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Shared patterns of human milk composition link mammary gland function to infant growth

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The mammary gland produces nutrient-rich human milk (HM), yet how mammary functional state shapes HM composition and infant outcomes remains poorly understood. We used HM multi-omics - metabolomics, proteomics, micronutrients, macronutrients, and HM oligosaccharides - across 1,543 samples from three cohorts, including two randomized trials, as a noninvasive readout of mammary functional state. Trajectory modeling and multi-omic integration showed that maternal supplementation improved recovery from early growth faltering and revealed a shared HM compositional axis linking maternal nutrition to infant growth. Machine learning, with Human Protein Atlas–informed proteomics, identified seven HM biomarkers reflecting variation across key mammary domains, indexing milk synthesis, barrier integrity, and immune/repair activity. Elevated valerylcarnitine and low pantothenic acid emerged as metabolic signals linking mammary functional state to infant growth.

The mammary gland is a highly specialized organ responsible for producing human milk (HM), a complex, adaptive system that supports infant survival, growth, and development (*1*–*6*). Reflecting the central role of HM in infant health, the WHO recommends exclusive breastfeeding for ~6 months, with continued breastfeeding for up to 2 years (*3*). Despite broad recognition that HM is a key mediator of maternal–infant health within the "mother–milk–infant triad" (*7*), the mammary gland itself remains understudied. Prior work has shown that HM-derived molecular data, including milk cells, transcriptomes, proteomes, and metabolomes, can provide a noninvasive window into mammary gland biology and lactation physiology (*8*–*11*). However, these approaches have rarely been integrated across diverse human cohorts to define

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shared HM signatures of mammary functional state linking maternal nutrition to infant growth.

Environmental factors—particularly maternal nutrition—influence specific HM components such as milk lipids, particularly in undernourished settings (*12–14*). Animal studies further show that changes in maternal nutritional status can alter milk energy, protein, and micronutrient content, while also affecting mammary gland development and gene-expression programs (*2*, *15–17*). These effects are likely mediated by mammary responses to changes in maternal metabolism: for example, energy depletion and nutrient deficiency reduce milk protein synthesis via AMPK/mTORC1 signaling in the gland (*18–20*). Yet in human lactation research, this pathway is rarely considered outside breast cancer, despite its direct relevance for populations experiencing nutrient deficiencies (*21*).

Prevailing views in high-income settings have often downplayed the impact of maternal nutrition on infant outcomes, arguing that mothers can mobilize body stores to buffer milk composition and thereby protect infant growth (*22–24*). However, this conclusion is based largely on short-term restriction studies in otherwise well-nourished populations and on analyses focused primarily on macronutrients, with relatively limited attention to other key HM components such as micronutrients. Evidence from settings of chronic undernutrition and sustained physiological stress suggests that buffering can be incomplete, particularly for micronutrients (vitamins and minerals) and other milk constituents that depend on maternal intake and stores (*25–28*). These discrepancies underscore the need for a more mechanistic, systems-level investigation of how maternal nutrition and health shape mammary function, HM composition, and infant growth in diverse settings.

More broadly, research on the human mammary gland has disproportionately focused on cancer, with relatively few mechanistic studies of lactation. This siloed approach is striking given that pre- and postnatal mammary development and breast cancer engage many of the same biological pathways, differing mainly in regulation versus dysregulation (*29*, *30*). Studies linking human mammary function to infant growth have largely centered on mastitis and other overt inflammatory or infectious states (*31–33*). However, emerging evidence suggests that mammary gland physiology more broadly - including metabolic and endocrine regulation of milk synthesis and secretion (such as protein, lipid, and oligosaccharide production), as well as immune and epithelial homeostasis - can shape milk composition even in the absence of overt infection and may thereby influence infant outcomes (*18–20*, *34*, *35*). As a result, the biological mechanisms linking variation in mammary gland functional state to milk composition - and to infant growth, particularly in the context of growth faltering - remain poorly defined.

Lactation depends on multiple integrated homeostatic functions of the mammary gland, including milk synthesis and maintenance of a selective blood–milk barrier. These functions are essential for sustaining infant nutrition and health but can be altered by perturbations such as infection or inflammation; regulated immune responses may be protective, whereas persistent or excessive activation can disturb glandular homeostasis (*36–39*).

The primary function of the mammary gland is milk production, characterized by high concentrations of lactose and milk-specific proteins. While some milk proteins are derived from blood, the major milk-specific proteins—such as casein and α-lactalbumin—are synthesized predominantly by secretory cells within the mammary epithelium, with approximately 80% of total milk amino acid content derived from casein (*18*, *39*, *40*). Human milk production is energetically demanding, requiring approximately 670 kcal/day during the first six months postpartum (*41*, *42*). In fact, the cumulative energy output from milk over the first four months of lactation is comparable to the total energy expenditure of pregnancy (*43*).

The selective transfer of nutrients, hormones, and immune factors into HM depends on the blood–milk barrier, composed of epithelial tight junctions and the underlying basement membrane and extracellular matrix (ECM) (*44*, *45*). Blood–milk barrier integrity is commonly assessed using milk sodium, potassium, and the Na:K ratio, with reduced tight junction selectivity leading to paracellular leak of sodium into and potassium out of milk, thereby elevating milk sodium and the Na:K ratio (*46–48*). These established markers have long been used to assess tight-junction permeability, delayed secretory activation, and mastitis-associated barrier disruption. Disruption of the blood-milk barrier leads to characteristic changes in HM composition, including the influx of blood-borne proteins and decreased lactose concentrations (*39*, *49–51*). Impairment of the mammary basement membrane can also indirectly reduce β-casein via ECM remodeling mediated by tenascin-C (*52*).

This barrier is highly sensitive to stress. Reactive oxygen species generated during inflammation or infection can compromise barrier integrity, increasing permeability to immune cells and serum proteins (*39*). Inflammation is often a protective response to infection, but sustained or excessive inflammatory activation can have complex effects on mammary function. Maternal inflammatory states trigger the up-regulation of mucosal immune factors - such as secretory IgA and calprotectin - which support recovery and confer passive immune protection to the infant (*53–56*). However, these responses can also disrupt core homeostatic functions, impairing milk protein synthesis and weakening the blood–milk barrier, both of which negatively impact milk nutrient content (*20*, *39*).

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Here, we use mammary gland functional state to refer to the coordinated status of the lactating gland across three interconnected programs: (i) milk biosynthesis and secretion (lactose and milk-specific proteins), (ii) selective blood–milk barrier function regulating transfer of serum-derived components, and (iii) regulated immune/repair responses to perturbations such as infection or inflammation. When used, mammary gland dysregulation refers specifically to sustained departures from lactational homeostasis, such as reduced biosynthetic output, increased barrier permeability, or persistent inflammatory activation.

Although the homeostatic functions of the mammary gland (milk synthesis, maintenance of barrier integrity) as well as its regulated immune/repair response are highly interconnected, they are often studied in isolation. A systems biology framework is needed to capture the complexity of mammary functional-state domains and their downstream effects on infant health. Given the mammary gland's central role in linking maternal physiology to HM composition and infant outcomes, we hypothesized that conserved nutritional and growth-related signals embedded within HM may serve as biomarkers of mammary gland functional state and dysregulation, as defined above. Specifically, we posited that, while HM composition varies across geographies, core maternal physiological processes—such as nutrient availability, immune regulation, and epithelial integrity—may be conserved and reflected in HM composition (*57*–*60*).

To test whether HM contains conserved nutritional and growth-related signals reflecting mammary gland functional state, we analyzed 1,543 longitudinal HM samples from three International Milk Composition (IMiC) Consortium studies spanning diverse nutritional and socioeconomic environments and distinct HM composition profiles (Fig. 1A and fig. S1). We used HM multi-omics to identify candidate biomarkers linking maternal nutrition, mammary gland functional state, and infant growth. We reasoned that HM could serve as a systems-level biological readout of the mother–mammary gland–infant axis, capturing milk-derived signals that connect maternal nutritional status with infant growth across diverse populations.

We asked whether maternal nutritional supplementation influences infant growth outcomes. We further sought to identify specific HM components shaped by maternal nutritional status that are also associated with infant growth and reflect core aspects of mammary gland functional state. Leveraging supervised and unsupervised machine learning across >25,000 HM analytes (Fig. 1)—including nutrients, proteins, immune factors, and metabolites—we uncovered shared compositional signals linking maternal physiology to HM composition and infant growth.

To interpret these associations mechanistically, we applied a systems biology framework informed by Human Protein Atlas annotations and HM proteomics to examine three key facets of mammary gland function: milk synthesis, epithelial barrier integrity, and immune/repair activity. Together, these analyses revealed candidate HM biomarkers that connect maternal metabolism to mammary gland function and infant growth and illustrate the broader utility of human milk multi-omics as a noninvasive strategy for probing human mammary gland processes.

Results

Integrating HM signals across diverse geographic contexts

To identify shared and cohort-specific HM biomarkers of mammary functional-state domains, we analyzed longitudinal HM samples from three IMiC studies: two randomized maternal balanced energy–protein (BEP) supplementation trials in Burkina Faso (MISAME III, n = 290; mixed nutritional status) and Pakistan (Mumta-LW, n = 150; undernourished) and the Canadian CHILD birth cohort (n = 400; all adequately nourished). These cohorts enabled cross-contextual analysis of human milk composition, maternal nutritional/metabolic factors, and infant growth.

In MISAME, milk was collected at 14-21 days, 1-2 months, and 3-4 months; in Mumta-LW, milk was collected at 42 days and at 56 days; in CHILD, milk was collected once between 3-4 months. Computational strategies were used to accommodate this sampling time heterogeneity. We profiled targeted and untargeted LC-MS metabolites (479 and 23,114 analytes, respectively), proteins (2,018), micronutrients (23), human milk oligosaccharides (HMOs; 21), and macronutrients (4).

We focused most of our analysis on the MISAME and Mumta-LW randomized trials, which capture nutritional variability and higher risk of undernutrition, micronutrient deficiencies, and infections that may impact mammary functions, as well as variability in infant growth outcomes (*53*, *61*–*63*). Consistent with prior studies on HM composition across geographic regions, we observed distinct HM profiles across cohorts, with between-study differences strongly exceeding within-study differences ($P < 2.2 \times 10^{-16}$ in all studies; fig. S1) (*59*, *60*). Compared to the CHILD cohort, HM samples from Mumta-LW showed greater within-cohort dispersion ($P = 0.013$), with a similar but weaker pattern observed in MISAME ($P = 0.051$).

Maternal nutritional intervention influences infant growth via milk composition

We first sought to establish that maternal nutritional supplementation influences infant growth outcomes. Because supplementation was administered to mothers during pregnancy (MISAME) and/or lactation (both MISAME and Mumta-LW), effects on growth could reflect prenatal influences, postnatal

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influences mediated through HM composition, or both; however, supplementation effects on milk composition appeared largely driven by postnatal BEP rather than prenatal exposure (fig. S2). To examine this, we applied unsupervised trajectory modeling to classify infant growth patterns and identify maternal and environmental factors associated with adverse outcomes. This approach enabled detection of supplementation benefits among high-risk infants that may be obscured by traditional mean-effect models (*64*, *65*).

Our primary infant growth outcome was weight-for-age Z-scores (WAZ), which capture both chronic and acute malnutrition and can serve as an early indicator of later stunting (*66*, *67*). In MISAME, we identified two groups with similarly low WAZ and BMI at delivery: one that diverged into a "recovered" trajectory with significantly higher BMI, growth velocity, length velocity, and head circumference at 6 months ($P = 1.5 \times 10^{-5}$, $P = 3.6 \times 10^{-5}$, $P = 0.0096$, $P = 0.0017$, respectively) relative to the nearest stable trajectory, and another "unrecovered" trajectory that remained stagnant (Fig. 2A). In Mumta-LW, the single perturbed trajectory resembled the unrecovered MISAME group, with low WAZ and BMI at 6 months and reduced length velocity ($P = 0.0044$) relative to the nearest stable trajectory (Fig. 2B).

To identify predictors of trajectory membership and transitions, we estimated risk ratios for developing, recovering from, or maintaining growth perturbations (Fig. 2, C and D, and see the supplementary text). Across both cohorts, key risk factors included maternal undernutrition, short stature, and season of birth, reflecting seasonal/environmental exposures. Postnatal BEP supplementation had a strong protective effect in both populations, increasing the likelihood of recovery in MISAME (Risk Ratio: 2.32, $\chi^2 = 4.44$, $P = 0.035$) and trajectory stability in Mumta-LW (Risk Ratio: 1.24, $\chi^2 = 10.22$, $P = 0.0014$). In contrast, prophylactic azithromycin administered to a subset of infants in the Mumta-LW trial was not significantly associated with trajectory stability.

We next aimed to directly link maternal supplementation and infant growth to HM composition. To identify conserved milk features connecting maternal nutrition to infant growth—and potentially reflecting mammary functional state—we integrated unsupervised and supervised machine learning across cohorts.

Using Joint-RPCA, a multi-modal dimensionality reduction method, we identified a shared signal along the first axis of HM variation in both MISAME and Mumta-LW that was significantly associated with both maternal nutrition (MISAME: $P = 0.0015$; Mumta-LW: $P = 0.0040$) and infant growth (WAZ at month 4, MISAME: $P = 0.042$; Mumta-LW: $P = 0028$; Fig. 3A). We ranked features along this axis and computed log-ratios of top versus bottom features within each modality (tables S1 and S2 and see the supplementary text), yielding a subset of 136 features per cohort (with 50.7% overlap), for a total of 203 distinct features—including targeted and untargeted metabolites, HMOs, and micronutrients—that robustly captured this shared signal. These log-ratios showed significant separation by both supplementation and growth categories across most data types and time points (Fig. 3B), and the shared feature set produced similar separation in both studies (fig. S3). Together with our trajectory modeling results, these findings support a link among maternal nutritional status, HM composition, and infant growth.

To refine the shared signal and identify specific molecular drivers, we next applied supervised machine learning. We trained study-specific XGBoost models to predict maternal supplementation status (binary) and infant growth (binarized 4-month WAZ). In our models, we used a wide-encoding approach for longitudinal samples, 10-fold cross-validation, and SHAP (SHapley Additive exPlanations) values to rank features by their contribution to prediction across model CV runs (see supplementary text).

Using SHAP-based feature selection, we identified 20 targeted HM components—including metabolites, HMOs, and micronutrients—and 21 untargeted LC-MS features that accurately predicted maternal supplementation in both MISAME and Mumta-LW (Targeted AUC = 0.86 for both; Untargeted AUC = 0.90 and 0.85, respectively; Fig. 4A and table S3). Similarly, 33 targeted and 29 untargeted features predicted infant WAZ across MISAME, Mumta-LW, and CHILD (Targeted AUCs: 0.67, 0.65, 0.64; Untargeted AUCs: 0.63, 0.84, 0.68, respectively). A broader set of 104 untargeted LC-MS features—prioritized by combining SHAP importance with univariate associations—demonstrated particularly strong cross-study WAZ prediction (AUCs: 0.73, 0.86, 0.86, for MISAME, Mumta-LW and CHILD respectively; Fig. 4A). These features were subsequently selected for MS2-based annotation (fig. S4, A and B).

To determine whether HM features contributed predictive signal beyond a baseline model, we compared each HM-based model with a naïve model without HM features. For all outcomes, HM-based models incorporating targeted, untargeted, or MS2-derived features significantly outperformed the naïve baseline in terms of ROC AUC, precision, and balanced accuracy ($P < 0.05$ for all comparisons; fig. S5). The naïve models performed near random chance, with ROC AUC values ranging from 0.39 to 0.56 across comparisons (Fig. 4A).

A core set of human milk components may constitute a conserved signature of mammary gland functional state

To identify HM components that may reflect conserved aspects of mammary gland biology across geographies, we first leveraged our machine learning results to find features

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capturing shared nutrition- and growth-related signals across independent study cohorts. To this end, we intersected (i) 53 HM components identified by supervised machine learning as predictive of either maternal supplementation status or infant WAZ in at least two of the three cohorts (MISAME, Mumta-LW, and CHILD; 20 targeted features predictive of maternal nutritional status and 33 predictive of infant growth) (table S3) with (ii) the 203 top- and bottom-ranked features along the first Joint-RPCA axis, which represents the dominant shared variation linking maternal supplementation and infant growth (shown in tables S1 and S2; features marked as *"Log_Ratio"*). This cross-signal integration yielded 23 HM components representing reproducible nutrition–growth features across modeling approaches and cohorts (Fig. 4B).

Because this shared maternal nutrition–infant growth axis could reflect either direct transfer of dietary constituents into milk or variation in the mammary gland's capacity to synthesize, remodel, and secrete milk, we next asked which of these 23 features also tracked proteomic signatures of mammary gland functional states. We performed ranked enrichment analyses in MISAME and Mumta-LW HM proteomes using protein lists ordered by correlation with each feature and Human Protein Atlas annotations representing secretory/glandular, ECM-remodeling, and immune-response signatures (supplementary text) (*68*).

Among the 23 cross-cohort nutrition–growth features, 18 showed significant enrichment in both MISAME and Mumta-LW for at least one mammary-related protein set (FDR<0.1; Fig. 4B). To define candidate biomarkers of mammary functional state, we next applied a stricter cross-study criterion, requiring at least two significant enrichments across the two studies (FDR < 0.1) with directions consistent with perturbed mammary function. Specifically, we selected features with positive links to mammary remodeling or immune-response activity, or features negatively associated with mammary secretory/glandular indicators or nutrient sufficiency. Features with opposite-direction associations were not retained. Using these criteria, six metabolites met the cross-study enrichment criteria: valerylcarnitine (C5), lysine, selenium, 6'-sialyllactose (6'SL), potassium and creatinine (Fig. 4B). We then prioritized features with literature-supported links to mammary gland biology or function. Four of the six candidates— C5 (*69–71*), lysine (*72–75*), selenium (*32*, *76–79*), and 6'SL (*80*, *81*) - had prior links to mammary biology and were therefore retained as the strongest data- and literature-supported candidates from the discovery stage, whereas creatinine and potassium were retained as exploratory (see the supplementary text and fig. S6).

These four prioritized metabolites converged on complementary aspects of mammary biology, spanning immune-response activity, metabolic remodeling, and milk biosynthetic capacity. Selenium has been linked to subclinical mastitis and mammary immune function (*32*, *76–79*). Sialylated HMOs such as 6'SL have been linked to maternal immune responses and to mastitis- and pathogen-related variation in milk (*80*, *81*). C5, a short-chain acylcarnitine involved in mitochondrial fatty acid transport and regulated by mTOR/AMPK signaling (*20*, *82*, *83*), accumulates in states of metabolic dysfunction, immune activation, and inflammation, including mastitis in cattle (*69–71*, *84–90*). Lysine, which is required for casein synthesis and is often nutritionally limiting in populations consuming corn- and rice-based diets (*72–75*), may reflect impaired milk protein synthesis when present in elevated free form. Together, these results identify C5, lysine, selenium, and 6'SL as cross-cohort candidate biomarkers linking the shared nutrition–growth axis to mammary immune response, metabolic remodeling, and milk synthesis/secretion.

We next asked whether a broader functional screen across the full set of targeted metabolites (micronutrients, HMOs, and targeted LC-MS features) would identify additional pathway-related or benchmark biomarkers not captured by the stricter nutrition–growth discovery set. We therefore extended the same ranked enrichment framework to all targeted HM features measured in MISAME (506 features) and Mumta-LW (508 features), including micronutrients, HMOs, and LC-MS metabolites. As in the previous analysis, we defined candidate mammary functional-state markers as metabolites showing at least two replicated enrichments across milk synthesis, mammary remodeling/barrier permeability, or immune response signatures across both cohorts at the prespecified FDR threshold (FDR<0.1; Fig. 4B). This expanded screen recovered 6'SL and C5 and additionally highlighted three related short-chain acylcarnitines - acetylcarnitine (C2), propionylcarnitine (C3), and butyrylcarnitine (C4) - as well as sodium, an established marker of delayed secretory activation and mastitis (*46–48*).

Because C2, C3, C4, and C5 participate in related acylcarnitine pathways linked to mammary metabolic and immune-response states, and because sodium is a well-established indicator of mammary barrier permeability, we incorporated these features into a focused biomarker panel for downstream analyses. We therefore carried forward: seven candidate biomarkers - C2, C3, C4, C5, lysine, selenium, and 6'SL - together with sodium, an established marker of mammary barrier permeability. This panel integrates discovery-stage nutrition–growth signals with pathway-consistent markers of mammary metabolic and immune-response states and an established comparator of mammary barrier function. We further considered the Na:K ratio as a separate established comparator rather than as a discovery-derived biomarker. Additional exploratory candidates with data-driven support but limited prior links to mammary biology are described in the supplementary text and fig. S6.

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Functional analysis links candidate biomarkers to mammary synthesis, remodeling, and immune-response axes

To define how the seven candidate HM biomarkers relate to mammary biology, we tested their associations with proteomic indicators of milk synthesis/secretion, ECM remodeling, and immune-response activity in MISAME and Mumta-LW. At the pathway level, biomarker–protein enrichment patterns were consistent with the proposed links between these HM candidates and mammary functional-state domains. Acylcarnitines showed the broadest cross-domain signal, spanning milk synthesis/secretion, ECM remodeling, and immune-response activity ($P < 0.05$ across all enrichments for at least one cohort), with C5 particularly linked to ECM remodeling ($P = 7.21 \times 10^{-6}$ in MISAME) and milk synthesis/secretion signatures ($P < 1 \times 10^{-4}$ in MISAME; Fig. 5A). Other biomarkers showed more domain-specific patterns: lysine aligned with reduced milk nutrient synthesis ($p < 0.001$ in MISAME), 6′SL with immune-response activity ($P < 0.001$ in Mumta-LW), and selenium with mammary glandular ($P < 0.05$ across most comparisons in both studies) and remodeling signatures ($P = 0.000488$ in MISAME; Fig. 5, A and B; fig. S7; and tables S4 and S5; see the supplementary text for details).

We also observed study-specific variation, particularly for immune cell and immunoglobulin proteins. These signatures showed significant associations with most biomarkers in Mumta-LW, with *P*-values ranging from 3.83×10^{-10} to 0.05, but only a single significant association in MISAME ($P = 0.0267$). This pattern is consistent with the higher underlying infection and inflammatory disease burden in the Mumta-LW study population (*91*), as also reflected by the elevated risk of reported infant illness in Mumta-LW compared with MISAME (RR = 5.28, 95% CI = 3.94 to 7.06, $P = 5.83 \times 10^{-37}$; fig. S8).

Benchmarking against sodium and Na:K highlighted both shared and distinct functional signals relative to these established barrier markers. Sodium and Na:K were most strongly associated with immune-response signatures, consistent with their established use as markers of tight-junction permeability and mastitis-associated barrier disruption, but neither showed significant enrichment for ECM-secreted proteins. In contrast, most candidate biomarkers, including C2, C3, C4, C5, selenium, and 6′SL, were associated with ECM remodeling ($p < 0.001$ in at least one cohort), suggesting that they capture mammary barrier or tissue-remodeling dimensions not reflected by sodium and Na:K alone (Fig. 5A and fig. S9; see the supplementary text for details). At the same time, all seven candidate biomarkers showed modest positive correlations with sodium ($R > 0.36$, IQR [0.38–0.46] in MISAME; $R > 0.33$, IQR [0.39–0.47] in Mumta-LW) and Na:K ($R > 0.22$, IQR [0.25–0.30] in MISAME; $R > 0.21$, IQR [0.24–0.36] in Mumta-LW), indicating shared variation in barrier permeability (fig. S9). Given that tight junction permeability has been linked to low milk production (*44*), these results are consistent with an altered mammary functional state, in which both barrier integrity and milk production may be jointly affected, potentially contributing to reduced infant growth outcomes.

Univariate protein-level analyses further linked elevated candidate biomarker levels to reduced milk synthesis/secretion and increased remodeling/immune activity. In MISAME, all seven biomarkers were negatively associated with total carbohydrates and β-casein ($R < -0.23$, $P < 0.01$ across all comparisons), with more restricted but directionally similar associations in Mumta-LW ($R < -0.23$, $P < 0.001$ for most biomarkers; Fig. 5B, fig. S10, tables S4 and S5; see the supplementary text for details). Candidate biomarkers were also positively associated with ECM remodeling proteins, including tenascin-C ($R > 0.2$, $P < 0.01$), and with immune-response markers such as IgA and calprotectin ($R > 0.25$, $P < 0.05$), supporting their interpretation as integrated indicators of altered mammary functional state (*52–56*).

To quantify the interconnectedness of these functional domains, we examined correlations among protein-level indicators of milk synthesis/secretion, ECM remodeling, and immune response activity used in Fig. 5B (fig. S11 and tables S6 and S7). ECM-remodeling and immune-response markers were positively correlated with each other ($R > 0.24$, $P < 0.05$ across all comparisons) and negatively correlated with β-casein and total carbohydrates ($R < -0.21$, $P < 0.05$ across most comparisons), indicating coordinated variation across these mammary functional-state domains. Together, these analyses indicate that the seven candidate biomarkers capture integrated variation across milk synthesis/secretion, barrier remodeling, and immune-response activity, with acylcarnitines—particularly C5—showing the broadest cross-domain signal.

Finally, given the role of acylcarnitine accumulation as a biomarker of metabolic stress or altered mitochondrial metabolism in other contexts (*82*, *84–86*, *88–90*, *92*), we evaluated whether elevated acylcarnitine concentrations were associated with reduced milk nutrient synthesis. High amounts of all four acylcarnitines (C2, C3, C4, and C5; top 20th percentile or higher) were consistently associated with reduced β-casein (MISAME: $P < 0.01$; Mumta-LW: $P < 0.001$ for all comparisons) and total carbohydrates (MISAME: $P < 0.01$; Mumta-LW: $P < 0.05$ for most comparisons) in both studies (Fig. 6A). Further, each of these acylcarnitines was positively associated with total milk protein ($P < 0.01$ across comparisons), consistent with prior reports linking elevated milk protein to low milk volume and reduced milk production, potentially reflecting increased influx of serum-derived proteins (fig. S12) (*93–95*). These findings link acylcarnitines,

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mitochondrial metabolism, inflammation, mammary biology, maternal nutrition, and infant growth (*19*, *20*, *69*, *71*, *96*).

The identified acylcarnitines were also consistently aligned with the negative end of PC1, a shared maternal nutrition–infant growth axis (Fig. 3), as reflected by enrichment among bottom-ranked targeted LC-MS metabolite features (Fig. 6B, and fig. S13A) and by some of the most negative PC1 feature loadings overall (Fig. 6C and fig. S13B). Consistent with this alignment, C5 showed the strongest association among the acylcarnitines with postnatal BEP intervention (fig. S13C). Together, these findings support the potential for short-chain acylcarnitines, particularly C5, to serve as multifaceted biomarkers connecting maternal metabolic status, altered mammary biology, and infant growth outcomes.

Pantothenic acid emerges as one nutritional route linking maternal nutrition, acylcarnitines, and mammary function

Given that short-chain acylcarnitines (C2-C5), and particularly C5, emerged as central biomarkers linking to mammary gland biology and impaired infant growth, we next asked what upstream nutritional or metabolic factors might regulate acylcarnitine profiles in HM. Acylcarnitine metabolism depends on acyl-CoA availability, which in turn requires coenzyme A (CoA) synthesized from pantothenic acid. When pantothenic acid availability is insufficient, CoA pools can decline, fatty acid oxidation may be impaired, and acylcarnitine profiles can become altered (*97*, *98*). Consistent with this biology, pantothenic acid (i) exhibited significant associations with C5 accumulation across both cohorts and multiple timepoints ($P < 0.05$; figs. S14 and S15) and (ii) emerged in our machine learning analyses as a prominent feature contributing to the prediction of both maternal nutritional intervention status and infant growth, prompting further investigation.

Although pantothenic acid is an essential metabolic cofactor and its demand increases during pregnancy, it has rarely been studied in human pregnancy and lactation because deficiency is generally assumed to be uncommon (*99*, *100*). We therefore examined the prevalence of low HM pantothenic acid concentrations using infant-age-appropriate vitamin reference percentiles from the MILQ study (*101*). Low pantothenic acid concentrations, defined as concentrations below the 50th MILQ reference percentile, were almost absent in the Canadian CHILD cohort (<1% of women), but were highly prevalent in MISAME and Mumta-LW, affecting 67.2% and 55.1% of women at one or more timepoints, respectively (Fig. 6D). These findings suggest that low HM pantothenic acid may be an overlooked component of maternal nutritional insufficiency in low- middle- income (LMIC) settings.

We next asked whether low pantothenic acid was associated with acylcarnitine accumulation within HM signatures linked to infant growth and altered mammary biology. Using MILQ reference percentiles, we defined severe pantothenic acid deficiency as HM concentrations in the bottom 25th percentile and compared acylcarnitine levels across deficiency strata. Milk from mothers in the bottom 10th percentile exhibited elevated levels of C2, C3, C4, and C5 in MISAME ($P < 0.05$), and elevated C5 in Mumta-LW ($P < 0.05$), even relative to women with concentrations between the 10th and 25th percentiles (Fig. 6E). This pattern is consistent with the central role of pantothenic acid in supporting CoA-dependent metabolism, including the citric acid cycle and β-oxidation, and with prior animal studies showing that pantothenic acid deficiency promotes acylcarnitine accumulation (*98*, *102*, *103*).

As pantothenic acid deficiency is unlikely to occur in isolation and may reflect broader B-vitamin insufficiency, we tested whether other B-vitamins showed similar relationships with acylcarnitine accumulation. Among the B-vitamins measured, pantothenic acid showed the most consistent relationship between severe deficiency and short-chain acylcarnitine accumulation across cohorts, although vitamin B6 also showed significant associations with all four acylcarnitines in MISAME ($P < 0.05$; figs. S14 and S15). Thus, while pantothenic acid may not represent the only nutritional pathway involved, it emerged as a prominent and biologically plausible axis connecting maternal nutritional status to HM acylcarnitine profiles.

Given the strong relationship between B-vitamins and maternal nutritional intervention in our data, we next evaluated whether BEP supplementation reduced the risk of severe B-vitamin deficiency (Fig. 6F). Postnatal BEP significantly reduced severe deficiency risk for most B-vitamins tested ($P < 0.05$; χ^2 test). The reduction was particularly consistent for severe pantothenic acid deficiency across all timepoints for both MISAME and Mumta-LW. Because the BEP intervention contained pantothenic acid along with other nutrients, these findings do not isolate pantothenic acid-specific effects, but support pantothenic acid as one supplementation-responsive component of the broader nutritional intervention.

C5 was also consistently associated with BEP across both MISAME and Mumta-LW ($P < 0.05$; Fig. 6G and fig. S13C). Among the short-chain acylcarnitines, C5 showed the strongest links to pantothenic acid deficiency, with significant associations across all comparisons in both cohorts ($P < 0.05$; Fig. 6D and fig. S13C) and also showed the strongest enrichment within the milk synthesis/secretion domain (Fig. 5A). Together, these results suggest that C5 captures an intersection between maternal nutritional status, CoA-dependent mitochondrial metabolism, and mammary functional signatures.

Building on these multi-cohort findings and prior evidence linking acylcarnitine accumulation, mTOR/AMPK signaling, and CPT1-mediated fatty acid transport in the

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mammary gland (*18*, *19*, *96*, *104–108*), we propose a mechanistic model connecting pantothenic acid, C5, and metabolic regulation in mammary tissue (Fig. 6H). In this model, pantothenic acid insufficiency reduces CoA availability, constraining acyl-CoA formation and β-oxidation. These resulting shifts in acylcarnitine profiles, including C5 accumulation, may reflect reduced metabolic capacity in the mammary gland, with downstream consequences for milk synthesis/secretion and inflammatory tone (*98*, *102*). mTOR/AMPK cross-talk, acting in part through CPT1, provides a plausible regulatory axis coupling nutrient status to acylcarnitine flux, fatty acid oxidation, and mammary immune responses (*16*).

In sum, across multiple geographic contexts, we identified convergent HM signals linking maternal nutritional intervention, acylcarnitine metabolism, mammary functional signatures, and infant growth. Within these signals, C5 emerged as a central acylcarnitine biomarker, while pantothenic acid emerged as a potentially modifiable nutritional axis that may contribute to acylcarnitine accumulation and altered mammary metabolism. These findings nominate the pantothenic acid–CoA–C5 axis as one plausible route through which maternal nutrition may influence mammary gland functional state and infant growth.

Discussion

In this study, we examined how mammary gland functional state relates to infant growth by analyzing HM composition across diverse populations. We hypothesized that, despite variation in HM composition across settings, core mammary programs that shape milk synthesis/secretion, barrier function, and immune/repair activity are conserved and can be reflected in HM composition. To test this, we analyzed 1,543 HM samples from three studies within the IMiC Consortium—including two randomized controlled trials of postnatal supplementation—using a comprehensive multi-omics approach spanning macronutrients, micronutrients, HMOs, proteins, immune factors, and targeted and untargeted metabolites.

We applied a systems biology framework that integrates HM composition with infant growth outcomes and maternal factors to investigate mammary gland functional state. Through supervised and unsupervised machine learning, we identified conserved HM compositional signals that capture maternal nutritional status and infant growth. Building on these signals and the Human Protein Atlas, we interrogated three central facets of mammary gland function—milk synthesis, barrier integrity, and regulated immune/repair response—within a unified framework to evaluate their collective impact on infant growth. Our analysis uses HM-derived multi-omic data, across over 1,500 samples, as a non-invasive window into mammary gland function, consistent with prior work (*8–11*, *109*), and leverages this framework to connect mammary gland functional states to infant growth outcomes. This study underscores the value of systematically analyzing HM as an integrated biological system linking maternal nutritional status, mammary gland functional state, milk composition, and infant growth across diverse cohorts.

We identified seven candidate biomarkers (C2, C3, C4, C5, lysine, 6'SL, selenium) and compared them to two established biomarkers, sodium and the Na:K ratio, which benchmark established axes of mammary barrier permeability and secretory activation. While the established biomarkers mainly captured processes linked to infection, immune response, or electrolyte effects, the set of seven candidate biomarkers captured a broader signal of mammary gland biology. Specifically, our study identified biomarkers capturing biological signals including mammary metabolism and ECM regulatory processes not reflected in established markers. All four acylcarnitines were associated with all three mammary functional-state domains (reduced milk nutrient synthesis, reduced barrier function, and immune activation) in our functional analysis.

At the nexus of these mammary gland functional domains, the short-chain acylcarnitine C5 emerged as a central biomarker of altered mammary functional state, and low pantothenic acid levels as a candidate upstream nutritional factor linked to acylcarnitine accumulation and altered mammary metabolism. The link between acylcarnitines and metabolic stress has been established in other contexts relevant to fetal, neonatal, and infant outcomes, such as mastitis and mammary metabolism in cows (*69–71*, *110*), adverse pregnancy outcomes (*87*, *88*), and neonatal health outcomes (*111*, *112*). However, this work systematically characterizes acylcarnitines as biomarkers of mammary gland functional state or lactation-related biology in human milk, particularly in relation to maternal nutritional status and infant growth, addressing this critical gap.

We suggest that elevated HM C5 levels reflect a maternal–mammary metabolic state shaped by chronic stressors such as malnutrition, inflammation, or sustained immune activation. During such conditions, the mammary gland may shift metabolic resources away from milk biosynthesis toward cellular maintenance and repair, leading to increased glucose utilization and lipolysis, and decreased production of milk proteins and lactose (*20*). Thus, prolonged states of malnutrition, chronic infections, or inflammation may constrain maternal metabolic reserves and reduce the capacity to sustain sufficient milk synthesis and secretion, particularly if TCA cycle and/or beta-oxidation rates are impaired due to deficiencies in micronutrients such as pantothenic acid. Similar metabolic effects are observed when mice are deprived of cysteine, which induces a dual state of increased lipolysis and reduced CoA levels (*113*). These mice exhibit rapid weight loss

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as well as increased urinary excretion of TCA cycle intermediates and C5. Further, beta-oxidation is normally suppressed in the mammary gland during lactation to support lipogenesis and lipid transfer to HM, suggesting that elevated C5 may mark altered mitochondrial and lipid metabolic flux in the lactating gland (*114*). In the context of the mother-milk-infant triad, elevated C5 levels may therefore identify a modifiable maternal–mammary metabolic state relevant to lactation physiology and infant growth.

C5 is negatively associated with pantothenic acid, which exhibited strong positive links to growth and nutritional intervention across the results from machine learning, Joint-RPCA, and univariate analyses. HM pantothenic acid levels below reference values were highly prevalent in MISAME and Mumta-LW, impacting over half of the mothers across both cohorts. Although pantothenic acid is often overlooked in human nutrition because its deficiency is considered rare in high-income countries, due to its widespread presence in a variety of foods, nutritional requirements are elevated during pregnancy and lactation (*99*). In addition to its direct role in metabolism, pantothenic acid deficiency can also trigger systemic reductions in metabolism at the level of gene regulation, further amplifying its impact on mitochondrial function, with potential downstream impacts on milk production and infant growth (*98*). Because the nutritional interventions studied here were not pantothenic-acid-specific, these findings do not establish pantothenic acid as the sole causal driver of the acylcarnitine signal; rather, they nominate pantothenic acid insufficiency as one plausible and potentially modifiable nutritional axis linking maternal nutrition to mammary metabolism.

Overall, our results support a model wherein maternal and mammary health, aided by nutritional supplementation, support milk synthesis and milk composition profiles that promote infant growth outcomes. In this model, the maternal environment dynamically influences mammary gland function, with important implications for basic biology and potentially for broader mammary biology. For example, C5 and other putative biomarkers exhibited associations with SOSTDC1, a key regulator of the Wnt β-catenin pathway that plays roles in mammary development, stem cell signaling, and breast cancer progression, as well as with other mammary gland development proteins (*30*, *115*, *116*). This broader connection should be interpreted as hypothesis-generating and warrants further study.

This model also challenges the view that mothers can fully buffer human milk composition - especially macronutrients - against sustained nutritional constraint by mobilizing body stores. Further, although multiple studies show that supplementation can improve infant growth, the extent to which these benefits are mediated through changes in milk composition and mammary function has remained less clear (*117*–*119*). Our results warrant further investigation into the mechanistic links between altered mammary functional state and maternal nutrition and health, including other contexts such as obesity and gestational diabetes mellitus, as well as the potential for prenatal supplementation to improve gland development and milk nutritional composition. The findings in this study also demonstrate the utility of leveraging human milk proteomics as a noninvasive strategy for probing human mammary gland processes.

From a clinical and public health perspective, our identification of short-chain acylcarnitines, pantothenic acid, and related metabolic markers as key indicators of maternal–mammary metabolic state points to tangible opportunities to strengthen maternal nutrition interventions in LMICs. Targeted strategies to support micronutrient sufficiency during lactation may not only safeguard maternal health but also support the mammary gland's capacity to produce nutrient-rich milk and provide adequate milk volume, ultimately supporting infant growth and development across diverse global settings. Further, we demonstrate the potential for acylcarnitines such as C5 as biomarkers in lactation, expanding their clinical utility as metabolic biomarkers across multiple contexts such as adverse pregnancy outcomes and diabetes. These insights underscore the value of HM multi-omics data as a critical link within the interconnected mother-milk-infant triad to facilitate biological and clinical discoveries.

Materials and methods

Study design

Human milk (HM) samples and mother–infant dyad data, including infant growth metrics, were sourced from three IMiC Consortium studies (Trial registration: NCT05119166): two randomized controlled trials–the Micronutriments pour la Santé de la Mère et de l'Enfant 3 (MISAME) trial (*120*) and the Mumta Lactating Women (Mumta-LW) trial (*121*)–and the Canadian Healthy Infant Longitudinal Development (CHILD) study (*122*). MISAME and Mumta-LW assessed the impact of balanced energy–protein (BEP) supplementation versus an iron-folic acid (IFA)-only control on infant growth outcomes; postnatal BEP was received by 53.1% of women in MISAME and 66.7% in Mumta-LW. In MISAME, IFA tablets contained 65 mg iron and 0.4 mg folic acid. In Mumta-LW, IFA was not provided as part of the trial intervention; however, antenatal IFA supplementation is standard of care in Pakistan and was therefore available through routine care (*123*, *124*). The CHILD study is an observational cohort conducted in Canada, a high-resource setting.

The MISAME study, conducted in rural Burkina Faso, is a randomized controlled trial evaluating the impact of balanced energy-protein (BEP) supplementation during pregnancy and lactation on pregnancy outcomes and infant growth (Trial registration: NCT03533712). Human milk

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samples were obtained from 290 mother-infant dyads at three postpartum intervals (14–21 days, 1–2 months, and 3–4 months) and infant growth was tracked throughout the first 12 months of life.

The Mumta-LW study is a randomized controlled trial conducted in peri-urban Karachi, Pakistan, evaluating the effect of balanced energy–protein (BEP) supplementation during lactation on infant growth outcomes (Trial registration: NCT03564652). The trial specifically enrolled undernourished women, defined by a maternal mid–upper arm circumference (MUAC) of 23 cm or less. In a subgroup of BEP-supplemented mothers, the impact of administering azithromycin to infants at 42 days of age on growth was also investigated. HM samples were obtained from 150 mother–infant dyads at two postpartum time points (42 and 56 days, with a 7-day window per timepoint)—and infant growth was tracked over the first 12 months of life.

The CHILD study is an ongoing prospective birth cohort investigating how environmental and genetic factors influence the development of asthma and allergies in children across four Canadian sites. From a subset of 400 mother-infant dyads, human milk samples were collected at 3 months postpartum, with follow-up data gathered through 12 months postpartum.

Maternal and neonatal data from all sites were curated and harmonized to generate an IMiC standardized dataset for each study, under the oversight of the Gates Foundation's K_i Data Curation team. Datasets were structured according to predefined formats consistent with the Clinical Data Interchange Standards Consortium Study Data Tabulation Model (CDISC SDTM). Further details on study design, inclusion criteria, human milk collection, and harmonization procedures for each study are available in our protocol paper (*125*).

Ethics

The IMiC project, as described in our protocol, encompassing secondary analysis of HM collected by the participating studies, was reviewed and approved by the Human Health Research Ethics Board (HREB) at the University of Manitoba in 2020 (Approval ID: HS23767) (*125*). For each participating study, ethical review and approval was received at their primary institution(s), including protocols for the collection of HM and metadata. All participants provided voluntary informed consent at or prior to enrollment, with procedures described in individual study protocols. When possible, participants provided written signed consent in the native language, or in cases of illiteracy in Mumta-LW and MISAME-3, participants gave consent through a witnessed thumb impression. Ethical approval for the CHILD Cohort Study, including the oversight of the CHILD biological samples and the CHILD database (CHILDdb), was obtained from the local Research Ethics Board of each study site: the University of British Columbia, the University of Alberta, the University of Manitoba, the Hospital for Sick Children, and McMaster University.

Multi-omics quantification of HM samples

HM samples from each study site were shipped to a central biorepository at the Manitoba Interdisciplinary Lactation Center (MILC) in Canada on dry ice. There, samples were distributed to multiple laboratories for analytical quantification. Minerals as well as other micronutrients, including B-vitamins and fat-soluble vitamins (vitamins A and E as well as carotenoids), were quantified using standardized protocols employed across the Mothers, Infants, and Lactation Quality (MILQ) study (*126*). Macronutrient analysis, which included measurements of total fat, protein, and carbohydrates, was conducted using near-infrared spectroscopy (NIR) with a SpectraStar XT device calibrated specifically for HM samples (*127*). Human milk oligosaccharides (HMOs) were extracted through high-throughput solid-phase extraction, followed by fluorescent labeling and quantified using high-performance liquid chromatography with fluorescence detection (HPLC-FLD), as previously described (*128*, *129*). Lipid profiling of HM was performed using flow injection analysis-tandem mass spectrometry (FIA-MS/MS), while targeted metabolite analyses were conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the MxP® Quant 500 kit (Biocrates Life Sciences), which was optimized and validated for HM matrices (*126*, *130*, *131*). Untargeted metabolomics were performed with rapid liquid chromatography-mass spectrometry (rLC-MS) at Sapient Bioanalytics, as previously detailed (*132*, *133*), with compound identification achieved via spectral alignment with reference data from open-source and restricted access spectral collections. Bioactive proteins (BAPs), such as IgA and calprotectin, were quantified using high-performance electrochemiluminescence with assay kits obtained from Meso Scale Discovery (*134*).

Untargeted proteomics analysis was performed for a subset of milk samples, including 150 samples at 56 days postpartum from the Mumta-LW study, 145 samples at 1–2 months postpartum from the MISAME study, and 100 samples at 3 months postpartum from the CHILD study. Analysis was performed via data-independent acquisition mass spectrometry (DIA-MS) using an S-Trap digestion workflow tailored for HM matrices (*135*).

Further details on the methodologies used for each omics dataset have been reported previously (*125*).

Data preprocessing and statistical analyses

All assay values were normalized using technical replicates from pooled human milk (HM) samples prior to harmonization, following the methods outlined in the protocol paper (*125*). After harmonization, further preprocessing steps were

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applied in this manuscript and are described below. For the targeted LC/MS dataset, features were excluded if 20% or more of their values were below the limit of detection (LOD; see fig. S16 and table S8 for distributions). Samples with over 50% of values below the LOD were also excluded from the targeted metabolomics analysis. Taurine levels were removed due to samples exceeding the calibration range. Values below the LOD were imputed using a logspline distribution fitted between 50% of the LOD and the LOD itself. Pooled HM samples were used to normalize the micronutrient datasets, with measurements converted to micromolar units. Missing values were imputed by generating random samples from a uniform distribution ranging from one-tenth to the full minimum observed value within the dataset. Vitamin B1 was calculated as the sum of thiamine, thiamine monophosphate, and thiamine pyrophosphate; vitamin B2 was derived from the sum of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide; vitamin B3 came from nicotinamide, nicotinamide adenine dinucleotide, nicotinamide mononucleotide, and nicotinamide riboside; and vitamin B6 was calculated as the sum of pyridoxal, pyridoxamine, pyridoxine, and pyridoxal 5′-phosphate. Macronutrient units were converted from percentages (g/100 mL) to kcal/L using established energy values of 4 kcal/g for protein and carbohydrates and 9 kcal/g for lipids.

For the untargeted LC/MS data, features from studies measured as separate batches were aligned to identify 1-to-1 matches, with best matches were defined based on retention time differences. In total, we identified 23,114 features with 1-to-1 matches between all three studies. Additional batch correction was not necessary as samples were independently analyzed per study. Missing values were then imputed by random sampling from a uniform distribution bounded between one-tenth and the full minimum observed value within each dataset.

Statistical analyses were performed in R and python. Unless otherwise stated, all pairwise statistical comparisons in this study were performed via Wilcoxon Rank Sum Test, and Spearman's rank correlation coefficient was calculated for all correlational analyses. These non-parametric tests were chosen for their robustness to deviations from distributional assumptions. Descriptions of multi-omics features (targeted and untargeted metabolites and proteins) included in this study are described in tables S9 to S11.

Comparing HM composition across studies

To enable comparison across studies, a low-dimensional representation of the HM data was generated using Joint-RPCA (Robust Principal Component Analysis), a robust compositionally aware dimensionality reduction method designed to identify underlying global patterns in multi-omics datasets (as a separate analysis from the one described above). Four modalities, each measured with different instruments, were selected for analysis: micronutrients, HMOs, macronutrients, and targeted LC/MS data. Untargeted features were excluded from this study-level comparison as the MISAME samples were quantified separately from the other studies and were thus not directly comparable. To ensure temporal alignment across the studies, the latest timepoint in each sample was selected for study-level comparison. Power transformation and centering was applied to each data type prior to Joint-RPCA analysis using the joint_rpca() function of the Gemelli Python library (v. 0.012).

Joint-RPCA outputs feature and subject ordinations (calculated across the last timepoint for each study), as well as a subject-level distance matrix which was used to calculate whether between-study differences were greater than within-study differences via Wilcoxon test. Differences in sample-level dispersion between studies were calculated as pairwise combinations of all three studies using the permdisp function of the scikit-bio python library (v. 0.5.9) (*136*).

Anthropometric calculations

Weight-for-age Z-scores (WAZ) from the harmonized IMiC dataset were used as the primary growth outcome in our analysis as it is a clinically relevant outcome capturing changes in height as well as body mass which may serve as an early indicator of later stunting (*66*, *67*). We focused on the month 4 WAZ outcome in MISAME and Mumta-LW as this timepoint captured cumulative effects from all milk timepoints, and the month 3 WAZ outcome in CHILD as infant growth measurements were recorded quarterly. Descriptions for all metadata variables included in this study are available in table S12.

We developed a binary growth outcome classification designed to contrast individuals most and least likely to reflect the biological or environmental determinants of adverse growth outcomes and minimize noise arising from normal variation near the mean. For MISAME, we selected thresholds of month 4 WAZ above 0 and below -1, excluding infants with values between 0 and -1. Similar thresholds were chosen based on month 3 WAZ values in CHILD. In Mumta-LW, due to down-shifted distributions compared to the other studies (median month 4 WAZ in Mumta-LW was -1.08 vs. -0.22 in MISAME and -0.17 in CHILD), we selected thresholds of month 4 WAZ values above -1 and below -2 (fig. S17). This binarization yielded the following group distributions: MISAME (WAZ < −1: n = 57; WAZ > 0: n = 111), Mumta-LW (WAZ < −2: n = 34; WAZ > −1: n = 68), and CHILD (WAZ < −1: n = 100; WAZ > 0: n = 176).

In addition, we also examined the BMI metric from the harmonized data, as well as the following derived variables calculated in this study: growth velocity, length velocity, and head-circumference-for-age Z-scores (HCAZ). Growth

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velocity was calculated according to the Patel exponential method with the following formula:

$$\text{growth velocity}\left(\text{g/kg/d}\right) = 1000 \times \frac{\log\left(\frac{\text{final weight}\left(\text{kg}\right)}{\text{initial weight}\left(\text{kg}\right)}\right)}{\text{infant age}\left(\text{d}\right)}$$

and length velocity as the change in length (cm) divided by the age of the infant in months (*91*, *137*):

$$\text{length velocity}\left(\text{cm/mo}\right) = \frac{\Delta\text{length}\left(\text{cm}\right)}{\text{infant age}\left(\text{d}\right) \times 30.437\left(\text{d/mo}\right)}$$

For comparison of trajectory groups (see next section), velocity measurements were calculated between birth and 6 months of life. For comparison with milk components, velocity was calculated between birth and three months to capture signals from recent HM samples using measurements available across all three studies. HCAZ measurements were calculated at delivery and six months using the addWGSR function of the zscorer R package (v.0.3.1), which uses the WHO Growth Reference chart to compute z-score measurements (*138*).

We also examined several maternal factors associated with malnutrition. We classified undernourished mothers using a maternal mid-upper arm circumference (MMUAC) threshold of 23 cm or below, which is often used as a cutoff for malnutrition (*91*, *139*, *140*). As the MMUAC < 23 cm threshold was used as an enrollment criterion in the Mumta-LW study, we used the threshold of 21 cm to identify severely undernourished women as was applied previously in this dataset (*91*). To assess the impact of maternal height on infant growth trajectories, we used a cutoff of 155 cm, which is often employed in LMIC nations and has known associations with infant outcomes such as stunting (*141*–*143*). Differences in rates of infantile illness (defined as any reports of vomiting, coughing, hospitalization, diarrhea, or fever reported by the mother) were compared using the epitools (v.0.5-10.1) R package (*144*).

Group-based trajectory modeling

Trajectory groups were assigned using group-based trajectory modeling, an unsupervised approach for clustering longitudinal datasets using the gbmt function of the gbmt R package (v. 0.1.4) (*145*). Essentially, this function fits multiple polynomial mixture models to longitudinal datasets to identify clusters of similar trajectories. Monthly WAZ measurements collected over the first 6 months of life in MISAME and Mumta-LW were used as inputs for group-based trajectory analysis. One pattern with unrealistic WAZ changes (defined as downward trends falling below -6) was excluded from the MISAME analysis.

Trajectory classifications were used to evaluate the influence of various risk factors (delivery season, prematurity, MMUAC below 23 cm (21 cm in Mumta-LW), maternal height MHTCM below 155 cm, and low birth weight on the relative risk of developing and adverse growth trajectory in MISAME and Mumta-LW using the epitools (v.0.5-10.1) R package (*144*). A similar approach was used to evaluate factors promoting growth trajectory recovery in MISAME or stability in Mumta-LW. The relative effect of postnatal BEP supplementation was compared to study-specific intervention (prenatal BEP in MISAME, maternal azithromycin administration in Mumta-LW), as well as delivery season and maternal factors (only in MISAME; MHTCM>155 cm, MMUAC>23 cm). Delivery season was examined due to its impact on food availability, as fresh foods are more abundant outside of the dry "summer" (November-April) season in Burkina Faso (*146*) and dietary diversity in Pakistan is greatest during the winter months (*147*). Maternal factors were not evaluated as stability-promoting factors in Mumta-LW to prevent redundancy, as the growth stability comparison represented the inverse of growth perturbation. Descriptions for risk factors used in this analysis are also provided in table S13.

Accounting for sampling time variability

To address sampling-time heterogeneity across sites, we used a site-stratified analytical framework in which each cohort was modeled independently to identify human milk features associated with maternal nutritional intervention and infant growth. For both Joint-RPCA and supervised machine learning analyses (see sections below), we incorporated all available timepoints within each study, with the goal of identifying signals that were reproducible across cohorts and analytical methods. We note that alternative longitudinal modeling approaches (for example, mixed-effects or time-aligned trajectory models) would require harmonized sampling schedules across cohorts, which is not the case in these datasets. Our approach was therefore designed to prioritize cross-cohort robustness and interpretability without requiring artificial temporal alignment across studies with different sampling schedules.

For Joint-RPCA, temporal variation across modalities was incorporated by fitting the model to samples from all available timepoints within each cohort, allowing study-specific latent structure to be estimated before comparing top-ranked signals across cohorts. For supervised machine learning, each analyte was represented using a longitudinal wide-encoding scheme, in which separate variables were created for that feature at each measured timepoint within a study. This approach is analogous to previously described multiple-instance / temporally aware learning frameworks (*148*, *149*). Features were prioritized for downstream functional analysis only if they were identified across multiple cohorts by

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supervised learning and were also supported by Joint-RPCA, thereby reducing sensitivity to cohort-specific sampling structure and increasing confidence that retained signals reflected reproducible biology.

Identification of joint nutritional and growth signals in HM

Joint-RPCA was used to determine whether there was overlap between nutrition and growth-associated signals in MISAME and Mumta-LW. Analyses were performed separately for each study and spanned all timepoints, as well as five modalities: micronutrients, HMOs, macronutrients, targeted LC/MS, as well as a subset of 104 untargeted LC/MS metabolites which were selected for additional examination (refer to "Supervised Machine Learning" below). Power transformation and centering was applied to each data type prior to Joint-RPCA analysis, although due to the non-compositional nature of untargeted metabolomics data, transformation was applied across features rather than samples.

To identify which features contributed the most to the joint supplementation and infant growth signal captured by Joint-RPCA's first principal component (PC1), the model's feature loadings were examined. These loadings, which indicate how strongly each feature relates to PC1, were ranked from highest to lowest. For HMOs and micronutrients, the top and bottom five features were used. For targeted and untargeted LC/MS, the top and bottom 10% features were used as these modalities contain a larger number of features. The same number of features was applied in both cohorts to maintain consistency. These top and bottom features from the sorted PC1 feature loadings were selected for a log-ratio analysis, where the sum of top features (numerator) is compared to the sum of the bottom features (denominator) by taking their logarithmic ratio. These ratios serve as a composite score for each sample and validate the signals captured by Joint-RPCA.

To identify consistent patterns within features negatively associated with maternal supplementation status and infant growth, we performed an enrichment analysis on targeted LC-MS PC1 denominator features in MISAME and Mumta-LW. For the enrichment analysis, hypergeometric tests were performed across metabolite categories represented by at least three metabolites within the denominator set. Enrichment analysis was performed relative to the full set of targeted LC-MS features included in the Joint-RPCA analysis outlined above.

Supervised machine learning

To identify core sets of HM metabolites that accurately predict infant growth and maternal nutritional intervention across geographic contexts, we developed XGBoost models individually tailored to each study using the XGBClassifier function of the xgboost Python package (v.2.0.3) (*150*). Models trained on sets with sample imbalances were adjusted the max_delta_step and scale_pos_weight parameters. The following hyperparameters were manually tuned using a different set of cross-validation seeds: n_estimators, max_depth, alpha, lambda, and gamma. Maternal nutritional intervention was predicted as the binary outcome "BEP" representing postnatal BEP supplementation status, and adverse infant growth outcomes were predicted in terms of the binarized WAZ variables as described in the anthropometric calculation section. Subject counts for machine learning analysis were as follows: MISAME (BEP: n=290, WAZ: n = 168), Mumta-LW (BEP: n=150, WAZ: n=102), and CHILD (WAZ: n = 386). Resulting class distributions were as follows: MISAME and Mumta-LW: ~2.0:1.0; CHILD: ~1.7:1.0.

To account for sampling time variability within our longitudinal dataset, we applied a longitudinal wide-encoding approach, in which each repeated measurement per subject was treated as a separate feature, analogous to a Multiple Instance Learning approach (*148*, *149*). Ten Monte-Carlo cross-validation iterations with 80:20 train-test splits were run per model. To ensure robustness, all train/test splits were performed at the subject level, preventing data leakage across timepoints. In addition, analyses were conducted in a site-stratified manner, and features were retained only if they recurred across independent cohorts and analytical approaches. This design reduces sensitivity to cohort-specific sampling schedules and mitigates instability due to correlated longitudinal measurements.

Because many HM analytes are biologically correlated, supervised feature importance was interpreted at the level of recurrent signals across runs and cohorts, rather than as definitive evidence for a uniquely important analyte within a correlated feature set. Features identified via SHAP in at least two studies were considered "cross-study" feature sets and were used for subsequent analysis. Top features per study were identified using SHAP (SHapley Additive exPlanations) values computed for each seed of each supervised machine-learning model using the *shap* package (v0.45.1) (*151*). For each predictive model, SHAP values were computed per seed and ranked to generate feature lists based on maximum and mean SHAP values across subjects. A cutoff value was applied to both rankings to generate two lists of N top features per seed (ranked by max and by mean SHAP rankings), with cutoffs optimized to yield approximately 20-40 final cross-study features. Features appearing in multiple studies through this process were designated as cross-study features, ensuring robust identification of reproducible signals.

For untargeted data (>23,000 features), a cutoff of 50 features per seed was applied to both mean and max rankings. The union of all features meeting this cutoff across seeds was generated, and for WAZ predictions, this combined list was

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ranked by mean or max SHAP values (depending on the type of list) calculated across the seeds and filtered to the top 400. Features appearing in multiple studies through this process were designated as cross-study untargeted features. For targeted data (MISAME: n=507, Mumta-LW: n=506, CHILD: n=485), cutoffs of 60 features per seed for BEP predictions and 40 for WAZ predictions were applied. Features recurring across at least three seeds in either the max- or mean-ranked feature sets were retained, and those appearing in multiple studies were designated as cross-study targeted features. Of 23,114 untargeted features, 21 and 29 consistently predicted BEP and WAZ across studies, respectively. Among targeted features (MISAME: P = 507, Mumta-LW: P = 508, CHILD: P = 485), 20 and 33 features consistently predicted BEP and WAZ, respectively. Mean and max SHAP values calculated across all ten cross-validation seeds are provided in tables S14 and S15, respectively.

Results for the untargeted machine learning analysis were used to identify features for identification via MS2 annotation. This set encompassed: (1) 21 cross-study BEP features, (2) 13 cross-study WAZ features: cross-study WAZ features with significant univariate association with the binarized WAZ outcome via Wilcoxon test $P<0.05$ (WAZ binary outcome), (3) 21 of the top CHILD-specific growth-associated features determined by machine learning, (4) 49 additional features identified via linear regression predicting WAZ outcomes adjusted for the following covariates: health center, maternal factors (age, BMI, height, hemoglobin, maternal mid-upper arm circumference, and parity), as well as the gestational age of the infant as covariates. Of this set of 104, annotations were assigned to 41 features (see table S9 for a complete list of annotations). Predictions stratified by annotation status are shown in fig. S4A and tended to be greater for annotated features, particularly in CHILD. SHAP values for predicting CHILD WAZ across all 104 MS2 features stratified by annotation status confirm this effect (fig. S4B).

Final models leveraging untargeted (BEP: 21, WAZ: 29) and targeted (BEP: 20, WAZ: 33) cross-study features as well as the 104 features used for MS2 annotation were compared to naïve baseline models to evaluate the robustness of each model via Wilcoxon test. As only HM compositional data were used for the machine learning models outlined above, naïve models only contained labels and a randomly assigned integer (ranging from 0-1000) which was used for predictions, and thus represent a random baseline. AUC, ROC, and balanced accuracy statistics were calculated across all seeds, with values reported in the text reflecting average values unless otherwise indicated.

Mammary gland functional analysis

HM proteomics data and the Human Protein Atlas (HPA) (*68*, *152*) were leveraged to assess relationships between HM components and mammary gland function. Initial screening for associations to mammary gland functional state were performed for 23 growth and nutrition-associated features, as well as for the set of all targeted features (micronutrients, HMOs, and targeted LC-MS features) measured in MISAME (P = 506) and Mumta-LW (P = 507). Associations between each feature and relevant mammary functions were screened via ranked enrichment analysis using the fgsea R package (GSEA test) (v. 1.28.0) (*153*), and were evaluated across all proteins with fewer than 50% missing values detected in each study. Ranked enrichment P-values were FDR-corrected using the Benjamini-Hochberg procedure.

Candidate biomarkers exhibiting multiple ranked enrichments consistent with mammary functional state in both studies, as well as the sodium:potassium ratio benchmark, proceeded to unranked enrichment analysis. Proteins with positive or negative Spearman correlations with each candidate biomarker with FDR-corrected P-values (calculated via Benjamini-Hochberg procedure) < 0.05 were identified for enrichment analysis (proteins with greater than 50% missing values were removed prior to analysis). To identify associations to mammary functional state, enrichment analyses were conducted on proteins positively and negatively correlated with each candidate biomarker against processes linked to adverse and normal mammary function, respectively. Enrichment analysis was performed relative to the full set of proteins quantified in the corresponding cohort-specific human milk proteomics datasets (MISAME and Mumta-LW).

Enrichment categories linked to mammary functional state used for ranked and unranked enrichment analyses were derived or extracted from the HPA as follows: (1) Proteins secreted to ECM or female reproductive system as well as immunoglobulin proteins were identified based on 'Secretome' labels within the HPA and (2) Proteins enriched in breast glandular cells or immune cells were calculated from single-cell expression data downloaded from the HPA. The percent expression of each protein within its top-expressing cell type was calculated and compared to the distribution of percent expression values for all genes within that same cell type. This comparison was used to compute a P-value, assessing whether the observed expression level was significantly higher than expected by chance. Proteins with a significant enrichment (P < 0.05) as well as an expression value above 5uTPM were assigned a tissue-type-specific enrichment, which was used to identify proteins with enriched expression in breast glandular cells as well as any immune cell type.

To better define how the seven candidate HM biomarkers identified above relate to distinct domains of mammary biology, we extended our functional analysis using unranked enrichment analyses leveraging the same functional categories. For each potential biomarker, we tested whether proteins

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with negative Spearman correlations with biomarker abundance (FDR < 0.05) were enriched for functions related to each mammary gland functional state enrichment category. Positive and negative protein sets were analyzed separately, and enrichment was calculated relative to the full set of proteins quantified in the corresponding cohort-specific HM proteomics dataset.

Individual proteins representing each mammary gland function were selected and analyzed for associations using univariate Spearman correlation. Milk synthesis proteins were identified as proteins enriched in breast glandular cell expression that were also secreted to the female reproductive system. Total carbohydrate measurements were also included to compare to lactose production (as lactose represents the predominant carbohydrate in HM (*154*, *155*)). Within proteins annotated as secreted to the ECM in the HPA, tenascin-C was highlighted due to its direct role in ECM remodeling within the mammary gland, and Agrin(AGRN), lysyl hydroxylase 3 (PLOD3), and the collagen monomers COL4A2 and COL3A1 were selected for their roles in basement membrane organization and collagen modification or stabilization, due to the roles of these processes in maintenance of the blood-milk barrier (*52*, *156*–*159*). To represent immune function, IgA, and calprotectin levels measured via targeted proteomics as well as an average of the Spearman associations between all immunoglobulin genes were selected. Proteins associated with mammary gland development were identified based on annotation with GO:0030879 (mammary gland development) or descendent GO terms. Associations between functional indicators of mammary gland function were also calculated via Spearman correlation within each study.

Acylcarnitine accumulation analysis

As acylcarnitine accumulation is a clinical biomarker associated with several diseases, we compared the effect of high levels of C3, C4, and C5 on WAZ outcomes. To represent accumulation, the top quintile (samples with highest 20% of expression calculated per timepoint per study) was compared to samples in the bottom quintile of each tested acylcarnitine. Associations between acylcarnitine levels and supplementation were also assessed via Wilcoxon test.

B-vitamin deficiency analysis

Human milk reference values (RV) for B-vitamins were obtained from the mothers, infants, and lactation quality (MILQ) study spanning measurements in Bangladesh, Brazil, Denmark, and The Gambia (*101*, *126*). Reference values for each percentile (10%, 25%, 50%, 100%) were selected based on values for each B-vitamin in alignment with the sampling times per timepoint in each study. For MISAME, the 18-31 day MILQ values were chosen for the 14-21 day sampling timepoint, 1-2 month MILQ values for the 1-2 month timepoint, and 3-4 months MILQ values for the 3-4 month timepoint. For Mumta-LW, the 1-2 month MILQ timepoint was selected for both timepoints (42 Days and 56 Days). 25th percentile cutoff values were used to define severe deficiency. The impact of postnatal BEP on the risk of developing severe deficiencies for the B-vitamins B1, B2, B3, pantothenic acid, B6 and Biotin were evaluated via risk ratio calculations using the epitools (v.0.5-10.1) R package (*144*).

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Diversity, equity, ethics, and inclusion: The CHILD Cohort Study emphasizes participant and public involvement in all stages of its research. Research questions are informed and generated based on relevance to participants and their families to study those affected by childhood health conditions. Efforts are made to ensure participants' involvement is meaningful, comfortable, and aligned with their preferences. Study results will be shared with participants through various channels, including newsletters and online platforms. Academic and public health publications will also communicate findings. We acknowledge and thank all participant advisers and study participants for their valuable contributions. **Competing interests:** MBA has received speaking honoraria from non-profit organizations that support breastfeeding (Institute for the Advancement of Breastfeeding & Lactation Education, Thai Breastfeeding Centre, UK Baby Friendly), and companies that produce human milk-related products (Prolacta Biosciences, Medela). She is a scientific advisor to TinyHealth (an infant microbiome testing company) and has consulted for DSM and AIIG (food ingredient companies). MJ and KL hold equity and a position at Sapient Bioanalytics, LLC. MBA also holds advisory roles in Human-Milk.com and PumpKin Baby Inc. DG has served on the Scientific advisory board for Medela AG. N.A. is a member of the Scientific Advisory Boards of January AI, Parallel Bio and WellSim Biomedical Technologies, is a cofounder of Takeoff41 and is a paid consultant for MaraBio Systems. LB is a co-inventor on patent applications related to the use of HMOs in preventing NEC and other inflammatory diseases. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. **Data, code, and materials availability:** Our data analysis code is deposited in https://github.com/Shenhav-Lab/IMiC_mammary_gland and on Zenodo (*190*). Multi-omics human milk metabolomics (MSV000102534) and proteomics (MISAME: MSV000100366,

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Mumta-LW: MSV000100367, CHILD: MSV000100364) datasets are available on MassIVE. Due to participant privacy protections and the terms of study consent across the IMiC studies, other individual-level data cannot be deposited in a public repository. De-identified datasets and accompanying metadata will be made available to qualified investigators upon reasonable request, contingent on approval by the relevant institutional review boards and execution of appropriate data-sharing agreements. No new materials were created in this study. Human milk multi-omics datasets (proteomics, metabolomics, micronutrients, macronutrients, and human milk oligosaccharides) are available from the International Milk Composition (IMiC) Consortium under a data-sharing agreement with the University of Manitoba. Data access inquiries should be sent to imic@umanitoba.ca. **License information**: Copyright © 2026 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. https://www.science.org/about/science-licenses-journal-article-reuse

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.aee7240
Supplementary Text
Figs. S1 to S17
Tables S1 to S15
References (*160–189*)
MDAR Reproducibility Checklist

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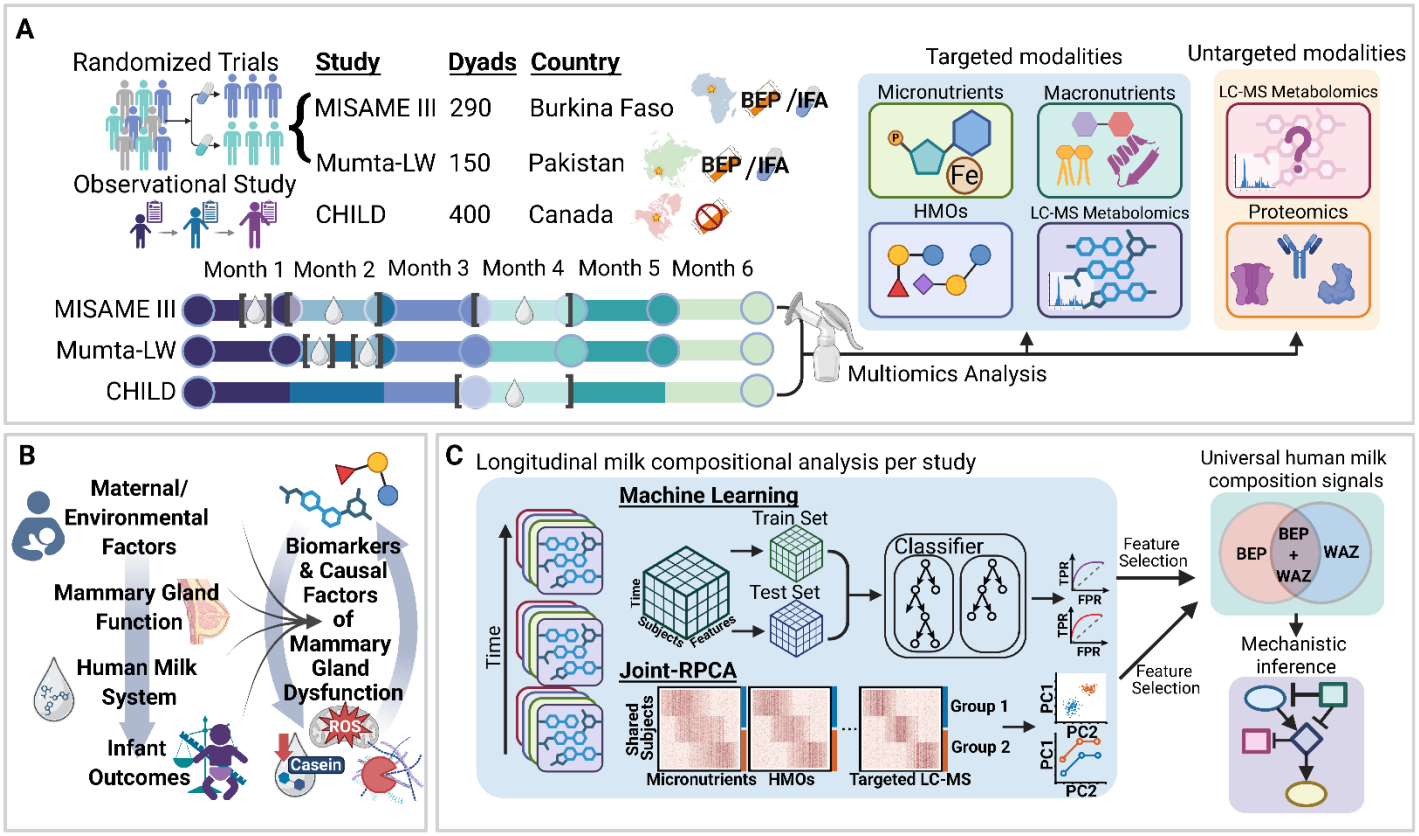

Fig. 1. Study design and methodology. (**A**) Samples were collected from three clinical studies, including two randomized controlled trials investigating the effect of maternal supplementation vs. an Iron-Folic Acid control (IFA) on infant growth (MISAME and Mumta-LW), and one observational cohort study (CHILD). Timeline shows human milk sample collection intervals in brackets, and infant anthropometric measurement time points as circles. Multiomics compositional analysis, including targeted and untargeted metabolomics, was performed on samples collected from all three studies. (**B**) Overview of our approach to identify biomarkers and candidate upstream factors linked to mammary gland functional state and impact infant growth outcomes. (**C**) For each study, samples were analyzed in parallel, leveraging both tailored machine learning algorithms and the Joint-RPCA dimensionality reduction tool (example data are shown for illustrative purposes, refer to tables S1 and S2 for Joint-RPCA ordinations). Results per study and across computational approaches were integrated to identify shared compositional signals associated with infant WAZ, supplementation, and the overlapping signal of both in human milk (BEP = balanced energy-protein supplementation, Joint-RPCA = Joint-Robust Principal Components Analysis, WAZ = weight-for-age z-score). [Figure created with BioRender.com]

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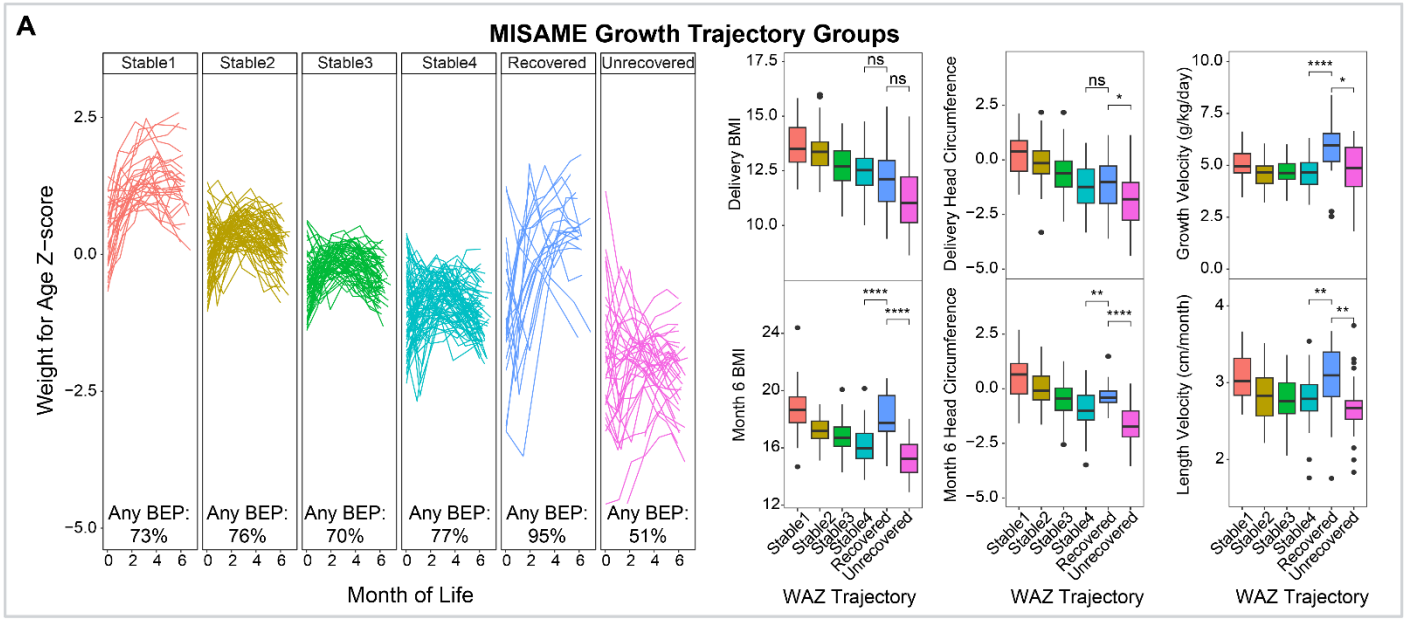

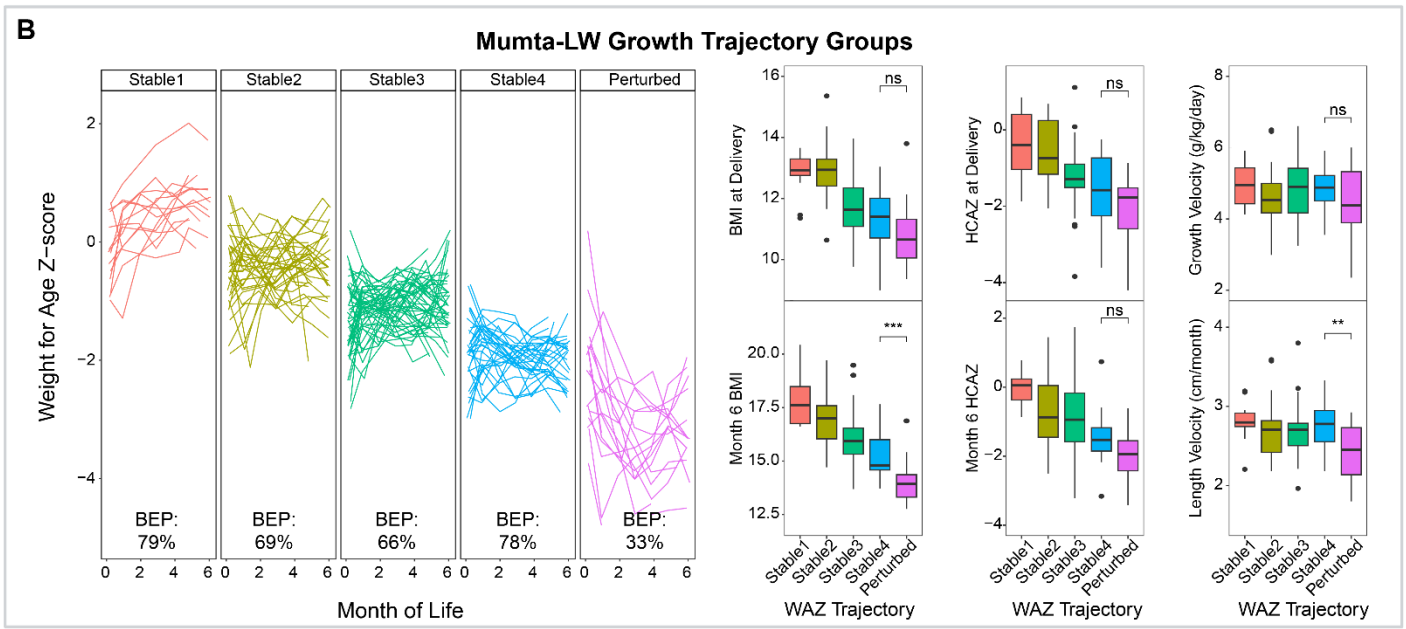

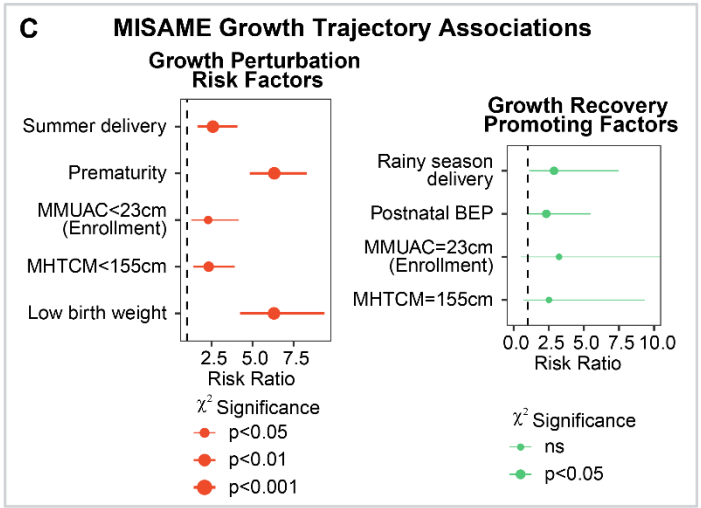

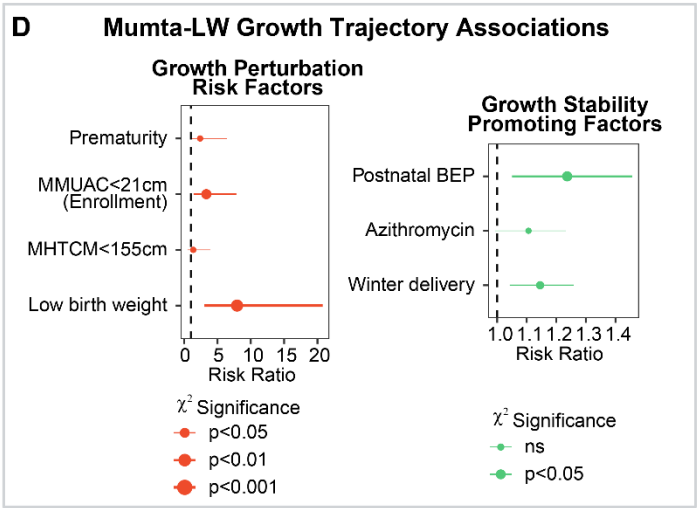

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Fig. 2. Maternal nutritional intervention influences infant growth via milk composition. (**A**) Infant WAZ trajectories for n=289 infants in MISAME over the first six months of life were clustered into six trajectory categories using group-based trajectory modeling representing four stable (stable1-4), one initially perturbed trajectory with recovery ('recovered'), and a perturbed trajectory without recovery ('unrecovered'). The proportion of mothers receiving supplementation is shown in text. (**B**) Infant WAZ trajectories in Mumta-LW (n=150) were clustered into five groups, representing four stable (stable1-4) and a 'perturbed' trajectory. (**C**) Risk factors for developing a 'perturbed' (recovered or unrecovered) trajectory vs. one of the four stable trajectories (stable1-4), and for developing a recovered vs. unrecovered trajectory in MISAME. (**D**) Risk factors for developing a perturbed growth trajectory (perturbed vs. stable1-4) and factors promoting growth trajectory stability (stable1-4 vs. perturbed) in Mumta-LW. (WAZ = weight-for-age z-score, HCAZ = head circumference for-age z-score, MHTCM = maternal height (cm); MMUAC = maternal mid-upper arm circumference in cm, Wilcoxon rank-sum tests were used for continuous group comparisons in panels (A) and (B), unless otherwise indicated. Risk ratios are shown in panels (C) and (D), with significance assessed using chi-square tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns = not significant).

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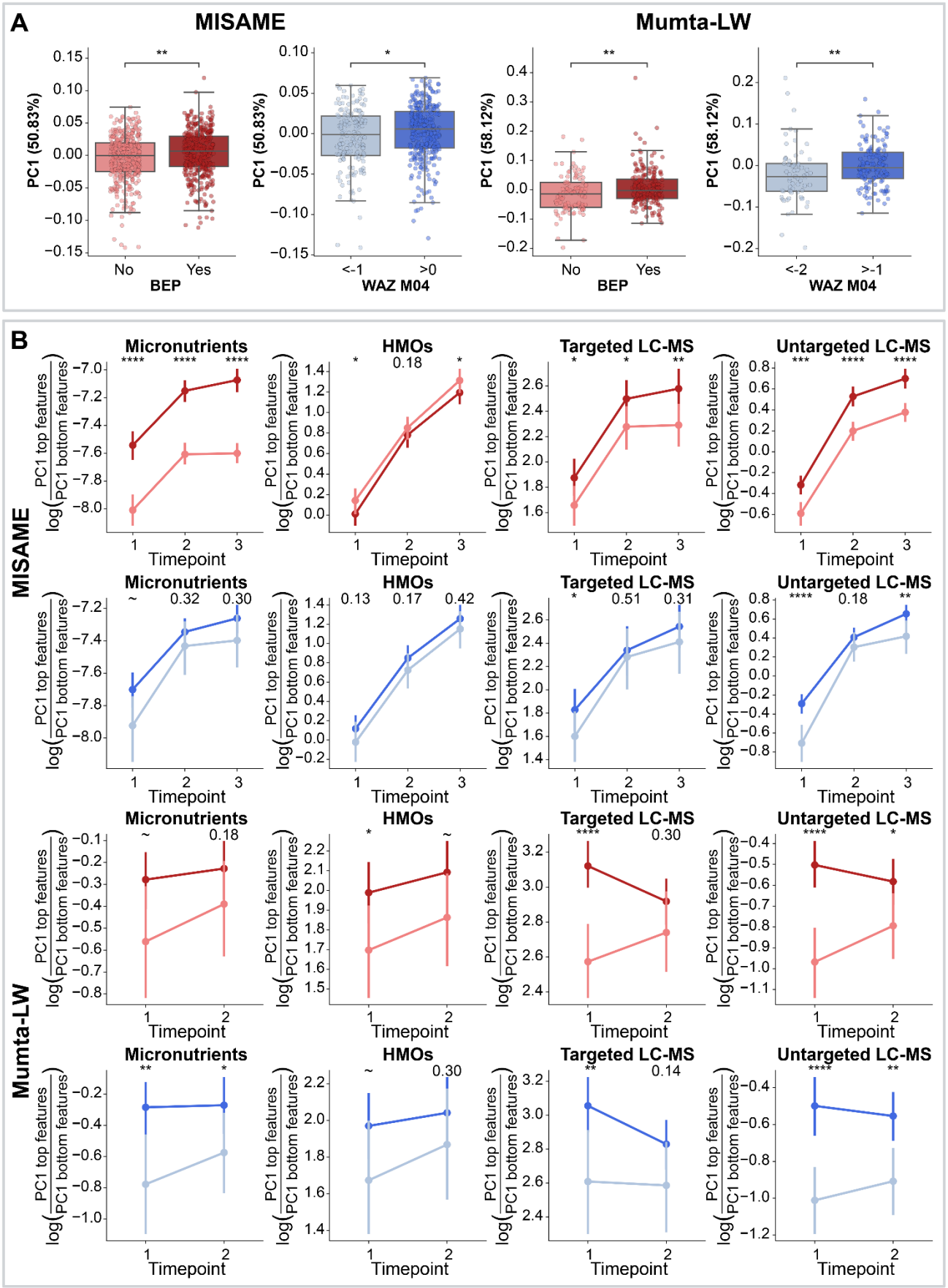

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Fig. 3. Shared HM compositional signal links maternal nutritional supplementation to infant growth. (**A**) Joint RPCA was used to identify the main axes of variation in HM composition performed across all infants in MISAME (n=290) and Mumta-LW (n=150). Associations between milk composition, maternal supplementation, and infant WAZ are consistently highlighted along the first axis of variation ('PC1') in MISAME and Mumta-LW. Boxplots comparing PC1 loadings across maternal supplementation and infant WAZ are shown. (**B**) Log ratios of the top micronutrients, HMOs, targeted and untargeted LC-MS metabolites, identified via Joint-RPCA in both studies, separate infants across both maternal supplementation and WAZ (refer to the "Data_Category" column in tables S1 and S2 for feature sets included for each comparison). Top rows represent differences among supplementation groups: supplemented mothers are shown in dark red and those without are shown in salmon. Bottom rows compare across infant WAZ (month 4). Infants with higher WAZ (WAZ >0 in MISAME and > -1 in Mumta-LW) are shown in dark blue, while those with lower (WAZ <-1 in MISAME and <-2 in Mumta-LW) are shown in light blue. (Timepoints: MISAME: 1=14-21 Days, 2=1-2 Months, 3=3-4 Months; Mumta-LW: 1=42 Days, 2=56-Days; Joint-RPCA = Joint-Robust Principal Components Analysis, BEP = balanced energy-protein, WAZ = weight-for-age z-score, HMO = human milk oligosaccharide; Wilcoxon rank-sum test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, ~*P* < 0.07).

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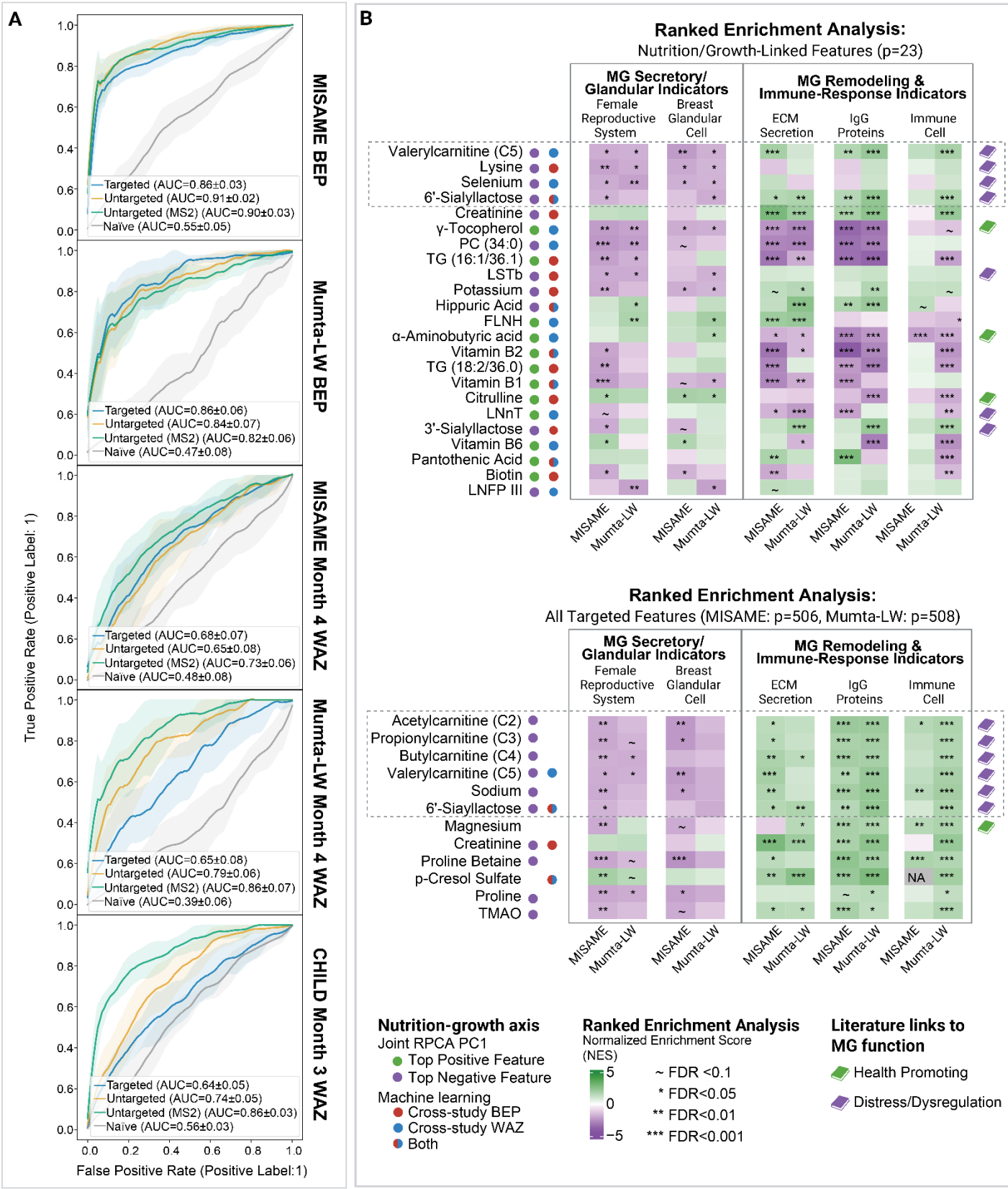

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Fig. 4. Core sets of human milk components outperform naïve baseline models for prediction of maternal intervention and infant growth outcomes. (**A**) Receiver operating characteristic (ROC) curves for supervised machine learning models predicting maternal supplementation status (BEP) and infant growth outcomes (WAZ) across MISAME, Mumta-LW, and CHILD cohorts. Models were trained using targeted human milk features (micronutrients, HMOs, and selected metabolites) and untargeted LC-MS metabolomics features, as well as a subset of 104 untargeted features further identified using MS2 analysis. Performance is compared to a naïve baseline model (gray), which excludes HM features and provides a reference for baseline predictive performance given class distributions. Shaded regions denote standard deviation across cross-validation folds. Subject counts for supervised machine learning analysis were as follows: MISAME (BEP: n = 290, WAZ: n = 168), Mumta-LW (BEP: n = 150, WAZ: n=102), and CHILD (WAZ: n = 386). (**B**) Ranked enrichment analysis of proteins associated with human milk features was performed for milk samples with available proteomic data (MISAME: n= 102, Mumta-LW: n = 147), which were all collected at timepoint 2 (MISAME: 1-2 Months, Mumta-LW 56 Days). Heatmaps show normalized enrichment scores (NES) for associations between HM-derived features and protein sets representing mammary gland secretory/glandular indicators (breast glandular cell–enriched proteins, female reproductive system secretome) and remodeling and immune-response indicators (ECM remodeling, immune cell–associated proteins). Features are ordered by their contribution to the shared nutrition–growth axis. Enrichment significance calculated using the GSEA test is indicated by ~ (FDR < 0.1), * (FDR < 0.05), ** (FDR < 0.01), and *** (FDR<0.001). This analysis links candidate biomarkers to functional pathways reflecting mammary functional state, including milk synthesis/secretion, tissue remodeling, and immune-response activity.

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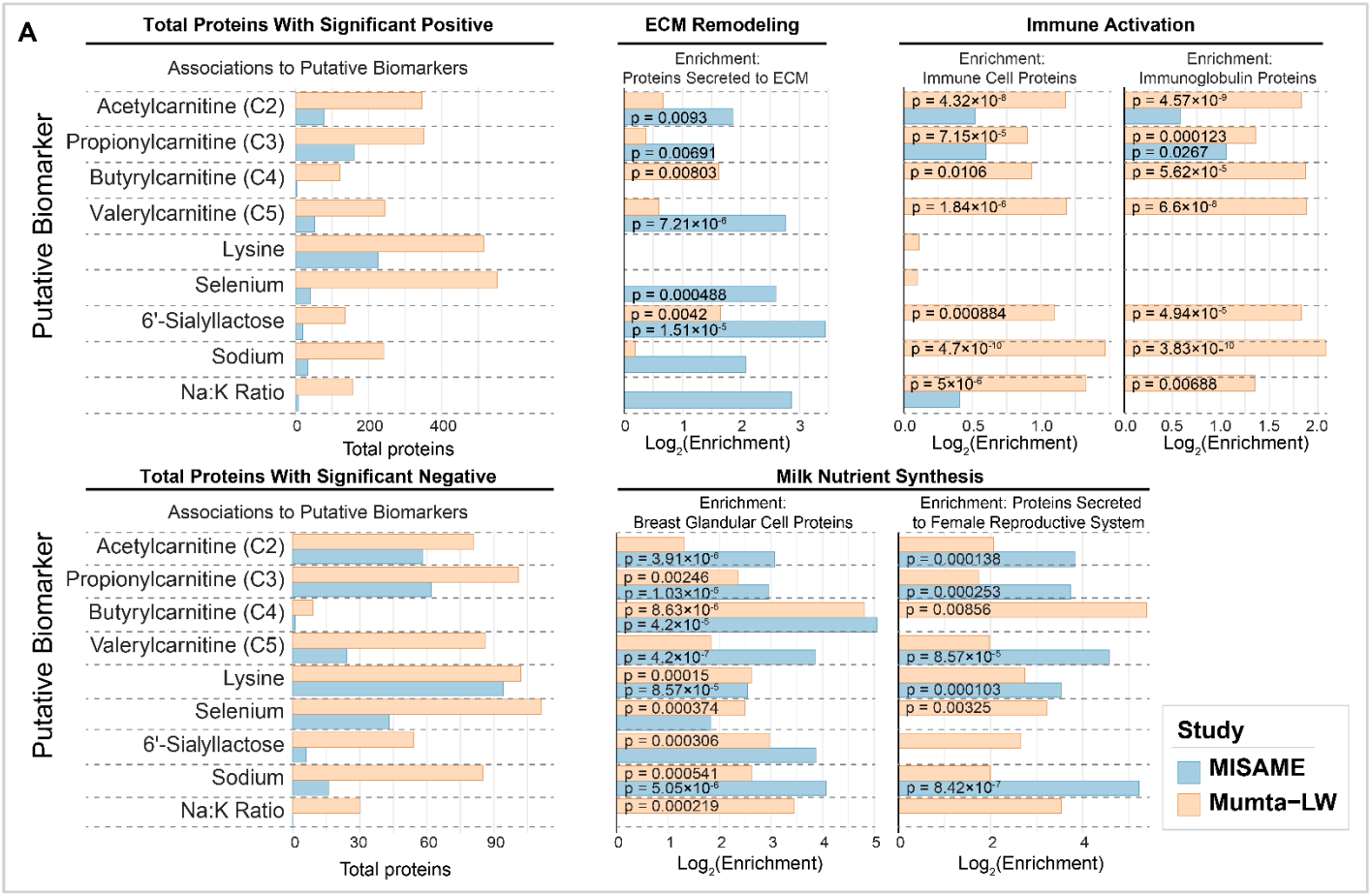

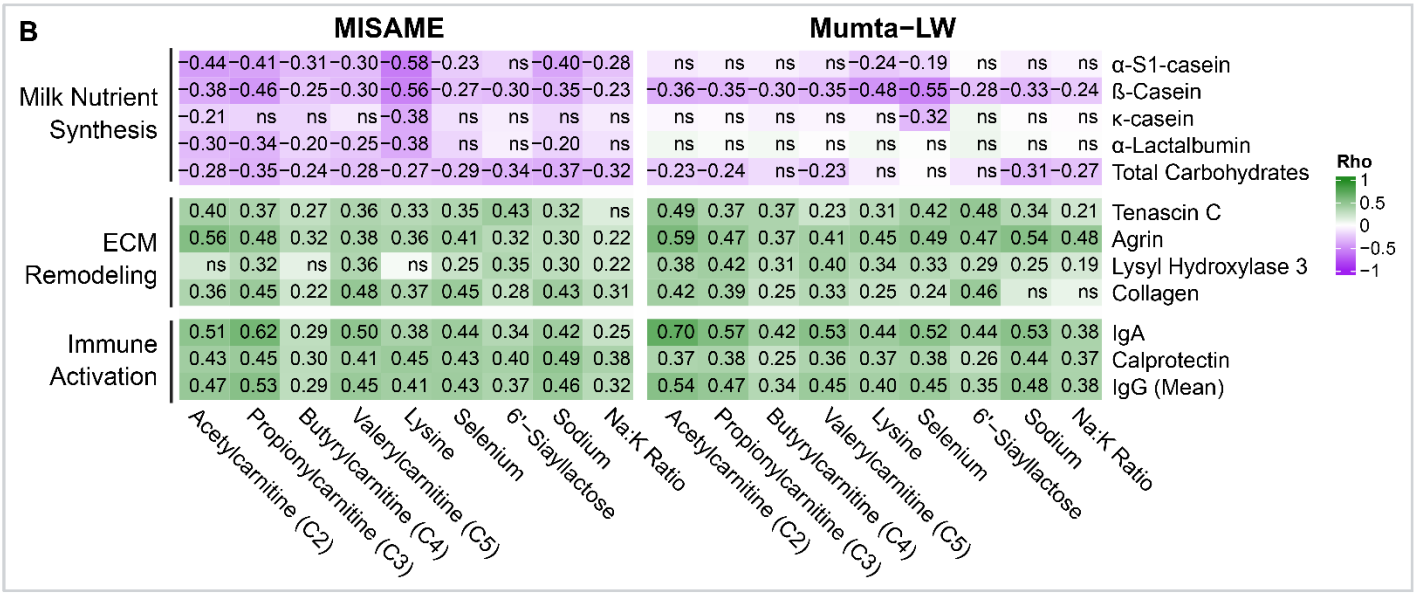

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Fig. 5. A set of seven HM candidate biomarkers are associated with key mammary gland functional-state domains. (**A**) Enrichment results for proteins significantly associated (positively or negatively; FDR<0.05, Spearman's rank correlation) with the identified set of seven HM candidate biomarkers as well as two established benchmarks (sodium and the sodium:potassium ratio). Total counts of proteins with significant positive (top) or negative (bottom) associations to each biomarker are shown on the left-most panels. Breast glandular cell-specific and immune cell-specific proteins were identified using expression data from the HPA. "Female secretion", "Secreted to ECM," and "Immunoglobulin gene" lists were directly obtained from the HPA. Only significant *P*-values (hypergeometric test) are shown. Enrichment was calculated relative to the full set of proteins quantified in the respective study. (**B**) The set of seven HM candidate biomarkers (columns) as well as sodium and Na:K; The panel of seven exhibited consistent as well as study-specific associations with proteins representing mammary synthesis/secretion, ECM remodeling, and immune-response domains (rows), as well as total carbohydrate levels (additional associations are shown in figs. S10 and S11).

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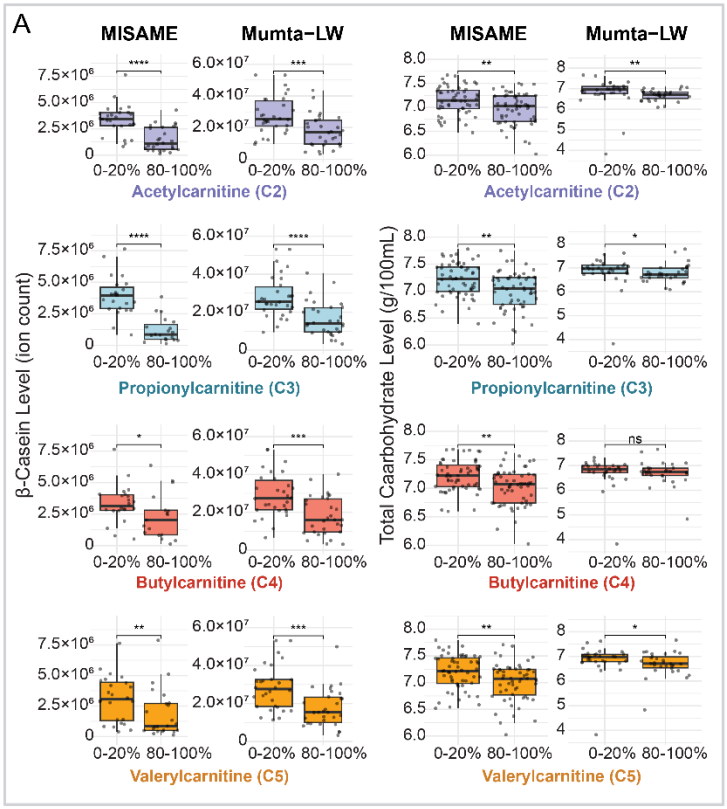

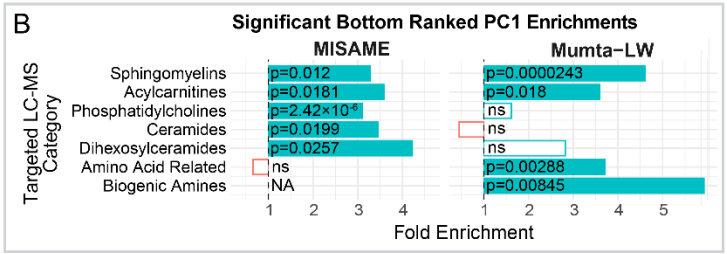

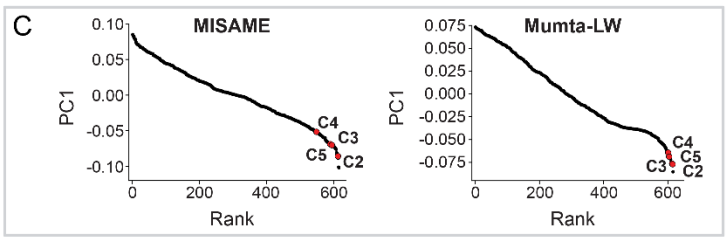

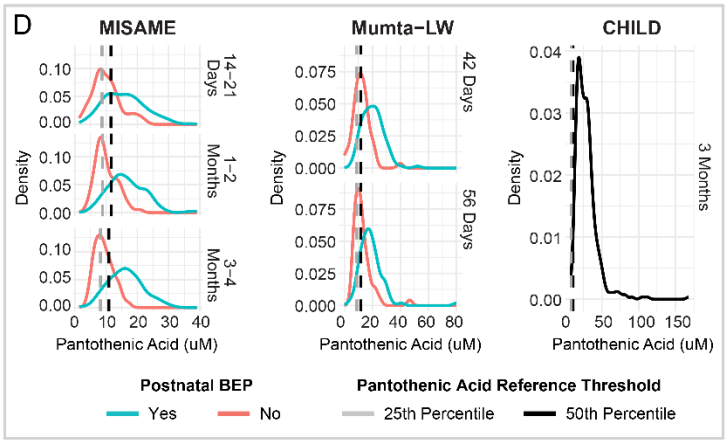

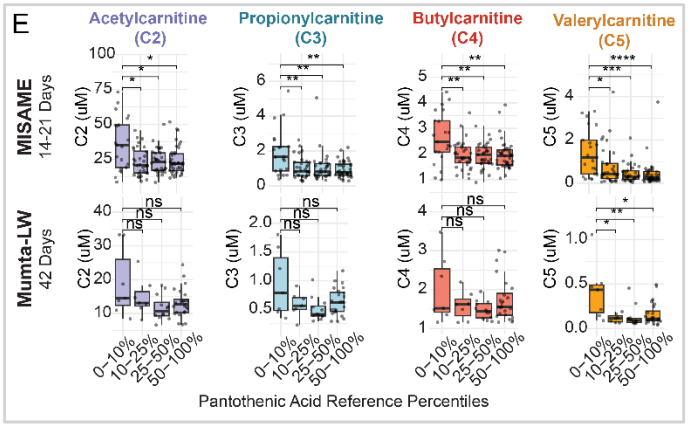

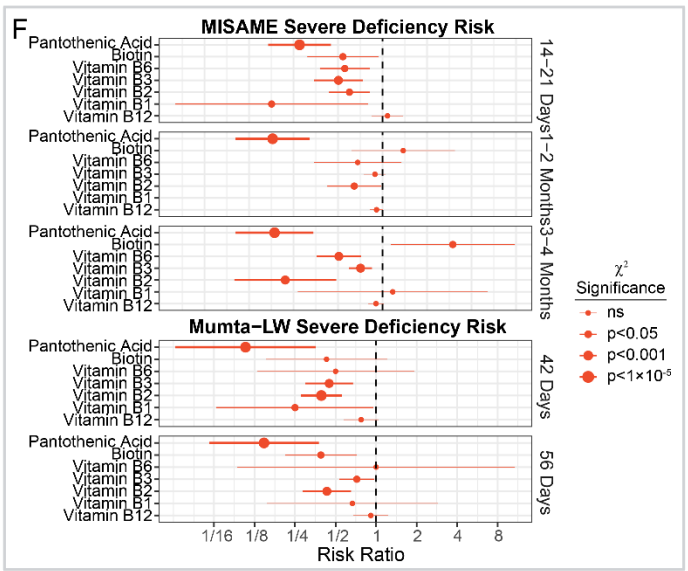

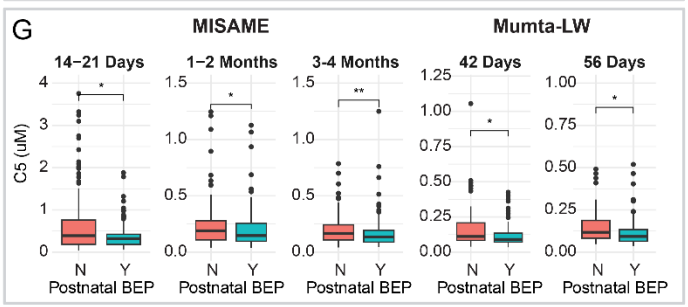

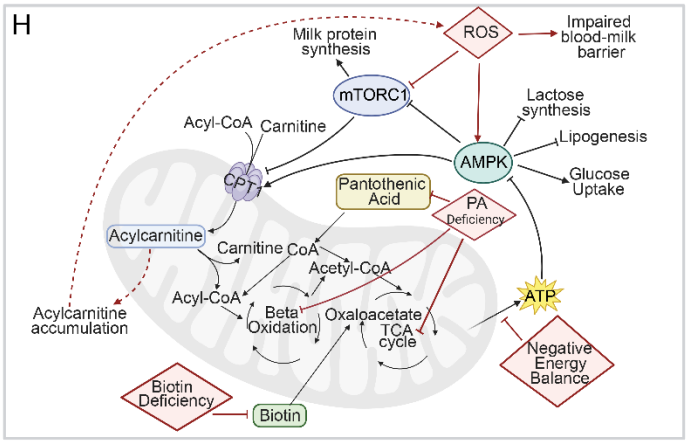

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Fig. 6. C5 links acylcarnitine metabolism, mammary functional state, maternal nutrition, and infant growth. (**A**) Lower milk concentrations of short-chain acylcarnitines C2–C5 were associated with higher concentrations of milk nutrients linked to mammary synthesis/secretion in both cohorts. Analyses used samples with paired proteomics data (MISAME: 1–2 months, n = 102; Mumta-LW: 56 days, n = 147). (**B**) Acylcarnitines were enriched among bottom-ranked targeted LC-MS features along PC1 (hypergeometric test; features with ≥1 significant association shown; see fig. S13A for all comparisons). (**C**) C2–C5 ranked among the most negative PC1 features in both cohorts. (**D**) Short-chain acylcarnitines were increased in milk from mothers with severe pantothenic acid deficiency in MISAME; C5 showed consistent associations across both cohorts (see fig. S14 and S15 for B-vitamin comparisons). Analyses used unsupplemented mothers at the earliest available timepoint per study (MISAME: n = 129; Mumta-LW: n = 50). (**E**) Milk pantothenic acid distributions by postnatal BEP status. Blue and red lines indicate supplemented and unsupplemented mothers, respectively; dashed lines indicate MILQ reference percentiles for pantothenic acid: 50th percentile (black) and 25th percentiles (gray). (**F**) Postnatal BEP supplementation reduced the risk of severe deficiency for pantothenic acid and other B-vitamins in both cohorts. Severe deficiency was defined as HM concentrations <10th percentile based on MILQ reference values. (**G**) C5 levels were consistently negatively associated with postnatal BEP supplementation across timepoints in both cohorts. (**H**) Proposed model linking pantothenic acid insufficiency to constrained CoA-dependent mitochondrial metabolism, acylcarnitine accumulation in HM, and altered mammary functional state, including reduced milk protein/lactose synthesis, immune/inflammatory activity, and blood–milk barrier permeability/remodeling. Analyses included all subjects in MISAME (n = 290) and Mumta-LW (n = 150) unless otherwise indicated. Wilcoxon rank-sum test unless otherwise indicated; risk-ratio analysis was used for panel (F) with significance computed via chi-squared test ; $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, ns = not significant. C2, acetylcarnitine; C3, propionylcarnitine; C4, butyrylcarnitine; C5, valerylcarnitine; BEP, balanced energy–protein supplementation. [Panel (H) created with BioRender.com]

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Shared patterns of human milk composition link mammary gland function to infant growth

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