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Original Article

Effects of parenteral nutrition and breastfeeding on the dynamic expression of serum ghrelin in premature infants

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Abstract: Objective: This study aims to explore the effects of parenteral nutrition and breastfeeding on the dynamic expression of serum ghrelin, growth, and immune function in premature infants. Methods: We randomly assigned 200 premature infants (with a gestational age between 32 to 35 weeks) to a control group and an observation group, in a 1:1 ratio. Infants in the control group were breastfed, and those who required more nutrition than breast milk were given additional electrolyte solutions according to relevant specifications. Infants in the observation group received early parenteral nutrition combined with breastfeeding. ELISA assay was employed to measure the dynamic expression of ghrelin in the femoral venous blood of infants at 24 h, 72 h, and 7 d after the delivery. Weight gain, albumin, and globulin levels were compared between the two groups at each time point. Results: Serum ghrelin expression at 24 h, 72 h, and 7 d after the delivery, weight gain, albumin level, and globulin level were markedly higher in the observation group than in the control group ($P<0.05$). Conclusion: Early parenteral nutrition combined with breastfeeding can significantly increase the expression level of serum ghrelin in premature infants, promote infant growth, and strengthen immune function.

Keywords: Parenteral nutrition, breastfeeding, premature infant, ghrelin, infant's growth, immune function

Introduction

Progress in perinatal medicine in China results in higher birth rates and survival rates of premature infants. Having underdeveloped physiological functions, especially poor digestive systems and immune systems compared to full-term infants, premature infants have low absorption efficiency of breast milk or artificial milk, are prone to intolerance of breast milk or artificial milk [1], giving rise to malnutrition, higher risks of infection and other complications, as well as sometimes having impaired physical and intellectual development [2]. The safe and practical combination of parenteral nutrition and breastfeeding based on well-developed parenteral nutrition supply technology can significantly improve the nutritional deficiency of premature infants, promote infant's growth, and enhance digestive system function [3]. Serum ghrelin was found to be closely related to feeding intolerance, malnutrition, and growth retardation in premature infants [4]. Huang Jingwei stated that early detection of serum ghrelin levels had certain value in predicting feeding intolerance in premature infants, and reckoned that exogenous ghrelin supplementation might improve intestinal feeding tolerance in premature infants [5]. Ghrelin is mainly secreted by gastric mucosal gland cells and is present in small amounts in the placenta, small intestine, hypothalamus, and cerebral cortex. Affected by hormones and nutritional status, ghrelin expression is negatively correlated with the growth of premature infants [6, 7]. So far, few studies have investigated the effects of parenteral nutrition or breastfeeding on serum ghrelin expression and its dynamic changes, or investigated the effects of serum ghrelin levels on the growth and immune function of premature infants. Therefore, this study will explore the effects of parenteral nutrition combined with breastfeeding on the dynamic expression of serum ghrelin, growth, and immune function in premature infants.

Table 1. Comparison of baseline data of infants and their mothers between the two groups

Group	Control group (n=100)	Observation group (n=100)	t/χ^2	P
Infant				
Boy/girl	49/51	47/53	0.080	0.777
Gestational age	34.2±1.3	34.0±1.4	0.526	0.423
Birth weight (g)	2342.5±156.8	2325.7±144.9	0.726	0.312
Birth head size (cm)	31.8±0.6	31.7±0.5	0.253	0.864
Mother				
Spontaneous delivery/caesarean section	32/68	30/70	0.094	0.760
Age of pregnancy (year)	32.5±4.6	33.8±4.9	0.432	0.569
Body mass index (kg/m^2)	23.5±1.9	23.4±1.7	0.632	0.357

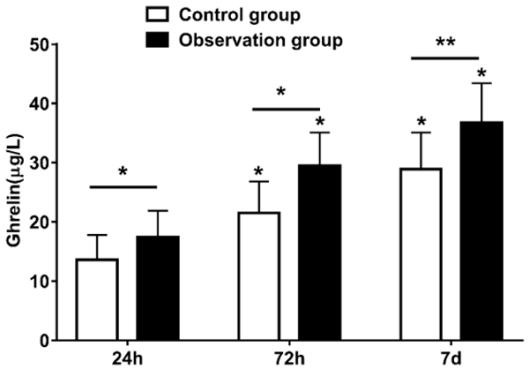

Figure 1. Detection of serum Ghrelin levels in two groups at different time by ELISA. Compared with control group, *P<0.05; Compared with control group, **P<0.01.

Materials and methods

Basic information on subjects

We collected 200 premature infants (with a gestational ages between 32 to 35 weeks) who were admitted to The People's Hospital of Langfang City from June 2018 to June 2019 as the study subjects. Inclusion criteria: 1. Live singleton pregnancy with a gestational age of less than 37 weeks, a bodyweight of less than 2.5 kg, and a head size of less than 33 cm. 2. Premature infants receiving nutritional supply according to the grouping with obtained informed consent from their parents. Exclusion criteria: 1. Infants with neonatal asphyxia, congenital gastrointestinal malformation, genetic metabolic disease, neonatal respiratory distress syndrome, or birth injury. 2. Infants with a hospitalization time of less than 7 days. 3. Infants whose mothers had pregnant gestational complications, such as preeclampsia and gestational diabetes mellitus.

Methods of nutrition supply

Infants in the control group were given oral or nasal breastfeeding based on the sucking, swallowing, and gastrointestinal tolerance of the infants, and those requiring more nutrition than the given breast milk, as suggested by the "water amount for newborns and premature infants at different ages" were given additional glucose solution and electrolyte [8]. The collection, storage, and transfer of breast milk were carried out in strict accordance with relevant requirements. Breastfeeding was started within 12 hours after the birth and infants were awakened and fed several times. The amount of breast milk was from an initial volume of 1 mL/kg to a final 150 mL/kg, in a stepwise increase of 2 mL each time. The amount of breast milk was adjusted in time if excessive feeding, abdominal distension, and vomiting occurred or as required by the abdominal circumference and abdominal X-rays. Infants were kept in warm conditions at a room temperature of 28 to 30°C and were monitored for blood oxygen, heart rate, and breathing and monitored for infection and bleeding. Non-nutritive sucking of a pacifier for 30 minutes was also performed 3 hours after breastfeeding. The infants were caressed on the head, face, chest, abdomen, back, hands, feet, and limbs with a stepwise ramp-up in the massage strength and rubbed on their large muscle groups, 30 minutes before the breastfeeding when infants were awake and quiet, twice a day. The massage time was gradually extended from 1 minute to 15 minutes.

In addition to breastfeeding and infant touch designed for the control group, infants in the observation group also received early paren-

Table 2. Comparison of the dynamic expression of serum ghrelin between the two groups (ug/L)

Group	Control group	Observation group	t	P
Number	100	100		
24 h	46.8±9.2	86.7±13.5	23.432	<0.001
72 h	223.5±35.4	403.6±65.7	35.264	<0.001
7 d	652.3±68.7	896.7±86.7	65.325	<0.001
$t_{72\ h/24\ h}/t_{7\ d/72\ h}$	4.277/4.955	5.313/4.762		
$P_{72\ h/24\ h}/P_{7\ d/72\ h}$	0.024/0.015	0.006/0.017		

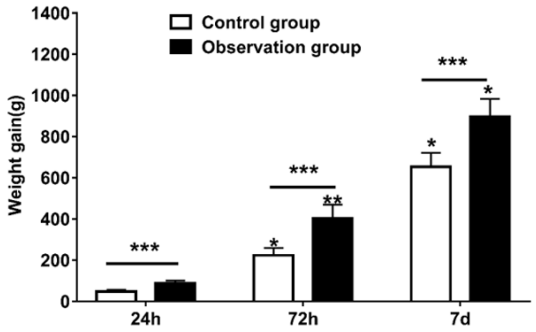

Figure 2. Comparison of weight gain between the two groups at different time. Compared with control group, *P<0.05; Compared with control group, **P<0.01; Compared with control group, ***P<0.001.

teral nutrition through the peripherally inserted central catheter (PICC). Infants were given an intravenous drip of Paediatric Compound Amino Acid Injection (50 mL, Shanghai Changzheng Fumin Jinshan Pharmaceutical Co., Ltd.) at a dose of 35-50 mL/kg/day at a slow drip rate, as well as a slow intravenous drip of parenteral nutrition solution at an appropriate concentration which was made from carbohydrates (70 mL/kg), fat (1.0 g/kg), and a proper amount of vitamins and trace elements. Parenteral nutrition input was stopped when intestinal nutrition reached 110-130 kCal/kg per day.

Outcome measures

ELISA assay was employed to measure the dynamic expression of ghrelin in the femoral venous blood of infants at 24 h, 72 h, and 7 d after the delivery. Weight gain, albumin, and globulin levels were compared between the two groups at each time point. Approximately 6 mL of fasting peripheral venous blood was drawn from infants and stored in an anticoagulant tube and was centrifuged at 2500 r/min for 15 minutes to collect the upper serum. Ghrelin reagent was purchased from Jiangsu Beyotime Technology Co., Ltd. and its detection was performed according to the reagent instructions. Radioimmunoassay was used to detect albumin and globulin using Hitachi Fully automatic 3700 biochemical detector (Japan) and supporting reagents.

Statistical analysis

Statistical analysis was performed by SPSS 20.0 statistical software. The measurement data were expressed by the mean ± standard deviation and their pairwise comparison was analyzed by the LSD-t test. The count data were expressed by the case number or percentages and their pairwise comparison was analyzed by the χ^2 test. The difference was statistically significant when P<0.05.

Results

Comparison of baseline data of infants and their mothers between the two groups

Premature infants were randomly divided into either the control group or the observation group, with 100 cases in each group. The two groups of infants were comparable in sex, gestational age, birth weight, birth head size, delivery mode, age of the mother in pregnancy, body mass index of the mother, and the primipara/multipara ratio (P>0.05). More details are shown in **Table 1**.

Comparison of the dynamic expression of serum ghrelin between the two groups

Serum ghrelin expression at different time points in the two groups was measured by ELISA assay. The serum ghrelin expression underwent a stepwise increase in the premature infants at 24 h, 72 h, and 7 d in both groups, and the serum ghrelin expression was markedly higher in the observation group than in the control group at each time point (P<0.05). More details are shown in **Figure 1** and **Table 2**.

Comparison of weight gain between the two groups

In both groups, the body weights of infants at 24 h, 72 h, and 7 d were higher than the birth

Table 3. Comparison of weight gain between the two groups (g)

Group	Control group	Observation group	t	P
Number	100	100		
24 h	46.8±9.2	86.7±13.5	23.432	<0.001
72 h	223.5±35.4	403.6±65.7	35.264	<0.001
7 d	652.3±68.7	896.7±86.7	65.325	<0.001
$t_{72\ h/24\ h}/t_{7\ d/72\ h}$	4.277/4.955	5.313/4.762		
$P_{72\ h/24\ h}/P_{7\ d/72\ h}$	0.024/0.015	0.006/0.017		

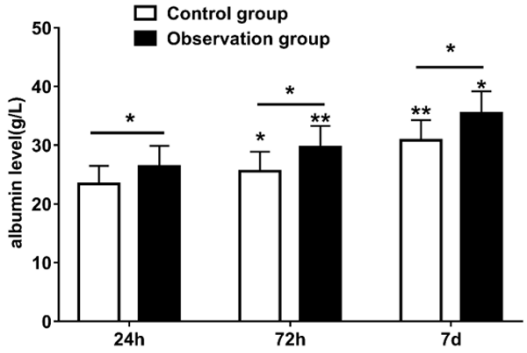

Figure 3. Comparison of serum albumin levels between the two groups. Compared with control group, *P<0.05; Compared with control group, **P<0.01.

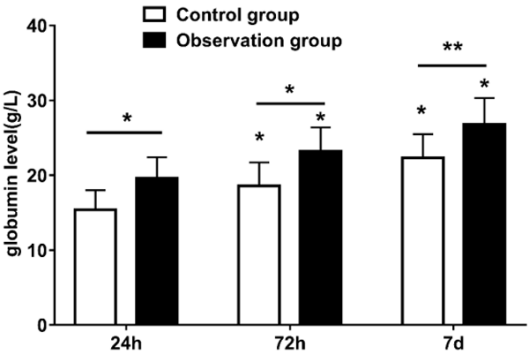

Figure 4. Comparison of globulin levels between the two groups. Compared with control group, *P<0.05; Compared with control group, **P<0.01.

weight, and the weight gain in the observation group at each time point (compared with the birth weight) was significantly higher than that in the control group (P<0.05). More details are shown in **Figure 2** and **Table 3**.

Comparison of serum albumin and globulin levels between the two groups

The serum albumin and globulin levels underwent a stepwise increase in the premature infants at 24 h, 72 h, and 7 d in both groups, and the serum albumin and globulin levels were markedly higher in the observation group than in the control group at each time point (P<0.05). More details are shown in **Figures 3**, **4** and **Tables 4**, **5**.

Discussion

As compared with full-term infants, premature infants have underdeveloped gastrointestinal structure and function, less gastrointestinal hormones and digestive enzymes, lower absorption and metabolic efficiency of nutrients, and higher risks of abdominal distension, vomiting, and feeding intolerance [9, 10]. To prevent complications such as hypothermia and jaundice, the vital signs of premature infants should be closely monitored in an independent space, which can lead to postponed first breastfeeding, irregular feeding, inappropriate amount of breast milk, and delayed nutritional supply [11, 12]. Parenteral nutrition support is the main method to solve this problem. The parenteral nutrition solution, containing a variety of vitamins, electrolytes, glucose, amino acids, and trace elements, can provide premature infants who are incapable of normal feeding with the same nutrients as provided in the uterus, ensuring a proper trophic basis outside the uterus. Unlike breastfeeding that may be insufficient in the milk volume, early parenteral nutrition can adjust the supply according to the nutritional needs of premature infants at different ages to ensure normal physical growth [13, 14]. Jin Xia stated that total parenteral nutrition could significantly enhance nutrient absorption, promote behavioral development, and strengthen immune function in premature infants [15]. Parenteral nutrition solution can mimic the composition of breast milk to promote the absorption efficiency in premature infants [16] and reduce the risk of feeding intolerance and parenteral nutrition associated cholestasis (PNAC) [17]. PNAC is more common in premature infants than in full-term infants, especially in those receiving parenteral nutrition (an incidence of approximately 18-24%), severely suppressing the normal physical and mental development of infants [18, 19]. Chen Xu found that early minimal feeding combined with parenteral nutrition could significantly improve the clinical condition of premature infants, relieve the symptoms of vomiting and

Table 4. Comparison of serum albumin levels between the two groups (g/L)

Group	Control group	Observation group	t	P
Number	100	100		
24 h	23.4±3.1	26.4±3.5	3.659	0.025
72 h	25.6±3.3	29.7±3.6	3.926	0.02
7 d	30.8±3.5	35.4±3.8	4.125	0.018
$t_{72\ h/24\ h}/t_{7\ d/72\ h}$	4.113/5.523	5.076/4.205		
$P_{72\ h/24\ h}/P_{7\ d/72\ h}$	0.022/0.003	0.005/0.020		

Table 5. Comparison of globulin levels between the two groups (g/L)

Group	Control group	Observation group	t	P
Number	100	100		
24 h	15.4±2.6	19.6±2.8	4.256	0.016
72 h	18.6±3.1	23.2±3.2	4.468	0.011
7 d	22.3±3.2	26.8±3.5	4.986	0.007
$t_{72\ h/24\ h}/t_{7\ d/72\ h}$	3.776/4.008	3.245/3.779		
$P_{72\ h/24\ h}/P_{7\ d/72\ h}$	0.031/0.023	0.033/0.030		

abdominal distension, promote recovery, and shorten the time of hospital stay [20].

Ghrelin, a peptide hormone composed of 28 amino acids, is mainly secreted by gastric mucosal gland cells and is present in small amounts in the placenta, small intestine, hypothalamus, and cerebral cortex. Ghrelin level is affected by hormones and nutritional status and is found to be negatively correlated with the growth of premature infants [21]. Studies have also proved the close relationship between ghrelin and feeding intolerance, malnutrition, and growth retardation in premature infants. A previous study holds that properly designed feeding modes for different premature infants, combined with enteral nutrition, non-nutritive sucking, and soft skin touch, could effectively boost the growth and development of infants, increase the expression of ghrelin, and enhance the function of T lymphocytes to achieve catch-up-growth [22]. So far few studies have investigated the effects of parenteral nutrition or breastfeeding on serum ghrelin expression and its dynamic changes, or investigated whether the change in serum ghrelin levels affects the growth and immune function of premature infants [23, 24]. This study focused on the effects of early parenteral nutrition combined with breastfeeding in premature infants. We found that the serum ghrelin expression at 24 h, 72 h, and 7 d after the delivery, weight gain, albumin levels, and globulin levels were markedly higher in the observation group than in the control group ($P<0.05$). Such findings suggest that the use of early parenteral nutrition in premature infants who cannot be breastfed in time or who have low breastfeeding efficiency, can increase the ghrelin expression, improve physical development and neurobehavioral ability, and strengthen immune function. Parenteral nutrition may regulate serum ghrelin concentration in premature infants, but the specific underlying mechanism is not yet clear. Previous studies have confirmed that parenteral nutrition can affect serum ghrelin levels and regulate its synthesis and secretion or its receptor expression in premature infants, improve energy metabolism and feeding tolerance, rationalize and upgrade the nutrition supply, and promote infant growth, providing guidance for the treatment of premature infants [25, 26].

The novelty of this study lies in noting the role of ghrelin in the growth and development of premature infants. Early parenteral nutrition or enteral nutrition in premature infants who cannot be breastfed in time or have low breastfeeding efficiency can increase ghrelin expression, boost the development of digestive system function, facilitate energy absorption, and promote catch-up-growth and intellectual development. This study is limited by the small sample size and short observation time, so further research should be made to verify the results of this study.

In summary, early parenteral nutrition combined with breastfeeding can significantly increase the expression level of serum ghrelin in premature infants, promote infant growth, and strengthen immune function.

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Disclosure of conflict of interest

None.

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References

[1] Lewis KA and Brown SA. Searching for evidence of an anti-inflammatory diet in children: a systematic review of randomized controlled trials for pediatric obesity interventions with a focus on leptin, ghrelin, and adiponectin. Bio Res Nurs 2017; 19: 511-530.

[2] Gonzalez KW, Weaver KL, Biondo DJ, Lim JD and Hendrickson RJ. Cycling parenteral nutrition in a neonatal surgical patient: an argument for increased utilization. J Pediatr Surg Case Rep 2017; 16: 1-4.

[3] William W Hay. Optimization of nutrition in premature infants. Chin J Contemp Pediatr 2017; 19: 1-21.

[4] Qi G, Juan ZY and Ying TX. Effect of addition time of breast milk enhancer on early growth and development and incidence of complications in very low birth weight infants. J App Clin Ped 2017; 32: 528-531.

[5] Wei HJ, Li WL and Ping QY. Study on the correlation between serum ghrelin level and feeding intolerance in preterm infants. Anhui Med J 2013; 34: 877-879.

[6] Ye Z, Mei RY and Yan LY. Strategies of parenteral nutrition for premature infants. Chin J Gen Pract 2017; 16: 122-127.

[7] Vasquez-Garibay EM, Larrosa-Haro A, Guzman-Mercado E, Munoz-Esparza N, Garcia-Arellano S, Munoz-Valle F and Romero-Velarde E. Serum concentration of appetite-regulating hormones of mother-infant dyad according to the type of feeding. Food Sci Nutr 2019; 7: 869-874.

[8] Dan S, Nan LY and Na JY. Effect of different feeding methods on the growth and development of premature infants, ghrelin and the level of cellular immunity. Mate Child Health Care Chin 2019; 34: 1778-1781.

[9] Zhang DL, Du Q, Djemli A, Julien P, Fraser WD and Luo ZC. Cord blood insulin, IGF-I, IGF-II, leptin, adiponectin and ghrelin, and their associations with insulin sensitivity, beta-cell function and adiposity in infancy. Diabet Med 2018; 35: 1412-1419.

[10] Kucuk N, Orbak Z, Karakelloglu C and Akcay F. The effect of therapy on plasma ghrelin and leptin levels, and appetite in children with iron deficiency anemia. J Pediatr Endocrinol Metab 2019; 32: 275-280.

[11] Lin H, Jie L and Mei ZC. Progress in prevention and treatment of parenteral nutrition-associated cholestasis in premature infants. Chin J Reprod Health 2018; 29: 293-296.

[12] Lane E and Murray KF. Neonatal cholestasis. Pediatr Clin N Am 2017; 64: 621-639.

[13] Bi ZD, Ting DY and Mei HX. Effect of early parenteral nutrition on post-discharge growth and development of premature low birth weight infants. Lab Immun Clin Med 2019; 26: 511-515.

[14] Özdemir ZC and Akşit MA. The association of ghrelin, leptin, and insulin levels in umbilical cord blood with fetal anthropometric measurements and glucose levels at birth. J Matern Fetal Neonatal Med 2020; 33: 1486-1491.

[15] Xia J. To explore the effect of total parenteral nutrition on nutrition, immune function and behavioral development of premature infants. Chin Pract Med 2016; 11: 102-104.

[16] Huang LL, Yang F and Xiong F. Association of leptin, adiponectin, and ghrelin in breast milk with the growth of infants with exclusive breastfeeding. Zhongguo Dang Dai Er Ke Za Zhi 2018; 20: 91-96.

[17] Ming LM. Effect of parenteral nutrition combined with early enteral nutrition on nutritional status of premature infants. J Parenter Enteral Nutr 2016; 23: 358-360, 364.

[18] Warchol M, Wojciechowska M, Kupsz J, Sot-Szewczyk MH, Michalak M, Kolodziejski P, Pruszynska-Oszmalek E and Krauss H. Association of cord blood ghrelin, leptin and insulin concentrations in term newborns with anthropometric parameters at birth. J Pediatr Endocrinol Metab 2018; 31: 151-157.

[19] Khodabakhshi A, Mehrad-Majd H, Vahid F and Safarian M. Association of maternal breast milk and serum levels of macronutrients, hormones, and maternal body composition with infant's body weight. Eur J Clin Nutr 2018; 72: 394-400.

[20] Xun C and Qing DZ. Clinical analysis of early minimal feeding combined with parenteral nutrition in premature infants. Chin Med Pharm 2019; 9: 87-89.

[21] Ohkawa N, Shoji H, Ikeda N, Suganuma H and Shimizu T. Relationship between insulin-like growth factor 1, leptin and ghrelin levels and catch-up growth in small for gestational age infants of 27-31 weeks during neonatal intensive care unit admission. J Paediatr Child Health 2017; 53: 62-67.

[22] Kara M, Orbak Z, Doneray H, Ozkan B and Akcay F. The relationship between skinfold thickness and leptin, ghrelin, adiponectin, and

resistin levels in infants of diabetic mothers. Fetal Pediatr Pathol 2017; 36: 1-7.
[23] Gomez-Diaz RA, Gomez-Medina MP, Ramirez-Soriano E, Lopez-Robles L, Aguilar-Salinas CA, Saucedo R, Zarate A, Valladares-Salgado A and Wacher NH. Lower plasma ghrelin levels are found in women with diabetes-complicated pregnancies. J Clin Res Pediatr Endocrinol 2016; 8: 425-431.
[24] Andreas NJ, Hyde MJ, Herbert BR, Jeffries S, Santhakumaran S, Mandalia S, Holmes E and Modi N. Impact of maternal BMI and sampling strategy on the concentration of leptin, insulin, ghrelin and resistin in breast milk across a single feed: a longitudinal cohort study. BMJ Open 2016; 6: e010778.
[25] Yu X, Rong SS, Sun X, Ding G, Wan W, Zou L, Wu S, Li M and Wang D. Associations of breast milk adiponectin, leptin, insulin and ghrelin with maternal characteristics and early infant growth: a longitudinal study. Br J Nutr 2018; 120: 1380-1387.
[26] Chen J, Miao X and Rong WG. Relationship between serum ghrelin and severity of disease and gastrointest dysfunction in children with hand, foot and mouth disease. Zhongguo Er Tong Bao Jian Za Zhi 2019; 7: 1-4.