This month’s issue features preventing bone loss, human-edible plant protein, milk and migration, and oligosaccharides in human milk.

Preventing Bone Loss With Dairy and Vitamin D

- Although vitamin D regulates calcium absorption, vitamin D supplementation alone is not associated with improved bone mineral density in older adults.
- The results of a new prospective cohort study indicate that dairy foods demonstrate a protective effect on bone mineral density but only in participants that were taking vitamin D supplements.
- Vitamin D supplementation may work best in conjunction with calcium, phosphorus, and protein provided by dairy foods to reduce age-related bone loss.

With age comes wisdom. That’s the good news. Age, unfortunately, also comes with less desirable traits, including bone loss. Soon after the human skeleton reaches its maximum bone mass, sometime between 20 and 30 years of age, the rate of bone breakdown begins to exceed the rate of bone formation. Although this physiological process is an inevitable part of aging, it can be slowed to avoid the development of osteoporosis, a bone disease currently affecting more than 10 million Americans over the age of 50 [1].

Of particular interest to clinicians is the potential of nutritional supplements to increase the rate at which calcium is incorporated into bones in older adults. Because a deficiency of vitamin D results in softer bones (better known as rickets in children and osteomalacia in adults), it stands to reason that increased vitamin D intake would result in a stronger skeleton. Indeed, vitamin D supplementation has been a staple in the prevention and treatment of osteoporosis for several decades [2]. It may seem surprising, then, that neither a recent randomized clinical trial [3] nor a large-scale meta-analysis of the literature [2] found any evidence of a benefit for vitamin D supplementation on bone mineral density.

But before you throw your bottle of vitamin D supplements away, use that age-earned wisdom and take note of newly published results [1] from a prospective cohort study on the relationship between dairy foods and bone loss. Sahni and colleagues [1] report that specific dairy foods (i.e., milk, yogurt, and cheese) demonstrate a protective effect against bone loss but only among participants taking vitamin D supplements. Vitamin D, it seems, may be more of a team player than an individualist, working best in combination with the unique set of nutrients—including calcium, phosphorus, and high-quality protein—provided by dairy foods.

It’s Time for a Remodel

The human skeleton should have its own show on HGTV because it is always undergoing a renovation project. Bone remodeling, the process of releasing (or resorbing) old bone and forming new bone, occurs throughout the life course and is essential for preserving the strength of the skeleton and maintaining homeostasis of calcium and other minerals throughout the body [4]. However, as humans age, each remodeling event results in more demolition than improvements—the rate at which bone is resorbed by cells called osteoclasts outpaces the rate at which bone is formed by cells called osteoblasts. This process is further accelerated in women undergoing menopause, where decreases in estrogen levels are associated with increased rates of bone resorption [1-3].

At peak bone mass, the structure of bone is often described as resembling a honeycomb in structure. As bone ages, it starts to look more like a spider’s web, with larger holes where minerals have been released into the circulation. It does not take a structural engineer to understand why larger holes and decreased mineral content make for weaker and more fragile skeletons.

Although it might not be possible to maintain the more densely packed skeleton of a 20-year-old into the retirement years, age-related bone loss need not progress to osteoporosis. Osteoporosis is a bone disease characterized by very low bone mineral density and associated with an increased risk of falls and bone fractures [1-3]. And yet, the International Osteoporosis Foundation estimates that nearly 10 million Americans, and 100s of millions more worldwide, suffer from osteoporosis. If the disease is preventable, why is its incidence so high?

One obvious reason is that many populations are not eating enough of the nutrients that help maintain bone. Bone formation requires calcium, so it follows that diets low in this mineral, and vitamin D, the hormone responsible for calcium absorption, would...
be associated with poor bone health. The current U.S. dietary guidelines recommend that females over 50 and males over 71 consume 1200 mg of calcium and 800 IU (international units) of vitamin D per day, but the most recent Dietary Guidelines for Americans [5] estimates that the majority of Americans in these age groups are missing the mark on both nutrients. Could better bone health be as simple as taking a daily supplement?

Tenacious D

The short answer to this question is, unfortunately, no. Calcium supplements do show a protective effect on bone loss, suppressing bone turnover by approximately 20% and improving bone density [6]. However, the safety of calcium supplementation has been questioned, and numerous studies [e.g., 7-9] report an increased risk associated with calcium supplements for heart attack and stroke. With an unclear relationship between calcium supplementation and cardiovascular health, many researchers and clinicians are instead recommending meeting daily calcium requirements exclusively through the diet. Unfortunately, the results from studies that looked at the relationship between dietary calcium intakes and bone health also are conflicting—although bone density showed slight improvements, meeting the 1200 mg RDA of calcium through the diet had no association with fracture risk [10].

The results from research on vitamin D supplementation are equally perplexing. Vitamin D is not a mineral incorporated into bones, but rather it is a hormone responsible for augmenting intestinal absorption of calcium. Calcium digestion is rather tricky, and not all calcium consumed in foods (or supplements) will make its way into the circulation. In its metabolically active form (referred to as calcitriol), vitamin D influences the expression of calcium-binding protein, which is responsible for the active transport of calcium from the intestines into the circulation (precisely where calcium needs to be in order to be transferred to the bones). Although a randomized control trial of vitamin D supplementation in 230 postmenopausal women under 75 years of age [2] found that supplemented groups did indeed increase calcium absorption, supplementation was not associated with any improvements in bone mineral density or decreased risk of falls compared with the placebo group. These findings are echoed by a systematic review of the literature [3] on effects of vitamin D supplementation on bone mass. Reviewing 23 studies involving just over 4000 participants (92% of whom were postmenopausal women), Reid and colleagues [3] report that the number of positive results (i.e., improved bone density) was not much better than what would have been expected by chance.

One possible explanation for these findings is that vitamin D supplementation may only be effective in getting calcium from the gut to the blood but has little influence on getting that calcium into bones—that is, in promoting bone mineralization [3,11]. This perspective helps reconcile why insufficient vitamin D can predispose individuals to osteoporosis, by limiting the amount of available calcium to make new bone, whereas vitamin D supplements do little to improve bone density.

Better Together—Dairy and Vitamin D

Vitamin D alone may not be very effective for bone health, but what if vitamin D supplements were working with other nutrients? A team of researchers from Harvard, University of Massachusetts, and the Institute for Aging in Boston investigated whether vitamin D supplementation would have any influence on the association between dairy intake and bone density [1]. To do so, they utilized data from the Framingham Osteoporosis Study, an offshoot of the famous Framingham Heart Study. This study included two bone mineral density measurements (taken four years apart) on of the femoral neck (trochanter), lumbar spine, and radius, allowing them to track longitudinal changes in bone mass in more than 600 men and women. The Framingham Study also provided dietary information on the weekly intake of milk, yogurt, cheese, and cream, permitting an analysis of specific dairy foods and bone density.

The study authors hypothesized that the intake of all dairy foods except for cream would be associated with higher bone mineral density and lower bone loss [1]. They further speculated that the benefits of dairy foods on bone health would be greater in those individuals taking vitamin D supplements. They were surprised to find no significant association between dairy intake, quantified as servings per week, and bone mineral density [1]. However, higher intakes of milk, milk + yogurt, and milk+yogurt+cheese were associated with higher bone density in the lumbar spine when they singled out vitamin D users. Moreover, intakes of milk+yogurt+cheese among vitamin D users demonstrated a protective effect against bone loss in the trochanter; for every serving increase of milk+yogurt+cheese per week by vitamin D supplement users (but not non-users), the study identified a 0.23% increase in trochanter bone mineral density [1].

What is it about dairy that brings out the best in vitamin D? Calcium is certainly part of the story. But previous studies that compared bone mass changes from calcium supplementation with those from calcium and vitamin D supplementation found the effects to be indistinguishable [3], suggesting vitamin D had very little, if any, effect when taken solely with calcium. In addition to calcium, dairy foods also provide bioactive proteins that are implicated in bone formation. For example, the protein insulin-like growth factor 1 (IGF-1), a hormone that (as its name suggests) plays a role in bone growth in children and adolescents, is also implicated in adding bone mineral in adults. Moreover, dairy’s casein proteins bind calcium and phosphorus, delivering these two bone-building minerals at the same time. Phosphorus promotes bone mineralization, and thus increases the chances that absorbed calcium will end up in the skeleton. Dairy foods may need sufficient vitamin D to ensure optimal calcium absorption, but the vitamin D needs dairy’s ingredients to be sure that the absorbed calcium is transferred to the skeleton.
Putting It to the Test

The results from the Framingham study indicate vitamin D supplementation may indeed be important for bone health but only when delivered with other nutrients. The next step in vindicating vitamin D will be randomized controlled trials (the gold standard for evaluating the effectiveness of an intervention) that examine specific dairy foods with various levels of vitamin D supplementation. It will also be important to evaluate other, perhaps more relevant, clinical outcomes of osteoporosis besides bone mineral density, such as number of falls or fractures. Here’s hoping that most of us reading this now can reap the rewards of this type of research for better bone health in our future!


Contributed by
Dr. Lauren Milligan Newmark
Research Associate
Smithsonian Institute

Humans and Dairy Cows Compete for Human-edible Plant Protein

- Dairy cattle obtain most of their protein nutritional requirements from feed that is inedible by humans.
- Intensive dairying uses additional feed supplements containing plant protein that is edible by humans.
- The ratio of human-edible protein produced in cow’s milk to human-edible protein ingested by the cow is proposed as an index of the sustainability of feed supplements in a future world with increasing competition for protein resources.
- Dairy cows currently produce more human-edible protein than they consume.
- Future assessments of sustainability will also need to take protein quality into account because the quality of milk protein is higher than plant protein for human nutrition.

Much More Food Required by 2050

An expert panel that contributed to a 2009 Food and Agriculture Organization of the United Nations (FAO) report entitled “How to Feed the World in 2050” calculated that world food production would need to increase 70% by 2050 to meet the challenge of feeding 9.7 billion people [1, 2]. For many of today’s agricultural scientists, this is a daunting, though some say achievable, number [3].

The 2009 FAO report indicated that although additional arable land in the world could become available for growing more crops for human consumption by 2050, this land will be limited in quantity and would likely support only a narrow range of agricultural species [1]. The report concluded that food production and its utilization will need to become much more efficient. Inevitably there will be competition for resources between different agricultural industries and questions raised regarding the sustainability of some agricultural practices. There may be greater attention paid to the net benefit to humans of using human-edible plant protein in livestock feed supplements [3]. The livestock industries need extensive protein-use efficiency data to enable future decisions on the optimum use of resources for plant and animal protein production.

Competition for Dietary Protein
The human demand for dietary animal protein is growing, not just as a result of population increase, but also because there is increased demand per individual within the more affluent and growing middle classes of many countries [4]. Dairy products are rich sources of protein for human consumption. Another FAO report concluded that global milk production will need to increase by 58% by 2050 to meet demand [5]. This will be a challenge for the dairy industry, although a similar percentage increase of milk yield in the USA was achieved over the last 30-40 years [6].

Grazing ruminants like cattle have the remarkable ability to transform pasture unsuitable for other agricultural purposes into energy- and protein-dense meat and milk suitable for human consumption. The cow’s dietary protein obtained from pasture is inedible by humans; however, intensive dairying now uses plant-based feed supplements containing human-edible protein to support the increasing demand [3]. In a recent publication, Christian Swensson and colleagues from the Swedish University of Agricultural Sciences and five other Swedish institutions suggest that this practice will generate competition between the intensive dairy industry and humans for agricultural resources producing human-edible plant protein [3]. Ultimately, the practice raises a question about the sustainability of intensive dairying in a much larger world population directly competing for human-edible protein.

The research group led by Christian Swensson investigated production data from intensive dairying occurring in five locations in Sweden, each with different types of feed supplements [3]. The researchers calculated the ratio of human-edible protein produced in cow’s milk per unit of human-edible protein in the feed intake of the cow. A ratio larger than one is favorable for the dairy industry. The main conclusion was that five of the six tested regions had ratios greater than one, i.e., those regions contained cows that created more human-edible protein than they consumed. However, the investigators noted that higher milk yield tended to decrease this ratio regardless of diet. In biology, there are no free lunches.

The important progress made by the researchers was to distinguish between human-edible and human-inedible protein used in a variety animal feed supplements. The researchers also included data in their calculations relating to the digestibility of human-edible plant protein in the feed supplements. They also suggested a need for future adjustments in their calculations to compensate for the lower nutritional quality (to humans) of human-edible plant proteins in feed supplements compared with the quality of the milk protein output.

**Plant and Animal Proteins Do Not Have the Same Nutritional Value**

Scientists have reported in many investigations that plant and animal proteins are different in their nutritional value to humans [7-9]. Humans and livestock break down dietary protein into its twenty component amino acids in the intestinal tract. Animals then absorb the amino acids into their body where tissues use them to synthesize new proteins that contribute to all cellular structures and functions. For humans and cows, there are nine amino acids (essential amino acids) that they cannot synthesize in the body from other molecules and therefore must come from the diet. The amino acid composition of animal protein, like milk proteins, perfectly suits the protein nutritional needs of humans.

Plant proteins in dairy feed supplements often have low levels of some essential amino acids. Therefore, the quality of these plant proteins for animal nutrition, including human nutrition, may not be optimal. For example, feed supplements containing only soy protein are deficient in the essential amino acids methionine and lysine, grains are deficient in lysine and legumes are deficient in methionine and cysteine. Dairy producers often use combinations of plant feed supplements to partially compensate for these deficiencies.

Future assessments of the sustainability of using human-edible protein in dairy feed supplements will require consideration of protein nutritional quality in the protein efficiency calculations. These adjustments may show even greater advantages of using the dairy cow to efficiently create protein suitable for human nutrition from an optimum balance of pasture and feed supplements containing human-edible protein.

traces are scraped from the ceramic remains and analyzed molecularly and can be found attached to pottery fragments that were once vessels used to carry or process milk and other animal products. Molecules in the fat and how they are arranged into their structural lipids or fats that are characteristically found in milk. The composition of fat is determined by the concentrations of different fatty acids, but the pattern of use varied, so that in some societies further to the east, meat predominated or was perhaps used exclusively.

Evidence for dairy milk as a food is seen at least as early as 9000 years ago, and has been interpreted to mean that dairy had its origin soon after herding began and animals were domesticated, sometime between 10,000 and 11,000 years ago in the regions of the fertile crescent of the Near East [2]. The migration of farming people from the Near East into Europe occurred during the mid-late Neolithic period and coincided with the movement of herds of domesticated animals and the rise of farming communities. Subsistence of these communities is the subject of intensive investigation by scientists.

The relative importance of milk exploitation in the subsistence diets of migrating communities as the Mediterranean basin was populated has been an active area of discussion and debate. A study published in November 2016 in PNAS [1] presents new evidence that milk was a regular part of diets as far west as the Iberian Peninsula (modern-day Spain) as far back as 8000 years ago, but the pattern of use varied, so that in some societies further to the east, meat predominated or was perhaps used exclusively.

There are two approaches that scientists can use to detect or infer dairy farming. One method involves the direct measurement of lipids or fats that are characteristically found in milk. The composition of fat is determined by the concentrations of different molecules in the fat and how they are arranged into their structural backbone. Traces of these fats survive for thousands of years and can be found attached to pottery fragments that were once vessels used to carry or process milk and other animal products. The traces are scraped from the ceramic remains and analyzed molecule by molecule using modern methods for separation and natural isotope detection. This has the advantage of direct measurement of the milk fats, and also relates the findings to the pottery used by communities at various stages of cultural development. The age of the pottery provides evidence of the stage of cultural development and complements other methods of determining age, e.g., carbon dating.

What about communities where pottery was not in use or not yet available? The study by Spiteri et al. [1] also used a method based on archaeological analysis of bones. The advantage of using animal bones is that they are concentrated in locations that were used for slaughter, and they can provide data on the age range and species of origin. The data can be used to interpret the main purpose of the animal’s use; e.g., the age of slaughter will reflect whether an animal was used primarily for meat, or whether it was consistent with the use of animals for milk. These data can also provide information about farming practices and diet in communities where there was no use of pottery. By combining these methods, the scientists were able to build a picture of the relative use of caprine (goats and sheep) or bovine (cattle) sources of milk in 82 archaeological sites across the northern Mediterranean and Near East that dated to between 7,000 and 9,000 years ago.

The data analysis revealed that most communities harvested milk for dietary purposes. This was greatest around the Sea of Marmara in modern-day Turkey, where over 70% of samples analyzed contained dairy fats, but even in the region around modern-day southern France and Spain, 60% of samples contained dairy fats. Another feature of the study was the range of sites from where samples were collected. Some of these sites suggest that the farmers engaged in early forms of intensive dairying, mostly in caves...
that were suited to restraining or protecting the dairy animals.

Milk was not used extensively in all communities surveyed. The most striking variation to the pattern was in northern Greece and the Aegean, where animal bones were mostly from pigs, and where other bones from ruminants indicated they were clearly used for meat. The varied proportions of meat use compared to milk reflects a diverse mixture of animal use across the region. The reason for this is not evident, but the variation probably arose from a complex range of factors that were related to terrain, climate, and social customs.

Interestingly, the adoption of milk as a food predates the emergence of the lactase persistence gene variant that provided human adults with the capacity to break down the milk sugar lactose [3]. However, it has been inferred from previous studies that cultured milk, or some form of cheese, was present from early herding communities. The treatment of milk in this way makes it digestible even without the capacity to break down lactose, so it is reasonable to think that this dairy technology traveled with the migrating farmers, or was adopted by people who learned from the migrating farmers.

It is fascinating to contemplate the sophistication in herding and farming from such an early age that emerged alongside, or perhaps even initiated the birth of, social structures along the northern Mediterranean fringe. The mobility of these farming practices opened new frontiers, apparently seeding and sustaining societies by providing subsistence dietary requirements. But more than that, farming became a vehicle for technological advances that were to provide an impetus for the development of more complex human cultures.


**Contributed by**

Professor Peter Williamson

Associate Professor, Physiology and Genomics

University of Sydney, Australia

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**The Oligosaccharides in Human Milk Vary by Population**

- A new study finds that the normal levels of oligosaccharides in human milk vary across human populations.
- The study analyzed 19 oligosaccharides in milk samples from 11 populations that are located in North and South America, Africa and Europe.
- The work is important in understanding the links between the specific components of human milk and infant health, as well as potential environmental influences on human milk constituents.

Human milk often contains a higher concentration of medium-length sugar molecules called oligosaccharides (HMOs) than it does protein. That may seem a little odd, given that these sugars, which number well over 150 different types, are generally undigested by the infants who consume them. Nonetheless, HMOs can confer impressive benefits on infant health. Some, for example, stop viruses finding a footing on intestinal cells. And more generally, the HMO profile of a mother’s milk appears to impact her infant’s metabolism, via changes in her offspring’s gut bacteria, as well as her infant’s lean and fat body mass. Scientists, however, know relatively little about what HMOs are normally produced by mothers in different parts of the world.

A recent publication in the American Journal of Clinical Nutrition seeks to improve this state of affairs [1]. It details the HMO profiles of human milk from 11 populations scattered across the globe. Between 2014 and 2016, the team of authors—drawn from most of the countries in which the populations of interest are located—set about finding about 40 healthy, breastfeeding women from each population who were willing to donate a sample of their milk to the project. In many of the 11 populations, the idea was to find women with similar genetic ancestry: the Swedish subjects all had Nordic grandparents, the Southern Californians classified themselves as Hispanic, and the Gambian and Ethiopian women self-identified as Mandinka and Sidama, respectively.

So what did the researchers find? They looked for 19 HMOs in each of the samples, using techniques called high-performance liquid chromatography (to separate the sugars) and mass spectrometry (to pinpoint their identity). The concentrations of all but one of the HMOs differed across populations.

The amounts of one particular HMO, called 2’-fucosyllactose, varied a great deal. Women who produce milk containing a high concentration of 2’-fucosyllactose were far more common in the outskirts of Lima, Peru, and in Southern California, than they were in rural Gambia. This matters because separate research into the diarrhea incidence of Mexican infants [2] and also into the allergies of Finnish 2 to 5 year olds [3] finds that drinking human milk
with high levels of 2’-fucosyllactose as an infant seems to protect against these maladies. But it wasn’t only population that predicted 2’-fucosyllactose levels. Environment also played a role. Indeed, heavier women everywhere tended to make more of this HMO than women with lower body mass indexes (BMI). And environment appeared important in the levels of several other HMO differences found among the milks of Mandinka women and those of Sidama women. Although it is not known what about the environment was driving these differences, different maternal diets could play a role, perhaps by inducing epigenetic changes. Similarly, epigenetics could lie behind correlations found between the levels of different HMOs and maternal age—as well as between HMO levels and the number of children a woman had given birth to during her lifetime—and how long it was since she last gave birth.

There is plenty more research to do in this area, beyond better understanding what is driving these correlations, and how specific HMOs affect infant health. For one thing, this study did not analyze milk from any Asian population. But, as the authors claim, their work does provide a baseline of typical HMO profiles of healthy women, against which future studies into the HMO profiles of women with health problems like malnutrition, diabetes and HIV can be compared.


Contributed by
Anna Petherick
Professional science writer & editor
www.annapetherick.com

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