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Minireview

Nutrition, population growth and disease: a short history of lactose

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Summary

Food and nutrition have played a crucial role in biological evolution. Lactation in mammals was one key invention. A central role in milk is played by lactose, otherwise an exotic sugar in nature. Lactose digestion needs the induction of specialized gut enzymes. This enzyme is shut off in a precisely timed developmental step leading to lactose malabsorption promoting weaning in the young and ovulation in the mother. The lactose–lactase system could thus regulate optimal birth spacing in land mammals. The domestication of cattle promoted milk as a food item also for adult nutrition. This was only possible by two further key inventions: the concomitant domestication of lactic acid bacteria which ferment the non-digestible lactose to the easily absorbed lactic acid and the mutation to lactase persistence (LP) in adults from dairy societies. This mutation represents one of the strongest selected loci of the human genome. Since no crucial nutritional selective advantage is conferred by LP, its dominance might be the result of indirect effects like the spread of cattle pathogens into humans. Lactase is also temporarily lost in rotavirus and *Escherichia coli* childhood diarrhoea and persistent diarrhoea is consequently best treated with lactose-free diets.

From eggs to milk

'In the Beginning was the Egg, and the Egg was with all Animals and all Animals were initially Egg' in these words a biologist could paraphrase a famous text to underline the importance of this key invention in biological evolution. Eggs serve first and foremost in the transfer of genetic information from one generation to the next, but this task can also be achieved with tiny cells as seen with animal sperm. The egg in contrast is the largest cell of the human body and can be seen with the naked eye. This size points to another function of the egg: it contains nutrients (yolk) – a food investment by the parental generation in support of its offspring. This invention in animal evolution was since never abandoned although it experienced a myriad of modifications.

The insightful Harvard zoologist A.S. Romer (1968) spoke about 'the triumph of the egg' in describing vertebrate evolutionary trends. In vertebrates the egg is no longer just a large reproductive cell with a big cytoplasm filled with food storage molecules. The egg of amniotes (reptiles, birds, mammals) became a world of its own composed of different tissues specialized for feeding (yolk sac), excretion (allantois) and respiration. The amniotic cavity carries the last remnant of the salt sea in which the embryo swims and with that invention vertebrates became independent from water for early development and thus true land animals. Sharing a successful trait is not enough in evolution – if an animal wants to stay ahead of competition it is condemned to make new inventions. New imaginative ways of food provision is a good target for invention. Brooding – now commonly practiced by all birds and some reptiles (e.g. the python) (Hutchison *et al.*, 1966) – was according to fossil evidence already tried by reptiles [oviraptor, the 'egg robber', became the nesting dinosaur (Varricchio *et al.*, 2008)]. Feeding the young after hatching from the egg was a key invention made by birds, although not maintained by all of them (precocious birds quickly flee from the nest after hatching). Feeding the young was in exceptional cases also tried by more basal vertebrates: for example, caecilian amphibians produce a nutritious secretion from their skin with which they feed the young (Kupfer *et al.*, 2006). However, only mammals made two further key inventions caring for the young. Mammals discovered the 'mobile' egg – first as seen in Echidna, a monotreme, a real egg maintained in an abdominal pouch which also contains the mammary

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gland. In higher mammals the egg implants in the placenta of the uterus. Here the embryo becomes connected to the circulatory system of the mother for an extended intra-uterine feeding period. In this way the egg indirectly obtained eyes and feet to run away, and claws and teeth to defend. However, vivipary is not an exclusive invention of mammals – many animals found this solution. For zoologists mammals are characterized by two traits: hair and breast milk. However, milk production might predate the evolution of mammals (Oftedal, 2012). Hair serves as thermic insulation, a key requirement for warm-blooded animals (birds invented feathers for this purpose). The name-giving term mammal refers, however, to the mammary gland, and for good reason. Breast feeding has several advantages over the feeding of the young by birds. Instead of insects and plant material fed by birds, breast milk is a food item specially adapted to provide optimal nutritional support for the early development of the newborn. Milk production is dynamic and milk matures with the development needs of the young. This is most dramatically seen in cattle where colostral milk is a vehicle for the passive transfer of maternal antibodies across a still 'open' gut of the calf (Jochims *et al.*, 1994) and only after the closure of the gut for macromolecules, the mature milk becomes food for the calf. Milk is essentially an emulsion of fats in an aqueous solution containing sugar, proteins and crucial electrolytes. The composition of the milk is adapted to the particular nutritional needs of a given species. Human milk, for example, contains 7.1 g lactose sugar, 4.5 g of fat and 0.9 g of protein per 100 ml milk, and has an energy content of 70 kcal. In comparison, cow's milk contains more proteins, calcium and phosphorus, but less lactose than human milk. Children can be raised on cow's milk, but the food industry tries to 'humanize' the composition of cow's milk for infant formula underlining the importance of adapted milk. Milk from marine mammals, for example, is much richer in fat content than that of land mammals because a thick fat layer plays a crucial role in thermal insulation of marine mammals.

The difficulty with lactose

Milk contains a special sugar, lactose. This sugar is so familiar to dairy microbiologists that they forget that lactose is an exotic compound in nature, found outside of milk only in forsythia flowers and some tropical shrubs.

Lactose is not an obvious biochemical choice: the synthesis of this disaccharide of two common sugars (glucose and galactose) poses a biochemical challenge. Human galactosyl transferase (subunit A of the lactose synthetase) does not possess enough affinity for glucose as acceptor to allow lactose synthesis. It needs subunit B for the enzyme to accept glucose and hormonal adjustments (prolactin up, progesteron down) have to occur in women to allow lactose synthesis.

In addition to these biosynthetic difficulties, lactose synthesis is energetically demanding. Galactose must first be phosphorylated by adenosin triphosphate and the sugars must be activated by uridine triphosphate which demands further chemical energy. Once produced, the difficulties with lactose continue. Beta-galactosidases and the lactose operon are historical signposts for microbiologists and we take it for granted that every organism can deal with lactose. However, this is not the case. Lactase is not a constitutively expressed enzyme in the small intestine of mammals (Lee *et al.*, 2002). The newborn human baby has still very low levels of lactase. This is not so surprising since mothers must first induce lactose synthesis, and breast milk production must be stimulated by the suckling of the newborn at the breast. Mothers do not express milk on the first day and start with producing 50 ml milk on the second day, increasing the production gradually by 50 ml for every day paralleling the increase of lactase expression in the intestine of the newborn. In fact, during the first 2 days the energy needs of the newborn are covered by autophagy of the no longer needed embryonal tissues (Kuma *et al.*, 2004). Obstetricians give the newborn sometimes a glucose solution since the glucose transporter is already expressed and remains so throughout life.

In view of these difficulties, one might ask why nature experimented with lactose and not glucose as energy carrier for breast feeding (in humans lactose represents about 30% of the milk calories). In fact, only few exceptional mammals, such as the primitive platypus and some marine mammals (Reich and Arnould, 2007), do not use lactose in milk. When biological systems use energy-costly devices, one might suspect regulatory needs behind it (e.g. futile cycles in the control of biochemical pathways). Regulatory needs might also be behind the lactose story. Lactase in humans and many other mammals is tightly regulated during development.

Lactase – a regulatory enzyme?

Lactase expression is not only gradually increased in the gut of the newborn after delivery, gut lactase activity also gradually decreases in older children. For example, at 18 months of age 60% of Bangladeshi children show lactose maladsorption, this prevalence is > 80% after the third year of life (Brown *et al.*, 1979). In Bangladesh these lactose maladsorption rates mirror the weaning rates. One potential argument about lactose and its regulatory role goes as follows. As long as the baby suckles the breast, milk is produced by the mother and this state can be maintained over years. The suckling initiates a neuroendocrine circuit that goes up to the hypothalamus where it suppresses the gonadotropin release, which in turn suppresses ovulation and the menstrual cycle. This circuit makes evolutionary sense: women would go beyond their physical capacities when providing large amounts of calories both to a baby via breastfeeding and a fetus developing in the uterus. Not only breastfeeding suppresses fertility – any nutritional stress can lead to amenorrhoea.

However, there is a problem with this regulatory cycle. If you install a negative feedback, you must also think on a circuit how to get out of the loop at an appropriate time. Early in human history the population size was very small. Therefore, optimal birth spacing had to be achieved: not too narrow because it would overstretch the feeding capacity or bodily food reserves of the women; and not too long because this strategy would compromise the growth of the tribe. One could hypothesize that the developmental regulation of lactase expression is the timing device for optimal birth spacing in humans (Brüssow, 2007). The mechanistic basis for this regulatory device could be as follows. When lactase expression is downregulated in the gut of children, lactose from breast milk would no longer be absorbed in the small intestine. Lactose would then reach the large intestine where it could induce osmotic diarrhoea when still not absorbed or bloating and flatulence when absorbed by bacteria breaking down lactose to volatile metabolic end-products. Both events would be recognized by the mother who might then start to introduce alternative food items. This behavioural change will be reinforced when she observes a mitigation of the symptoms of lactose intolerance by weaning food. Then the decreased frequency of the breast feeding would take away the major driver stimulus for milk production. Although this looks like a plausible explanation, it is still a hypothesis. Supportive evidence would come from a temporal correlation between lactase downregulation and the onset of weaning in other mammalian species and altered birth spacing in populations with a loss of lactase downregulation phenotype.

Lactose and the first farmers

Key breakthroughs in human cultural evolution were the use of animal furs to keep the body temperature in the cold (which allowed the loss of body hair and thus better heat dissipation by sweating) and the use of stone tools and fire, crucial for food acquisition and food processing. The recent transition from food collection to food production was called the Neolithic Revolution. Domestication of animals and plants occurred independently at different places, but that which started in eastern Anatolia was special: it was the earliest, was associated with the development of the proto-Indoeuropean language and transformed still in Turkey into a dairy culture as deduced from milk residues on potsherds (Dudd and Evershed, 1998). Significantly, the economic term ‘pecuniary’ derives from the old Latin term ‘pecu’ which signifies both the cow and money. Archaeologists have retraced the spread of the Neolithic Revolution through Europe. Geneticists are still undecided whether it was based on a process of cultural adaptation (transfer of the food technology and language in a joint cultural package by learning) or by the physical migration of the early farmers (and their genes) who replaced the hunter-gatherer populations (Chikhi *et al*., 2002). Cereals and cattle quickly replaced the diet of the hunter-gatherers. The high esteem for these agronomic novelties can still be deduced from the deification of the domesticated cereals and the bull: Ceres became a Roman goddess and the bull became the incarnation of the God-Father of the Greek pantheon.

Cattle have many uses. They are a source of meat and hide, and can function as traction animals for the transport of heavy goods or ploughing. Meat is a one time food source, but there are more sustainable uses of cattle which do not kill the animal off which you want to live. Some pastoral cultures bleed cows (Massai), most cattle cultures, however, use cow’s milk as important food source and became thus dairy cultures. Even when accounting for the lower productivity of prehistoric cows and the milk needed for raising the calves, it was estimated that about 200 kg of milk were then a surplus per cow and per weaning period (Gerbault *et al*., 2011). Over a 10-year period more calories could be extracted from the milking cow than contained in its body mass and you could still kill the bull calf and finally also the milking cow when milk productivity decreases with age. Economically, dairy culture makes a lot of sense. Dairy culture also has ecological assets: the waste of cows became fertilizers for the crop fields. Yet, when considered from a logical viewpoint, this scenario presents a problem. How does this emergence of dairy cultures fit with lactose intolerance that develops in humans after the weaning period? Milk could not have been an easily digestible food for the early farmers. Drinking more than a pint of milk should lead to digestion problems in adults and this is indeed the case for most contemporary Asian populations. It needed two further events to allow the development of dairy cultures. One is a further domestication event of an organism that is so small that it is easily overlooked and the other event is a mutation in the human genome that has fascinated geneticists and archaeologists alike.

Domestication of lactic acid bacteria

In fact, there is a simple solution to the lactose conundrum: lactic acid bacteria (LAB). If you leave raw milk under appropriate environmental conditions, the milk might either turn or experience spontaneous fermentation by LAB naturally contained in the milk leading to a sour milk or yoghurt-like fermentation product. The early cattle farmers will have quickly detected that inoculating fresh milk with such a spontaneous fermentation product would increase the likelihood of a fermentation process. As practiced before with wild plants and animals, empirically selecting the right source of inoculation material would create distinct mixtures of bacterial cultures leading to differentiated milk fermentation products. Recipes for cheese production were found on Sumerian cuneiform tablets, the first written documents. Early Babylonian and Egyptian texts even contain recipes for more complicated food technologies like beer brewing. We can thus safely, but indirectly deduce that milk fermentation technologies predate the historical period. Archaeologists investigating ceramic pottery for chemical traces of fermented milk residuals found indeed supportive evidence that dairy technology was already practiced when the early farmers or their technology had spread from its East Anatolian origin to Western Turkey at 8500 BP. This region today shows only a low prevalence of lactase persistence (LP) in adults, the mutation which allows the drinking of unfermented milk. Skeletal remains from the prehistoric Linear Band Keramik (LBK) people who practiced dairy agriculture at 5500 BP showed that LP had not yet evolved at that point of time and space (Burger *et al*., 2007). The processing of milk into cheese would be an invention that solved the lactose digestion problem while at the same time producing a food stable at ambient temperature and easier to transport than milk. Potsherds pierced with small holes appeared in temperate Europe in the sixth millennium BC in the region of the LBK culture. Archaeologists had interpreted them as 'cheese-strainers', but sceptics argued that these pottery vessels were used for filtering fruit juice. Now analytical chemists have demonstrated abundant milk fat in these specialized vessels thus providing good evidence that these vessels were used to separate fat-rich milk curds from lactose-containing whey (Salque *et al*., 2013). LAB are therefore a fitting missing link to solve the conundrum and should therefore be added to the list of domesticated organisms from the early farmers that were acquired shortly after the domestication of the milk-producing animals. North African rock art showed the life of nomadic cattle herders in the then still green Sahara, rare pictures showed evidence of milking. Analytical chemists demonstrated milk fat also in excavated Saharan ceramics that were securely dated in the fifth millennium BC (Dunne *et al*., 2012). Apparently, dairying was an obvious invention that spread from the Near East into both Central Europe and North Africa.

Fermented milk products are easily digested by lactase-deficient subjects (Savaiano and Levitt, 1987). This statement applies not only to food items that experience a long fermentation process like cheese where practically all lactose is fermented into lactic acid and further downstream metabolites. Lactic acid is in fact easily absorbed by the human small intestine via an organic/carboxylic acid transporter protein. Also products of short fermentation processes like yogurt where the majority of milk lactose is still present in the dairy product are digestible for lactase-deficient subjects due to the presence of a the corresponding bacterial enzyme (beta-galactosidase) produced by LAB and consumed together with the yogurt. This bacterial enzyme can cleave lactose *in situ* in the small intestine. This trick is not different from the ingestion of biotechnologically produced lactase which allows lactase-deficient subjects to consume raw milk in quantities that would otherwise lead to symptoms like bloating and flatulence (Montalto *et al*., 2006). In its fermented form, cow's milk not only represents a food supplement to the lactase-possessing suckling baby, but also relieves a nutritional stress of the mother (allowing shorter lactation period) and represents a fallback food item in times of food shortage. These technological developments could have led to closer birth spacing due to an earlier onset of new ovulations without compromising the nutritional state of the mother or the milk dependence of the baby. This way, dairy cultures could reproductively out-compete the hunter-gather societies and non-dairy agriculturalists. In its fermented form, milk could also become a valuable food source for adults in the early farming societies. It is perhaps not a chance event that fermented milk is still today a very popular beverage (ayran) and food item (yoghurt) in regions which are close to the early spreading of the cattle farmers (Turkey, Persia, Balkan).

It is therefore probably not farfetched to speculate that LAB belonged, together with the domesticated cattle, wheat and a language, to a cultural package spreading through Europe with the early farmers (Douglas and Klaenhammer, 2010). Dairy culture takes, thus, a double meaning – bacteria as starters of food fermentation and the language and technology of this early agrarian society. Yet how good is the evidence that LAB were domesticated during the Neolithic revolution? Bacteria do not leave fossils and ancient DNA from prehistoric pottery was not yet reported. The only way to deduce such events is by comparison of the genomes of extant dairy LAB to their cousins which persisted in their original environment. 'Domestication' of bacteria would correspond to a specialization to a narrow niche, milk. After a niche change many genes become obsolete and the genome will undergo a process of reductive evolution through gene elimination (Mira *et al*., 2001). Spanish microbiologists argued that mobile elements such as insertion elements greatly increase in number after 'domestication'. The insertion sequence (IS) elements will inactivate genes that are unnecessary in the new environment, which leads to their ultimate deletion. Dramatic IS expansions occurred in bacteria which 'discovered' humans as profitable hosts when the population size increased in the early farmer societies. Human-specific pathogens like *Shigella flexneri* in contrast to *Escherichia coli* or *Bordetella pertussis* in contrast to *B. bronchiseptica* showed this pattern as well as pathogens of the expanding crop plants like *Pseudomonas syringiae* in contrast to *P. aeruginosa* and notably also the classical dairy starter *Lactococcus lactis*, which might have been a pioneer for early cheese fermentation (Mira *et al*., 2006). This subject was also investigated by dairy microbiologists who investigated more than 70 *L. lactis* strains by multilocus sequence typing. They distinguished 'environmental' strains – *L. lactis* occurs naturally on plants – from 'domesticated' dairy strains, which were subject to a recent genetic bottleneck. In the absence of a reliable molecular clock in bacteria, it is difficult to infer divergence times, but the authors argued that the inferred bottleneck reflects more likely industrial starter strain selection in the 20th century than early lactococcal domestication 10 000 years ago (Passerini *et al*., 2010). Other researchers inferred that three dairy groups of *L. lactis* have arisen from plant *L. lactis* strains. Adaptation to the dairy environment resulted in loss of functions leading to a smaller chromosome and acquisition of genes via plasmids that facilitated growth in milk (Kelly *et al*., 2010). Prominent plasmid-encoded functions were lactose utilization and oligopeptide permease systems (Siezen *et al*., 2005). A plant-derived *L. lactis* was also propagated for 1000 generations in milk to simulate the domestication process. Genome sequencing revealed the loss of a conjugative transposon encoding pathways involved in the utilization of complex plant polymers and the gain of a plasmid carrying an extracellular protease which improved growth in milk because of amelioration in nitrogen metabolism. Overall, the evolved strains showed increased acidification rates and biomass yields in milk. The transition from a plant to a milk environment can thus be achieved without much genetic changes (Bachmann *et al*., 2012). Also the genome of *Lactobacillus bulgaricus*, which in cooperation with *Streptococcus thermophilus* is the dominant starter strain combination for yoghurt production, shows clear signs of genome reduction in its transition from a plant-associated habitat to the stable protein and lactose-rich milk environment. The genome reduction was documented in *L. bulgaricus* by a substantial number of pseudogenes, incomplete metabolic pathways and few regulatory functions (van de Guchte *et al*., 2006). The closest phylogenetic relatives of *S. thermophilus* are *S. salivarius*, oral commensals of mammals, and pathogenic streptococci. Also *S. thermophilus* showed a striking level of gene decay and many genes involved in carbon utilization are non-functional. Adaptation to the nutrient-rich milk niche was again through reductive evolution which conferred rapid growth in milk, but total loss of virulence genes (Bolotin *et al*., 2004; Goh *et al*., 2011). The domestication of the three major dairy starters can thus be easily read from their present genomes, but whether this reductive evolution started in the Neolithic revolution or is a result of the Industrial revolution can at the moment not be decided.

The far reach of the lactase mutation

Older medical textbooks refer to adult lactose maldigestion as a mild disease even if this is the normal physiological situation. These textbooks were written in Europe or North America where the mutant phenotype of LP into adulthood became so frequent that the medical writers took this as the normal situation. In reality, 65% of the adult world population lacks this LP mutation defining it as the prevailing condition. This mutation is a fascinating detective story of modern human genetics. By classical phenotypic tests, geneticists located the peak areas of adult LP in Northern Germany and Denmark. Notably, this area covers also the highest diversity of cattle milk genes in Europe suggesting a geographical focus for the development of dairy farming (Beja-Pereira *et al*., 2003). As this area corresponds to the distribution of the Funnel Beaker Culture, one could tentatively place this event into the third millennium BC. Human geneticists elucidated the molecular basis for the LP phenotype: two point mutations located 14 and 22 kb upstream of the lactase (LCT) gene (Enattah *et al*., 2008). When single nucleotide polymorphisms were typed around the lactase gene, a common haplotype was identified that extends largely undisrupted over 1 Mb of DNA. Geneticists developed a time frame for the origin of this mutation: it is not older than 20 000 years and not younger than 5000 BP (Gerbault *et al*., 2011). This short time frame is strikingly recent for a haplotype found at such high frequency. What is more: The LP phenotype was also observed in pastoralists from Africa and Asia (Tishkoff *et al*., 2007). Genetic analysis demonstrated similar, but molecular distinct point mutations upstream of the lactase gene occurring over the same time frame. We have here a striking case for convergent evolution in the fast lane. Geneticists and nutritionists looked for plausible explanations as to why LP should confer such a selective advantage to come to dominance in such a short time period. The culture-historical hypothesis maintains that LP developed after milk production spread (Holden and Mace, 1997) while the reverse-cause hypothesis proposes that only populations with high enough LP frequency developed dairy cultures. However, these are questions of historical timing (where current evidence favours the first explanation) which do not address the question of the advantage of being LP.

Why should the capacity to drink milk as an adult be a question of life and death (Gerbault *et al.*, 2011)? One hypothesis states that milk is a reserve food resource in times of famine or just before the next harvest when food reserves are at its annual low. Epidemiologists have observed a correlation between latitude and LP frequency and suggested a calcium assimilation hypothesis (Flatz and Rotthauwe, 1973). Calcium assimilation depends on vitamin D which is produced in the skin upon sun exposure. Since the sun exposure is low in high latitudes, a calcium-rich food like milk could compensate for a lesser sun exposure. However, hunter-gatherers when they met the early farmers relied heavily on marine food which is rich in vitamin D (Richards *et al.*, 2005). This observation takes the driving force out of the argument. In classical arguments, only deadly diseases (e.g. malaria selecting for the sickle cell haemoglobin or the glucose 6-phosphate dehydrogenase) are strong enough selection mechanisms to achieve such a homozygosity as seen with the LP mutations. Malaria was again proposed as a selective force, but data from Sardinia did not support this hypothesis (Meloni *et al.*, 1996). Still another hypothesis states that milk is a relatively clean liquid in regions where water is scarce and fecally contaminated leading to many diarrhoeal diseases. The lactase argument might indeed be indirect. It was argued that many important human diseases were acquired from cattle. The early dairy farmers who survived this first wave of infection were either genetically selected for resistance to these new infections or developed increasing immunity to these pathogens, while any non-dairy population coming in contact with them would be fully susceptible to the new diseases spread by the dairy farmers and succumb to them (Diamond, 1997; Brüssow, 2009). In this hypothesis LP could be of relatively small nutritional advantage to its carrier, but could still have dramatic effects by eliminating non-carriers for this mutation from reproduction due to their susceptibility to veterinary pathogens dairy farmers were shedding.

Still other hypotheses envision human niche construction as the driving force for the spread of this LP mutation (Gerbault *et al.*, 2011). The niche construction model postulates culturally transmitted behaviours [e.g. milk drinking as prestige class behaviour in a highly hierarchical dairy society (Simoons, 1970)] together with features of the environment shaped by ancestral populations that led to striking human gene-culture co-evolution. The jury is clearly still out on whether any of these hypotheses explain the dramatic expansion of the LP phenotype in some, but not in other regions of the world. The very fact that the LCT locus is one of the most selected regions of the human genome and shows the result of a selection pressure as great as on the major histocompatibility region underlines the importance of the question and at the same time the degree of our ignorance about basic aspects of the impact of nutrition on human health. Without exaggeration one might state that understanding LP means understanding a major piece in the nutrition and health puzzle.

Lactose and diarrhoea

The reproductive success of a population is determined by the childhood mortality rate. The leading causes of childhood mortality in developing countries are respiratory infections and diarrhoeal diseases, complicated by malnutrition (Baqui *et al.*, 1998). In prehistoric societies the burden of childhood diarrhoea was probably even greater than today. Common diarrhoeal pathogens of childhood like rotavirus or enteropathogenic *Escherichia coli* (EPEC), which do not cause invasive disease, damage the enterocytes, the outermost cell layer in the intestinal mucosa (Kaper *et al.*, 2004). EPEC leads to an effacing of its microvilli, rotavirus to a desquamation of the entire cell. In both cases, the enzymes located in the microvilli are lost until new enterocytes migrate from the crypt to the tip of the villus to resume function. The time of this migration correlates with average duration of uncomplicated childhood diarrhoea (5 days). Since the enterocyte is key to the absorption of nutrients in the intestine, valuable nutrients are lost during this period and diarrhoea leads to a transient arrest in weight gain. Not enough with that: A prominent enzyme of the enterocyte membrane is lactase. Children with diarrhoea become thus transiently lactase deficient. Lactose is no longer absorbed and adds via its water drag to additional osmotic water loss enhancing diarrhoea. This led in the past to recommendations to stop formula and breast feeding to avoid lactose. Today, paediatrician recommend continued breast feeding in diarrhoea since the caloric gains from feeding are for the nutrition of the child more important than the negative osmotic effects of lactose. For formula-fed children, a lactose-reduced formula is recommended.

In about 5–10% of children from developing countries diarrhoea continues for more than 2 weeks (Persistent diarrhoea, PD) with disastrous consequences for the nutritional state of the infant (Moore, 2011). Diarrhoea becomes thus a driver for malnutrition which makes the child more susceptible to another bout of diarrhoea creating a vicious cycle. After the introduction of oral rehydration solutions (which fix the electrolyte problems, but not the diarrhoea and the nutrition problem), mortality from acute diarrhoea has decreased. PD became thus the major cause for diarrhoeal mortality in children from developing countries. The pathophysiology of PD is not yet clear, but lactose seems to play a crucial role. A trial conducted on several continents showed that replacement of a milk-based diet by locally available products with reduced lactose content (like rice, yoghurt, lentils and vegetable oil for Pakistan) ameliorated the condition in 60% of PD patients. Some refractory PD patients responded to a lactose-free diet (like rice, chicken, glucose, oil in Pakistan) (Anonymous, 1996). Lactose-reduced diets like yoghurt are still today the treatment of choice in children with PD from South America (de Mattos *et al*., 2009), while a trial from Bangladesh showed that a lactose-free green plantain diet was even better than a yoghurt diet (Alvarez-Acosta *et al*., 2009). These dietary treatments are superior to medical treatment of PD. Antibiotics are, as first line treatment, inefficient and not recommended.

Outlook

Food and nutrition have always played a crucial role in biological evolution. Nutrition also played a big role when the basis of the medical sciences where laid by early physicians like Hippocrates and Galen. With the development of a pharmaceutical industry, drugs dominated medical treatment and prevention measures. Nutrition is now increasingly perceived as an important factor for human health and – as we saw in the last section – human disease. The story of the modest disaccharide lactose is thus a strong reminder of the impact of simple nutrients on human biology.

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References

Alvarez-Acosta, T., León, C., Acosta-González, S., Parra-Soto, H., Cluet-Rodriguez, I., Rossell, M.R., and Colina-Chourio, J.A. (2009) Beneficial role of green plantain [*Musa paradisiaca*] in the management of persistent diarrhea: a prospective randomized trial. *J Am Coll Nutr* **28:** 169–176.

Anonymous (1996) Evaluation of an algorithm for the treatment of persistent diarrhoea: a multicentre study. International Working Group on Persistent Diarrhoea. *Bull World Health Organ* **74:** 479–489.

Bachmann, H., Starrenburg, M.J., Molenaar, D., Kleerebezem, M., and Vlieg, J.E. (2012) Microbial domestication signatures of *Lactococcus lactis* can be reproduced by experimental evolution. *Genome Res* **22:** 115–124.

Baqui, A.H., Black, R.E., Arifeen, S.E., Hill, K., Mitra, S.N., and al Sabir, A. (1998) Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. *Bull World Health Organ* **76:** 161–171.

Beja-Pereira, A., Luikart, G., England, P.R., Bradley, D.G., Jann, O.C., Bertorelle, G., *et al*. (2003) Gene-culture coevolution between cattle milk protein genes and human lactase genes. *Nat Genet* **35:** 311–313.

Bolotin, A., Quinquis, B., Renault, P., Sorokin, A., Ehrlich, S.D., Kulakauskas, S., *et al*. (2004) Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. *Nat Biotechnol* **22:** 1554–1558.

Brown, K.H., Parry, L., Khatun, M., and Ahmed, G. (1979) Lactose malabsorption in Bangladeshi village children: relation with age, history of recent diarrhea, nutritional status, and breast feeding. *Am J Clin Nutr* **32:** 1962–1969.

Brüssow, H. (2007) *The Quest for Food. A Natural History of Eating*. New York, NY, USA: Springer Publisher.

Brüssow, H. (2009) Europe, the bull and the Minotaur: the biological legacy of a Neolithic love story. *Environ Microbiol* **11:** 2778–2788.

Burger, J., Kirchner, M., Bramanti, B., Haak, W., and Thomas, M.G. (2007) Absence of the lactase-persistence-associated allele in early Neolithic Europeans. *Proc Natl Acad Sci USA* **104:** 3736–3741.

Chikhi, L., Nichols, R.A., Barbujani, G., and Beaumont, M.A. (2002) Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci USA* **99:** 11008–11013.

Diamond, J. (1997) *Guns, Germs and Steel. The Fates of Human Societies*. New York, USA: Norton.

Douglas, G.L., and Klaenhammer, T.R. (2010) Genomic evolution of domesticated microorganisms. *Annu Rev Food Sci Technol* **1:** 397–414.

Dudd, S.N., and Evershed, R.P. (1998) Direct demonstration of milk as an element of archaeological economies. *Science* **282:** 1478–1481.

Dunne, J., Evershed, R.P., Salque, M., Cramp, L., Bruni, S., Ryan, K., *et al*. (2012) First dairying in green Saharan Africa in the fifth millennium BC. *Nature* **486:** 390–394.

Enattah, N.S., Jensen, T.G., Nielsen, M., Lewinski, R., Kuokkanen, M., Rasinpera, H., *et al*. (2008) Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *Am J Hum Genet* **82:** 57–72.

Flatz, G., and Rotthauwe, H.W. (1973) Lactose nutrition and natural selection. *Lancet* **ii** (7820): 76–77.

Gerbault, P., Liebert, A., Itan, Y., Powell, A., Currat, M., Burger, J., *et al*. (2011) Evolution of lactase persistence: an example of human niche construction. *Philos Trans R Soc Lond B Biol Sci* **366:** 863–877.

Goh, Y.J., Goin, C., O'Flaherty, S., Altermann, E., and Hutkins, R. (2011) Specialized adaptation of a lactic acid bacterium to the milk environment: the comparative genomics of *Streptococcus thermophilus* LMD-9. *Microb Cell Fact* **10** (Suppl. 1): S22.

van de Guchte, M., Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., *et al*. (2006) The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. *Proc Natl Acad Sci USA* **103:** 9274–9279.

Holden, C., and Mace, R. (1997) Phylogenetic analysis of the evolution of lactose digestion in adults. *Hum Biol* **69:** 605–628.

Hutchison, V.H., Dowling, H.G., and Vinegar, A. (1966) Thermoregulation in a brooding female Indian python, *Python molurus bivittatus*. *Science* **151:** 694–696.

Jochims, K., Kaup, F.J., Drommer, W., and Pickel, M. (1994) An immunoelectron microscopic investigation of colostral

IgG absorption across the intestine of newborn calves. *Res Vet Sci* **57:** 75–80.
Kaper, J.B., Nataro, J.P., and Mobley, H.L. (2004) Pathogenic *Escherichia coli*. *Nat Rev Microbiol* **2:** 123–140.
Kelly, W.J., Ward, L.J., and Leahy, S.C. (2010) Chromosomal diversity in *Lactococcus lactis* and the origin of dairy starter cultures. *Genome Biol Evol* **2:** 729–744.
Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., *et al*. (2004) The role of autophagy during the early neonatal starvation period. *Nature* **432:** 1032–1036.
Kupfer, A., Müller, H., Antoniazzi, M.M., Jared, C., Greven, H., Nussbaum, R.A., and Wilkinson, M. (2006) Parental investment by skin feeding in a caecilian amphibian. *Nature* **440:** 926–929.
Lee, S.Y., Wang, Z., Lin, C.K., Contag, C.H., Olds, L.C., Cooper, A.D., and Sibley, E. (2002) Regulation of intestine-specific spatiotemporal expression by the rat lactase promoter. *J Biol Chem* **277:** 13099–13105.
de Mattos, A.P., Ribeiro, T.C., Mendes, P.S., Valois, S.S., Mendes, C.M., and Ribeiro, H.C. Jr (2009) Comparison of yogurt, soybean, casein, and amino acid-based diets in children with persistent diarrhea. *Nutr Res* **29:** 462–469.
Meloni, T., Colombo, C., Ogana, A., Mannazzu, M.C., and Meloni, G.F. (1996) Lactose absorption in patients with glucose 6-phosphate dehydrogenase deficiency with and without favism. *Gut* **39:** 210–213.
Mira, A., Ochman, H., and Moran, N.A. (2001) Deletional bias and the evolution of bacterial genomes. *Trends Genet* **17:** 589–596.
Mira, A., Pushker, R., and Rodríguez-Valera, F. (2006) The Neolithic revolution of bacterial genomes. *Trends Microbiol* **14:** 200–206.
Montalto, M., Curigliano, V., Santoro, L., Vastola, M., Cammarota, G., Manna, R., *et al*. (2006) Management and treatment of lactose malabsorption. *World J Gastroenterol* **12:** 187–191.
Moore, S.R. (2011) Update on prolonged and persistent diarrhea in children. *Curr Opin Gastroenterol* **27:** 19–23.
Oftedal, O.T. (2012) The evolution of milk secretion and its ancient origins. *Animal* **6:** 355–368.
Passerini, D., Beltramo, C., Coddeville, M., Quentin, Y., Ritzenthaler, P., Daveran-Mingot, M.L., and Le Bourgeois, P. (2010) Genes but not genomes reveal bacterial domestication of *Lactococcus lactis*. *PLoS ONE* **5:** e15306.
Reich, C.M., and Arnould, J.P.Y. (2007) Evolution of Pinnipedia lactation strategies: a potential role for α-lactalbumin? *Biol Lett* **3:** 546–549.
Richards, M.P., Jacobi, R., Cook, J., Pettitt, P.B., and Stringer, C.B. (2005) Isotope evidence for the intensive use of marine foods by Late Upper Palaeolithic humans. *J Hum Evol* **49:** 390–394.
Romer, A.S. (1968) *Notes and Comments on Vertebrate Paleontology*. Chicago, IL, USA: The University of Chicago Press.
Salque, M., Bogucki, P.I., Pyzel, J., Sobkowiak-Tabaka, I., Grygiel, R., Szmyt, M., and Evershed, R.P. (2013) Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature* **493:** 522–525.
Savaiano, D.A., and Levitt, M.D. (1987) Milk intolerance and microbe-containing dairy foods. *J Dairy Sci* **70:** 397–406.
Siezen, R.J., Renckens, B., van Swam, I., Peters, S., van Kranenburg, R., Kleerebezem, M., and de Vos, W.M. (2005) Complete sequences of four plasmids of *Lactococcus lactis* subsp. *cremoris* SK11 reveal extensive adaptation to the dairy environment. *Appl Environ Microbiol* **71:** 8371–8382.
Simoons, F.J. (1970) Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. *Am J Dig Dis* **15:** 695–710.
Tishkoff, S.A., Reed, F.A., Ranciaro, A., Voight, B.F., Babbitt, C.C., Silverman, J.S., *et al*. (2007) Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet* **39:** 31–40.
Varricchio, D.J., Moore, J.R., Erickson, G.M., Norell, M.A., Jackson, F.D., and Borkowski, J.J. (2008) Avian paternal care had dinosaur origin. *Science* **322:** 1826–1828.

