



### SPLASH! milk science update

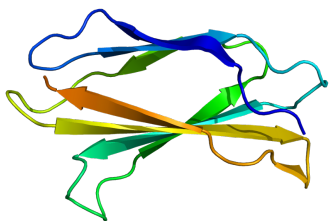
February 2015 Issue

This month's issue features a milk protein that may prevent HIV, the 1000 bulls genome project, improving brain development with certain milk fats, and how milk protein influences consumer height.

## The Future of HIV, with Tenascin-C

- **A protein called tenascin-C is known to attach to HIV and make it much harder for the virus to infect immune cells.**
- **Tenascin-C is found in breast milk—and also in cow's milk— but more research into dairy-derived tenascin-C is needed to determine its usefulness.**
- **Lately, scientists have made a recombinant version that shows strong levels of activity.**
- **New research is exploring how to make naturally occurring tenascin-C more effective, how to increase levels of antibodies that support its function, and the potential benefits of adding tenascin-C to food and microbicide gels.**

It has been two decades since breast milk researchers realized that some chemical details of their favorite liquid of inquiry are protective against HIV infection. The protection is far from complete, but it is evident in a statistical anomaly: more than 90% of infants exposed to HIV through the breast milk they drink—their mucosal membranes doused with the virus every few hours, for about two years—never catch it.



Back in 1995, a multi-purpose protein, called lactoferrin, was found to contribute to this effect[1]. More recent studies have focused on smaller sorts of molecules, such as sugars called oligosaccharides[2, 3]—and on antibodies. Indeed, it was while they were investigating antibodies that Sallie Permar and her team from Duke University Medical Center in Durham, North Carolina, realized they were missing the point.

To be sure, the control milk samples in their experiments—the samples lacking the antibodies under investigation—couldn't quite muster the same level of HIV-neutralizing activity as those containing these antibodies, but these control samples somehow still seemed able to get the job done. After some head scratching, the team shifted focus. They separated the contents of their control milk samples into groups of proteins, or fractions, to try to isolate what caused the effect. Soon enough, the answer became clear: only one protein appeared in the fraction that could neutralize HIV and never appeared in any of the other, ineffective fractions. "That was a bit of luck," admits Permar. This protein was tenascin-C, a wound-healing molecule. The academic literature had already noted that it occurred in breast milk, but, until this moment, no one really knew what it was doing there.

Of course, there are many more steps before this could become a common HIV-prevention strategy. A key concern, for example, is whether the immune systems of HIV-positive mothers, potentially already badly damaged by the virus, will be able to generate lots of antibodies in response to immunization. But if they can, a simple nasal vaccine has the potential to prevent many infant infections annually, partly because vaccines can be a one-off affair.

By comparison, anti-retroviral drugs (ARVs) come with the chore of daily adherence. "It is a severe misconception to think that anti-retroviral prophylaxis has taken care of mother-to-child transmission—and I don't think that access to the drugs is the problem," says Permar. "We still have a quarter million infants becoming infected annually—and that's despite having ARVs widely available." Often HIV-infected women are highly motivated to stick doggedly to their drug regimens during pregnancy, but adherence tends to drop off rapidly after they've given birth. This tendency is as true for the US as it is for the developing world—but only in the poorest corners of the planet does an absence of clean drinking water make infant formula more likely to kill a young child than giving him or her breast milk that is laced with HIV.

A problem with the current global ARV-centered strategy to prevent mother-child transmission of HIV, says Permar, is that it pays little attention to the possibility that HIV-negative mothers might catch the virus over the course of pregnancy, or while they are breastfeeding. "If you're breastfeeding for two years, that's a long window of time during which you can acquire the virus.... [As a result] it may be worth immunizing every mother in some areas."

The vaccination idea[6] is certainly not the only way of using tenascin-C's activity to cut infection rates. The protein works by sticking to an 'envelope protein' on the outside of the virus; when this happens, the envelope protein changes slightly at another location—the part that it uses to attach to immune system cells (or, put simply, to infect people).

Rather than focusing on boosting tenascin-C activity through vaccination, tenascin-C could be added separately to food, as another method of increasing protection. That might be particularly helpful in cases where individual mothers happen to produce relatively small amounts of it in their milk. (The natural variation in tenascin-C's concentration in breast milk has about a ten-fold range.) For a food supplement to be a practical option, however, a cheap and ready source of tenascin-C would be necessary.

This is where most of the team's effort has gone since their original discovery. "We've come up with some methods to purify it from breast milk, so we know that *is* possible," points out Permar. It's also present in cow's milk, but the team has yet to figure out exactly how closely the cow-version of the protein matches its human cousin in function. That aside, they have already managed to create—and patent—a recombinant version of tenascin-C that works pretty well.

Yet the main use for this might be elsewhere. HIV-blocking food supplements are all well and good for infants, but adults almost never catch HIV through their digestive systems—rather, they catch it through sexual activity. It is in this vein that research into tenascin-C could lead to its most important manmade application.

Microbicides are creams or gels that are applied inside the vagina or rectum to protect against sexually transmitted diseases. It seems likely, if tenascin-C were to be added to them, that these creams might prove to be better at fending off HIV. That is an exciting idea because there is plenty of room for improvement; recently, a microbicide trial was much-fêted for reporting[7] a reduction in HIV risk of 39% compared to placebo. There are, of course, many concerns—but toxicity, obviously, should not be one of them. "It should be exquisitely safe," says Permar, given that human bodies produce tenascin-C anyway. "I mean, babies drink it every day!"

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## Towards Individualized Genomes for Dairy Cows

- **The 1000 Bulls Genome Project is an international collaborative partnership to sequence the DNA of key bulls.**
- **Growth in dairy production results from breeding the best cows.**
- **Genomic selection uses new DNA technologies to predict the best cows.**
- **The 1000 Bulls Genome Project will make the new methods robust so they can be applied to national breeding programs.**
- **Eventually, we will have individualized genome sequences for dairy cows.**

International cooperation between scientists has really taken off in genomic sciences, and now, in a program that has grown out of dairy genetic research, one thousand bulls are set to be immortalized by having their DNA sequenced. The so-called 1000 Bulls Genome Project is an international collaboration between scientists in Europe, USA, and Australia. The project began in 2010, when scientists were looking for a way to share the huge cost of sequencing many entire genomes. The result was the 1000 Bulls Genome Project, which spreads the costs and shares the resources to help geneticists apply their collective knowledge of cattle to improve the productivity of cattle herds.

Why are dairy scientists so interested in generating so much information about bulls? After all, it is surely the cows that are important for milk production. Well, ever since artificial insemination became the method of choice for breeding dairy cattle, a number of national programs were established to select the best bulls. The semen from these bulls is then sold to

farmers who are developing and improving their herds. Through these programs, one bull may give rise to many thousands of daughters. Milk production of many of the daughters is then tested on a regular basis and the information sent to a central analysis centre to calculate the genetic value of the bull. Farmers use this information to select the bulls that they want for their breeding programs. So bulls have become the center of selective breeding practices, and over time, farmers have been able to increase the returns from their herds by using the best bulls.

The potential for accelerating the rate of improvement was given an enormous boost with the completion of the bovine genome sequence, which was officially released in 2009. Although this was a reference sequence derived from a single animal, the project spawned a huge number for related research projects, and resulted in an enormous database of information about diversity in cattle. As a result, it became possible to apply the new information to detect and analyze the genetic differences between individual animals that affects their productivity. Dairy scientists were at the forefront of this effort, primarily because they were armed with mountains of data from decades of performance measurements, and the outcomes of breeding programs. With this information already collected, they were able to quickly develop new methods.

The ultimate goal for selective breeding using these DNA based methods, is based on a theoretical model proposed in 2001 by Meuwissen et al[1]. The model outlined a framework to develop a system for what is now referred to as genomic selection. The process divides the entire genome into intervals that can be compared between bulls to accurately predict how much milk their daughters will produce, as well as the milk quality. Because of the bovine genome sequencing project and related studies, the potential to develop this approach to selective breeding has rapidly become a reality. The major question being asked by scientists became: which genomic markers are the best ones to use to define the intervals? The question has generated a great flourish of activity in recent years in an attempt to define and test the model against sets of markers, and to develop accurate predictions that can be applied for herd improvement[2-5].

So, why do we need the genomes of one thousand bulls to be completely sequenced? Even though the data supported the original models and molecular breeding values have now been calculated for dairy programs across the world. However, scientists soon discovered that within a few generations, or for distantly related animals, the capacity to predict the productivity of the cows fell quickly. Not to be deterred, the scientists have regrouped. They reasoned that the best strategy is to use markers that are found in genes that control the most important physiological functions that drive milk production. The search is now on to identify just these markers, but it is not such a simple task, and requires a lot more detailed sequence data, hence the 1000 bulls project. Choosing one thousand bulls meant that the most important gene sequences will be found, and their precise location in the bovine genome will be known. The international consortium for the 1000 bulls project reported its first outcomes (using 234 bulls) in a recent publication [6]. The study reports the first success in extracting detailed information from the sequencing efforts, and confirmed the identity of over 23 million sites to explore – including significant mutations in genes that affect milk fat content. In addition, the scientists used a very clever technique called imputation [7-9]. Imputation allows them to fill in the unknown sequence information between two sites in the genome of an individual cow based on the relationship of the animal to one of the 1000 bulls. In this way, the value of the 1000 bulls project is amplified enormously.

The exciting news is that more recent reports have indicated that many more than 1000 bulls have been sequenced. The project has gained momentum and will become a major international resource for selective breeding programs for many decades into the future.

Very soon, individual dairy cows will have their genome sequence recorded in an ever-growing international dairy cattle database. In the future, cows may even have their individual DNA sequence recorded on their ear tag or in a microchip attached to their body. Farmers will use this information to improve the health and productivity of their cows.

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## Fats, Formula, and Brainy Babies

- **Research indicates that breastfed babies have better cognitive abilities than formula-fed babies.**
- **Some fats naturally present in breast milk are linked to neurological development and function.**
- **A new study found that supplementing formula with certain fats—phospholipids and gangliosides—improved spatial learning and affected brain growth and composition in newborn piglets.**



Can infant formula be boosted to prevent formula-fed babies from missing out on the brain-stimulating ingredients of breast milk? Seeking to answer this important question, a new study found that a supplement of naturally occurring milk fats improved the brain development and certain cognitive abilities in newborn piglets [1].

Among the pile of data indicating that ‘breast is best’ when it comes to infant nutrition and health, there is a fair amount of evidence to suggest that breastfed babies turn out smarter than formula-fed babies. Smarter, that is, according to certain measures of brain development, including spatial learning and memory. One reason for this is thought to be the high amounts of certain fats in breast milk, compared with those in formula, as discussed in past issues of this newsletter [2, 3]. [Fat molecules are critical structural components of the brain](#), and [particular parts of some fats](#) have been linked to neurological development and function, learning and memory.

While—so far—breast milk beats formula, a majority of babies in the US are not breastfed for as long as recommended by the WHO [4]. That’s one reason scientists have long tried to create an infant formula that mimics breast milk and its effect on brain development, among other health aspects.

Demonstrating specific effects of formula supplements in studies involving human babies, however, is not straightforward because of compounding factors such as environment and genetics. Therefore, researchers often use animal models to examine diet-specific effects in a more precise and controlled way. Recently, scientists from the US and China used piglets to assess the effect of certain dietary fats on neurodevelopment, a process that is very similar in newborn piglets and human babies [1].

### Little pig, little fatty... where’s your chocolate milk?

In the study, 24 piglets were divided into ‘control’ and ‘test’ groups and fed different diets. The control piglets received a standard, nutritionally complete formula. The test piglets received the same formula supplemented with fats called phospholipids and gangliosides, which are naturally present in both human and sow milk, and known to be important for brain development and function. After receiving one of these diets for the first four weeks of life, the piglets were put through all sorts of tests.

One of the tests assessed how good the piglets were at learning and remembering where inside a maze they would get a special treat—chocolate milk. As it turned out, the piglets receiving the fat supplement made more correct choices in the maze than the control piglets; they also spent less time making their choices.

At the end of the trial period, the researchers found that the piglets receiving the fat supplement had five per cent higher brain weight than the control piglets. The heavier brains of the test piglets had larger volume and more gray and white matter in several areas—notably, areas linked to motor control, cognition, and learning behavior.

In summary, the study showed that dietary phospholipids and gangliosides improved spatial learning and affected brain growth and composition in newborn piglets. The researchers suggested that adding these fats to infant formula may affect brain development and improve some aspects of cognition in human babies who miss out on the brain-stimulating ingredients of breast milk.

As the authors [1] conceded, however, a lot more research is required to fully understand and close the gap in learning differences between breastfed and formula-fed babies. The exquisite complexity of breast milk needs to be investigated in

a holistic way, because—as emphasized before in this newsletter—[no single group of milk constituents determines neurodevelopment and cognition \[2\]](#).

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## Milk Protein in Diet Predicts Human Height

- **Socioeconomic and nutritional factors interact with genes to influence human growth and adult height.**
- **Biologists study variations in human height to identify environmental factors that allow populations to reach their genetic potential for stature.**
- **A new study reports on genetic and environmental influences on height from 42 European countries, the U.S., Australia, and New Zealand.**
- **The strongest predictor of adult male height was dietary protein consumption, specifically the “protein index.”**
- **Taller populations had a higher proportion of dietary protein from animal sources, especially milk and pork, compared to vegetable sources.**
- **Increasing high quality animal protein intake during childhood may have positive impacts on growth and population health outcomes.**

The Dutch and Montenegrins are the tallest men in Europe, measuring in at an average height of just over six feet (1.83 m). Separated by nearly 2000 miles, what might these two countries have in common that explains their above-average stature? The results of a new study [1] suggest their height may have as much to do with what is on their dinner plate as what is in their DNA. Using data from 42 European nations, as well as the U.S., New Zealand, and Australia, Graggruber et al. [1] found that the strongest predictor of male adult height was the population’s protein index - the amount of protein consumed from animal sources, such as dairy and pork, compared to proteins consumed from vegetable sources, such as wheat. If height is a marker for the health of a population, could the answer to improving health outcomes be as simple as dining like the Dutch?

### Reaching your potential

Height is a complex trait, resulting from the interaction of the genes you inherit from your parents and the environment in which you grow and develop. While genetic variation explains between 60-80% of the variation in human height [2], the influence of the environment should not be underscored. Despite what the genes may code for, a child’s growth may fail to reach its genetic potential in an environment with nutritional and immunological challenges. This was clearly demonstrated over 100 years ago by anthropologist Franz Boas who found that children born in the U.S. were taller than their foreign-born parents. Indeed, numerous studies have described this secular trend in height among immigrants from developing to developed countries [3].

Based on his findings, Boas argued that the study of human growth was analogous to the study of the human condition. This thinking still predominates today. The United Nations (through the World Health Organization) uses height as a measure of health in developing countries and at-risk populations in developed countries. Human biologists study variation in height across populations in order to identify environmental factors that allow certain populations to maximize their genetic potential.



### The tall and the short of it



Grasgruber et al. [1] are just such human biologists. They are not the first to investigate sources of variation in human height in Europeans, but their study is the first to include countries in Eastern Europe, including the Balkan states and former Soviet republics. And the quantity of data compiled is nothing short of impressive. They tracked down height data collected in the last 10 years for 42 European countries, as well as the U.S., Australia, and New Zealand. Socioeconomic factors examined (by country) included: gross domestic product (GDP), health expenditure, children's mortality, total fertility, urbanization, the Gini Index (a measure of wealth distribution or inequality), and nutrition (from data on food consumption provided by FAOSTAT via the United Nations). Lastly, they also pulled genetic data (specifically, Y haplogroups – shared gene clusters on the Y chromosome) from the literature for 43 of the 45 countries.

While the methods were quite complicated, their research questions were simple: what factors are causing the modern increase in stature among some European countries? Not surprisingly, height correlated positively with GDP and health expenditure, and negatively with children's mortality. Because protein is well known to be the most important nutrient for skeletal growth, it also was not surprising that male stature positively correlated with animal protein consumption, particularly milk and cheese (both of which had higher correlation coefficients than pork and fish).

What the authors had not anticipated, however, was the strong, albeit negative influence of vegetable proteins. The Dutch are the tallest in Europe, as well as in the world. Their extra centimeters were best explained, not by genes on their Y chromosome, but by a nutritional factor called the protein index – the ratio of animal proteins to vegetable proteins. Furthermore, the results were identical when they simplified the protein index to include only milk proteins versus wheat proteins. The tallest populations were those that consumed more protein from milk, and less protein from wheat.

### Wealthy, but unhealthy?

A country's nutrition cannot be separated from the various socioeconomic factors that were examined in this study. Access to high quality proteins (based on amino acid structure and digestibility) is certainly influenced by wealth, but several wealthy countries, including the U.S., have much lower protein indexes than would be predicted by their GDP [1]. At an average height of 179 cm (around 5' 9"), U.S. males (of European ancestry) are more than 2 cm shorter than Bosnians and Serbians, 3 cm shorter than Czechs, and nearly 6 cm shorter than men from the highlands of Herzegovina. A lower protein index, along with less health expenditure per capita than predicted by the GDP, best explained the shorter stature of white males in the U.S.[1].

### Ham and Gouda, hold the bread?

The study's authors took this result as good news, because it implies access to proteins in milk, and other animal sources may be influenced more by personal choices, than finances [1]. What's more, nutritional recommendations, such as increasing milk protein consumption in growing children and adolescents, are likely much easier to implement than a major overhaul to a country's health care system (just ask Obama). The importance of milk protein for [growth](#) and [bone health](#) has been demonstrated in numerous other studies. This study further highlights dairy as an essential component for optimal growth.

Finally, it is important to emphasize that the data collected only allows the authors to discuss what has been the most important influence on height in contemporary European populations. Social equality, access to healthcare, and generally higher standards of living have obviously been important and can explain height increases across Europe, the U.S., Australia, and New Zealand over the last 150 years [1] and may currently be as important as nutrition in explaining variations in height in other parts of the world.

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***Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium.***