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REVIEW ARTICLE

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Bioactive compounds in mothers milk affecting offspring outcomes: A narrative review

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Summary

Background: Compared to the exhaustive study of transgenerational programming of obesity and diabetes through exposures in the prenatal period, postnatal programming mechanisms are understudied, including the potential role of breast milk composition linking maternal metabolic status (body mass index and diabetes) and offspring growth, metabolic health and future disease risk.

Methods: This narrative review will principally focus on four emergent bioactive compounds [microRNA's (miRNA), lipokines/signalling lipids, small molecules/metabolites and fructose] that, until recently were not known to exist in breast milk. The objective of this narrative review is to integrate evidence across multiple fields of study that demonstrate the importance of these compositional elements of breast milk during lactation and the subsequent effect of breast milk components on the health of the infant.

Results: Current knowledge on the presence of miRNA's, lipokines/signalling lipids, small molecules/metabolites and fructose in breast milk and their associations with infant outcomes is compelling, but far from resolved. Two themes emerge: (1) maternal metabolic phenotypes are associated with these bioactives and (2) though existing in milk at low concentrations, they are also associated with offspring growth and body composition.

Conclusion: Breast milk research is gaining momentum though we must remain focused on understanding how non-nutritive bioactive components are affected by the maternal phenotype, how they subsequently impact infant outcomes. Though early, there is evidence to suggest fructose is associated with fat mass in the 1st months of life whereas 12,13 diHOME (brown fat activator) and betaine are negatively associated with early adiposity and growth.

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KEYWORDS
bioactives, growth, lactation, metabolic programming, milk, obesity

1 | INTRODUCTION

Though breast milk is a complex biological fluid with known benefits for maternal and infant health as compared to formula, the compositional components responsible for these effects are little known. A recent (November 2021) PubMed search revealed ~20% more published articles on bovine milk composition as compared to human milk composition. This only highlights the gap that currently exists in the field and the accumulating evidence linking specific milk components with infant growth and the potential to protect from obesity and diabetes later in life and overall long-term health. Despite attention to breastfeeding and health, considerable gaps currently exist between what is shown epidemiologically about the benefits of human milk and the scientific evidence demonstrating the specific features of milk underlying its purported benefits.[1] It is important that the scientific community continues to conduct rigorous analyses and reviews of the current state of the field so that a better understanding of the cross-talk between maternal factors, human milk composition and infant outcomes can be determined. Building upon prior reviews, this narrative review will synthesize the current state of 'novel' human milk component research, specifically focusing on nutrients and bioactive components that until recently were not known to exist in human milk.[2,3]

1.1 | Outline of review

Topics covered in this narrative review are as follows: (1) the theoretical framework for lactational programming of infant organ development, growth and later health outcomes; (2) possible mechanisms for absorption of milk 'bioactives' in the infant's gut; (3) review of keystone animal cross-fostering studies that demonstrate plausibility for compounds in milk that are altered with maternal metabolic dysfunction and are bioactive for the offspring; (4) review of recent 'novel' milk bioactives including microRNA's, lipokines/signalling lipids, small molecules/metabolites and fructose and (5) recommendations for study approaches and new research areas needed to advance the field. This section will not discuss hormones, adipokines or human milk oligosaccharide (HMO)'s given they have been the most studied and reported on to date.

2 | LACTATIONAL PROGRAMMING OF OFFSPRING METABOLIC HEALTH

The concept of programming during critical windows of development was first hypothesized by Barker describing the 'developmental origins of health and disease'.[4] The field of developmental programming has rapidly expanded with both animal models and human studies identifying complex influences across the perinatal and postnatal period with a focus on the first 1000 days of life.[2,5] Within this period of rapid growth and development of critical and vulnerable organ systems, a major factor in long-term programming for metabolic disease risk is postnatal nutrition.[6,7] The lactation window defines a key period where offspring survival is dependent on maternal milk with milk providing dynamic bioactive factors and nutrients that contribute to programming and future health (Figure 1).[2] Bartol termed the early exposure of biologically active factors through milk consumption and the importance on offspring health, the 'lactocrine hypothesis'.[8]

The lactational period is a critical period open to insults, with differentiation of fragile organ-systems continuing during the early postnatal period, therefore setting the stage for heightened risk of future disease.[2] Offspring organ development spans the prenatal through the postnatal period including key developmental and maturation events occurring in the first days to months after birth ranging from completion of adipocyte development in the first months to ongoing islet and hepatic development until beyond ~2 years of life, after weaning has occurred.[2,9] Regulation of energy homeostasis, metabolism and appetite is governed by the ongoing development of the hypothalamus, liver, muscle, pancreas, gut and adipose tissue that continues during the postnatal stage, but with timing that varies between altricial (incapable of movement after hatching, needing extensive parental care) and precocial (capable of movement upon hatching) species.[10] While organ development and differentiation are well-organized, controlled and regulated processes, disruptions or alterations in signalling events precipitated by milk factors can impact downstream development.[11] Proposed programming factors in breast milk include, but are not limited to, macronutrients, lipid species, amino acids, hormones (insulin, leptin, ghrelin, adiponectin and cortisol), adipokines, growth factors, metabolites, prebiotics, probiotics, cytokines, chemokines, anti-microbials, pro-inflammatory and anti-inflammatory signalling molecules, vitamins, toxins, cells, milk fat globule membranes, microRNAs, nucleotides and oligosaccharides.[12,13] Emergent data are beginning to explore and identify mechanisms by which these milk components may potentially serve as mediators in programming offspring tissues and organ systems. The proposed mechanisms include epigenetic modifications,[10,14] induction of oxidative stress,[15] alterations in gene expression and translation through microRNAs[16,17] and infant intestinal microbial colonization.[18]

3 | MECHANISTIC FRAMEWORK FOR THE SYSTEMIC BIOACTIVITY OF MILK COMPONENTS IN THE INFANT

For non-nutrient milk components to play a causal role in infant health, they must remain 'bioactive' after ingestion. First, although

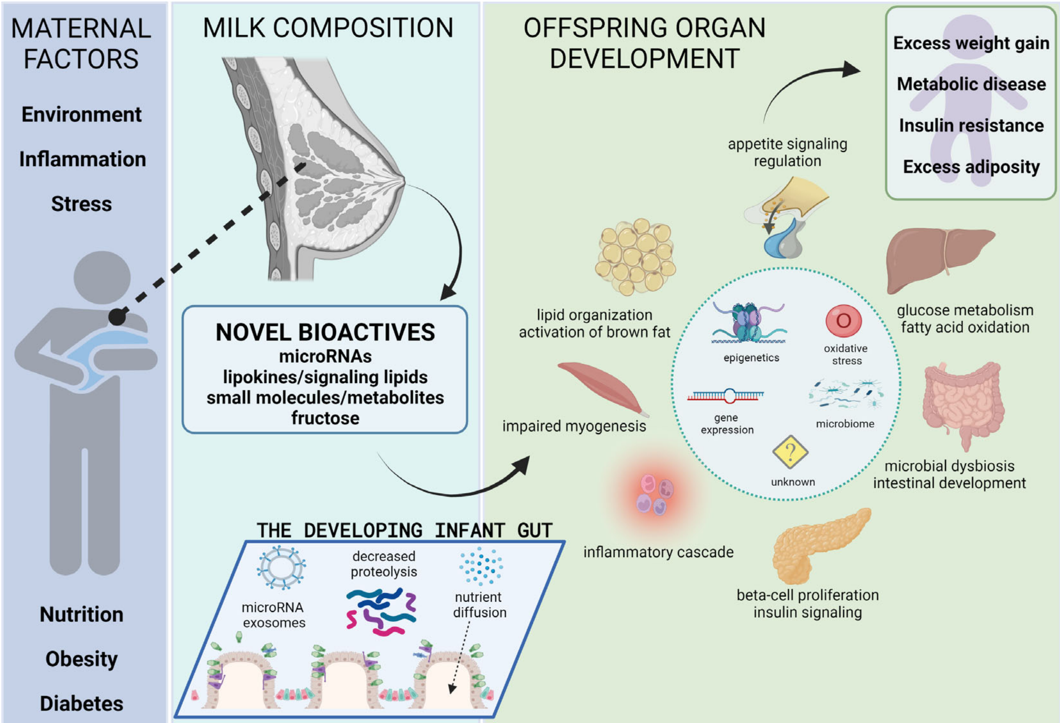

FIGURE 1 Overview of lactational programming. Created with BioRender.com

most macromolecules are degraded by digestive enzymes and are absorbed in the form of nutrients, some do resist both the low pH of the gastric fluid and proteolytic enzyme hydrolysis, allowing them to reach the small intestinal lumen. This is particularly the case for infants, in that the infant's gut resists proteolysis due to early milk being high in protease inhibitors, immaturity of pancreatic enzymes and the infant stomach having a higher pH than in later life.[19] Second, the paracellular pathway allows diffusion of small molecules through tight junction pores and other structures in between the intestinal epithelial cells.[20] Paracellular diffusion is a particularly important non-digestive absorptive pathway characteristic of the neonatal gut for macronutrients.[21] Third, transcellular transport of large particles, including microbes, has been ascribed to intestinal M cells as well as dendritic cells that can extend dendrites between epithelial cells to ample soluble antigens in the lumen allowing transport into the circulation.[20] Finally, certain milk components, like microRNAs, are protected in exosomal vesicles which are also resistant to degradation.[22] Collectively, these biological/physiological pathways/mechanisms demonstrate unique aspects of newborn and milk physiology that allow a variety of large and small molecules in human milk to retain function and bioactivity in the infant's gut and to be transported into the infant circulation.

4 | CROSS-FOSTERING STUDIES

The study of the role of breast milk in infant development is complicated given the dynamic and multifaceted composition of milk, which is influenced by maternal health, maternal nutrition, stress, environmental exposures, stage of lactation, time of day and individualization based on offspring needs.[23] Animal models allow the specific study of exposures during discrete time periods during lactation, which is challenging to demonstrate in human studies. The granularity of these animal models provides conclusive evidence that milk composition confers metabolic signals that can either prevent or drive diabetes/obesity in the offspring.

Through maternal diet alteration (fat, carbohydrate, proteins and specific fatty acids), artificial rearing, cross-fostering, litter size reduction and direct neonatal diet supplementation, researchers are able to narrow their focus to outcomes that result from exposures solely during the period of lactation in animal models.[24–27] Mouse models have demonstrated milk composition is altered in the setting of maternal nutrition during lactation and maternal disease.[23,28–30] We performed a recent review looking at these outcomes in depth.[2] Since that time, additional mouse and rat models have shown that dietary exposures during lactation alter milk composition. Maternal high fat diet isolated to the lactation period, for example, resulted in milk alterations with increased milk fat, energy, cholesterol, omega-6/omega-3 polyunsaturated fatty acid ratio and altered long-chain fatty acid composition. In lactational high fat diet models offspring had resulting abnormal lipid accumulation in white adipose tissue and liver, adipocyte hypertrophy, insulin resistance and divergent gut microbial diversity levels, all signs of offspring metabolic compromise.[18,24–26,31,32] Monks et al set out to dissect the effects of maternal obesity versus maternal high-fat diet during the lactation period. They found that pups reared by lean dams on a high-fat diet during lactation had the fastest rate of growth with early increased visceral fat accumulation and inflammation, implicating maternal diet having a stronger impact on metabolic outcomes than maternal obesity in this mouse model.[32] On the other hand, a rat model of caloric restriction during lactation identified higher levels of adiponectin and myo-inositol in milk.[27,33] The changes in milk composition due to caloric restriction lead to changes in offspring's long term health outcomes including lower body weight and better glucose control. These experiments carefully demonstrate the strong impact of maternal diet during lactation on rodent offspring.

Cross-fostering models looking at the effect maternal obesity plays during lactation, show a clear and consistent effect of increased obesity in offspring from control mothers fostered onto obese dams.[34–36] These studies all reported an increased absolute offspring body weight with some even showing an increased relative fat mass (i.e., %fat) when obese dams suckled pups of lean dams. The main culprits in the increased somatic growth of the offspring suckled by obese dams are thought to be increased breast milk insulin, with leptin showing mixed findings.[37,38] A possible target for these changes may be neuronal and lead to long-term alterations in food intake. Pups exposed to maternal diabetes during lactation by cross-fostering onto a gestational diabetes mellitus mother at birth had abnormal programming of both structure and function of hypothalamic neurons that resulted in a delayed growth pattern during the neonatal period.[39] Collectively these cross-fostering studies (and others) demonstrate exposure to 'obesogenic-diabetic' milk during lactation to be linked with negative offspring future health. Confirmation is bi-directional in that maternal health via milk composition is a stronger driver (perhaps the strongest) in conferring the pup phenotype than in utero exposure. Gorski et al reported offspring from obese dams fostered onto lean dams had improved insulin sensitivity (though no changes in body weight were observed).[35]

Translating these animal model findings to human research is challenging, however, given the limited ability to control for confounders such as maternal environmental exposures and offspring exposures during pregnancy in humans. A direct study of infant diets is also difficult as there is an inability to randomize to alternative nutrition interventions for infants, given the known benefits of providing human milk.

5 | REVIEW OF NON-NUTRITIVE COMPONENTS

Extensive evaluation of the complex components in human milk and their associations with infant metabolic outcomes remains limited in current research. Previous review articles have importantly highlighted the roles of nutritive (fat, protein and carbohydrate) and non-nutritive (insulin, leptin, adiponectin, ghrelin, interleukin-6 (IL-6), tumour necrosis factor-alpha and toxins) components of human milk in lactational programming through animal models and human studies. Of the more thoroughly studied hormones, leptin (an appetite regulator), has shown early inverse associations with an infant growth trajectory. However, the influences on human offspring outcomes remain inconsistent for many other non-nutritive factors which are limited by the small sample size, inability to control confounding factors, and a limited number of studies. Beyond these factors, there are newly discovered human milk components that appear to have more consistent relationship to infant growth. This review will synthesize the evidence for emerging bioactive compounds in human milk including microRNA's (miRNAs), lipokines/signalling lipids, small molecules/metabolites and fructose. Table 1 summarizes predominantly human studies of these novel factors focusing on studies during the window of lactation and programming outcomes.

5.1 | miRNAs

A rather new class of bioactive components discovered in human milk and now being investigated are miRNAs. miRNAs are small, non-coding, single-stranded RNAs that modulate gene regulation by inhibiting mRNA translation and protein synthesis. Genes targeted by human milk miRNAs can impact many pathways, particularly carbohydrate and energy metabolism, immune function and brain development.[40] Human milk is one of the richest sources of RNA and miRNAs among all body fluids.[17] Most human milk miRNAs are transported and encapsulated by the lipid bilayer of extracellular vesicles, predominantly in exosomes. Exosomal miRNAs in breast milk are thereby protected from the enzymatic degradation that would occur in the digestive tract,[41] allowing uptake by intestinal epithelial cells.[22,42]

Multiple factors such as maternal nutrition, exercise, and maternal disease states (e.g., obesity and diabetes) can impact the abundance and constituency of human milk miRNAs. Studies investigating the role of maternal metabolic status on human milk miRNA composition are sparse. A study performed by Xi et al shows that some miRNAs involved in adipogenesis (miRNAs-let-7a, 378 and 30b) are negatively correlated with maternal pre-pregnancy body mass index (BMI).[43] Further, a study performed by Zamanillo et al showed that maternal obesity altered the breast milk supply of some miRNAs, their association with milk bioactive factors such as leptin and adiponectin, and their impact on infant BMI at 2 years of age.[44] Our group has recently shown the abundance of selected miRNAs involved in important metabolic pathways such as insulin signalling and adipogenesis (miRNAs-148a-3p and 30b-5p) are decreased in human milk exosomes obtained from overweight or obese women.[45] In the same study, these miRNAs were also associated with infant weight and adiposity at 1 month of age suggesting a potential role of miRNAs in regulating infant growth and body composition. Mechanistic studies into the effects of human milk miRNAs on growth, development and early infant programming are lacking. Future mechanistic studies are needed to examine the uptake and targets of ingested human milk miRNAs, not only in the infant's gut but also in the metabolically active tissues such as the liver, pancreas identify their functional role.

5.2 | LIPOKINES AND SIGNALLING LIPIDS

Based on accumulating data over the last 15 years, certain lipids are now recognized as having hormone-like effects in the regulation of

TABLE 1 Summary of predominantly human studies of novel factors focusing on studies during the window of lactation and programming outcomes

Bioactive component	Author (Year)	Species (*n*)	Milk collection standardized	Lactation time point (s)	Maternal influence	Infant outcomes	Weakness/limitations
miRNA	Xi (2016)	Human ($n = 86$)	No	2–5 days, 3 months	miRNA-30B, let-7a, miRNA-378 in colostrum and mature milk are negatively correlated with maternal BMI (pre-pregnancy and late pregnancy), weight Let-7a in mature milk is negatively correlated with late pregnancy maternal weight No miRNA differences with gestational diabetes mellitus or gestational age.	miRNA-30B and miRNA-378 were higher in colostrum for girls	No maternal diet data No infant anthropometrics
	Zamanillo (2019)	Human ($n = 59$)	Yes	1 months, 2 months, 3 months	NW mothers had negative correlations between milk miRNAs expression Leptin/adiponectin levels observed in NW but not in OW/OB mothers	In NW mothers, infant BMI at 2 years negatively correlated with miRNA at 2 m (miR-103, miR-17, miR-181a, miR-222, miR-let7c and miR-146b). No infant growth correlations with OW/OB mothers	No maternal diet data Infant growth at 2 years and maternal BMI were obtained by the maternal recall
	Shah (2021)	Human ($n = 60$)	Yes	1 months and 3 months	Decreased miRNA (miR-148a, miR-30b) abundance at 1 months in OW/OB mothers compared to NW mothers	miR-184a was negatively associated with infant weight, FM and FFM at 1 m miR-32 was negatively associated with 1 m infant weight miR-30b abundance was positively associated with 1 m weight, %fat and fat mass. No significant associations were observed with 1 m milk miRNA and 6 m infant anthropometrics, or between 3 m milk miRNA and infant anthropometrics at 3 m or 6 m	No maternal diet data
Lipokines and signalling lipids	Brezinova (2018)	Human ($n = 53$) and Mouse	No	72 h	PAHSAs levels were positively associated with weight gain in pregnancy. Obese mothers had lower total PAHSA and 5-PAHSA levels	No outcomes examined	No maternal diet data. No infant outcomes examined
	Bruun (2018)	Human ($n = 100$)	No	4 months	Only adjusted for maternal BMI	The low weight z-score group had higher concentrations of oleoylethanolamide, steroylethanolamide, palitoylethanolamide versus	No early lactation samples. No maternal diet data. Storage techniques altered the concentration of *N*-acylethanolamides.

(Continues)

TABLE 1 (Continued)

Bioactive component	Author (Year)	Species (n)	Milk collection standardized	Lactation time point (s)	Maternal influence	Infant outcomes	Weakness/limitations
						infants in the high weight z-score. Lower skinfold thickness and weight gain per day were associated with higher milk steroylethanolamide	
	Wolfs (2021)	Human ($n = 58$)	Yes	1 months, 3 months, 6 months	12,13-diHOME positively associated with height, however no association with maternal pre-pregnancy BMI or gestational weight gain 12,13-diHOME concentration increased in milk after exercise at 1 months postpartum	12,13-diHOME was positively associated with birth weight for length and BMI z-score, and negatively associated with BMI and weight for length z-score over the first 6 months of life 12,13-diHOME was negatively associated with subcutaneous and overall adiposity at 1 months No association between infant sex and milk 12,13-diHOME levels High milk succinate was associated with lower infant BMI at 6 months	No maternal diet data.
Small molecules and metabolites	Isganaitis (2019)	Human ($n = 35$)	Yes	1 months, 6 months	Maternal BMI was associated with 10 differing metabolites at 1 months 20 differing metabolites at 6 months 1mo, maternal obesity was linked to differences in human milk orotate and 1,5-anhydroglucitol 6 months maternal obesity was associated with increased acylcarnitine, monosaccharide, and 1,5-anhydroglucitol	Adenine positively correlated with infant weight at 1 months, while X-19656 (an unidentified metabolite) was negatively correlated 1 month, 5-methylthioadenosine was positively correlated with infant %fat and maternal BMI. No correlations between milk metabolites with infant weight or adiposity and maternal BMI at 6 months	No maternal diet data.
	Prentice (2019)	Human ($n = 619$)	Yes	4–8 weeks	SCFAs were not associated with maternal BMI Mothers exclusively breastfeeding had higher milk butyrate	Butyrate was negatively associated with infant weight between 3 and 12 months Milk formic acid levels were negatively associated with BMI over the first 2 years of life Acetate was negatively associated with skinfold thickness at 3 months	No maternal diet data.

TABLE 1 (Continued)

Bioactive component	Author (Year)	Species (*n*)	Milk collection standardized	Lactation time point (s)	Maternal influence	Infant outcomes	Weakness/limitations
	Saben (2020)	Human ($n = 159$)	Yes	2 weeks, 2 months, 6 months	Maternal BMI and %fat positively correlated with the amount of human milk monosaccharides and sugar alcohols	6 months milk mannose, lyxitol and shikimic acid showed a positive association with infant fat mass and %fat	No maternal diet data.
	Ribo (2021)	Human ($n = 143$) and mouse	Yes	1 months	Did not report maternal influence on milk betaine	Human milk betaine concentration was inversely associated with infant weight-for-length z-score at 1 and 12 months	No maternal diet data
					In mice supplemented with betaine, a fivefold increase in betaine milk content was observed	In mice, early exposure to betaine in lactation was associated with decreased adiposity and improved glucose homeostasis in adult mice. Betaine intake transiently increases offspring *Akkermansia* species during early life, which is linked to lower gut inflammation in mice	
Fructose	Goran (2017)	Human ($n = 25$)	Yes	1 months, 6 months	Maternal BMI was used as a covariate	Milk fructose was associated with high infant body weight, lean mass, FM, weight for length z-score, and bone mineral content at 6 months	No maternal diet data.
	Berger (2018)	Human ($n = 41$)	Yes	6 weeks	HFCS-sweetened beverage increased milk fructose for up to 5 h without an impact on milk glucose or lactose.	None were collected	No infant outcomes No maternal diet data
	Berger (2020)	Human ($n = 88$)	NA	NA	No differences in maternal fructose or sugar-sweetened beverage + juice intake based on maternal pre-pregnancy BMI	Infant 24 months cognitive development scores were inversely correlated with fructose consumption by their mothers at 1 months postpartum No association at 6 months postpartum	Homogenous sample (limited to Hispanic mother-infant dyads) No milk compositional analysis was performed

Note: Novel Bioactive Compounds in Human Milk.

insulin sensitivity, pancreatic beta-cell function, inflammation and other cellular processes, leading Hotamisligli and colleagues to coin the term 'lipokine'.[46] Recent studies indicate that human milk is an abundant source of lipokines and is associated with infant growth and metabolism. For example, fatty acid esters of hydroxy fatty acids (FAHFAs) are endogenous lipids with known insulin-sensitizing potential and anti-inflammatory properties.[47,48] Recently, Brezinova et al studied the role of a specific FAHFA, palmitic acid hydroxystearic acid in the milk of normal weight and obese women.[49] Their findings were quite interesting in that: (a) for the first time the presence of FAHFs in breast milk were detected (previously, it was thought FAHFAs only existed in serum and white adipose tissue) and (b) palmitic acid hydroxystearic acid was significantly lower in milk from obese mothers. These findings perhaps represent a pathway where milk from obese mothers who were shown to have lower concentrations of anti-inflammatory and pro-insulin sensitizing FAHFAs provide a double-hit to their offspring, with the first hit occurring in utero and the second during lactation.

The regulation of food intake and appetite are in part regulated by palmitoylethanolamide (PEA), N-acylethanolamine lipids oleoylethanolamide (OEA) and stearoylethanolamide (SEA).[50] Human milk concentrations of these appetite regulators were studied in mother/infant dyads from the Odense Child Cohort (an unselected, prospective birth cohort in Odense, Denmark).[51] Breast milk was collected at 4-months in 100 infants divided equally into one of two groups, low weight-for-age Z-score (L-WAZ score) or high weight-for-age Z-score (H-WAZ score). Key findings from this study were (a) OEA, SEA and PEA concentrations were lower in the H-WAZ group versus the L-WAZ group ($p < .05$) and (b) higher concentrations of SEA were associated with lower triceps skinfold thickness and lower weight gain per day (birth to date of testing). These data demonstrate that key appetite regulators are not only present in breast milk but may play a part in driving growth and fat deposition.

More recently, certain lipokines have been shown to play a central role in the regulation of thermogenesis and brown adipose tissue function. For example, the oxylipin 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) was shown to be released into plasma in response to cold exposure and to stimulate brown adipose tissue fuel uptake and thermogenesis.[52] Our group recently identified 12,13-diHOME in human milk and reported an inverse correlation between the abundance of this oxylipin and infant adiposity.[53] Interestingly, maternal moderate exercise resulted in a significant increase in milk 12,13-diHOME, raising the intriguing possibility that maternal interventions might enhance milk content of obesegenic-protecting factors.[54] Further reinforcing the importance, the emerging connection between breastmilk and adipose tissue metabolism in infancy, the lab of Tamas Roszer recently described an elegant mechanism by which alkylglycerol-type ether lipids in human milk play a role in downregulating the shift from brown-fat like metabolically active 'beige' adipocytes to lipid-storing 'white' adipocytes.[55] Alkylglycerol lipids are metabolized by adipose tissue macrophages, triggering beige adipocyte development in the infant via a signalling cascade involving IL-6 and STAT3. Notably, such alkylglycerol lipids are unique to human milk, and are largely absent from infant formulas and adult diets. Together, these data suggest that human milk influences childhood obesity risk via lipid-dependent effects on adipose tissue metabolism.

5.3 | SMALL MOLECULES AND METABOLITES

With the advent of high throughput 'metabolomics' platforms, which allow the comprehensive quantification of up to 1000 small molecules and metabolic intermediates in a small biological sample, there has been increasing interest in how the human milk metabolome may be influenced by maternal phenotype, and how these small molecules, in turn, may regulate infant growth and metabolism. Metabolomic analyses have proved to be a valuable approach for biomarker discovery in developmental programming and metabolism research.[56] Considered 'downstream' from the genome, transcriptome and proteome, the metabolome also can be influenced by the nutritional environment and the gut microbiome. As such, metabolomics integrates multiple aspects of metabolic health, providing a sensitive research tool. Analyses of human milk have to date identified an association of maternal BMI with the milk metabolome. The first report on the human milk metabolome, published in 2013 by Smilowitz and colleagues, detected 65 low-molecular-weight metabolites including carbohydrates, oligosaccharides, amino acids, fatty acids, vitamins and nucleotides. A key finding was wide inter-individual variation in the amount and composition of human milk oligosaccharides.[57] This is especially intriguing given the observation that human milk oligosaccharides play a role in immune regulation, bacterial defences and infant growth.[58]

More recently, our group used an untargeted metabolomics platform to analyse associations among the human milk metabolome, maternal obesity and infant body composition.[59] At 1 month postpartum, only 10 of 275 detectable metabolites differed between overweight/obese and lean women at a significance threshold of $p < .05$; at 6 months postpartum 20 of 275 metabolites were altered with $p < .05$. Although the number of altered metabolites was relatively small, it was notable that many of the metabolites that were altered by maternal obesity belonged to the same chemical class, with enrichment of purine nucleotide derivatives and human milk oligosaccharides. Indeed, milk content of the nucleotide adenine was positively correlated both with maternal BMI, and with infant adiposity and fat accrual (DXA) between 1 and 6 months postpartum. This is intriguing considering clinical trial data and meta-analyses indicating that nucleotide supplementation of cow's milk-based infant formulas results in accelerated postnatal weight gain.[60] Thus, human milk nucleotides may represent an 'obesigenic' factor in human milk.

In 2021, Saben et al. published the largest study on the metabolomic composition of human milk reported to date.[61] The metabolome of human milk was analysed at 2 weeks, 2 months and 6 months postpartum in women with normal (BMI < 25.0) versus obese (BMI ≥ 30.0) pre-pregnancy BMI. This repeated-sampling approach allowed the authors to identify a time-dependent effect of maternal obesity on milk composition; they report a significant interaction of maternal BMI and time on milk content of metabolites related to amino acid metabolism. Their findings suggest that maternal BMI alters the evolution of human milk during the postnatal period from 'transitional' to 'mature' milk. Notably, 111 metabolites, or nearly a third of the 355 metabolites that were identified using their GC–MS approach, were significantly associated with maternal BMI ($p < .05$), with milk from obese mothers having a higher abundance of monosaccharides and sugar alcohols. In turn, a proportion of the differentially abundant metabolites (e.g., mannose, lyxitol and shikimic acid) were associated with infant adiposity. These results support the possibility that components of human milk may be mediators of mother–child transmission of obesity risk, although these relationships would need to be validated using experimental studies in in vitro or in vivo systems.

From a mechanistic perspective, the human milk metabolome has the potential to influence infant growth via several distinct mechanisms, including direct interactions between individual milk biochemicals and infant intestinal or immune cells, nutritional effects (e.g., providing higher or lower amounts of essential amino acids or vitamins), effects of absorbed metabolites on distant organs, effects on the developing infant microbiome and other pathways. The possibility of metabolome—microbiome interactions is supported by a recent study by Ribo et al, which found reduced levels of betaine (a one-carbon metabolite previously linked to cardiovascular and diabetes risk[62,63]) in milk from obese mothers.[64] Betaine supplementation of mouse dams resulted in increased betaine content in milk, and long-term improvements in metabolic health of offspring including decreased adiposity and improved glucose tolerance, via a microbiome-dependent mechanism. The role of milk components in regulating offspring energy metabolism is suggested by Prentice et al who showed that milk short-chain fatty acid butyrate content was protective against excess infant weight gain and adiposity development in the first year of life.[65]

In sum, the milk metabolome is altered in the setting of maternal obesity and many metabolites have been linked to infant health outcomes. The extent to which modifiable risk factors such as gestational weight gain, glucose homeostasis or exercise might affect the milk metabolome, and whether metabolomic fingerprints of maternal obesity might be reversible, will be important areas for future studies.

5.4 | FRUCTOSE

There is evidence that maternal dietary intake during lactation may modify and/or introduce nutrients into human milk that are then transmitted to the nursing infant. However, recent data in this realm are limited, as few studies have collected detailed dietary assessments of mothers during the postnatal period. Dynamic changes in the food environment may inadvertently expose nursing infants to novel constituents of Western-style diets that may not be ideal at this life stage. For example, a randomized cross-over trial revealed that mothers who consumed a sugar-sweetened beverage containing fructose had significantly higher concentrations of milk fructose, as compared to those consuming a control beverage.[66] Indeed, mothers who consumed the fructose-containing beverage had increased concentrations of human milk fructose above baseline which persisted up to 5 h after intake.[66] Infant exposure to milk fructose through human milk, even at low levels, has been associated with greater fat mass and lean mass in nursing infants at 6 months of age[67] as well as poorer neurodevelopmental outcomes at 2 years.[68] These findings may inform dietary recommendations for mothers during the period of exclusive human milk feeding, as high fructose corn syrup is not a naturally occurring sugar in human milk.

6 | CURRENT STATE AND RECOMMENDATIONS TO ADVANCE THE FIELD

Based on the current state of the literature we have identified five domains in human milk research that limit our collective knowledge and need to be breached to propel the field forward. Theoretically, milk is of immense importance as a carrier of metabolic cues between parental and offspring generations, over and above as its importance as a nutrient source. While human data is still limited, results from animal models are more conclusive,[2] suggesting numerous impacts during the highly plastic period of early development, including the shaping of the early infant gut, immune system and key metabolic systems responsible for glucose and weight regulation. In the hopes of propelling the field forward, we propose some solutions to identified weaknesses in the field.

1. *Limited statistical power*. To date, most studies in the literature are sorely underpowered, do not take into account important covariates and many times are samples of convenience. These issues decrease inference and the ability to make definitive conclusions.
   Action needed: Adequately and appropriately powered studies to definitively answer the study hypothesis. Consortia of individual studies with similar sampling methods should be established and funded to combine harmonizable data to address the above hypotheses in large numbers of mothers and infants/children.
2. *Cross-sectional study designs limit the determination of temporality*. Currently, most studies capture one-time point or in some cases two over several months (though rarely) but few are carried out past 3 months. We acknowledge these sorts of studies are hard to implement and execute but are sorely needed to advance the field and understand compositional changes in milk over time.
   Action needed: Conduct longitudinal studies that include colostrum, transitional milk and mature milk to 6–12 months (but 6 months if at all possible), with repetitive and synchronized collections of both maternal serum (to gauge maternal inflammation, nutrient status, etc.), human milk and infant outcomes.
3. *Poor coordination between basic animal models and human study designs*. Experimental studies in animals provide needed mechanistic precision and causal support for lactational programming, where

randomization of women to most dietary or lactational interventions would be unethical. However, human studies are always needed given species variation in milk composition, infant nutrient requirements, metabolic physiology, early growth and development and life history characteristics. Further, human studies are needed to appropriately judge the relative and absolute contributions on the infant of the clustered and socially determined maternal conditions mentioned above (e.g., obesity, diet quality and breast feeding duration). Animal models should also take into consideration mixed feedings and the timing of the introduction of solid foods, both of which are important influences for many babies.

Action needed: Basic scientists and human clinical scientists must work more closely together on shared aims, with iterative and cross-species replication/experimentation, to build a base for causal evidence of lactational programming and make mechanistic discoveries that are properly rooted in human epidemiology and have the potential for translation.

4. *Lack of consideration for maternal metabolic phenotypes (gestational weight gain, obesity status and diabetes status) during pregnancy and lactation*. Few studies take into account maternal obesity and/or diabetes status and their impact on the composition of breastmilk. Expansion and cooperation between pregnancy studies and infant prospective cohorts would also enhance the information that can be gathered from these costly and time-intensive study designs. This information would also allow investigators to parse out lactational versus gestational programming effects and how the lactation exposures may modify gestational stressors.

   Action needed: Per routine, all milk studies would collect clinically relevant pre-pregnancy and pregnancy data, such as BMI, weight gain, glucose control and other metabolic markers.

5. *Poor and or lack of collection of maternal diet assessment during lactation*. Though the scientific community as a whole recognizes the importance of the use of nutritional surveys, the practical assessment in the dietary intake on an individual level is challenging in the best of circumstances, partly due to the complexity and frequently changing nature of dietary habits.[69] The ultimate goal of dietary assessment is to better understand the impact of dietary intake of specific nutrients on the composition of milk and the outcome in the infant.

   Action needed: Human milk researchers should partner with registered dietitians or other nutrition researchers to determine how to sample maternal dietary habits in prospective mother-infant studies.

7 | CONCLUDING STATEMENTS

It is important that as the field moves forward, we remain in unwavering pursuit of detailed knowledge of breast milk, the complex suite of factors/signals impacting its composition, and the molecular roles that identified and yet to be identified human milk factors play on child obesity and other aspects of long-term offspring health.

CONFLICT OF INTEREST

No conflict of interest was declared.

AUTHOR CONTRIBUTIONS

David A. Fields and Brigid Gregg designed the basic framework and purpose of the review. Lindsay Ellsworth, Greg Pavela, Elvira Isganaitis and Ellen W. Demerath contributed to the development and refinement of the project. Gregory Pavela did the initial literature review. Brigid Gregg, Lindsay Ellsworth, Elvira Isganaitis, Gregory Pavela, Kruti Shaw, Ellen W. Demerath and David A. Fields assisted in writing and editing the manuscript. Brigid Gregg, Lindsay Ellsworth, and Sheri VanOmen generated the tables and figures. All the authors read and approved the final manuscript.

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