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The Influence of Leptin, Adiponectin and Insulin in Human Milk on the Growth of Children Exposed to Adverse Intrauterine Environments: A Prospective Cohort Study

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Abstract

Background:

Human milk (HM) acts as a metabolic signaling pathway between mother and infant, influencing appetite regulation and child growth. This function is partly determined by the hormones leptin, adiponectin, and insulin, present in HM. This study aimed to evaluate the relationship between these hormones and somatic growth from birth to preschool age, considering pre-pregnancy maternal body mass index (BMI) and fetal exposure to different adverse intrauterine environments.

Methods:

This cohort study recruited postpartum women and their newborns from three hospitals in southern Brazil. Mother–child dyads were allocated into five groups according to intrauterine environment: diabetes mellitus (DM), systemic arterial hypertension, smoking, intrauterine growth restriction (IUGR), and a control group. From birth to preschool age, seven assessments were conducted, during which BMI-for-age Z-scores were recorded. Pre-pregnancy BMI was also recorded. HM samples were collected within 48 hours postpartum and at 30 days for hormone analysis. Interactions between hormone concentrations over time and between groups were analyzed using generalized estimating equations and Spearman's correlation (ρ).

Results:

The number of HM samples ranged from 5 to 60, depending on group and collection time. Adiponectin concentrations differed between groups ($p = 0.007$), while insulin levels varied across collection periods ($p < 0.001$). In the smoking group, pre-pregnancy maternal BMI correlated with changes in leptin ($\rho = 0.574$; $p = 0.032$) and adiponectin concentrations ($\rho = 0.732$; $p = 0.002$). In the DM group, early postpartum HM leptin correlated with neonatal BMI-for-age Z-scores at 7 ($\rho = 0.886$; $p = 0.019$) and 15 days ($\rho = 0.900$; $p = 0.037$), while leptin at 30 days correlated with BMI-for-age Z-score at six months ($\rho = 0.811$; $p = 0.004$). In the IUGR group, this latter correlation was negative ($\rho = -0.709$; $p = 0.015$). In the early postpartum period, HM adiponectin concentration correlated with neonatal BMI-for-age Z-score at 7 days in the control group ($\rho = 0.783$; $p = 0.003$), while HM insulin concentration at 30 days correlated with infant BMI-for-age Z-score at six months in the smoking group ($\rho = 0.697$; $p = 0.025$).

Conclusions:

Variations in HM insulin and adiponectin concentrations were positively associated with pre-pregnancy maternal BMI in the smoking group, while specific correlations between HM hormones and children's BMI-for-age Z-scores were identified across groups.

BACKGROUND

During pregnancy, placental nutrition is essential for fetal development, and after birth, human milk (HM) plays an equally important role, not only in newborn nutrition but also in protection against several diseases throughout life [1]. HM appears to act as a metabolic signaling pathway between mother and infant; its composition, which includes a variety of nutrients and bioactive substances, influences appetite regulation and child growth [2]. Among the components studied are the hormones leptin, adiponectin, and insulin, which are important for the regulation of food intake, energy expenditure, and glycemic control [3, 4], thereby contributing to protection against obesity [2].

Leptin plays an important role in the regulation of body fat reserves, influencing eating behavior, metabolism, the autonomic nervous system, and energy balance [5]. First identified in HM in 1997 [6], leptin has receptors in the intestine, suggesting that it may be absorbed by the infant and act in short-term regulation of food intake [7]. Its concentration in HM is positively correlated with maternal BMI [8, 9]; however, findings remain inconsistent regarding variations in leptin concentration between colostrum and mature milk [8, 10–12]. Higher leptin exposure through HM intake may reduce appetite and modulate weight gain during the first years of life [2, 13], although results remain inconclusive, as some studies have reported no such associations [14, 15].

Adiponectin is secreted by adipose tissue and is involved in several physiological processes, including increased cellular uptake of blood glucose [16]. It was identified in HM in 2006 [17], where it plays important roles, such as anti-inflammatory activity and contributions to reductions in infant weight and BMI [18]. Like leptin, adiponectin has shown an inverse association with child growth, particularly with weight and length [13]. Higher adiponectin levels in HM have been associated with lower weight-for-age and weight-for-length Z-scores at six months of age [19]. However, a longitudinal study that assessed adiponectin concentrations in HM collected at three months postpartum and followed child growth up to 17 years of age concluded that adiponectin has no long-term effect on BMI or cardiometabolic outcomes during childhood [15].

In the context of lactation, among these three hormones, insulin was the one discovered earliest, in 1975 [20], and has attracted increasing interest due to its potential role in the prevention of metabolic diseases. Studies have shown that oral administration of insulin promotes intestinal maturation and reduces intestinal permeability to macromolecules, potentially contributing to the primary prevention of type 1 diabetes mellitus [21]. In addition, Yadav and Rustogi [22] suggested that insulin is one of the most important endocrine regulators in early postnatal life. In HM, it has been negatively associated with neonatal anthropometric indicators such as weight, lean mass, and BMI-for-age and weight-for-length Z-scores [23]. Moreover, consumption of HM with high insulin concentrations during the first month of life has been associated with lower weight gain and reduced accumulation of lean mass [24]. A study by Christensen et al. [19], which assessed the concentrations of appetite-regulating hormones in HM among participants from four countries, found that the highest insulin concentrations were present in samples from Brazilian mothers, the country that also showed the highest prevalence of maternal overweight at the time of sample collection.

Currently, there is a growing global increase in obesity among both children and adults, alongside accumulating evidence supporting the importance of leptin, adiponectin, and insulin in HM. Accordingly, the aim of this study was to evaluate the influence of the concentrations of these hormones in HM on child growth from birth to preschool age, assessed using BMI-for-age Z-scores, according to children's exposure to adverse intrauterine environments resulting from different gestational clinical conditions and pre-pregnancy maternal BMI.

Methods

This cohort study is part of the IVAPSA Project Phase I (2011–2016), entitled "Impact of Perinatal Different Intrauterine Environments on Child Growth and Development in the First Six Months of Life" [25], and Phase II (2017–2019), entitled "Impact of Perinatal Different Intrauterine Environments on Child Growth and Development in the First Five Years of Life".

A convenience sample of postpartum women with recent delivery (24–48 hours before the interview) and their live-born newborns with a - age between 37 and 42 weeks was used. Mother–child dyads were allocated into adverse intrauterine environment groups according to different maternal gestational clinical conditions:

- DM: postpartum women diagnosed with type 1 diabetes mellitus, type 2 diabetes mellitus, or gestational diabetes mellitus;
- Systemic arterial hypertension (SAH): postpartum women diagnosed with hypertensive disorders, including preeclampsia, eclampsia, preeclampsia superimposed on chronic hypertension, chronic SAH, or gestational hypertension;
- Maternal smoking (smokers): postpartum women who answered affirmatively to smoking during pregnancy at the first interview;
- IUGR: postpartum women who delivered term newborns classified as small for gestational age, defined as below the 5th percentile according to the Alexander growth curve parameters [26];
- Control: postpartum women who did not present any of the conditions described above.

Postpartum women presenting more than one clinical condition were excluded from the analysis. Participant recruitment took place in the rooming-in wards of three public hospitals in Porto Alegre, Rio Grande do Sul, Brazil. These institutions provide obstetric care through the Brazilian Unified Health System (Sistema Único de Saúde, SUS) for both low- and high-risk pregnancies and serve as reference centers in the municipality. More detailed information on study protocols, assessments, and the IVAPSA project can be found in previous publications [25, 27].

Seven postpartum interviews were conducted: within the first 48 hours after birth; at 7 and 15 days; at 1, 3, and 6 months postpartum; and when the child reached preschool age. At the first interview, written informed consent was obtained from the participants, while at the final assessment in Phase II, a new informed consent form was signed by the child's legal guardians. Each mother–child dyad was assigned a unique identification number corresponding to questionnaires and assessments, thereby ensuring participant anonymity.

The inclusion criteria for IVAPSA Phase I required postpartum women who delivered at the three hospitals previously described to be residents of the municipality of Porto Alegre, Rio Grande do Sul, Brazil. Exclusion criteria included HIV-positive mother, multiple births, and preterm newborns or those with congenital malformations, or who required neonatal hospitalization. For IVAPSA Phase II, all mother–child dyads that had participated in the interview conducted at six months of age were included.

During follow-up interviews, anthropometric measurements of children's weight and length were performed in duplicate according to a standardized protocol [28]. The World Health Organization (WHO) software programs Anthro (version 3.2.2) and AnthroPlus (version 1.0.4) were used to calculate and standardize BMI-for-age Z-scores. Pre-pregnancy maternal BMI was calculated according to WHO criteria [29] using pre-pregnancy weight obtained from the prenatal care card or hospital records and height measured at the first interview.

Human milk (HM) collection for analysis of leptin, adiponectin, and insulin concentrations was performed in the early postpartum period (within 48 hours after delivery) and at 30 days of the child's life. Postpartum women were instructed to wash their hands with soap and water before manual expression and to cleanse the breasts using potable water only [30]. After collection, HM samples (1–5 mL), aliquoted into 1.5 mL tubes, were labeled and stored at − 80°C. Before analysis, HM samples (colostrum and mature milk) were thawed and centrifuged at 15,000 rpm for 30 minutes at 4°C. Once the fat layer was isolated to prevent interference with the measurements, it was discarded. No protease inhibitors were added. After the initial study period (six months), samples were discarded in appropriate containers for biological materials. Hormone concentrations were quantified using ELISA kits (Millipore®), and all samples were analyzed in duplicate.

Information on whether mothers breastfed immediately before HM collection or were fasting was not recorded. This decision was based on previously published findings showing no significant differences in leptin [2, 31] and insulin [2] concentrations between HM samples obtained at the beginning and at the end of a breastfeeding session.

For statistical analysis, variables were described according to their distribution as mean ± standard deviation, median (interquartile range), or n and percentage (%). Comparisons among the five study groups (DM, SAH, smoking, IUGR, and control) were performed using one-way analysis of variance (ANOVA) for normally distributed data, followed by Bonferroni-corrected post hoc tests. When normality was not met, the nonparametric Kruskal–Wallis test was applied, with multiple comparisons conducted using Dunn's test with Bonferroni adjustment. Categorical variables were analyzed using Pearson's chi-square test in conjunction with adjusted residuals analysis.

To analyze the interaction between leptin, adiponectin, and insulin at two points in time (early postpartum and at 30 days) and across groups, a generalized estimating equations (GEE) model with a Tweedie distribution and a log link function was applied, complemented by the least significant difference (LSD)

test. Associations between variables were assessed using Spearman's correlation coefficient.

Variables were adjusted for mode of delivery, newborn birth weight and sex, maternal age, education, pre-pregnancy maternal BMI and number of children, marital status, and family income. This adjustment was performed through the construction of a Directed Acyclic Graph (DAG) to control for potential confounding factors that could influence both maternal exposures and child growth (Supplementary Material). Information on maternal race/skin color, Apgar score, total duration of breastfeeding, and child age at the last interview was also collected. Statistical analyses were conducted considering a significant level of 5% ($p < 0.05$) and a 95% confidence interval. Data was analyzed using SPSS (Statistical Package for the Social Sciences), version 31.0. The IVAPSA project was approved by the Research Ethics Committee of HCPA (protocol numbers 11/0097 and 17/0107) and by the Ethics Committee of GHC (protocol number 11/027). The project was registered on the Brazilian National Research Ethics Platform (Plataforma Brasil) under registration number CAAE 65190217500005327. All methods were performed in accordance with the latest guidelines and current regulations of the National Health Council of the Ministry of Health of Brazil (resolutions no. 466/2012 and no. 580/2018).

Results

Initially, 342 mother–child dyads were included in the study and distributed into five groups (55 DM, 19 SAH, 86 smoking, 31 IUGR, and 151 control). However, many mothers did not provide HM samples, and in some cases the sample volume was insufficient for all planned analyses. Consequently, the number of HM samples ranged from 5 to 60, depending on the study group and the time point of collection.

Table 1 presents the maternal sociodemographic and clinical characteristics, as well as newborn and preschool characteristics, according to groups defined by different gestational clinical conditions associated and their adverse intrauterine environments. A statistically significant difference was observed only for newborn birth weight ($p = 0.014$), with infants in the IUGR group presenting lower birth weight compared with those in the control group (2707.86 g ± 505.82 g vs. 3431.33 g ± 474.02 g).

Hormone concentrations in HM were highly heterogeneous. In the early postpartum period, leptin concentrations ranged from 0 to 3.45 ng/mL, and at 30 days from 0 to 14.37 ng/mL, while adiponectin concentrations ranged from 2.99 to 34.34 ng/mL in the early postpartum period and from 4.25 to 38.98 ng/mL at 30 days. Insulin concentrations ranged from 5.39 to 328.11 μU/mL in the early postpartum period and from 4.91 to 126.44 μU/mL at 30 days.

No significant interaction effect between hormone concentrations, study group, and time was observed ($p > 0.05$). That is, hormone concentrations did not differ between groups at two points in time evaluated (within the first 48 hours postpartum and at 30 days). Adiponectin concentrations were higher in HM from the smoking and IUGR groups compared with the control group within the first 48 hours after delivery ($p = 0.007$), and at 30 days, higher values were observed in the IUGR group than in the SAH, smoking, and control groups. On the contrary, insulin concentrations decreased between 48 hours and 30 days postpartum ($p < 0.001$) in all groups except the smoking group (Table 2).

A statistically significant positive association was observed between pre-pregnancy maternal BMI and the change (delta) in leptin ($\rho = 0.574$; $p = 0.032$) and adiponectin concentrations ($\rho = 0.732$; $p = 0.002$) between 48 hours and 30 days postpartum, exclusively in the smoking group (Figure 1). This finding indicates that, among smokers, higher pre-pregnancy maternal BMI was directly proportional to HM hormone concentrations over the studied period. No association was observed for insulin across groups.

To evaluate each HM hormone as a potential predictor of BMI-for-age at different time points, Spearman's correlation analyses were performed. Leptin concentration measured in the early postpartum period showed a positive correlation with neonatal BMI-for-age at 7 ($\rho = 0.886$; $p = 0.019$) and 15 days ($\rho = 0.900$; $p = 0.037$) in the DM group, while leptin concentration measured at 30 days was positively correlated with BMI-for-age at six months in the DM group ($\rho = 0.811$; $p = 0.004$) and negatively correlated in the IUGR group ($\rho = -0.709$; $p = 0.015$). Postpartum adiponectin concentration showed a positive correlation with BMI-for-age measured at 7 days in the control group ($\rho = 0.783$; $p = 0.003$), and insulin concentration measured at one month was positively correlated with infant BMI-for-age at six months in the smoking group ($\rho = 0.697$; $p = 0.025$). Tables containing detailed data are available as Supplementary Material.

Table 1. Sociodemographic and clinical characteristics of mothers, newborns, and preschool children, according to groups defined by different gestational c

	DM	SAH	SMOKI
Maternal characteristics			
Maternal age, years (n=41)	26.33±6.80	27.00±7.21	27.31±
Number of children (n=33)	3(3-3)	2(2-2)	3(2-4)
Maternal race/skin color (n=41)			
white	1(33.3%)	2(66.7%)	7(53.8
black	2(66.7%)	0	4(30.8
brown (parda)	0	1(33.3%)	2(15.4
Marital status: married (n=41)	3(100%)	2(66.7%)	10(76.
Years of education (n=41)	9(9-9.5)	7(6-9)	10(7-1
Family income, BRL(n=37)	700(700-1100)	1200(1100-1600)	1200(9
Pre-pregnancy maternal BMI, kg/m² (n=39)	26.95(23.80-33.15)	29.97(29.11-30.99)	23.53(
Newborn characteristics			
Female sex (n=41)	3(100%)	3(100%)	7(53.8
Apgar score at 1 minute (n=40)	10(9-10)	9(9-9)	9(9-9)
Apgar score at 5 minutes (n=40)	10(9.5-10)	10(10-10)	10(9-1
Cesarean delivery (n=41)	2(66.7%)	2(66.7%)	3(23.1
Birth weight, g (n=40)	3446.67±658.96[ab]	3498.33±301.71[ab]	3192.0
Preschool child characteristics			
Preschool age, years (n=13)	unchanged	5(5-5)	4.5(4-5
Breastfeeding duration, months (n=12)	unchanged	42(24-60)	5(2-8)

Only participants with available data on human milk leptin, adiponectin, and insulin were included in this analysis. DM, diabetes mellitus; SAH, systemic arte
Wallis test; X^2 Pearson's chi-square test. Different letters indicate statistically significant differences between groups. Data are presented as mean ± standar

Table 2 – Mean concentrations of leptin, adiponectin, and insulin in human milk according to groups with different gestational clinical conditions associated with adverse intrauterine environments, assessed at birth and at 30 days of life, with effects estimated by GEE. IVAPSA Cohort Phases I and II – Porto Alegre (RS), Brazil.

Variables	DM	SAH	Smoking	IUGR	Control	GEE effects*		
						Group	Time	Group x Time
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE			
Leptin (ng/ml)						0.978	0.078	0.753
Birth	0.56±0.18	0.56±0.26	0.48±0.13	0.66±0.27	0.66±0.20			
30 days	0.41±0.10	0.43±0.13	0.51±0.13	0.31±0.12	0.45±0.09			
Adiponectin (ng/ml)						**0.007**	0.082	0.583
Birth	9.86±3.18^{a,ef}	12.5±4.55^{a,ef}	13.3±1.63^{a,f}	16.5±2.04^{a,f}	9.33±1.27^{a,e}			
30 days	11.0±1.61^{a,ef}	8.33±1.85^{a,e}	9.93±1.24^{a,e}	13.6±1.62^{a,f}	8.57±1.23^{a,e}			
Insulin (uU/ml)						0.460	**<0.001**	0.280
Birth	85.3±23.9 ^{b,e}	73.2±24.9^{b,e}	68.3±19.1^{a,e}	126.5±51.8^{b,e}	60.0±15.3^{b,e}			
30 days	40.2±5.73^{a,e}	29.8±7.89^{a,e}	42.3±9.82^{a,e}	27.6±4.19^{a,e}	25.5±5.20^{a,e}			

Adjusted for sex, mode of delivery, maternal education, marital status, maternal age, number of children, pre-pregnancy BMI, family income, and birth weight; ^{a,b,c,d} identical letters do not differ according to the LSD test at a 5% significance level (intragroup comparison); ^{e,f,g,h} identical letters do not differ according to the LSD test at a 5% significance level (intergroup comparison). BMI: body mass index; DM: diabetes mellitus; SAH: systemic arterial hypertension; IUGR, intrauterine growth restriction; GEE: generalized estimating equations; SE: standard error; LSD: Least Significant Difference.

Discussion

The present study demonstrated differences in HM insulin concentrations between the two collection times (within the first 48 hours postpartum and at 30 days) in the DM, SAH, IUGR, and control groups. In contrast, HM adiponectin concentrations at birth were higher in the smoking and IUGR groups, while at 30 days postpartum higher concentrations were observed only in the IUGR group. Pre-pregnancy maternal BMI was positively associated with changes (delta) in HM leptin and adiponectin concentrations in the smoking group.

Like other studies [8, 10, 32], a wide variation in HM leptin concentrations was also observed. When assessing this hormone in HM at birth and at 30 days postpartum in a sample of 15 mother–infant pairs, Doneray, Orbak, and Yildiz [10] reported an increase in leptin concentrations from 1.5–15.4 ng/mL in colostrum to 2.6–46.3 ng/mL in mature milk. In contrast, other studies have reported a reduction in HM leptin levels, including a 33.7% decrease from the first to the sixth month of life [8], and a decline from 3.35 ± 0.25 ng/mL on the second day of life to 1.63 ± 0.18 ng/mL on day 25 [11]. Pontes et al. [33] found no differences in HM leptin or insulin concentrations between 2 and 119 days postpartum. Consistent with these findings, the present study also did not observe differences in HM leptin concentrations between the evaluated time points; however, a reduction in HM insulin concentrations was identified in all groups, except for the smoking group.

Regarding adiponectin concentrations, although mean values appeared to be lower at 30 days postpartum, except in the DM group, this reduction was not statistically significant. In contrast, Martin et al. [17] observed a decrease in HM adiponectin concentrations from the first to the seventh month of lactation. Similarly, Bronsky et al. [34] reported a reduction up to three months postpartum, followed by an increase up to 12 months, which the authors attributed to the introduction of complementary feeding and to longer intervals between HM feeds. Considering the available evidence in the literature, the lack of statistical significance observed in the present study may be attributed to the relatively small sample size.

In relation to differences between groups, we found that mean adiponectin concentrations at birth were higher in the smoking and IUGR groups compared with the control group, while at 30 days postpartum they were higher in the IUGR group than in the SAH, smoking, and control groups. Although several studies have examined the isolated effects of maternal clinical conditions on HM hormones, we did not identify studies that simultaneously evaluated the five groups compared in the present analysis. Among the available evidence, for example, mothers with gestational DM (GDM) showed decreased adiponectin and increased insulin concentrations in HM at three and 90 days postpartum compared with non-GDM mothers [35]. Conversely, another study reported lower mean HM insulin concentrations at 30 and 90 days postpartum in women with GDM than in those without GDM [36], highlighting the inconsistency of the findings.

With respect to the influence of pre-pregnancy BMI on the concentrations of hormones present in HM, the present study showed a moderate and a strong correlation with the change (delta) in leptin and adiponectin concentrations, respectively, but only in the smoking group. Overall, these findings are in line with the systematic review by Andreas et al. [37], which did not identify any studies reporting an association between adiponectin concentrations and pre-

pregnancy BMI. More recently, however, an inverse association between these variables has been reported [38]. Studies that included maternal smoking as a covariate did not find an association with adiponectin, leptin, or insulin concentrations in HM [14], and similarly, no reduction in adiponectin concentrations was observed with increasing numbers of cigarettes smoked up to the sixth week postpartum [35].

Related to leptin, Andreas et al. [38] identified a positive association in 11 of the 15 studies reviewed, which was corroborated by Balcells-Esponera et al. [41], who reported a strong correlation between leptin concentrations in HM and pre-pregnancy BMI ($\rho = 0.648$, $p < 0.0001$). These findings are biologically plausible, given that leptin is produced predominantly by adipose tissue. More recently, Pontes et al. [33] observed leptin concentrations that were 88.8% higher between 28 and 50 days postpartum and twice as high between 88 and 119 days postpartum in women with overweight or obesity compared with eutrophic women ($p < 0.0001$). Adjusted results from Sadr Dadres et al. [39] also support this association ($\beta = 0.494$; $p < 0.001$). Higher leptin concentrations in HM during the first week of life have been associated with lower infant weight gain up to six months of age [42]. This effect, likely mediated by appetite regulation, may represent a protective mechanism against excess weight gain, highlighting the importance of breastfeeding among women with obesity in promoting a healthy growth pattern [8].

Regarding insulin, the present study did not show a correlation with pre-pregnancy BMI, in line with previous studies [23, 33]. However, other investigations have reported that insulin concentrations in HM six weeks postpartum were higher among mothers with pre-pregnancy overweight/obesity compared with eutrophic mothers [39]. Additionally, higher pre-pregnancy BMI has been associated with insulin concentrations in mature HM, but not in colostrum [40]. A prospective cohort conducted in the United States, which included pregnant women and subsequently analyzed 130 HM samples, demonstrated a statistically significant, albeit weak, association between pre-pregnancy BMI and HM insulin concentrations ($\beta = 0.144$, $p = 0.030$) [38]. In a similar way, Sims et al. [41] reported significantly higher insulin concentrations in HM up to the ninth postpartum month among women with overweight, implying greater infant exposure to this hormone.

One previous longitudinal study has evaluated the change (delta) of a HM hormone, relating it to infant BMI [10]. In that study, the authors assessed leptin delta in 15 postpartum women between the 1st and 30th days of the infant's life and observed an inverse correlation between leptin delta and infant BMI over the same period. In contrast, the present study analyzed changes in HM hormone concentrations and their association with pre-pregnancy BMI, as this temporal analytical approach controls for part of the existing variability and, therefore, captures changes occurring throughout lactation.

The literature reports largely consistent findings regarding an inverse association between HM leptin and infant growth, particularly during the first months of life. In the present study, leptin showed punctual correlations with BMI-for-age Z-scores over time. Classic studies have described a negative association between leptin concentrations during the first month of lactation and infant BMI at 18 and 24 months of age [9], as well as an association between higher leptin concentrations six weeks postpartum and lower infant BMI up to approximately five months of age [42]. However, Logan et al. [42] also observed that higher leptin levels could be linked to accelerated BMI gain during the first two years of life. Similar results were reported by Brunetto et al. [43], in which leptin was associated with lower BMI-for-age Z-scores at three and six months. A recent systematic review reinforces this pattern, showing that in more than half of the studies, leptin was negatively associated with weight, length, BMI, and body fat percentage during the first two years of life, although inconsistencies remain [13]. Multicenter data further confirm that this association may vary according to context: in The Gambia, West Africa, higher leptin levels were associated with lower weight-for-age Z-scores, whereas no such association was observed in other countries [19]. In addition, a pioneering study investigating this relationship demonstrated a negative correlation between leptin concentrations in mature milk and BMI delta, and between changes in HM leptin concentrations (from colostrum to mature milk) and BMI delta [10].

In this study, different adiponectin concentrations were observed across groups, with higher mean levels in the smoking and IUGR groups, which were also those with lower birth weight. This finding supports the role of adiponectin in the negative regulation of ponderal growth [44]. In the literature, the presence of adiponectin in HM has been associated with lower weight gain during the first three months of life and with lower BMI extending into adolescence among children more exposed to this hormone [15]. A systematic review [13] identified that most studies reported inverse associations between HM adiponectin and infant anthropometric measures [15, 35, 44–47]. Conversely, one study reported positive associations with infant fat mass [48]. In a multicenter study, higher adiponectin levels were associated with lower weight-for-length Z-scores in The Gambia and with lower weight-for-age Z-scores in Brazil, whereas this relationship was not observed in other countries evaluated [19]. A large Canadian birth cohort study (CHILD – Canadian Healthy Infant Longitudinal Development) found no association between HM adiponectin and infant anthropometry up to one year of age [14]. Taken together, these findings suggest that the effects of adiponectin may be modulated by both the maternal metabolic context and the environment.

Regarding insulin, the literature provides more limited evidence, although some associations have been described. Birth weight was negatively correlated with insulin concentrations in hindmilk samples collected one week postpartum, whereas at three months postpartum, insulin showed a negative correlation with infant length measured at seven days of life [2]. In the multicenter study by Christensen et al. [19], higher HM insulin levels were positively associated with length-for-age Z-scores among infants in The Gambia, but not in other countries. In the present study, insulin measured in HM at 30 days of life was associated with BMI-for-age Z-scores at six months among infants in the smoking group. Regarding maternal smoking, one study reported a twofold higher prevalence of overweight among children born to mothers who smoked during pregnancy [49]. An interesting finding was reported by Chan et al. [14], who observed a U-shaped association between HM insulin and infant body composition, with intermediate concentrations predicting the lowest weight-for-age and BMI-for-age Z-scores at four and 12 months. The authors suggested that these intermediate insulin concentrations may optimally support infant metabolism while the immature pancreas develops its capacity to produce insulin, whereas insufficient or excessive insulin exposure may impair this process.

It is worth noting that, although infant formula constitutes a safe nutritional substitute, it does not replicate the complexity of bioactive components present in HM, including hormones and adipokines [13]. This highlights the importance of understanding the role of these substances in infant metabolic programming and of striving to standardize studies on HM components, as existing research is highly heterogeneous regarding collection procedures and analytical methods, which hinders comparability across studies [13, 50].

In terms of study limitations, the sample size was relatively small, which limits the statistical power of the analysis. This is a common limitation in research on bioactive components in HM, particularly in prospective longitudinal designs such as the present study. For example, Pontes et al. [33] evaluated only six mother–infant dyads with collections at three time points up to 119 days postpartum, illustrating the difficulty of maintaining prospective follow-up with HM sampling. Another limitation concerns the lack of standardization regarding HM collection (foremilk versus hindmilk); however, previous studies have reported no significant differences related to this factor [31, 51, 52].

As a strength of this study, it is worth highlighting the simultaneous comparison of four groups with different gestational clinical conditions and adverse intrauterine environments, in addition to a control group and in a cohort study, this design includes conditions frequently excluded from other studies, such as maternal smoking and SAH, thereby increasing the relevance of the findings and enabling a more comprehensive understanding of different risk contexts.

Conclusions

In this study, differences in the concentrations of leptin, adiponectin, and insulin in HM were evaluated among women with different gestational clinical conditions and their adverse intrauterine environments. These hormones were measured within the first 48 hours and at 30 days of the infant's life, and their concentrations were associated with pre-pregnancy BMI and infant and preschool BMI-for-age Z-scores. No significant interaction was observed in hormone concentrations across the five groups or between the different collection times within groups. Changes (delta) in insulin and adiponectin concentrations were positively associated with pre-pregnancy BMI in the smoking group, whereas only punctual correlations were identified between hormone concentrations and BMI-for-age Z-scores.

Although leptin, adiponectin, and insulin present in HM are well known to be influenced by maternal characteristics such as BMI and to be related to infant body composition, findings across numerous studies remain inconsistent. Methodological differences and variations in the populations studied likely account for this heterogeneity. Nevertheless, the results of the present study reinforce the potential role of these hormones in metabolic programming and infant growth, highlighting the need for future investigations with larger sample sizes and longitudinal follow-up.

Abbreviations

BMI: Body mass index

BMI-for-age (BMI/A): Body mass index–for-age

CAAE: Certificate of Presentation for Ethical Consideration

DAG: Directed acyclic graph

DM: Diabetes mellitus

ELISA: Enzyme-linked immunosorbent assay

GEE: Generalized estimating equations

GDM: Gestational diabetes mellitus

GHC: Grupo Hospitalar Conceição

HCPA: Hospital de Clínicas de Porto Alegre

HM: Human milk

IUGR: Intrauterine growth restriction

IVAPSA: Impact of Variations in the Perinatal Environment on Newborn Health during the First Six Months of Life

LSD: Least Significant Difference

RPM: Revolutions per minute

ρ: Spearman correlation coefficient

SAH: Systemic arterial hypertension

SD: Standard deviation

SE: Standard error

SPSS: Statistical Package for the Social Sciences

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

The IVAPSA project was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (HCPA) (protocols no. 11/0097 and no. 17/0107) and by the Research Ethics Committee of the Grupo Hospitalar Conceição (GHC) (protocol no. 11/027). The project was registered on Plataforma Brasil, the Brazilian National Research Ethics Registry, under registration number CAAE 65190217500005327. All methods were performed in accordance with the latest guidelines and current regulations of the National Health Council of the Ministry of Health of Brazil (resolutions no. 466/2012 and no. 580/2018).

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GK participated in literature review, data analysis, and manuscript writing, and also performed the statistical analysis.

CHS contributed to the conception, study design, and coordination, and assisted in manuscript preparation.

DRB critically reviewed the manuscript.

NCV critically reviewed the manuscript.

MZG contributed to the conception, study design, and coordination, and assisted in manuscript preparation.

JRB participated in conception, data analysis, and manuscript writing.

All authors read and approved the final manuscript.

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Figures

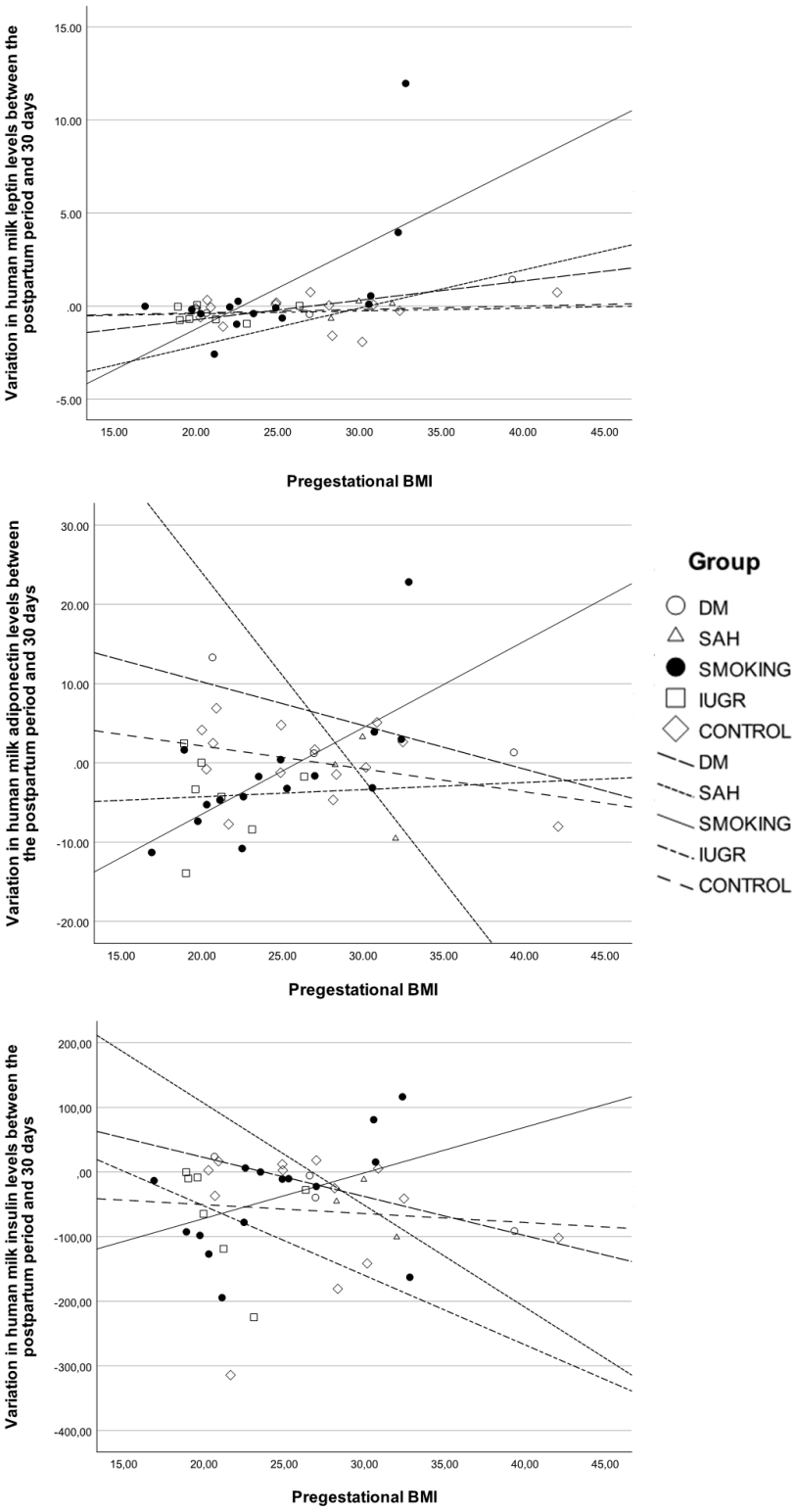


Figure 1

Pre-pregnancy BMI and hormone concentrations in HM between birth and 30 days of life.

DM: diabetes mellitus; SAH: systemic arterial hypertension; BMI: body mass index; HM: human milk; IUGR: intrauterine growth restriction.

Supplementary Files

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