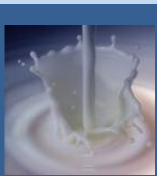
SPLASH! milk science update: October 2014 Issue



SPLASH! milk science update

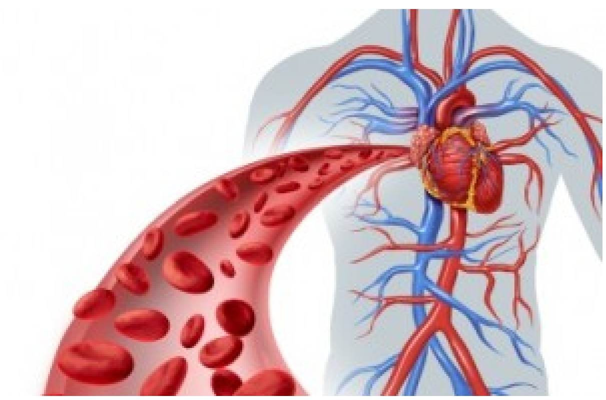
October 2014 Issue

This month's issue features articles about milk sugars and immune development, bone health, protein and obesity risks, and genetic insights into milk production in cows.

Milk Sugars Enter Circulation

- **Sugars called oligosaccharides have been detected in the blood plasma of infants who have consumed them as a naturally occurring component of breast milk.**
- **Although present at very low concentrations, oligosaccarides may be able to affect the development of the brain and the immune system.**
- **Evidence is being gathered as to whether some of these sugars are absorbed from the gut into the blood at higher levels than others.**

For years, researchers have wondered out loud about the possible roles of a group of sugars found in breast milk in the development of the brain and the immune system. Relatively high levels of these sugars in mothers' milk have been linked to less frequent upper respiratory infections in infants. Autopsies have revealed that consuming breast milk, as opposed to formula, increases the concentration of a certain acid that forms part of many of these sugars—sialic acid—in infants' brains. This, in turn, has been shown to improve learning and memory in animal experiments. The evidence for systemic effects of these sugars may seem impressive, but it is undermined by a missing link. Strengthening the immune response in the lungs, and indeed, improving memory in the brain, requires first traveling from the gut into the blood. However, whenever researchers have tried to detect these sugars in breast-fed infants' blood, they have failed.



That is, they have failed until quite recently. A few labs have now reported finding various members of this group of sugars in infant blood. The sugars in question are called oligosaccharides, or oligos, for short.

In one paper published in July, Karen Goehring and her colleagues provide evidence that the relative levels of different oligos in the blood correspond to their relative levels in breast milk. They find that even though the oligos' proportions are roughly the same in both fluids, oligos are present at just 0.01% of their breast milk concentration in blood plasma (and at 4% of their milk concentration in urine). Meanwhile, another paper, also published in July, suggests that some oligos are likely to be absorbed into the bloodstream at higher concentrations than others, or possibly even made by the infant's gut. Specifically, Renee Ruhaak and colleagues working in the Lebrilla lab at UC Davis propose that those oligos with a sialic acid section—the acid that is purportedly good for brain development—occur at higher levels in blood than in milk.

The papers use different methods, which likely explain their differing conclusions. The paper by Goehring et al focuses on representative structures of different types of oligos. For example, they clearly state that their techniques are unable to distinguish the different forms of the particular oligos commonly used to supplement infant formula. Perhaps the strongest argument for sialic acid-containing oligos receiving some sort of preferential treatment as they move from the infant gut to the blood (compared, that is, to other oligos) comes from detection of a new kind of sialic acid structure. This was found by the UC Davis group. The new sialic acid structure is only found in blood, which suggests a more active process than the passivity implied by sialic acid-containing oligos' presence in the same proportions in blood as in milk.

The broader question is whether the low concentrations of oligos in blood plasma are sufficient to influence brain and immune system development. If the sialic acid oligos are accumulating slowly in the brain over months and months, then such low levels are potentially sufficient, although the case is far from proven, says Lars Bode, an oligo expert at the University of California, San Diego, who was not involved in either of these studies. Goehring et al. argue strongly that the concentrations they report do meet those that have been shown in laboratory experiments to affect immune system components. For example, they make the point that oligo concentrations of 12.5 µg/mL can have an anti-inflammatory effect by inhibiting the sticking together of white blood cells. Research using isotopically labeled oligos suggests that oligos appear in urine a good 36 hours after they are taken in as part of breast milk—implying a decent period spent swimming around in the body to exert influence on their consumer's biochemistry.

The field is coming closer to filling the hole in its theories of these sugars' wider health effects. To better understand what is going on, blood samples from larger samples of infants would be helpful (these studies both examine the plasma of fewer than 20 individuals)—as well as more work on the relative concentrations of oligos in blood. As detection techniques are improved, the number of oligos that can be found in blood is likely to rise—probably, quite quickly.

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2. Ruhaak R. L., Stroble, C., Underwood M. A., Lebrilla, C. B. (2014) Detection of milk oligosaccharides in plasma of infants.Anal Bioanal Chem doi: 10.1007/s00216-014-8025-z.

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Boning Up on Dairy and Skeletal Health

- **Many environmental factors, including nutrition, influence bone health.**
- **Dairy foods are high in calcium, but also contain protein, phosphorus, and other nutrients that are essential for bone health.**
- **Both observational and controlled clinical studies have demonstrated a positive effect of consumption of dairy foods on bone health, particularly in adolescent females.**
- **The current dietary recommendation for dairy intake (3 servings per day) provides sufficient amounts of nutrients used for building and maintaining healthy bones.**

For its weight, bone is the strongest material in nature. Developing and maintaining strong bones is heavily influenced by your genetic makeup, but nutritional and other environmental factors can make or break your chances of reaching your genetic potential. The role of calcium in supporting bone growth and preventing bone loss is well known (1), but overall, bone health depends on more than just calcium. A new paper (2) reviews more than two decades of research on the relationship between consumption of dairy foods, such as milk, cheese, and yogurt, and markers of bone health. Because dairy provides calcium along with other micronutrients, macronutrients, and bioactive factors involved in bone metabolism, it has been demonstrated to be an important dietary source for improving bone health throughout the lifespan.

Reaching the Highest Peak

While it may be as strong as steel, bone is a living tissue that is constantly being built up and replaced over time. In infants, children, and young adults, the formation of new bone exceeds the amount of bone that is lost, or resorbed. Sometime between the ages of 30-35, we reach our peak bone mass (the most bone we will ever have in our lifetime). After this point, the rate of resorption exceeds the rate in which new bone is being made (1, 2).

Although we think of bone loss as a pathology associated with conditions such as osteoporosis, it is actually a natural part of aging. As such, reaching the highest possible peak bone mass is critical for maintaining strong bones in later life. Indeed, measures of peak bone mass can be used to assess future fracture risk and the development of osteoporosis (1 – 3).

So how do you reach the highest peak and reduce bone loss? Genes have a lot to say about bone mass and bone loss throughout your lifetime (1,2), but as the vast literature on dietary supplementation to improve bone health can attest, the influence and importance of nutritional factors should not be downplayed. After all, bone is built from collagen, a protein, and hydroxyapatite, a mineral compound made from calcium and phosphorus. Our bodies cannot manufacture calcium or phosphorus, so these bone-building minerals must be provided by the diet. It is not surprising, then, that insufficient dietary calcium has been associated with both lower bone mass in children and adolescents and increased rate of bone loss in older adults (1,2,4).

Calcium et al.

Calcium may be the superstar of bone health research, but its supporting characters – including protein, phosphorus, and vitamin D – may deserve a little more of the limelight. Like insufficient calcium intake, diets low in these nutrients can influence the genetic potential for reaching peak bone mass (2). Dairy is often promoted for bone health because of its high calcium content, but Rizzoli's review paper (2) highlights the benefits to bone health provided by dairy's full cast of characters.

Dairy foods are a good source of protein, which many believe to be as essential as calcium to osteoporosis prevention (2, 5). High protein diets are associated with an increase in bone mineral density, while diets with insufficient protein have been associated with loss of bone mass and decreased bone strength (2, 5). Further, dietary protein has been shown to increase blood levels of hormones, such as insulin-like growth factor 1 (IGF-1). This hormone, as its name suggests, plays a role in bone growth in children and adolescents, but also has been shown to play a role in adding bone mineral in adults (5,6).

In addition, dairy foods contain nutrients that can increase the amount of calcium the body can absorb. One we are probably all familiar with is vitamin D, a steroid hormone responsible for enhancing intestinal absorption of calcium. But even in dairy foods not fortified with vitamin D, calcium absorption can be increased by the milk sugar lactose (7). Differences in study results between dairy and calcium supplementation (2) may reflect the action of these additional nutrients.

The Data on Dairy

Almost all of the studies that Rizzoli highlights in his review (2) involve children and adolescents, as the evidence for the influence of dairy foods on reducing (or preventing) fracture in adults is less established than that for increasing bone mass. However, intervention or supplementation studies during growth represent an opportunity to influence both longitudinal bone growth (adult stature) and peak bone mass, the latter of which can influence bone mass later in life.

Outcome measures (e.g., bone mineral density, bone mineral content, growth, serum IGF-1) and study design (e.g., retrospective, observational, randomized, cross-sectional, longitudinal) varied widely, making it difficult to directly compare results. However, taken together, the findings of these numerous studies from both Western and non-Western populations indicate dairy's positive effect on markers of bone health. Here are some examples of specific findings:

- Supplementing females (10-12 yr) with low dietary calcium intake with cheese to provide 1000 mg/d calcium was associated with a greater gain in bone mineral content than with a tablet containing 1000 mg/d calcium.
- Adolescent females receiving calcium supplementation and those with a high consumption of dairy products both showed increases in bone mineral density of the hip and forearm, but only the high dairy consumers also increased bone mineral density in their spine.
- In a randomized study of over 700 adolescent Chinese females (10-12 yr), those that received calcium-fortified milk at school over a two-year period had significant increases in total body bone mineral content and bone mineral density, as well as height.

Current Recommendations

What most of the studies reviewed by Rizzoli indicate is that you don't need to consume large quantities of dairy in order to reap the benefits for bone health. In the Chinese school milk intervention study, girls were given 330 ml, or about 1.5 cups, of fortified milk per day. To obtain 1000 mg of calcium, provided by cheese (or calcium tablets) in the previously highlighted intervention study, is in line with current recommended daily intakes (RDI) for calcium (these vary depending on age and sex, but in U.S. they are between 700-1300 mg/day). This amount of calcium could be met through consumption of 3 – 4 servings of dairy per day, which simultaneously provides the RDI of other nutrients that have demonstrated positive effects on bone health, such as phosphorus and protein.

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(2) Rizzoli R. 2014. Dairy products, yogurts, and bone health. Am J Clin Nutr 99 (suppl): 1256S-62S.

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(5) Bonjour JP. 2005. Dietary Protein: an essential nutrient for bone health. J Am Coll Nutr 6 (Suppl): 526S-36S.

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(7) Kwak HS, Lee W-J, Lee M-R. 2012. Revisiting lactose as an enhancer of calcium absorption. Int Dairy J 22: 147-151.

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Protein for Babies: Too Much of a Good Thing

- **Protein is important for the growth and maintenance of our tissues and organs; however, do we know how much is enough for babies?**
- **High protein intake early in life has been associated with obesity later in life.**
- **Breast milk contains low amounts of protein, which match the growth and developmental requirements of the human infant, whereas standard infant formulae have higher protein content.**
- **A new study demonstrated beneficial growth outcomes of a low protein formula compared to the standard high protein formula for infants born to overweight or obese mothers.**
- **These findings highlight the need to lower the protein content of infant formulae for babies for whom breast milk is not readily available or that are at greater risk of obesity at birth.**

Intuitively, most of us would think that a high protein intake would be advantageous for babies and that the more of it would be better, as protein helps build and maintain our muscles and different tissues. However, in the long term it may actually be the opposite. Recent reports indicate that a high protein intake in infancy is associated with a greater risk of obesity later in life. Given the money that governments spend on managing the serious health issues related to the obesity epidemic, we might rethink the quantities of protein that are being fed to babies.

Key agencies around the globe, including the World Health Organization (WHO), and the Research Institutions and Governments, are warning us about the need to adopt eating habits that help maintain our weight within the normal range. This is not for aesthetic reasons, but rather a matter of life and death. Obesity is accompanied by a range of additional health issues, including heart disease, diabetes, and cancer, to name a few. Alarmingly, it now affects not only adults, but also an increasing population of children, reaching over 40 million (WHO). Obesity problems, increasing in both the developed and developing world, put enormous pressure on health professionals as well as federal health budgets.

Surely, there must be something we can do. A number of studies are suggesting early life programming, starting in utero and continuing postnatally in infancy. Obese mothers give birth to babies that are at higher risk of becoming overweight or obese later in life. Postnatally, breastfeeding seems to protect against future obesity. There are a number of potential explanations for this observation. Protein intake seems to be a key player in the way our body programs its appetite controlling system.

Higher protein intake during infancy has been associated with higher insulin metabolism and greater release of IGF-1. In turn, this results in a greater deposition of fat, more rapid weight gain and a higher body mass index (BMI) into childhood and adulthood. The problem of higher than the requirement protein intake for infants is introduced when formula is used to feed babies. Breast milk's protein content, although low, appears to match the growth requirements of the human infant. Moreover, breast milk composition is dynamic and changes in response to feeding and during lactation, supporting the changing growth and developmental needs of the baby as it grows. This does not happen with formula.

To address the importance of these protein differences for infant growth, Martin et al. conducted a study in Chile comparing the effects of a standard (i.e. high) and a low protein formula provided after month 3 of age in children born to overweight or obese mothers. Breastfeeding was also compared with the two formulae. Interestingly, it was shown that babies fed the low protein formula had slower growth than those fed the high protein formula. Breastfed babies in this study showed faster growth than the WHO Child Growth Standards, potentially because they were born to overweight or obese mothers, and were therefore predisposed to faster growth. Metabolomic analysis in the infants' stools and urine revealed that formula-fed infants were metabolically different from their breastfed counterparts, irrespective of the type of formula used. A key difference was found in the fermentation of milk oligosaccharides, which was higher in breastfed compared to formula-fed infants. This further highlights the importance of this breast milk component in gut development and function early in life.

Infants born to overweight or obese mothers already have a higher risk of obesity. Obese mothers have difficulties breastfeeding and thus often use formula. The findings of this study are very important because they demonstrate that the extra protein fed to these babies postnatally places them at an even greater risk of becoming overweight or obese later in life.

It therefore becomes clear that giving more protein to a baby may facilitate faster growth, but that is not necessarily a good thing. In fact, it may cause more problems than it solves by contributing to the development of obesity, affecting the individual's life quality in the long term as well as the society as a whole. Breast milk is the natural food for infants, providing maximal nutritional, developmental and protective benefits, in addition to responding to the infant's changing needs. Replacing the gold standard with a suboptimal source of nutrition at a period that is critical for the development of tissues and organs may have detrimental, and potentially irreversible health effects. However, in cases where breast milk is not readily available, infants may benefit from the use of formulae with low protein content similar to that of human milk. This may be particularly important for babies born to overweight or obese mothers. These babies may need special support early in life to overcome their higher risk of obesity imparted to them in utero.

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CNVs Linked to Milk Production

- **The genomes of individual cows can differ by the number of "copies" of genome segments.**
- **This genetic variation is known as Copy Number Variation (CNV).**
- **CNVs are part of the genetic variation within a dairy herd population.**
- **Inclusion of these variants in genetic analyses revealed a link between CNV regions and dairy traits.**
- **For the dairy industry, these results mean that the genetics of milk production can be better managed with new genetic testing that includes CNVs as well as SNPs.**

You may be surprised to learn that you can have many more copies of a particular gene than your neighbor, and yet you both still function like normal human beings (or not!). With each new generation of cows (or humans), DNA is copied from parents to offspring. During this copying process, whole segments of the genome can be missed or duplicated such that the offspring have fewer or more copies of the segment than normal. This variation in the number of copies of a genomic segment is called "copy number variation" (CNV). Just as for other forms of genetic variation, CNVs can be linked to or actually cause a physiological change that is seen as a different phenotype.

CNVs have attracted more attention as the details of genome sequence have been revealed. First, it became clear that the genome had very large numbers of this kind of variation. Researchers discovered that these variants could account for phenotypic variation of interest for health and production characteristics. In a recent paper by Xu et al, CNVs were investigated in dairy cattle. This study showed that CNVs were linked to milk production traits [1].

Most studies of genetic association in dairy cattle over recent years have focused on analyses that use single nucleotide polymorphisms (SNPs) as a means of defining variation between individual animals. The advantage of using SNPs is that they could be mined more easily from large scale DNA sequence data. Furthermore, they are distributed at relatively regular intervals across the genome, thereby providing good coverage to analyze association with traits from all regions of each chromosome. The very large majority of SNPs are functionally silent. That is, they have no direct effect on biological variation, but may be genetically linked to nearby mutations that do have an effect. This linkage has been used to great success in many genome-wide association studies in cattle, as well as other species. Meanwhile, scientists interested in functional genomics have been following the SNP leads to undertake the more painstaking task of trying to relate positive associations to physiological outcomes. There has been debate on the need to identify such links in dairy cattle, where the primary purpose is to use genomic information for selective breeding, which is advancing on the basis of SNP analysis. However, it now seems likely that the accuracy of this approach will erode more rapidly with each generation than initially thought. So, researchers continue to look for functional interpretation of genetic association data. One way to achieve this using large-scale analysis is by looking at CNV data.

CNVs involve more DNA sequence than SNP and are more likely to lead to variations that produce a functional change. One of the most informative analyses of CNVs in this regard relates to the association with variation in levels of gene expression. These studies, performed in mice and humans, show that CNVs accounted for up to 30% of gene expression polymorphism [2] [3] [4]. This is a very significant number when we consider that the majority of functional variation arises from altered levels of gene expression. CNVs may directly influence gene expression where gene duplication events lead to an increase in the number of functional genes coding for one protein – a gene dose effect. Alternatively, CNVs may lead to alterations in sequences involved in the regulation of gene expression.

Based on this rationale, Xu et al., first undertook an analysis of CNV location in more than 26,000 dairy bulls and cows from the Cooperative Dairy DNA repository, the National Center for Genetic Resource Preservation, ARS, and the USDA. They identified 99 CNVs for further association analysis with five dairy traits. From this analysis, they found that 34 were significantly associated with at least one of the five traits: milk yield, milk protein yield/percentage or milk fat yield/percentage. Some of these were clearly present at very high frequency in the tested animals, indicating that they have been under positive selection in the past. When the scientists compared their results to other research results that have been published, they found that they were strongly supported in other international dairy herds. They were also interested in determining whether analysis using SNP versus CNV gave the same information. Many CNVs were captured by linkage to SNP that were located close to the CNV site, but 25% were not captured using the commonly used Bovine50SNP method. This may partially explain why it has been difficult to identify the heritability of milk production traits using SNPs alone.

When trying to determine what lies behind a champion dairy cow, looking at CNVs may be a winner. The dairy industry is now moving beyond the Bovine50SNP chip, and should consider including CNVs while developing any new tests. Each year, scientists get a little closer to understanding why one cow produces more or different milk from its neighbor.

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