Dental Time Machines: Tartar Provides Direct Evidence of Dairy Consumption in Africa

- Scientists can use tartar stuck on fossilized teeth to reconstruct the exact foods an individual consumed during their lifetime.
- A new study looked for milk-specific proteins in tartar and ancient genetic data on lactose digestion to understand the origins of dairying in Northeast Africa.
- The earliest direct evidence for dairy consumption in Africa is dated to approximately 6,000 years ago and was not associated with genetic adaptations for lactose digestion.
- In Northeast Africa, dairy consumption preceded the genetic changes related to lactase persistence.

Six-thousand-year-old tartar is a dental hygienist’s nightmare but an archaeologist’s dream. That’s because the same yellow, cement-like deposits that have to be manually scraped off during a dental visit are also dietary time capsules [1]. Like an insect preserved in amber, food particles from a lifetime of meals get trapped in tartar’s mineral matrix and become part of the fossil record. Rather than infer what past populations might have eaten, researchers can analyze ancient plaque and say what one particular individual actually ate.

The ability to identify specific food proteins in tartar (or dental calculus) has been a game-changer for scientists reconstructing the origins of dairying. Consumption of animal milk represents a major nutritional and cultural transition in human history. Milk was an important source of protein, fat, and essential vitamins and minerals, as well as a potential weaning food for infants and a critical source of hydration for humans living in arid environments [2, 3]. Multiple lines of evidence suggest that humans have included milk or milk-derived foods such as cheese and yogurt in their diet for at least 8,500 years [2, 3]. But several key questions about the origins and practice of dairying remain unanswered, including the anthropological chicken and egg: which came first, dairy consumption or the genetic variant that allowed for dairy consumption into adulthood? Did populations start consuming dairy, thereby driving positive selection for lactase persistence (LP), or were there already low frequencies of the LP gene variant present during the transition to agriculture that encouraged dairy consumption [1]?

Hoping to solve this evolutionary riddle, a new study [2] led by researchers at the Max Planck Institute for the Science of Human History linked diet with genetics by analyzing ancient proteins in dental calculus from 41 prehistoric individuals from across Sudan and Kenya, many of which also provided ancient genetic information (aDNA) on LP. This region has been called the cradle of humanity for human origins and it could potentially be the same for dairy culture; it has the earliest archaeological evidence in Africa for pastoralism, and three of the five known LP variants are believed to have originated in either northeastern or eastern Africa [2].

The team focused on the identification of proteins specific to milk, particularly the whey protein β-lactoglobulin (BLG) [2, 3]. Although not the most common protein in mammalian milk, BLG has proven to be the easiest to detect in ancient samples [2, 3]. They identified BLG and other milk-specific proteins in dental calculus from eight of the 41 individuals, with the earliest direct evidence for dairy consumption coming from a 6,000-year-old burial in Sudan [2]. Some of the ancient proteins were so well preserved in...
the dental calculus, the researchers were even able to identify BLG to the species level (goat) from a Sudanese burial. At 4,000 years before the present (BP), this finding is the earliest direct evidence of goat milk consumption in Africa [2]. The earliest direct evidence for dairy consumption from Kenya dates to approximately 3,500 years BP. Although not quite as ancient as those in Sudan, these dates are the earliest direct evidence for pastoralism in Southern Kenya [2].

Despite their teeth demonstrating they were dairy consumers, none of the individuals that had complementary aDNA data had any known LP variants [2]. This finding supports data from other regions of the world that suggest dairy consumption came first, preceding natural selection for LP by perhaps thousands of years [2]. Like modern-day populations without genetic adaptations, these early dairy consumers may have benefited from gut microflora or fermentation practices that help break down at least some of the lactose and limit digestive issues associated with lactose intolerance. Researchers speculate that selection for LP in East Africa may have increased when populations increased their dependence on dairy consumption, perhaps during periods of drought [2]. Thanks to the lack of toothbrushes and dentists in the Neolithic (and some pretty nifty scientific analyses in the present day), it is possible that this speculation could soon be validated.


Contributed by
Dr. Lauren Milligan Newmark
Researcher, Science Writer

**Immune System-stimulating Proteins Influence the Development of the Neonatal Microbiome and Immune System**

- Interactions between the immune system and gut microbiota play an important role in early immune development and maturation.
- A new study investigates the role of certain immune system-stimulating proteins found at high levels in breast milk—S100A8 and S100A9—in the development of the neonatal gut microbiota and immune system.
- S100A8 and S100A9 are also found at high fecal levels in infants compared with adults, and are crucial host factors influencing the co-development of beneficial intestinal microbiota and gut immunity in infants.
- Intestinal S100A8 and S100A9 deficiency increases the risk of newborn individuals developing unfavorable gut microbiota and associated long-term disorders such as obesity.
- Feeding mice S100A8 at birth prevented many of the unfavorable outcomes of low intestinal S100A8 and S100A9, and the researchers suggest that nutritional supplementation with these proteins could potentially help immune development and prevent microbiota-related disorders in preterm infants.

The saying “you are what you eat” may be especially true for newborns. For instance, breastfeeding is known to have long-term impacts on health and immunity, and several components present in breast milk are known to influence both infant immunity and their gut microbiome.

In a new study, Dr. Viemann Dorothee of Hannover Medical School and her colleagues investigated the role of certain proteins found at high levels in breast milk, S100A8 and S100A9, in the development of the microbiome and early immune responses [1]. Breast milk contains extremely high levels of S100A8 and S100A9, and these proteins are also found at high levels in healthy breast-fed infants [2,3]. Physiologically, these proteins form a complex (S100A8-A9) known as calprotectin [4,5].
Early immune development and maturation are critical to infant health, and it has become increasingly clear that the infant gut microbiome plays a crucial role in these processes. As microbes initially colonize the infant gut, their interactions with the intestinal immune system help in its early development and maturation [6,7]. If all goes well, the gut microbiome and host immune system develop a balance, known as “homeostasis,” but disruptions in this balance can increase an individual’s risk of inflammatory and metabolic diseases [8–12]. However, it’s still unclear what host factors influence the interactions between intestinal immunity and initial gut colonization to ensure homeostasis.

Previous studies showed that in healthy neonates high serum concentrations of S100A8-A9 can dampen immune reactions to microbes in a process known as “stress tolerance” [13–15]. However, when S100A8-A9 is released later in life in inflammatory settings, it has an amplifying effect, and fecal calprotectin serves as a biomarker of inflammation in Inflammatory Bowel Disease [16].

To elucidate the role that calprotectin plays in the development of infant intestinal immunity and microbial colonization, Dorothee and her colleagues collected stool samples from both full-term and preterm infants over different time points during the first year of life and analyzed fecal microbiomes and levels of S100A8–A9. They also studied the development of the neonatal intestinal microbiota and immune system in mice lacking calprotectin.

The researchers found that fecal calprotectin levels were significantly higher in healthy term babies during the first three months of life than in adults. Initial fecal S100A8-A9 levels were significantly lower in preterm infants than in term infants but increased during the first month of life. In both preterm and term infants, fecal S100A8-A9 levels were higher after vaginal delivery than after a Caesarian section delivery.

The researchers first looked at how S100A8-A9 influenced the microbiome. High fecal S100A8-A9 levels during infancy—from 30 days to 1 year of age—relative to the cut-off for normal adults of 50 μg/g correlated with an increase in certain beneficial gut microbiota such as Bifidobacteriaceae and reduction of other microbiota such as Enterobacteriaceae that have been linked to chronic inflammation and disease. These taxonomic changes suggest that an increase in calprotectin is linked to an increase in favorable infant gut microbiota and beneficial changes to gut metabolism, including an increase in the availability of short-chain fatty acids. Additionally, the researchers found a higher abundance of Enterobacteriaceae in the fecal microbiomes of mice lacking S100A8-A9.

The researchers then explored how S100A8-A9 influences intestinal immunity. They found that S100A8 and S100A9 proteins were expressed by immune cells known as lamina propria macrophages (LPMPs) in intestinal tissues from infants at higher levels than in intestinal tissues from adults. In addition, high levels of fecal S100A8-A9 in the mouse neonatal gut influenced LPMPs to increase levels of immune cells known as T regulatory cells. T regulatory cells can turn promote favorable changes to the gut microbiota, thus providing a mechanistic link between S100A8-A9 levels and the microbiota.

To understand the clinical relevance of S100A8-A9 fecal levels, the researchers also investigated the effects of S100A8-A9 on necrotizing enterocolitis and late-onset sepsis, two diseases of preterm infants associated with dysregulation of the gut microbiota, known as gut dysbiosis. They found virtually no S100A8-A9 expression in the intestine of necrotizing enterocolitis patients, and low fecal S100A8-A9 levels reliably predicted a high risk of late-onset sepsis, suggesting that fecal S100A8-A9 deficiency is associated with necrotizing enterocolitis and sepsis.
In addition, a low level of S100A8 and S100A9 proteins in infant fecal samples was associated with the development of sepsis and obesity by age 2, suggesting a link between these proteins and long-term dysbiosis-associated diseases. Previous studies have shown that long-term gut dysbiosis is a risk factor for the development of obesity [5,17,18].

When the researchers looked at neonatal mice lacking S100A8-A9, they found that these were also more likely to weigh more and develop fatal sepsis than normal mice. However, feeding mice S100A8 at birth reduced body weight and death from neonatal sepsis and beneficially changed the gut microbiome and intestinal immunity.

The findings indicate that S100A8-A9 plays a clinically relevant role in regulating newborn intestinal immunity and could help prevent dysbiosis-related diseases. In addition, data from mouse studies indicate the potential for nutritional supplementation of S100A8-A9 to prevent such diseases. The researchers suggest the need for clinical studies to evaluate whether S100A8 and S100A9 supplementation might aid in the development of gut immunity in preterm infants and prevent dysbiosis-associated disorders later in life.


Contributed by
Dr. Sandeep Ravindran
Freelance Science Writer
Sandeepr.com
The Early Influence of Breastfeeding on the Infant Immune Response

- Breastfeeding is known to influence health and immunity, but its very early impacts on the neonatal immune system are still unclear.
- A new study finds that immune cells known as T regulatory cells increased during the first three weeks of life, and were nearly two-fold higher in breastfed infants compared with formula-fed infants.
- Breastfeeding also increased infants’ immune tolerance for maternal cells and led to subtle differences in the microbiome at three weeks compared with formula feeding.
- The study shows that breastfeeding influences the neonatal immune response within the first three weeks of life.

Breastfeeding is known to have several long-term impacts on health and immunity, including a lower incidence of allergy, asthma, diabetes, and multiple sclerosis [1-4]. But researchers still know relatively little about the development of the immune system within the first few weeks of life, and about the effect of breastfeeding on this early immune development [5-9].

"If you think about what babies need to do from the immunological point of view when they're first born, they need to learn to respond to pathogens, but they also need to learn to tolerate environmental antigens and things that the immune system shouldn't be reacting to," says Dr. Gergely Toldi of Birmingham Women's and Children's Hospital. In a new study, Toldi and his colleagues investigated the development of the neonatal immune system and how it is affected by breastfeeding [10].

The Earliest Immune Responses

Studying the immune system of newborn babies wasn’t easy. “Working on blood samples taken from babies is always a challenge because of the small quantity of the samples that we can take, but it also provides a lot of interesting information, and with today’s technologies a lot can be done from just tiny amounts of blood,” says Toldi.

Toldi analyzed the development of the immune system between birth and three weeks of age in a cohort of 38 healthy neonates born by Caesarean section (C-section). “We know that labor and delivery induce an inflammatory response both in mom and baby, so this is why we very purposefully chose a cohort of all babies born from Caesarian section to rule that influence out,” he says. “They were all healthy and only sampled for research purposes, so they didn’t have any other sort of medical problems that could have influenced the function of the immune system,” says Toldi. “The first three weeks of life was also very carefully selected because as these babies grow and receive immunizations or acquire infections, these influence the immune response,” he says.

Toldi and his colleagues also explored how breastfeeding influenced neonatal immune responses. “We know from epidemiological studies that those early influences, whether someone receives breast milk or formula milk, can have quite long-lasting consequences during the individual's lifetime,” says Toldi. "In particular, immune-mediated disorders are known to present with variable prevalence in those who are breastfed compared to those who are formula-fed,” he says.

The researchers compared the immune repertoire and function between breastfed and formula-fed infants, and also investigated the microbiome from neonatal stool samples. “We first wanted to characterize the kinetics and changes of the T cell compartment of the immune system, which is the adaptive arm of the immune system and includes the cells and the networks that learn to recognize pathogens,” says Toldi. "The first step was basically just looking at how these cells evolve in the first three weeks, how the complexity of this cell subset changes, and what different subtypes evolve over time,” he says.
Toldi and his colleagues found that certain immune cells known as T regulatory cells expanded substantially in the first three weeks of life. “We found that there is an increase in the population of the T regulatory cells, which is quite a special type of cell responsible for regulating the response from all the other T cells,” says Toldi. “So it's basically a brake on the immune system and controls inflammation or the immune response when it's not needed anymore,” he says.

Maternal Cells and Infant Immunity

The researchers then investigated how the neonatal immune system responded to maternal cells. “The next step was to see how the baby's and mother’s immune systems recognize each other because they are not perfectly separated,” says Toldi. “Before birth, there is some cell exchange through the placenta and after birth in a breastfed baby the maternal cells do enter babies' guts and eventually their circulation through the breast milk,” he says. “It is an interesting question that hasn't really been studied before, to see how an immune tolerance is maintained towards those maternal antigens that otherwise would be recognized as foreign from the baby immune system's point of view,” says Toldi.

The researchers found that tolerance of maternal cells was much better in babies who were breastfed. "Physiologically that makes perfect sense, because even if there's probably not that many maternal immune cells entering a baby's system in a single feed, over time it adds up to quite a lot of antigen exposure and antigenic load,” says Toldi. “This was an interesting finding that there is this tolerance and it's stronger in babies who are breastfed,” he says.

Toldi and his colleagues then investigated the mechanisms of these early immune responses. “This is where the T regulatory cells come into the picture again, because in breastfed babies we found almost twice as many T regulatory cells as in non-breastfed babies, and they are actually mechanistically responsible for maintaining this immune tolerance,” says Toldi. “So when we depleted these cells from the immunological experiments that we conducted using moms’ and babies’ cells, then this tolerance did not occur,” he says. “The tolerance is also very specific towards moms' antigens, because it was not seen when we repeated the experiments with an unrelated, healthy persons' cells,” says Toldi.

The researchers then analyzed stool samples to look at changes to the microbiome. “By three weeks we did find subtle differences, with specific strains of bacteria called Gemella and Veillonella more abundant in breastfed babies’ stool samples,” says Toldi. “The way this fits in with the immunological findings is that we know that these strains of bacteria produce large amounts of short-chain fatty acids, and so they support the function of the T regulatory cells,” he says. “Whether the increase in these bacteria is a consequence of having higher numbers of T regulatory cells or whether these bacteria actually contribute to that elevation is not quite clear yet, and that’s something we would like to clarify in future studies,” says Toldi.

Toldi is also interested in future studies looking at babies born vaginally, although this will require finding ways to rule out the compounding effects of labor and delivery on the immune response. “From the microbiome point of view, there would definitely be differences in how these babies develop in the first three weeks compared with the C-section-born babies,” he says. "Another interesting question would be to see how long-lasting these changes are and whether these things balance out in later life at some stage,” says Toldi. This would require following up on the cohort and seeing how the children develop. “That would allow us to also explain whether there’s a direct link between these early immune changes and the epidemiological findings that support different incidence of things like autoimmune diseases between breastfed and formula-fed children,” he says.

Toldi’s findings suggest that breastfeeding directly promotes the development of T regulatory cells that suppress immune recognition of maternal cells, and these effects could potentially confer lifelong benefits. The results improve our understanding of how breastfeeding very early in life can influence health and immunity. "What surprised me was that it’s such a well-balanced system and it all kind of fits together very nicely,” says Toldi.

Success of African Cattle Linked to Admixture Event 1,000 Years Ago

- A new genomic analysis found that most modern-day African cattle breeds are the result of a genetic admixture event between *Bos taurus* and *Bos indicus* dated to approximately 1,000 years.
- The genome of today’s African cattle reflects selection for traits related to inflammation, immune function, and heat stress inherited from both species.
- Crossbreeding increases genetic variation and can be implemented to improve modern-day cattle productivity.

If cattle had ancestry.com or 23andMe (or, make that 31andMe), it would look a lot like this study [1]. An international team of researchers sequenced the DNA of 172 cattle from 16 breeds indigenous to Africa to understand their genetic history and identify genetic markers for traits related to the cattle’s survival and success (and that of the pastoralist populations who rely on them) across the continent over the last several millennia.

Kim and colleagues [1] discovered that today’s African cattle owe their resilience to an admixture event that occurred 150 generations (750–1,050 years) ago between two species, *Bos taurus* and *Bos indicus*. Gene flow between these two species increased genetic variation, giving both the humans that bred them and nature a chance to select for traits that enhanced survival and productivity [1].

Cattle Family Tree

The majority of today’s cattle breeds in Africa trace their ancestry to both *B. taurus* (taurine cattle) and *B. indicus* (indicine cattle) [1]. *B. taurus* were domesticated in the Near East [2] and their earliest archaeological evidence in Africa dates to between 6,000 and 7,000 years ago in Egypt and Sudan [1]. *B. indicus* were domesticated on the Indian subcontinent and had a much later arrival in the Horn of Africa, circa 700 A.D. [1, 3, 4].

To figure out how long it took for humans to cross these two species after *B. indicus* arrived in Africa, Kim et al. [1] had to work backward. First, they determined the level of taurine and indicine admixture found today in each of the 16 African breeds surveyed. Then, they calculated (with the help of some fancy genetics statistical software) how many generations it would take to establish these varied genetic patterns.

Kim et al.’s calculations suggested the two species first swapped genes approximately 150 generations
researcher, science writer

Dr. Lauren Milligan Newmark

Contributed by

Researcher, Science Writer

ago [1]. Assuming a generation time of 5–7 years, this would equate to approximately 750–1,050 years ago [1], or about 300 years after B. indicus arrived on the African continent. This means that the mosaic of genomes seen across today’s indigenous African cattle breeds took a mere 1,000 years to evolve. Such rapid evolutionary change (described as an “evolutionary jolt” by study co-author Stephen Kemp [5]) strongly points at selection—natural and artificial—as humans and their cattle moved into new habitats.

**Beefed Up Genomes**

African cattle have over 20,000 genes. If one of those genes is evolutionary neutral (that is, it neither increases nor decreases survival and reproductive success), it is just as likely to have come from B. taurus as it is from B. indicus. Genes under selection, however, favor a particular ancestry. Kim et al. [1] identified three genetic regions that showed an excess of taurine ancestry and 13 that showed an excess of indicine ancestry.

Further genetic sleuthing revealed protein-coding genes within these regions related to the inflammatory response, immunity, and heat stress. B. taurus contributed several genes believed to be associated with increased tolerance to the parasitic infection trypanosomosis (aka sleeping sickness), known to be a major obstacle to livestock productivity in Africa [1]. This would have provided an advantage in humid environments where tsetse flies were more common. In contrast, B. indicus passed along genes related to heat stress and water conservation that would have been beneficial in Africa’s more arid environments [1]. Finally, they identified a handful of immune-related indicine genes that may play a role in resistance or tolerance to ticks and tick-borne diseases [1]. Although neither species is native to Africa, B. taurus and B. indicus both brought genes from their ancestral homes that proved advantageous in many environments across the African continent.

**Using the Past to Inform the Future**

Modern-day African cattle now know that they got their parasitic infection tolerance from their B. taurus ancestors, and their tolerance to heat stress and tick infection from their B. indicus ancestors. Although this knowledge may be of little use to cows, it is extremely useful to modern-day pastoralists and those working in livestock management. Specifically, Kim et al. [1] highlight the importance of genetic diversity. As global temperatures increase and the demand for milk and meat grows, they suggest that breeding indigenous African cattle with exotic cattle could help increase adaptability, productivity, and sustainability in modern-day livestock.


Contributed by

Dr. Lauren Milligan Newmark

Researcher, Science Writer
Editorial Staff of SPLASH® milk science update:

Dr. Danielle Lemay, Executive Editor
Dr. Katie Rodger, Managing Editor
Dr. Anna Petherick, Associate Editor
Dr. Lauren Milligan Newmark, Associate Editor
Dr. Sandeep Ravindran, Associate Editor
Cora Morgan, Editorial Assistant
Dr. Elieke Deemer, Content Strategist
Sydney Sullivan, Copy Editor

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

The views and opinions expressed in this newsletter are those of the contributing authors and editors and do not necessarily represent the views of their employers or IMGC sponsors.