4.0% (water). According to these authors [19], the differences can be explained by the highly speculative mechanism that milk lipids may alter pulmonary gas exchange in asthmatic persons mediated by prostaglandins.

In a prospective, randomized, double-blind, placebo-controlled crossover study, 25 asthma patients who were neither allergic to cows' milk nor lactose intolerant were randomly assigned to ingest milk (10 g of whole milk powder dissolved in 60 mL placebo) or placebo (60 mL of strawberry-flavoured mocha mix). Some changes in the parameters FEV_1 or FEV_1/FVC were measured 30 minutes, 60 minutes and 7 hours after consumption (Table 5). However, no clinically significant decrease occurred. This author defined a clinically significant decrease as a decrease in FEV_1 or FEV_1/FVC of ≥ 20% [46]. Further investigations were conducted by Woods *et al.* [47] in a randomized, cross-over, double-blind, placebo-controlled trial on 20 asthmatic adults (aged 18 to 65 years) with no positive skin prick test to cows' milk. Ten of them reported that their asthma worsened after the consumption of dairy products. All subjects received either 300 mL of cows' milk or of a placebo (rice milk) (both products were ultra-heat treated and supplemented with sugar, decaffeinated coffee, citric acid and the placebo with rice syrup). The mean group data of FEV_1 and peak expiratory flow (PEF) were not statistically significant between the dairy challenge and the placebo (treatment effects), between the sequence of administration (period or order effects), or between positive and negative perceivers (perception effects). None of the subjects reported an increase in cough or sputum production after the dairy challenge. No significant treatment effects were found for the group as a whole. On an individual basis, nine subjects had a decline in ventilatory function greater than 15% from baseline after one or both challenges, which is defined as a "likely positive" challenge (Table 6). The authors concluded that they were unable to demonstrate convincingly that the consumption of milk induced a bronchoconstrictor effect in a group of adult subjects with asthma.

Influence of a Change of Dairy Nutrition on Asthma

In a double-blind crossover design, 15 adult patients with moderate asthma received twice daily 225 g yogurt with or without *Lactobacillus acidophilus.* The study tested the hypothesis that the consumption of yoghurt containing living lactic acid bacteria leads to some clinical benefits such as improved immune and clinical responses. The experiment was conducted over two 1 month-phases. Among the immune and clinical parameters measured, interferon gamma increased, but the mean daily peak flow did not show any difference and the spirometric values did not change [48].

In a single blind prospective study, 22 children with asthma (13 in the experimental and 9 in the control group; age between 3 and 14 years) received an egg- and milk-free diet for eight weeks. After this period the children of the experimental group exhibited distinctly decreased IgG-antibody-concentrations toward ovalbumin and β-lactoglobulin. In 5 children of the experimental group the PEF rate was notedly increased compared to the findings in 5 children on the control group. Based on these results lung function in asthmatic children seem improvable by eliminating egg and milk from the diet [49]. However, the findings have to be confirmed in a trial with more subjects before such a diet restriction can be recommended for the management of asthma in children.

Table 6. Individual Challenge Results of Subjects with Asthma after Consumption of an Equivalent of 300 mL of Skim Milk or 300 mL of Placebo [47]

Subj. No.	Gender	Perception[1]	Baseline % predicted FEV_1 and (PEF)	Maximum % decrease in FEV_1 and (PEF)		Symptom scores Day-/ Night-time	
				Skim milk	Placebo	Skim milk	Placebo
1	m	−	67 (71)	11.4 (12.9)	−5.0 (6.3)	0/0	0/0
2	m	−	84 (70)	8.4 (12.9)	11.4 (19.8)	0/0	2/0
3	f	−	90 (72)	27.1[a] (27.6[a])	23.4[a] (24.7[a])	2/2	2/0
7	m	−	106 (103)	1.8 (15.2[a])	2.9 (7.8)	0/1	1/0
8	m	−	73 (63)	9.0 (3.3)	18.1[a] (15.3[a])	0/0	0/0
14	f	−	70 (66)	20.6[a] (16.8[a])	22.6[a] (16.8[a])	2/1	2/0
16	f	−	101 (116)	6.9 (5.2)	4.7 (7.0)	0/0	0/0
17	f	−	133 (102)	9.3 (5.7)	14.7 (10.4)	2/1	2/1
18	m	−	129 (115)	2.8 (1.6)	4.8 (4.0)	0/0	0/0
20	m	−	110 (116)	1.3 (4.9)	3.3 (9.1)	1/2	2/2
4	f	+	107 (99)	9.3 (11.9)	5.1 (8.8)	1/0	0/0
5	f	+	90 (98)	3.3 (5.7)	14.6 (13.9)	0/0	1/1
6	f	+	76 (73)	6.0 (3.6)	4.2 (6.6)	0/0	0/0
9	f	+	94 (102)	19.1[a] (23.9[a])	26.2[a] (36.7[a])	3/0	3/0
10	f	+	116 (109)	4.7 (0.4)	9.4 (10.2)	0/0	1/0
11	m	+	122 (120)	6.5 (6.9)	7.4 (9.2)	1/0	1/0
12	F	+	107 (71)	7.5 (19.5[a])	6.0 (−1.2)	2/0	0/0
13	F	+	113 (124)	9.2 (14.4)	4.3 (8.2)	1/0	0/0
15	f	+	82 (90)	14.8 (18.5[a])	16.4[a] (18.5[a])	3/0	2/0
19	f	+	102 (88)	37.0[a] (40.5[a])	3.4 (0.3)	5/2	1/1

FEV_1 = forced expiratory volume in 1 second; PEF = peak expiratory flow

[1]perception means reported be the subjects

[a]change in spirometry of more than 15%. Score: 0 (day- and night-time) = no symptoms; 5 (daytime) = symptoms so severe that normal tasks could not be performed; 4 (night-time) = did not sleep at all. Perception: the subjects were asked to describe the perceived effect on their asthma after they ingested dairy products. Positive = one glass of milk was sufficient to induce asthma symptoms between 5 minutes and 2 hours after consumption.

CONCLUSION

The belief that milk consumption leads to an increased mucus production is present among some members of the public. The following conclusions can be drawn from the results of the different investigations: People who believe that milk increases mucus formation are more likely to report changes in sensory perceptions related to mucus after drinking milk than those who do not hold the same belief. In a double blind trial, symptoms of increased mucus formation were detected by healthy adults after consumption of both cow's milk and a non-milk beverage with similar sensory properties. Furthermore, persons who were convinced of mucus formation due to milk consumption showed more respiratory symptoms. It is possible that aggregation after mixing of an emulsion such as milk with saliva can partly explain this sensation.

Recommendations to abstain from dairy products due to the belief that they induce symptoms of asthma are not supported by the body of research evidence on the relationship between dairy consumption and occurrence of asthma. Furthermore, in general, there is no evidence to explain an underlying mechanism linking dairy and asthma. Therefore, people with asthma do not need to avoid the consumption of dairy products to control symptoms. There have been a few documented cases in which humans with an IgE-mediated cow's milk allergy presented with asthma symptoms, but these do not apply to most people with asthma. Milk and milk products are the main source of calcium in the diet, and they contain eight additional essential nutrients. Needless avoidance of dairy products can lead to limited intakes of these essential nutrients.

REFERENCES

1. Lopez-Vidriero MT: Mucus as a natural barrier. Respiration, 55 (Suppl 1):28–32, 1989.
2. Silberberg A, Meyer FA: Structure and function of mucus. Adv Exper Med Biol 144:53–74, 1982.
3. Wu AM, Csako G, Herp A: Structure, biosynthesis, and function of salivary mucins. Mol Cell Biochem 137:39–55, 1994.
4. Farber JM, Finberg L: Milk effect on mucus production during upper respiratory tract infection. J Am Med Assoc 266:1289, 1991.
5. NN: Die richtige Ernährung. Amsterdam: Time-Life Books B.V., 1997.
6. Rosner F.: Moses Maimonides' treatise on asthma. J Asthma 21:119–129, 1984.
7. Temelie B: Ernährung nach den Fünf Elementen. Sulzberg: Joy Verlag GmbH, 1999.
8. Carroll D: The complete book of natural medicines. New York: Summit Books, p. 97, 1980. Cited after Haas et al. (19).
9. McBean L: The benefits of dairy foods in health promotion. Dairy Council Dig 75:13–18, 2004.

10. Pinnock CB, Graham NM, Mylvaganam A, Douglas RM: Relationship between milk intake and mucus production in adult volunteers challenged with rhinovirus-2. Am Rev Resp Dis 141:352–356, 1990.
11. Pinnock CB, Martin AJ, Mylvaganam A: Cross-over trial of a high milk diet in asthmatic children. Proc Nutr Soc Australia, 14:131, 1989; cited after Arney, Pinnock (12).
12. Arney WK, Pinnock CB: The milk mucus belief: sensations associated with the belief and characteristics of believers. Appetite 20:53–60, 1993.
13. Bus AEM, Worsley A: Consumers' health perceptions of three types of milk: a survey in Australia. Appetite 40:93–100, 2003.
14. Lee C, Dozor AJ: Do you believe milk makes mucus? Arch Ped Adolesc Med 158:601– 603, 2004.
15. Pinnock CB, Arney WK: The milk mucus belief: sensory analysis comparing cow's milk and a soy placebo. Appetite 20:61–70, 1993.
16. Blumberger W, Glatzel H, Rettenmaier G: Untersuchungen zur Kreislauf- und Speichelwirksamkeit der Milch. Nahrung 9:152–153, 1965.
17. Vingerhoeds MH, Blijdenstein TBJ, Zoet FD, van Aken GA: Emulsion flocculation induced by saliva and mucin. Food Hydrocolloids 19:915–922, 2005.
18. Cosman MP: A feast for Aesculapius: historical diets for asthma and sexual pleasure. Ann Rev Nutr 3:1–33, 1983.
19. Haas F, Bishop MC, Salazar-Schicchi J, Axen KV, Lieberman D, Axen K: Effect of milk ingestion on pulmonary function in healthy and asthmatic subjects. J Asthma 28:349–355, 1991.
20. Orenstein, SR, Orenstein, DM: Gastroesophageal reflux and respiratory disease in children. J Ped 112:847–858, 1988.
21. Janahi IA, Elidemir O, Shardonofsky FR, Abu-Hassan MN, Fan LL, Larsen GL, Blackburn MR, Colasurdo GN: Recurrent milk aspiration produces changes in airway mechanics, lung eosinophilia, and goblet cell hyperplasia in a murine model. Ped Res 48:776–781, 2000.
22. Spock B: "Spock's Baby and Child Care." New York: Pocket Books, 1998.
23. Dawson KP, Ford RPK, Mogridge N: Childhood asthma: what do parents add or avoid in their children's diet? New Zeal Med J 103:239–240, 1990.
24. Woods RK, Weiner JM, Abramson M, Thien F, Walters EH: Patients' perceptions of food-induced asthma. Austr New Zeal J Med 26:504–512, 1996.
25. Becker AB, Chan-Yeung M: Primary prevention of asthma. Curr Opin Pulmon Med 8:16–24, 2002.
26. Rumsaeng V, Metcalfe DD: Asthma and food allergy. Nutr Rev 56:S153–S160, 1998.
27. Novembre E, de Martino M, Vierucci A: Foods and respiratory allergy. J Allergy Clin Immunol 81:1059–1065, 1988.
28. Bernaola G, Echechipia S, Urrutia I, Fernandez E, Audicana M, Fernandez de Corres L: Occupational asthma and rhinoconjunctivitis from inhalation of dried cows milk caused by sensitization to alpha-lactalbumin. Allergy 49:189–191, 1994.
29. Senti G, Leser C, Wal JM, Bernard H, Wüthrich B: Severe asthma and anaphylaxis in a cow's milk-allergic patient. Sheep's, goat's or horse's milk as an alternative for milk-protein-allergic persons. Allergologie 25:333–337, 2002.
30. Rancé F, Dutau G: Asthme et allergies alimentaires: à propos de 163 observations pédiatriques. Arch Péd 9 (Suppl 3):402S–407S, 2002.
31. Wüthrich B: Zur Nahrungsmittelallergie. Häufigkeit der Symptome bei 402 Patienten— Kuhmilchallergie — Nahrungsmittel und Neurodermitis atopica. Allergologie 16:280–287, 1993.
32. Nagel G, Linseisen J: Dietary intake of fatty acids, antioxidants and selected food groups and asthma in adults. Eur J Clin Nutr 59:8–15, 2005.
33. Blötzer IC, Wüthrich B: IgE-vermittelte Nahrungsmittelallergien - Klassifikation nach dem Sensibilisierungsweg anhand von 87 Fällen des Jahres 1998. Allergologie 27, 191–202, 2004.
34. Liccardi G, De Falco F, Gilder JA, D'Amato M, D'Amato G: Severe systemic allergic reaction induced by accidental skin contact with cow milk in a 16-year-old boy. A case report. J Investig Allergol Clin Immunol 14:168–171, 2004.
35. Stöger P, Wüthrich B: Type I allergy to cow milk proteins in adults. A retrospective study of 34 adult milk- and cheese-allergic patients. Int Arch Allergy Immunol 102:399–407, 1993.
36. Woods RK, Thien F, Raven J, Walters EH, Abramson M: Prevalence of food allergies in young adults and their relationship to asthma, nasal allergies, and eczema. Ann Allergy Asthma Immunol 88:183–189, 2002.
37. Roberts G, Patel N, Levi-Schaffer F, Habibi P, Lack G: Food allergy as a risk factor for life-threatening asthma in childhood: A case-controlled study. J Allergy Clin Immunol 112:168–174, 2003.
38. Woods RK, Walters EH, Raven JM, Wolfe R, Ireland PD, Thien FCK, Abramson MJ: Food and nutrient intakes and asthma risk in young adults. Am J Clin Nutr 78:414–421, 2003.
39. Onorato J, Merland N, Terral C, Michel FB, Bousquet J: Placebo-controlled double-blind food challenge in asthma. J Allergy Clin Immunol 78:1139–1146, 1986.
40. Bierman CW, Shapiro GG, Christie DL, VanArsdel PP, Furukawa CT, Ward BH: Allergy grand rounds. Eczema, rickets and food allergy. J Allergy Clin Immunol 61:119–127, 1978.
41. David TJ, Waddington E, Stanton RH: Nutritional hazards of elimination diets in children with atopic eczema. Arch Dis Childhood 59:323–325, 1984.
42. Davidovits M, Levy Y, Avramovitz T, Eisenstein B: Calcium-deficiency rickets in a four-year-old boy with milk allergy. J Ped 122:249–251, 1993.
43. Wijga AH, Smit HA, Kerkhof M, de Jongste JC, Gerritsen J, Neijens HJ, Boshuizen HC, Brunekreef B: Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the Piama Birth Cohort Study. Thorax 58:567–572, 2003.
44. Hijazi N, Abalkhail B, Seaton A: Diet and childhood asthma in a society in transition: a study in urban and rural Saudi Arabia. Thorax 55:775–779, 2000.
45. Tockham MS, Khoury MJ, Cohen BH: Milk drinking and possible protection of the respiratory epithelium. J Chron Dis 39:207–209, 1986.
46. Nguyen MT: Effect of cow milk on pulmonary function in atopic asthmatic patients. Ann Allergy Asthma Immunol 79:62–64, 1997.
47. Woods RK, Weiner JM, Abramson M, Thien F, Walters EH: Do dairy products induce bronchoconstriction in adults with asthma? J Allergy Clin Immunol 101:45–50, 1998.

48. Wheeler JG, Shema SJ., Bogle ML, Shirrell MA., Burks AW, Pittler A, Helm RM: Immune and clinical impact of *Lactobacillus acidophilus* on asthma. Annals Allergy Asthma Immunol 79, 229–233, 1997.
49. Yusoff NAM, Hampton SM, Dickerson JWT, Morgan JB: The effects of exclusion of dietary egg and milk in the management of asthmatic children: a pilot study. J Royal Soc Promot Health 124:74–80, 2004.

Received September 9, 2005

Review

Dairy Product Consumption and the Risk of Breast Cancer

Peter W. Parodi, PhD

Human Nutrition and Health Research, Dairy Australia, Melbourne, AUSTRALIA

Key words: breast cancer, dietary fat, insulin-like growth factor-1, estrogens, growth hormone, rumenic acid, calcium

It has been suggested in some reports that dairy product consumption may increase the risk of breast cancer. This review gives a brief overview of the etiology of breast cancer and in particular the roles of fat, bovine growth hormone, insulin-like growth factor-1 and estrogens. Evidence from animal studies and epidemiology does not support a role for fat in the etiology of breast cancer. The daily intake of insulin-like growth factor-1 and biologically active estrogens from dairy products is minute in comparison to the daily endogenous secretion of these factors in women, whereas bovine growth hormone is biologically inactive in humans. On the other hand, milk contains rumenic acid, vaccenic acid, branched chain fatty acids, butyric acid, cysteine-rich whey proteins, calcium and vitamin D; components, which have the potential to help prevent breast cancer. Evidence from more than 40 case-control studies and 12 cohort studies does not support an association between dairy product consumption and the risk of breast cancer.

Key teaching points:

- The etiology of breast cancer is still largely undetermined. A women's reproductive history provides the most consistent evidence for risk, but the relative risk for most risk factors is close to the null value of 1.
- More than 40 case-control and 12 cohort studies do not suggest that dairy product consumption is associated with the risk of breast cancer.
- It has been suggested by some researchers that dairy products may increase the risk of breast cancer due to their content of fat, insulin-like growth factor-1, estrogens or growth hormone. However, the available evidence does not support this association.
- Animal studies and epidemiology do not suggest a role for fat in the etiology of breast cancer. Bovine growth hormone is biologically inactive in humans. Daily intake of insulin-like growth factor-1 and biologically active estrogens is insignificant compared to daily endogenous secretion in women.
- Milk contains rumenic, vaccenic, butyric and branched chain fatty acids, whey protein, calcium and vitamin D, which have the potential to protect against breast cancer.

INTRODUCTION

Breast cancer is the most common - and most feared - malignancy in women living in developed countries, and is second only to lung cancer as a cause of cancer death. There is large international variation in breast cancer rates. In developed countries the age-standardized incidence rates are around 100/100,000 women with mortality rates about 25/100,000. These rates are up to 5-fold higher than those reported from Asian regions, which have the lowest incidence of breast cancer [1]. Breast cancer is rarely found before the age of 25 years. Thereafter, the incidence increases with age until menopause when the rate of increase is less pronounced. About three-quarters of diagnosed cases are in postmenopausal women [2–7].

Despite extensive research to find the cause of breast cancer the etiology is largely undetermined. It is estimated that around 75% of women who present with this malignancy have no established risk factors other than age and living in a western society [2]. When women migrate from a region of low incidence for breast cancer to one with a high incidence their risk does not immediately assume the rate in the host country. However, the risk in their descendants approaches that of their adopted country after two to three generations, which indicates that environmental factors are of greater importance than genetic factors [4,5,8,9]. Nevertheless, breast cancer is known to cluster in

Address reprint requests to: Dr Peter Parodi, 9 Hanbury St., Chermside, 4032, Queensland, AUSTRALIA. E-mail: peterparodi@uq.net.au

Journal of the American College of Nutrition, Vol. 24, No. 6, 556S–568S (2005)
Published by the American College of Nutrition

families and having a first-degree relative (mother, sister, daughter) with breast cancer, especially at a young age, can double the risk of developing this cancer. Two high-penetrant genes, *BRCA1* and *BRCA2* account for the majority of inherited breast cancer, however, mutations in these and other low-penetrant susceptibility genes account for less than 5 to 10% of breast cancer cases [5,10].

From the mass of epidemiological data generated over the years, characteristics of a woman's reproductive history provide the most consistent evidence for the risk of breast cancer. Early onset of menarche, a late menopause, delayed childbirth, nulliparity and low cumulative lactation time all increase the risk of breast cancer [2,4,5]. It is believed these factors reflect a longer lifetime exposure to endogenous steroid hormones. This is supported by observations that women with bilateral oophorectomy at an early age have a decreased risk of breast cancer compared with women who had a natural menopause [4,11]. Further, there is a small increase in risk of breast cancer associated with long-term use of oral contraceptives and hormone replacement therapy (HRT) [3–5]. However, most of these risk factors are weak and the relative risk (RR) or odds ratio (OR), indices used to indicate the strength of risk, are seldom much greater than the null value of 1 [11].

A number of other important, although minor, risk factors have been noted. Women exposed to excessive levels of radiation, especially at a young age, are at increased risk of breast cancer [5,12]. Increased mammographic breast density is associated with increased risk [5,13]. Obesity is associated with a decreased risk of breast cancer in premenopausal women and an increased risk in postmenopausal women [4,11,14]. Physical activity decreases risk [4,5]. Height is a risk factor [4], and risk increases with increasing birth weight [15]. Most of this group of risk factors may influence or be influenced by steroid hormones. Although the role of diet in the etiology of breast cancer has been studied extensively there is no clear indication that any dietary item, apart from alcohol, is associated with breast cancer risk [16].

Special interest groups, media articles, books and some scientific papers have suggested that dairy product consumption can increase the risk of developing breast cancer. The rationale for this claim is that dairy products are a source of fat, including saturated fatty acids; insulin-like growth factor, a mitogen; estrogenic hormones, which are weak carcinogens and mutagens, and growth hormone [17–20]. The validity of these assertions is now examined.

DAIRY PRODUCT CONSUMPTION AND BREAST CANCER RISK: EPIDEMIOLOGY

Some 41 case-control studies together with 12 cohort and case-control studies nested within cohort studies have determined the associations between total dairy product or specific dairy item consumption and the risk of breast cancer. Knekt and Jarvinen [21] give a description and results of studies published up to 1998, and summarize in table form the strength of association for the various studies. As part of a meta-analysis on dietary fat and breast cancer risk, Boyd et al. [22] included two dairy categories, milk (16 studies) and cheese (12 studies), which showed ORs with associated 95% confidence intervals (CIs) of 1.12 (0.88–1.43) and 1.26 (0.96–1.66), respectively. Missmer et al. [23] conducted a pooled analysis of primary data from eight large prospective studies as part of the Pooling Project of Prospective Studies of Diet and Cancer. No relation was found with dairy products analyzed as total dairy fluids, total dairy solids, ten sub-groups, or seven specific dairy foods and the risk of breast cancer.

Recently, Moorman and Terry [24] summarized the results of ten cohort and 36 case-control studies that evaluated the association between dairy product consumption and breast cancer risk. They concluded that the available epidemiological evidence dose not support a strong association between the consumption of milk or other dairy products and the risk of breast cancer. Since this report [24] results have appeared for two case-control studies and two cohort studies. One case-control study found a significant negative association between high milk intake and breast cancer risk [25]. The other study [26] found a significant negative association between a high intake of total dairy and low-fat dairy intake and the risk of breast cancer, but high-fat dairy consumption was nonsignificantly associated with risk. In the Nurses' Health Study II [27] women with a high consumption of low-fat dairy products during their premenopausal years had a nonsignificant negative association with breast cancer risk. However, total dairy intake was nonsignificantly associated, and high-fat dairy intake was positively associated with risk. The other cohort study [28] assessed the risk of adolescent diet and the risk of breast cancer and will be discussed separately.

Adolescent Diet and the Risk of Breast Cancer

Exposure to initiating events during childhood, adolescence and early adulthood, when the mammary gland is attaining adult-stage morphology, may influence the risk of breast cancer in later life. Indeed, several studies show that the risk of breast cancer associated with alcohol consumption and cigarette smoking increases with decreasing age at which exposure to these practices commenced [29]. For women treated with high doses of ionising radiation for tuberculosis, acute postpartum mastitis, enlarged thymus and Hodgkin's disease, the risk of breast cancer increased with decreasing age at exposure [12]. Long-term follow-up studies of the incidence of breast cancer among atomic bomb survivors from Hiroshima and Nagasaki also show increased risk with decreasing age at exposure [12].

Three cohort and four case-control studies have examined the consumption of dairy products during adolescence and the subsequent risk of breast cancer. The results of these studies are presented in Table 1. Of the 12 associations listed, ten showed a

Table 1. Summary of Data from Cohort and Case-Control Studies Evaluating the Association between Adolescent Dairy Product Intake and The Subsequent Risk of Breast Cancer

Study	Cases	Controls or cohort size	Menopausal status at diagnosis	Product evaluated	Results OR[1] or RR[1] (95% CI[2])
COHORT					
Frazier et al. [28]	361	47,355	94.8% premenopausal	Total dairy (less butter)	0.83 (0.56–1.24)
				High-fat dairy	1.11(0.76–1.62)
				Low-fat dairy	0.88 (0.60–1.29)
Shin et al. [139]	327	–[3]	Premenopausal	Milk	0.81 (0.51–1.28)
	1509	–[3]	Postmenopausal	Milk	1.02 (0.82–1.26)
Hjartaker et al. [140]	317	48,844	Premenopausal	Milk	0.64 (0.22–1.87)
CASE-CONTROL					
Shu et al. [141]	1459	1556	Mixed	Milk	0.76 (0.59–0.98)
Potischman et al. [142]	1647	1501	Premenopausal	Dairy products	0.98 (0.8–1.2)
Pryor et al. [143]	99	101	Premenopausal	Milk fat	0.4 (0.1–1.1)
	70	88	Postmenopausal	Milk fat	0.2 (0.0–0.8)
Hislop et al. [114]	263	306	Premenopausal	Whole milk	0.71 (0.40–1.27)
	392	435	Postmenopausal	Whole milk	0.75 (0.49–1.13)

[1]Odds ratio or relative risk for the highest category of intake vrs.the lowest. The fully adjusted models are presented.
[2]Confidence interval.
[3]Not given in text.

negative association between intake of dairy products and the risk of breast cancer, but only one achieved statistical significance.

FAT, FAT TYPE AND BREAST CANCER RISK

For many years it was considered that fat intake provided the strongest dietary link with breast cancer risk. This belief was based largely on two lines of evidence; strong correlation between *per capita* consumption of fat and breast cancer mortality in international comparison studies; and animal experiments that showed a high fat diet increased the incidence of chemically induced mammary tumors [16].

It is now realized that in cancer studies there is an interrelationship between dietary fat and calories. In studies using rodent models of carcinogenesis in which the effects of calorie intake were separated from those of the fat content, the fat content of the diet did not significantly influence tumor development. On the other hand, calorie restriction inhibited tumor development [30,31]. Because fat intake is highly correlated with energy intake it is essential to adjust for energy intake in epidemiological studies that assess associations between dietary fat intake and the risk of breast cancer.

Most international comparison (ecologic) studies show strong positive correlation between *per capita* fat consumption and mortality from breast cancer [32,33]. Ecological studies are a poor format for determining causality. Dietary information based on national food disappearance data is a poor reflection of individual consumption and tells nothing about the diets of individuals who develop cancer and those who do not. Other dietary, environmental and reproductive patterns can vary widely between countries, and are not adjusted for in this type of study [34].

Within-population epidemiological studies can avoid much of the confounding found in ecological studies. Goodwin and Boyd [35] reviewed the published results from 14 case-control studies that examined the relationship between the intake of total fat or fat containing foods and the risk of breast cancer. Eight studies examined the relationship between total fat intake and breast cancer risk. Only one study found a statistically significant positive association. Results were inconsistent in the six studies that examined the risk for various fat containing foods. Howe et al. [36] conducted a pooled analysis of the original data from 12 case-control studies of diet and breast cancer that represented 4427 cases. The RR for the highest vs. lowest quintile of total fat was 1.13 (non-significant) for premenopausal women and 1.48 (significant) for postmenopausal women. This analysis did not include the then largest study of 2024 cases [37], or a subsequent study with 2564 cases [38], both of which did not find an association between fat intake and the risk of breast cancer.

The accuracy of associations generated by case-control studies can be affected by dietary measurement error due to unreliable nutrient databases, inaccurate assessment of past diet, and dietary recall bias by subjects who have breast cancer. Inappropriate selection of control subjects can also introduce bias [34]. Prospective (cohort) studies largely overcome these biases, because diet is assessed before cancer diagnosis, and at a time closer to its initiation. In addition, control subjects belong to the same community as cases [34].

Hunter et al. [39] conducted a collaborative-pooled analysis of original data from seven large prospective studies published up to 1995 that represented 4980 cases. The analysis found no

evidence of an association between the intake of cholesterol or total, saturated, monounsaturated or polyunsaturated fat and the risk of breast cancer. There was no reduction in risk among women whose energy intake from fat was less than 20% of total energy intake. What is more, for the small number of women reporting less than 15% of energy from fat, the risk of breast cancer increased more than two-fold. A follow-up pooled analysis by Smith-Warner et al. [40], with 7,329 cases, confirmed the lack of association between total fat, fat class or animal or vegetable fat intake and the risk of breast cancer. In addition, no survival advantage was found for consumption of a low fat diet or type of fat, after diagnosis of breast cancer in participants from the Nurses' Health Study [41]. High correlations between various dietary fatty acids in epidemiological studies reduce the ability to detect an independent association with cancer risk. Nevertheless, there is no convincing evidence from epidemiological studies that any individual fatty acid is associated with the risk of breast cancer [42].

Of the dietary items thought to protect against breast cancer, fruit and vegetables and fiber have received the most attention. However, a pooled analysis of cohort studies suggests that fruit and vegetable consumption, at least during adulthood, is not significantly associated with reduced breast cancer risk [43]. Likewise, evidence from well-conducted epidemiological studies does not suggest a protective effect for dietary fiber [16]. In contrast, there is consistent epidemiological evidence that alcohol consumption is positively associated with breast cancer risk [16]. Overall, there is no convincing evidence that fat intake is associated with the risk of breast cancer. The RRs and related confidence intervals associated with nearly all dietary items in the epidemiological studies are close to the null value of 1. This suggests that diet does not play an important role in the etiology of breast cancer.

INSULIN-LIKE GROWTH FACTOR AND BREAST CANCER

The Insulin-Like Growth Factor System

Insulin-like growth factors (IGFs) belong to a larger family of insulin related peptides, which include insulin, IGF-1 and IGF-2. Together with binding proteins, binding protein proteases and receptors they form the IGF system. IGFs are mitogens that play an important function in almost every organ of the body, where they regulate cell proliferation, differentiation and apoptosis [44, 45]. IGFs, particularly IGF-1, are required for normal mammary gland development, but it is also implicated in breast cancer development [46,47]. IGF-1 exerts its biological actions by interacting with a specific type 1 IGF-1 receptor (IGF-IR) associated with the cell membrane [45,46]. The bioactivity of IGF-1 depends on complex physiological regulation. Only a small portion circulates in the free form; the remainder is regulated by a series of six IGF-binding proteins (IGFBP-1 through IGFBP-6), which have a somewhat stronger affinity for IGF-1 than the receptor. More than 90% of serum IGF-1 is bound in a ternary complex with IGFBP-3 and an acid-labile subunit (ALS). This complex cannot leave the circulation and serves to both increase the half-life of IGF-1 and at the same time inhibit its mitogenic effect. The presence of IGFBP proteases in tissues can cleave the binding protein and liberate free IGF-1 [44,45,47–49]. IGFBP-3 can also modulate the IGF-1 signaling pathway independently of its IGF-1-binding ability. In mammary tissue, IGFBP-3 may interact with its own membrane receptor to inhibit growth, induce apoptosis and mediate cell growth arrest induced by other molecules [47–50].

Most IGF-1 and IGFBPs are produced in the liver under control of growth hormone, and levels can be influenced by nutritional factors. Non-hepatic tissues can also produce IGF-1 and IGFBP-3, where they exert autocrine and paracrine effects [44,45]. In the breast, IGF-1 is expressed in stromal cells adjacent to normal or malignant epithelial cells. The extent to which circulating versus endogenously produced IGF-1 is important for mammary gland development and in tumorigenesis is still to be resolved [46,51,52].

Determinants of Circulating IGF-1 and IGFBP-3 Levels

Serum IGF-1 levels are low at birth, rise during childhood and reach a peak at puberty. Thereafter, values decline with age. The age-specific distribution of IGFBP-3 and ALS is similar to the distribution for IGF-1 [44,47,53]. There is considerable heterogeneity in adult serum IGF-1 levels, with a range of 80 to 425 μg/L [53], however, an individual's circulating level of IGF-1 and IGFBP-3 is relatively constant. Thissen et al. [54] and Yu and Rohan [47] have reviewed the determinants of circulating IGF and IGFBPs. The most consistent determinant of IGF-1 levels is dietary protein. Levels are markedly lowered by severe protein and energy restriction, with essential amino acid deficiency having a severe depressive effect. Over nutrition has the opposite effect, but not to the same extent as under nutrition. There have been few studies on dietary micro- and macronutrients, and the results are conflicting. Associations between serum IGF-1 levels and other factors, such as physical activity, energy intake within normal limits, smoking, BMI and anthropometric indices have provided divergent results [47,54].

Epidemiological Studies

Many epidemiological studies have examined the association between circulating levels of IGF-1 and IGFBP-3 and the risk of breast cancer. Recently, three meta-analyses of these studies, using different exclusion criteria, were published [55–57]. Overall, there was a marginally significant association between high levels of circulating IGF-1 and increased risk of breast cancer in premenopausal women, but not in postmenopausal women. Surprisingly, there was no protective effect for

IGFBP-3, and high levels were associated with a marginally increased risk of premenopausal breast cancer.

Breast cancer cells can produce IGF-1 [46,47,58]. Also, because breast cancer cells secrete IGFBP-3 proteases, this can alter circulating levels of free IGF-1 without increasing its production [59], and breast cancer tissue exhibits higher IGF-1R levels than adjacent normal tissue [44,46,47]. An interesting sequential serum IGF-1 study was conducted in a nested case-control of prostate cancer, a hormone-related epithelial malignancy with a common pathogenic framework to breast cancer. In the prostate cancer cases serum IGF-1 levels were significantly higher at the time of diagnosis than in previous samples drawn 2 to 5 years before diagnosis [60]. Thus elevated IGF-1 levels in breast cancer patients may be a marker of, rather than a cause of the disease. Further, the positive association between serum IGFBP-3 levels and the risk of breast cancer may be a consequence of the production of IGFBP-3 by breast cancer cells [61].

IGF-1 in Milk

The IGF-1 content of bovine milk varies with the stage of lactation. A recent study showed colostrum had a level of 300ng/mL and the content dropped to 7ng/mL at 1 week postpartum. Thereafter the levels dropped further to below 2ng/mL. IGFBP-3, which inhibits the mitogenic effect of IGF-1, is by far the most abundant binding protein in milk and content varies throughout lactation in a manner similar to IGF-1 [62]. At any given stage of lactation, IGF-1 levels can vary widely between cows due to many factors including parity and farm practise [63]. The level of IGF-1 in milk is not affected by pasteurisation [64].

Milk IGF-1 and Breast Cancer

Because milk contains IGF-1, which has an identical amino acid sequence to human IGF-1 [65], it has been suggested its consumption may be linked to breast cancer [17,18]. The evidence presented to justify this connection does not stand up to serious scientific scrutiny. Firstly, the amount of IGF-1 consumed daily from milk products is minute compared to endogenous production. Based on a milk content of 4ng/mL, milk product consumption equivalent to 1.5L milk/day would contribute 6,000ng IGF-1 to the gastrointestinal tract. The gastrointestinal tract also receives considerable exogenous IGF-1 from saliva, biliary fluid, pancreatic juice and secretions from the intestinal mucosa, estimated to total 380,000ng/day [66,67]. In addition, it is estimated that in adults the liver and extra-hepatic tissues produce 10^7ng IGF-1/day [68]. Thus, milk-derived IGF-1 would contribute less than 0.06% of total daily IGF-1 production if it escaped proteolysis during intestinal passage, and was absorbed by the intestine and passed to the circulation. This is unlikely, as considerable, if not total, digestion of IGF-1 should take place in the small intestine [69].

Studies cited to justify absorption of IGF-1 from the intestine [17] used suckling rats. This is an inappropriate model, because neonates do not have a fully developed protease/peptidase system and intestinal closure has not occurred, which allows enhanced permeability of macromolecules. Even so, evidence from neonatal animal studies suggests that feeding IGF-1 results in negligible intestinal absorption [70]. Of greater significance, recent studies that fed human adults up to 60g/d of a concentrated bovine colostrum protein powder for up to 8 weeks did not find an increase in serum IGF-1 levels [71–73]. These studies provide compelling evidence that IGF-1 in dairy products is not implicated in the etiology of breast cancer.

Diet and Serum IGF-1 Levels

In an oft-cited study by Heaney et al. [74], subjects with habitual low dairy product consumption consumed their usual diet or their usual diet plus three servings of dairy per day. After 12 weeks serum IGF-1 levels increased by 12% in the milk drinkers, and decreased by 2% in the non-milk drinkers. However, the increase in IGF-1 levels in milk drinkers was accompanied by an increase in total protein intake and energy compared to non-milk drinkers. Total energy intake and protein consumption are the major determinants of circulating IGF-1 [47–54]. In a nested case-control study from the Physician's Health Study there was a modest increase in serum IGF-1 levels with increasing skim or low-fat milk consumption. Non-significant increases were found for poultry and fish consumption [75]. In a randomised double blind study, healthy men consumed 40g of soy protein (often associated with protection from breast cancer) or milk protein daily for 3 months. Serum IGF-1 levels increased from baseline with both protein supplements, but were significantly higher only for soy protein [76]. Animal studies suggest that the essential amino acid content of dietary protein may be the important determinant for IGF-1 level [77].

SEX HORMONES AND BREAST CANCER

Established risk factors for breast cancer are predominantly associated with a woman's reproductive history, which suggests they are markers for exposure to endogenous ovarian hormones, the estrogens and progestins [3,14]. Support for the concept that cumulative exposure to estrogens is a major determinant of breast cancer risk comes from several epidemiological studies and clinical observations. Women with bilateral oophorectomy have a lower risk of breast cancer than women who have a natural menopause. The younger the age of oophorectomy, the lower the risk [3,4,11]. The antiestrogenic drug tamoxifen is successful in the prevention and treatment of breast cancer, especially in women with estrogen receptor (ER) positive tumours [78]. In addition, aromatase inhibitors, which

prevent the aromatase enzyme catalysing the final step in estrogen biosynthesis, are also successful in the prevention and treatment of breast cancer [79].

Use of oral contraceptives slightly increases the risk of breast cancer in young women. The risk increases with increasing duration of use, and after age 45 years. [3–5,80] Epidemiological studies show there is a modest increase in risk of breast cancer associated with hormone replacement therapy (HRT). Combined estrogen and progestogen use appears to be related to a higher risk for breast cancer than estrogen alone. Overall, the risk associated with HRT use for a year is comparable to delayed menopause for the same period of time. Risk is higher for long-term users, but risk falls when use ceases [3,81,82].

Estrogens as Carcinogens

A number of lines of evidence suggest that estradiol, the most potent estrogen, is a weak carcinogen and mutagen, although the molecular mechanisms are still incompletely understood [83–85]. Estrogens function in cells by diffusing passively through cell membranes binding to nuclear ERs and stimulating transcription of genes involved in cell proliferation. This increases the opportunity for accumulation of DNA damage that may lead to carcinogenesis. There is also accumulating evidence that estradiol can be metabolised to genotoxic compounds like 16α-hydroxy estradiol and the catechol estrogen quinones that directly damage DNA [83,85]. Estrogens act in concert and interact synergistically with elements of the IGF-1 axis. In breast cancer cells estrogens induce the expression of IGF-1 and enhance its mitogenic effect. Estrogens stimulate production of IGF-1Rs, repress synthesis of IGFBP-3 and increase the synthesis of cathepsin D, an IGFBP-3 protease. [47,86,87].

Serum Sex Hormone Level and Breast Cancer Risk

Because of the important role for sex hormones in the etiology of breast cancer, numerous studies have investigated the association between circulating sex hormone levels, particularly estradiol, and the risk of breast cancer. The physiologically significant estrogens in order of potency are estradiol (17β-estradiol), estrone and estriol in a ratio of about 100:10:4. Most circulating estradiol is bound to plasma proteins, sex hormone-binding globulin (SHBG) or albumin, which renders them biologically inactive [14].

Premenopausal Women. Key [88] lists four prospective studies that reported on estrogens and breast cancer in premenopausal women. Together, they do not suggest that a higher level of serum estradiol is associated with an increased risk of breast cancer. However, a single blood sample may not represent a woman's habitual hormone status because of large variation in hormone level during the menstrual cycle. Estradiol level varies from 6ng/100mL in the early follicular phase to 33 to 70ng/100mL in the late follicular phase, and a value around 20ng/100mL in the mid luteal phase [89].

Postmenopausal Women. About three-quarters of diagnosed breast cancer occurs in postmenopausal women. After menopause ovarian estrogen production ceases and the major circulating estrogen is estrone (30pg/mL), which is formed by aromatization of the steroid hormone androstenedione in peripheral tissues, primarily adipose tissue. Some estrone, in turn, is metabolized to estradiol (15pg/mL) [14,90].

The Endogenous Hormones and Breast Cancer Collaborative Group [91] conducted a pooled analysis of the original data from nine prospective studies. In postmenopausal women they found a statistically significant increase in the risk of breast cancer with increasing concentrations of all sex hormones examined. Interestingly, the association between the different levels of estrogens and breast cancer risk was stronger in never uses of HRT than users.

Determinants of Serum Estrogen Levels

Overweight, obese and sedentary postmenopausal women have elevated concentrations of circulating estrogens, and lower concentrations of SHBG [14,92]. Exercise can reduce serum estrogen and increase SHBG levels, but the effect is dependent on loss of body fat [92]. There is no clear association between obesity and estrogen levels in premenopausal women [14]. Many studies have investigated the role of diet on serum estrogen levels, but the results are inconclusive [14]. A relationship between dietary fat and serum estrogen levels is unclear [14,34]. Dietary fiber intake may be inversely related to concentrations of serum estrogen [14].

Estrogen Metabolism in Breast Tissue

Are high circulating levels of estrogens a cause of breast cancer, or a correlate, or a consequence of the disease? There is no simple linear relationship between serum levels and tissue concentrations of estrogens [93,94]. The levels of estradiol in normal and malignant breast tissue are similar for both premenopausal and postmenopausal women, even though serum estrogen levels are up to 50-fold lower in postmenopausal women [93,95,96]. However, estradiol levels are significantly higher in breast cancer tissue than in normal tissue for both premenopausal and postmenopausal women [93]. Levels of estrone sulphate, the major form of circulating estrogen in postmenopausal women, were significantly higher in their breast tumors than in those of premenopausal women [94].

The concentration of estrogens in breast tissue is far higher than in circulating plasma [94,97,98], which suggests that local production of estrogens in breast tissue is far more important than uptake of estrogens from the circulation [85,99]. Breast tissue contains all the enzymes necessary to synthesize the biologically active estradiol from circulating precursors. Firstly, aromatase, which converts androstenedione to estrone; secondly, estrone sulfatase that hydrolyses biologically inactive estrone sulphate to estrone; and thirdly 17β-hydroxysteroid dehydrogenase, which reduces the weakly bioactive estrone to

estradiol [85]. Human breast cancer cells can adapt to a deprivation in estradiol stimulation by developing enhanced estrogen sensitivity to the residual levels of estradiol present [100] or to the precursors of estrogen by increasing the levels of estrogen synthesizing enzymes [96].

Contribution of Milk Estrogens to Circulating Levels in Women

Steroid hormones are widely distributed in the animal and vegetable products we consume [101]. Milk contains estrone and estradiol, but the concentration varies considerably during the estrous cycle and during pregnancy, especially in estrone sulfate [102,103].

As part of a German market basket survey Hartman et al. [101] purchased samples of dairy products and determined their content of estrone and estradiol. Based on previously published national nutritional data they calculated that a woman would consume about 0.05μg/day of estrogens from dairy products, with about 90% represented by the weakly bioactive estrone. These estrogens are largely conjugated and a large proportion of injested hormones are inactivated by the first-pass effect of the liver [101]. In contrast, during the late follicular phase of the menstrual cycle a woman produces up to 1mg/day of estradiol and 0.7 mg/day of estrone [89]. Postmenopausal women produce between 40 and 200μg/day of estrone from androstenedione, depending on their weight [90]. Thus, the contribution of dairy product consumption to a woman's estrogen status is infinitesimal and cannot be considered a risk for breast cancer.

GROWTH HORMONE

Growth hormone (GH) or somatotropin is secreted by the anterior pituitary gland, and regulates growth in most tissues from birth to puberty, although GH still has important metabolic effects in adults. GH levels are low in infancy, increase slightly during childhood and peak during puberty. Thereafter levels progressively decrease with age, but there is considerable inter-individual variation. There is also considerable intra- individual variation in GH levels, which are low during most of the day with bursts occurring after meals, exercise and emotional stress, but mostly during the first few hours of sleep. This pulsatile pattern of GH release by the pituitary gland is controlled by the hypothalamic factors; growth hormone-releasing hormone, which stimulates release of GH, and growth hormone-inhibitory hormone (somatostatin) that inhibits the release of GH [104].

GH is an essential factor in the development of the mammary gland. Acting through its receptor, GH induces stromal cells to synthesize IGF-1, which can stimulate proliferation and differentiation in adjacent epithelial cells in a paracrine manner [105]. Estradiol enhances the stimulatory effect of GH and IGF-1 on mammary gland development and in breast cancer cells [51,87]. The GF/IGF-1 axis also plays a role in mammary tumorigenesis. GH binds to receptors in the liver to induce IGF-1, thereby elevating circulating IGF-1 levels. On the other hand, GH also increases IGFBP-3 levels [106]. Autocrine production of GH in mammary carcinoma cells can promote cell proliferation, transcriptional activation and prevention of apoptosis. Autocrine produced GH is believed to be a more potent stimulator of mammary carcinoma cell spreading than exogenously administered GH [107].

Despite the mitogenic activity of GH, relatively few studies have addressed the role of GH in the etiology of breast cancer. Animal studies using transgenic mice that over or under express GH show that GH deficiency is associated with less tumor growth, whereas over expression of GH increases tumor development [87,108,109]. Serum GH levels in breast cancer patients were higher than in control subjects in what appears to be the only study that examined the relationship between GH level and the risk of breast cancer [110]. However, an independent role for GH in breast cancer etiology is difficult to establish because of its effect on the GH/IGF-1 axis.

Milk Derived GH

Commercial use of recombinant bovine GH (rbGH) to increase milk yield and efficiency in dairy cows commenced in the United States in 1994 [111]. This event provoked considerable debate among special interest groups, the media and in the scientific literature, as to whether milk from treated cows would cause adverse health effects [17,18,112–114].

Bovine milk naturally contains less than 1ng/mL of GH [115], whereas humans secrete 500 to 875 μg of GH per day [104]. There is no significant increase in bGH levels in milk from cows treated with rbGH [113,115]. Pasteurization of milk destroys about 90% of bGH [113]. Because bGH is a protein it is hydrolyzed in the intestinal tract during the digestion process. Should any bGH survive digestion it will have no effect on human biology, because the human GH receptor does not respond to bGH [63,116].

Administration of rbGH to cows increases the level of IGF-1 in milk, but overall the impact is minimal when considered against the large variations influenced by stage of lactation, parity, nutrition and herd environment [63,111,113]. What is more, when IGF-1 levels increase so do the levels of IGFBP-3 and ALS [111]. The unlikely survival of dietary IGF-1 in the intestinal tract to produce a biological response in humans was discussed in a previous section.

COMPONENTS OF MILK WITH THE POTENTIAL TO PREVENT BREAST CANCER

The assertion that consumption of milk and its products could increase the risk of developing breast cancer because of

their content of fat, IGF-1, estrogens and GH is ill founded. On the other hand, milk contains a number of components with the potential to help prevent breast cancer.

Calcium and Vitamin D

Both calcium and vitamin D play an important role in the regulation of cell growth. In addition, vitamin D, through its active metabolite 1,25-dihydroxy vitamin D_3($1,25(OH)_2D_3$), is important for calcium homeostasis and absorption into cells [117,118]. Animal studies suggest that hyperproliferation and hyperplasia in mammary epithelial cells can be reduced by dietary calcium and vitamin D [117].

There are a number of possible mechanisms for the anti-proliferative action of calcium. Calcium may neutralize fatty acids and mutagenic bile acids, which can rapidly pass from the intestine to the breast where they can affect ERs and induce estrogen-regulated protein in a manner similar to estradiol [119]. Human breast cancer cells express elevated levels of fatty acid synthase [FAS], the major enzyme required for endogenous fatty acid biosynthesis, a process that has been linked to cell proliferation. Treatment of breast cancer cell lines with cerulenin, an inhibitor of FAS activity, resulted in rapid growth inhibition that was associated with apoptosis [120]. Zemel [121] recorded that high-calcium diets suppressed $1,25(OH)_2D_3$-induced calcium influx into adipocytes - the predominant cells in the breast - and inhibited FAS activity.

Increased mammographic breast density is strongly associated with the risk of breast cancer [5]. A recent study showed that an increased intake of calcium and vitamin D was associated with decreases in mammographic breast density [13]. Boyapati et al. [122] recently reported that dietary calcium intake was negatively associated with the risk of breast cancer in both premenopausal and postmenopausal women. These authors also tabulated the results of seven other case-control and two cohort studies, all of which found negative associations between calcium intake and the risk of breast cancer. In the Nurses' Health Study both calcium and dairy product intake was associated with a survival benefit for women with breast cancer [41].

Rumenic and Vaccenic Acids

Rumenic acid (RA) is the predominant natural isomer of conjugated linoleic acid (CLA), and milk fat is the richest natural source. Vaccenic acid (VA), the major *trans*-monounsaturated fatty acid in milk fat can be converted to RA in animals and humans by the enzyme Δ^9 - desaturase [123]. In normal rat mammary epithelial cells, RA inhibited cell growth and induced apoptosis [124]. At physiological concentrations RA, VA and milk fat all arrested cell growth in breast cancer cells [125,126]

When added to the diet of rats at a level of 1% or less, RA is a potent inhibitor of mammary tumor development. Tumor inhibition is independent of the amount or type (saturated or polyunsaturated) of fat in the diet, and is particularly effective when fed only during the period of mammary gland development to adult stage morphology. Feeding RA during this period resulted in a decrease in epithelial density associated with a reduced proliferation of the epithelial cells within the terminal end buds and lobular epithelium, areas where most tumors develop [124]. The anti-tumor action of RA is possibly additionally mediated by induction of apoptosis and inhibition of angiogenesis associated with decreased serum and glandular levels of vascular endothelial growth factor and its receptor Flk-1 [124,127]. RA is a potent inhibitor of FAS in human breast cancer cell lines [128,129]. As part of a CLA mixed isomer supplement, RA reduced serum IGF-1 levels in rats [130].

Epidemiological Studies. The initial case-control study found a significant inverse association between dietary intake of RA and the risk of breast cancer in Finnish postmenopausal women. Serum levels of RA and VA also showed a significant inverse relationship to breast cancer risk [131]. A study conducted in New York [132] found that there was a nonsignificant inverse association between intake of RA and incidence of breast cancer in premenopausal but not postmenopausal women. The benefit was more apparent in women with the more aggressive ER negative tumors. Three other studies did not find a relationship between RA and breast cancer risk. The methodological limitations in these, and other RA/VA studies, have been discussed [123].

Branched-Chain Fatty Acids

Branched long-chain fatty acids (BCFA) are synthesized by rumen bacteria, and iso- and anteiso-BCFAs, particularly those with a chain length of 13 to 17 carbon atoms, are found in milk fat [123]. Initially, Yang et al. [133] reported that 13-methyltetradecanoic acid (13-MTDA) induced cell death in human breast cancer cells by rapid induction of apoptosis. Recently, Wongtangtintharn et al. [129] tested the antitumour activity of a series of iso-BCFA in two human breast cancer cell lines. The highest antitumour activity was found with iso-16:0, and the activity decreased with an increase or decrease in chain-length from iso-16:0. Anteiso-BCFAs were also cytotoxic. Interestingly, cytotoxicity of 13-MTDA was comparable to RA. Both 13-MTDA and RA inhibited FAS.

Butyric Acid

Butyric acid, uniquely present in milk fat, is a potent anticancer agent, which induces differentiation and apoptosis and inhibits proliferation and angiogenesis. Although butyrate has a short half-life in the circulation this can be increased when butyrate is present as a derivative. In the case of milk fat, butyrate is esterified as a triacylglycerol, and about one-third of all milk fat triglycerides contain butyrate. Synergy with other dietary anticancer agents like vitamin A, vitamin D and resveratrol reduce the plasma concentration of butyrate required to

modulate cell growth [123]. Two studies showed that dietary butyrate significantly inhibited chemically induced mammary tumor development in rats [134,135].

Milk Proteins

Evidence from animal studies and *in vitro* studies with human breast cancer cells suggest that milk proteins, especially those associated with the whey fraction, have anticarcinogenic properties [136,137]. Whey protein is a rich source of cysteine, which is essential for the synthesis of glutathione. Glutathione is a potent cellular antioxidant and also acts by itself or by its related enzymes as a detoxifying agent that facilitates the elimination of mutagens, carcinogens and other xenobiotics from the body [136]. Results from a recent nested case-control study from within the prospective Nurses' Health Study [138] show that women with higher plasma concentrations of cysteine had a significantly reduced risk of breast cancer.

CONCLUSION

The etiology of breast cancer is still largely undetermined, although a woman's reproductive history is considered an important determinant. A role for diet in breast cancer is not well established. An examination of the results from more than 40 case-control studies and 12 cohort studies does not support an association between dairy product consumption and the risk of breast cancer. The research that addresses theories about an association between dairy product consumption and breast cancer via fat, IGF-1, GH and estrogens was examined, however, the weight of evidence does not support a link. Although estrogens and the GH/IGF-1 axis play a critical role in the development of the mammary gland and in breast cancer, the mechanisms are complex and cancer is probably influenced more by autocrine/paracrine secretion than by circulating levels. Nevertheless, the daily contribution of these factors from dairy product consumption is far too small compared to daily endogenous secretion to exert a physiological effect. The presence of rumenic, vaccenic, butyric and branched chain fatty acids, cysteine-rich whey proteins, calcium and vitamin D in milk has the potential to help prevent breast cancer.

REFERENCES

1. Greenlee RT, Murray T, Bolden S, Wingo PA: Cancer statistics, 2000. CA Cancer J Clin 50:7–33, 2000.
2. Fisher B, Osborne CK, Margolese R, Bloomer W: Neoplasms of the breast. In Holland JF, Frei E, Bast RC, Kufe DW, Morton DL, Weichselbaum RR (eds): "Cancer Medicine," 3rd ed. Philadelphia: Lee and Febiger, pp 1706–1774, 1993.
3. Pike MC, Spicer DV, Dahmoush L, Press MF: Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 15:17–35, 1993.
4. Kuller LH: The etiology of breast cancer - From epidemiology to prevention. Public Health Rev 23:157–213, 1995.
5. Kelsey JL, Bernstein L: Epidemiology and prevention of breast cancer. Annu Rev Public Health 17:47–67, 1996.
6. Lacey JV, Devesa SS, Brinton LA: Recent trends in breast cancer incidence and mortality. Environ Mol Mutagen 39:82–88, 2002.
7. Marsden J: The menopause, hormone replacement therapy and breast cancer. J Steroid Biochem Mol Biol 83:123–132, 2003.
8. Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura AMY, West DW, Wu-Williams AH, Kolonel LN, Horn-Ross PL, Rosenthal JF, Hyer MB: Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 85:1819–1827, 1993.
9. Lopez-Otin C, Diamandis EP: Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. Endocr Rev 19:365–396, 1998.
10. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ, Easton DF: A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8:843–854, 1999.
11. Harris JR, Lippman ME, Veronesi U, Willett WC: Breast cancer. N Engl J Med 327:319– 328, 1992.
12. Land CE: Studies of cancer and radiation dose among atomic bomb survivors. JAMA 274:402–407, 1995.
13. Berube S, Diorio C, Verhoek-Oftedahl W, Brisson J: Vitamin D, calcium, and mammographic breast densities. Cancer Epidemiol Biomarkers Prev 13:1466–1472, 2004.
14. Bernstein L, Ross RK: Endogenous hormones and breast cancer risk. Epidemiol Rev 15:48–65, 1993.
15. Michels KB, Trichopoulos D, Robins JM, Rosner BA, Manson JE, Hunter DJ, Colditz GA, Hankinson SE, Speizer FE, Willett WC: Birthweight as a risk factor for breast cancer. Lancet 348: 1542–1546, 1996.
16. Willett WC: Diet and Breast Cancer. J Intern Med 249:395–411, 2001.
17. Outwater JL, Nicholson A, Barnard, N: Dairy products and breast cancer: the IGF-1, estrogen, and bGH hypothesis. Med Hypoth 48:453–461, 1997.
18. Epstein SS: Re; Role of the insulin-like growth factors in cancer development and progression. J Natl Cancer Inst 93:238, 2001.
19. Li XM, Ganmaa D, Sato A: The experience of Japan as a clue to the etiology of breast and ovarian cancers: relationship between death from both malignancies and dietary practices. Med Hypoth 60:268–275, 2003.
20. Qin L-Q, Xu J-Y, Wang P-Y, Ganmaa D, Li J, Wang J, Kaneko T, Hoshi K, Shirai T, Sato A: Low-fat milk promotes the development of 7,12-dimethylbenz(a)anthracine (DMBA)-induced mammary tumors in rats. Int J Cancer 110:491–496, 2004.
21. Knekt P, Jarvinen R: Intake of dairy products and breast cancer risk. In Yurawecz MP, Mossoba MM, Kramer JKC, Pariza MW, Nelson GJ (eds): "Advances in Conjugated Linoleic Acid Research, Volume1," Champaign, Illinois: AOAC Press, pp 444–470, 1999
22. Boyd NF, Stone J, Vogt KN, Connelly BS, Martin LJ, Minkin S: Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. Br J Cancer 89:1672– 1685, 2003.
23. Missmer SA, Smith-Warner SA, Spiegelman D, Yaun S-S, Adami H-O, Beeson W.L., van den Brandt PA, Fraser GE,

Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, Miller AB, Potter JD, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter D J: Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. Int J Epidemiol 31:78–85, 2002.

24. Moorman PG, Terry PD: Consumption of dairy products and the risk of breast cancer: a review of the literature. Am J Clin Nutr 80:5–14, 2004.
25. Hirose K, Takezaki T, Hamajima N, Miura S, Tajima K: Dietary factors protective against breast cancer in Japanese premenopausal and postmenopausal women. Int J Cancer 107:276–282, 2003.
26. Shannon J, Cook LS, Stanford JL: Dietary intake and risk of postmenopausal breast cancer. Cancer Causes Control 14:19–27, 2003.
27. Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA, Willett WC, Premenopausal fat intake and risk of breast cancer. J Natl Cancer Inst. 95:1079–1085, 2003.
28. Frazier AL, Li L, Cho E, Willett WC, Colditz GA: Adolescent diet and risk of breast cancer. Cancer Causes Control 15:73–82, 2004.
29. Colditz GA, Frazier AL: Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. Cancer Epidemiol Biomark Prev 4:567–571, 1995.
30. Beth M, Berger MR, Aksoy M, Schmahl, D: Comparison between the effects of dietary fat level and of calorie intake on methylnitrosourea-induced mammary carcinogenesis in female SD rats. Int J Cancer 39:737–744, 1987.
31. Ip C: Quantitative assessment of fat and calorie as risk factors in mammary carcinogenesis in an experimental model. In Mettlin CJ, Aoki K (eds): "Recent Progress in Research on Nutrition and Cancer." New York: Wiley-Liss, Inc, pp 107–117, 1990.
32. Carroll KK: Experimental evidence of dietary factors and hormone-dependent cancers. Cancer Res 35:3374–3383, 1975.
33. Rose DP, Boyar AP, Wynder EL: International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer 58:2363–2371, 1986.
34. Willett WC: "Nutritional Epidemiology," 2nd ed. Oxford: Oxford University Press, 1998.
35. Goodwin PJ, Boyd NF: Critical appraisal of the evidence that dietary fat intake is related to breast cancer risk in humans. J Natl Cancer Inst 79:473–485, 1987.
36. Howe GR, Hirohata T, Hislop TG, Iscovich JM, Yuan J-M, Katsouyanni K, Lubin F, Marubini E, Modan B, Rohan T, Toniolo P, Shunzhang Y: Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 82:561– 569, 1990.
37. Graham S, Marshall J, Mettlin C, Rzepka T, Nemoto T, Byers T: Diet in the epidemiology of breast cancer. Am J. Epidemiol 116:68–75, 1982.
38. La Vecchia C, Negri E, Franceschi S, Decarli A, Giacosa A, Lipworth L: Olive oil, other dietary fats, and the risk of breast cancer (Italy). Cancer Causes Control 6:545–550, 1995.
39. Hunter DJ, Spiegelman D, Adami H-O, Beeson L, van den Brandt PA, Folson AR, Fraser GE, Goldbohm RA, Graham S, Howe GR, Kushi LH, Marshall JR, McDermott A, Miller AB, Speizer FE, Wolk A, Yaun S-S, Willett WC: Cohort studies of fat intake and the risk of breast cancer. N Engl J Med 334:356–361, 1996.
40. Smith-Warner SA, Spiegelman D, Adami H-O, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham, S, Kushi LH, Miller AB, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter DJ: Types of dietary fat and breast cancer: a pooled analysis of cohort studies. Int. J Cancer 92:767–774, 2001.
41. Holmes MD, Stampfer MJ, Colditz GA, Rosner B, Hunter DJ, Willett WC: Dietary factors and the survival of women with breast carcinoma. Cancer 86:826–835, 1999.
42. Willett WC: Specific fatty acids and risks of breast and prostate cancer: dietary intake. Am J Clin Nutr 66:1557S–1563S, 1997.
43. Smith-Warner SA, Spiegelman D, Yaun, S-S, Adami H-O, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Miller AB, Potter JD, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter DJ: Intake of fruit and vegetables and risk of breast cancer. JAMA 285:769–776, 2001.
44. Le Roith D: Insulin-like growth factors. N Engl J Med 336:633–640, 1997.
45. Jones JI, Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34, 1995.
46. Rajaram S, Baylink DJ, Mohan S: Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and function. Endocr Rev 18:801–831, 1997
47. Yu H, Rohan T: Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 92:1472–1489, 2000.
48. Ricort J-M, Binoux M: Insulin-like growth factor (IGF) binding protein-3 inhibits type 1 IGF receptor activation independently of its IGF binding affinity. Endocrinology 142:108– 113,2001.
49. Oh Y, Muller HL, Lamson G, Rosenfeld RG: Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. J Biol Chem 268:14964–14971, 1993.
50. Ruan W, Kleinberg DL: Insulin-like growth factor 1 is essential for terminal end bud formation and ductal morphogenesis during mammary development. Endocrinology 140:5075–5081, 1999.
51. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE: The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev 21:215–244, 2000.
52. Wood TL, Yee D: IGFs and IGFBPs in the normal mammary gland and in breast cancer. J Mammary Gland Biol Neoplasia 5:1–5, 2000.
53. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE: Serum insulin-like growth factor-1 in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 78:744–752, 1994.
54. Thissen J-P, Ketelslegers J-M, Underwood LE: Nutritional regulation of the insulin-like growth factors. Endocr Rev 15:80–101, 1994.
55. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M: Insulin-like growth factor (IGF)-1, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363:1346–1353, 2004.
56. Shi R, Yu H, McLarty J, Glass J: IGF-1 and breast cancer: a meta-analysis. Int J Cancer 111:418–423, 2004.
57. Sugumar A, Liu Y-C, Xia Q, Koh Y-S, Matsuo K: Insulin-like

growth factor (IGF)-1 and IGF-binding protein 3 and the risk of premenopausal breast cancer: a meta-analysis of literature. Int J Cancer 111:293–297, 2004.
58. Sachdev D, Yee D: The IDF system and breast cancer. Endocr Relat Cancer 8:197–209, 2001.
59. Salahifar H, Baxter RC, Martin JL: Insulin-like growth factor binding protein (IGFBP)-3 protease activity secreted by MCF-7 breast cancer cells: Inhibition by IGFs does not require IGF-IGFBP interaction. Endocrinology 138:1683–1690, 1997.
60. Woodson K, Tangrea JA, Pollak M, Copeland TD, Taylor PR, Virtamo J, Albanes D: Serum insulin-like growth factor 1: Tumor marker or etiologic factor? A prospective study of prostate cancer among Finnish mean. Cancer Res 63:3991–3994, 2003.
61. Martin JL, Coverley JA, Pattison ST, Baxter RC: Insulin-like growth factor-binding protein-3 production by MCF-7 breast cancer cells: stimulation by retinoic acid and cyclic adenosine monophosphate and differential effects of estradiol. Endocrinology 136:1219– 1226, 1995.
62. Sejrsen K, Pedersen LO, Vestergaard M, Purup S: Biological activity of bovine milk. Contribution of IGF-1 and IGF binding proteins. Livest Prod Sci 70:79–85, 2001.
63. Etherton TD, Bauman DE: Biology of somatotropin in growth and lactation of domestic animals. Physiol Rev 78:745–761, 1998.
64. Collier RJ, Miller MA, Hildebrandt JR, Torkelson AR, White TC, Madsen KS, Vicini JL, Eppard PJ, Lanza GM: Factors affecting insulin-like growth factor-1 concentration in bovine milk. J Dairy Sci 74:2905–2911, 1991.
65. Honegger A, Humbel RE: Insulin-like growth factors I and II in fetal and adult bovine serum. J Biol Chem 261:569–575, 1986.
66. Chaurasia OP, Marcuard SP, Seidel ER: Insulin-like growth factor 1 in human gastrointestinal exocrine secretions. Regul Pept 50:113–119, 1994.
67. FAO/WHO Joint Expert Committee on Food Additives (JECFA): Toxicological evaluation of certain veterinary drug residues in food, WHO Food Additives Series 41, pp 125–146, 1998.
68. Guler H-P, Zapf J, Schmid C, Froesch ER: Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh) 121:753–758, 1989.
69. Alpers DH: Digestion and absorption of carbohydrates and proteins. In Johnson LR (ed): "Physiology of the Gastrointestinal Tract," 3rd ed. New York: Raven Press, pp 1723–1749, 1994.
70. Burrin DG: Is milk-borne insulin-like growth factor-1 essential for neonatal development? J Nutr 127:975S–979S, 1997.
71. Coombes JS, Conacher M, Austen SK, Marshall PA: Dose effects of oral bovine colostrums on physical work capacity in cyclists. Medicine & Science in Sports & Exercise 34:1184– 1188, 2002.
72. Kuipers H, van Breada E, Verlaan G, Smeets R: Effects of oral bovine colostrum supplementation on serum insulin-like growth factor-1 levels. Nutrition 18:566–567, 2002.
73. Buckley JD, Brinkworth GD, Abbott MJ: Effect of bovine colostrum on anaerobic exercise performance and plasma insulin-like growth factor I. J. Sports Sci 21:577–588, 2003.
74. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI, Rosen CJ: Dietary changes favorably affect bone remodeling in older adults. J Am Diet Assoc 99:1228–1233, 1999.
75. Ma J, Giovannucci E, Pollak M, Chan JM, Gaziano JM, Willett WC, Stampfer MJ: Milk intake, circulating levels of insulin-like growth factor-1, and risk of colorectal cancer in men. J Natl Cancer Inst 93:1330–1336, 2001.
76. Khalil DA, Lucas EA, Juma S, Smith BJ, Payton ME, Arjmandi BH: Soy protein supplementation increases serum insulin-like growth factor-1 in young and old men but does not effect markers of bone metabolism. J Nutr 132:2605–2608, 2002.
77. Ammann P, Laib A, Bonjour J-P, Meyer JM, Ruegsegger P, Rizzoli R: Dietary essential amino acid supplements increase bone strength by influencing bone mass and bone microarchitecture in ovariectomized adult rats fed on isocaloric low-protein diet. J Bone Miner Res 17:1264–1272.
78. Jordan VC: Estrogens and antiestrogens. In Holland JF, Frei E, Bast RC, Kufe DW, Morton DL, Weichselbaum RR (eds): "Cancer Medicine," 3rd ed. Philadelphia: Lee and Febiger, pp 857–865, 1993.
79. Campos SM: Aromatase inhibitors for breast cancer in postmenopausal women. Oncologist 9:126–136, 2004.
80. Collaborative Group on Hormonal Factors in Breast Cancer: Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Lancet 347:1713–1727, 1996.
81. Collaborative Group on Hormonal Factors in Breast Cancer: Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. Lancet 350:1047–1059, 1997.
82. Stahlberg C, Pedersen AT, Lynge E, Ottesen B: Hormone replacement therapy and risk of breast cancer: the role of progestins. Acta Obstet Gynecol Scand 82:335–344, 2003.
83. Liehr JC: Is estradiol a genotoxic mutagenic carcinogen? Endocr Rev 21:40–54, 2000.
84. Clemons M, Goss P: Estrogens and the risk of breast cancer. N Engl J Med 334:276–285, 2001.
85. Russo J, Russo IH: Genotoxicity of steroidal estrogens. Trends Endocrinol Metab 15:211–214, 2004.
86. Martin MB, Stoica A: Insulin-like growth factor-1 and estrogen interactions in breast cancer. J Nutr 132:3799S–3801S, 2002.
87. Laban C, Bustin SA, Jenkins PJ: The GH-IGF-1 axis and breast cancer. Trends Endocrinol Metab 14:28–34, 2003.
88. Key TJ: Serum oestradiol and breast cancer risk. Endocr Relat Cancer 6:175–180, 1999.
89. Hsueh AJW, Billig H: Ovarian hormone synthesis and mechanism of action. In DeGroot LJ (ed): "Endocrinology," 3rd ed. Philadelphia: WB Saunders, pp 2019–2030, 1995.
90. Odell WD: The menopause and hormone replacement. In DeGroot LJ (ed): "Endocrinology," 3rd ed. Philadelphia: WB Saunders, pp 2128–2139, 1995.
91. The Endogenous Hormones and Breast Cancer Collaborative Group: Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 94:606–616, 2002.
92. McTiernan A, Tworoger SS, Ulrich CM, Yasui Y, Irwin ML, Rajan KB, Sorensen B, Rudolph RE, Bowen D, Stanczyk FZ, Potter JD, Schwartz RS: Effect of exercise on serum estrogens in postmenopausal women: a 12-month randomized clinical trial. Cancer Res. 64:2923–2928, 2004.

93. Thijssen JHH, van Landeghem AAJ, Poortman J: Uptake and concentration of steroid hormones in mammary tissue. Ann NY Acad Sci 464:106–116, 1986.
94. Pasqualini JR, Chetrite G, Blacker C, Feinstein M-C, Delalonde L, Talbi M, Maloche C: Concentrations of estrone, estradiol, and estrone sulphate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. J Clin Endocrinol Metab 81:1460–1464, 1996.
95. van Landeghem AAJ, Poortman J, Nabuurs M, Thijssen JHH: Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. Cancer Res 45:2900–2906, 1985.
96. Yue W, Santner SJ, Masamura S, Wang J-P, Demers LM, Hamilton C, Santen RJ: Determinants of tissue estradiol levels and biological responsiveness in breast tumors. Breast Cancer Res Treat 49:1S–7S, 1998.
97. Geisler J: Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators. J Steroid Biochem Mol Biol 86:245–253, 2003.
98. Nakata T, Takashima S, Shiotsu Y, Murakata C, Ishida H, Akinaga S, Li P-K, Sasano H, Suzuki T, Saeki T: Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients. J Steroid Biochem Mol Biol 86:455–460, 2003.
99. Yue W, Santen RJ, Wang J-P, Hamilton CJ, Demers LM: Aromatase within the breast. Endocrine-Related Cancer 6:157–164, 1999.
100. Masamura S, Santner SJ, Heitjan DF, Santen RJ: Estrogen deprivation causes estradiol hypersensitivity in human breast cancer cells. J Clin Endocrinol Metab 80:2918–2925, 1995.
101. Hartmann S, Lacorn M, Steinhart H: Natural occurrence of steroid hormones in food. Food Chem 62:7–20, 1998.
102. Henderson KM, Karanikolas M, Kenealy L, Macmillan KI: Concentrations of oestrone sulphate during pregnancy in milk from Jersey and Friesian dairy cows differing in milk yield and composition. NZ Vet J 42:89–92, 1994.
103. Wilson SJ, Marion RS, Spain JN, Spiers DE, Keisler DH, Lucy MC: Effects of controlled heat stress on ovarian function of dairy cattle.1. Lactating cows. J Dairy Sci 81:2124–2131, 1998.
104. Biller BMK, Daniels GH: Neuroendocrine regulation and diseases of the anterior pituitary and hypothalamus. In Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL (eds): "Harrison's Principles of Internal Medicine," 14th ed. New York: McGraw-Hill, pp 1972–1999, 1998.
105. Walden PD, Ruan W, Feldman M, Kleinberg DL: Evidence that the mammary fat pad mediates the action of growth hormone in mammary gland development. Endocrinology 139:659–662, 1998.
106. Cohen P, Clemmons DR, Rosenfeld RG: Does the GH-IGF axis play a role in cancer pathogenesis? Growth Hormone & IGF Res 10:297–305, 2000.
107. Kaulsay K, Zhu T, Bennett WF, Lee K-O, Lobie PE: The effects of autocrine human growth hormone (hGH) on human mammary carcinoma cell behavior are mediated via the hGH receptor. Endocrinology 142:767–777, 2001.
108. Yang X-F, Beamer WG, Huynh H, Pollak M: Reduced growth of human breast cancer xenografts in hosts homozygous for the *lit* mutation. Cancer Res. 56:1509–1511, 1996.
109. Swanson SM, Unterman T: The growth hormone-deficient spontaneous dwarf rat is resistant to chemically induced mammary carcinoma. Carcinogenesis 23:977–982, 2002.
110. Emerman JT, Leahy M, Gout PW, Bruchovsky N: Elevated growth hormone levels in sera from breast cancer patients. Horm Metabol Res 17:421–424, 1985.
111. Bauman DE: Bovine somatotropin and lactation: from basic science to commercial application. Domest Anim Endocrinol 17: 101–116, 1999.
112. Daughaday WH, Barbano DM: Bovine somatotropin supplementation of dairy cows. Is milk safe? JAMA 264:1003–1005, 1990.
113. Juskevich JC, Guyer CG: Bovine growth hormone: human food safety evaluation. Science 249:875–884, 1990.
114. Collier RJ, Bauman DE: Re: Re: Role of the insulin-like growth factors in cancer development and progression. J Natl Cancer Inst 93:876, 2001.
115. Torkelson AR, Dwyer KA, Rogan GJ, Ryan RL: Radioimmunoassay of somatotropin in milk from cows administered recombinant bovine samatotropin. J Dairy Sci 70 (Suppl 1):146, 1987.
116. Souza SC, Frick GP, Wang X, Kopchick JJ, Lobo RB, Goodman HM: A single arginine residue determines species specificity of the human growth hormone receptor. Proc Natl Acad Sci USA 92:959–963, 1995.
117. Lipkin M, Newmark HL: Vitamin D, calcium and prevention of breast cancer: a review. J Am Coll Nutr 18:392S–397S, 1999.
118. Colston KW, Hansen CM: Mechanisms implicated in the growth regulatory effects of vitamin D in breast cancer. Endocr Relat Cancer 9:45–59, 2001.
119. Javitt NB, Budai K, Miller DG, Cahan AC, Raju U, Levitz M: Breast-gut connection: origin of chenodeoxycholic acid in breast cyst fluid. Lancet 343:633–635, 1994.
120. Pizer ES, Jackisch, C, Wood FD, Pasternack GR, Davidson NE, Kuhajda FP: Inhibition of fatty acid synthesis induces programmed cell death in human breast cancer cells. Cancer Res 56:2745–2747, 1996.
121. Zemel MB, Role of dietary calcium and dairy products in modulating adiposity. Lipids 38:139–146, 2003.
122. Boyapati SM, Shu XO, Jin F, Dai Q, Ruan Z, Gao Y-T, Zheng W: Dietary calcium intake and breast cancer risk among Chinese women in Shanghai. Nutr Cancer 46:38–43, 2003.
123. Parodi PW: Milk fat in human nutrition. Aust J Dairy Technol 59:3–59, 2004.
124. Ip MM, Masso-Welch PA, Ip C: Prevention of mammary cancer with conjugated linoleic acid: role of stroma and the epithelium. J Mammary Gland Biol Neoplasia 8:103–118, 2003.
125. O'Shea M, Devery R, Lawless F, Murphy J, Stanton C: Milk fat conjugated linoleic acid (CLA) inhibits growth of mammary MCF-7 cancer cells. Anticancer Res 20:3591–3602, 2000.
126. Miller A, McGrath E, Stanton C, Devery R: Vaccenic acid (*t*11–18:1) is converted to *c*9,*t*11-CLA in MCF-7 and SW480 cancer cells. Lipids 38:623–632, 2003.
127. Masso-Welch PA, Zangani D, Ip C, Vaughan MM, Shoemaker SF, McGee SO, Ip MM: Isomers of conjugated linoleic acid differ in their effects on angiogenesis and survival of mouse mammary adipose vasculature. J Nutr 134:299–307, 2004.
128. Oku H, Wongtangtintharn S, Iwasaki H, Toda T: Conjugated linoleic acid (CLA) inhibits fatty acid synthetase activity *in vitro.* Biosci Biotechnol Biochem 67:1584–1586, 2003.

129. Wongtangtintharn S, Oku H, Iwasaki H, Toda T: Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. J Nutr Sci Vitaminol 50:137–143, 2004.
130. Buison A, Ordiz F, Pellizzon M, Jen K-LC: Conjugated linoleic acid does not impair fat regain but alters IGF-1 levels in weight-reduced rats. Nutr Res 20:1591–1601, 2000.
131. Aro A, Mannisto S, Salminen I, Ovaskainen M-L, Kataja V, Uusitupa M: Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in post menopausal women. Nutr Cancer 38:151–157, 2000.
132. McCann SE, Ip C, Ip MM, McGuire MK, Muti P, Edge SB, Trevisan M, Freudenheim JL: Dietary intake of conjugated linoleic acids and risk of premenopausal and postmenopausal breast cancer, Western New York Exposures and Breast Cancer Study (WEB Study). Cancer Epidemiol Biomark Prev 13:1480–1484, 2004.
133. Yang Z, Liu S, Chen X, Chen H, Huang M, Zheng J: Induction of apoptotic cell death and *in vivo* growth inhibition of human cancer cells by a saturated branched-chain fatty acid,13-methyltetradecanoic acid. Cancer Res 60:505–509, 2000.
134. Yanagi S, Yamashita M, Imai S: Sodium butyrate inhibits the enhancing effect of high fat diet on mammary tumorigenesis. Oncology 50:201–204, 1993.
135. Belobrajdic DP, McIntosh, GH: Dietary butyrate inhibits NMU-induced mammary cancer in rats. Nutr Cancer 36:217–223, 2000.
136. Parodi PW: A role for milk proteins in cancer prevention. Aust J Dairy Technol. 53:37–47, 1998.
137. Hakkak R, Korourian S, Shelnutt SR, Lensing S, Ronis MJJ, Badger TM: Diets containing whey proteins or soy protein isolate protect against 7,12-dimethylbenz(a)anthracine-induced mammary tumors in female rats. Cancer Epidemiol Biomark Prev 9:113–117, 2000.
138. Zhang SM, Willett WC, Selhub J, Manson JE, Colditz GA, Hankinson SE: A prospective study of plasma total cysteine and risk of breast cancer. Cancer Epidemiol Biomark Prev 12:1188–1193, 2003.
139. Shin M-H, Holmes MD, Hankinson SE, Wu K, Colditz GA, Willett WC: Intake of dairy products, calcium, and Vitamin D and risk of breast cancer. J Natl Cancer Inst 94:1301–1311, 2002.
140. Hjartaker A, Laake P, Lund E: Childhood and adult milk consumption and risk of premenopausal breast cancer in a cohort of 48,844 women - the Norwegian Women and Cancer Study. Int J Cancer 93:888–893, 2001.
141. Shu XO, Jin F, Dai Q, Wen W, Potter JD, Kushi LH, Ruan Z, Gao Y-T, Zheng W: Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. Cancer Epidemiol Biomark Prev 10:483–488, 2001.
142. Potischman N, Weiss HA, Swanson CA, Coates RJ, Gammon MD, Malone KE, Brogan D, Stanford JL, Hoover RN, Brinton LA: Diet during adolescence and risk of breast cancer among young women. J Natl Cancer Inst 90:226–233, 1998.
143. Pryor M, Slattery ML, Robison LM, Egger M: Adolescent diet and breast cancer in Utah. Cancer Res 49:2161–2167, 1989.
144. Hislop TG, Coldman AJ, Elwood JM, Brauer G, Kan L: Childood and recent eating patterns and risk of breast cancer. Cancer Detect Prev 9:47–58, 1986.

Received September 9, 2005

Review

The Myth of Increased Lactose Intolerance in African-Americans

Katherine G. Byers, RD, and Dennis A. Savaiano, PhD

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana

Key words: lactose intolerance, lactose maldigestion, African-Americans, calcium

In the United States, approximately three fourths of African-Americans have the potential for symptoms of lactose intolerance because lactose digestion depends on the presence of the enzyme lactase-phlorizin hydrolase which is reduced by up to 90–95% in individuals with lactase nonpersistence. The 'African-American diet' is more likely to be low in a variety of vitamins and minerals, including calcium. African-Americans consume low amounts of dairy foods and do not meet recommended intakes of a variety of vitamins and minerals, including calcium. Low intake of calcium and other nutrients put African-Americans at an increased risk for chronic diseases. The 2005 Dietary Guidelines recommend consuming three servings of dairy foods per day to ensure adequate calcium intake, among other nutrients, and the National Medical Association has recently published a similar recommendation of three to four servings of dairy per day for the African-American population. Research has shown that lactose maldigesters, including African-American maldigesters, can consume at least one cup (8 oz) of milk without experiencing symptoms, and that tolerance can be improved by consuming the milk with a meal, choosing yogurt or hard cheeses, or using products that aid in the digestion of lactose such as lactase supplements or lactose-reduced milks.

Key teaching points:

- African-Americans are at high risk for a number of chronic diseases that may be ameliorated by adequate calcium intake.
- Lactose maldigesters, including African-American maldigesters, can consume one cup (8 oz) of milk in one meal setting without experiencing symptoms.
- Lactose intolerance can be limited by drinking milk with meals.
- Yogurts and hard cheeses are well tolerated.
- African-Americans, like other Americans, should not avoid consumption of dairy products due to concerns about lactose intolerance.

INTRODUCTION

The risk for a number of chronic diseases is elevated among African-Americans. The debate over the cause of this elevated risk continues as additional data accumulate regarding unique genetic, social and environmental factors affecting African-Americans. Hypertension, heart disease and other illnesses affect African-Americans at a rate which is higher than the average for the US population [1]. As a group, African-Americans consume a diet that is lower in recommended nutrients and meet fewer of the national recommendations than the average American. In comparison to national recommendations, the African-American diet is more likely to be low in vitamins and minerals, including calcium, and higher in fat [2,3]. Additionally, the food pattern in the African-American diet includes more meat and fats, while being lower in fruits, vegetables and dairy foods [4,5]. This pattern is markedly different than the recent recommendations of the Dietary Guidelines Committee, and the advice of numerous other nutrition guidelines. The increased consumption of fruits, vegetables and low fat dairy foods are among the most common recommendations currently being promoted to improve the American diet [6].

Adequate calcium in the US diet is typically associated with the consumption of 3 or more servings of dairy foods per day [7]. Dairy foods are, of course, an excellent source of calcium

Address reprint requests to: Dennis A. Savaiano, PhD, Purdue University, College of Consumer and Family Sciences, 701 West State Street, West Lafayette, IN 47907. E-mail: savaiano@purdue.edu.

Journal of the American College of Nutrition, Vol. 24, No. 6, 569S–573S (2005)
Published by the American College of Nutrition

(along with several other nutrients) and provide about 73% of the calcium in the US diet [8]. One perceived barrier to the consumption of dairy foods among African-Americans is the potential for lactose intolerance. Approximately three fourths of African-Americans are lactose maldigesters, and thus have the potential for symptoms of lactose intolerance [9,10]. Primary acquired hypolactasia, more commonly referred to as lactase nonpersistence (LNP), is estimated to affect approximately 75% of the world's population. In LNP there is a 90–95% reduction in activity of the enzyme lactase-phlorizin hydrolase (LPH) which is synthesized in enterocytes controlled by the LPH gene on chromosome 2 [11]. Alternatively, congenital lactase deficiency, where lactase is completely absent at birth, does exist, however this condition is very rare. Recently, some special interest groups have suggested that national recommendations to include three servings of dairy foods in the diets of all Americans are racially biased because of the high incidence of lactose intolerance among African-Americans. In contrast, the National Medical Association (the nation's oldest and largest medical association that represents physicians of African descent in the US and Caribbean with over 30,000 members) has recently reviewed the scientific literature on health risks, diets and dairy foods in relation to the African-American population and concludes that African-Americans should consume a minimum of 3 to 4 servings of dairy foods per day in order to improve their diets, especially in relation to adequate calcium consumption [12]. We, therefore, are reviewing the literature to determine if evidence exists to support the hypothesis that African-Americans experience increased intolerance to lactose and thus should limit dairy foods that are high in lactose.

DEFINING LACTOSE INTOLERANCE

Lactose intolerance is the reduced ability to digest lactose due to decreased lactase activity in the small intestine [13]. There is substantial research evaluating tolerance to lactose among lactose maldigesters, and numerous reviews have been published evaluating these studies [11,13,14,15]. These studies evaluated a variety of ethnic groups including Asian-Americans and Hispanic-Americans and may or may not include African-Americans as subjects. Typically, most studies have selected subjects based on estimates of maldigestion, using breath hydrogen, blood glucose, or other clinical tests, rather than race or ethnic background. However, it is clear from blinded experimental trials that most, if not all, lactose maldigesters can consume at least one 8 ounce glass of milk [16,17,18] without experiencing physiologic symptoms. Tolerance is further improved when milk is consumed with a meal, such that stomach emptying and intestinal transit are slowed, facilitating gastrointestinal digestion of lactose [19,20]. In addition, tolerance is dose-dependent. When more than one glass of milk is consumed in the fasting state, symptoms of intolerance exceed baseline symptoms. These symptoms are most likely to be excessive flatulence and stomach discomfort. Acute diarrhea occurs much less frequently. Since many dairy foods, including hard cheeses, ice cream, yogurts, cottage cheese and even soft cheeses, contain reduced amounts of lactose, these foods present less potential for symptoms of intolerance [21,22]. Yogurt does not necessarily have a reduced amount of lactose when compared with the same volume of milk. However, the improved tolerance to lactose observed with yogurt is likely due to autodigestion of lactose in the intestine by the starter culture bacteria in the yogurt [22]. Finally, tolerance is also improved with repeated exposure to lactose in the diet, presumably due to colon microbial adaptation which enhances fermentation and reduces gas production [23,24].

STUDIES OF LACTOSE INTOLERANCE IN AFRICAN-AMERICANS

What about African-Americans? Do they experience increased symptoms of lactose intolerance? Does lactose intolerance among African-Americans prevent them from consuming moderate amounts of milk in a mixed diet? In 1966, Bayless and Rosensweig studied 20 African-American and 20 Caucasian prisoners for LNP and intolerance [25]. As confirmed by subsequent studies, approximately 70% of the African-Americans were LNP based on a lactase assay of mucosal biopsy samples and a blood glucose assay for maldigestion. In this study, lactose in a water solution was administered orally in very high doses. Each subject received a large dose of lactose based on body size (50 gm/sq m of body surface). Some doses were the equivalent of up to 1¾ quarts of milk. Subjects almost uniformly experienced symptoms of intolerance to this dose of lactose. However, the authors note that 'six subjects had to drink a quart of milk at one time before these symptoms developed' and 'amounts less than one or two glasses of milk, as in cereal or coffee were well tolerated'. Since only one non African-American maldigester was studied, this report does not provide direct comparisons of tolerance between African-Americans and Caucasians. However, the study does provide some evidence for the dose-response relationship between lactose consumption and tolerance at levels (1–2 cups of milk) that are similar to studies of non African-American maldigesters [26].

More directly pertinent to this review, in 1971, Paige et al [27] addressed the question of the ability of African-American children to consume a moderate amount of milk: the half pint quantity served in schools. This observational study was conducted on two different school days and researchers categorized children as either milk drinkers or non-milk drinkers. After passing through the food line and consuming their food, the students surrendered their meal trays and researchers weighed the amount of milk remaining in the container. A milk

drinker was defined as one who consumed 50% or more of their milk by weight and a non-milk drinker was one who consumed less than 50% of their milk. Researchers found that a greater proportion of African-American children failed to consume 50% of the milk served (20% of African-American children vs. 10% of Caucasian children) and concluded that milk rejection among African-American children was significantly higher than Caucasian children. Maldigestion was determined using blood glucose. Lactose intolerance symptoms were measured using a lactose load of 50 gm/sq m body surface, an amount much higher than a standard 8 oz portion of milk containing 12 gm lactose. It does appear from this study that the African-American children who were maldigesters appear more likely to be non-drinkers. Given that only five Caucasian children were maldigesters, the data is insufficient to determine if African-American maldigesters experienced greater or lesser symptoms of intolerance.

Marrs reported on the milk drinking habits of the elderly in 1978 [28]. The investigators provided 240 ml of milk, as part of a meal, in a congregate dining situation. Participants self-selected the type of milk: whole, skim, chocolate and buttermilk as well as alternate beverages such as coffee and water. Following the meal, participants completed a questionnaire regarding the type of milk they preferred, milk acceptability and perceived milk tolerance. Seventy-five/81 Hispanics, 123/139 African-Americans and 109/117 'Anglos' reported that they drank the milk and were symptom-free. Two of the Hispanics, 7 of the African-Americans and 1 'Anglo' drank the milk and reported some symptoms. Six Hispanics, 2 African-Americans and 3 'Anglos' did not drink the milk because of symptoms. Clearly, the vast majority of African-Americans tolerated one glass of milk in this study. Interestingly, only 1.4% of the African-Americans listed 'symptoms' as their reason for avoiding milk, compared with 6.6% of Hispanics and 2.5% of 'Anglos'. Participants could also select 'dislike' as their reason for avoiding milk. Five percent of African-Americans selected this response in comparison to 8.8% and 3.4% of Hispanic-Americans and Caucasians, respectively.

Rorick and Scrimshaw [29] reported on tolerance among the elderly to 240 ml (8 ounces) of milk vs. lactose-free milk. The researchers found no differences in symptomatic response under double-blind conditions to the lactose-containing and lactose-free milks. Using the breath collection technique for lactose tolerance testing, 23 maldigesters were identified in the study. Five had symptoms following both treatments; two had symptoms exclusively after the lactose-free treatment and none had symptoms exclusively following the lactose treatment. Only 5 of the 23 were African-American and the authors did not delineate the specific responses for these five subjects. None-the-less, the lack of response to the lactose challenge by all subjects, including the 5 African-American subjects, suggests African-Americans are not different in their response.

Johnson et al [30] studied adolescent and young adult African-Americans to evaluate lactose digestion in a group of subjects who claimed to be lactose intolerant. One hundred and sixty-four subjects, who were 12 to 40 years of age and claimed they experienced some gastrointestinal symptoms after consuming a cup of milk, participated. Hence, the population selected was biased toward a subgroup of African-Americans who might experience symptoms. Stage 1 involved a lactose challenge test of 25 g lactose suspended in 200–300 ml water. Breath samples were collected and analyzed using gas chromatography. As breath samples were collected, subjects also reported gastrointestinal symptoms. Only 58% (95/164) were determined to be maldigesters. Eighty-two of the 95 (86%) maldigesters reported some symptoms following the 25 g challenge.

In stage 2 of the investigation [30], only those individuals who had an increase in hydrogen concentration of >20 parts per million (ppm) or more (maldigesters) were invited to participate. Forty-five subjects chose to participate in this double-blind, crossover test for milk intolerance. Subjects were offered a lactose-containing or lactose-free dairy drink on 3 different days. The lactose challenge test, breath samples, and symptoms record were repeated as in stage 1. In stage 2, 30 subjects reported symptoms when they consumed the lactose-containing beverage and 15 reported symptoms when they consumed either beverage. Thus, 15/45 subjects experienced no symptoms following the consumption of 25 g of lactose. The authors concluded that factors other than lactose are also important in determining symptomatic response among individuals who believe that they are milk intolerant. No comparisons to non African-American populations were made, but the relative incidence of symptoms following this dose (equivalent to drinking two 8 ounce glasses of milk on an empty stomach) is consistent with the incidence observed in non African-American maldigesters.

In a follow up study, Johnson et al [31] challenged 25 African-American maldigesters who were found to be intolerant to lactose-containing milk via double-blind crossover study with increasing amounts of lactose. The lactose in low-fat milk was hydrolyzed using lactase, and the hydrolyzed milk was mixed proportionately with untreated low-fat milk to produce milks containing varying amounts of lactose. There was no requirement for overnight fast in this study and subjects received the milk in the morning hours of each weekday. Subjects were asked to record their symptoms and keep a daily food record. Initially subjects received a milk drink containing 5 g lactose. If a subject did not report experiencing symptoms from a certain dose of lactose for a period of 2–4 days, the lactose content of the milk was increased by 1 g by changing the proportion of lactose-hydrolyzed and untreated milk. The amount of lactose was increased until a dose was reached that produced gastrointestinal symptoms. Then, the subject was given the same lactose dose over a period of days until the symptoms became negligible. Over the 6–12 week period of the study, 17 (77%) of the 22 who completed the study tolerated 12 g or more of lactose. Of these subjects who were able

to tolerate 12 g lactose or more, 10 had hydrogen concentrations ≥20 ppm. This is consistent with data in other populations of maldigesters who claim intolerance [16] demonstrating a high likelihood of tolerance to lactose when it is consumed in normal serving size amounts. Again, no direct comparison of racial groups or random sampling of the population was made. But, the data indicate substantial tolerance in this African-American group, similar to other maldigesters who claim intolerance.

In 1999, Klesges et al [3] reported on the milk drinking habits of 32,144 Air Force recruits. Regardless of race or maldigestion status, only 17% reported consuming three or more servings of milk per day. Slightly more than half reported consuming less than one serving per day. Milk consumption was positively associated with fruit and vegetable consumption. The most interesting finding relative to this review was that the self-reported incidence of milk-related gastric distress was similar between African-American and Asian recruits. Additionally, a significant trend of experiencing distress was observed for African-Americans. Caucasians in this study reported lower gastric distress than both African-Americans and Asians. Perceived milk intolerance was highest among older African-American women (51.4%), second only to older Asian men (60.4%).

In a study to determine adaptability to a dairy-rich diet, a lactose challenge test was administered twice to a group of 17 African-American girls, aged 11 to 15 [32]. The girls were participants in a 21-day calcium metabolism study in which all subjects lived in a supervised environment for the duration of the study and consumed 1,211 +/− 76 mg calcium per day. The subjects consumed approximately four servings of dairy foods daily containing an estimated 33 g lactose/day. Prior to the study the subjects consumed approximately 17 g lactose per day. On the first day of the intervention subjects were challenged with 0.35 g lactose/kg body weight which was presented as 1% milk. Breath samples were collected at baseline, following milk consumption, and hourly for 8 hours. This lactose challenge process was repeated on day 21 of the intervention. Symptoms were recorded hourly using a self-reported record sheet. Subjects were asked to rate symptoms (abdominal pain, bloating, flatulence, diarrhea/loose stools, and headache) a score of 0 to 5 depending on severity of symptoms.

The breath samples were analyzed for carbon dioxide and hydrogen concentrations. Girls who had an increase of ≥20 ppm of breath hydrogen were classified as having lactose maldigestion [32]. Fourteen of the 17 subjects who participated in the lactose challenge were classified as lactose maldigesters. From the time the test was administered on the first day of the study to the second administration on day 21, there was a significant decrease in the amount of hydrogen produced, suggesting colonic adaptation to lactose throughout the 21-day intervention. Most importantly, it was noted that during both challenges, and during the 21 day period on the high dairy diet, subjects reported minimal or no gastrointestinal symptoms. Thus, this African-American population could consume a dairy rich diet, and meet adolescent calcium needs, without symptoms of intolerance.

CONCLUSIONS

Direct comparisons of the relative tolerance to lactose from African-Americans as compared to other lactose maldigesters are not available. However, the information presented here demonstrates that African-American maldigesters, like all maldigesters, experience symptoms in a dose-response fashion. Further, there is a range of symptoms that appear in a given population fed the same dose of lactose. Milk consumed in a mixed meal, and in single portion sizes (8 ounces of milk containing approximately 12 g lactose) is unlikely to cause symptoms of intolerance among African-Americans. Additionally, Suarez et al [18] demonstrated that an 8 ounce portion of milk can be consumed twice per day, as compared with once per day, with no additive effect on symptoms, even in people who claim severe lactose intolerance. Other strategies for consuming lactose containing foods while avoiding or minimizing intolerance symptoms include utilizing products that aid in the digestion of lactose, and choosing yogurt or hard cheeses [14]. The literature on African-Americans, though limited, reflects the findings in the overall body of literature on lactose intolerance in maldigesters. Thus, there is little reason to believe that African-Americans are especially lactose intolerant. African-Americans should not avoid dairy products due to concerns about lactose intolerance and should follow dietary recommendations from the 2005 Dietary Guidelines and the National Medical Association.

REFERENCES

1. American Heart Association: “Heart Disease and Stroke Statistics—2005 Update.” Dallas: American Heart Association, 2005.
2. Looker AC, Loria CM, Carroll MD, McDowell MA, Johnson CL: Calcium intakes of Mexican Americans, Cubans, Puerto Ricans, non-Hispanic whites, and non-Hispanic blacks in the United States. J Am Diet Assoc 93:1274–1279, 1993.
3. Klesges RC, Harmon-Clayton K, Ward KD, Kaufman EM, Haddock CK, Talcott GW, Lando HA: Predictors of milk consumption in a population of 17- to 35-year-old military personnel. J Am Diet Assoc 99:821–826, 1999.
4. Wiecha JM, Fink AK, Wiecha J, Hebert J: Differences in dietary patterns of Vietnamese, white, African-American, and Hispanic adolescents in Worcester, Mass. J Am Diet Assoc 101:248–251, 2001.
5. Sharma S, Murphy SP, Wilkens LR, Shen L, Hankin JH, Monroe KR, Henderson B, Kolonel LN: Adherence to the food guide pyramid recommendations among African-Americans and Latinos: Results from the multiethnic cohort. J Am Diet Assoc 104:1873–1877, 2004.

6. Dietary Guidelines for Americans 2005. United States Department of Agriculture.
7. National Dairy Council: "Lactose Intolerance and Minorities: The Real Story." Rosemont, IL: National Dairy Council, 2005.
8. Gerrior S, Bente L: "Nutrient Content of the U.S. Food Supply, 1901–99: A Summary Report." U.S. Department of Agriculture, Center for Nutrition Policy and Promotion. Home Economics Research Report No. 55, 2002.
9. Sahi T: Genetics and epidemiology of adult-type hypolactasia. Scand J Gastroenterol 29:7–20, 1994.
10. National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. "Lactose Intolerance." National Institutes of Health Publication No. 03-2751, March 2003.
11. Savaiano D, Hertzler S, Jackson KA, Suarez FL: Nutrient considerations in lactose intolerance. In Coulston AM, Rock CL, Monsen ER (eds): "Nutrition in the Prevention and Treatment of Disease." San Diego: Academic Press, pp 563–575, 2001.
12. Wooten WJ, Price W: The role of dairy and dairy nutrients in the diet of African Americans. J Natl Med Assoc 96:1S–31S, 2004.
13. Jackson KA, Savaiano DA: Lactose maldigestion, calcium intake and osteoporosis in African-, Asian-, and Hispanic-Americans. J Am Coll Nutr 20:198S–207S, 2001.
14. Scrimshaw NS, Murray EB: The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance. Am J Clin Nutr 48S:1083–1159, 1988.
15. Savaiano D: Lactose intolerance: A self-fulfilling prophecy leading to osteoporosis? Nutr Rev 61:221–223, 2003.
16. Suarez FL, Savaiano DA, Levitt MD: A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. N Engl J Med 333:1–4, 1995.
17. Vesa TH, Korpela RA, Sahi T: Tolerance to small amounts of lactose in lactose maldigesters. Am J Clin Nutr 64:197–204, 1996.
18. Suarez FL, Savaiano D, Arbisi P, Levitt MD: Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance. Am J Clin Nutr 65:1502–1506, 1997.
19. Martini MC, Savaiano DA: Reduced intolerance symptoms from lactose consumed during a meal. Am J Clin Nutr 47:57–60, 1988.
20. Dehkordi N, Rao DR, Warren AP, Chawan CB: Lactose malabsorption as influenced by chocolate milk, skim milk, sucrose, whole milk, and lactic cultures. J Am Diet Assoc 95:484–486, 1995.
21. Martini MC, Kukielka D, Savaiano DA: Lactose digestion from yogurt: influence of a meal and additional lactose. Am J Clin Nutr 53:1253–1258, 1991.
22. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J: Probiotics—compensation for lactase insufficiency. Am J Clin Nutr 73:421S–4219S, 2001.
23. Hertzler SR, Savaiano DA: Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. Am J Clin Nutr 64:232–236, 1996.
24. Florent C, Flourie B, Leblond A, Rautureau M, Bernier JJ, Rambaud JC: Influence of chronic lactulose ingestions on the colonic metabolism of lactulose in man (an in vivo study). J Clin Invest 75:608–613, 1985.
25. Bayless TM, Rosensweig NS: A racial difference in incidence of lactase deficiency. JAMA 197:968–972, 1966.
26. Hertzler SR, Huynh BL, Savaiano DA: How much lactose is low lactose? J Am Diet Assoc 96:243–246, 1996.
27. Paige DM, Bayless TM, Ferry GD, Graham GG: Lactose malabsorption and milk rejection in Negro children. Johns Hopkins Med J 129:163–169, 1971.
28. Marrs DC: Milk drinking by the elderly of three races. J Am Diet Assoc 72:495–498, 1978.
29. Rorick MH, Scrimshaw NS: Comparative tolerance of elderly from differing ethnic backgrounds to lactose-containing and lactose-free dairy drinks: A double-blind study. J of Gastroenterol 34:191–196, 1979.
30. Johnson AO, Semenya JG, Buchowski MS, Enwonwu CO, Scrimshaw NS: Correlation of lactose maldigestion, lactose intolerance, and milk intolerance. Am J Clin Nutr 57:399–401, 1993.
31. Johnson AO, Semenya JG, Buchowski MS, Enwonwu CO, Scrimshaw NS: Adaptation of lactose maldigesters to continued milk intakes. Am J Clin Nutr 58:879–881, 1993.
32. Pribila BA, Hertzler SR, Martin BR, Weaver CM, Savaiano DA: Improved lactose digestion and intolerance among African-American adolescent girls fed a dairy-rich diet. J Am Diet Assoc 100:524–528, 2000.
33. Zemel MB, Richards J, Milstead A, Campbell P: Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res 13:1218–1225, 2005.
34. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N: For the DASH Collaborative Research Group: A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 336:1117–1124, 1997.

Received September 9, 2005.

Review

Newer Perspectives on Calcium Nutrition and Bone Quality

Robert P. Heaney, MD, and Connie M. Weaver, PhD

Creighton University, Omaha, Nebraska (R.P.H.), Purdue University, West Lafayette, Indiana (C.M.W.)

Key words: calcium, dairy, bone quality, bone remodeling, fracture, growth

It is now generally accepted that an adequate calcium intake is important for building and maintaining a skeleton that expresses *quantitatively* the full genetic program and reduces lifetime fracture risk. In this brief review we focus mainly on a new and growing body of evidence indicating a benefit of adequate calcium intake on *qualitative* features of the skeleton that, independent of the quantity of bone, themselves influence skeletal strength and fragility.

Change in bone mass and size during growth are dependent on both calcium intake and exercise, with the largest differences being observed in prepubertal children who have both high exercise levels and high calcium intakes. Much of this benefit is expressed as increased bone diameter (and hence stiffness). Fracture risk peaks at about the time of puberty and is inversely related to bone mass. However, even prepubertally, children with low calcium intakes have been reported to have a fracture rate 2.7× that of their birth cohort.

Bone remodeling triples from age 50 to 65 in typical women and is now recognized to have primarily a homeostatic basis. While remodeling improves bone strength by repairing acquired defects, homeostatic remodeling, while necessary to maintain blood calcium levels, contributes only structural weakness to bone. High calcium intakes in postmenopausal and older women reduce this homeostatic remodeling to approximately pre-menopausal values and improve bone strength immediately, well prior to any appreciable change in bone mass.

Key teaching points:

- Low bone mass is associated with increased fracture risk in children, just as in adults.
- Low dairy intake is one of the causes of reduced bone mass during growth.
- Physical activity and calcium intake interact during growth, with the largest accumulation of bone being concentrated in children with high physical activity and high calcium intakes.
- Bone remodeling, necessary to repair or reshape bone, also serves calcium homeostasis; on prevailing diets, homeostatic remodeling is larger than structural remodeling, tripling in magnitude from the premenopausal years to age 65.
- Homeostatic remodeling, while it provides needed calcium ions to the extracellular fluid, weakens bone locally, wherever in the skeleton it occurs. Available evidence suggests that excessive remodeling is a major cause of osteoporotic bony fragility.
- Reduction in bone remodeling by high calcium intakes produces an immediate reduction in fracture risk, well before perceptible change in bone mass can occur.

Introduction

Calcium serves two major functions for bone. First, calcium is the bulk cation out of which bone mineral is constructed. As such it must be absorbed in sufficient quantity from ingested foods to build a skeleton during growth and to maintain skeletal mass in maturity (the latter by offsetting obligatory losses from the body). Second, calcium serves as an indirect regulator of skeletal remodeling. The first function has dominated the attention of the clinical nutrition community through most of the past century and provides the foundation for an impressive array of calcium nutritional policy statements [1–5]. The second is only now emerging as an important contributor to bone strength.

Address reprint requests to: Robert P. Heaney, M.D., Creighton University Medical Center, 601 N. 30th St., Suite 4841, Omaha, NE 68131. rheaney@creighton.edu

Journal of the American College of Nutrition, Vol. 24, No. 6, 574S–581S (202005)
Published by the American College of Nutrition

Although there remain some isolated pockets of disagreement (e.g., ref. 6), there is now a broad consensus that a calcium intake of 1000–1500 mg/d is needed to ensure skeletal optimization across the population at all ages after childhood. The policy statements cited review the now massive body of evidence supporting this consensus. Our purpose here is to highlight new information on the relation of calcium intake to childhood fractures, on the interaction of dietary calcium and physical activity in skeletal health, and on the still evolving understanding of the role played by bone remodeling in bony fragility and its interaction with calcium intake.

Dietary Calcium and Childhood Fractures

Adequate dietary calcium has long been recognized to play an important role in building peak bone mass as a strategy to decrease incidence of fracture later in life [7]. More recently, it has become apparent that even childhood fractures are also related to low bone mass, and that childhood bone mass in turn is influenced by diet and physical activity.

Childhood fractures are often attributed mainly to the "clumsiness" and risky behaviors of youth. However, Goulding's report [8] on the association of fracture with low bone density in 3–15 year old girls living in New Zealand showed that fracture incidence even during childhood was related to a property of bone, i.e. massiveness, modifiable by lifestyle choices. Although calcium intakes in children with fractures and healthy controls were not significantly different for Goulding's cohort of girls or in a subsequent cohort of boys [9], Goulding's group subsequently reported that children under age 10 who were milk avoiders had significantly less bone and were shorter than a birth cohort of more than 1000 from the same city (10). In her population, the odds ratio for a fracture in those with low bone density compared to matched controls was 2.3 for the radius, 2.4 for the spine, and 2.0 for the hip. The milk avoiders had total skeletal bone mineral content (BMC) Z-scores averaging −0.45, which was significantly different than the distribution in the healthy population (Z-scores represent deviation from the age-adjusted mean normative data). A subsequent evaluation of their relative fracture incidence showed that one in three of the 50 milk avoiders had reported fractures, with 18 of their 22 fractures occurring before age 7 [11]. This fracture rate was 175% greater in the milk avoiders than expected from their birth cohort. Interestingly, the milk avoiders also had a higher risk of being overweight. Given that the most common site of fracture was the forearm, being overweight could exacerbate the impact load on the arm during a fall.

Vulnerability to fracture is not uniform across childhood. There is a transient increase in porosity of cortical bone during puberty as a result of a phase lag between achievement of peak height and peak bone mass [12]. The timing of this decrease in bone density was recently characterized in a group of Canadian children studied longitudinally by annual bone density scans through puberty [13]. In girls, average peak height velocity occurred at age 11.8 and average peak BMC velocity occurred at age 12.4, a lag of 0.7 y. Similarly, in boys the lag occurred between an average peak height velocity of 13.4 y and to a peak BMC velocity of 14.1 y.

Fig. 1 profiles the incidence of forearm fracture with age in the Midwestern U.S. [14]. The peak incidence of fracture occurs slightly before the period of increased bone porosity predicted by Bailey et al. [15]. In girls, the highest rate of bone turnover occurs during the 2 years preceding onset of menses and declines after onset of menses [16]. Bone strength expressed as fracture incidence may relate as much to bone turnover rate as to bone mass, as we discuss later. The peak incidence of fracture in girls aged 8–11 and boys aged 11–14 would fall close to peak bone turnover rates associated with pubertal growth. However, neither a dip in bone mineral density (BMD) nor accelerated bone turnover, suffice to explain

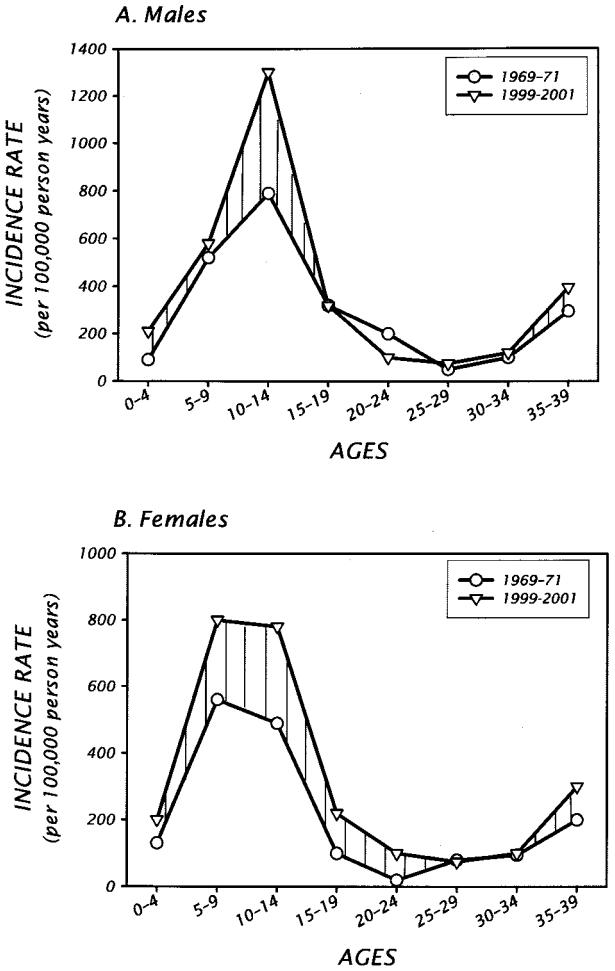

Fig. 1. Plots of incidence of distal forearm fractures in males (A) and females (B) from the data of Khosla et al. [14] among residents of Rochester, Minnesota. The lower line for both panels represents fractures reported in 1969–1971 and the upper line represents fractures reported in 1999–2001. The shaded zones represent the increases in childhood fracture in 3 decades.

the frequency of fracture at ages younger than 7 years in milk-avoiding New Zealand children [11].

Also apparent in Fig. 1 is the increase in forearm fracture in children over the last 3 decades (56% for girls and 32% for boys). The largest increase occurred at the same age as that of peak incidence. The authors attributed part of this increased incidence of fracture to increased participation in recreational activities. However, milk consumption in children has also declined during this period, a change that has been associated with increased fracture during childhood [8, 17] and later in life [17]. The impact of the interaction between dietary calcium and physical activity on bone strength may be stronger than either factor alone.

Dietary Calcium, Physical Activity, and the Growing Skeleton

Recent advances in imaging techniques to evaluate bone geometry have contributed to our understanding of the interplay of calcium intakes and physical activity on the growing skeleton. At the beginning of the decade, we knew from intervention studies that bone mass could be improved with both calcium or milk powder supplements and exercise [18]. In postmenopausal women, subjects with calcium intakes over ~1 g/day randomized to exercise intervention had improved BMD at the spine [19] and tibia and hip [20] compared to calcium alone. However, the interaction between dietary calcium and physical activity in the growing skeleton remained uncertain because of lack of intervention trials and the inability of then available bone densitometry to capture bone geometric characteristics (beyond measurement of BMD and BMC) which contribute to strength in the growing skeleton.

Two important intervention trials have been reported since 2002 that shed light on the interaction of dietary calcium and physical activity in growing bone. Specker and Binkley [21] studied 239 children aged 3–5 y for 1 year who were randomized to 1 g/d calcium or placebo and to two exercise regimens, gross motor (weight bearing) or fine motor (sitting). Leg BMC gain, determined by dual energy X-ray absorptiometry (DXA), was significantly higher only in the combined calcium and weight-bearing exercise group. However, peripheral quantitative computed tomography (pQCT) of the 20% tibia, which measures geometry of the leg, gave additional information about bone strength.

As shown in Fig. 2, weight-bearing exercise alone increased tibia periosteal and endosteal circumferences ($P = 0.05$) which raised bone strength by increasing cross-sectional moment of inertia, even though there was no increase in bone mass. Cross-sectional moment of inertia is a measure of the distribution of material around a given axis. The contribution of bone mass to strength is proportional to its squared distance from the axis around which bending occurs. Thus small increases in diameter can have profound positive effects on the bending strength of a bone. There was a significant interaction between

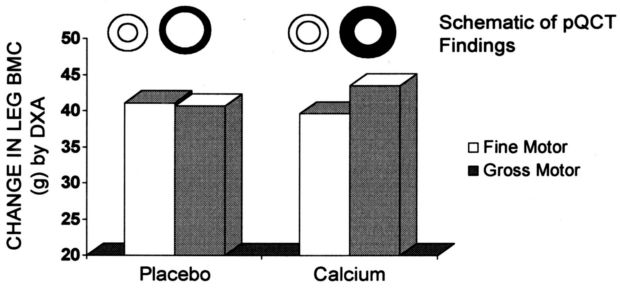

Fig. 2. Twelve month changes in 20% tibia cross-section by pQCT and leg BMC by DXA in 3–5 y olds randomized to calcium supplementation or placebo and fine motor vs. gross motor exercise in a 2 × 2 factorial design. There was a significant interaction between activity and Ca supplementation in BMC ($P = 0.05$). There were significant ($P \leq 0.05$) activity effects in perisoteal and endosteal circumferences by pQCT and significant Ca × activity interactions for cortical area ($P = 0.01$) and cortical thickness ($P = 0.02$). Reproduced with permission from reference 22.

weight-bearing exercise and calcium supplementation for leg BMC ($P = 0.05$) and tibial cortical thickness and cortical area ($P \leq 0.02$), resulting in the largest bone gain. With only BMC from DXA, the strength advantage from greater bone circumferences due to exercise alone was not apparent. The increased calcium intake allowed greater bone mineralization of the larger bone area stimulated by exercise. This insight was achieved through the use of a factorial design and bone imaging technology.

A second randomized trial using a factorial design, in 66 older girls aged 8.8 ± 0.1 years, found a positive interaction of milk mineral supplements and moderate impact exercise for 20 minutes 3 times per week for 8.5 months on some bone sites but not others [23]. High impact exercise alone increased bone mass at the loaded site (tibia-fibula) and calcium alone increased bone mass at non-loaded sites (humerus and ulna-radius). A significant ($P < 0.05$) exercise-calcium interaction was detected at the femur, but not the tibia-fibula.

Main effects of calcium intake and physical activity on bone gain have been reported in a number of randomized, controlled trials in children [7]. The effects may differ at bone sites which differ in cortical vs. trabecular bone, the stage of maturity of the growing skeleton, or the interdependency of calcium intake and physical activity. Cortical-rich bone regions have responded more to calcium supplementation in most trials than trabecular-rich regions [24]. On the other hand, activity trials in children have shown significant increases in trabecular bone [25] as well. Mechanical loading stimulates trabecular number and size [26]. Activity trials usually are more effective in prepubertal children possibly because of a synergistic activity between exercise and growth hormone [27]. Findings on the benefits of calcium supplementation in prepubertal vs. pubertal children have been inconsistent. In the only calcium supplementation trial that has spanned puberty, the benefits of calcium on bone were greater during the pubertal growth spurt than during bone

consolidation [28]. The lack of main effects of calcium and exercise and positive interaction of the two in the Specker and Binkley [21] study suggest that part of the inconsistency among trials of either calcium or activity alone may be the failure to appreciate this interaction.

Calcium, Bone Remodeling, and Skeletal Fragility

Broadly speaking, remodeling of bone serves two, closely linked purposes: 1) the repair of fatigue damage and the reshaping of bone to accommodate growth and altered usage; and 2) a source and sink for calcium in the protection of extracellular fluid (ECF) [Ca^{2+}]. In both, small packets of bone are resorbed by osteoclasts, and the released bone mineral either recycled or used to offset excretory losses. The first role of remodeling is generally divided into two types: i) "remodeling" properly considered, i.e., the replacement of damaged structures, and ii) "modeling", i.e., the reshaping of bone. In the first, bony resorption and formation occur at the same skeletal site, though separated in time (resorption first, followed by formation); while in the second, formation and resorption occur on different surfaces (e.g., periosteal, endosteal), but simultaneously. During growth both processes are active, while after growth, when adult skeletal shape is approximately stable, true remodeling predominates.

Both types share a common feature: bone mineralization in the formation phase of remodeling takes calcium and phosphorus out of the circulating blood, creating a mineral deficit in the ECF which constitutes the principal systemic basis for stimulating parathyroid hormone (PTH) secretion. PTH in turn is the principal determinant of the quantity of bone resorption occurring throughout the skeleton. In this sense, bone mineralization "pulls" bone resorption. In parathyroidectomized animals and in humans with hypoparathyroidism, total bone remodeling drops to levels less than one-sixth the value found in intact organisms. The result, however, is usually hypocalcemia.

During periods of fasting or low calcium intake, PTH secretion rises, and with it bone resorption (and, thereby, total remodeling). From a homeostatic perspective, such remodeling provides the calcium needed to maintain ECF [Ca^{2+}]. However, structurally, homeostatic remodeling contributes only weakness, since bone at sites being remodeled is reduced in mass and hence in strength. This strength reduction is illustrated diagrammatically in Fig. 3, which makes the point that a resorption cavity in the side of a load-bearing bone trabecula produces local weakness out of proportion to the modest reduction in mass. Over the short term, this loss in strength is trivial, but if inadequate calcium intake is continuous, then remodeling remains high and fragility increases. The numbers of these compromised trabeculae accumulate and ultimately bone mass declines as well. It is important to note that the increase in fragility precedes appreciable loss of mass, and is due, as Fig. 3 illustrates, to compromised structures.

Until recently the major emphasis in the field of clinical bone biology had been on the ultimate effects of remodeling on bone mass, which explains why calcium balance, or change in BMD (or BMC) has been the primary outcome variable in many studies of nutritional interventions (e.g., calcium and vitamin D). Virtually all such studies show that increasing calcium intake to or above age-specific threshold values leads in the young to greater bone gain, and in the elderly to decreased age-related bone loss [29]. But the matter is more complex than that. When an intervention that reduces PTH-mediated remodeling is first started, it produces a prompt, one time increase in bone mass that has been termed a "remodeling transient" [30]. The reason is that resorption slows immediately when PTH levels drop, while older remodeling loci, now in their mineralizing phase, come back into service at the rate of their creation months earlier. The result is an effective reclaiming of some of the bone taken out of service because of remodeling - a phenomenon called "closure of the remodeling space".

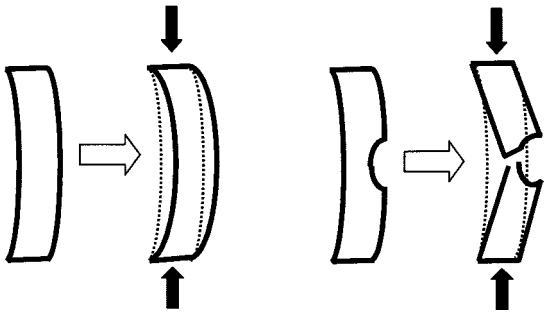

Fig. 3. Diagrammatic illustration of the fact that vertical trabeculae bow slightly when loaded. Resorption pits in the side of such trabeculae serve as stress concentrators, since the prior load must now be borne by a smaller cross-section. The result is a tendency to snap with usual load-bearing activities. Hundreds of such healed or healing trabecular fractures can be found in osteoporotic bone by micro-dissection. (Copyright Robert P. Heaney, 2005. Used with permission.)

The remodeling transient has to be factored into any interpretation of the results of interventions that alter bone remodeling, particularly if one is interested in the effects of the intervention on steady state bone balance [31, 32]. But until recently, the transient was seen mainly as something that got in the way of discerning the "true" effect of the agent on bone [32]. It is now likely that the remodeling change is substantially more important than the mass change - at least over the short term when the remodeling change is fully expressed but the mass change is just getting under way.

This conclusion first became apparent in the analysis of osteoporosis treatment trials, in which BMD change was found to explain less than half of the fracture reduction at the end of the trial [33]. Even more to the point, the fracture reduction produced by bisphosphonates and selective estrogen receptor modulators (SERMs) was noted to begin immediately after starting treatment, before there was time for an appreciable mass difference to develop [34,35]. But calcium also functions

as an antiresorptive agent. It does not antagonize PTH action on bone as do estrogen, the SERMs, and the bisphosphonates, but reduces remodeling by directly reducing PTH secretion. McKane et al., for example, showed that high calcium intakes in healthy postmenopausal women reduced 24-hr PTH levels by 40% [36]. Moreover, analysis of the fracture risk curves reported for two major calcium and vitamin D intervention studies [36, 37] shows clearly that the fracture risk reduction occurs almost immediately after starting treatment. Fig. 4 is a replot of some of the fracture data of these two trials, showing forcefully the prompt reduction in fracture risk that is produced by supplemental calcium and vitamin D.

What this means, in the practical order, is that individuals with substantial bony deficits, when given an adequate calcium intake, experience an immediate reduction in fragility, without having to wait for the mass deficit to be fully repaired (which is often not feasible, at least by nutritional means). Further, the benefit consists of an absolute reduction in fracture risk, not simply a slowing of the progressive fragility of aging that had originally been judged to be the goal of stopping age-related bone loss. In truth, both effects occur. For example, in the study by Chapuy et al. [37] bone loss that amounted to greater than 3%/yr at the hip in the control subjects, was stopped entirely in the calcium and vitamin D supplemented subjects. At the same time, as Fig. 4 shows, fracture rate dropped well before change in bone mass could be expressed.

Although most of the data on this effect of remodeling have been developed in studies of the elderly, similar conclusions seem applicable to studies in young people. Fig. 5 is a schematic redrawing of the data from the calcium intervention trial of Johnston et al. in adolescents [39], and its follow-up, post-intervention, by Slemenda et al. [40]. The figure shows the curvilinear positive remodeling transient at the onset of supplementation, and the corresponding negative transient at its withdrawal. Bone mass in both the treated and untreated groups was increasing, as these were rapidly growing young people. The research question had been "Would this bone accumulation be greater in the calcium-supplemented group?". Such gain would have consisted of a combination of coincident growth, calcium augmentation (if any), and the remodeling transient, with the latter now recognized to be the largest of the three, at least over the short term. Unfortunately, the remodeling transient had not entered into design considerations at the time this trial was performed, and it was the *total* increase that was the design endpoint.

As the figure suggests, the slope of the BMC curve was slightly greater for the supplemented than for the unsupplemented twins, and the final value one year after supplementation ceased was higher for the supplemented than for the

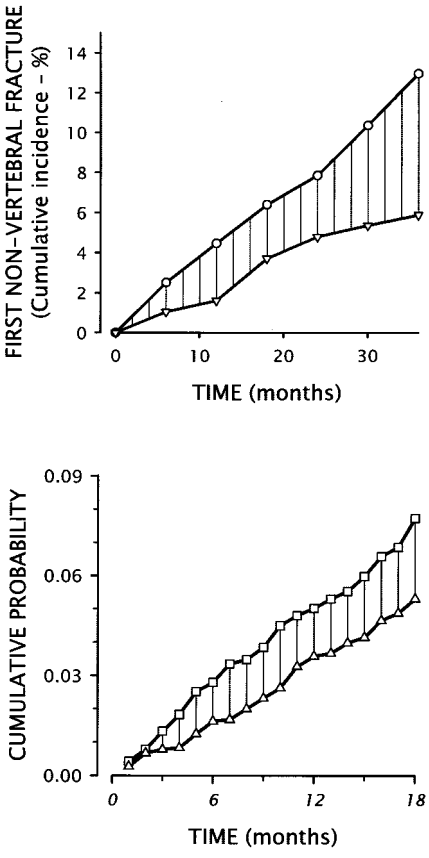

Fig. 4. Plots of the cumulative incidence of fractures, redrawn from the studies of Chapuy et al. [37] (bottom) and Dawson-Hughes et al. [38] (top). In both cases, the upper line represents the placebo control subjects, and the lower line represents the calcium and vitamin D-treated subjects. The shaded zones represent the reduction of fracture risk, which, as can be readily seen, starts with the very beginning of treatment. (Copyright Robert P. Heaney, 2004. Used with permission.)

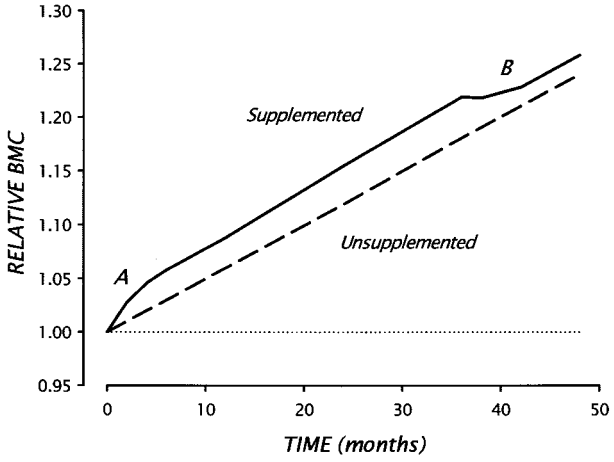

Fig. 5. Schematic redrawing of the change in BMC in the compliant subjects in the study of Johnston et al. [39], with the post-treatment follow-up data from the report of Slemenda et al. [40]. (Data supplied by Dr. C.C.Johnston.) ***A*** represents the positive remodeling transient at the beginning of supplementation, and ***B***, the negative transient at its withdrawal. (Copyright Robert P. Heaney, 2005. Used with permission.)

unsupplemented twins as well (both compatible with augmentation by calcium). However, neither difference was statistically significant. Unfortunately the study was powered to find the *total* bone mass difference at the end of the intervention, but not to evaluate the slopes of the two curves, nor the mass difference (if any) after the inevitable negative transient following withdrawal of the supplement. As the figure suggests, much of the augmented gain of the supplemented group was due to the transient, and thus the study was unable to address the issue behind the original research question, i.e., steady-state bone balance.

As this example illustrates, the transient has come to be seen mainly as an important confounding factor. However, with the insight derived from the fracture efficacy trials in the elderly, it now seems clear that, in both young and old, the transient itself, or more properly, the remodeling suppression that produces it, is a part of the benefit - and indeed, perhaps the larger part [41]. Both the increased mass and the reduced remodeling during calcium augmentation are now understood to increase bony strength.

Wastney et al. [16], using short duration calcium kinetic studies in children, showed that increases in calcium intake suppress bone resorption without affecting bone formation (at least over the life of one remodeling cycle). The role of remodeling adjustment in calcium homeostasis was beautifully exemplified in this study, as increased absorption from food was matched, milligram for milligram, by decreased calcium release from bone by decreased resorption.

There are two features of remodeling suppression that deserve special comment. First, the symmetry of the two remodeling transients, i.e., going on and coming off supplementation (shown for example, in Fig. 5), has been used to argue that the bone gain on supplementation should not be considered evidence that the calcium requirement is higher than prevailing intakes. The bone gain is not permanent - so the argument goes - and thus the response to supplementation is not a true nutrient effect. This argument limps at very best. Supplying a needed nutrient to a deficient individual will always result in a benefit that is only temporary if the nutrient is subsequently withdrawn and the deficiency state returns. As virtually everyone knows, nutritional health is an ongoing affair.

The second feature is the level of remodeling itself, and the associated questions of what rate is optimal, and whether suppressing remodeling is a good thing to do. In adults, bone turns over at a rate estimated to be in the range of 8–12%/yr, with cancellous bone regions in contact with red marrow being replaced at 2–3× that average rate, and the cortical bone of long bone shafts, at perhaps half that rate or lower. Remodeling is known to repair fatigue damage and hence has generally been considered to be a positive factor for bone strength, overall. Moreover, remodeling had been assumed initially to be driven largely by this need for structural repair. Thus, reduced remodeling, by allowing fatigue damage to accumulate, had been predicted to increase bony fragility. For this reason it came as a surprise when reduced remodeling was found not to increase fragility, but to reduce it, and in fact to be the probable reason for reduced fracture risk [33,41] in the osteoporosis treatment trials.

The explanation now considered most likely is that most remodeling in First World adults is homeostatic, not structural. Homeostatic remodeling, as already noted, while it contributes calcium, decreases local bone strength. Moreover, recent research quantifying remodeling has shown that cancellous bone remodeling doubles across menopause, and by the mid-60s is about 3× the premenopausal level [42]. This change, almost certainly not driven by mechanical need, is now thought to be the likely cause in postmenopausal women of the greatly increased fragility of that life stage. The premenopausal rate, measured histomorphometrically, is about 6–7%/yr at the iliac crest. By contrast, Parfitt has recently estimated that a remodeling rate of 2%/yr should be sufficient to repair fatigue damage [43]. Whatever the optimal structural rate may be, it now seems certain that there is a relatively large excess of remodeling in ostensibly healthy, First World, adult humans that has its basis not in structural repair, but in calcium homeostasis. To the extent that this remodeling is a source of weakness, it follows that remodeling reduction will strengthen bone - which is what the data show.

The reasons for what is now recognized as a high level of homeostatic remodeling are only partially understood. Two explanations, pertinent to the focus of this paper, are low calcium and vitamin D intakes. Both, as already noted, lead to elevated PTH secretion and hence to increased bone remodeling. Thus it is logical and, in retrospect, predictable, that elevating calcium and vitamin D intakes should promptly decrease bony fragility. It is worth recalling that PTH secretion drops immediately when extra calcium and vitamin D are given, and bone resorption responds virtually immediately, as well [16]. Thus, pre-existing resorption cavities are filled in day by day, while new ones are being created at a reduced rate, leading to an improvement in strength within days of starting remodeling suppressive therapy.

But contemporary low intakes of these two key nutrients can be only a part of the explanation for high remodeling. The study of McKane et al. [36], previously mentioned, pushed total calcium intakes in healthy postmenopausal women to 2400 mg/d, and did succeed in lowering 24-hr average PTH and bone remodeling rates - but only to premenopausal levels which, if Parfitt is correct, are still substantially higher than needed to maintain mechanical integrity of the skeleton.

An additional, possible explanation is the shift to a seed-based diet at the time of the agricultural revolution. Seed foods today account for about two-thirds of the energy intake of the global population, while our hunter-gatherer ancestors typically got less than 5% of total calories from such sources. (This is probably the largest shift in diet in the history of the human race.) Seed foods are typically low in calcium and potassium,

and high in sulfur-containing amino acids; all these characteristics are known to be associated with increased PTH secretion. Abbott et al. [44], examining static remodeling indices in skeletal remains from pre- and post-agricultural populations, found an approximate doubling of remodeling across the agricultural revolution. Additionally, the agricultural revolution, by producing surplus energy, permitted a human population explosion that forced migration to higher latitudes, where vitamin D status became problematic.

Whether these factors, taken together, constitute a fully adequate explanation for the elevated remodeling of modern humans is uncertain. Nevertheless the new appreciation of the importance of remodeling enhances the rationale for ensuring an adequate calcium intake.

Conclusions

Several aspects of the importance of calcium for bone are now clear that had not been understood as recently as five years ago. Dietary calcium can augment the ability of physical activity to strengthen growing bone through allowing increased bone mineralization of larger bone sizes. Furthermore, because high calcium intakes can reduce homeostatic bone remodeling, they are likely to improve skeletal strength even if they have no appreciable effect on bone mass or bone balance.

REFERENCES

1. Consensus Conference on Osteoporosis. JAMA 252:799–802, 1984.
2. Consensus Conference on Optimal Calcium Intake. JAMA 272: 1942–1948, 1994.
3. "Dietary Reference Intakes for Calcium, Magnesium, Phosphorus, Vitamin D, and Fluoride. Food and Nutrition Board, Institute of Medicine." Washington, DC: National Academy Press, 1997.
4. Bone Health and Osteoporosis: A Report of the Surgeon General. DHHS (PHS), 2004.
5. Nutrition and Your Health: Dietary Guidelines for Americans, 2005. USDA.
6. Lanou AJ, Berkow SE, Barnard ND: Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Pediatrics 115:736–743, 2005.
7. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C: Peak bone mass. Osteoporos Int 11:985–1009, 2000.
8. Goulding A, Cannan R, Williams SM, Gold EJ, Taylor RW, Lewis-Barned NJ: Bone mineral density in girls with forearm fractures. J Bone Miner Res 13:143–148, 1998.
9. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM: Bone mineral density and body composition in boys with distal forearm fractures. A dual energy X-ray absorptiometry study. J Pediatr 139:509–515, 2001.
10. Black RE, Williams SM, Jones IE, Goulding A: Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. Am J Clin Nutr 76:675–680, 2002.
11. Goulding A, Rockell JEP, Black RE, Grant AM, Jones IE, Williams SM: Children who avoid drinking cow's milk are at increased risk for prepubertal bone fractures. J Am Diet Assoc 104:250–253, 2004.
12. Parfitt AM: The two faces of growth: benefits and risk to bone integrity. Osteoporos Int 4:382–298, 1994.
13. Bailey DA, Martin AD, McKay AA, Whiting S, Miriwald R: Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Minera Res 15:2245– 2250, 2000.
14. Khosla S, Melton III LJ, Delutoski MB, Achenbach SJ, Oberg Al, Riggs BL: Incidence of childhood distal forearm fractures over 30 years. JAMA 290:1479– 1485, 2003.
15. Bailey DA, Wedge JH, McCullough RG, Martin AD, Bernhardson SC: Epidemiology of fractures of the distal end of the radius in children as associated with growth. J Bone Joint Surg 71A:1225–1231, 1989.
16. Wastney ME, Martin BR, Peacock M, Smith D, Jiang X-Y, Jackman LA, Weaver CM: Changes in calcium kinetics in adolescent girls induced by high calcium intake. J Clin Endocrinol Metab 85:4470–4475, 2000.
17. Kalkwarf HJ, Khoury JC, Lanphear BP: Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am J Clin Nutr 77:257–265, 2003.
18. Weaver CM: Calcium requirements of physically active people. Am J Clin Nutr 72:579S–584S, 2000.
19. Specker BL: Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. J Bone Miner Res 11:1539–1544, 1996.
20. Prince R, Devine A, Dick I, Criddle A, Kerr D, Kent N, Price R, Randell AS: The effects of calcium supplementation (milk powder or tablets) and exercise on bone density in postmenopausal women. J Bone Miner Res 10:1068–1075, 1995.
21. Specker B, Binkley T: Randomized trial of physical activity and calcium supplementation on bone mineral content in 3- to 5-year old children. J Bone Miner Res 18:885–892, 2003.
22. Specker BL, Schoenau E: Quantitative bone analysis in children: Current methods and recommendations. J Pediatr 146:726–731, 2005.
23. Iuliano-Burns S, Saxon L, Naughton G, Gibbons K, Bass SL: Regional specificity of exercise and calcium during skeletal growth in girls: A randomized controlled trial. J Bone Miner Res 18:156–162, 2003.
24. Wosje KS, Specker BL: Role of calcium in bone health during childhood. Nutr Rev 58:253–268, 2000.
25. French S, Fulkerson J, Story M: Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescent: a review of intervention trials. Prevent Med 3:22–31, 2000.
26. Rubin C, Turner AS, Muller R, Mittra E, McLeod K, Lin W, Qin YX: Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. J Bone Miner Res 17:349–357, 2002.
27. Bass SL: The prepubertal years: A uniquely opportune stage of growth when the skeleton is most responsive to exercise. Sports Med 30:73–78, 2000.
28. Matkovic V, Goel PK, Badenhop-Stevens WE, Landoll JD, Li B, Illich JZ, Skugor M, Nagode LA, Mobley SL, Ha E-J, Hangartner TN, Clairmont A: Calcium supplementation and bone mineral

density in females from childhood to young adulthood: a randomized controlled trial. Am J Clin Nutr 81:175–188, 2005.
29. Heaney RP: Ethnicity, bone status, and the calcium requirement. Nutr Res 22:153– 178, 2002.
30. Heaney RP: The bone remodeling transient: implications for the interpretation of clinical studies of bone mass change. J Bone Miner Res 9:1515–1523, 1994.
31. Heaney RP, Yates AJ, Santora AC II: Bisphosphonate effects and the bone remodeling transient. J Bone Miner Res 12:1143–1151, 1997.
32. Heaney RP: The bone remodeling transient: interpreting interventions involving bone-related nutrients. Nutr Rev 59:327–333, 2001.
33. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM: Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. Am J Med 112:281–289, 2002.
34. Maricic M, Adachi JD, Sarkar S, Wu W, Wong M, Harper KD: Early effects of raloxifene on clinical vertebral fractures at 12 months in postmenopausal women with osteoporosis. Arch Intern Med 162:1140–1143, 2002.
35. Roux C, Seeman E, Eastell R, Adachi J, Jackson RD, Felsenberg D, Songcharoen S, Rizzoli R, Di Munno O, Horlait S, Valent D, Watts NB: Efficacy of risedronate on clinical vertebral fractures within six months. Curr Med Res Opin 20:433–439, 2004.
36. McKane WR, Khosla S, Egan KS, Robins SP, Burritt MF, Riggs BL: Role of calcium intake in modulating age-related increases in parathyroid function and bone resorption. J Clin Endocrinol Metab 81:1699–1703, 1996.
37. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ: Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med 327:1637–1642, 1992.
38. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE: 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, 1997.
39. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, Peacock M: Calcium supplementation and increases in bone mineral density in children. N Engl J Med 327:82–87, 1992.
40. Slemenda CW, Peacock M, Hui S, Zhou L, Johnston CC: Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. J Bone Miner Res 12:676–682, 1997.
41. Heaney RP: Is the paradigm shifting? Bone 33:457–465, 2003.
42. Recker RR, Lappe JM, Davies KM, Heaney RP: Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. J Bone Miner Res 19: 1628–1633, 2004.
43. Parfitt AM: What is the normal rate of bone remodeling? Bone 35:1–3, 2004.
44. Abbott S, Trinkaus E, Burr DB: Dynamic bone remodeling in later Pleistocene fossil hominids. Am J Phys Anthropol 99:585–601, 1996.

Received September 9, 2005.

Review

Cow's Milk Allergy: A Complex Disorder

Ross G. Crittenden, PhD and Louise E. Bennett, PhD

Food Science Australia, Werribee, Victoria, AUSTRALIA

Key words: CMA, milk proteins, allergy, breast-feeding

Cow's milk allergy (CMA) is a complex disorder. Numerous milk proteins have been implicated in allergic responses and most of these have been shown to contain multiple allergenic epitopes. There is considerable heterogeneity amongst allergic individuals for the particular proteins and epitopes to which they react, and to further complicate matters, allergic reactions to cow's milk are driven by more than one immunological mechanism. Finally, the incidence and dominant allergic mechanisms change with age, with IgE-mediated reactions common in infancy and non-IgE-mediated reactions dominating in adults. The complexity of CMA has lead to many public misconceptions about this disorder, including confusion with lactose intolerance and frequent self-misdiagnosis. Indeed, the prevalence of self-diagnosed CMA in the community is 10-fold higher than the clinically proven incidence, suggesting a sizable population is unnecessarily eschewing dairy products. Avoidance of dairy foods, whether for true or perceived CMA, carries with it nutritional consequences and the provision of appropriate nutritional advice is important. In this review, the epidemiology and natural course of CMA is discussed along with our current understanding of its triggers and immunological mechanisms. We examine current strategies for the primary and secondary prevention of allergic sensitization and the ongoing search for effective therapies to ultimately cure CMA.

Key teaching points

- Cow's milk allergy is an inflammatory response to milk proteins and is distinct from lactose intolerance.
- CMA is more prevalent in infants (2–6%) than in adults (0.1–0.5%), and the dominant immunological mechanisms driving allergic reactions change with age.
- The prevalence of self-diagnosed CMA in the community is substantially higher than the incidence reported in blinded and controlled challenge trials, suggesting that a proportion of the population is unnecessarily eschewing dairy products
- Breast-feeding is the best preventative strategy, although it cannot eliminate the risk of allergic sensitization in infants.
- Management of CMA involves avoidance of dairy during the duration of the disease, and the provision of appropriate nutritional advice is important to prevent nutritional deficiencies, particularly for parents of young children who have dairy withdrawn from their diet due to either diagnosed or perceived CMA.

INTRODUCTION

Epidemiology and Natural History of CMA

Cow's milk allergy (CMA) is a complex and often misunderstood disorder. A frequent misconception among the general public is confusion between CMA and cow's milk intolerance, which is mainly intolerance to lactose (Fig. 1). While consumers often use these terms synonymously and interchangeably they are distinct disorders driven by different aetiological mechanisms. Hence, they require separate methods of diagnosis and distinct strategies for management and treatment (Table 1). It is the involvement of the immune system in the adverse reaction that defines food allergies. In CMA, the immune system is incorrectly programmed to react to innocuous milk proteins. Allergy symptoms result from the collateral damage to tissues caused by the immune system's aberrant inflammatory response. Some individuals are exquisitely allergic to cow's milk proteins and the reactivity threshold can be as little as 0.1 mL of milk [1].

Abbreviations: CMA = cow's milk allergy, CMI = cow's milk intolerance, DBPCFC = double-blind, placebo-controlled food challenge, eHF = extensively-hydrolyzed formulas, GALT = gut-associated lymphoid tissue, IgE = immunoglobulin E, IL-10 = interleukin-10, pHF = partially-hydrolyzed formulas, RAST = radioallergosorbant test, SPT = skin prick test, TGF-β = transforming growth factor-beta, Th1 = T helper cell-type1, Th2 = T helper cell-type 2, T reg = regulatory T cell.

Address reprint requests to: Ross G. Crittenden, PhD, Food Science Australia, Private Bag 16, Werribee VIC 3030, AUSTRALIA. E-mail: ross.crittenden@csiro.au

Journal of the American College of Nutrition, Vol. 24, No. 6, 582S–591S (202005)
Published by the American College of Nutrition

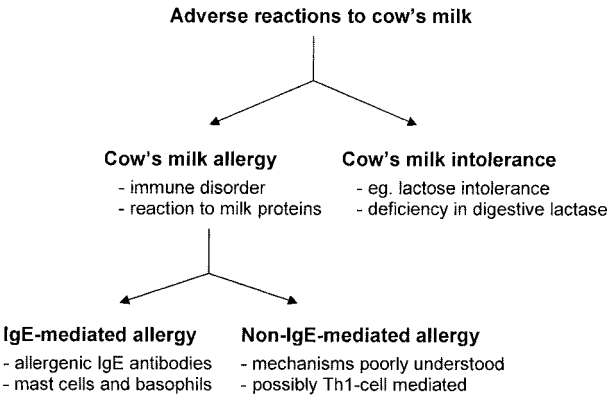

Fig. 1. Cow's milk allergy is distinct from cow's milk intolerances such as lactose intolerance and is caused by an aberrant inflammatory immune response to milk proteins. CMA is also not a single disease, but possibly involves a spectrum of immunological mechanisms. It is generally classified into IgE-mediated allergy and non-IgE-mediated allergy.

Cow's milk is a member of the so-called "Big-8" food allergens, ranking alongside egg, soy, wheat, peanuts, tree nuts, fish and shellfish in terms of prevalence [2–5]. The incidence of CMA varies with age. Cow's milk is the most frequently encountered dietary allergen in infancy when the immune system is relatively immature and susceptible to sensitization from environmental antigens. Hence, CMA is the dominant food allergy in babies [6]. The reported prevalence of CMA in infants and adults varies between studies, in part due to the difficulties in accurate diagnosis, differences in the age of study populations, and the clinical assessment criteria used. However, it is clear that CMA is most prevalent in early childhood, with figures generally reported between 2 and 6% [7–9], and decreases into adulthood to an incidence of 0.1–0.5% [10–11]. The long-term prognosis for the majority of affected infants is good, with 80–90% naturally acquiring tolerance to cow's milk by the age of 5 years [6, 12]. However, there remains a strong trend in infants who recover from CMA to develop atopic symptoms such as asthma, hay fever, or dermatitis to inhalant allergens later in life: the so-called "atopic career" or "atopic march" [12–13]. CMA appears to be an early indicator of atopy.

Perception versus Reality

Of concern is that the prevalence of self-diagnosed CMA in the community is significantly higher than the incidence supported by evidence from randomised, controlled, food challenge trials. Woods et al. [10] demonstrated that the self-diagnosed incidence of CMA in an Australian population was 10-fold higher than the clinically diagnosed prevalence. A similar pattern has been observed with other food allergies in other Western populations [14]. The reasons underlying this large discrepancy between the incidences of perceived and clinically proven milk allergy remain to be explored. Either dairy intolerance and/or allergy extends beyond the current diagnostic criteria, or more likely, many people misdiagnose

Table 1. Differences among the Most Prevalent Adverse Reactions to Cow's Milk

	Lactose intolerance	IgE-mediated cows' milk allergy	non-IgE-mediated cows' milk allergy
Prevalence	high	low	low
Racial variation	high	low	unknown
Common age	adolescence/adulthood	infancy	infancy and adulthood
Offender	lactose	milk proteins	milk proteins other components?
Mechanism	metabolic disorder - intestinal lactase deficiency	immunologic - IgE	immunologic - cell-mediated - immune complex - others?
Symptoms	gastrointestinal (GI)	one or more of GI, skin, respiratory, anaphylaxis	mainly GI and/or respiratory
Time of onset post ingestion	0.5–2 hours	<1 hour	>1 hour to days
Diagnostics	lactose tolerance test; breath test; stool acidity test; intestinal biopsy	skin-prick test; RAST	no simple diagnostic tests; DBPCFC
Prevention			
-primary	—	Breast-feeding. Milk protein avoidance in infancy (0–6 months)	unknown
-secondary	Avoid lactose	Avoid intact milk proteins	Avoid intact milk proteins
Processing options	Lactose hydrolysis or chromatographic lactose removal	Remove allergenic epitopes. Milk protein hydrolysis	Remove allergenic epitopes. No suitable products available

themselves without clinical evaluation and unnecessarily eschew dairy products. This carries with it nutritional implications, particularly for adequate vitamin and calcium intake and bone health [15–17]. Misdiagnosis of CMA by parents and restriction of dairy intake in young children without adequate dietetic supervision can lead to poor nutritional outcomes for growth, bone density and, where unorthodox alternative diets are implemented, inadequate protein and energy intake [15–19].

Understanding the Mechanisms of CMA

Perhaps contributing to the prevalent public perception of allergy is the lack of simple and reliable diagnostic tests for many individuals with CMA. In addressing why this is the case it is important to dispel another common misconception about CMA. That is, while it is often thought of as a single disease, CMA is in fact driven by at least two, and possibly more, distinct immune pathologies. Allergies to milk are often broadly classified into immunoglobulin E (IgE)-mediated allergy and non-IgE-mediated allergy (Fig. 1) [7, 20]. The immunopathological mechanisms of non-IgE-mediated allergy in particular remain poorly understood, and this has hindered the development of simple and reliable diagnostics. Recognising that understanding mechanisms is critical to the development of diagnostics and effective management, treatment and therapeutic strategies, numerous research groups are now focusing on acquiring an understanding of the molecular and cellular mechanisms of CMA. The following sections outline what we currently know about the triggers and immunology of CMA and how this knowledge is being applied in disease management, prevention and treatment.

BACKGROUND

Milk Protein Allergens

While some similarities exist between the protein composition of bovine and human milk (Table 2), there are substantial differences in the types of proteins and their homologies that provide ample scope for cow's milk proteins to be recognized as foreign by the human immune system [21–22]. In most people the immune system is able to recognise the milk proteins as harmless and tolerate them. However, in allergic individuals the immune system becomes sensitized to the milk proteins and mounts a damaging inflammatory response. The reasons why an unfortunate few develop CMA are not well understood. There appears to be a hereditary predisposition, but the phenotypic expression of allergy depends on a complex interaction between genetic and environmental factors [23] and the fundamental mechanisms of sensitization remain unclear.

Table 2. Typical Compositions of the Major Proteins in Human and Cow's Milk.

Protein	Human (mg/mL)	Cow (mg/mL)
α-lactalbumin	2.2	1.2
α-s1-casein	0	11.6
α-s2-casein	0	3.0
β-casein	2.2	9.6
κ-casein	0.4	3.6
γ-casein	0	1.6
immunoglobulins	0.8	0.6
lactoferrin	1.4	0.3
β-lactoglobulin	0	3.0
lysozyme	0.5	trace
serum albumin	0.4	0.4
other	0.8	0.6

In contrast, our understanding of the number and nature of allergenic determinants in milk is rapidly improving. It is known that both the allergy triggers in milk and the immune responses to those triggers in allergic individuals are multifarious. For example, most major cow's milk proteins (more than 30 so far) have been implicated in allergic responses, including both casein and whey proteins [21]. Epitope mapping of a number of milk proteins has revealed multiple allergenic epitopes within each protein, both for B cells that produce antibodies, and for T cells that direct both antibody and cell-mediated immune responses [22, 25–30]. Additionally, there is considerable heterogeneity amongst allergic individuals for the particular proteins and epitopes to which they react [21]. While there is scope for further epitope mapping of milk proteins, the complexity of antigenic determinants in milk is already apparent, as is the scale of the challenge to selectively eliminate them.

Immunological Mechanisms in CMA

Since different mechanisms are involved in driving CMA, different approaches are required for diagnosis and eventual treatments. A basic appreciation of the immunology of CMA is helpful in understanding the basis of strategies for prevention and therapies currently under investigation.

IgE-Mediated CMA (Immediate Hypersensitivity). IgE-mediated allergy is the best-understood allergy mechanism and, in comparison to non-IgE-mediated reactions, is relatively easily diagnosed. Since the onset of symptoms is rapid, occurring within minutes to an hour after allergen exposure, IgE-mediated allergy is often referred to as "immediate hypersensitivity" [31]. In healthy immune systems, this type of inflammatory response has evolved to target multicellular parasites such as worms [31]. Allergic responses occur when benign environmental antigens, such as food proteins, are incorrectly targeted.

The development of IgE-mediated CMA occurs in two stages. The first, "sensitization", occurs when the immune system is aberrantly programmed to produce IgE antibodies to milk proteins. These antibodies attach to the surface of mast cells and basophils, arming them with an allergen-specific trigger. Subsequent exposure to milk proteins leads to "activation" when the cell-associated IgE binds the allergenic epitopes on the milk proteins and triggers the rapid release of powerful inflammatory mediators leading to allergy symptoms (Fig. 2).

The symptoms associated with IgE-mediated CMA include one or more of cutaneous (eczema; urticaria; angioderma), gastrointestinal (oral allergy syndrome; nausea; vomiting; diarrhoea) or respiratory manifestations (rhinoconjunctivitis; asthma) [7]. Life-threatening anaphylactic reactions to cow's milk may also occur, but are fortunately rare [32]. Since reactions to cow's milk proteins can occur on contact with the mouth or lips, strategies to reduce allergenicity by improving protein digestibility in the gut are unlikely to be effective for all allergic individuals.

Simple diagnostic procedures, such as skin-prick tests (SPT) and RAST (radioallegosorbant test), can be used to identify individuals with IgE-mediated CMA, although both of these tests produce false-positive results in some individuals [33]. Food elimination and challenge testing are sometimes required to confirm milk allergy, and double-blind, placebo-controlled, food challenge (DBPCFC) testing remains the gold standard diagnostic. IgE-mediated reactions account for an estimated half of the CMA cases in young children [7, 34], but are rare in adults. Woods et al. [10] reported an incidence of 0.1% challenge-confirmed IgE-mediated CMA in a randomised population of more than 3000 Australian adults, a finding that was recently supported in a study of German adults [5].

Non-IgE-Mediated CMA (Delayed Hypersensitivity). A significant proportion of infants and the majority of adults with CMA do not have circulating milk protein-specific IgE and show negative results in skin prick tests and RAST [7, 35, 36]. These non-IgE-mediated reactions tend to be delayed, with the onset of symptoms occurring from 1 hour to several days after ingestion of milk. Hence, they are often referred to as "delayed hypersensitivity" [37]. As with IgE-mediated reactions, a range of symptoms can occur, but are most commonly gastrointestinal and/or respiratory in nature [7]. The gastrointestinal symptoms, such as nausea, bloating, intestinal discomfort and diarrhoea, mirror many of those that are symptomatic of lactose intolerance, complicating self-diagnosis. Anaphylaxis is not a feature of non-IgE mediated mechanisms [37]. IgE and non-IgE mediated reactions are not mutually exclusive and reactions to milk can involve a mixture of immunological mechanisms [37]. Adults with non-IgE-mediated allergy to milk tend to suffer ongoing allergy without the development of milk tolerance.

The precise immunopathological mechanisms of non-IgE-mediated CMA remain unclear. A number of mechanisms have been implicated, including type-1 T helper cell (Th1) mediated reactions (Fig. 2) [38–44], the formation of immune complexes leading to the activation of Complement [45], or T-cell/mast cell/neuron interactions inducing functional changes in smooth muscle action and intestinal motility [46–48].

There appears to be a discrepancy between reportedly higher rates of natural recovery during childhood from non-IgE-mediated CMA (compared to IgE-mediated CMA) [6, 12, 49], and the predominance of non-IgE-mediated CMA in adult populations [10, 35–36]. This suggests that a non-IgE-mediated CMA population emerges later in life. In a study of different

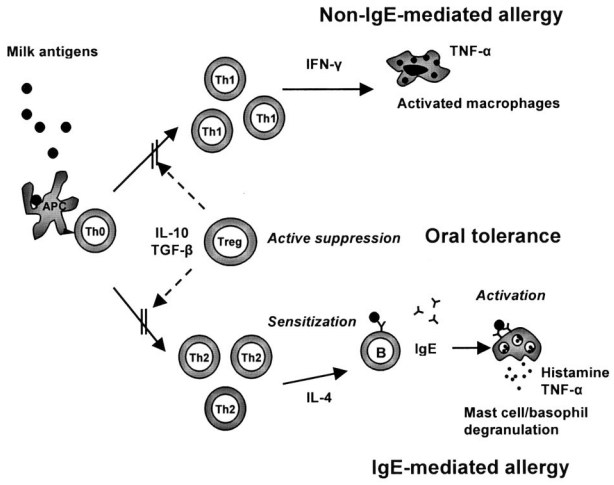

Fig. 2. Mechanisms of allergic reactions to milk proteins. Milk proteins are pinocytosed by antigen presenting cells (APC) and peptide epitopes are presented to T cells. Dendritic cells are an important class of APCs with a strong ability to program naive T cells. In IgE-mediated allergy, Th2 effector T cells signal B cells via interleukin-4 (IL-4) to class switch antibody production to allergenic milk protein-specific IgE, which then binds to, and arms, mast cells (sensitization). Milk proteins cross-linking the IgE on armed mast cells cause cell degranulation and rapid release of powerful inflammatory mediators (activation). Non-IgE-mediated mechanisms are poorly understood, but may involve activation of inflammatory cells via interferon-gamma (IFN-γ). Oral tolerance is achieved by T cell anergy, or by the action of regulatory T cells (T reg) that suppress the action of effector T cells (Th1 and Th2) via interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), or cell-to-cell contact.

age groups in Germany, Zuberbier et al. [5] reported an increase in the incidence of non-IgE-mediated food allergies with increasing age. However, the emergence of a new CMA population in adults remains to be conclusively demonstrated. Good epidemiological data for non-IgE-mediated CMA in both adults and children remains scarce because tedious DBPCFC trials remain the only truly conclusive diagnostic tests to confirm this form of allergy [50]. In many cases, gastrointestinal food allergy remains undiagnosed or is classified as irritable bowel syndrome.

Dysfunctional Tolerance. Even in the midst of a discussion on allergy it should be remembered that the majority of infants and adults are not allergic to cow's milk proteins. Understanding how this tolerance is mediated is central to developing strategies to prevent or treat allergy. Food antigens contact the immune system throughout the intestinal tract via the gut associated lymphoid system (GALT), where interactions between antigen presenting cells and T cells direct the type of immune response mounted (Fig. 2). Unresponsiveness of the immune system to dietary antigens is termed "oral tolerance" and is believed to involve the deletion or switching off (anergy) of reactive antigen-specific T cells and the production of regulatory T cells (T reg) that quell inflammatory responses to benign antigens [51–53].

CMA is believed to result from the failure to develop these tolerogenic processes or from their later breakdown. In the case of IgE-mediated CMA, a deficiency in regulation and a polarisation of milk-specific effector T cells towards type-2 T helper cells (Th2) lead to signalling of B-cells to produce milk protein-specific IgE [24, 40] (Fig. 2). Non-IgE-mediated reactions may be due to Th1 mediated inflammation [7]. Dysfunctional T reg cell activity has been identified as a factor in both allergy mechanisms [54–55]. Additionally, the induction of tolerance in children who have outgrown their CMA has been shown to be associated with the development of T reg cells [56–57]. Much research is currently focused on manipulating the activity of dendritic cells (specialised antigen presenting cells important in programming immune responses) to induce T reg cells and/or to redress Th1/Th2 imbalances in order to promote tolerance to allergenic foods.

DESCRIPTION OF SUBJECT

Management and Treatment of CMA

There is currently no cure for CMA and the only effective management strategy is avoidance of intact cow's milk proteins throughout the duration of the disease. The homologies between various mammalian milk proteins means that milks from other species (for example, goats and sheep) share many allergenic epitopes with cow's milk proteins and are often not reliable hypoallergenic cow's milk substitutes [58–60]. Individuals with CMA are also often allergic to a number of foods including soy, which is one of the "Big-8" allergens [61]. Hence, soymilk is often not a suitable alternative, and is especially not recommended for young infants (< 6 months) who are more susceptible to allergic sensitization [62]. Hypoallergenic infant formulas are available for CMA infants who cannot be breast-fed, while for adults with CMA the inclusion of milk proteins in an ever-expanding array of processed foods provides an increasing challenge to the management of their conditions.

Intervention strategies in CMA have been targeted at three levels; 1) primary prevention of initial sensitization; 2) secondary prevention of the triggering of allergic reactions; and 3) induction of tolerance in already sensitized individuals (specific immunotherapy, SIT). While there is general scientific agreement on how to manage the triggering of allergic reactions, debate on the most effective strategies to avoid initial sensitization remains intense, and more fundamental research into allergy and tolerance mechanisms is required to allow targeted strategies to induce tolerance.

Primary Prevention of Sensitization

CMA has a strong hereditary prevalence and currently familial history of atopy is the best predictive test for identifying children at risk of developing CMA. The precise point at which infants become sensitized to milk proteins is still controversial, which contributes to the sometimes fierce debate as to the best methods to prevent sensitization. There is emerging evidence from studies of cord bloods that both sensitization and the acquisition of tolerance can begin *in utero* [8, 63]. The window of main danger for sensitization to food proteins extends prenatally, remaining most critical during early infancy when the immune system and intestinal tract are still maturing.

Breastfeeding Is the Best Preventative for CMA. Although sensitization may perhaps begin *in utero,* there is no conclusive evidence to support the restriction of dairy intake in the maternal diet *during pregnancy* in order to prevent CMA. It is generally not recommended since the drawbacks in terms of loss of nutrition out-weigh the benefits [64–65]. Breastfeeding during the first 4–6 months is the most protective strategy known against the development of CMA [66]. Traces of cow's milk proteins ingested by the mother can be transferred to the sucking infant through breast milk [8], and exclusive breastfeeding does not completely eliminate the risk [67]. For at-risk infants, there are indications that maternal avoidance of dairy proteins during lactation can further minimize the risk of infant sensitization [65]. However, further randomised, controlled trials are required to examine if dietary exclusion by lactating mothers can truly minimize risk to a significant degree and if any reduction in risk is out-weighed by deleterious impacts on maternal nutrition.

For a variety of reasons, some babies cannot be breast-fed and require infant milk formulas. Evidence from a number of prospective studies indicates that the use of hydrolyzed formulas in early infancy provides better protection than the use of formulas with intact cow's milk proteins, especially in at-risk infants (having at least one atopic parent) [8, 66, 68–69]. It remains to be seen if these hydrolyzed formulas provide any protection against the later development of atopic disease [70]. A Cochrane analysis of studies comparing soy to hydrolyzed cow's milk formula found a significant increase in infant and childhood allergy cumulative incidence and infant eczema in infants fed soy formula [71]. The authors concluded that soy formula should not be recommended for the prevention of allergy or food intolerance in infants at high risk of allergy or food intolerance.

Partially Hydrolyzed Formulas (pHF). The proteins in hypoallergenic cow's milk infant formulas are extensively hydrolyzed in order to destroy allergenic epitopes. While these extensively hydrolyzed formulas (eHF) remove allergenicity, the loss of immunogenicity also prevents the immune system from developing tolerance to milk proteins. Partially hydrolyzed cow's milk formulas (pHF) have been developed with the aim of minimizing the number of sensitizing epitopes within milk proteins, while at the same time retaining peptides with sufficient size and immunogenicity to stimulate the induction of oral tolerance. Since they contain larger peptides than eHF, pHF trigger activation of symptoms in a relatively large percentage of already sensitized infants [72] and are therefore not

recommended where there is a risk of severe CMA symptoms [64]. Human intervention studies in at-risk infants have shown that pHF reduce the incidence of atopic dermatitis in the first 2 years compared to intact cow's milk protein formulas [8, 73–74]. However, despite animal studies indicating that pHF have an increased capacity to induce tolerance [75–77], there remains no clear evidence from human studies that they are better than eHF in preventing CMA [23, 70]. Further prospective human feeding studies are required to establish if they can play a useful role in preventing CMA.

Probiotics. Epidemiological evidence shows that allergy is more common in industrialized countries than in developing nations and more frequent in urban compared to rural communities [78]. This has lead to the development of the "hygiene hypothesis", which speculates that a decline in Th1-inducing exposure to pathogens and parasites contributes to the Th2-skewed immunity seen in IgE-mediated allergies [79–80]. Providing a microbial challenge in the form of dietary probiotic bacteria (live *Lactobacillus* and *Bifidobacterium* cultures used in fermented dairy products) has redressed Th1/Th2 imbalances and induced regulatory T cell activity in animal studies [70, 81–82]. Interestingly, controlled feeding studies using probiotics in human infants have produced clinically significant ameliorations of atopic dermatitis [83–84] that have been maintained up to the age of 4 years [85]. Probiotics are now included in some infant formulas, together with oligosaccharides (prebiotics), which can induce the development of a *Bifidobacterium*-dominated intestinal microbiota, replicating the effect of human breast milk. Although still in its infancy, the use of probiotics, prebiotics and components of intestinal parasites in the prevention of allergy [86] is an exciting and burgeoning area of research.

Immune Factors in Milk. Regulatory cytokines in human milk, such as transforming growth factor-beta (TGF-β), play an important role in promoting appropriate responses to food antigens during early infancy when the gut immune system is still developing [87]. However, cow's milk-based infant formulas are generally deficient in regulatory cytokines [88]. Using a rodent model, Penttila et al. [89] reported that supplementing infant formulas with cow's milk fractions rich in immunoregulatory factors enhanced the development of oral tolerance to food antigens. In the future, replicating the immunoregulatory capacity of human breast-milk may prove a valuable strategy to promote the tolerogenicity of cow's milk formulas.

Secondary Prevention: Making Cow's Milk Proteins Less Allergenic

For individuals who are already sensitized to cow's milk, CMA is managed by the avoidance of intact cow's milk proteins. The sheer number of allergenic epitopes and their conformational and sequence-based nature preclude the use of genetic selection or protein denaturation processes such as heating to remove allergenicity. Manufacturers of hypoallergenic infant milk formulas have approached the problem by destroying allergenic epitopes through extensive hydrolysis of milk proteins to peptides typically smaller than 1500 Da [66, 90]. These extensively hydrolyzed formulas (eHF) successfully prevent the triggering of allergy symptoms in the majority of allergic infants [90] and are evidently effective for both IgE-mediated and non-IgE-mediated reactions. In a small percentage of cases, even eHF trigger symptoms in highly sensitive infants and amino acid-based formulas are required [90]. While extensive hydrolysis eliminates allergenicity, it also destroys the physical and biological functionalities of milk proteins, and the search for alternative methods to produce hypoallergenic milks continues [91–96].

Curing Allergy: Immunotherapy

Specific immunotherapy (SIT) aims to induce immune regulation in sensitized individuals through controlled exposure to the allergen, which is often modified to prevent the triggering of adverse reactions. To date, trials of SIT for CMA have been limited largely to experimental animal models. Systemic immunizations using milk proteins, or recombinant milk protein fragments with appropriate adjuvants, have induced tolerogenic responses in Th2 skewed rodent [97–98] and dog models of CMA [99]. Similarly, the use of a DNA vaccine using a bacterial plasmid encoding the milk protein β-lactoglobulin has also been effective in inducing tolerance in a mouse model [100]. Recombinant bacteria expressing milk proteins and peptides have also been developed for oral vaccinations [97, 101], although they have not yet been effective in inducing tolerance to cow's milk proteins.

A recent report has detailed a protocol for oral desensitization in older children with severe IgE-mediated CMA [102]. The experiment showed that gradually increasing the daily oral dose of milk protein over a period of months improved tolerance to cow's milk in the majority of patients (15 of 21). This preliminary result requires confirmation in larger, double-blind, placebo-controlled studies. However, it shows that SIT has the potential to benefit food allergy sufferers in a similar way to its current effective use for desensitizing people against aeroallergens.

CONCLUSION

Recent years have seen major advances in our understanding of the immunological processes involved in the development of CMA and importantly, oral tolerance to food antigens. They have revealed the complexity of CMA in terms of the number of allergenic epitopes, the heterogeneity of allergic responses, and the potential diversity in immunological pathways leading to allergy symptoms. The epidemiology of CMA requires further investigation, but it is clear that it is more

frequent in young children (2–6%) and then decreases in prevalence among adults (0.1–0.5%). Importantly, the prevalence of self-diagnosed CMA in the community far exceeds the clinically proven incidence leading to unnecessary avoidance of dairy foods with nutritional consequences in terms of inadequate calcium and vitamin intake. While the mechanisms of IgE-mediated allergy are fairly well understood, the immunology and variety of non-IgE mediated reactions remains largely unknown. A better understanding of these allergy mechanisms is a prerequisite to the development of improved diagnostics, which in turn will facilitate an improved understanding of the epidemiology of CMA, particularly for non-IgE-mediated reactions. It will also aid the development of hypoallergenic dairy products, especially for adults with CMA for whom there is currently a dearth of suitable low-allergenic dairy products.

Some of the risk factors for the development of CMA have been identified, with a familiar history of atopy one of the main determinants. However, the mechanisms of allergic sensitization and the precise interactions between genetics and various environmental factors leading to CMA remain to be elucidated. The first few months of life, during which the immune system is still maturing, appears to be a critical risk period for the allergic sensitization. For at-risk infants with at least one atopic parent, breast-feeding during this period is currently the best identified preventative strategy, with the use of hydrolyzed formulas recommended for babies who cannot be breast-fed. The use of immunomodulatory dietary adjuvants such as probiotics is an emerging approach with considerable promise for primary prevention.

For CMA sufferers, avoidance of dietary milk proteins remains the only effective management strategy, but carries with it nutritional implications, particularly for adequate vitamin and calcium intake, and protein and energy intake where unorthodox alternative diets are implemented. A growing understanding of the molecular and cellular mechanisms of oral tolerance is underpinning advances in potential therapies for food allergies and is pivotal to eventually curing allergy in sensitized individuals. Unravelling the links between innate and adaptive immunity and the roles of dendritic cells and T cells in directing immune responses and homeostasis to environmental antigens are likely to remain a focus of fundamental food allergy research in coming years.

ACKNOWLEDGMENT

Financial support provided by Dairy Australia for milk protein allergy research at Food Science Australia is gratefully acknowledged.

REFERENCES

1. Morisset M, Moneret-Vautrin DA, Kanny G, Guénard L, Beaudouin E, Flabbée J, Hatahet R: Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. Clin Exp Allergy 33:1046– 1051, 2003.
2. Taylor SL, Hefle SL: Food Allergies and Other Food Sensitivities. Food Technology 55:68–83, 2001.
3. Madsen C: Prevalence of food allergy/intolerance in Europe. Environ Toxicol Pharmacol 4:163–167, 1997.
4. Schäfer T, Böhler S, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, Filipiak B, Wichmann H-E, Ring J: Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. Allergy 56:1172–1179, 2001.
5. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, Roehr CC, Bergmann KE, Niggermann B: Prevalence of adverse reactions to food in Germany—a population study. Allergy 59:338–345, 2004.
6. Wood RA: The natural history of food allergy. Pediatrics 111: 1631–1637, 2003.
7. Hill DJ, Hosking CS, Zhie CY, Leung R, Baratwidjaja K, Iikura Y, Iyngkaran N, Gonzalez-Andaya A, Wah LB, Hsieh KH: The frequency of food allergy in Australia and Asia. Environ Toxicol Pharmacol 4:101–110, 1997.
8. Exl B-M, Fritsché R: Cow's milk protein allergy and possible means for its prevention. Nutr 17:642–651, 2001.
9. Garcia-Ara MC, Boyano-Martinez MT, Diaz-Pena JM, Martin-Munoz MF, Martin-Esteban M: Cow's milk-specific immunoglobulin E levels as predictors of clinical reactivity in the follow-up of the cow's milk allergy infants. Clin Exp Allergy 34:866–870, 2004.
10. Woods RK, Thien F, Raven J, Walters EH, Abramson MA: Prevalence of food allergies in young adults and their relationship to asthma, nasal allergies, and eczema. Ann Allergy Asthma Immunol 88:183–189, 2002.
11. Bindels JG, Hoijer M: Allergens: latest developments, newest techniques. Bull Int Dairy Fed 351:31–32, 2000.
12. Høst A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K: Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. Pediatric Allergy Immunol 13 (Supp 15): 23–28, 2002.
13. Isolauri E: Cow-milk allergy. Environ Toxicol Pharmacol 4:137–141, 1997.
14. Sicherer SH: Food allergy. Lancet 360:701–710, 2002.
15. Henriksen C, Eggesbo M, Halvorsen R, Botten G: Nutrient intake among two-year-old children on cows' milk-restricted diets. Acta Paediatrica 89:272– 278, 2000.
16. Black RE, Williams SM, Jones IE, Goulding A: Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. Am J Clin Nutr 76:675–680, 2002.
17. Hidvegi E, Arato A, Cserhati E, Horvath C, Szabo A: Slight decrease in bone mineralization in cow milk-sensitive children. J Pediatr Gastroenterol Nutr 36:44–49, 2003.
18. Liu T, Howard RM, Mancini AJ, Weston WL, Paller AS, Drolet BA, Esterly NB, Levy ML, Schachner L, Frieden IJ: Kwashiorkor in the United States: fad diets, perceived and true milk allergy, and nutritional ignorance. Arch Dermatol 137:630–636, 2001.
19. Carvalho NF, Kenney RD, Carrington PH, Hall DE: Severe nutritional deficiencies in toddlers resulting from health food milk alternatives. Pediatr 107:E46, 2001.

20. Eigenmann PA, Frossard CP: The T lymphocyte in food-allergy disorders. Curr Opin Allergy Clin Immunol 3:199–203, 2003.
21. Wal J-M: Cow's milk proteins/allergens. Annal Allergy Asthma Immunol 89: 3–10, 2002.
22. Järvinen KM, Chatchatee P, Bardina L, Beyer K, Sampson HA: IgE and IgG binding epitopes on alpha-lactalbumin and beta-lactoglobulin in cow's milk allergy. Int Arch Allergy Immunol 126:111–118, 2001.
23. Halken S: Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. Pediatr Allergy Immunol 15 (Suppl 16): 4–5, 9–32, 2004.
24. Schade RP, Tiemessen MM, Knol EF, Bruijnzeel-Koomen CA, van Hoffen E: The cow's milk protein-specific T cell response in infancy and childhood. Clin Exp Allergy 33:725–730, 2003.
25. Nakajima-Adachi H, Hachimura S, Ise W, Honma K, Nishiwaki S, Hirota M, Shimojo N, Katsuki T, Ametani A, Kohno Y, Kaminogawa S: Determinant analysis of IgE and IgG4 antibodies and T cells specific for bovine alpha(s)1-casein from the same patients allergic to cow's milk: existence of alpha(s)1-casein-specific B cells and T cells characteristic in cow's-milk allergy. J Allergy Clin Immunol 101:660–671, 1998.
26. Inoue R, Matsushita S, Kaneko H, Shinoda S, Sakaguchi H, Nishimura Y, Kondo N: Identification of beta-lactoglobulin-derived peptides and class II HLA molecules recognized by T cells from patients with milk allergy. Clin Exp Allergy 31:1126–1134, 2001.
27. Chatchatee P, Järvinen KM, Bardina L, Beyer K, Sampson HA: Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: differences in patients with persistent and transient cow's milk allergy. J Allergy Clin Immunol 107: 379–383, 2001.
28. Chatchatee P, Järvinen KM, Bardina L, Vila L, Beyer K, Sampson HA: Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow's milk allergic patients. Clin Exp Allergy 31:1256–1262, 2001.
29. Busse PJ, Järvinen KM, Vila L, Beyer K, Sampson HA: Identification of sequential IgE-binding epitopes on bovine alpha(s2)-casein in cow's milk allergic patients. Int Arch Allergy Immunol 129:93–96, 2002.
30. Cocco RR, Jarvinen KM, Sampson HA, Beyer K: Mutational analysis of major, sequential IgE-binding epitopes in alpha s1-casein, a major cow's milk allergen. J Allergy Clin Immunol 112:433–437, 2003.
31. Roitt I, Brostoff J, Male D: "Immunology," 6th ed., New York: Mosby, 2001.
32. Eigenmann PA: Anaphylaxis to cow's milk and beef meat proteins. Ann Allergy Asthma Immunol 89 (Suppl 1):61–64, 2002.
33. Fiocchi A, Bouygue GR, Restani P, Bonvini G, Startari R, Terracciano L: Accuracy of skin prick tests in IgE-mediated adverse reactions to bovine proteins. Annal Allergy Asthma Immunol 89:26–32, 2002.
34. Heine RG, Elsayed S, Hosking CS, Hill DJ: Cow's milk allergy in infancy. Curr Opin Allergy Clin Immunol 2:217–225, 2002.
35. Pelto L, Laitinen I, Lilius E-M: Current perspectives on milk hypersensitivity. Trends Food Sci Technol 10:229–233, 1999.
36. Pelto L, Impivaara O, Salminen S, Poussa T, Seppänen R, Lilius E-M: Milk hypersensitivity in young adults. Eur J Clin Nutr 53:620–624, 1999.
37. Sabra A, Bellanti JA, Malka Rais J, Castro HJ, Mendez de Inocencio J, Sabra S: IgE and non-IgE food allergy. Annal Allergy Asthma Immunol 90:71–76, 2003.
38. Augustin M, Karttunen TJ, Kokkonen J: TIA1 and mast cell tryptase in food allergy of children: increase of intraepithelial lymphocytes expressing TIA1 associates with allergy. J Pediatr Gastroenterol Nutr 32:11–18, 2001.
39. Veres G, Helin T, Arato A, Färkkilä M, Kantele A, Suomalainen H, Savilahti E: Increased expression of intercellular adhesion molecule-1 and mucosal adhesion molecule alpha4beta7 integrin in small intestinal mucosa of adult patients with food allergy. Clin Immunol 99:353–359, 2001.
40. Beyer K, Castro R, Birnbaum A, Benkov K, Pittman N, Sampson HA: Human milk-specific mucosal lymphocytes of the gastrointestinal tract display a Th2 cytokine profile. J Allergy Clin Immunol 109:707–713, 2002.
41. Osterlund P, Smedberg T, Schröder J, Järvinen KM: Expression of intercellular adhesion molecules on circulating lymphocytes in relation to different manifestations of cow's milk allergy. Clin Exp Allergy 33:1368– 1373, 2003.
42. Osterlund P, von Willebrand M, Andersson LC, Suomalainen H: T-cell signal transduction in children with cow's milk allergy - increased MAP kinase activation in patients with acute symptoms of cow's milk allergy. Pediatr Allergy Immunol 14:163–168, 2003.
43. Walker-Smith J: Cow's milk allergy: a new understanding from immunology. Annal Allergy Asthma Immunol 90:81–83, 2003.
44. Yuan Q, Furuta GT: Insights into milk protein allergy: Microenvironment matters. Gastroenterol 124:259–261, 2003.
45. Høst A, Jacobsen HP, Halken S, Holmenlund D: The natural history of cow's milk protein allergy/intolerance. Eur J Clin Nutr 49 (Suppl 1): S13– 18, 1996.
46. Stenton GR, Vliagoftis H, Befus AD: Role of intestinal mast cells in modulating gastrointestinal pathophysiology. Annals Allergy Asthma Immunol 81:1–15, 1998.
47. Bauer O, Razin E: Mast Cell-Nerve Interactions. News Physiol Sci 15:213– 218, 2000.
48. Mekori YA, Baram D: Heterotypic adhesion-induced mast cell activation: biological relevance in the inflammatory context. Mol Immunol 38: 1363– 1367, 2001.
49. Vanto T, Helppila S, Juntunen-Backman K, Kalimo K, Klemola T, Korpela R, Koskinen P: Prediction of the development of tolerance to milk in children with cow's milk hypersensitivity. J Pediatr 144:218–22, 2004.
50. Niggemann B, Reibel S, Roehr CC, Felger D, Ziegert M, Sommerfeld C, Wahn U: Predictors of positive food challenge outcome in non-IgE-mediated reactions to food in children with atopic dermatitis. J Allergy Clin Immunol 108:1053–1058, 2001.
51. Groux H: An overview of regulatory T cells. Microbes Infection 3:883–889, 2001.
52. Schmidt-Weber CB, Blaser K: T-cell tolerance in allergic response. Allergy 57: 762–768, 2002.
53. Curotto de Lafaille MA, Lafaille JJ: CD4+ regulatory T cells in autoimmunity and allergy. Cur Opinion Immunol 14:771–778, 2002.
54. Tiemessen MM, van Hoffen E, Knulst AC, van der Zee JA, Knol EF, Taams LS: CD4+CD25+ regulatory T cells are not functionally impaired in adult patients with IgE-mediated cows milk allergy. J Allergy Clin Immunol 110: 934–936, 2002.

55. Pérez-Machado MA, Ashwood P, Thomson MA, Latcham F, Sim R, Walker-Smith JA, Murch SH: Reduced transforming growth factor-beta1-producing T cells in the duodenal mucosa of children with food allergy. Eur J Immunol 33: 2307–2315, 2003.
56. Karlsson MR, Rugtveit J, Brandtzaeg P: Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 199:1679–1688, 2004
57. Tiemessen MM, Van Ieperen-Van Dijk AG, Bruijnzeel-Koomen CA, Garssen J, Knol EF, Van Hoffen E: Cow's milk-specific T-cell reactivity of children with and without persistent cow's milk allergy: key role for IL-10. J Allergy Clin Immunol 113: 932–939, 2004.
58. Pessler F, Nejat M: Anaphylactic reaction to goat's milk in a cow's milk-allergic infant. Pediatr Allergy Immunol 15:183–185, 2004.
59. Restani P, Beretta B, Fiocchi A, Ballabio C, Galli CL: Cross-reactivity between mammalian proteins. Ann Allergy Asthma Immunol 89:11–15, 2002.
60. Speurgin P, Walter M, Schiltz E, Deichmann K, Forster J, Mueller H: Allergenicity of alpha-caseins from cow, sheep and goat. Allergy 52:293–298, 1997.
61. Ahn KM, Han YS, Nam SY, Park HY, Shin MY, Lee SI: Prevalence of soy protein hypersensitivity in cow's milk protein-sensitive children in Korea. J Korean Med Sci 18:473–477, 2003.
62. Dupont C, De Boissieu D: Formula feeding during cow's milk allergy. Minerva Pediatrica 55:209–216, 2003.
63. Hauer AC, Riederer M, Griessl A, Rosegger H, MacDonald TT: Cytokine production by cord blood mononuclear cells stimulated with cows milk proteins *in vitro:* interleukin-4 and transforming growth factor β-secreting cells detected in the CD45RO T cell population in children of atopic mothers. Clin Exp Allergy 33: 615–623, 2003.
64. Zeiger RS: Food allergen avoidance in the prevention of food allergy in infants and children. Pediatr 111:1662–1671, 2003.
65. Kramer, MS; Kakuma, R: Maternal dietary antigen avoidance during pregnancy and/or lactation for preventing or treating atopic disease in the child. Cochrane Database Systematic Rev (4):CD000133, 2003.
66. American Academy of Pediatrics Committee on Nutrition: Hypoallergenic Infant Formulas. Pediatr 106:346–349, 2000.
67. Saarinen KM, Juntunen-Backman K, Jarvenpaa AL, Kuitunen P, Lope L, Renlund M, Siivola M, Savilähti E: Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: A prospective study of 6209 infants. J Allergy Clin Immunol 104:457–461, 1999.
68. Gore C, Custovic A: Can we prevent allergy? Allergy 59:151–161, 2004.
69. Osborn DA, Sinn J: Formulas containing hydrolysed protein for prevention of allergy and food intolerance in infants. Cochrane Database Systematic Rev (4):CD003664, 2003.
70. Fiocchi A, Martelli A, De Chiara A, Moro G, Warm A, Terracciano L: Primary dietary prevention of food allergy. Ann Allergy Asthma Immunol 91: 3–13, 2003.
71. Osborn DA, Sinn J: Soy formula for prevention of allergy and food intolerance in infants. Cochrane Database Systematic Rev (3):CD003741, 2004.
72. Giampietro PG, Kjellerman N-IM, Oldaeus G, Wouters-Wesseling W, Businco L: Hypoallergenicity of an extensively hydrolyzed whey formula. Periatr Allergy Immunol 12:83–86, 2001.
73. Chan YH, Shek LPC, Aw M, Quak SH, Lee BW: Use of hypoallergenic formula in the prevention of atopic disease among Asian children. J Pediatr Child Health 38:84–88, 2002.
74. von Berg A, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D: German Infant Nutritional Intervention Study Group. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. J Allergy Clin Immunol 111:467–470, 2003.
75. Fritsché R, Pahud JJ, Pecquet S, Pfeifer A: Induction of systemic immunologic tolerance to beta-lactoglobulin by oral administration of a whey protein hydrolysate. J Allergy Clin Immunol 100:266–273, 1997.
76. Pecquet S, Leo E, Fritsché R, Pfeifer A, Couvreur P, Fattal E: Oral tolerance elicited in mice by beta-lactoglobulin entrapped in biodegradable microspheres. Vaccine 18:1196–1202, 2000.
77. Pecquet S, Pfeifer A, Gauldie S, Fritsche R: Immunoglobulin E suppression and cytokine modulation in mice orally tolerized to beta-lactoglobulin. Immunol 96:278–285, 1999.
78. Sheikh A, Strachan DP: The hygiene theory: fact or fiction? Curr Opin Otolaryngol Head Neck Surg 12:232–236, 2004.
79. Yazdanbakhsh M, Kremsner PG, van Ree R: Allergy, parasites, and the hygiene hypothesis. Science 296: 490–494, 2002.
80. Matricardi PM, Bouygue GR, Tripodi S: Inner-city asthma and the hygiene hypothesis. Ann Allergy Asthma Immunol 89:69–74, 2002.
81. Cross M, Stevenson LM, Gill HS: Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria? Int Immunopharmacol 1:891–901, 2001.
82. Prioult G, Pecquet S, Fliss I: Stimulation of Interleukin-10 Production by Acidic beta-Lactoglobulin-Derived Peptides Hydrolyzed with *Lactobacillus paracasei* NCC2461 Peptidases Clin Diagn Lab Immunol 11:266–271, 2004.
83. Isolauri E, Arvola T, Sutas S, Moilanen E, Salminen S: Probiotics in the management of atopic eczema. Clin Exp Allergy 30:1604–1610, 2000.
84. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E: Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 357:1076–1079, 2001.
85. Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E: Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 3611869–1871, 2003.
86. Matricardi PM, Björkstén B, Bonini S, Bousquet J, Djukanovic R, Dreborg S, Gereda J, Malling HJ, Popov T, Raz E, Renz H, Wold A: Microbial products in allergy prevention and therapy. Allergy 58:461–471, 2003.
87. Penttila IA, Flesch IE, McCue AL, Powell BC, Zhou FH, Read LC, Zola H: Maternal milk regulation of cell infiltration and interleukin 18 in the intestine of suckling rat pups. Gut 52:1579–1586, 2003.
88. Donnet-Hughes A, Duc N, Serrant P, Vidal K, Schiffrin EJ: Bioactive molecules in milk and their role in health and disease: the role of transforming growth factor-beta. Immunol Cell Biol 78:74–79, 2000.

89. Penttila IA, Zhang MF, Bates E, Regester G, Read LC, Zola H: Immune modulation in suckling rat pups by a growth factor extract derived from milk whey. J Dairy Res 68:587–599, 2001.
90. Walker-Smith J: Hypoallergenic formulas: are they really hypoallergenic? Annal Allergy Asthma Immunol 90:112–114, 2003b.
91. Jost R, Fritsche R, Pahud JJ: Reduction of milk protein allergenicity through processing. In Somogyi JC, Muller HR, Ockhuizen T (eds) "Food Allergy and Food Intolerance. Nutritional Aspects and Developments." 48:127–137, 1991.
92. Leary HL: Nonclinical testing of formulas containing hydrolysed milk protein. J Pediatr 121 (Suppl): S42–S46, 1992.
93. Sutas Y, Soppi E, Korhonen H, Syvaoja EL, Saxelin M, Rokka T, Isolauri E: Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes. J Allergy Clin Immunol 98: 216–224, 1996.
94. Lee JW, Kim JH, Yook HS, Byun MW, Kang SY, Hwang HJ: Effects of gamma radiation on the allergenic and antigenic properties of milk proteins. J. Food Prot 64: 272–276, 2001.
95. Bonomi F, Fiocchi A, Frokiaer H, Gaiaschi A, Iametti S, Poiesi C, Rasmussen P, Restani P, Rovere P: Reduction of immunoreactivity of bovine beta-lactoglobulin upon combined physical and proteolytic treatment. J Dairy Res 70:51–59, 2003.
96. Ehn BM, Ekstrand B, Bengtsson U, Ahlstedt S: Modification of IgE binding during heat processing of the cow's milk allergen beta-lactoglobulin. J Agric Food Chem 52:1398–1403, 2004.
97. Chatel J-M, Nouaille S, Adel-Patient K, Le Loir Y, Boe H, Gruss A, Wal J-M, Langella P: Characterization of a *Lactococcus lactis* Strain That Secretes a Major Epitope of Bovine Beta-Lactoglobulin and Evaluation of Its Immunogenicity in Mice. Appl Environ Microbiol 69:6620–6627, 2003.
98. Afuwape AO, Turner MW, Strobel S: Oral administration of bovine whey proteins to mice elicits opposing immunoregulatory responses and is adjuvant dependent. Clin Exp Immunol 136:40–48, 2004.
99. Frick OL, Teuber SS, Buchanan BB, Morigasaki S, Umetsu DT: Allergen immunotherapy with heat-killed *Listeria monocytogenes* alleviates peanut and food-induced anaphylaxis in dogs. Allergy 60:243–250, 2005.
100. Adel-Patient K, Creminon Ch, Boquet D, Wal JM, Chatel JM: Prevention of an IgE response to bovine beta-lactoglobulin by gene immunization in mice [French]. Allerg Immunol (Paris) 34:77–81, 2002.
101. Bernasconi E, Germond JE, Delley M, Fritsché R, Corthésy B: *Lactobacillus bulgaricus* proteinase expressed in *Lactococcus lactis* is a powerful carrier for cell wall-associated and secreted bovine beta-lactoglobulin fusion proteins. Appl Environ Microbiol 68: 2917–2923, 2002.
102. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG: A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 59:980–987, 2004.

Received September 9, 2005.