

Systematic Review:

Relationship Between Breast Milk Ghrelin and Infants' Serum Ghrelin and Growth in Breastfeeding Infants: A Systematic Review and Meta-Analysis



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ABSTRACT

Background: There is positive correlation between infant serum ghrelin and the growth of infants in breastfed infants.

Objectives: The present study aimed to evaluate of the relationship between breast milk ghrelin and serum ghrelin and growth in breast-milk-fed infants by systematic review and meta-analysis approaches.

Methods: Electronic resources used in the current search were Scopus, Cochrane Library, Science Direct, PubMed, and EMBASE. The English language full-text articles and abstracts published from 1999 to July 2019 were collected. Only the articles that explored infants under two years of age and receiving breast milk exclusively were reviewed. Papers related to healthy mothers and infants were entered in this study. The results of 6 papers regarding the effect of serum ghrelin on the growth of infants, i.e., evaluated by meta-analysis using Stata software. The data addressing the effect of breast milk ghrelin on growth were reported by a systematic review manner; three articles were found in this area. We used Preferred Reporting Items for systematic reviews and meta-analyses (prisma) checklist for performing this study.

Results: In this study, the association of infants' serum ghrelin with infants' growth was evaluated by meta-analysis. Newcastle-Ottawa Scale (NOS) value was ≥ 7 scores for the 6 explored meta-analysis articles. The present meta-analysis research suggested a positive and significant correlation between infant serum ghrelin and the growth of infants ($r=0.163$).

Conclusions: The random-effect model data demonstrated a positive and significant correlation between infant serum ghrelin and the growth of infants. However, in the study of the effect of breast milk ghrelin on infants' growth, the number of articles was limited and contradictory results were reported. Accordingly, no general conclusion was reached.

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1. Introduction

Obesity and weight gain are health-related risk factors with short- and long-term effects in infants (1). The increase in Body Mass Index (BMI) enhances the risk of diabetes (2), metabolic syndrome (3), cardiovascular disease (4), and numerous other complications. Prior research suggested that nourishment is effective in controlling weight gain and appetite in short and long terms. Comparing formula-fed and breast-fed infants indicated a better control of weight in the former (5, 6). Previous studies inferred the presence of adipokines in the breast milk, which protect and modulate weight and appetite in the infants (7, 8). Ghrelin is an adipokine in the breast milk and the human body. This adipokine predominantly contains 28 amino acids; it is a natural antagonist for leptin (9). There are two forms of ghrelin, i.e., acylated and de-acylated (10). Ghrelin is a Growth Hormone (GH) secretion stimulant, mainly secreted from the abdomen and the epsilon cells of the pancreas (11). In addition, ghrelin is among the most orexigenic peptides that stimulates hunger (12). The concentration of this hormone increases before eating and decreases after that (12, 13). Ghrelin is involved in regulating energy metabolism (14), carbohydrates and lipids (15), body growth and development, and food intake (16). Studies have investigated the relationship between breast milk feeding and infants' serum ghrelin.

According to Aydin et al., there was a significant correlation between breast milk and infants' serum ghrelin (17). Yesim Ozarda Ilcol and Banu Hizli observed a positive association between breast milk ghrelin and serum ghrelin in infants ($r=0.331$, $P<0.05$) (18). Moreover, there exist different reports regarding the effect of serum ghrelin on the growth and weight gain of infants. Based on F. Savino et al., serum ghrelin levels were negatively correlated with weight gain ($r=-0.302$, $P=0.003$) (19). Furthermore, Gokhan Cesur indicated a significant and positive relationship between breast milk, and serum ghrelin and weight gain in infants ($r=0.576$, $P=0.025$) (20). Ghrelin plays different roles; one of which is in controlling appetite and energy consumption and consequently, body weight. This hormone has been the subject of numerous research studies in various fields, including obesity and food intake. Breastfed infants receive ghrelin hormone through breast milk. However, data on the effects of serum and breast milk ghrelin on infants' growth and weight gain are contradictory. Thus, this study investigated the relationship between breast

milk ghrelin, serum ghrelin, and growth in infants that fed with breast milk, exclusively. This investigation was performed through a systematic review and meta-analysis.

The present study aimed to review the articles that illustrated the correlation between breast milk ghrelin concentration, weight gain, and growth in the infants through a systematic review. Moreover, we explored the relationship between serum ghrelin concentration and the growth, weight gain, and anthropometric characteristics of the infants through a meta-analysis study.

2. Methods

Electronic resources considered in the current search were Scopus, Cochrane Library, Science Direct, PubMed, and EMBASE. The articles in English and the articles with an English abstract published from 1999 to July 2019 were collected. The search mesh terms in PubMed and Scopus and text words included the following: "infant, BMI, growth, weight gain, anthropometric parameters, ghrelin, serum, adipokines, and breast milk". Moreover, the references of the extracted articles were re-examined to find uncited papers by an electronic search.

Only the articles were reviewed that investigated infants <2 years of age and receiving breast milk, exclusively. Therefore, the main source of ghrelin in these infants was breast milk. Papers related to healthy mothers and infants were enrolled in this study. The studied mothers did not smoke, consume alcohol, or use medications. All analyses were conducted based on the information of previous published articles. Two authors collected the following data: authors' names, journal's name, the country of research conduction, the year of publication, the sample size, and the mean concentration value of the hormone level in breast milk and serum. The ghrelin level in breast milk and serum of infants was measured by one of the following approaches: Enzyme-Linked Immunosorbent Assay (ELISA), Radio-Immunoassay (RIA), and MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel. The relevance between breast milk, serum ghrelin, and the anthropometric (BMI & weight gain) properties of the studied infants was expressed by correlation coefficient (r).

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist and the Cochrane Recommendations were used in this meta-analysis and systematic review study. Three authors selected articles in accordance with the meta-analysis standard guidelines. Two authors independently collected all research

papers' data. Disagreements between the researchers were solved by consultation with a third author.

The quality of the articles was determined using the Newcastle-Ottawa Scale (NOS) checklist. The Review Manager Software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) was used in this study. The Standardized Incidence Rate (SIR)/Relative Risk (RR), and 95% CI were estimated to evaluate the effect of serum ghrelin on the anthropometric factors of the studied infants. Cochran's test and random effects model were used, as well. Begg's funnel plot was used to detect publication bias.

3. Results

In this study, in section A, the correlation between breast milk ghrelin and infants' weight gain was determined by a systematic review method. In section B, a meta-analysis was performed to evaluate the effect of infant serum ghrelin on the growth and weight gain of the studied infants.

Initially, the relationship between breast milk ghrelin and weight gain was investigated. The characteristics of the studies are listed (in form of three papers) in Table 1. Khodabakhshi et al. (2014) evaluated 40 healthy-weight infants and 40 obese infants in 2-5 months (21). They observed that the mean breast milk ghrelin value in the mothers with healthy-weight infants was higher than those with obese infants (137.5 vs. 132 pg/mL). Infants' weight and breast milk ghrelin level were measured at birth, as well as at 2, 4, and 6 months of age. The correlation between breast milk ghrelin and healthy-weight infants was evaluated at birth ($r=-0.108$), 2 (0.195), 4 (0.012), and 6 months (-0.053) of age (21).

Gokhan Cesur et al. assessed the relationship between breast milk ghrelin and weight gain in infant among 25

mothers and their infants in 2012. Six mothers and infants were excluded from the analysis at 4 months after baseline. The mean breast milk ghrelin score was 2876.75-1626.35 pg/mL; the correlation coefficient between breast milk ghrelin level and weight gain in infants was 0.157 at 4 months of age (20). Huang Li-Liand et al. explored 67 infants and concluded that ghrelin level in breast milk was a key indicator related to body weight in them ($\beta=0.161$) (22). The Newcastle-Ottawa Scale (NOS) score was equal to 7 in two articles.

In this study, PRISMA checklist was used for the qualitative evaluation of the collected articles (Figure 1). Four keywords, namely: "infant, ghrelin, growth, and serum infant" were used to search the articles. Keywords were selected based on Patient, Intervention, Comparison, Outcome, Time, Study (PICOTS) method. One hundred eight articles were extracted while searching the databases. After studying the titles and abstracts of the collected articles, 29 full-texts were assessed and 23 articles were excluded from the study. Six articles met the criteria for meta-analysis (19, 20, 23-26). The excluded articles from the study ($n=23$) included the following: 5 articles presented the effect of breast milk ghrelin on growth in infants (6, 18, 21, 22, 27), 8 articles expressed no correlation between infants' serum ghrelin and growth (2, 17, 28-33), 3 articles were of descriptive nature (34-36), and 6 articles had an intervention group (37-42).

This study considered a correlation between serum ghrelin and growth in infants. The characteristics of the selected studies are listed in Table 2. This study included three cohort (20, 23, 26) articles and three cross-sectional (19, 24, 25) studies on infants aged <2 years for meta-analysis. Immunoassay methods were used for measuring ghrelin in the serum of infants. BMI, weight gain, and weight-for-length ratio were considered for

Table 1. Characteristics of the studies included in the systematic review of correlation between breast milk ghrelin and growth in infants

Study	Country	Mean Value of Ghrelin in Breast Milk	Statistical Index	Correlation between Ghrelin in Breast Milk and Weight Gain	NOS*	Reference Number
Adeleh Khodabakhshi (2014)	Iran	137.50 pg/mL	Spearman's correlation coefficient	$r=-0.053$	7	21
Gokhan Cesur(2012)	Turkey	2,876.75±1626.35 pg/mL	Spearman's correlation coefficient	$r=0.51$	7	20
Huang li-li(2017)	China	2.6 (2.1,3.7) ng/mL	-	$\beta=0.161$	-	22

*NOS: Newcastle-Ottawa Scale.

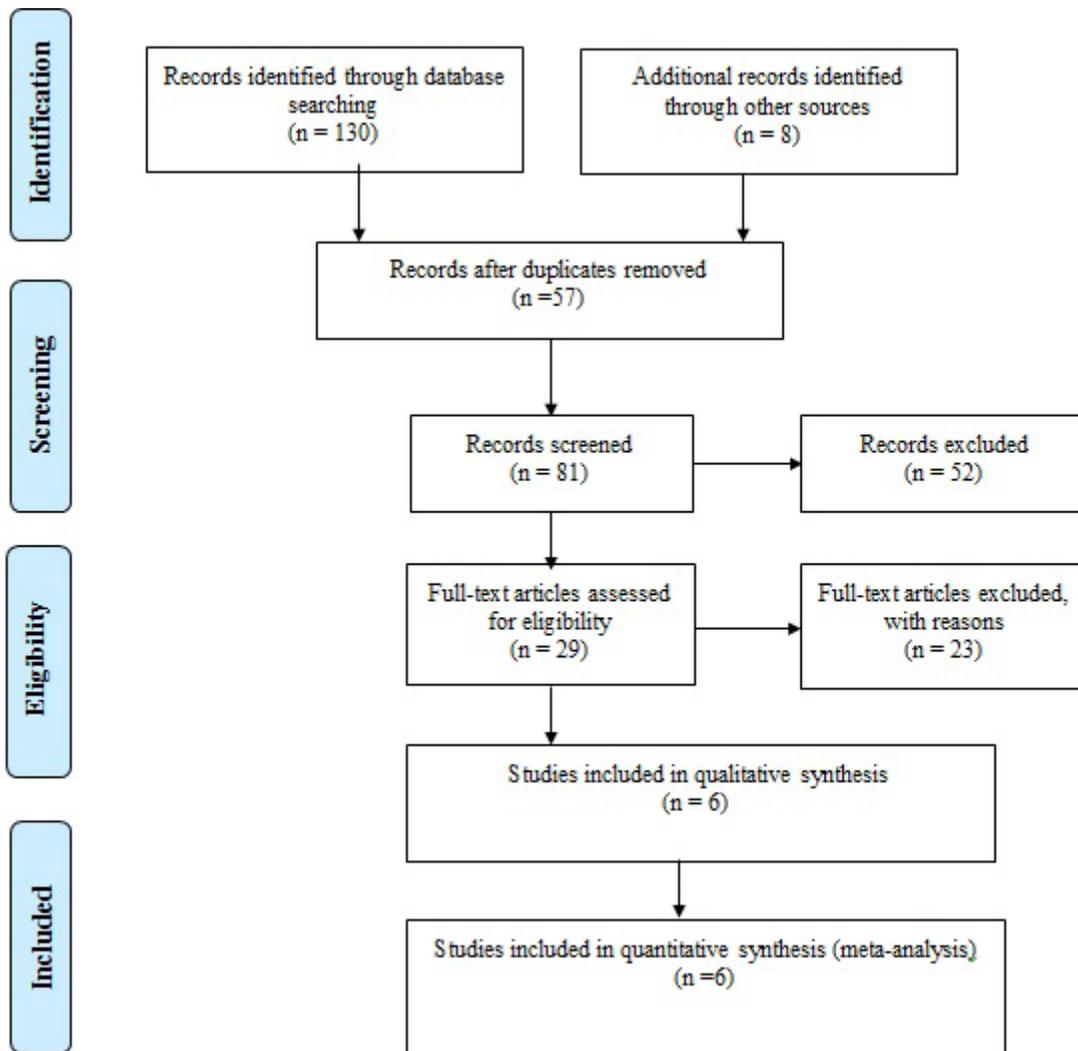


Figure 1. PRISMA process flowchart of study selection

infant growth index. The correlation between growth index and infants serum ghrelin was expressed with Spearman's correlation coefficient in 4 studies along with Pearson's correlation coefficient.

The total number of investigated study participants was 554. A correlation coefficient (r) was used to express the relationship between infants' serum ghrelin and growth. The quality assessment data by the

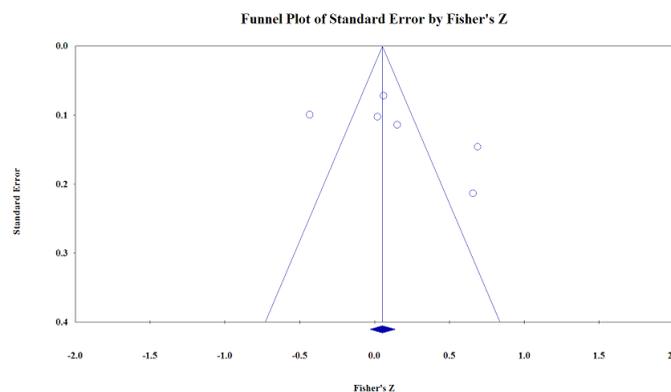


Figure 2. Publication bias of the effect of ghrelin serum on growth (weight gain & BMI) in the investigated infants

Table 2. The characteristics of the studies included in the meta-analysis of correlation between ghrelin serum levels and growth in infants

Study (year of publication)	Country	Comparative Factor	Method	Sample Size	Mean of Breast Milk Ghrelin (pg/mL)	Type of Study	Statistical Index	Correlation	NOS	Reference
F. Savino (2005) [33]	Italy	Weight gain	RIA	104	2981.65 6 1444.96	Cross-sectional	-0.407	Negative	8	19
Piero Pirazzoli (2005) [23]	Italy	BMI	RIA	98	889.2 (450-2047)	Cohort	0.02	Positive	7	23
Adeleh Khodabakhshi (2018) [24]	Iran	Weightgain	ELISA	80	673 (580-1066)	Cross-sectional	0.15	Positive	8	24
Gokhan Cesur (2012) [20]	Turkey	-	RIA	25	5,188.00±951.00	Cohort	0.576	Positive	7	20
Hasan Kahveci (2015) [25]	Turkey	Weight gain	ELISA	50	1976.8±639.7(650.0-3290.0)	Case-control study	0.597	Positive	8	25
Laura M. Breij (2017) [26]	Netherlands	Weight-for-length	MILLIPLEX MAP	197	33.2	Cohort	0.06	Positive	8	26

*NOS: Newcastle-Ottawa Scale.

NOS were ≥7 scores (Table 1). Sensitivity analysis and emission bias analysis were performed using funnel diagrams and Begg’s test. The publications indicated no significant bias in this meta-analysis (Figure 2).

The heterogeneity of the selected studies was assessed using Q-test and 12 index (I-Squared=90). Due to the heterogeneity of the collected studies, the random effects model was used to combine the results of the investigations. According to Figure 3, the results of random-effect model demonstrated a direct and significant correlation between the serum ghrelin and growth of infants (r=0.163).

4. Discussion

The present study evaluated the effect of infants’ serum ghrelin on growth by a meta-analysis approach. The obtained data suggested a direct and significant effect in this area. Contradictory results were obtained regarding the effect of breast milk ghrelin on the studied infants’ weight gain.

Studies have reported that adipokines are essential hormones in regulating energy, appetite, and weight in the short and long term (43). Ghrelin (the first known controlling appetite hormone) is an adipokine discovered in 1999; its’ mechanism remains undiscovered (44). Prior research suggested that the main activities of ghrelin include growth hormone secretion, appetite

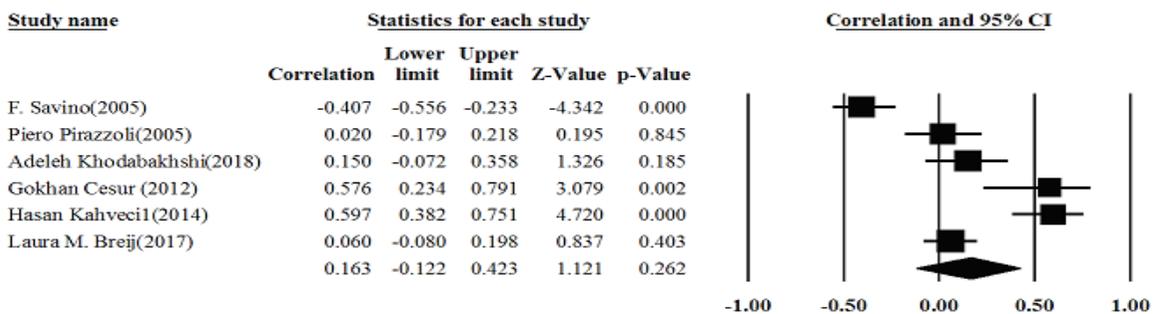


Figure 3. ORs and 95% CIs of individual studies of the effect of ghrelin serum on growth in infants

stimulation, food intake, changes in gastric acid secretion, and changes in endocrine and exocrine pancreatic secretions. Furthermore, it directly affects corticotropin and lactotrophic hormone secretion, controlling the axons of male hypogonadism, polycystic ovary syndrome, and H-pylori gastric helicobacter pylori (44-46). Research has suggested that ghrelin impacts short-term energy regulation; it is reduced in positive energy balance conditions (e.g., satiety time), and increased in negative energy balance conditions (e.g., hunger). Further observed was the effect of ghrelin on long-term energy regulation. The results of several studies have demonstrated that ghrelin is higher in obese individuals with a usual positive energy balance, and lower in those with anorexia nervosa with a frequent negative energy balance (47-49).

Ghrelin is the only known peptide that can cross the blood-brain barrier. The human ghrelin gene is on the short arm of chromosome 3 at position P 26-25 (50). The ghrelin precursor is in the form of pre-pro-ghrelin that becomes active with post-translation changes (the acidification of third serine residue hydroxyl group by 8-carbon fatty acid by ghrelin – O- acyl transferase [GOAT]) (13). Mainly, ghrelin is in des-acyl form (90%), and to lesser extent, in acyl form; moreover, it is very restricted to the C-terminal of proghrelina peptide form in the bloodstream (46, 50). Acyl and des-acyl forms of it are created by the cells of the hypothalamic arcuate nucleus (50). Ghrelin is a ligand for the Growth Hormone Secretagogue Receptor (GHS-R). The major Ghrelin Receptor (GHS-R) is mainly expressed from the hippocampus, pituitary, and hypothalamus whose gene is on chromosome 3 at position q26-27 (47).

There are two ghrelin receptor types: GHS-R type 1a, a seven-domain GPCR, acting as a receptor for ghrelin receptor, and GHS-R cDNA, type 1b; the second type receptor, i.e., pharmacologically inactive (51, 52). Ghrelin is mainly produced by the pancreatic cells in the fetus; however, after birth, ghrelin is mainly secreted from gastric endocrine X/A cells (13). Furthermore, ghrelin is expressed in numerous tissues of the body, including the lung, colon, duodenum, jejunum, ileum, heart, testis, pancreas, and the pituitary gland in adolescence (50). Ghrelin, secreted from the stomach, enters the bloodstream, then the hypothalamus and pituitary. Ghrelin binds to its receptor on the surface of pituitary somatotroph cells, which activates phospholipase C and produces IP3 and diacyl glycerol; accordingly increasing intracellular calcium levels (53). Increased intracellular calcium enhances pituitary GH production, appetite, and food absorption. Growth Hormone-Re-

leasing Hormone (GHRH) is a natural analog of ghrelin in the human body. Besides, it activates adenylate cyclase and increases intracellular cAMP by binding to its receptor on the surface of pituitary cells.

GHRH is valuable as a secondary messenger for activating protein kinase A. Moreover, increased intracellular cAMP leads to the enhanced production of pituitary GH hormones as well as body metabolism (54). GHSs are synthetic analogs for ghrelin with similar mechanisms. In humans, 133 homologs have been identified for ghrelin (50). Studies have revealed that ghrelin, by affecting the arcuate nucleus of the hypothalamus, produces NPY/AgRP neurons; orexigenic neurons reduce fatty acid oxidation, and increase hunger and food absorption in the body. On the other hand, NPY neurons, by affecting the Paraventricular Nucleus (PVN) cells of the hypothalamus, suppress the activity of gamma-aminobutyric acid neurotransmitters, increase the production of Corticotropin-Releasing Hormones (CRH), and eventually enhance the production of cortisol and Adrenocorticotrophic Hormones (ACTH) (50, 53, 54). POMC is a hormone in the body that suppresses appetite. The effect of ghrelin on hypothalamic cells activates NPY/AgRP neurotransmitter neurons. The NPY/AgRP neurons suppress POMC, consequently increasing appetite and the absorption of nutrients in the body (53).

The analyzed studies investigated the effect of breast milk ghrelin on infants' growth. In some studies, the exact level of correlation was not mentioned or a correlation was not observed; therefore, they were excluded from this study. An example of these articles was Yis U et al.'s research in 2010 that demonstrated higher serum ghrelin in breast-fed infants. They also indicated its' role in their faster growth rate during the first three months of life; however, they concluded that breast milk ghrelin was not correlated with the respective serum ghrelin or anthropometric data in the breast-fed group (28). Francesco Savino et al. measured ghrelin, leptin, and IGF-I levels in breast-fed infants in the first year of life (33). They expressed IGF-I, leptin, and ghrelin in breast milk were effective in the growth of infants up to 4 months of age; however, the exact correlation was disregarded (33).

A limitation of this study was the limited number of articles on the subject. In addition, the articles were in English language, and the relationship between breast milk, serum ghrelin, growth, and weight gain was not addressed in some published articles. It is suggested that more studies be conducted to evaluate the effect

of breast milk ghrelin and serum ghrelin on growth and weight gain in infants.

5. Conclusion

According to the present meta-analysis study data, serum ghrelin had a direct and significant correlation with the growth of infants under two years of age. Increased ghrelin in infants' serum level enhanced appetite, food absorption, and growth. Three articles with contradictory results were found regarding the relationship between breast milk and weight gain in infants; thus, more studies are required to provide further relevant details.

Ethical Considerations

Compliance with ethical guidelines

This article is a meta-analysis with no human or animal sample.

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Authors' contributions

All authors contributed in designing, running, and writing all parts of the research.

Conflicts of interest

The authors declared no conflicts of interest.

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