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REVIEW ARTICLE

Human milk as "chrononutrition": implications for child health and development

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Human biology follows recurring daily rhythms that are governed by circadian cues in the environment. Here we show that human milk is a powerful form of "chrononutrition," formulated to communicate time-of-day information to infants. However, 85% of breastfed infants in the US consume some milk that does not come directly from the breast but is pumped and stored in advance of feeding. Expressed milk is not necessarily circadian-matched (e.g., an infant might drink breastmilk pumped in the evening on the following morning). Ingesting mistimed milk may disrupt infants' developing circadian rhythms, potentially contributing to sleep problems and decreased physiological attunement with their mothers and environments. Dysregulated circadian biology may compromise infant health and development. Despite wide-ranging public health implications, the timing of milk delivery has received little empirical study, and no major pediatric or public health organization has issued recommendations regarding the circadian-matching of milk. However, potential adverse developmental and health consequences could be ameliorated by simple, low-cost interventions to label and circadian-match stored milk. The current paper reviews evidence for human milk as chrononutrition and makes recommendations for future research, practice, and policy.

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INTRODUCTION

Health-care organizations unanimously recommend that human milk be the sole source of infant nutrition for the first 6 months of life, with continued human milk feeding for at least 1 year as appropriate solids are introduced.[1–3] Numerous best practice guidelines regarding the handling, storage, and provisioning of human milk have been issued.[1,3–7] These recommendations, however, overlook one important fact— that milk composition changes dramatically over the day.

Recent research suggests that milk is a powerful form of "chrononutrition," formulated by evolutionary processes to communicate time-of-day information to infants.[8,9] For example, human milk contains higher levels of cortisol and activity-promoting amino acids during the day, which likely function to promote alertness, feeding behavior, and catabolic processes in infants.[10,11] Night milk, by contrast, contains low levels of these activity-promoting compounds, instead delivering high levels of melatonin and tryptophan to foster sleep, relax digestion, and support cell restoration.[8,12] When infants feed directly from the breast, they reap the benefits of milk that matches maternal circadian rhythms and, potentially, other circadian cues, such as light–dark cycles. However, most infants in industrialized societies consume at least some milk that has been previously expressed via breast pump and then frozen or refrigerated for later use. For example, in the United States, 85% of breastfeeding mothers feed their infants with previously expressed human milk at least some of the time, and 25% of these infants drink previously expressed milk daily.[13] Approximately 6% of breastfeeding mothers in the US never feed their infant at the breast, only feeding their infants expressed milk.[13]

Infancy represents an important stage for circadian programming, and human milk may have evolved to help facilitate the development of stable circadian rhythms. As such, it is plausible that mistimed milk may disrupt or delay the development of circadian rhythms, with potentially significant effects on infant sleep and health. Surprisingly, this issue has received almost no empirical study. Although health-care organizations typically recommend feeding infants milk matched to their developmental stage (e.g., providing colostrum, transitional milk [milk produced from days 3–5 until day 10–14 postpartum], and mature milk to infants in succession), we could find no recommendations from any health-care organization regarding the timing of human milk delivery across the day,[1,2,7,14] with the exception of one paper by a pediatrician advocating the use of circadian-matched milk in Neonatal Intensive Care Units (NICUs).[15] Policy statements on breastfeeding from the American Academy of Pediatrics,[3,4] the National Association of Neonatal Nurses,[1] the Academy of Breastfeeding Medicine,[5,6] and the World Health Organization[7] do not mention the circadian nature of human milk composition nor advise circadian-matching of expressed milk. Further, we found no studies investigating whether mistimed milk dysregulates the infant circadian clock. This gap is particularly striking because potential interventions are low cost and easy to implement (e.g., changing the labeling and organization of stored milk).

Further, to our knowledge, no studies have compared the development of circadian cortisol patterns in breastfed versus

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formula-fed infants. Most studies have either only sampled exclusively breastfeeding infants[16,17] or provide no information on whether infants were breastfed.[18,19] We hypothesize that circadian biology will develop more quickly in infants exclusively fed milk directly from the breast than those exclusively formula-fed, given that the former group would receive consistent circadian signals in milk while the latter would receive no time-of-day milk signals. In addition, no prior study has reported whether mothers fed their infant directly from the breast (certain to provide circadian-matched milk) or used previously pumped milk, which may or may not be circadian-matched.[20] We hypothesize that the emergence of stable circadian rhythms may be delayed in infants who receive more of their feedings through mistimed expressed milk compared to those who receive circadian-matched milk (e.g., milk from either the breast or circadian-matched expressed milk).

This review aims to motivate vital research into the implications of mistimed milk for infant health and development. First, we describe research documenting diurnal changes in human milk composition over the day. Next, we review evidence linking disruption of circadian biology to poor infant health and discuss pathways through which circadian variation in milk may help to foster healthy infant circadian biology. We conclude with recommendations for future research and discuss implications for clinical practice and public policy.

CHANGES IN MILK COMPOSITION OVER THE DAY

Here we review an emerging literature documenting nutritional, hormonal, and immunological fluctuations in milk composition across the day (see Fig. 1). Where human studies are lacking, we discuss relevant animal models that may inform understanding of milk composition in humans.

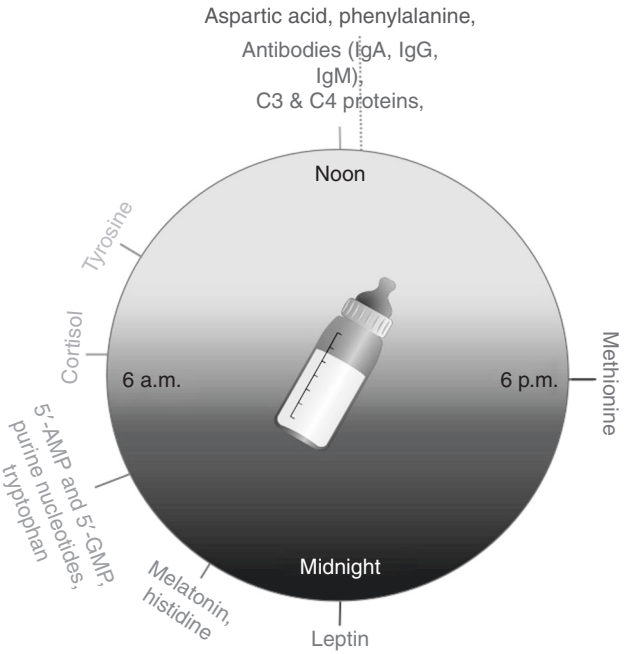

Fig. 1 Circadian variation in human milk. Reported peak levels of compounds in human mature milk. The following sources were used to give peak levels of compounds in milk to create this figure: nucleotides (Sánchez et al.[21]), amino acids (Sánchez et al.[11]), leptin (Cannon et al.[35]), melatonin (Illnerova et al.[12]), cortisol (Pundir et al.[10]), immune components (Franca et al.[46])

Nutritional composition over the day

Amino acids, mineral concentrations, and micronutrients vary in milk across the day. For example, the daily rhythms of various nucleotides in milk were characterized in a study of 30 healthy mothers followed over 24 h.[21] Nucleotides serve as the building blocks of nucleic acids in DNA and are precursors to energy-rich compounds that control biosynthesis in all cells. Human milk contains higher nocturnal levels of 5′AMP and 5′GMP—purine nucleotides known to be important for the release of GABA and melatonin, respectively.[21] Higher levels of activity-promoting neuroactive amino acids (tyrosine, a precursor of norepinephrine and epinephrine; methionine, an essential amino acid and precursor to acetylcholine; phenylalanine, an essential amino acid; aspartic acid and glycine, neurotransmitters implicated in activity) are all at peak levels in day milk compared to night milk.[11] Although only methionine and tryptophan show daily variation in transitional milk,[11] most amino acids show circadian variation in mature milk.[11] In addition, mature milk expressed at night has higher total fat content than milk expressed during the day in mothers of both full-term[22–25] and preterm infants.[26] Iron in milk peaks at around noon; vitamin E peaks in the evening[27]; magnesium, zinc, and potassium are all at their highest levels in the morning[28]; and sodium levels are the highest in the early morning hours.[29]

Hormonal composition over the day

The endocrine components of human milk change dramatically over the day. Circadian variation in cortisol and melatonin in milk have received the most attention by researchers, in part, because these endocrine factors are key mediators of metabolism[30] and immune activity.[31] Circadian shifts in milk cortisol are particularly notable. In a recent study, 23 exclusively breastfeeding mothers pumped milk before and after each breastfeeding session over 24 h, and all mothers' milk showed distinct diurnal variation in cortisol.[10] Cortisol levels were an average of 330% higher in morning milk (2.97 ng/ml between 0400 and 1000 h) compared to late afternoon and evening milk (0.69 ng/ml between 1600 and 2200 h). Cortisol in milk is transferred from maternal plasma,[32] and therefore follows the same diurnal cycle detectable with other measures of cortisol (i.e., plasma and saliva). Melatonin in milk similarly tracks nocturnal patterns found in maternal circulation. Although undetectable during the day, melatonin levels in milk rise before nighttime sleep, peaking in the early morning hours.[8,12] Regular daily variation in milk melatonin emerges within the first days after birth.[11]

Circadian patterns in several other hormones have been detected in human milk, although their daily variations are less well characterized. For example, leptin regulates energy balance by inhibiting hunger and is present in human milk.[33,34] Leptin evinces a subtle bimodal rhythmicity.[35] In a study of 19 mothers who expressed milk samples before and after each feeding for 24 h, leptin levels were significantly higher in milk collected between 10 p.m. and 4 a.m. than milk collected between 4 a.m. and 10 p.m.[35] This pattern corresponds with the previously reported nocturnal rise in human plasma leptin.[36] Rodent studies suggest that leptin levels may vary over the course of lactation. For example, a "leptin surge" has been observed in the milk of lactating rat dams at approximately 10 days following birth, which corresponds to the timing of previously observed increases of plasma leptin in rat pups.[37,38] This surge in milk leptin around the midpoint of lactation has been hypothesized to contribute to the programming of offspring metabolic development.[37,38] Prolactin is also present in human milk[39] and thought to aid infant intestinal nutrient absorption.[40] At the time of writing, only one study of 15 nursing mothers 2–3 days postpartum could be found examining circadian variation in the hormone prolactin in human milk. The authors reported that prolactin tended to be higher in morning

than in night milk in a mild trend that did not attain statistical significance.[41]

Although numerous other hormones with circadian rhythms have been identified in human milk [e.g., insulin,[42] adiponectin,[41] ghrelin,[43,44] and thyroid hormones[45]] to our knowledge, circadian variation in these hormones has yet to be examined in humans. Rodent studies, however, may provide clues to their variation in human milk. In a study of 30 lactating rat dams collecting 24-h milk samples on days 5, 10, and 15 postpartum, adiponectin showed significant rhythmicity at days 5 and 10, while ghrelin exhibited rhythmicity only at day 5.[37] At day 5, both hormones displayed bimodal patterns, with ghrelin concentrations peaking at 16:00 and 4:00, and adiponectin peaking at 12:00 and 20:00. With respect to day 10, there were spikes in milk adiponectin levels at 12:00 and 16:00.[37] Follow-up work is needed to examine these hormones in human mothers and attempt to clarify the potential functional outcomes on infant development of these rhythms should they be observed.

Immune composition over the day

A small but growing literature suggests that key immune factors display circadian rhythms in human milk, with generally higher concentrations found during the day, particularly in the early postnatal period. In the most comprehensive study, 36 mothers provided morning and evening milk samples at day 3 (colostrum), day 10 (transitional milk), and day 30 (mature milk) postpartum, revealing that immune components were generally higher in the diurnal than in the nocturnal period.[46] Specifically, in colostrum, diurnal milk (relative to nocturnal milk) had higher levels of the antibody IgA, C3 and C4 proteins of the complement system, and polymorphonuclear phagocytes. Transitional diurnal milk contained elevated concentrations of antibodies and C3 and C4 proteins compared to nocturnal milk. In mature milk, greater numbers of antibodies, C3 and C4 proteins, and phagocytes were found in diurnal than in nocturnal milk. Consistent with these results, higher levels of the cytokine interferon-γ have been found in mature day milk versus night milk (although the opposite pattern emerged in transitional milk).[47] Another study of 24 mothers who provided milk samples at 3 days postpartum (colostrum) and 30 days postpartum (mature milk) found differences in cytokine levels based both on time of day and also maturational stage.[48] Specifically, interleukin (IL)-6 levels were higher in diurnal vs. nocturnal colostrum, and tumor necrosis factor-alpha was higher in diurnal colostrum than in mature milk.[48]

Summary

Ample evidence indicates that the nutritional, hormonal, and immunological composition of human milk varies over the day. Although we do not know the functional significance of these circadian patterns, it seems plausible that they evolved to help mothers entrain circadian rhythms in infants. Additional yet-to-be-discovered compounds in milk may also exhibit diurnal changes that shift postpartum to match the needs of developing infants. In the following section, we discuss the importance of circadian biology for infant health and the role that diurnal changes in milk composition may play in supporting infant circadian biology.

Circadian biology and health

The circadian clock controls circadian rhythms in sleep–wake cycles, respiratory rate, body temperature, digestion, metabolism, hormone release, and other important physiological functions.[31] In adults, dysregulated circadian rhythms have been linked with a host of physical and mental health problems, including poor immune function,[49,50] sleep problems,[51–53] and psychological disorders.[54,55] We know far less about the link between circadian biology and health in infancy. Babies with colic have blunted cortisol rhythms, characterized by lower morning and higher evening cortisol, compared to infants without colic.[56] Infants in the NICU deprived of regular 12-h light/dark patterns (associated with circadian entrainment) exhibit reduced feeding, retarded growth, and delayed NICU discharge.[57–60]

In healthy adults and children, the biological clock follows a regular 24-h cycle, synchronizing with environmental cues (e.g., light). In mammals, the central circadian clock resides in the suprachiasmatic nucleus of the brain and is primarily regulated by light/dark signals detected by the retina.[31] The central clock regulates temporal changes in the whole organism by synchronizing multiple peripheral clocks that induce changes in virtually every cell in the body.[61] For example, the suprachiasmatic nucleus triggers a hormonal cascade in the hypothalamic–pituitary–adrenal (HPA) axis, the major peripheral branch of the circadian clock, that leads to the release of glucocorticoids (e.g., cortisol in humans) and catecholamines (i.e., epinephrine and norepinephrine) from the adrenal glands that then circulate throughout the body.[62] Catecholamines act via alpha-adrenergic and beta-adrenergic receptors, which can have diverse effects on the immune system and metabolism.[63] Cortisol helps to regulate the genetic clock within cells by acting through the glucocorticoid receptor, which is expressed by almost all mammalian cells. Other peripheral clocks include daily changes in melatonin and leptin.[61] Melatonin is synthesized by the pineal gland at night under the control of suprachiasmatic nucleus. Melatonin has a mild sedating effect in humans[64] and helps the body adjust to seasonal changes in day length.[65] Leptin, synthesized by adipocytes, increases at night to inhibit food intake.[61] Leptin does not impact the central clock directly but can sensitize the central clock to light-induced phase shifts.[66] These peripheral hormonal clocks can also alter the hormonal rhythms of one another. For example, glucocorticoid administration in adrenalectomized rats can phase shift the leptin rhythm.[67] Notably, these time-keeping hormones vary in milk over the day, which may serve as environmental cues to facilitate development of infant circadian biology.

The emergence of circadian biology in infancy

Infants are not born with a fully operational circadian clock.[17] Although daily fluctuations in body temperature appear almost immediately after birth in full-term infants, other rhythms, including rest–activity, sleep–wake, and hormonal cycles, typically develop slowly over the first months of life.[68] Research shows that exposure to light/dark intervals,[57,69] temporal variation in social interaction,[16] and other fluctuating sources of environmental stimulation (e.g., noise)[70] all influence the timing of infant circadian biology development.

Studies disagree as to when circadian patterns in the key peripheral time-keeping hormone cortisol are first detectable in infancy, with estimates ranging from as early as 2 weeks to as late as 9 months.[17–20,71–74] Daily variations in cortisol appear key to establishing stable circadian processes in glucose release, inhibition of insulin, and inhibition of growth hormone.[75] Moreover, normal morning peaks and nightly troughs in cortisol tend to appear in tandem with infant milestones like predictable sleep and feeding routines.[72,76] In a study of 130 full-term infants that assessed diurnal cortisol monthly from birth through the first year of life,[17] detectable circadian cortisol rhythms emerged on average at around 4 weeks, with a strikingly high degree of variability. Approximately 35% of the sample showed stable morning/evening differences within a few days after birth, whereas 10% of infants had yet to develop morning/evening differences by the age of 6 months. The heterogeneity in the emergence of detectable cortisol rhythms may owe in part to differential exposure to regular circadian signals in human milk. To date, no studies have directly compared the onset of circadian cortisol patterns in breastfed versus formula-fed infants or in

infants exposed to circadian-matched expressed milk versus non-circadian matched expressed milk.

Potential mechanisms through which milk entrains infant circadian biology

Circadian variation in cortisol in milk may be a particularly important mechanism through which mothers' milk bolsters the fledgling circadian biology of infants. In rodents, ingested milk glucocorticoids readily cross the intestinal epithelial barrier into neonatal plasma and the brain.[77] Consistent with the fact that milk cortisol passes into infant circulation, studies in humans have shown that milk cortisol predicts changes in infant development. For example, higher (compared to lower) milk cortisol exposure at 3 months after birth prospectively predicts heightened stature and lower body mass index percentile at 2 years.[78] Further, human infants whose mothers' milk is higher in cortisol display more fearful temperaments.[79] Studies of non-human primates echo these results, and rodent studies provide compelling causal demonstrations that glucocorticoid signals in milk can shape infant development.[80–82] Administering corticosterone (the rodent equivalent of cortisol) to a lactating dam, for example, elevates corticosterone concentrations in maternal plasma and milk, leading to enhanced learning and altered fear responding in offspring.[80–82] Additionally, early corticosterone exposure via milk has been shown to alter the number of glucocorticoid receptors in the hippocampus in rodents after weaning,[81] which may explain why behavioral and cognitive effects persist into adulthood. Exogenous glucocorticoid administration in rodents and humans can phase shift the circadian clock,[83] suggesting that milk cortisol could represent a plausible, yet untested, modulator of the infant circadian clock in humans.

Circadian variation in milk melatonin is another likely regulator of infant circadian biology. Rodent studies show that melatonin in milk also rapidly crosses the intestinal barrier and diffuses into many tissues, including liver, kidney, and brain.[84] In support of the importance of melatonin in milk for circadian development in humans, breastfed infants show more regular nocturnal increases in 6-sulfatoxymelatonin, the metabolite of melatonin excreted in urine, than formula-fed infants,[85] a difference that may explain why breastfed infants have higher sleep efficiency and less fragmented sleep in early life compared to formula-fed infants.[8,85,86] Studies that randomly assigned 2–6-month-old infants to receive tryptophan-enriched infant formula at night and non-enriched formula in the day report improvements in sleep and increased synthesis of serotonin to melatonin.[9,86] Throughout most of human evolution, newborns could rely on ingesting the melatonin they needed from milk, thus it is unsurprising that infants' immature neuroendocrine and metabolic systems do not perform the protein synthesis needed to convert the essential amino acid L-tryptophan from food into serotonin and then melatonin until approximately the third month of life.[87]

In sum, chronosignals in breast milk constitute a plausible—yet largely unrecognized—environmental signal that might help to explain the large degree of heterogeneity in the development of circadian HPA activity. Just as feeding directly from the breast may promote maternal–infant neuroendocrine circadian attunement, mistimed feedings may disrupt circadian attunement.

FURTHER IMPLICATIONS OF INGESTING MISTIMED MILK

In the ancestral past, infants would only receive milk containing misaligned diurnal rhythms if their mothers' circadian biology was severely disrupted (e.g., by exposure to environmental stressors). Accordingly, if infants evolved to utilize hormonal signals in milk as environmental cues, time-discordant hormonal signals in expressed milk could resemble the signals received by infants in particularly volatile environments.[88–91] Relatedly, studies in both rodents and humans indicate that infants exposed to unpredictable stressors during pregnancy or early infancy (e.g., random light/dark cues, intermittent loud noises or electric shocks, disorganized or fragmented maternal behavior) show developmental delays[92–97] and adult phenotypes often characterized by hypervigilance,[96,98] earlier sexual maturation,[99] and reduced longevity.[100] These changes in response to early life uncertainty may represent "faster" life history strategies, wherein the organism gains a fitness advantage by attuning the HPA axis to threat and reproducing early at the expense of later health.[101–106] Of course, the extent to which human infants rely on bioactive signals in milk to make developmental trade-offs is unknown. However, exposure to milk spiked with high levels of glucocorticoids at random intervals may potentially convey unreliable information to infants about the stability and safety of their environments.

Exogenous hormone administration experiments may provide indirect evidence of the potential implications of ingesting non-circadian-matched milk. Current human milk banking practices aggregate away circadian variation in milk because large volumes of milk expressed at different times of day are batched. This practice conceivably exposes infants who rely on batched milk to tonic levels of time-keeping hormones like cortisol over 24-h periods. Although we do not know what effect exposure to tonic milk signals could have on peripheral tissue in humans, studies with adult mice show that administering low-dose synthetic glucocorticoids continuously throughout the day impedes healthy circadian genetic oscillations in the muscles and liver.[107] Similarly, as exogenous melatonin administered to human adults in the morning produces sedating effects,[64] feeding infants melatonin-rich night milk in the morning may similarly induce drowsiness. Importantly, inferences about milk feeding should be drawn with caution from studies of supraphysiologic doses of hormones, which are typically far higher than those naturally occurring in human milk. Nonetheless, these studies demonstrate that exposure to tonic or acute doses of hormones can impact circadian biology and highlight the need for direct research in humans testing how exposure to unvarying milk signals or to acute, unpredictable doses of milk signals impact infant development. Given the urgency of this situation, we next outline several research priorities and discuss possible policy implications.

FUTURE DIRECTIONS AND POLICY IMPLICATIONS

A number of pressing questions warrant empirical attention. First, we must better understand the basic circadian signals in milk and how they vary across milk stages. Moreover, the epigenetic effects of circadian variation in milk on development (e.g., metabolic programming and future health outcomes) warrant greater study. Circadian epigenetic effects appear highly plausible given that ingesting human milk can lead to epigenetic changes in the organism.[108] For example, in vitro studies show that human breast milk suppresses inducible IL-8 promoter gene activation in human intestinal cells by inhibiting the activation of the transcriptional nuclear factor κB.[109] Compounds in human milk can also influence epigenetics indirectly through the microbiome, as in the case of the indigestible oligosaccharides in human milk that primarily function as prebiotics for health-promoting bacteria (e.g., lactobacillus and bifidobacteria) that can upregulate the secretion of secretory IgA (sIgA) excretion in the infant gut.[110] Future research should investigate how the amount, composition, and time of day that milk is ingested[111,112] regulates genes on daily and long-term timescales.

Beyond basic research, randomized controlled trials are needed to determine whether providing circadian-matched milk improves child health. Because dysregulated circadian biology can adversely impact almost every process in the body, circadian-matched milk may improve health in multiple areas (e.g., time spent in active feeding in the day, growth rate, sleep consolidation, and

neurocognitive development). Interventions to circadian-matched milk are negligible in risk and readily implemented. Mothers and other care providers could be advised to label expressed milk with the time of day that it was expressed, and select the stored milk that best corresponds with the current time when bottle-feeding infants. Large-scale implementation of this intervention could begin rapidly and at little expense. Conceptually analogous interventions focused on using exogenous environmental light cues in the NICU to entrain infant circadian biology have already been shown to improve infant growth,[58–60] hasten time to oral feeding,[59] improve cardiorespiratory function,[59,113] reduce length of hospital stay,[57] and enhance the ratio of night/day activity.[114]

A greater basic understanding of the daily rhythms in the bioactive components of human milk could reveal new opportunities for interventions to enhance infant health. For example, infants who are at particularly high risk of infection or who are currently fighting infection might benefit from milk selectively collected during the day, as key immune factors are generally higher in milk expressed during the day compared to the night.[46,47] Human diurnal milk is particularly rich in sIgA antibodies,[46] which provide protection against bacterial and viral infections, neutralize toxins, and act as opsonins by increasing free radical levels.[115] Day (compared to night) colostrum and mature milk also contain larger quantities of phagocytes[46] that help to engulf and destroy harmful microorganisms, foreign particles, and cellular debris, in addition to playing an important role in maintaining cell lineages that facilitate long-term defense against specific pathogens.[115] Hence, milk banks could conceivably create special morning milk batches with high levels of these and other immune components that could be selectively fed to the sickest infants, should the immunological benefits be determined to outweigh the countervailing costs in other areas (e.g., sleep/wake cycling).

In addition to changing the labeling and provisioning of expressed milk, research on milk chrononutrition has implications for maternity leave and workplace policy. Public policy-makers have pushed for "breastfeeding-friendly workplaces," which are equipped with lactation rooms and provide time for scheduled pumping breaks. There is evidence that such workplaces improve mothers' intentions to breastfeed and lengthen breastfeeding duration.[116] However, facilitating milk expression is only a partial solution. Genuinely supportive policies might allow women to take longer maternity leaves (in the U.S., 25% of new mothers return to work within 2 weeks after childbirth[117]) or access childcare facilities on site at their workplaces. Such policies would permit direct feeding—allowing milk production and delivery to be perfectly circadian-matched.

Conclusion

Most infants in industrialized societies are routinely fed previously expressed human milk without regard for the time of expression.[13] The impact of this practice on infant circadian biology is alarmingly understudied and dimly understood given the far-reaching potential effects on infant health, including sleep, metabolism, and neurocognitive development.

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J.H.-H. and L.G. conceptualized the research concept and wrote the initial draft of the manuscript. D.S., C.S., and C.B. helped revise the article critically for important intellectual content. All authors gave their final approval of this paper for publication.

ADDITIONAL INFORMATION

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