

Potential sex differences in human milk leptin and their association with asthma and wheeze phenotypes: Results of the Ulm Birth Cohorts

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Running title: Leptin in human milk, asthma and wheeze phenotypes

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Word count: 4188

Number of Tables and Figures: 5

Supplementary Materials: Table S1: Characteristics of all mothers (n=1066) and children (n=1090) included in the Ulm Birth Cohort Study as well as those who had a complete set of human milk leptin, wheeze phenotype or asthma data available. Table S2: Characteristics of all mothers and children included in the Ulm Birth Cohort Study as well as those who had a complete set of human milk leptin, wheeze phenotype or asthma data available. Table S3: Number of children with data on wheeze phenotypes and doctor-diagnosed asthma at age 17 years in the Ulm Birth Cohort Study (UBCS). Table S4: Associations of human milk leptin measured at 6 weeks of lactation with wheeze

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/PAI.13586](#)

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phenotypes (6 and 17 years) and doctor-diagnosed asthma (11 and 17 years) in the Ulm Birth Cohort Study (UBCS). **Table S5:** Associations of human milk leptin measured at 6 weeks of lactation with wheeze phenotypes and doctor-diagnosed asthma in the Ulm SPATZ Health Study. **Table S6:** Sex-specific associations of human milk leptin measured at 6 weeks of lactation with wheeze phenotypes and doctor-diagnosed asthma at 17 years in the Ulm Birth Cohort Study (UBCS). **Table S7:** Associations of human milk leptin measured at 6 weeks of lactation with doctor-diagnosed asthma in the Ulm SPATZ Health Study adjusted for pre-pregnancy body mass index (BMI). **Table S8:** Associations of human milk leptin measured at 6 weeks of lactation with doctor-diagnosed asthma in the Ulm SPATZ Health Study adjusted for maternal body mass index (BMI) concurrent to time of milk sampling

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Article type : Original Article

i. **Conflict of Interest:** J.G. is the project manager of unrestricted grants from Danone Nutricia Research to Ulm University and Leipzig University for research on other aspects of human milk composition in the Ulm birth cohort studies.

ii. **Financial support:** The Ulm Birth Cohort Study was supported by grants of the German Research Council (BR 1704/3-1, BR 1704/3-2, BR 1704/3-3). The Ulm SPATZ Health Study was funded through an unrestricted grant by the Medical Faculty of Ulm University.

iii. **Abstract**

Background: The hormone leptin has been suggested to play a role in the respiratory and immune systems. Evidence on sex-specific concentrations of leptin in human milk and sex-specific associations with the development of asthma and wheeze has been put forward but is still scarce.

Objective: To investigate whether male and female infants receive different levels of leptin through human milk and whether leptin is implicated in the development of asthma and wheeze in a sex-dependent manner using data from the two Ulm birth cohort studies.

Methods: Leptin data were available from human milk samples collected at 6 weeks [Ulm Birth Cohort Study (UBCS, n=678; Ulm SPATZ Health Study, n=587)], and, in SPATZ only, at 6 months (n=377) and 12 months (n=66) of lactation. Sex-specific associations with doctor-diagnosed asthma and wheeze phenotypes were assessed in crude and adjusted models using logistic regression. Adjustments were made for maternal allergy, exclusive breastfeeding, infant age at the time of milk sampling and child BMI z-score.

Results: At 6 weeks, leptin levels (median [min, max], in ng/l) were higher in the milk for girls (197 [0.100, 4120]) than in milk for boys (159 [1.02, 3280], $p=0.045$) in UBCS. No significant sex differences were observed in SPATZ ($p=0.152$). There were no significant associations of leptin with asthma or wheeze in both studies, even in a sex-dependent manner ($p>0.05$).

Conclusion: It remains unclear whether male and female infants receive different levels of leptin through human milk. However, leptin in human milk may not be associated with history and development of asthma and wheeze in a sex-specific manner.

Key message

Male and female infants could benefit from sex-specific nutritional strategies and further investigations are needed to determine whether leptin in human milk, amongst other hormones, could contribute to the development of asthma, wheeze and other immune health outcomes in a sex-specific manner.

Keywords: human milk, leptin, sex-specific associations, asthma, wheeze

iv. Main text

Introduction

Human milk is considered the ideal source of nutrition during early infancy, a critical period for growth and development, which potentially influences health throughout life^{1,2}. Human milk contains bioactive compounds and hormones, which contribute individually and in combination with other human milk components to various growth and health outcomes^{3,4}. Interest has spiked in human milk leptin, a hormone widely supposed to play a role in metabolic programming during early infancy⁵⁻⁷. Leptin in human milk has been studied extensively in relation to short term regulation of food intake, infant growth and adiposity⁷⁻⁹ but also maternal adiposity¹⁰⁻¹². However, emerging evidence suggests that circulating leptin also plays a potential role in the respiratory and immune systems^{13,14}.

Evidence suggests that leptin is either transferred from maternal circulation into human milk or synthesised by mammary gland epithelial cells and subsequently secreted into human milk¹⁵⁻¹⁷. Therefore, it is plausible that leptin in human milk not only reflects the amount in the maternal bloodstream but also the amount produced in the epithelial cells. On one hand, there is some evidence that the composition of human milk could be influenced by the infant's sex¹⁸. On the other hand, the evidence for sex-specific secretions and concentrations of leptin in human milk is conflicting, with some studies reporting dependence on child sex^{19,20} and others not^{10,15,16,21}.

Regarding health outcomes, serum leptin has been associated with the development of asthma²². Potentially underlying mechanisms include the induction of oxidative stress and inflammation in the airways^{13,23}. Furthermore, sex differences in asthma prevalence, incidence and severity are widely documented^{24,25}. These sex differences are suggested to be a result of narrower airway calibre in males than females in early life due to effects of different hormones including leptin²⁶. Yet, studies investigating sex dependence of human milk leptin and the potential sex-specific associations with long-term health outcomes, such as asthma and wheeze, in early and late childhood are scarce.

We are aware of only one recent study²⁷ that has investigated sex-specific associations of human milk leptin (amongst other hormones) with probable asthma at the age of 3 years. Although the sample size of infants included in the study was relatively large (n=428), human milk was only sampled once at 3-4 months postpartum from each participant. Also, this study focused on asthma assessed at 3 years of age, a period during which asthma is only

unreliably diagnosed²⁸. Research suggests a potential need for sex-specific nutritional strategies for male and female infants¹⁸. Therefore, to contribute to this area of research, we investigated whether the hormone leptin in human milk is related to the infant's sex and whether these sex differences are associated with doctor-diagnosed asthma or wheeze phenotypes at ages 6, 11 and 17 years using data from the two Ulm birth cohort studies.

Methods

Data were obtained from the Ulm Birth Cohort Study (UBCS) and the Ulm SPATZ Health Study (SPATZ). Both UBCS and SPATZ used similar methodology and included mothers and their live newborns from the general population at the University Medical Center in Ulm, southern Germany (the only maternity clinic in Ulm, a city of approximately 100,000 inhabitants), in 2000-2001 (UBCS) and 2012-2013 (SPATZ)²⁹. In UBCS, exclusion criteria were outpatient delivery, maternal age < 18 years, insufficient knowledge of the German, Russian or Turkish language, gestational age < 32 weeks, birth weight < 2500g and transfer of the new-born to intensive care immediately after delivery. At baseline, the cohort included 1090 new-borns of 1066 mothers (67% of 1593 eligible families). Exclusion criteria in SPATZ were outpatient delivery, maternal age < 18 years, insufficient knowledge of the German language, stillbirth and transfer of the new-born to intensive care immediately after delivery. At baseline, 1006 new-borns of 970 mothers (49% of all 1999 eligible families) were included in SPATZ. For the purpose of this analysis, the study populations were restricted to singletons.

Ethical approval for both cohort studies was obtained from the ethics board of Ulm University (UBCS: No. 98/2000; SPATZ: No. 311/11) and for UBCS additionally from the Physicians' boards of the states of Baden-Wuerttemberg and Bavaria. Participation in both studies was entirely voluntary and written informed consent was obtained from all mothers participating in the studies.

Data collection and measurements

Demographic information, information on feeding practices, maternal and birth-related data (child sex, age, education, pre-pregnancy and maternal body mass index (BMI; calculated as [weight (kg) / height (m)²])) and maternal and infant history of allergic disease were collected using self-administered questionnaires, hospital records and records from routine screening examinations during pregnancy. At each follow-up, mothers were asked whether the child was still receiving human milk at the time of sample collection and whether they had

introduced additional foods or liquids. Exclusive breastfeeding was considered when the mother reported no additional liquid, semi-solid or solid food was given up to that moment. This was then cross-validated and derived based on maternal recall at each time point when mothers were asked about the age at which additional foods or liquids were introduced. Data on breastfeeding method, time of collection, and time from last feeding were only available in SPATZ. Maternal allergy was self-reported and classified as mothers who reported a history of physician-diagnosed hay fever, neurodermatitis or asthma. Non-allergic mothers were classified as mothers who reported the absence of an allergic disease. Infant body weight and length were obtained from the routine examination documentation during scheduled pediatric appointments at 3 to 10 days, 4 to 5 weeks, 3 to 4 months, 6 to 7 months, 10 to 12 months, and 21 to 24 months of age. For the purpose of this study, assessments recorded at 4 to 5 weeks and 6 to 7 months of age were used in correspondence to the 6 weeks (UBCS and SPATZ) and 6 months (SPATZ only) human milk leptin assessments. Further follow-ups in UBCS were conducted with self-administered questionnaires at ages 1, 2, 3, 4, 6, 8, 11, 13, 15 and 17 years. Yearly follow-ups are currently ongoing in SPATZ with data available up to the age of 6 years.

Wheeze and asthma definitions

In UBCS, parents were asked to report wheeze and/or use of asthma-related medication in the previous 12 months³⁰. Wheezing phenotypes were classified into four categories³¹ as follows: *Transient* wheezers were children who had wheeze before 3 years of age and none later in life. *Persistent* wheezers were those who started to wheeze before 3 years of age and continued to wheeze later in life. *Late-onset* wheezers were children with wheeze symptoms starting after 3 years of age. The reference group consisted of *never* wheezers who never reported wheeze in any questionnaire. Doctor-diagnosed asthma cases were defined as positive report of asthma-related medication or a response of “bronchial asthma” to the question “Has a doctor diagnosed your child with one of the following diseases since their previous birthday?” in the 6-, 8-, 11-, 13-, 15- or 17- year follow-up. We used the UBCS data to investigate wheeze phenotypes using the data up to age 6 years comparable to SPATZ, see below. In addition, we explored the long-term outcomes of doctor diagnosed asthma using information available up to age 11 years and age 17 years as well as wheeze phenotypes up to age 17 years.

In SPATZ, a parental report of child wheeze in the past 12 months was assessed consecutively from age 1 year at each yearly follow-up until the age of 6 years by self-administered questionnaires. Wheezing phenotypes were determined as described above for UBCS. Diagnosed asthma was assessed by separate parent and paediatrician reports of doctor-diagnosed asthma on separate self-administered questionnaires also at each yearly follow-up from age 1 to age 6 years. Children with at least one positive report of doctor-diagnosed asthma (from the parents or the paediatricians) at ages 5 or 6 were considered as plausible asthma cases.

Human milk sample collection and analysis

Human milk samples were collected from consenting mothers who were actively breastfeeding at 6 weeks (both UBCS and SPATZ), 6 months and 12 months (SPATZ only). To minimize potential variations resulting from times of expression and feed, human milk (fore-milk) samples were collected using the same strict protocol in both cohort studies. Mothers were instructed to wash their hands and clean the breast prior to milk sampling and manually express or pump human milk into collection tubes provided by the study. Mothers were to express or pump 20 mL of human milk (stored in two 10 mL collection tubes) between 9am and 12 noon, after breakfast and before lunch, but at least one hour after the infant's last human milk feed. In some instances, although rare, and where necessary, trained study nurses helped mothers with expression or pumping human milk into the collection tubes. Mothers were further instructed to keep the samples refrigerated, until collection by trained study nurses who transported them directly to a central laboratory. Samples were collected on the same day if milk was expressed between 9 am and 12 pm (76.9%, SPATZ), or on the next day if milk was expressed in the evening (after 12pm, 9%; and before 9am, 13.8%, all in SPATZ). The samples were then divided into aliquots, frozen (-80 °C) within 48 hours of collection, and stored until analysis. Data on time of milk sample collection were only available in SPATZ.

Leptin in human milk was measured in skimmed milk in both studies, as previously described^{8,10}. Briefly, absolute leptin concentrations [ng/l] were measured in 6 weeks samples from UBCS and in 6 weeks, 6 months and 12 months samples from SPATZ using commercially available ELISA (R & D Systems). All measurements were done in the same lab, by the same technician and after approximately the same storage duration of the frozen human milk samples (3.5–4.5 years for UBCS, 3–4 years for SPATZ). In UBCS, absolute leptin measurements [ng/l] were available for 754 human milk samples at 6 weeks. In

SPATZ, absolute leptin measurements [ng/l] were available for 666, 442 and 69 human milk samples at around 6 weeks, 6 months and 12 months of lactation, respectively. Human milk samples with a complete set of standardized leptin values (complete data set) and child outcomes were available for 678 at 6 weeks in UBCS and 587, 377 and 66 lactating women at 6 weeks, 6 months and 12 months in SPATZ, respectively. This complete data set was used for all analyses unless otherwise stated.

Statistical analysis

The Wilcoxon rank sum test for two unpaired samples was used to evaluate differences ($p < 0.05$) in demographics and lifestyle as well as sex-differences of leptin concentrations at 6 weeks (UBCS and SPATZ), 6 months and 12 months (SPATZ only). Leptin values were log-transformed prior to analysis. A nonlinear association between leptin in human milk and maternal BMI concurrent to time of human milk sampling has been reported previously¹⁰. To account for this association, as well as associations with breastfeeding frequency per day and human milk fat content, leptin z-scores were calculated using spline regression as previously described^{8,10}. Maternal BMI concurrent to the time of human milk sampling was not available in UBCS, thus pre-pregnancy BMI was used. Leptin values (standardized leptin) used for subsequent analyses were standardized based on pre-pregnancy BMI for UBCS, maternal BMI concurrent to milk sampling (i.e. 6 weeks or 6 months) for SPATZ, and breastfeeding frequency and human milk fat concentration. Child BMI measurements were also converted to z-scores based on the respective study population (UBCS or SPATZ) using separate linear models for each measurement period and adjusted for the precise age at assessment (days) and child's sex⁸. The derivation of child BMI z-scores was based on the relative study population rather than from standardized growth charts in order to ensure that the associations reported were reflective of the source population³². Logistic regression was used to assess associations between standardized leptin and wheeze phenotypes as well as doctor-diagnosed asthma. The associations were assessed as follows: (1) crude unadjusted association, (2) adjusted model accounting for maternal allergy, exclusive breastfeeding and infant age at the time of milk sampling and (3) additional adjustment for child BMI z-score. In the last model, age of the child was excluded because child BMI was already adjusted for age. These covariates were selected to be included in the models comparable to the one previous study evaluating these associations²⁷. Due to the reduced number of samples available at 12 months ($n = 66$) of lactation in SPATZ, only 6 weeks and 6 months samples

were included for sex-stratified analysis. All statistical analyses were performed using R (version 3.5.1; R Foundation for Statistical Computing).

Results

The complete data set used for the current analysis comprised human milk samples from 678 (63.6 %) women at 6 weeks participating in UBCS, 587 (60.5%) and 377 (38.9%) lactating women at 6 weeks and 6 months participating in SPATZ (**Table 1**). The prevalence of wheeze phenotypes and doctor-diagnosed asthma was higher in boys than in girls, in both cohort studies. Lactating women in SPATZ (the more recent cohort) were older, more highly educated and had a higher pre-pregnancy BMI compared to lactating women in UBCS (**Supplementary Table S1-S2**). In both UBCS and SPATZ, the majority of infants received human milk exclusively. Additional data on wheeze phenotypes and doctor-diagnosed asthma up to 17 years were also available from UBCS (**Supplementary Table S3**).

In UBCS, crude leptin (absolute concentrations) measurements were available from 754 human milk samples at 6 weeks (381 boys, 373 girls). In SPATZ, crude leptin measurements were available from 666 (350 boys, 316 girls), 442 (236 boys, 206 girls) and 69 (39 boys, 30 girls) lactating women at 6 weeks, 6 months and 12 months of lactation, respectively. Comparing these crude leptin values, leptin was higher in the milk for girls than that for boys at 6 weeks in both cohorts (**Figure 1**). These sex differences observed at 6 weeks were also significant when the sample size was restricted to exclusively breastfed infants in UBCS ($p=0.024$) and SPATZ ($p=0.018$). In SPATZ, the differences at 6 months were close to statistical significance ($p=0.051$) while there was no statistically significant difference at 12 months ($p=0.875$).

The difference in leptin levels with regard to the infant's sex at 6 weeks were also statistically significant ($p=0.045$) in UBCS when comparing leptin concentrations standardized for pre-pregnancy BMI, breastfeeding frequency per day, and breast milk fat concentration (**Table 2**). This standardization led to attenuation of the aforementioned crude differences in SPATZ which were no longer statistically significant. Likewise, when the sample size was restricted to exclusively breastfed infants, standardized leptin values were significantly higher in the milk for girls than that for boys in UBCS ($p=0.0375$) but not in SPATZ ($p=0.152$). A multi-level interaction test using log-transformed leptin values did not show any significant potential infant sex differences in the changes in leptin concentrations over lactation at 6 months ($p=0.720$) and at 12 months ($p=0.533$) in SPATZ.

Standardized leptin values were used in models evaluating associations with doctor-diagnosed asthma or wheeze phenotypes. Overall and irrespective of infant sex, higher leptin was associated with lower odds of persistent wheeze [OR (95% CI), 0.66 (0.47, 0.91), $p=0.014$] at 6 years and higher odds of developing asthma [1.40 (1.00, 1.99), $p=0.056$] up to 11 years of age, in UBCS (**Supplementary Table S4**). However, these associations were not statistically significant following adjustments for maternal history of allergy, exclusive breastfeeding, maternal age, and standardised child BMI. None of these associations were statistically significant in SPATZ (**Supplementary Table S5**). There were no significant sex-specific associations observed between standardized leptin and child asthma or wheeze phenotypes in UBCS (**Table 3**) or SPATZ (**Table 4**). Further analysis including wheeze phenotypes and doctor-diagnosed asthma up to age 17 years, also did not show sex-specific associations (**Supplementary Table S6**). These associations were also not statistically significant when the sample size was restricted to exclusively breastfed infants (not shown).

Discussion

In this study, we investigated sex differences of absolute and standardized leptin concentrations in human milk and evaluated potential sex-specific associations with asthma and wheeze phenotypes up to the age of 17 years using data from two methodologically similar birth cohort studies. We show significant sex differences in absolute human milk leptin concentrations at 6 weeks, in both UBCS and SPATZ. However, when using leptin concentrations standardized for important determinants of these concentrations¹⁰, these sex differences at 6 weeks were statistically significant in UBCS only and not replicated in SPATZ. In addition, UBCS infants receiving human milk with higher leptin, were less likely to develop wheeze until the age of 6 years. In contrast, they were more likely to develop asthma until the age of 11 years. Of note, these associations were not statistically significant following adjustments for maternal history of allergy, exclusive breastfeeding, and child BMI.

The prevalence of doctor-diagnosed asthma at 11 and 6 years in UBCS and SPATZ, respectively, as well as of wheeze phenotypes was higher in boys than in girls like in many previous studies. A trend towards higher prevalence in boys, although not statistically significant, was also seen in UBCS at 17 years of age. This is in contrast to previous reports from other large cohorts^{24,25,33} which show changing sex-specific prevalence of childhood asthma with age, from male to female predominance during adolescence. However, UBCS

follow-up may have been too short to observe the complete change in sex-specific prevalence, which has been reported to occur by age 18^{24,34,35} or 20 years³³. Moreover, UBCS sample size was limited with 19 girls and 33 boys with asthma at age 17 years.

In addition, the absolute human milk leptin concentrations measured at 6 weeks of lactation tended to be higher in the milk for girls than that for boys in both cohort studies. Some evidence¹⁸ suggests that human milk for boys will differ in composition compared to human milk for girls and we show a sex difference in crude leptin concentrations which had been previously reported for the UBCS study²⁰. Similarly, a trend ($p=0.14$) towards higher leptin in the milk for girls has been previously reported although sex differences were not statistically significant²⁷. However, we have previously shown an important nonlinear association between maternal BMI and human milk leptin¹⁰. Following standardization of leptin concentrations for this major determinant, leptin concentrations remained differing by infant sex in UBCS while this became statistically non-significant in SPATZ. These results were the same for both cohort studies when the sex differences were assessed amongst exclusively breastfed infants. Of note, several other studies^{10,15,16,19,21} did not find statistically significant differences in leptin in the milk for boys and that for girls. Overall, and irrespective of sex, absolute leptin values were much higher in SPATZ than in UBCS, despite the equal sample collection, processing, freezing and analysis. However, these differences were not no longer visible following standardization⁸. Nonetheless, we cannot rule out the effect of differences in other exposures which include but are not limited to differences in the genetic makeup in other human milk components between the two studies. Still, there is no clearly defined recommendation for what constitutes an adequate supply of leptin through human milk to ensure the most benefit for infants. Furthermore, we did not find significant sex-specific associations between standardized leptin and asthma or wheeze phenotypes in early and late childhood extending into adolescence. No significant associations were observed when the analysis was restricted to the exclusively breastfed infants either. Despite these findings, it is important to note that at least one recent study²⁷ reported that girls receiving human milk higher in leptin and insulin were more likely to develop possible or probable asthma by the age of 3 years. Several differences to that study²⁷ should be considered when interpreting our results. Firstly, we previously argued that accounting for maternal pre-pregnancy BMI (as done in CHILDS) rather than maternal BMI concurrent to the time of human milk sampling (as done in SPATZ) may result in residual confounding¹⁰. Secondly, unlike simple adjustment for linear effects, we account for nonlinear effects of

maternal BMI concurrent to milk sampling which potentially more precisely account for maternal adiposity^{8,10}. Nonetheless, we did observe small differences between models when using log-transformed leptin values and simple adjustment for pre-pregnancy BMI (as in the CHILD study) or maternal BMI concurrent to time of human milk sampling (**Supplementary Tables S7-S8**). Still, these differences could also be driven by several other factors which were not assessed in the current study.

Thirdly, we adjusted for child BMI at milk sampling while the CHILD study adjusted for child BMI at the time at which asthma was assessed. We previously reported associations between leptin and child BMI at milk sampling, as well as some evidence that higher leptin was associated with child BMI during the first two years⁸. Finally, we employed more specific definitions of doctor-diagnosed asthma and wheeze. Therefore, children with unclear patterns of wheezing throughout childhood or a doctor's diagnosis of asthma reported up to age 4 years when asthma is difficult to diagnose²⁸ were excluded from the case definition. Moreover, the inclusion of doctor-diagnosed asthma assessment up to 11 and 17 years of age in UBCS allowed us to explore potential long-term associations of human milk leptin measured at 6 weeks of lactation with asthma and wheeze.

Leptin is suggested to have important physiological functions³⁶ and together with its receptors are expressed in the lung tissue. Their expression levels have been shown to be associated with the development and severity of asthma^{22,37,38}. Additionally, leptin is apparently expressed differently between boys and girls, a phenomenon that may neither be solely explained by sex hormones nor the sex-dependent distribution of body fat^{13,14,39}. Thus, physiologic sex-specific requirements of leptin may regulate human milk leptin concentrations. However, granted that other human milk components and maternal factors could influence human milk composition, human milk leptin should be investigated in combination with milk concentrations of leptin-binding protein^{6,39}. Although sex plays a role in the natural history of asthma development²⁴, there's limited, if any, evidence that leptin in human milk is secreted in concentrations specific for infant sex and it remains unclear how sex-specific responses to nutritional stimuli could contribute to asthma and wheeze development.

Furthermore, effect estimates in SPATZ were directionally opposite when using leptin measured at 6 weeks and 6 months of lactation. We previously showed significant and stronger correlations between proximal leptin measures across lactation¹⁰. In light of this,

leptin concentrations were lower at 6 months compared to 6 weeks samples. Thus, human milk samples collected at a single time point of lactation may not be generalizable to both infants who receive human milk for a shorter or longer duration. Likewise, while earlier exposure may be important in reducing the odds of the outcome, as the infant grows older, other risk factors and triggers may predominate, which could modify the associations observed. We also observed associations of lower odds of persistent wheeze with higher milk leptin, compared to higher odds of asthma with higher milk leptin in UBCS. However, these associations were not statistically significant in subsequent adjusted and sex-stratified models, thus, suggesting positive confounding in our study. Similarly, higher cord leptin was associated with lower risk of wheeze in girls⁴⁰, and previous studies have shown increased leptin in children with atopic^{41,42} and severe asthma⁴³. Therefore, these several direct and indirect effects of leptin highlight the need for more mechanistic studies to investigate the specific and distinct mechanisms by which leptin may be implicated in the incident of asthma or wheeze in children.

A limitation of our study is the recruitment of participants from the general population in Ulm, Southern Germany, subsequently leading to smaller numbers of wheeze and doctor-diagnosed asthma cases compared to what a high-risk cohort would report. While asthma is a prevalent disease and our cohorts are generally medium-sized and of large size when it comes to collected human milk samples, the lower sample size of children with doctor-diagnosed asthma and wheeze phenotypes could have limited the statistical power to detect previously reported²⁷ sex specific associations. As a result of this limitation, we also could not explore these associations in relation to human milk leptin concentrations measured at 12 months of lactation. Still, we provide much larger sample size from two independent cohorts in comparison to the one previous study²⁷ on the sex-specific association of human milk leptin with childhood asthma. Lastly, circadian variations in human milk leptin are well documented⁴⁴. However, due to lack of data on time of milk sampling in UBCS, we could not determine exact biological variation between these two study populations. Nonetheless, there were no significant intraindividual changes in leptin z-scores (standardized for BMI, breastfeeding frequency and milk fat concentration) among mothers between the sampling times in SPATZ¹⁰. Thus, the strict and similar protocols in the sampling of human milk were mostly helpful in reducing diurnal variations between individuals. We also cannot rule out the synergistic effect of other additional biological components in human milk.

In conclusion, the findings from our study suggest that leptin in human milk may not be associated with a history of asthma and wheeze development in a sex-specific manner. More research is needed to validate or confirm these findings and determine whether male and female infants could benefit from sex-specific nutritional strategies.

v. **Acknowledgements**

We thank the midwives, nurses and obstetricians of the Department of Gynaecology and Obstetrics, University Medical Centre Ulm and the caring paediatricians and mothers and their families for their study support and participation. The authors would also like to thank Mrs. Gerlinde Trischler for providing excellent technical assistance.

Author contributions: L.P.S. and J.G. conceived the study question. L.P.S. conducted the statistical analyses. L.P.S. and J.G. interpreted the data and wrote the manuscript. H.B. and D.R. conceived and designed the Ulm Birth Cohort Study, D.R. and J.G. conceived and designed the Ulm SPATZ Health Study. W.K. contributed to data collection. All authors including E.A. critically reviewed the manuscript and contributed to its final version.

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viii. Tables

Table 1: Characteristics of lactating mothers who had a complete set of human milk leptin, wheeze phenotypes or asthma data available in the Ulm birth cohort studies.

	UBCS		SPATZ			
	6 weeks samples (n = 678)		6 weeks samples (n = 587)		6 months samples (n = 377)	
	Girls (n = 332)	Boys (n = 346)	Girls (n = 283)	Boys (n = 304)	Girls (n = 178)	Boys (n = 199)
Maternal age (years)	31.6 (4.7)	31.3 (4.9)	33.4 (4.1)	33.0 (4.7)	33.7 (3.5)	33.6 (4.6)
Maternal age category						
< 25 years	35 (10.5)	43 (12.5)	8 (2.8)	12 (4.0)	2 (1.1)	7 (3.5)
25 – 36 years	232 (69.9)	244 (70.7)	205 (72.4)	210 (69.3)	134 (75.3)	127 (64.1)
> 36 years	65 (19.6)	58 (16.8)	70 (24.7)	81 (26.7)	42 (23.6)	64 (32.3)
Delivery mode						
Vaginal	287 (86.4)	286 (82.7)	225 (79.5)	239 (78.6)	147 (82.6)	159 (79.9)
Caesarean	45 (13.6)	60 (17.3)	58 (20.5)	65 (21.4)	31 (17.4)	40 (20.1)
Educational level						
Low	60 (18.2)	75 (21.7)	13 (4.6)	20 (6.7)	7 (4.0)	5 (2.6)
Intermediate	132 (40.1)	120 (34.7)	71 (25.1)	74 (24.8)	39 (22.0)	39 (20.0)
High	137 (41.6)	151 (43.6)	199 (70.3)	204 (68.5)	131 (74.0)	151 (77.4)
Pre-pregnancy BMI						
Underweight (BMI < 18.5)	8 (2.4)	13 (3.8)	4 (1.4)	6 (2.0)	1 (0.6)	4 (2.0)
Normal ($18.5 \leq \text{BMI} < 25$)	222 (66.9)	249 (72.0)	177 (62.5)	216 (71.1)	118 (66.7)	145 (72.9)
Overweight ($25 \leq \text{BMI} < 30$)	83 (25.0)	61 (17.6)	66 (23.3)	53 (17.4)	43 (24.3)	30 (15.1)
Obese (BMI ≥ 30.00)	19 (5.7)	23 (6.6)	36 (12.7)	29 (9.5)	15 (8.5)	20 (10.1)
Maternal allergy						
No	242 (72.9)	257 (74.3)	201 (71.0)	210 (69.3)	121 (68.0)	132 (66.7)
Yes	90 (27.1)	89 (25.7)	82 (29.0)	93 (30.7)	57 (32.0)	66 (33.3)
Exclusive breastfeeding						
No	47 (14.2)	59 (17.1)	70 (24.7)	72 (23.7)	46 (25.8)	66 (33.2)
Yes	285 (85.8)	287 (82.9)	213 (75.3)	232 (76.3)	132 (74.2)	133 (66.8)
Doctor-diagnosed asthma*						
No	257 (94.8)	278 (93.6)	90 (90.9)	73 (83.9)	60 (90.9)	56 (80.0)
Yes	14 (5.2)	19 (6.4)	9 (9.1)	14 (16.1)	6 (9.1)	14 (20.0)
Wheeze phenotype						
Never	213 (64.2)	207 (59.8)	189 (66.8)	164 (53.9)	115 (64.6)	96 (48.2)
Transient	49 (18.7)	52 (20.1)	66 (25.9)	105 (39.0)	44 (27.7)	73 (43.2)
Persistent	28 (11.6)	39 (15.9)	37 (16.4)	31 (15.9)	27 (19.0)	30 (23.8)
Late onset	18 (7.8)	28 (11.9)	10 (5.0)	25 (13.2)	7 (5.7)	20 (17.2)

Data presented as n (%) for categorical variables and mean (SD) for continuous variables. *Doctor-diagnosed asthma using follow up data up to 6 years and 11 years for SPATZ and UBCS, respectively. Abbreviations: BMI-body-mass index; UBCS-Ulm Birth Cohort Study; SPATZ-Ulm SPATZ health study;

Table 2: Absolute human milk leptin concentrations (ng/l) measured at 6 weeks (both Ulm birth cohort studies), 6 months and 12 months (SPATZ only) from the complete data set stratified by infant's sex.

UBCS				
	Milk for boys	Milk for girls	Overall	P value
6 weeks (<i>n</i> = 678)				
Mean (SD)	272 (379)	292 (399)	282 (389)	0.045
Median [min, max]	159 [1.02, 3280]	197 [0.100, 4120]	172 [0.100, 4120]	
SPATZ				
6 weeks (<i>n</i> = 587)				
Mean (SD)	349 (336)	392 (370)	370 (353)	0.108
Median [min, max]	231 [15.7, 2480]	285 [15.1, 2950]	259 [15.1, 2950]	
6 months (<i>n</i> = 377)				
Mean (SD)	269 (337)	263 (252)	266 (300)	0.452
Median [min, max]	146 [0.260, 2080]	209 [3.66, 1640]	171 [0.260, 2080]	
12 months (<i>n</i> = 66)				
Mean (SD)	358 (403)	290 (273)	328 (351)	0.276
Median [min, max]	176 [22.2, 1480]	245 [15.5, 1250]	223 [15.5, 1480]	

P values derived from Wilcoxon rank sum test comparing standardised leptin concentrations in the milk for boys versus milk for girls at each stage of lactation in UBCS and SPATZ separately. Leptin values used to determine differences (i.e. p values) were pre-pregnancy BMI-standardized leptin z-scores in UBCS and maternal BMI-standardized leptin z-scores. Level of statistical significance is $\alpha = 0.05$.

Table 3: Sex-specific associations between human milk leptin measured at 6 weeks of lactation with wheeze phenotypes (6 years) and doctor-diagnosed asthma at 11 years in the Ulm Birth Cohort Study (UBCS).

	UBCS 6 weeks samples				
	Boys (n = 346)		Girls (n = 332)		P value
	OR	95% CL	OR	95% CL	
Doctor-diagnosed asthma					
Crude	1.45	(0.88, 2.49)	1.42	(0.76, 2.79)	0.96
Model 2	1.44	(0.87, 2.46)	1.41	(0.75, 2.81)	0.99
Model 3	1.44	(0.88, 2.48)	1.38	(0.74, 2.7)	0.86
Transient wheeze					
Crude	0.78	(0.57, 1.05)	0.88	(0.62, 1.24)	0.61
Model 2	0.79	(0.58, 1.07)	0.88	(0.62, 1.26)	0.67
Model 3	0.78	(0.57, 1.07)	0.89	(0.62, 1.28)	0.61
Persistent wheeze					
Crude	0.88	(0.62, 1.25)	0.80	(0.56, 1.15)	0.70
Model 2	0.92	(0.65, 1.32)	0.79	(0.56, 1.14)	0.57
Model 3	0.90	(0.63, 1.31)	0.88	(0.61, 1.33)	0.96
Wheeze late onset					
Crude	1.14	(0.75, 1.79)	1.05	(0.61, 1.85)	0.82
Model 2	1.11	(0.72, 1.77)	1.07	(0.61, 1.95)	0.86
Model 3	1.11	(0.71, 1.78)	1.02	(0.56, 1.87)	0.75

Abbreviations: CL-confidence limits; OR-odds ratio. Leptin values used for the analyses were pre-pregnancy BMI-standardized leptin z-scores. Associations determined by logistic regression. Model 2 adjusted for maternal allergy, exclusive breastfeeding at stage of lactation and infant age at time of human milk sampling. Model 3 adjusted for maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment. P values shows significance level of sex interaction. Level of significance $\alpha = 0.05$.

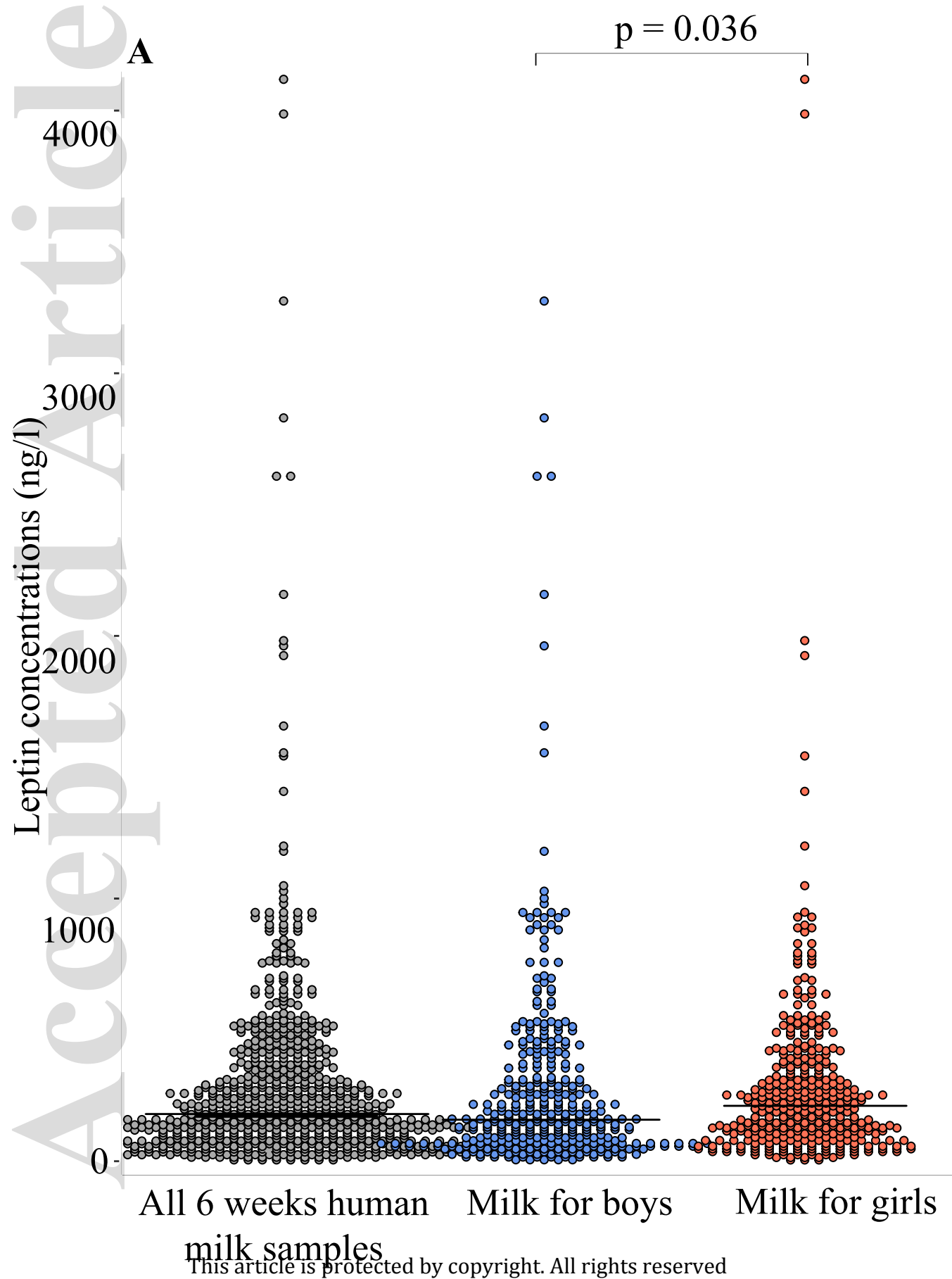
Table 4: Sex-specific associations between human milk leptin measured at 6 weeks and 6 months of lactation with wheeze phenotypes and doctor-diagnosed asthma at 6 years in the Ulm SPATZ Health Study.

	SPATZ 6 weeks samples					SPATZ 6 months samples				
	Boys		Girls		p	Boys		Girls		p
	OR	95% CL	OR	95% CL		OR	95% CL	OR	95% CL	
Doctor-diagnosed asthma										
Crude	0.84	(0.45, 1.60)	0.76	(0.44, 1.37)	0.81	1.58	(0.85, 3.23)	2.02	(0.69, 8.28)	0.73
Model 2	0.86	(0.44, 1.72)	0.78	(0.44, 1.43)	0.76	2.07	(1.0, 4.94)	2.02	(0.61, 9.64)	0.80
Model 3	0.80	(0.41, 1.57)	1.03	(0.49, 2.34)	0.67	1.69	(0.85, 3.73)	2.13	(0.70, 9.66)	0.93
Transient wheeze										
Crude	1.10	(0.84, 1.43)	1.03	(0.78, 1.37)	0.74	1.19	(0.91, 1.59)	1.08	(0.72, 1.64)	0.71
Model 2	1.08	(0.83, 1.41)	1.03	(0.78, 1.37)	0.70	1.19	(0.9, 1.6)	1.20	(0.77, 1.89)	0.93
Model 3	1.11	(0.84, 1.47)	1.17	(0.86, 1.61)	0.79	1.17	(0.87, 1.62)	1.13	(0.74, 1.77)	0.94
Persistent wheeze										
Crude	0.97	(0.66, 1.44)	1.02	(0.73, 1.45)	0.84	1.13	(0.8, 1.68)	1.03	(0.62, 1.74)	0.76
Model 2	0.98	(0.66, 1.48)	1.00	(0.71, 1.44)	0.93	1.15	(0.81, 1.72)	1.08	(0.63, 1.90)	0.78
Model 3	0.98	(0.65, 1.50)	1.19	(0.81, 1.79)	0.39	1.13	(0.77, 1.72)	0.99	(0.58, 1.73)	0.73
Late on set wheeze										
Crude	0.97	(0.62, 1.55)	1.09	(0.58, 2.17)	0.77	1.14	(0.75, 1.86)	1.35	(0.54, 3.83)	0.75
Model 2	0.93	(0.59, 1.49)	1.08	(0.56, 2.25)	0.84	1.12	(0.73, 1.83)	1.35	(0.50, 4.01)	0.71
Model 3	0.91	(0.57, 1.49)	1.23	(0.64, 2.55)	0.65	1.16	(0.74, 1.97)	1.40	(0.56, 4.01)	0.81

Abbreviations: CL-confidence limits; OR-odds ratio; Leptin values used for analysis were maternal BMI-standardized leptin z-scores. Associations determined by logistic regression. Model 2 adjusted for maternal allergy, exclusive breastfeeding at stage of lactation and infant age at time of human milk sampling. Model 3 adjusted for maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment and 6 to 7 months for the 6 months assessment. P values show significance level of sex interaction. Level of significance $\alpha = 0.05$.

ix. Figure legends

Figure 1: All human milk samples with data for absolute leptin concentrations (ng/l) available in (A) the Ulm Birth Cohort Study (UBCS) and (B) the Ulm SPATZ Health study (SPATZ). P values derived from Wilcoxon rank sum test comparing absolute human milk leptin concentrations in the milk for boys and milk for girls at 6 weeks (both cohorts), 6 months and 12 months (SPATZ only) for all human milk samples with crude leptin measures available. Level of significance $\alpha = 0.05$.



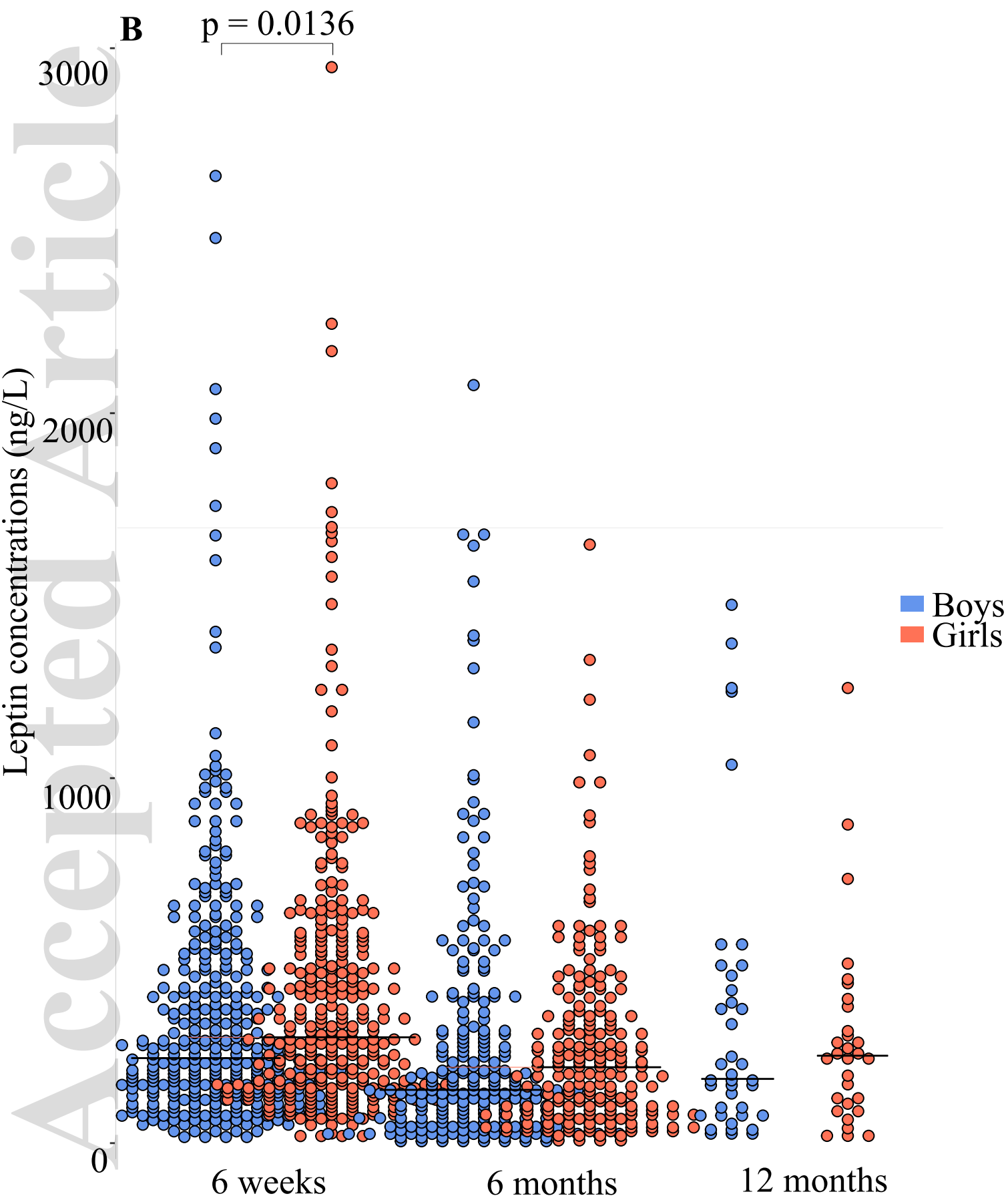


Table S1: Characteristics of all mothers (n=1066) and children (n=1090) included in the Ulm Birth Cohort Study as well as those who had a complete set of human milk leptin, wheeze phenotype or asthma data available.

	UBCS	
	Total Study Population	6 weeks samples (n = 678)
Infant sex		
Girls	537 (49.4)	332 (49.0)
Boys	551 (50.6)	346 (51.0)
Maternal age	30.91 (5.23)	31.48 (4.83)
Maternal age category		
< 25	166 (15.9)	78 (11.5)
25 - 36	689 (66.2)	476 (70.3)
> 36	186 (17.9)	123 (18.2)
Delivery mode		
Vaginal	875 (84.0)	573 (84.5)
Caesarean	167 (16.0)	105 (15.5)
Education level		
Low	248 (24.5)	135 (20.0)
Intermediate	386 (38.1)	252 (37.3)
High	380 (37.5)	288 (42.7)
Pre-pregnancy BMI		
Underweight (BMI < 18.5)	33 (3.2)	21 (3.1)
Normal (18.5 ≤ BMI < 25)	696 (67.4)	471 (69.5)
Overweight (25 ≤ BMI < 30)	218 (21.1)	144 (21.2)
Obese (BMI ≥ 30.00)	86 (8.3)	42 (6.2)
Maternal allergy		
Yes	277 (26.6)	179 (26.4)
No	765 (73.4)	499 (73.6)
Exclusive breastfeeding		
Yes	690 (63.3)	572 (84.4)
No	400 (36.7)	106 (15.6)
Doctor diagnosed asthma at 11 years		
Yes	40 (4.2)	33 (5.8)
No	902 (95.8)	535 (94.2)
Doctor diagnosed asthma at 17 years		
Yes	67 (7.1)	52 (9.2)
No	874 (92.9)	514 (90.8)
Wheeze phenotype at 6 years		
Never	715 (65.5)	420 (61.9)
Transient	146 (17.0)	101 (19.4)
Persistent	96 (11.8)	67 (13.8)
Late on set	69 (8.8)	46 (9.9)
Wheeze phenotype at 17 years		
Never	687 (63.0)	395 (58.3)
Transient	193 (21.9)	132 (25.0)
Persistent	111 (13.9)	77 (16.3)
Late on set	96 (12.3)	73 (15.6)

Data presented as n (%) for categorical variables and mean (SD) for continuous variables. Sums may not add up to total due to missing data for some variables. Abbreviations: BMI-body-mass index; UBCS-Ulm Birth Cohort Study

Table S2: Characteristics of all mothers and children included in the Ulm Birth Cohort Study as well as those who had a complete set of human milk leptin, wheeze phenotype or asthma data available.

	Overall	6 weeks samples (n = 587)	6 months samples (n=377)
Infant sex			
Girls	524 (52.0)	283 (48.2)	178 (47.2)
Boys	483 (48.0)	304 (51.8)	199 (52.8)
Maternal age	32.71 (4.74)	33.21 (4.44)	33.62 (4.11)
Maternal age category			
< 25	60 (6.4)	20 (3.4)	9 (2.4)
25 - 36	643 (68.8)	415 (70.8)	261 (69.4)
> 36	231 (24.7)	151 (25.8)	106 (28.2)
Delivery mode			
Vaginal	697 (74.6)	464 (79.0)	306 (81.2)
Caesarean	237 (25.4)	123 (21.0)	71 (18.8)
Education level			
Low	90 (9.8)	33 (5.7)	12 (3.2)
Intermediate	280 (30.6)	145 (25.0)	78 (21.0)
High	546 (59.6)	403 (69.4)	282 (75.8)
Pre-pregnancy BMI			
Underweight (BMI < 18.5)	21 (2.3)	10 (1.7)	5 (1.3)
Normal ($18.5 \leq \text{BMI} < 25$)	554 (61.3)	393 (67.0)	263 (69.9)
Overweight ($25 \leq \text{BMI} < 30$)	209 (23.1)	119 (20.3)	73 (19.4)
Obese ($\text{BMI} \geq 30.00$)	120 (13.3)	65 (11.1)	35 (9.3)
Maternal allergy			
No	648 (69.9)	411 (70.1)	253 (67.3)
Yes	279 (30.1)	175 (29.9)	123 (32.7)
Exclusive breastfeeding			
Yes	582 (57.8)	445 (75.8)	323 (69.8)
No	425 (42.2)	142 (24.2)	40 (30.2)
Doctor diagnosed asthma at 6 years			
Yes	32 (13.2)	23 (12.4)	20 (14.7)
No	210 (86.8)	163 (87.6)	116 (85.3)
Wheeze phenotype at 6 years			
Never	667 (66.2)	353 (60.1)	211 (56.0)
Transient	249 (27.2)	171 (32.6)	117 (35.7)
Persistent	102 (13.3)	68 (16.2)	57 (21.3)
Late on set	51 (7.1)	35 (9.0)	27 (11.3)

Data presented as n (%) for categorical variables and mean (SD) for continuous variables. Sums may not add up to total due to missing data for some variables. Abbreviations: BMI-body-mass index; SPATZ- Ulm SPATZ Health Study.

Table S3: Number of children with data on wheeze phenotypes and doctor-diagnosed asthma at age 17 years in the Ulm Birth Cohort Study (UBCS).

	Girls (n = 332)	Boys (n = 346)
Wheeze phenotypes (n, %)		
Never	202 (60.8)	193 (55.8)
Transient	66 (24.6)	66 (25.5)
Persistent	34 (14.4)	43 (18.2)
Late on set	30 (12.9)	43 (18.2)
Doctor-diagnosed asthma (n, %)		
Yes	19 (7.1)	33 (11.1)
No	249 (92.9)	265 (88.9)

Table S4: Associations of human milk leptin measured at 6 weeks of lactation with wheeze phenotypes (6 and 17 years) and doctor-diagnosed asthma (11 and 17 years) in the Ulm Birth Cohort Study (UBCS).

	UBCS 6 weeks samples (n = 678)					
	Up to 11 years ¹			Up to 17 years ²		
	OR	95% CL	p value	OR	95% CL	p value
Doctor-diagnosed Asthma						
Crude	1.40	(1.00, 1.99)	0.056	1.34	(0.97, 1.87)	0.08
Model 2	1.41	(0.95, 2.13)	0.10	1.34	(0.97, 1.87)	0.08
Model 3	1.38	(0.93, 2.08)	0.12	1.38	(1.00, 1.94)	0.06
Wheeze transient						
Crude	0.82	(0.65, 1.02)	0.08	0.94	(0.76, 1.16)	0.57
Model 2	0.83	(0.66, 1.04)	0.11	0.96	(0.78, 1.19)	0.74
Model 3	0.83	(0.65, 1.05)	0.11	0.93	(0.74, 1.16)	0.51
Wheeze persistent						
Crude	0.66	(0.47, 0.91)	0.014	0.87	(0.69, 1.10)	0.24
Model 2	0.84	(0.66, 1.08)	0.16	0.87	(0.69, 1.11)	0.26
Model 3	0.88	(0.68, 1.1)	0.33	0.92	(0.72, 1.18)	0.49
Wheeze late onset						
Crude	1.09	(0.78, 1.54)	0.63	0.96	(0.73, 1.27)	0.76
Model 2	1.09	(0.78, 1.54)	0.64	0.96	(0.73, 1.28)	0.79
Model 3	1.05	(0.75, 1.5)	0.78	0.97	(0.72, 1.29)	0.81

Abbreviations: CL-confidence limits; OR-odds ratio. Leptin values used for analyses were pre-pregnancy BMI-standardized leptin z-scores. ¹Wheeze phenotypes at 6 years and doctor-diagnosed asthma up to 11 years. ²Wheeze phenotypes and doctor-diagnosed asthma up to 17 years. Associations determined by logistic regression. Model 2 adjusted for maternal self-reported history of allergy, exclusive breastfeeding at stage of lactation and infant age at time of human milk sampling. Model 3 adjusted for maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment. P values for the significance level of association. Level of significance $\alpha = 0.05$.

Table S5: Associations of human milk leptin measured at 6 weeks of lactation with wheeze phenotypes and doctor-diagnosed asthma in the Ulm SPATZ Health Study.

	SPATZ 6 weeks samples (n = 587)			SPATZ 6 months samples (n = 377)		
	OR	95% CL	p value	OR	95% CL	p value
Asthma						
Crude	0.81	(0.54, 1.22)	0.30	1.58	(0.92, 2.94)	0.12
Model 2	0.80	(0.53, 1.23)	0.30	1.58	(0.91, 3.00)	0.13
Model 3	0.88	(0.55, 1.41)	0.58	1.76	(0.98, 3.49)	0.08
Wheeze transient						
Crude	1.05	(0.87, 1.27)	0.62	1.13	(0.90, 1.43)	0.31
Model 2	1.04	(0.86, 1.26)	0.69	1.17	(0.93, 1.50)	0.20
Model 3	1.12	(0.91, 1.37)	0.28	1.09	(0.86, 1.390)	0.50
Wheeze persistent						
Crude	1.00	(0.78, 1.30)	1.00	1.08	(0.81, 1.48)	0.60
Model 2	0.99	(0.77, 1.29)	0.94	1.10	(0.82, 1.52)	0.54
Model 3	1.09	(0.83, 1.46)	0.54	1.07	(0.79, 1.49)	0.66
Wheeze late onset						
Crude	0.98	(0.68, 1.41)	0.90	1.12	(0.76, 1.76)	0.59
Model 2	0.96	(0.66, 1.39)	0.81	1.12	(0.74, 1.78)	0.62
Model 3	1.01	(0.69, 1.49)	0.97	1.09	(0.72, 1.74)	0.70

Abbreviations: CL-confidence limits; OR- odds ratio. Leptin values used for analyses were maternal BMI-standardized leptin z-scores. Associations determined by logistic regression. Model 2 adjusted for maternal self-reported history of allergy, exclusive breastfeeding at stage of lactation and infant age at time of human milk sampling. Model 3 adjusted for maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment and 6 to 7 months for the 6 months assessment. P values for the significance level of association. Level of significance $\alpha = 0.05$.

Table S6: Sex-specific associations of human milk leptin measured at 6 weeks of lactation with wheeze phenotypes and doctor-diagnosed asthma at 17 years in the Ulm Birth Cohort Study (UBCS).

	UBCS				
	Boys		Girls		p value
	OR	95% CL	OR	95% CL	
Doctor-diagnosed asthma					
Crude	1.33	(0.90, 2.01)	1.46	(0.85, 2.63)	0.80
Model 2	1.37	(0.92, 2.08)	1.44	(0.83, 2.62)	0.84
Model 3	1.37	(0.92, 2.10)	1.55	(0.88, 2.87)	0.73
Transient wheeze					
Crude	0.85	(0.64, 1.14)	1.06	(0.78, 1.46)	0.31
Model 2	0.88	(0.66, 1.17)	1.07	(0.78, 1.48)	0.40
Model 3	0.83	(0.61, 1.13)	1.05	(0.75, 1.48)	0.31
Persistent wheeze					
Crude	0.94	(0.66, 1.33)	0.82	(0.59, 1.15)	0.57
Model 2	0.98	(0.69, 1.39)	0.81	(0.58, 1.13)	0.44
Model 3	0.96	(0.68, 1.39)	0.88	(0.62, 1.29)	0.82
Late on set wheeze					
Crude	1.15	(0.81, 1.65)	0.96	(0.62, 1.49)	0.53
Model 2	1.16	(0.81, 1.69)	0.97	(0.61, 1.54)	0.50
Model 3	1.14	(0.79, 1.66)	1.02	(0.63, 1.66)	0.65

Abbreviations: CL-confidence limits; OR-odds ratio. Leptin values used for analyses were pre-pregnancy BMI-standardized leptin z-scores. Associations determined by logistic regression. Model 2 adjusted for maternal self-reported history of allergy, exclusive breastfeeding at stage of lactation and infant age at time of human milk sampling. Model 3 adjusted for maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment. P values for the significance level of sex interaction. Level of significance $\alpha = 0.05$.

Table S7: Associations of human milk leptin measured at 6 weeks of lactation with doctor-diagnosed asthma in the Ulm SPATZ Health Study adjusted for pre-pregnancy body mass index (BMI)

	All 6 weeks samples			Girls		Boys		p ²
	OR	95% CL	p ¹	OR	95% CL	OR	95% CL	
Doctor-diagnosed asthma								
Crude	0.92	(0.55, 1.54)	0.74	0.85	(0.40, 1.88)	0.93	(0.46, 1.90)	0.87
Model 2	0.78	(0.42, 1.48)	0.44	0.78	(0.32, 2.03)	0.78	(0.31, 1.95)	0.91
Model 3	0.91	(0.46, 1.82)	0.79	1.29	(0.42, 4.40)	0.71	(0.28, 1.77)	0.63

Abbreviations: CL-confidence limits; OR- odds ratio. Leptin values used for analyses were log-transformed. Associations determined by logistic regression. Model 2 adjusted for maternal self-reported history of allergy, exclusive breastfeeding at stage of lactation, pre-pregnancy body mass index (BMI) and infant age at time of human milk sampling. Model 3 adjusted for pre-pregnancy BMI, maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment and 6 to 7 months for the 6 months assessment. P values for the ¹significance level of association and ²significance level of sex interaction. Level of significance $\alpha = 0.05$.

Table S8: Associations of human milk leptin measured at 6 weeks of lactation with doctor-diagnosed asthma in the Ulm SPATZ Health Study adjusted for maternal body mass index (BMI) concurrent to time of milk sampling

	All 6 weeks samples			Girls		Boys		p ²
	OR	95% CL	p ¹	OR	95% CL	OR	95% CL	
Doctor-diagnosed asthma								
Crude	0.92	(0.55, 1.54)	0.74	0.85	(0.40, 1.88)	0.93	(0.46, 1.90)	0.87
Model 2	0.77	(0.41, 1.50)	0.95	0.76	(0.31, 2.05)	0.80	(0.30, 2.16)	0.95
Model 3	0.90	(0.44, 1.87)	0.60	1.28	(0.39, 4.70)	0.72	(0.27, 1.92)	0.60

Abbreviations: CL-confidence limits; OR- odds ratio. Leptin values used for analyses were log-transformed. Associations determined by logistic regression. Model 2 adjusted for maternal self-reported history of allergy, exclusive breastfeeding at stage of lactation, maternal body mass index (BMI) concurrent to time of milk sampling and infant age at time of human milk sampling. Model 3 adjusted for maternal BMI concurrent to time of milk sampling, maternal history of allergy, exclusive breastfeeding and child BMI assessed at 4 to 5 weeks for the 6 weeks assessment and 6 to 7 months for the 6 months assessment. P values for the ¹significance level of association and ²significance level of sex interaction. Level of significance $\alpha = 0.05$.