

Evolution of lactation: nutrition v. protection with special reference to five mammalian species

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The evolutionary origin of the mammary gland has been difficult to establish because little knowledge can be gained on the origin of soft tissue organs from fossil evidence. One approach to resolve the origin of lactation has compared the anatomy of existing primitive mammals to skin glands, whilst another has examined the metabolic and molecular synergy between mammary gland development and the innate immune system. We have reviewed the physiology of lactation in five mammalian species with special reference to these theories. In all species, milk fulfils dual functions of providing protection and nutrition to the young and, furthermore, within species the quality and quantity of milk are highly conserved despite maternal malnutrition or illness. There are vast differences in birth weight, milk production, feeding frequency, macronutrient concentration, growth rate and length of lactation between rabbits, quokkas (*Setonix brachyurus*), pigs, cattle and humans. The components that protect the neonate against infection do so without causing inflammation. Many protective components are not unique to the mammary gland and are shared with the innate immune system. In contrast, many of the macronutrients in milk are unique to the mammary gland, have evolved from components of the innate immune system, and have either retained or developed multiple functions including the provision of nourishment and protection of the hatchling/neonate. Thus, there is a strong argument to suggest that the mammary gland evolved from the inflammatory response; however, the extensive protection that has developed in milk to actively avoid triggering inflammation seems to be a contradiction.

Lactation: Evolution: Mammary glands: Breast-feeding: Milk

Background

The 'father' of taxonomy, Carolus Linnaeus, grouped animals with the capacity to produce milk for their young into one Class and chose the term Mammalia in 1758 to denote this Class of animals. The grouping of these animals into one Class united land quadrupeds with aquatic cetaceans (formally fish) into the new Class – Mammalia. The selection of the term mammal is unusual because it is only applicable to half of the animals in this Class (females). Yet there are a number of other traits, such as hair, sweat glands, bony palate, singular lower jawbone and three ear bones that are unique to both males and females in this Class. Wet-nursing the human infant, the equivalent to 'cuckoo care' of hatchlings, was a prevalent practice in the 'better classes' in Sweden and other European countries at this time. Linnaeus was opposed to wet-nursing and it is said

that he chose the term Mammalia because he wanted to emphasise that young mammals should be fed from their own mothers⁽¹⁾.

Currently there are more than 4000 species of mammal living in many and varied environments, from Weddell seals in the Antarctic seas to kangaroos in the central Australian deserts. Pond⁽²⁾ attributed this diversity in habitat to a primary advantage that lactation confers on mammals, that is, the ability to reproduce and nourish their young in any environment that supports the health and wellbeing of the adult. Thus, lactation allows for nutrients that are both distant in time and space to be transferred to the young. For example, brown bears nourish their young while estivating, baleen right whales nourish their young while fasting for several months (lactation strategies that only may be possible because of these animals' size) and primates lactate

Abbreviations: C/EBP, CCAAT enhancer-binding protein; Jak, Janus kinase; sIgA, secretory IgA; Stat, signal transducer and activator of transcription; XOR, xanthine oxidoreductase.

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for several years in tropical regions where fast-growing vegetation has a low nutritional value for the weanling.

The evolutionary origin of the mammary gland has been difficult to establish because little knowledge can be gained on the origin of soft tissue organs from fossil evidence. Although other animals such as pigeons, sharks, salamanders and skinks can nourish their young from various bodily secretions, mammals are the only animals that secrete a complex nutritive and protective fluid from complicated skin glands to provide the sole source of nourishment for the growth, development and protection for either their hatchlings or neonates. The development of a fully functional lactating mammary gland predates the origin of mammals; the primitive beginning for the mammary gland probably dates back more than 310 million years (the Pennsylvanian epoch) to when the Synapsida branch of the Amniota evolved to have a soft glandular integument rather than the scales of related taxa. Many theories have been advanced to explain the evolution of lactation, but recently Oftedal^(3,4) and Vorbach *et al.*⁽⁵⁾ have taken different innovative approaches to developing their theories on ancestral development of the mammary gland and lactation. Oftedal took a multi-functional approach looking for evidence in support of a developing role for lactation during the course of synapsid evolution that could have led to the development of mammals. This approach included identification of skin glands that could have been ancestral mammary glands, analysis of the comparative anatomy of existing 'primitive' mammals, including the platypus and kangaroo, and the conditions required for successful reproduction in animals that laid porous parchment-shelled eggs. Vorbach *et al.* examined the metabolic and molecular synergy between the highly conserved innate immune system and both milk composition and the regulation of the synthesis and secretion of milk. This theory also has the ancestral origin of the mammary gland dating back to the soft-skinned synapsids of the Pennsylvanian epoch. These theories initially appear to be quite distinct, but it is of interest to explore them in more detail to determine how both concepts relate to Darwin's theory of natural selection.

Oftedal theory

Oftedal first discussed in detail the development of the mammary gland⁽³⁾ and then considered how the gradual development of a maternal exocrine secretion could have assisted reproductive efficiency in the evolutionary line beginning with the parchment-shelled egg-laying synapsids⁽⁴⁾. He suggested that the mammary gland was derived from an ancestral apocrine-like gland associated with hair follicles and noted that this association is retained in the nipple-less mammary patch of monotremes and in the early ontogenetic development of marsupials. Furthermore, in contrast to the rigid-shelled eggs of birds, the parchment-shelled eggs are porous and therefore could easily become desiccated if subjected to low humidity and/or higher temperatures. Thus, Oftedal proposed that the evolutionary precursor to milk was secreted from hypertrophied skin glands that were associated with hair follicles and the secretion was favoured by natural selection because it prevented desiccation and microbial attack of the

parchment-shelled eggs of the synapsids. Furthermore, this secretion gradually became nutrient rich and this paralleled a progressive decline in egg size with a possible dual role of providing some transfer of nutrients during egg incubation and subsequently an enteral supply of nutrients for the hatchlings.

Brawand *et al.*⁽⁶⁾ provide further evidence for this concept by showing the progressive loss of function of vitellogenins, the proteins that transport nutrients to the egg yolk, during mammalian evolution. Interestingly, the two gene sequences related to vitellogenin identified in the genome of the egg-laying mammal, the platypus, indicate that their inactivation occurred after the evolution of lactation. Further analysis of the platypus genome revealed the presence of a κ -casein orthologue, a gene involved in micelle stabilisation that also prevents blockage of the milk ducts. They proposed that the evolution of lactation reduced the selective pressure to preserve yolk reserves and the subsequent development of placentation in marsupials and eutherians allowed for the loss of yolk-dependence.

Vorbach, Capecchi and Penninger theory

On the other hand, Vorbach *et al.* compared the biochemical connections between the dual functions of milk, the provision of both nutrition and innate immunological protection⁽⁵⁾. They provided new evidence in support of the theory that the mammary gland evolved from simple skin glands that secreted mucous containing a variety of antimicrobial molecules for the protection of damaged skin. They proposed that lactation partly evolved from the highly conserved inflammatory response to tissue damage and infection. Therefore they concluded that the sequence of development was first to provide protection and subsequently nutritional components evolved to nourish either hatchlings or newborn mammals. Xanthine oxidoreductase (XOR) and lysozyme were highlighted as two important antimicrobial enzymes of the innate immune system that became key regulators in the development of the nutritional components of milk. Due to gene sharing, the housekeeping enzyme XOR is involved in purine catabolism and is required for the secretion of the milk fat globules⁽⁷⁾. This discovery led to the investigation of the role of XOR in the evolution of the innate immune system⁽⁸⁾ and the mammary gland⁽⁵⁾. Multiple features of the innate immune system involve XOR including bactericidal activity, the production of reactive oxygen species as well as the recruitment and activation of neutrophils and macrophages. As XOR is multifunctional and predates the innate immune system Vorbach *et al.* suggest that it was a central molecule in the development of this system⁽⁸⁾. α -Lactalbumin, a key whey protein and a subunit of the lactose synthase that catalyses the synthesis of lactose, evolved from gene duplication of lysozyme (40% amino acid homology). Lactose provides both dietary carbohydrate and the osmotic gradient for the formation of the aqueous phase of milk in most mammals⁽⁵⁾. A further unification of this theory of the evolution of the protective and nutritional functions of milk was suggested by the central roles in inflammation and lactation of the two signalling pathways, NF- κ B and Janus

kinase/signal transducer and activator of transcription (Jak/Stat) (see Fig. 1).

NF- κ B is a transcription factor central to multiple inflammatory and developmental pathways, including the development of the mammary gland. The importance of NF- κ B in lactation was demonstrated in mice that contain an inhibitor of κ B kinase- α (IKK- α) knock-in mutation, which prevents IKK- α from phosphorylating the inhibitors of NF- κ B. During pregnancy, these transgenic mice display decreased differentiation of mammary epithelial cells, resulting in impaired lactation⁽⁹⁾. The functions of two crucial lactogenic hormones, prolactin⁽¹⁰⁾ and oxytocin⁽¹¹⁾, are also regulated by NF- κ B^(12,13). The promoter of the human oxytocin receptor gene contains three NF- κ B binding sites; however, whilst confirmation that NF- κ B alters the promoter activity of oxytocin receptor has been shown in human primary myocytes⁽¹³⁾, investigations of its role in regulation of the oxytocin receptor gene in the mammary gland are awaited with interest. Furthermore, one of the major inflammatory cytokines TNF- α activates the human prolactin promoter via the NF- κ B pathway⁽¹²⁾ and it is possible that this pathway also activates XOR⁽⁵⁾.

It is through the Jak/Stat pathway that prolactin as well as interferon- γ stimulates the expression of XOR⁽⁵⁾. Activation of Jak proteins (usually by receptor binding) results in the phosphorylation and activation of Stat proteins. Upon activation, Stat proteins translocate to the nucleus where they are able to regulate inflammatory gene expression. In the mammary gland, Jak2 is activated by prolactin, resulting in the activation and nuclear translocation of Stat5. Mice with double knock-outs of both Stat5 iso-forms have

reduced expression of milk proteins during lactation and defects in the expansion of macrophages in inflammatory exudates⁽⁵⁾.

The CCAAT enhancer-binding protein (C/EBP) family of proteins play essential roles in inflammation, adipogenesis, differentiation, metabolism and lactation⁽¹⁴⁾. C/EBP β -deficient mice show aberrant ductal morphogenesis and decreased secretory differentiation⁽¹⁵⁾, possibly due to their involvement in many lactation pathways. C/EBP β has been shown to activate the promoters of the human oxytocin receptor gene⁽¹³⁾ and the rat XOR gene⁽¹⁶⁾. Interestingly, C/EBP β was shown to co-immuno-precipitate with NF- κ B and when concentrations of these two molecules are simultaneously increased in cultured human myocytes the oxytocin-receptor gene promoter activity is dramatically increased⁽¹³⁾.

In mesenchymal stem cells, prolactin has been shown to increase the expression of C/EBP β and PPAR γ ⁽¹⁷⁾. In this connection it is of interest that C/EBP β and C/EBP δ also have the ability to stimulate the expression of PPAR γ ⁽¹⁸⁾, a gene that regulates fat cell differentiation and inhibits inflammation⁽¹⁹⁾. Altered PPAR γ expression has dramatic impacts on the young. Mice deficient for PPAR γ in haematopoietic and endothelial tissues have increased expression of lipid oxidation enzymes in the mammary gland and their milk contains inflammatory lipids that are toxic to the young⁽¹⁹⁾. PPAR γ expression is also affected by glucocorticoid and the response varies depending upon tissue type. In isolated human adipocytes glucocorticoid treatment resulted in an increase of PPAR γ ⁽²⁰⁾ expression whereas in the labyrinth zone of the placenta in mice PPAR γ expression decreased with glucocorticoid treatment⁽²¹⁾. In this connection, the fatty acid composition of the milk of preterm mothers who have received parturum glucocorticoid therapy requires investigation.

Darwin – evolution

Darwin⁽²²⁾ published his comprehensive book *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life* on theory of evolution in November 1859. He observed that man could make changes by the selection of particular variations in plants and animals to produce divergent domestic breeds and strains (domestic productions).

‘There is much difficulty in ascertaining how much modification our domestic productions have undergone; but we may safely infer that the amount has been large, and that the modifications can be inherited for long periods. As long as the conditions of life remain the same, we have reason to believe that a modification, which has already been inherited for many generations, may continue to be inherited for an almost infinite number of generations. On the other hand we have evidence that variability, when it has once come into play, does not wholly cease; for new varieties are still occasionally produced by our most anciently domesticated productions.

‘Man does not actually produce variability; he only unintentionally exposes organic beings to new conditions

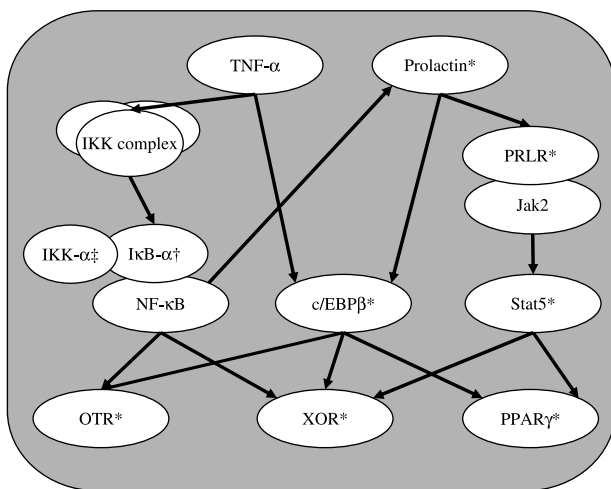


Fig. 1. Lactation-impaired transgenic models. Genes crucial to lactation and their interaction with the inflammation modulators; NF- κ B, TNF- α and prolactin. Impaired lactation phenotypes have been observed in transgenic mouse models when genes have either been knocked-out (*signal transducer and activator of transcription 5 (Stat5), PPAR- γ), over-expressed (†inhibitor of κ B- α (IkB- α)⁽⁵⁾) or mutated (‡IkB kinase- α (IKK- α)). The ability of these molecules to regulate the protein activity or RNA expression has been demonstrated in the mammary gland, or the potential to interact based on either promoter gene analysis or interactions has been observed in other tissues. PRLR, prolactin receptor; Jak2, Janus kinase 2; C/EBP β , CCAAT enhancer-binding protein β ; OTR, oxytocin receptor; XOR, xanthine oxidoreductase.

of life, and then nature acts on the organisation, and causes variability. But man can and does select the variations given to him by nature, and thus accumulate them in any desired manner. He thus adapts animals and plants for his own benefit or pleasure. He may do this methodically, or he may do it unconsciously by preserving the individuals most useful to him at the time, without any thought of altering the character of a breed by selecting, in each successive generation, individual differences so slight as to be quite inappreciable by an uneducated eye. This process of selection has been the great agency in the production of the most distinct and useful domestic breeds.'

Darwin reasoned that over geological time gradual alterations in the natural environment could similarly result in the development of new species by 'natural selection'.

'There is no obvious reason why the principles which have acted so efficiently under domestication should not have acted under nature. In the preservation of favoured individuals and races, during the constantly-recurrent Struggle for Existence, we see the most powerful and ever-acting means of selection.'

...
'With animals having separate sexes there will in most cases be a struggle between the males for possession of the females. The most vigorous individuals, or those which have most successfully struggled with their conditions of life, will generally leave most progeny. But success will often depend on having special weapons or means of defence, or on the charms of the males; and the slightest advantage will lead to victory.'

...
'This preservation of favourable variations and the rejection of injurious variations, I call Natural Selection.'

...
'We shall best understand the probable course of natural selection by taking the case of a country undergoing some physical change, for instance, of climate. The proportional numbers of its inhabitants would almost immediately undergo a change, and some species might become extinct.'

Contemporary considerations

It follows that an understanding of the physiology of lactation (the most vulnerable phase of the reproductive cycle) in wild mammals is vitally important to determine environmental changes that may, in time, threaten the survival of many of the 4000 species of mammals. It is possible to define lactation behaviour in wild animals in relation to suckling behaviour, feeding frequency and duration of lactation. However, there is little knowledge of the comparative complexities of milk composition in these species⁽²³⁾. In contrast to wild mammals, the transition to domestication began only about 11 000 years before the present. Due to their economic importance 'genetically superior' animals (traits preferred by humans) have been selected and their natural environment has been extensively

modified to maximise production. Although there is extensive research on the physiology of lactation in dairy animals such as the cow and the goat, much less is known about lactation in non-dairy domestic mammals such as beef cows and sows. In this context, women are neither wild nor domesticated. Therefore understanding the physiology of human lactation poses several difficult problems. First, it is difficult to define 'normal' behaviour for human lactation in terms of suckling behaviour, feeding frequency and duration of lactation. Second, the function of human lactation can be discussed in terms of protection, nutrition including functional food considerations, and breast-feeding in relation to the psychological development of the infant. However, compared with other mammals it is difficult to apply objective rational standards for breast milk intake and infant growth and development. Thus the norms for other mammals such as continuation of a genotype by either natural selection or economic value do not necessarily apply to human lactation and infant development.

From these considerations we have chosen to review the physiology of lactation by comparing the nutritional and protective roles of lactation in five mammalian species. We have selected two mammals that have evolved by natural selection (the quokka (*Setonix brachyurus*) and the rabbit), two mammals that have been domesticated and selected for special traits (the sow and the cow) and one mammal that is difficult to classify (the woman). The role of lactation will be discussed in relation to how milk production, milk removal, infant nutrition and protection of the young against pathogens relates to the two recent theories on the evolution of lactation.

Duration of lactation and neonatal growth

Lactation in the quokka⁽²⁴⁾ as in other macropod marsupials can be divided into four functional phases⁽²⁵⁾. The quokka gives birth to and suckles a single young (joey) for approximately 300 d⁽²⁶⁾. For the first 70 d of lactation the young is permanently attached to the teat, then from 70 to 180 d postpartum the joey remains in the pouch⁽²⁶⁾ and presumably suckles intermittently. From 180 to 200 d of lactation there is a transition period during which the young makes excursions from the pouch⁽²⁶⁾. After 200 d, the joey remains outside the pouch, returning to suckle until lactation ceases⁽²⁶⁾. It has been suggested that permanent pouch exit in marsupials is influenced by a combination of the mother controlling access to the pouch and changes induced by the development of the joey, for example, increased heat production^(27–29).

The quokka has a birth weight of about 0.3 g⁽³⁰⁾ and doubles its birth weight during the first 3 d of lactation (Department of Zoology, University of Western Australia, unpublished results). Its body weight continues to increase and by the end of the lactation period the average weight is 1689 (SE 27.4) and 2024 (SE 103) g for females and males, respectively. This represents a staggering 5600- and 6700-fold increase in birth weight for females and males, respectively, during lactation⁽²⁶⁾. In contrast, there is only a four- to six-fold increase in body weight in rabbit kittens, piglets, calves and human infants during lactation (Table 1). Furthermore, Miller⁽²⁶⁾ found that in the quokka the rate of

Table 1. Variation in the characteristics of lactation in rabbits, quokkas (*Setonix brachyurus*), pigs, cows and humans

	Quokkas	Rabbits	Pigs	Cows	Humans
Wild or domesticated	Wild	Wild	Domesticated	Domesticated	
Number of young	1	6	4–13	1	1
Number of nipples	4	8–10	12–16	4	2
Growth					
Birth weight (g)	0.3	70–100	1420	37 900	3300
Time to double birth weight (d)	3	6–7	8	36	105–126
Weaning weight/birth weight	5600–6700	4–6	4–6	6	4
Lactation					
Length of lactation (d)	300	28–35	56	180–240	
Daily milk production (g)	30 (165 d postpartum)	140–320	4500–5700	10 000	450–1126
Interval between feeding (h)	Permanently attached until 70 d postpartum	24	1	5	1–11
Protection					
Transfer of immunoglobulins during pregnancy	None	IgG	None	None	IgG
Immunoglobulin proportions in colostrums*	IgG > IgA	IgA > IgG > IgM	IgG > IgA > IgM	IgG > IgA, IgM	IgA > IgM > IgG
Total concentration (mg/ml)		7.1	72	58	20
Nutrition (mid-lactation)					
Fat concentration (g/l)	50	22	55	48	38
Fatty acid substrate	Acetate and glucose	Acetate and glucose	Acetate and glucose	Acetate	Glucose
Predominant fatty acids	LCFA	SCFA	LCFA	MCFA and LCFA	LCFA
Protein concentration (g/l)	60	103	56	32 (80 % casein)	9 (17 % casein)
β-Lactoglobulin	Yes	No	Yes	Yes	No
Predominant carbohydrate	Oligosaccharides	Lactose	Lactose	Lactose	Lactose
Lactose concentration (g/l)	30	22	55	48	70
Oligosaccharides concentration (g/l)	40				12.9

LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids.
* For graphical representation of immunoglobulin proportions, see Fig. 6.

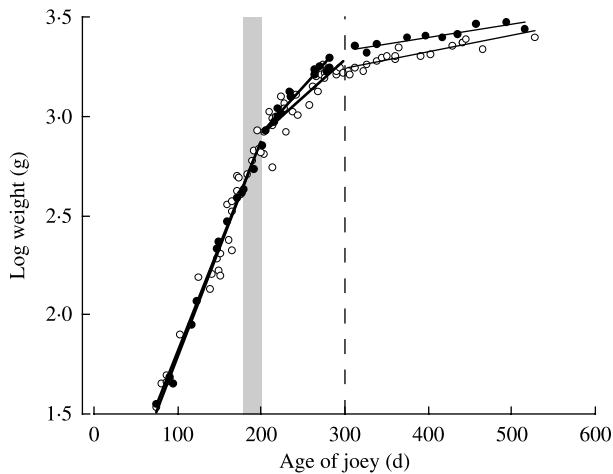


Fig. 2. The log plot of the body weights of female (○) ($n=78$) and male (●) ($n=39$) quokkas (*Setonix brachyurus*) for the first 550 d postpartum⁽²⁶⁾. (■), Transition period while the young is still returning to the pouch; (---), end of lactation.

increase in weight up to 200 d was linear (Fig. 2) and then declined significantly when the joey emerged from the pouch, clearly demonstrating the importance of lactation to the growth and development of the quokka. There is concomitant growth and development of the mammary gland in the quokka as in other macropods over this period. This could simplistically be attributed to a 'demand and supply' response of the mammary gland to the increasing appetite of the young joey; however, it has been demonstrated in the tamar wallaby that the mother exercises considerable control of the development of the young through inherent sequential changes in milk composition⁽³¹⁾.

In contrast, wild rabbits (*Oryctolagus cuniculus* (L.)) usually suckle about six young in a litter. The rabbit is also born in an altricial state (hairless and blind) and the newborn are protected in underground nests. The average birth weight of the young is 70–100 g, depending upon maternal body weight⁽³²⁾ and they double their birth weight in 6–7 d⁽³³⁾. The young begin to consume solid foods during the third week when they weigh 200–400 g (depending on maternal body weight) and are weaned between the age of 4 and 5 weeks. Unfortunately, most of the information on lactation and litter growth in rabbits has been derived from either experimental colonies of rabbits or commercial rabbit farms and therefore probably represents growth and milk production under optimal conditions. Nevertheless, the reproductive capacity of the rabbit allows it to respond relatively quickly to favourable environmental conditions to maximise its reproductive potential. Therefore, over the lactation period of 300 d that is required for a single quokka joey, the wild rabbit could produce three litters to independence⁽³⁴⁾. On the other hand, from an ecological perspective, the strategy of a low investment in pregnancy and the sustained investment in lactation enables the quokka to reproduce in areas that have limited resources over prolonged periods without endangering the wellbeing of their young⁽³⁴⁾. The duration of lactation in the rabbit and quokka as well as in other wild mammals is highly predictable and any displacement in these patterns would be viewed with concern by environmental ecologists.

The wild relative of the domestic pig (*Sus scrofa*) is not yet extinct, and therefore there is an opportunity to compare wild and domesticated lactation. Wild sows build a nest for their litters and the piglets remain in the nests for about 2 weeks and are weaned after an 8-week lactation. From the little that is known about lactation in the wild sows, it has been observed that groups (sounders) of up to four sows synchronise their pregnancies enabling mutual protection and care of the young that extends to cross-suckling between litters⁽³⁵⁾.

An approximate indicator of economic productivity in commercial piggeries is the number of piglets reared per sow per year. To increase profitability the length of lactation has been truncated to as short as 3 weeks and practices are employed to keep the interval between weaning to conception to a minimum. The average birth weight of the piglet is approximately 1.42 kg, this doubles within 8 d⁽³⁶⁾ and the average weight at 19 d is 4.21 kg⁽³⁷⁾. It is of interest that the success in obtaining the maximum numbers of piglets reared per sow per year, in part, depends upon successful cross-fostering of piglets soon after birth so that litter numbers are evened out and each piglet can gain access to a preferred teat. This management practice may have been facilitated by the communal behaviour of the wild sows.

In the dairy cow (*Bos taurus*) the aim is to produce one calf per cow per year and this also may have led to modification of the lactation period. Both human and domestic animal performances have increased over time. For example, there has been an increase in both human athletic performances in the Tour de France⁽³⁸⁾ and milk production in Holstein dairy cows in the USA⁽³⁹⁾ over the past 35 years (Fig. 3). At least in the latter case the improvement has been due to both genetic selection and physiological manipulation, so now cows can accomplish very high and sustained levels of milk production. As a result they are 'dried off' at quite high levels of milk production and consequently are exposed to a physiologically abrupt and difficult cessation of milk synthesis.

The newborn calf is precocious, being born well-developed and able to stand within a few minutes of birth⁽⁴⁰⁾. Beef cows at pasture suckle their calves for 6–8 months and achieve weaning weights of approximately 190–220 kg⁽⁴¹⁾, and calves take about 36 d to double their birth weight⁽⁴²⁾. Thus in both the domesticated sow and cow, management practices designed to maximise profit dictate the duration of lactation.

It is tempting to suggest that the breast-feeding in hunter-gatherer societies represents 'normal' lactation in women (*Homo sapiens*). However, as pointed out by Fischler⁽⁴³⁾:

'Man feeds not only on proteins, fats, and carbohydrates, but also on symbols, myths, and fantasies.'

That is, the selection of food is influenced not only by physiological requirements, and perceptual and cognitive mechanisms, but also on the basis of cultural and social influences. The latter habits are probably largely a result of arbitrary intrinsic coherence⁽⁴³⁾. Therefore, it is likely that cultural and social pressures in traditional societies have had a similar impact on lactation behaviour as has been experienced in Western societies. For example, women in most traditional societies consider colostrum to be bad milk

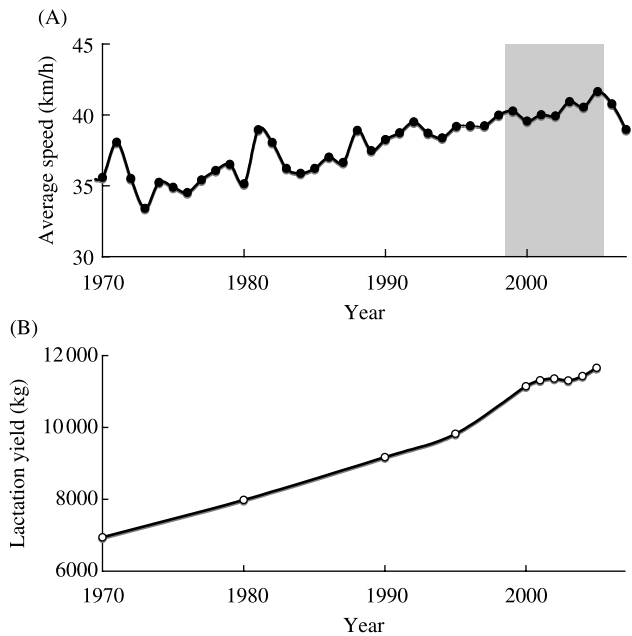


Fig. 3. (A) Performance improvements from 1970 to 2007 in cycling. The average speed of each winner of the Tour de France is plotted. (□), Years that Lance Armstrong won the Tour de France, including the fastest tour ever (2005). Armstrong's significant increase in performance after beating testicular cancer (1996) has recently been linked to hormonal changes associated with the removal of his left testicle. Permanent changes in serum hormone levels are reported to occur after orchiectomy that result in increased gonadotropins (including the lactogenic hormone prolactin) in an effort to maintain testosterone levels. Increased ratios of luteinising hormone, follicle-stimulating hormone and prolactin to testosterone could improve fuel metabolism, resulting in increased efficiency, delayed fatigue, enhanced recovery and subsequently increased performance in multi-stage races such as the Tour de France⁽¹⁷⁰⁾. (B) Performance improvements from 1970 to 2007 in the dairy industry. The average standardised lactation yields (kg) are plotted for Holstein cows from 1970 to 2006 from the USA.

and therefore do not commence breast-feeding until their milk 'comes in' about 2 d after birth⁽⁴⁴⁾. Unlike the piglet that is born with only about 2 % body fat, the human infant is born with more than 10 % body fat⁽⁴⁵⁾ and therefore the human baby is able to survive such nutritional insults. Nevertheless, in light of present knowledge, deprivation of the protective benefits of colostrum cannot be viewed as a beneficial cultural tradition.

The median birth weight of babies is reported by the WHO to be 3.3 (SD 0.5) kg and the time taken to double birth weight for human babies on the 50th percentile for growth is 18 weeks for girls and 15 weeks for boys⁽⁴⁶⁾. This is a very slow growth rate compared with other mammals (for example, quokka, rabbit, pig) that at least double their birth weight in the same period of time that it takes the human baby to regain its birth weight (Table 1).

The duration of breast-feeding in traditional societies varies greatly. Australian aboriginal babies are breast-fed into their sixth year of life⁽⁴⁷⁾ whereas the Hottentots only breast-feed for a few months⁽⁴⁸⁾. However, to be consistent with the duration of lactation in other primates, the average duration of lactation in traditional women would be

expected to be about 3–4 years⁽⁴⁸⁾. Yet some contemporary well-intentioned health professionals in Western countries have considered breast-feeding a 4-year-old to be child abuse. Thus, it is impossible to determine 'normal' weaning behaviour for both women and domesticated mammals because human cognitive input has resulted in artificial and generally abrupt termination of the lactation period based on either social and cultural 'acceptability' or economic expediency. This is in contrast to wild mammals⁽⁴⁹⁾ such as the quokka and the rabbit that have different but consistent weaning behaviour patterns. In general the young are weaned when they are able to consume and adequately digest adult foods.

There is obviously wide variation in the length of the lactation period and the rates of growth of suckling mammals. The interspecies variation in growth rate is probably due to multiple causes including phylogenetic constraints and, in particular, to adaptation to certain features of the animal's environment such as infant mortality rate, adult food requirements and food availability⁽⁵⁰⁾. Since infant growth rate and length of lactation have not been highly conserved across the mammals, these factors do not add greatly to our understanding of the evolution of lactation in mammals.

Milk removal

Suckling frequency

Observation from dawn to dusk of breast-feeding in the !Kung hunter-gatherers⁽⁵¹⁾ and village people in Papua New Guinea⁽⁵²⁾ found that babies were breast-fed more than once per h but for only a few minutes at a time. All of these mothers also breast-fed at night but observations on frequency could not be carried out. A major sociological intervention on the frequency of breast-feeding was initiated by William Cadogan in 1748 in his 'Essay upon the nursing and management of children from their birth to 3 years of age'. Cadogan strongly supported breast-feeding but was concerned about obesity in breast-fed babies and therefore recommended restricted breast-feeding⁽⁵³⁾ (Fig. 4).

By Night I

would not have them fed or suckled at all;
that they might at least be hungry in a Morn-
ing. It is this Night-feeding, that makes them
so over-fat and bloated.

Their Meals, and in my Opinion their sucking
too, ought to be at slated Times, and the same
every Day; that the Stomach may have Inter-
vals to digest, and the Appetite return.

Four times in four and twenty
Hours will be often enough to give it Suck;
letting it have as much as it will take, out of
both Breasts, at each time.

Fig. 4. Excerpts from Cadogan's 'An essay upon nursing, and the management of children, from their birth to three years of age'⁽⁵³⁾.

From this small beginning, by the early to mid-twentieth century scheduled breast-feeding became entrenched with the support of influential people such as Fredrick Truby-King⁽⁵⁴⁾ and was, in part, responsible for a gradual decline in the proportion of women choosing to breast-feed their babies. Extended intervals between breast-feeds compromised milk production in mothers with smaller breasts⁽⁵⁵⁾ because they have a smaller storage capacity⁽⁵⁶⁾. Indeed, Wickes^(48,57–60) in reviewing the history of infant feeding optimistically predicted that the revival of breast-feeding would follow the abandonment of scheduled feeding and its replacement with feeding on demand:

‘When this regime (demand feeding) becomes universally adopted, as it surely will, so then the last chapter on the history of infant feeding will be concluded.’

Feeding ‘on demand’ is a somewhat unfortunate phrase as it may be taken to imply that babies are demanding when, in fact, the concept is that the baby’s appetite should determine the frequency and quantity of milk consumed (Fig. 5). In this connection, there is considerable evidence to suggest that infants that are permitted to breast-feed to appetite have a healthy growth rate. This shows that the baby’s appetite control functions effectively from an early age. Indeed, it seems that appetite control operates well when parents do not know how much food (breast milk) the baby is consuming at each meal (breast-feed). Obesity problems in pre-school children seem to accelerate when other foods begin to be eaten and parents then consider that they are better judges of infant appetite.

More than three-fold differences were observed in the number of feeds per d and in night feeding in a study⁽⁶¹⁾ of women who were exclusively breast-feeding their infants from 1–6 months postpartum. Since only 14% of babies always consumed milk from both breasts at each suckling

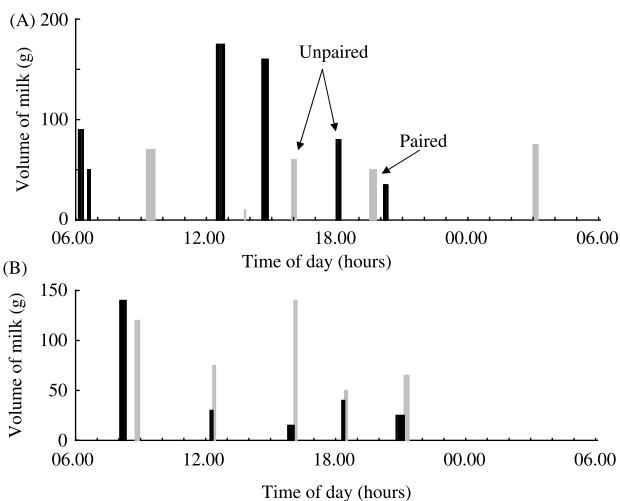


Fig. 5. Pattern of 24 h milk intake for two infants measured using the infant test-weigh method⁽¹⁷²⁾. The volume transferred from the left (□) or right (■) breast is plotted against the time and duration of each feed. Feeds were defined as paired if they occurred within 30 min of each other or unpaired if the interval was longer than 30 min. A 64-d-old infant had a combination of paired and unpaired feeds with variable intervals between feeds (A). The feeding pattern for an infant aged 165 d shows paired breast-feeds occurring regularly during the day (B).

and 28% of babies always consumed milk from only one breast at a suckling, a breast-feed was defined as the baby taking milk from a particular breast at a suckling. Thus if milk was taken from both breasts at a suckling it was considered to be two breast-feeds (Fig. 5). Based on this definition breast-feeding frequency varied from five to seventeen times in 24 h with no trend with stage of lactation and at 6 months of lactation 58% of the babies breast-fed at night.

In contrast to women, suckling patterns are relatively consistent within other species but highly variable between species (Table 1). The quokka remains attached to the teat for the first 70 d postpartum. On the other hand, seals have unusual feeding patterns, for example, the Antarctic fur seal suckles the young at about 6 h intervals for the first 7–8 d after birth and then returns to sea to feed for up to 4 weeks, returning to suckle the young for 2 d before returning to the sea to feed⁽⁶²⁾. This suckling/feeding pattern is repeated seventeen times and then the pup self-weans⁽⁶³⁾.

The calves of beef cows suckle on average 5.0 ± 0.1 times per d and the average time spent suckling in a 24 h period was 46 ± 1 min⁽⁶⁴⁾. On the other hand, traditionally, dairy cows are milked twice per d. Although increasing the frequency of milking from two to four times per d increases milk production⁽⁶⁵⁾, it is not sufficient to offset the extra cost involved in more frequent milking except in very high-producing cows. Piglets suckle every 50–60 min day and night with a very consistent behaviour pattern. A suckling lasts 2–5 min in piglets but the duration of milk flow during that period is <15 s⁽³⁶⁾. Sows in close proximity synchronise their suckling times; however, some sucklings are unsuccessful in that the piglets do not obtain milk. Under these circumstances the litter will return to suckle at a shorter interval and then gradually lengthen the interval and synchronise with the other sows over the next two to three sucklings⁽³⁶⁾. Compared with the pig, rabbits have long intervals of about 24 h between sucklings^(33,66). Suckling in the rabbit lasts 2–5 min during which time up to 250 g milk may be transferred to the litter⁽⁶⁷⁾. It is difficult to determine the exact suckling behaviour of the quokka because it remains permanently attached to the teat for the first 70 d postpartum and then remains confined to the pouch for a further 110 d and presumably suckles intermittently.

There is a wide range in the frequency of suckling between different species and behaviour patterns have evolved to suit the maternal physiology. Furthermore there is considerable flexibility in frequency of suckling in women. Similarly in cattle, the lower-producing beef cows are suckled five times per d, whereas in the dairy industry high-producing cows are traditionally milked twice per d without significant detrimental effects. Thus suckling frequency is not highly conserved between mammals and varies greatly both between and within species. Nevertheless, this variation in suckling frequency suggests that there must be intriguing control mechanisms regulating the rate of milk synthesis so that it can respond to variations in the offspring’s appetite and intervals between sucklings.

Milk ejection reflex

The milk ejection reflex is a neuro-hormonal reflex that is triggered by stimulation of the nipple or teat area activating

a neural pathway that brings about the systemic release of oxytocin from the posterior pituitary gland. Oxytocin, in turn, causes the contraction of the myoepithelial cells surrounding the alveolus and the expulsion of milk towards the nipple. Oxytocin is a small peptide (nine amino acids) that, in addition to causing milk ejection, has numerous functions related to reproductive and cardiac function⁽⁶⁸⁾ as well as modulating social, reproductive and aggressive behaviours⁽⁶⁹⁾. The milk ejection reflex is highly conserved across all species of mammal, from the echidna and quokka to women and sows, and is essential for successful lactation. Oxytocin is reported as one of the most highly conserved hormones, as oxytocin-like peptides exist in fish, reptiles, birds and mammals differing by only one or two amino acids⁽⁷⁰⁾. Thus bonding and maternal behaviours that are protective and regulated by oxytocin may have been present before lactation evolved. Subsequently, when lactation evolved oxytocin was co-opted to have an essential role in nutrition, by facilitating milk removal.

In women, sows, rabbits and quokkas little or no milk can be obtained without activation of the milk ejection reflex whereas in cows some milk can be removed from the udder without milk ejection because of the storage of milk in teat and gland cisterns⁽⁶⁷⁾. The importance of oxytocin in facilitating milk ejection has been demonstrated by gene knock-out studies in laboratory animals where the young of knock-out mothers die soon after birth⁽¹¹⁾. Furthermore, the important role of milk ejection in lactation is clearly illustrated by the characteristic behaviour pattern associated with suckling and the high level of control over milk ejection that is observed, for example, in the domestic sow. Initially there is vocalisation by the sow and jostling by the piglets to gain access to their preferred teat. This is followed by a period of nuzzling of the mammary glands and slow sucking. Then the amplitude and frequency of the sow's vocalisation increases as oxytocin is released, followed by rapid sucking by the piglets for a period of about 15 s, followed by slow sucking and nuzzling and then a return to a rest period. Since milk is only available for a short period (15 s) and flow ceases from all teats at the same time regardless of the volume of milk that has been removed, stronger piglets are unable to out-compete weaker piglets for milk and thus this strategy favours the survival of larger litters.

The universal requirement of milk ejection for successful lactation suggests that the evolution of the milk ejection reflex was central to the functional development of the mammary gland. In this connection, it is of interest that there is a shared afferent neural pathway from the nipple to the hypothalamus for the activation of both oxytocin and prolactin release⁽⁷¹⁾. Furthermore, at the molecular level, NF- κ B is not only important for the differentiation of mammary epithelial cells⁽⁵⁾ and rapid-response inflammatory pathways⁽⁷²⁾ but also is involved in regulation of the oxytocin receptor (Fig. 1).

Suckling action

Despite vast differences in maturity of young mammals at birth, within minutes of parturition all mammalian species are capable of removing milk from the nipple or teat of their mother. It is quite amazing to consider marsupials, such as

the quokka, which are born before organogenesis is complete, the equivalent of an approximately 10-week-old human embryo. Despite being extremely altricial, feeding is possible because they have developed the oral apparatus and major physiological systems much earlier than other species⁽⁷³⁾. In addition, despite differences in maturity and morphology, all mammalian species, with the exception of the platypus and echidna, suckle either a nipple or a teat to remove milk⁽⁷⁴⁾. There is compelling evidence that lactation evolved to prevent parchment shell egg desiccation by spreading a thin layer of fluid from glands associated with hair follicles over the egg^(3,4). Oftedal also hypothesised that nipples may have been incompatible with spreading fluid over the egg and that when viviparity evolved, protruding hairs may have interfered with suckling. Thus nipples may have evolved in lineages in which live-born neonates resulted⁽³⁾. Adaptations such as a secondary hard-palate and jaw changes allow neonates to suckle⁽³⁾, and nipples provide an easier attachment site compared with a mammary patch.

Suckling is a requirement for the survival of the neonatal mammal and involves the co-ordination of sucking, swallowing and breathing so that the efficient intake of milk is achieved while maintaining blood oxygen saturation levels and preventing food aspiration⁽⁷⁵⁾. Suckling has been studied in many species using various methods⁽⁷⁴⁾; however, controversy exists as to the role of suction *v.* compression in the removal of milk from the mammary gland. Ardran *et al.*^(76–78) used cineradiography to investigate sucking in infants, kid goats and lambs. They described a peristaltic stripping motion of the tongue that resulted in teat distortion and expression of milk. They also concluded that the function of the observed negative intra-oral pressure was to refill the teat. However, they also noted that the response of the young is dependent on the shape and texture of the teat used, as teat rigidity and flow resistance will influence the pressures applied by the young^(77,78). Thus, variation in suckling patterns during bottle feeding, combined with the problems associated with the imaging techniques used (unnatural positioning, milk indistinguishable from soft tissues), may explain some of the controversy regarding the function of vacuum.

Recently Geddes *et al.*⁽⁷⁹⁾ investigated infant sucking during breast-feeding by simultaneously recording ultrasound images of the intra-oral cavity and measuring intra-oral vacuum. The improvements in ultrasound technology have allowed for better imaging of oral structures, ducts in the nipple and milk flow, without compromising positioning and attachment of the infant. Thus the movement of the tongue and the changes in intra-oral vacuum could be related to the periods of milk flow. They observed that when the infant attached to the breast it created and maintained a seal (baseline vacuum, -64 (SD 45) mmHg) during both sucking bursts and pause periods. Baseline vacuum appears to be a general feature in mammals as there are many observations of the suckling young remaining attached when either the mother moves or the mother is lifted up. Indeed, this also can occur in women, as we are aware of one mother whose baby slipped while being breast-fed and remained fully suspended from her breast (replication of this experiment is not recommended). During a breast-feed the end of the nipple was positioned in the baby's mouth at a distance of 6.9

(SD 1.3) mm from the junction of the hard and soft palate and there was a rhythmic up and down movement of the tongue during sucking bursts. The milk ducts and milk flow were only visible as the tongue moved down, that is, at the time of increased vacuum (peak vacuum, -145 (SD ± 58) mmHg). Although at this time the nipple moved slightly towards the junction of the hard and soft palate, the lowering of the tongue created a space into which the milk flowed. The space was bounded ventrally by the top of the tongue, proximally by the end of the nipple, dorsally by the hard palate, and distally by a downward extension of the soft palate. As the tongue was raised the bolus of milk moved under the soft palate and was cleared to the back of the pharynx. When the tongue moved up the nipple became slightly compressed and milk flow ceased. A peristaltic stripping motion of the tongue was not observed and thus they concluded that vacuum was likely to play a major role in milk removal.

Few detailed studies on the removal of milk from the mammary gland by young of other mammals are available so it is difficult to make generalisations. However, most studies are in agreement that mammalian young use dorso-ventral movements of the tongue to remove milk⁽⁷⁴⁾. Thexton *et al.*⁽⁸⁰⁾ investigated the piglet removing milk from a bottle by simultaneously measuring intra-oral vacuum and tongue movements using cinefluoroscopy. They concluded that milk flow was associated with the downward movement of the posterior tongue that resulted in the creation of a vacuum (peak vacuum -3.4 mmHg). Whilst the magnitude of pressures observed were much smaller than those measured in breast-feeding infants, similar patterns of cyclical tongue movements and negative pressure were observed⁽⁸⁰⁾. Calf suckling has been investigated by simultaneous measurements of intra-oral pressure and teat cistern pressure⁽⁸¹⁾. The peak vacuum of the suckling calf's oral cavity (-75 to -457 mmHg) was much higher than observed for breast-feeding infants. It was concluded that the observed positive pressures (0 to 412 mmHg) that were measured in the teat cistern were caused by compression of the teat. The cow teat contains a large cistern that can store milk⁽⁶⁷⁾, a feature that is not present in either human⁽⁸²⁾ or sow nipples⁽⁸³⁾. Therefore, calves may be able to remove milk from the teat by a combination of compression and vacuum.

The similarities between the morphology of the structures related to feeding, both infant (oral anatomy) and maternal (mammary gland anatomy), suggest that there may be a common feeding mechanism. The mammary glands of most mammals have either a prominent nipple or teat upon which the young attaches to remove milk. Indeed, it appears that nipples and suckling may have been a later development in lactation and possibly coincided with the increased nutritive role. Nipples may have provided an advantage when milk production increased as they control leakage of milk and aid milk removal by providing a point of attachment. A number of the identifying features of mammals are contained either within or around the oral cavity (bony palate, singular lower jaw bone and three ear bones) and the newborn mammal has the instinctive ability to find and attach to the nipple or teat whereas suckling the newborn is a learned experience for the mother⁽⁵²⁾. For example, the very immature newborn quokka moves from the birth canal to the pouch and attaches unassisted⁽⁸⁴⁾ and the newborn piglet moves towards and

identifies the nipples, claiming the nipple from which they first obtain colostrum⁽³⁶⁾. Similarly, the newborn human infant when placed on the mother's stomach is able to move to the breast, attach to the nipple and suckle unaided⁽⁸⁵⁾ and facilitation of this behaviour results in a more successful lactation⁽⁸⁶⁾. These inter-species similarities suggest that the general mechanisms utilised by the young to remove milk may be consistent among most mammals but at present it is not possible to determine the extent of this linkage because there is insufficient comparative information. However, there are notable exceptions such as the Australian monotremes, the platypus and the echidna, that have milk patches instead of nipples and the young lick the milk from associated hairs⁽²⁴⁾.

Milk production

Diamond⁽⁸⁷⁾ confirmed Aristotle's theory that mammalian teat number is closely related to litter size, with most species containing twice as many teats as the average litter size and that the maximum litter size is equal to the teat number. This suggests that normally the maternal capacity to produce milk is in excess of the needs of the young. The finding that women can produce enough milk to either nourish twins⁽⁸⁸⁾ or to tandem feed younger and older infants⁽⁸⁹⁾ supports this suggestion. Similar observations have been made for other species, for example, larger than normal litter sizes are required in laboratory mammals to ensure the growth of the litter is dependent on maternal milk production. Furthermore, Dewey & Lonnerdal⁽⁹⁰⁾ demonstrated that pumping milk remaining after each breast-feed significantly increased maternal milk production. However, on cessation of pumping, the babies did not consume the extra milk that was available, demonstrating that the infant's appetite regulated milk intake. Consequently, whereas milk synthesis and secretion are relatively constant over the day, the frequency of suckling and the volume of milk taken in by the infant vary greatly from breast-feed to breast-feed.

In the past it was assumed that milk production was dependent on maternal nutrition because it had been clearly established that dietary intake was an important determinant of milk production in the dairy cow and the sow. Similar conclusions were made for laboratory animals (rats, mice and rabbits); however, litter numbers are adjusted in laboratory animals so that litter growth rate is dependent upon maternal milk yield. As a result of a similar finding in malnourished women⁽⁹¹⁾ the recommendation of 'feed the nursing mother, thereby the infant' was promoted. However, more recently the studies of Prentice *et al.*⁽⁹²⁾ showed that poorly nourished women in The Gambia produced as much milk as well-nourished women in Cambridge, UK and it is now clear that milk yield is relatively resistant to maternal nutritional status. The common link from these findings suggests that normally milk intake is regulated by the appetite of the young mammal rather than by the capacity of the mother to produce milk.

Milk composition

Although the sequence of the small physiological variations that ultimately drove the evolution of lactation has not been elucidated, the incredible complexity of the composition

and function of milk suggests that these variations were many and varied. Basically, milk components provide the classical macronutrients (protein, fat and carbohydrate; Table 1) in a highly digestible form together with the micronutrients (minerals, trace elements and vitamins) required for the growth and development of the young of each species. Indeed, breast milk is the only single food that meets the entire nutritional requirements for human life. Milk also contains an essential array of factors associated with the innate immune system that facilitates the neonate's transition from the relatively sterile environment of the mother's uterus to a postnatal environment containing a multitude of microbes including life-threatening pathogens. In addition, breast milk is a functional food⁽⁹³⁾ providing many growth factors, hormones and compounds that act as metabolic signals to the infant as well as enzymes and cofactors of a similar complexity to those in the cytosol of the lactocyte⁽⁹⁴⁾. Furthermore there are about 130 different oligosaccharides in human milk⁽⁹⁵⁾ and over 600 different fatty acid molecules in cows' milk⁽⁹⁶⁾. Thus milk is an extremely complex fluid containing many thousands of different molecules and a comparative review of the nutritional, protective and bioactive components in milk, even in a limited number of species, is a daunting task. However, there are unifying features that are common to most mammals.

Unique nutritional components

Although there is considerable variation between species in the organic and inorganic composition, the milks of almost all mammals contain common macronutrients (proteins, carbohydrates and fats) that are unique to the mammary gland. In most eutherian mammals (for example, cows, sows, rabbits and women) relatively small changes in milk composition occur after secretory activation⁽⁹⁷⁾ and the establishment of the lactation phase of milk secretion. This is in marked contrast to marsupials, where major changes in milk composition occur over the course of lactation, particularly at critical time points in the development of the young such as pouch emergence⁽⁹⁸⁾. The concentration of protein in milk varies greatly between species from only 9 g/l in human milk to 32 g/l in cows' milk and 60 g/l in the milk of the quokka mid-lactation^(26,99). The carbohydrate composition of milk in most mammalian species is predominantly lactose and this disaccharide varies from traces in the milk of fur seals to more than 80 g/l in some primates. In addition, the milk of some species including women and quokkas contains high concentrations of oligosaccharides (13 g/l⁽¹⁰⁰⁾ and 40 g/l mid-lactation⁽²⁶⁾, respectively). In most species studied the fat content of milk increases as the gland is drained of milk and therefore it is difficult to determine the average fat content of milk consumed by suckling young. However, values range from less than 10 g/l (for example, for some primates) to in excess of 500 g/l (for example, for some seals)⁽⁹⁹⁾.

Most of the protein in milk is synthesised in the lactocytes (80–90%) on the rough endoplasmic reticulum, transferred to the Golgi apparatus, packaged into secretory vesicles and secreted into the alveolar lumen by exocytosis⁽¹⁰¹⁾. Other proteins such as albumin, secretory IgA (sIgA), IgG

and insulin are taken up from the blood by endocytosis at the basolateral membrane and transported through the cytosol to the apical membrane of the lactocyte and either released directly into the alveolar lumen or secreted with the milk-specific proteins. The protein in milk is subdivided into two fractions, the caseins and whey proteins ('curds and whey'). The caseins are defined as the proteins that can be precipitated from milk at pH 4-6, while the whey proteins remain in solution. Whereas 80% of the protein in cows' milk is casein, human milk contains the lowest casein concentration (only 17% of the total protein) of all species⁽¹⁰²⁾. The caseins occur as α -, β - and κ -casein. κ -Casein is related structurally to γ -fibrinogen rather than the other caseins, and the absence of κ -casein results in lactation failure due to blockage of the alveolar lumen and milk ducts with protein aggregates⁽¹⁰³⁾. κ -Casein has an essential role in stabilising the other insoluble α - and β -caseinates into colloidal suspension forming casein micelles. The casein micelles contain high concentrations of calcium phosphate and due to the high proline content and lack of disulfide bonds of the caseins, the micelles do not have secondary and tertiary structure but rather exist as a tangled web⁽¹⁰⁴⁾. The micelles form porous structures (1.0 g casein occupies 4.0 ml) that aggregate on dehydration. It is possible that casein's initial role in low concentrations was to protect the parchment-shelled eggs from dehydration. Later the evolution of the κ -casein gene permitted the formation of casein micelles and the secretion of nutritionally significant quantities of these proteins, providing hatchlings and neonates with amino acids, phosphate and Ca. Therefore, the emergence of the κ -casein gene was a critical event in the evolution of lactation and loss of yolk nutrition⁽⁶⁾. κ -Casein is denatured in the stomach resulting in the milk fat globules being trapped in a firm casein curd in calves and rabbits. This firm curd allows a slow release of peptides, amino acids and fat⁽¹⁰⁵⁾ into the small intestine that sustains the young over longer intervals between sucklings. In contrast, the casein in human milk is present in low concentrations and forms a soft curd so that the human infant can suckle frequently without ill effects.

A number of whey proteins are unique to the mammary gland, including α -lactalbumin, β -lactoglobulin and whey acidic protein while others are not: serum albumin, insulin, and many enzymes and hormones. Quokka milk, as with the milk of other marsupials, contains many whey proteins that either are present over the entire lactation or appear at certain stages of lactation^(31,106–108). α -Lactalbumin is a mammary-specific protein that evolved from lysozyme⁽¹⁰⁹⁾. In human milk α -lactalbumin is the major milk protein, whereas it is not present in the milk of some seals⁽⁹⁹⁾. β -Lactoglobulin is a major whey protein in cow, sow and marsupial milk but is not present in either human or rabbit milk^(110,111). Although β -lactoglobulin has no clear function, on proteolysis peptides with antioxidant and antimicrobial activities are released⁽¹¹²⁾.

Lactose is the dominant carbohydrate in the milk of women, sows, cows and rabbits and regulates the aqueous phase of milk by the osmotic influence of its synthesis in the Golgi vesicles. For most species including women, cows, sows and rabbits, the initiation of lactation is associated with a surge of lactose synthesis that results in an increase in its

concentration and a concomitant increase in milk volume. Furthermore lactose is the dominant dietary carbohydrate in the milk of these species⁽⁹⁷⁾. Oligosaccharides contain two to ten monosaccharide units, combining to form many different molecules. In nutritional terms oligosaccharides can be classed as 'soluble fibre' as they are not readily digested.

Almost all the fat (98 %) in milk is present in the milk fat globule as TAG, consisting of three fatty acids bound to a glycerol backbone. Human milk contains over 200 different fatty acids, but only seven of these fatty acids are present in amounts in excess of 1 % of the total fatty acids. The conservation of the positional distribution of certain fatty acids on the glycerol backbone has been well documented and is unique to the milk fat of most mammals^(113,114), with oleate and linoleate at the *sn*-1 or *sn*-3 positions⁽⁹⁹⁾. The positional preference of fatty acids on the TAG molecule is highly conserved across mammals although the actual fatty acids change depending on the proportion of SCFA present (cow, rabbit) and whether hydrogenation of dietary fatty acids has occurred in the upper digestive tract (cow). In human milk, palmitic acid is preferentially esterified to the *sn*-2 position. Since pancreatic lipase hydrolyses ester bonds at the *sn*-1 and *sn*-3 positions, palmitic acids remains as a monoacylglycerol and is absorbed from the small intestine more efficiently than if it were present as the free acid⁽¹¹⁵⁾.

The milk fat globule membrane contains a number of proteins including XOR and butyrophilin (only expressed in the mammary gland), lactadherin and α -actalbumin. XOR and butyrophilin are essential for the normal secretion of the milk fat globule^(7,116,117). Milk fat globules vary in size, from less than 1 μ m up to 12 μ m in humans and the smaller milk fat globules increase the rate of gastric emptying as well as the rate of fat absorption⁽¹¹⁸⁾. In addition, the milk fat globule membrane contains sphingomyelins, neutral glycosylceramides and gangliosides that are synthesised in the lactocytes, make up <1 % of the milk fat, and are involved in central nervous system myelination and the development of the retina⁽¹¹⁹⁾. Fatty acids are derived from either the blood or synthesised in the mammary gland from either glucose (women) or acetate (cow) or both (rabbit, sow, quokka). The fatty acid composition of the milk of the quokka is similar to that of the sow^(26,120,121). This suggests that while some biohydrogenation occurs in the fore-stomach, the fatty acid metabolism of the quokka is more closely aligned to that of single-stomached mammals^(26,120–123) and the absence of SCFA in the milk of the quokka is not generally a feature of the milk fat of other herbivores, such as the rabbit^(26,120,124).

It is apparent that there is large consistency in the macronutrient composition, but not concentration, of the milk of diverse species of mammal and that most of these proteins (caseins, α -lactalbumin, β -lactoglobulin, and whey acidic protein), carbohydrates (lactose) and fats (TAG) are unique and specific to the mammary gland. Thus the nutrient content of milk may have evolved to provide a specific nutritional advantage to the newborn mammal.

Protection from pathogenic micro-organisms

The newborn mammal's immune system is immature at birth as it makes the transition from the relatively sterile environment of the mother's uterus to the microbial-rich maternal environment. The initial deficiencies of the neonate's immune system are compensated for by the transfer of maternal antibodies to the fetus and/or newborn through either the placenta and/or the colostrum (Table 1), respectively^(125,126). Human and rabbit neonates have some preparation for this transition with transfer of passive immunity from the mother to the fetus by the selective transport of IgG across the placenta in late pregnancy. However, calves and piglets are born with no passive immunity and absorb maternal antibodies from the intake of colostrum within the first hours after birth⁽¹²⁷⁾. Similarly, no immunoglobulins have been detected in the blood of the newborn quokka, but were detected after the ingestion of colostrum⁽¹²⁸⁾. In the sow the concentration of total whey proteins (including immunoglobulins) decreases by 70 % during the first day and reaches minimal concentrations by the end of week 2 after birth. The majority of immunoglobulins are transferred to piglets and calves during the first day after birth. However, in the quokka passive transfer of immunity from mother to young continues to 170–220 d postpartum^(129,130). This period equates to the time until the young is emerging from the pouch. In contrast to the sow and the cow the dominant immunoglobulin in human colostrum and milk is sIgA (Fig. 6), primarily providing protection to mucosal surfaces by binding to pathogens and preventing them from attaching⁽¹²⁵⁾. In women, this high concentration of sIgA and low volume of colostrum for up to 2 d after birth may permit this glycoprotein to more effectively coat the linings of the infant's respiratory and gastrointestinal tracts and thus provide a high level of protection against environmental pathogens.

In addition to maternal antibodies, milk contains many components that provide protection to the neonate. These components include lactoferrin, lysozyme, lactoperoxidase, xanthine oxidoreductase, oligosaccharides, glycoprotein,

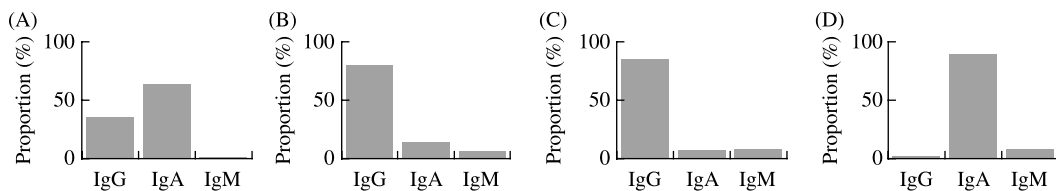


Fig. 6. Immunoglobulin proportions in colostrum in (A) rabbits, (B) pigs, (C) cows and (D) humans.

glycolipids, cytokines, growth factors, fatty acids, defensins, cathelicidins, lactadherin, antiseptory factor and leucocytes (neutrophils, macrophages and lymphocytes) that provide protection against pathogenic micro-organisms by either binding the microbes and preventing entry of the underlying tissues, engulfing and killing micro-organisms by phagocytosis, depriving microbes of essential nutrients or neutralising viruses and toxins. In addition some components function as anti-inflammatory factors (antioxidants, epithelial growth factors, cellular protective agents and enzymes that degrade mediators of inflammation), immunomodulators (nucleotides, cytokines and anti-idiotypic antibodies)⁽¹³¹⁾ and as prebiotics for symbiotic bacteria⁽¹³²⁾.

In contrast to the macronutrients in milk, most of the components that protect the neonate against pathogenic micro-organisms are not unique to the mammary gland but rather 'ply their trade' in many other parts of the body. Nevertheless milk provides a very potent and targeted defence against pathogenic micro-organisms. First, this defence system protects the mammary gland against infection. With the exception of dairy cows and Western breast-feeding women that have about a 20% incidence of mastitis, infection of the lactating mammary gland is indeed rare in all other suckling mammals including sows housed in intensive piggeries⁽¹³³⁾. Second, the defence system in milk protects the vulnerable neonate against a wide range of pathogenic micro-organisms. The degree of concentration of this protection into the lactating mammary gland can be appreciated from the observation that sIgA makes up the largest proportion of the total antibodies in human adults⁽¹³⁴⁾. Furthermore colostrum contains up to 12 g sIgA/l and mature milk contains 0.5–1.0 g sIgA/l⁽¹³⁵⁾. In other words, a 4-month-old exclusively breast-fed baby would consume approximately 75 mg sIgA/kg per 24 h compared with the production of 40 mg sIgA/kg per d for the non-lactating adult⁽¹³⁴⁾. Thus the exclusively breast-feeding mother provides almost twice the adult levels of sIgA to protect her baby's mucosal surfaces lining the respiratory, reproductive and gastrointestinal tracts. More specifically, in The Gambia weight loss associated with *Helicobacter pylori* was only in babies whose mothers did not have vacuolating cytotoxin A IgA antibodies in their milk⁽¹³⁶⁾.

'Altricial' neonatal metabolism

When mammals are born many of their metabolic pathways are functionally immature and milk supplements these deficiencies and enables the healthy growth and development of the young. This is clearly the case for the protection of the infant against pathogenic micro-organisms, where the components of the innate immune system in milk provide a shield for the young while it is developing its own specific immune defences⁽¹³⁵⁾. The initial selective advantage of a maternal secretory defence system against pathogenic micro-organisms may have arisen for the protection of soft-shelled eggs. The humid warm environment required for these eggs to hatch would be conducive to bacterial and fungal growth. On the other hand, a delay in immunological development in placental mammals may have developed

both because the uterus protects the fetus from most pathogenic micro-organisms, and to avoid untoward immunological reactions to maternal tissue during pregnancy⁽¹³¹⁾. This protection does not result in infants developing as 'germ-free' animals; indeed, there are many factors that encourage the appropriate colonisation of the neonate with commensal and symbiotic micro-organisms during the lactation period. For example, the close proximity of the birth canal and the anus encourages a controlled inoculation of the neonate with the commensal and perhaps symbiotic bacteria associated with the maternal gastrointestinal tract. Milk and salivary esterases together with gastrointestinal esterases and lipases hydrolyse milk TAG, releasing specific fatty acids that have potent antibacterial and antiviral activity⁽¹³⁴⁾.

Furthermore, milk contains many factors that facilitate the metabolism of the suckling offspring. The complexity of this symbiotic relationship between maternal milk components and the metabolism of the infant is clearly illustrated in two diverse species, the human infant and the quokka. The breast-fed infant benefits from a number of digestive enzymes (for example, bile salt-stimulated lipase) that have been found in human milk⁽¹³⁷⁾. The human infant is well adapted to lactose as a source of dietary carbohydrate. Intestinal β -galactosidase hydrolyses lactose to galactose and glucose, and while the uptake of glucose by the liver from the portal blood is limited because of the low activity of glucokinase, the high activity of galactokinase rapidly removes galactose ensuring adequate hexose for neonatal liver metabolism⁽¹³⁸⁾. Milk contains many hormones and tissue growth factors including erythropoietin, prolactin, insulin, growth hormone, oestrogens, prostaglandins and epidermal growth factor, and some of these compounds such as oestrogens, prostaglandins⁽¹³⁹⁾ and growth hormone⁽¹⁴⁰⁾ are synthesised in the mammary gland. The advantages afforded by the presence of the hormones and growth factors in milk only have to provide a marginal biological advantage for positive natural selection⁽¹⁴¹⁾ and therefore detection of all the evolutionary beneficial components in milk would be very difficult indeed.

In contrast to eutherian mammals, large changes in the concentration of milk components have been observed in marsupials at different stages of lactation. For example, when the pouch young of the quokka detaches from the teat but remains confined in the pouch, the protein, lipid and total solids content in the milk remain relatively constant⁽²⁶⁾. However, when the presence of herbage is first observed in the joey's forestomach (150d) the levels of total carbohydrate and lactose in milk begin to decline^(26,142). The decline in the concentration of lactose is accompanied by an increase in the concentration of galactose and glucose in the milk⁽²⁶⁾ (Fig. 7). The increase in these monosaccharides in the tamar wallaby (*Macropus eugenii*) has been associated with an appearance of a β -galactosidase in the milk⁽¹⁴³⁾. It has been suggested that the rise in monosaccharide levels in the milk may provide a readily absorbed source of energy required for initial pouch emergence, act as substrates for forestomach micro-organisms and/or prevent lactose intolerance resulting from a decline in intestinal lactase^(122,144). Many other

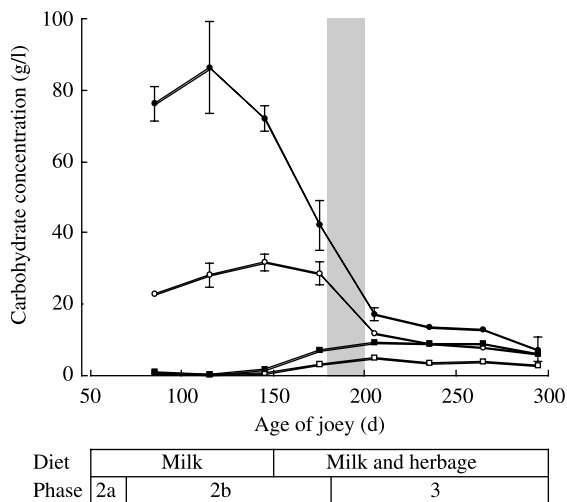


Fig. 7. The average concentration of total carbohydrate (g/l) (●), lactose (g/l) (○), galactose (g/l) (■) and glucose (g/l) (□) in the milk of the quokka (*Setonix brachyurus*) during lactation⁽²⁶⁾. (■), Transition period while the young is still returning to the pouch. Values are means, with standard errors represented by vertical bars.

stages of lactation-dependent changes in whey proteins have been reported for marsupials, including the quokka with α_2 -globulin increasing after 150 d postpartum (intake of herbage) and whey α_1 -globulin increasing after 200 d (permanent pouch exit)^(31,106,130).

More extensive studies have been carried out in the tammar wallaby and the complex pattern of maternally programmed increases and decreases in the secretion of milk components are related to the stage of lactation rather than by the sucking pattern of the young⁽³¹⁾. It has been found that when younger animals are fostered onto lactating females (that were at a more advanced stage of lactation) gross milk composition and secretion of specific milk proteins remained unchanged and the developmental rate of the fostered young dramatically increased⁽³¹⁾. Therefore, the female tammar regulates the functional development of the mammary gland during lactation and the synthesis and secretion of milk in turn regulates the development of the young regardless of its age. Thus it is possible that rather than the milk composition being uniquely tailored to circumvent the immaturity of various metabolic systems and pathways, it may be that in most mammals to a lesser or greater degree lactation has an active rather than passive input to guiding the development of the suckling young.

Multifunctional components

The multifunctional capacity of the components in milk is an amazing feature of lactation. The proteins, carbohydrates and fats in milk not only provide the suckling mammal with a complete and balanced diet but also double as functional foods⁽⁹³⁾ and provide protection against infections⁽¹³⁵⁾. Human casein is thought to be easily digested, providing amino acids, Ca and P for the baby. However, proteolytic hydrolysis of human β -casein results in the formation of N-terminal peptides containing phosphorylated amino acid

residues (casein phosphopeptides) that appear to keep Ca in a soluble form and thereby facilitate Ca absorption⁽¹⁴⁵⁾. The hydrolysis of β -casein in human and cows' milk releases peptides that have been shown to have both opioid (β -casomorphins) and anti-opioid activity. However, more studies are required to evaluate the physiological significance of these peptides⁽¹⁴⁵⁾. In addition, components of casein seem to prevent cellular adhesion of *Actinomyces* and streptococci, and κ -casein blocks *H. pylori*⁽¹³⁴⁾. Gastrointestinal digestion of casein also releases bioactive peptides that may lower the incidence of CVD, type 1 diabetes, autism, schizophrenia⁽¹⁴⁶⁾ and dental caries⁽¹⁴⁷⁾. α -Lactalbumin binds to β 1,4-galactosyl transferase to form lactose synthase. The formation of lactose from glucose and UDP-galactose in the Golgi vesicles is catalysed by lactose synthase⁽¹⁴⁸⁾. However, the concentration of α -lactalbumin in milk is much higher than that of β 1,4-galactosyl transferase. Therefore, in addition to its enzymic role, α -lactalbumin is present in nutritionally significant amounts and has a very high biological value as a dietary protein for infants. Furthermore, the hydrolysis of bovine α -lactalbumin with pepsin, trypsin and chymotrypsin releases three peptide fragments with bactericidal properties⁽¹⁴⁹⁾. As the name implies, lactoferrin has two high-affinity binding sites for Fe and it has been suggested that this ability facilitates the absorption of Fe from the infant's intestines. Since only a small fraction of the Fe-binding capacity of human lactoferrin (3–5%) is utilised, it has been proposed from *in vitro* studies that it may have a bacteriostatic effect by depriving bacteria of Fe⁽¹⁵⁰⁾. Many functions have been put forward for lactoferrin including the release of a bactericidal peptide (lactoferricin), a growth factor and an immunomodulatory factor. However, these proposed roles need further study⁽¹⁴⁵⁾.

Lactose promotes the absorption of Ca in the small intestine of young mammals. Furthermore, a significant proportion of dietary lactose escapes hydrolysis in the small intestine and, together with the oligosaccharides, facilitates the growth of the favourable bifidobacteria and lactobacilli flora in the large intestine. These bacteria provide 'colonisation resistance' against potential pathogens⁽¹³⁵⁾. In addition, oligosaccharides are an important source of the sialic acid that is required for brain development⁽⁹⁵⁾.

Studies have observed an improved immune function in breast-fed infants compared with infants fed artificial formula^(151,152). Certain of the fatty acids (8:0 to 12:0 and 18:2n-6) and their monoacylglycerols released from the hydrolysis of milk TAG in the gastrointestinal tract are antibacterial and can disrupt the envelope of viruses⁽¹⁵³⁾ and kill parasitic protozoa *in vitro*⁽¹⁵⁴⁾. Furthermore, the fat globule membrane in human milk contains mucin that binds certain bacteria as well as sIgA antibodies that may provide antimicrobial activity during intestinal passage⁽¹³⁴⁾. In addition, it has been speculated that butyrophilin may function as a component of the immune system either in the lactating mother or in the suckling neonate⁽¹⁵⁵⁾.

Conservation of function

Milk quality and quantity are highly conserved in each mammalian species and large variations of either quantity or

composition of the mother's diet have minimal effects on lactation. For example, milk production in women only seems to be affected by dehydration when the mother's life is threatened⁽¹⁵⁶⁾. Nevertheless, the exceptions to this generalisation are taken as the norm because most of the research has been directed towards mammals that are forced to lactate at the limits of their physiological capacity (dairy animals, sows and laboratory animals). On the other hand, in macropod marsupials the relatively long duration of lactation and slow growth of the young are well suited to a grazing existence in habitats that are frequently resource impoverished, without compromising the survival of the young^(34,157). Similarly, the limitation of growth is not seen in eutherians, where the supply of milk by the mother increases in response to the demand by the young and mothers have the physiological capacity to produce more milk than that required by the normal litter⁽¹⁵⁸⁾.

In women, poor maternal nutrition does not greatly affect the protein, carbohydrate, fat or energy content of breast milk⁽¹⁵⁹⁾ and the quantity and quality of breast milk are not compromised in women with common illnesses^(160,161). Furthermore, with the exception of Se, the minerals and trace elements are largely unaffected by the maternal diet. The metabolic mechanism that facilitates the secretion of Ca into milk is unresponsive to major changes in the Ca content of the mother's diet and even women with severe Fe-deficiency anaemia have a normal Fe concentration in their breast milk. With the notable exception of vitamin B₁₂, most of the water-soluble vitamins are unaffected by the maternal diet. On the other hand, the fat-soluble vitamins (except vitamin A) and the individual fatty acids are affected by the maternal diet⁽¹⁵⁹⁾.

Indeed, of the components in milk, fatty acids are the most responsive to changes in the maternal diet. In fact, in cows, the introduction of fish oil alters fatty acid composition as well as total milk fat⁽¹⁶²⁾ and feeding a high concentrate–low forage diet reduces the content of milk fat⁽¹⁶³⁾. This diet was shown to reduce not only milk fat synthesis but also the uptake of fatty acids from circulation. However, the variations in the fatty acid composition of milk are confined to within certain limits. For example, there is a close reciprocal relationship between the medium-chain fatty acids (12:0 and 14:0) and oleic acid (18:1) in human milk^(164–166). Furthermore, the signalling protein PPAR γ has a central role in controlling the composition of milk fat. Mice with a PPAR γ knock-out produce 'toxic' milk due to the incorporation of pro-inflammatory, oxidised fatty acids into milk fat⁽¹⁹⁾.

The mammary gland can also show compensatory responses in order to maintain milk production. Increasing the frequency of milking in cows as in other mammals increases milk production such that cows milked four times per d produce more milk than if they are milked twice per d. However, if all four glands of a cow are milked four times per d and then half of the udder is reduced to twice per d, milk production in these glands declines but there is a compensatory increase in milk production in the other half of the udder that continues to be milked four times per d⁽¹⁶⁷⁾. Similarly, in the sow, if twelve piglets are suckling twelve glands and then the piglets are restricted to having access to only six glands, there is a compensatory increase in milk

production in the six glands such that the growth rate of the piglets is maintained⁽¹⁶⁸⁾. Compensatory growth has also been observed in the remaining mammary gland of goats after hemi-mastectomy⁽¹⁶⁹⁾. It is suggested that similar compensation can occur in other reproductive organs such as the testes and, interestingly, this may explain the significant increase in Lance Armstrong's performance in the Tour de France after orchiectomy⁽¹⁷⁰⁾ (Fig. 3).

The major determinants in natural selection are reproductive success and survival of the young to reproductive maturity⁽¹⁴¹⁾; individuals that have advantageous traits for these determinants are favoured during evolution. Reproductive success in mammals is dependent upon, first, the synchronisation of birth and the initiation of copious milk production and, second, on the maintenance of lactation until the young is able to develop a competent host defence system and has matured so that it can be adequately nourished on the adult diet. Furthermore, despite the conservation of function, the energy demands of lactation are considerable and the lactating mouse produces its body weight in lipids in the 20 d lactation period⁽¹⁷¹⁾. Even in women, where infant growth is particularly slow, the lactating breast requires approximately 30% of resting energy. Thus lactation is a very critical period for the survival of the mammalian neonate and the conservation of the quantity and quality of milk under adverse conditions as well as the large energy commitment to lactation reinforces the evolutionary importance of lactation to mammals.

Investigations into the physiology of lactation in domestic species such as the cow and the sow are more clear-cut because there are measurable economic goals (the return on each litre of milk produced by cows and higher weaning weight of piglets). In contrast, for non-domestic species and for women in particular, comparisons are generally between breast-fed and non-breast-fed infants with no objective measurements of either the quantity of milk consumed or the composition of the milk. Furthermore, the normal ranges for healthy breast-fed babies in terms of growth rate, freedom from infection, as well as physical and cognitive development are very broad and of little use in determining the importance of all but the major lactation outcomes.

For example, a cross-sectional study of healthy exclusively breast-fed infants (1–6 months of lactation) found that there was no significant change in milk production over this period and that the average milk intake was 788 g/24 h, with a large variation between infants in milk intake (450–1126 g \pm 2 SD), feeding frequency and milk intake at each feed⁽⁶¹⁾.

Conclusion

The functions of the components of the innate immune system in milk appear to have been conserved. In contrast, a major proportion of the macronutrients in milk also has non-nutritional functions that are directed towards defence against pathogenic micro-organisms. In fact, the major nutritional components of milk that have evolved as proteins, carbohydrates and fats are not only unique to milk but share an ancestry with components of the innate immune system. Therefore, it is reasonable to assume that there was a close evolutionary link between nutrition

and protection. The fact that all of the nutritional components in milk have either potent or residual activity against pathogenic micro-organisms suggests that the nutritional components evolved from components of the innate immune system. This observation provides further support for the theory that the mammary gland evolved from the innate immune system⁽⁵⁾.

There is much synergy between the Oftedal and Vorbach *et al.* theories of the evolution of the mammary gland and its secretion. It is clear that the evolution of a secretion that could prevent both dehydration and microbial overgrowth of porous parchment-shelled eggs would provide a selective advantage to ancestral mammals.

While the antimicrobial function could be facilitated through components of the highly conserved innate immune system, the post-hatching or birth dietary role for lactation required the evolution of unique dietary macronutrients. A number of the major dietary components of milk have indeed evolved from the innate immune system⁽⁵⁾ and have either retained or developed multiple functions including the provision of nourishment and protection of the hatchling or neonate. Furthermore, the anatomical evidence suggesting that the mammary gland evolved from the glandular tissue associated with hair follicles⁽³⁾ is compelling. While there is a strong argument to suggest that the mammary gland evolved from the inflammatory response⁽⁵⁾, the extensive protection that has developed in milk to actively avoid triggering inflammation seems to be a contradiction. However, preventing tissue engagement by potential pathogens and toxins, and the consequences of inflammation including tissue damage and loss of appetite⁽¹³⁵⁾ are highly beneficial to the suckling mammal.

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