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Mother and infant Body Mass Index, breast milk leptin and their serum leptin values

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Abstract: Purpose: This study investigates correlations between mother and infant Body Mass Index, their serum leptin values and breast milk leptin concentration in early infancy. Subjects and Methods. We determined serum leptin values in 58 healthy infants and leptin values in their mothers' breast milk, using RIA. Infant and maternal anthropometrics were measured. Results. Median leptin concentration was 3.9 ng/ml (IQR:2.75) in infant serum, 4.27 ng/ml (IQR:5.62) in maternal serum and 0.89 ng/ml (IQR:1.32) in breast milk. Median maternal BMI and weight were 24 kg/m^2 (IQR:4.41) and 64 kg (IQR:15). Median infant BMI was 15.80 kg/cm^2 (IQR:4.02), while average weight was 5.130 kg (IQR:1.627). Infants serum leptin values positively correlated with infants' BMI (p=0.001; r=0.213) and breast milk leptin (p=0.03; r=0.285). Maternal serum leptin values positively correlated with maternal BMI (p=0.000, r=0.449) and breast milk leptin ones (p=0.026; r=0.322). Conclusion. Breast milk leptin and maternal BMI could influence infant serum leptin values. Further studies are needed to better elucidate the role of genetics and environment on infant leptin production and risk of obesity later in life.

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Keywords: mothers; serum leptin; BMI; breast milk; infancy

1. Introduction

Leptin is a polypeptide hormone, made of 167 amino acids and discovered by Zhang et al. in 1994 thanks to studies on ob/ob gene in mice [1]. This hormone is the product of the ob gene, located on chromosome 7q31.3. It circulates in plasma free or bound to proteins and it exerts its action through the sOB receptor [2]. The primary function of this hormone is to inhibit food intake and to promote energy expenditure by regulating neuronal activity in hypothalamic arcuate nuclei: leptin, in fact, activates anorectic POMC/CART neurons and hinders the activity of those which stimulate food intake (NPY/AgRP) [3].

It has been shown that higher serum leptin values correlate with lower body mass index (BMI) in childhood and with lower predisposition to develop metabolic disorders in adolescence and adulthood [4].

Schuster et al. suggests milk leptin could provide a link between maternal body composition and infant growth and development and also plays a role in regulating infant appetite and food intake during early infancy [5].

It is known that leptin is mainly produced by white adipose tissue; this is the reason why serum leptin values directly correlate with body fat stores [6]. During fasting or weight loss, leptin levels decrease, while during overeating, they increase [7]. Leptin is also released by hypothalamus, pituitary gland, skeletal muscle, stomach, liver, placenta and mammary gland [8,9].

Leptin is found in breast milk and, interestingly, it is not only related with infants' body fat mass, but also with their mothers' one [10]. It is produced by mammary epithelial cells and it is associated with fat globules. Studies conducted on mice have shown that this hormone is transferred from maternal blood to breast milk and that it is then transferred from milk to mice puppies' bloodstream. Interestingly, the presence of leptin receptors has been referred on gastric and intestinal epithelial cells of both humans and rats, suggesting that leptin may play a role in the regulation of GI functions [11]. It could be assumed that leptin taken by children with breast milk can directly pass into their bloodstream through gut since leptin receptor isoform has been found in brush border, basolateral membrane, and cytoplasm of enterocytes [12].

The aim of this study is to investigate correlations between mother and infant BMI, their serum leptin values and breast milk leptin concentration in early infancy.

2. Materials and Methods

2.1 Subjects

2.1.1 Infants

We enrolled 58 AGA healthy term infants who were admitted to the Department of Paediatrics of the University of Turin, Regina Margherita Children's Hospital, between June 2013 and July 2015. The infants underwent blood tests during routine outpatient examinations. The study protocol was approved by the local Ethical Committee at Ospedale Mauriziano - Ospedale Infantile Regina Margherita - S. Anna Torino, and infants' parents gave their written consent.

Criteria for enrollment were as follows:

Age: children from 10 days of life to 6 months and 15 days of life;

Gestational age: from 38 to 40 weeks;

Birth characteristics: we enrolled infants with birth weight from 2500g to 4500g, APGAR equal or above 7 and who had not suffered from neonatal diseases;

Nutrition: infants were fed with breast milk and they had not been weaned yet;

Clinical condition: at the time of blood sampling, infants did not have acute diseases and were afebrile.

At the time of sampling, infants were exclusively breastfed and they had not received any complementary feeding.

2.1.2 Mothers

58 caucasian mothers , belonging to a rural or urban setting, were enrolled with their children. Regarding delivery, 19 mothers underwent a Caesarian section, while 39 had a spontaneous delivery. Criteria for enrollment were as follows:

Mothers who delivered infants at 38 to 40 weeks' gestation;

Mothers who were planning to exclusively breastfeed;

Mothers who signed written informed consent. Eligibility criteria for mothers were no maternal medical complications, non-smoking mothers, normal response to a glucose tolerance test, no mastitis, no prescribed medication, no digestive disorders.

2.2 BMI measurement

Anthropometric measures were collected by two trained medical doctors with high intra-observer and inter-observer reliability.

Infants were weighed with an electronic integrating scale (SECA, model 757, Vogel & Halke, Hamburg, Germany), were measured in length with a stat meter and BMI was calculated as the ratio of body weight (kg) to the square of length (m2). Mothers were weighed with a scale (Wunder, Italy), measured in height with a stat meter (Holtain Limited, Crymych, Dyfed, UK) and BMI was calculated as above.

2.3 Blood sampling and hormone analysis

For the evaluation of leptin in serum, infants underwent four hours fasting before blood testing usually at 8.00 in the morning. The sample was stored in a refrigerator for 60 minutes and was then put in the refrigerated centrifuge at 4 °C at 4000 revolutions / minute for 10 minutes . The serum obtained was divided into 2 test tubes and was stored in a freezer at -30 ° C . The same procedure was carried out for mothers.

Hormone analysis was conducted with a commercially available radioimmunoassay (RIA) kit (LEP R-40, Multispecie-Leptin-RIA-Sensitive, Mediagnost, Reutlingen, Germany) with a sensitivity of 0.04 ng/ml (0.01 ng/ml with the procedure for increased sensitivity). The intra-assay variation was less than 5%, and the inter-assay variation did not exceed 7.6%.

2.4 Breast milk sampling and hormone analysis

About 5 ml of foremilk samples were collected from the lactating women by hand expression between 7 a.m and 9 a.m.. All milk samples were collected in tubes containing protease inhibitors (Sigma) and immediately frozen at -20°C. Samples were thawed at 4-6°C overnight and centrifuged at 2500 rev. at 4°for 20 min to separate the fat milk. Like serum leptin, 2 ml of skimmed breast milk leptin was analyzed with a RIA kit (LEP R-40, Multispecie-Leptin-RIA-Sensitive, Mediagnost, Reutlingen, Germany) with a sensitivity of 0.04 ng/ml (0.01 ng/ml with the procedure for increased sensitivity).

2.5 Statistical analysis

Statistical analyses were conducted using SPSS software (version 21.0, SPSS, Inc., Chicago, IL). First, we performed univariate descriptive analysis . The normal distribution of the variables was tested by the Shapiro-Wilk test. Continuous variables were expressed as median and interquartile range (IQR). Data that were not normally distributed were analysed with the Mann–Whitney U test and the Kruskal-Wallis test. Correlations are expressed by the Spearman correlation coefficient. All tests were done with two tails, with a fixed significance alpha = 5 % .

3. Results

Median leptin concentration was 3.9 ng/ml (IQR: 2.75) in infant serum, 4.27 (IQR: 5.62) ng/ml in maternal serum and 0.89 (IQR: 1.32) ng/ml in breast milk. Statistical significance was set at $p < 0.05$ and correlations were assessed using Spearman's rho.

We evaluated the impact of potential confounders on breast milk leptin values and maternal and infant serum leptin values. Particularly, we analyzed the effect of infant age and gender on leptin concentrations.

Regarding infant age, we divided our cohort into three age groups at enrollment. We obtained a median (IQR) leptin concentration of 2.87 (2.53) ng/ml in infant serum, 3.27 (5.38) ng/ml in maternal serum and 0.83 (1.17) ng/ml in breast milk in group 1 (< 2 months; n=30), of 4.54 (9.89) ng/ml in infant serum, 2.46 (1.49) ng/ml in maternal serum and 1.18 (1.29) ng/ml in breast milk in group 2 (< 4 months; n=18) and of 4.85 (7.51) ng/ml in infant serum, 3.21(2.25) ng/ml in maternal serum and 0.87 (3.55) ng/ml in breast milk in group 3 (4-6 months; n=10). No significant differences in breast milk and infant and serum leptin values were detected among the three groups ($p>0.05$).

We divided patients by gender into two groups: as concernes males (n=26), the median (IQR) leptin concentration was 2.83 (2.16) ng/ml in infant serum, 3.27 (5.13) ng/ml in maternal serum and 0.83 (1.32) ng/ml in breast milk; in females (n=32), the median (IQR) leptin concentration was 4.79 (8.46) ng/ml in infant serum, 2.84 (2.14) ng/ml in maternal serum and 0.93 (2.59) ng/ml in breast milk. With reference to gender, we did not observe any statistical differences in breast milk leptin values and maternal and infant serum leptin values (p>0.05).

Table 1. Infant anthropometric parameters and serum leptin values (median + IQR).

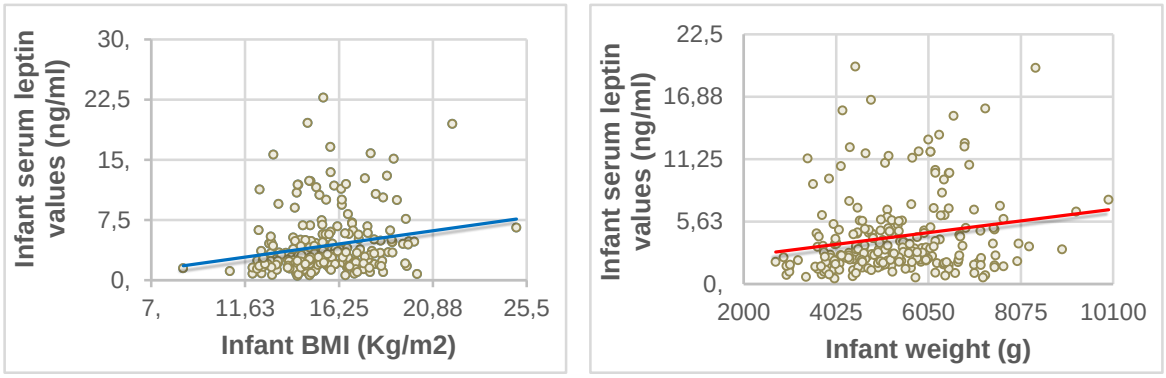
Parameters	Infants
	n = 58
Age (days)	61 (76.5)
Gestational Age (weeks)	39 (1.5)
Birth Weight (kg)	3.275 (0.622)
Birth Length (cm)	49.45 (2.2)
Birth Cranial Circumference (cm)	34.05 (1.5)
Weight (kg)	5.130 (1.269)
Height (cm)	55 (3.25)
Cranial Circumference (cm)	39 (3)
BMI (kg/m^2)	15.80 (2.47)
Serum Leptin (ng/ml)	3.9 (2.75)

Table 2. Maternal anthropometric parameters, serum leptin and BM leptin values (median + IQR).

Parameters	Mothers
	n = 58
Age (years)	28.5 (8)
Weight (kg)	64 (12.59)
Height (cm)	164 (0.064)
BMI (kg/m^2)	24 (4.52)
Serum Leptin (ng/ml)	4.27 (5.62)
Breast Milk Leptin (ng/ml)	0.89 (1.32)

3.1. Infant serum leptin values and infant BMI

Serum leptin values positively correlated with infants' weight (p=0.002; r=0.2) and BMI (p=0.001; r=0.213), as shown in Figure 1.



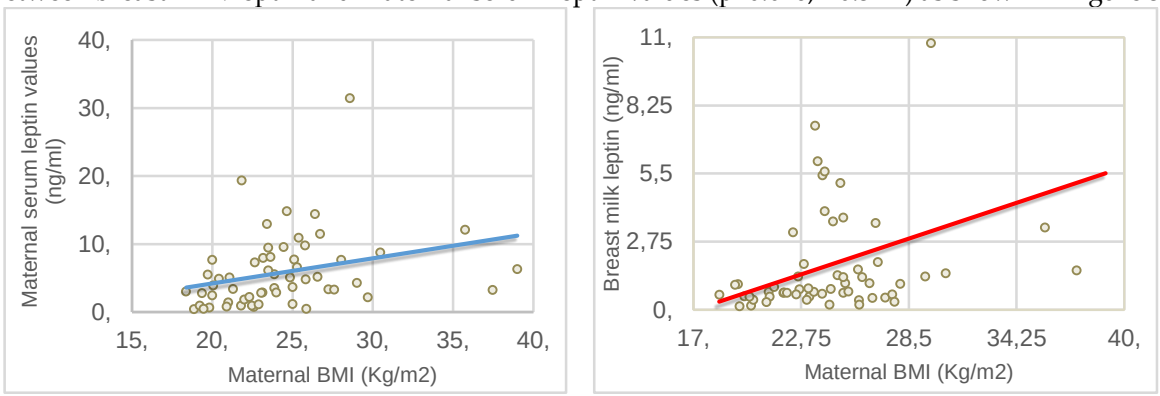
(**a**) (**b**)

Figure 1. Correlation between infant serum leptin values and infant BMI and weight. (**a**) Association between serum leptin values and BMI. (**b**) Association between serum leptin values and weight.

The positive correlation between infant serum leptin values and both infant BMI and weight suggests that leptin is directly related to body fat stores. This hormone is primarily released by adipocytes in adipose white tissue [6]. This is the reason why infants with higher BMI have higher serum leptin values [13].

3.2. Maternal serum leptin values, maternal BMI and breast milk leptin

Maternal BMI positively correlated with maternal serum leptin levels (p=0.000;r=0.449) and breast milk leptin (p=0.004;r=0.368) as illustrated in Figure 2. We found a significant correlation between breast milk leptin and maternal serum leptin values (p=0.026;r=0.322) as shown in Figure 3.



(**a**) (**b**)

Figure 2. Correlation between maternal BMI and maternal serum leptin and breast milk leptin. (**a**) Association between maternal BMI and maternal serum leptin values. (**b**) Association between maternal BMI and breast milk leptin.

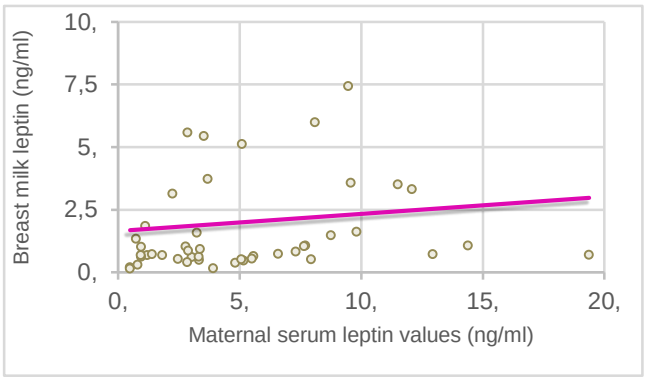


Figure 3. Correlation between maternal serum leptin and breast milk leptin.

We found a positive and significant correlation between BMI and serum leptin values. As shown for infants, mothers with higher BMI have higher serum leptin values, suggesting that leptin concentration is directly proportional to body fat mass percentage [13].

Regarding breast milk, it is interesting that a positive correlation exists between maternal BMI and leptin levels in breast milk [14]. It could be that not only breast milk leptin depends on the amount produced by mammary epithelial cells, but also on the amount released from maternal body fat stores.

A significant correlation was observed between maternal serum leptin values and breast milk leptin [15]. Also Weyermann et al. (2006) observed that leptin concentration in breast milk correlated positively with leptin in maternal serum [16].

3.3. Infant serum leptin values, maternal serum leptin values and breast milk leptin.

We did not find any significant correlation between maternal and infant serum leptin values (p>0,05), suggesting thus that further studies are required to investigate the possible role of maternal leptin in the regulation of infant metabolism [17]. Regarding breast milk leptin and infant serum leptin values, we obtained a positive correlation, as illustrated in Figure 4 (p=0.03;r=0.285).

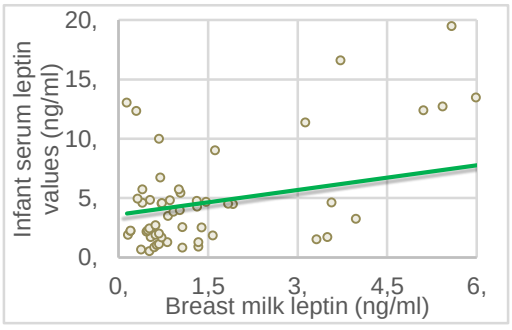


Figure 4. Correlation between breast milk leptin and infant serum leptin values.

The higher breast milk leptin concentration is, the higher infant serum leptin values are. These findings suggest a possible association between breast milk components and infant adiposity [18].

4. Discussion

This study presents data of a positive correlation between breast milk leptin and infant serum leptin values. Furthermore, our study is strengthened by the fact that we found that breast milk leptin

directly correlates with maternal serum leptin values. Actually, we did not obtain a significant association between maternal and infant serum leptin values. Regarding maternal and infant BMI, we showed that breast milk leptin and maternal serum leptin values directly correlate with materal BMI. In addition, we demonstrated a positive association between infant BMI and infant serum leptin values. We evaluated the possible impact of infant age and gender on infant and maternal serum leptin values and breast milk leptin concentrations. We did not obtain any significant differences in leptin values among the created groups.

4.1. Serum leptin values and BMI

Leptin is mainly produced by adipocytes; thus its levels are strictly associated to body fat mass percentage. During fasting, this hormone decreases; on the other hand, in overeating, its levels increase [19]. Both in infants and their mothers, we found that this hormone correlates with BMI and weight. Higher BMI correlates with higher serum leptin levels. It is known that people with an elevated BMI have high serum leptin levels not only because they have a larger amount of fat mass, but also because their adipocytes are bigger. What is more, Dusserre et al. showed that leptin values vary according to the type of adipose tissue that releases them: omental adipocytes express leptin mRNA less than subcutaneous adipocytes. [20]

4.2. Breast milk leptin and maternal serum leptin values

Casabiell et all. showed that leptin is transferred from maternal bloodstream to breast milk in mice [10]. We found a positive correlation between breast milk leptin values and maternal serum leptin ones. It is thus possible that leptin in breast milk depends not only on the amount produced by mammary epithelial cells, but also on the amount in maternal bloodstream. It would be interesting to evaluate if maternal leptin values represent a predictor for infant obesity [21].

4.3. Breast milk leptin and infant serum leptin values

Leptin receptors have been found on gastrointestinal epithelial cells, suggesting that this hormone could be absorbed from infant mucosa and then transferred to infant bloodstream. The significant correlation that we found between breast milk leptin and infant serum leptin values could indicate that leptin in children is influenced by both infant fat stores and breast milk leptin. In previous studies, we demonstrated that formula fed infants have lower leptin levels than breast milk fed ones [22]. Data on the presence of leptin in infant formula are still controversial [23], however more investigations are needed to detect if hormones present in breast milk might have a beneficial effect on obesity later in life [24,25].

4.4 Study limitations

This study has several limitations. We could not assess the influence of leptin circadian variations since we do not have daily access to serum leptin samples, nor were we able to assess daily changes in breast milk leptin.

Moreover, we did not measure serum leptin at the same age time in all subjects enrolled.

However, baseline characteristics were similar among the infants in the study group.

Further, we were unable to measure fat mass at the same time of leptin sampling in mothers and infants.

Finally, since this study is observational it is important to interpret our correlations with caution.

Our findings are consistent with the possibility that breast milk leptin could have an influence on infant health later in life and open new implications for research such as the role of breastfeeding and infant metabolic response.

Therefore, a follow-up of our patients, based on these results, will help us build a stronger overall evidence base and fill the gap in knowledge.

It is known that early nutrition may play an important role in the development of metabolic diseases in adolescence and adulthood. It has been shown that breast-fed infants are at lower risk to become obese than formula-fed ones [26]. The positive correlation observed between maternal serum leptin concentration and maternal BMI is strictly linked to breast milk leptin values, suggesting that the amount of leptin in breast milk is influenced not only by mammary gland, but also by leptin released from maternal fat storages [27]. Interestingly, in a previous study, we demonstrated that infant serum leptin values are correlated to maternal BMI, thus showing that infants breast-fed by mothers with high BMI receive higher amounts of leptin [28]. Children with obese mothers seem to be at higher risk to become obese themselves [29]. The protective effect of breastfeeding against early childhood obesity may differ with race and ethnicity [30].

Many factors related to breastfeeding may influence childhood weight outcomes and obesity such as breastfeeding duration [31]; however, it should be considered that, ingesting high amounts of leptin, infants with obese mothers become leptin resistant and have alterations in appetite regulation [32-34]. In animal models it has been shown that obese phenotype can be transmitted by mothers to the following generations [35]. Since recently it has been observed that higher perinatal leptin is associated with lower adiposity at 3 years of life [36], leptin could be a key to understand the relationship between maternal BMI and infant growth and development.

Interesting data showed that breast feeding could affect infants' self-regulation of milk intake during late infancy [37].

5. Conclusion

The existing data of the effects of breast milk leptin on infant growth and adiposity are controversial.

In this study we investigated the possible correlations between maternal and infant serum leptin values, breast milk leptin concentrations and infant and maternal BMI. We demonstrated a positive correlation between infants serum leptin concentrations and both maternal and infant BMI.

Regarding breast milk leptin values, we obtained a positive association with maternal and infant serum leptin values and maternal BMI.

There was no association between infant and maternal serum leptin concentrations.

Overall, our findings show that breast feeding and maternal BMI could influence infant serum leptin values. Further studies are needed to better elucidate the role of genetics on infant leptin production and risk of obesity later in life.

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Author Contributions: FS conceived the study and designed the research, and wrote the manuscript. AS performed the experiments and wrote the manuscript. LR performed the experiments; analyzed the data. AS searched references and revised the manuscript. LS conceived the study and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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