

Beta Endorphin Concentrations in Human Milk

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ABSTRACT

Background: The source and regulatory mechanisms that elevate beta-endorphin (β -EP) approximately twofold higher than circulating plasma levels in the colostrum of lactating mothers are still unknown, and no studies have examined β -EP availability previously during maturation phases of human milk. Therefore, the aim of this study was to determine whether concentrations of β -EP vary over time between colostrum, transitional, and mature breast-milk and to evaluate whether this depends on the method of delivery.

Methods: Mothers of healthy full-term and pre-term newborn infants who planned to breast-feed their newborn infants were considered for this study. They were consecutively recruited in one of 3 groups of 14, according to delivery method: group 1, vaginal delivery at term (gestational age 40.2 ± 0.3 weeks; birth weight, 3.48 ± 0.09 kg); group 2, preterm vaginal delivery (gestational age, 35.6 ± 0.3 weeks; birth weight, 2.49 ± 0.08 kg); and group 3, at-term elective cesarean section (gestational age, 39.0 ± 0.3 weeks; birth weight, 3.32 ± 0.14 kg). Three consecutive breast milk samples were obtained on the fourth day after birth, before each mother's discharge, and thereafter on the 10th and 30th postpartum days, close to expression of the colostrum, transitional, and mature milk production phases, respectively, to test β -EP concentrations (β -Endorphin ^{125}I RIA; INCSTAR Corporation, Stillwater, MN). Data are presented as mean \pm standard deviation. Statistical comparison of β -EP concentration among the three lactating mother groups was performed using the Kruskal-Wallis nonparametric test. In addition, to test the hypothesis of a trend toward smaller values

with time of β -EP, the authors computed within each mother group a *P* value per trend (Kruskal-Wallis test) of β -EP concentration averages on the 4th, 10th, and 30th days, respectively. Student's *t* test for independent samples was used for the analysis of the other data. The 0.05 significance level was used in the statistical analysis. All computations were made by computer.

Results: Colostrum β -EP concentrations on the fourth postpartum day of group 1 and group 2 mothers who were delivered of a neonate vaginally, at term, or prematurely were significantly higher ($P < 0.01$) than colostrum levels of group 3 mothers who underwent cesarean section. Group 2 mothers who were delivered of a neonate vaginally and prematurely presented the highest β -EP concentrations ($P < 0.05$), lasting until the transitional milk phase (10th day). No significant differences were found across all 3 groups of lactating mothers in mature milk (30th day) β -EP concentrations. In addition, the β -EP trend toward smaller values with time within each of the three groups on days 4, 10, and 30 was statistically significant ($P < 0.01$ per trend).

Conclusions: It is hypothesized that elevated β -EP concentrations in colostrum and transitional milk of mothers who were vaginally delivered of infants may contribute to postnatal fetal adaptation, to overcoming birth stress of natural labor and delivery, and at the same time to the postnatal development of several related biologic functions of breast-fed infants. *JPGN* 33:160–164, 2001. **Key Words:** Beta endorphin—Full-term—Human milk—Labor pain—Longitudinal—Premature. © 2001 Lippincott Williams & Wilkins, Inc.

The milk composition of breast-feeding mothers develops and changes from colostrum to mature milk to guarantee the satisfaction of the different needs of growing newborn infants. However, the change during the course of lactation in many bioactive compounds has not yet been considered fully (1).

The source and regulatory mechanisms that make available elevated beta-endorphin (β -EP) concentrations,

approximately twofold higher than circulating plasma levels, in colostrum of lactating mothers are still unknown, and no studies have examined β -EP availability previously during maturation phases of maternal milk in relation to method of delivery (2,3).

The possibility that the gastrointestinal tract of suckling mammals, which absorbs various proteins and opioid peptides derived from human β -casein (4), may preserve the opioid properties of these substances cannot be excluded (5,6). Thus, the presence of β -EP in human milk may be of importance in overcoming stressful perinatal situations along with vaginal delivery and in the postnatal development of several related biologic func-

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tions of breast-fed infants, like analgesia, steroidogenesis, cardiovascular and endocrine functions, neuroimmunomodulation, sleep-wake patterns, and behavior (7,8).

The purpose of the current study was to investigate whether concentrations of β -EP vary over time between colostrum, transitional, and mature milk and to determine whether this depends on the method of delivery.

MATERIALS AND METHODS

Participants

Mothers of healthy at term and preterm newborn infants who planned to breast-feed their newborn infants were considered for this study. They were recruited consecutively in one of 3 groups of 14, according to delivery method: group 1, vaginal delivery at term (gestational age, 40.2 ± 0.3 weeks; birth weight, 3.48 ± 0.09 kg); group 2, preterm vaginal delivery (gestational age, 35.6 ± 0.3 weeks; birth weight, 2.49 ± 0.08 kg); and group 3, at-term elective cesarean section (gestational age, 39.0 ± 0.3 weeks; birth weight, 3.32 ± 0.14 kg).

Inclusion criteria for mothers were absence of any complication of pregnancy, delivery, and puerperium (particularly hypertension, eclampsia, infection, duration of stage 1 labor >24 hours, dystocia, or agalactia), and in the neonates, absence of fetal distress, birth asphyxia, sepsis, major congenital abnormalities, or other significant disease.

On admission, the women received a minimum of 30 minutes of continuous electronic fetal heart rate monitoring to assess fetal status. Electronic fetal heart-rate monitoring or intermittent auscultation was performed every 30 minutes in the first stage and every 15 minutes in the second stage of labor of vaginal delivery.

Mothers underwent at-term elective cesarean section (contracted pelvis, breech presentation, or previous cesarean section) in the absence of labor and apparent antepartum fetal distress. For conventional spinal analgesia, the women received 2 ml of 0.5% bupivacaine in the form of bolus doses. After initiation of analgesia, electronic fetal heart rate monitoring was continued for at least 30 minutes.

In an attempt to eliminate the influence on maternal milk β -EP concentrations of other factors other than method of delivery, exclusion criteria were oxytocin administration to augment labor or to stimulate uterine contractions, and cases of preterm cesarean section for fetal or maternal delivery complications. Mothers of near-term premature infants incapable of autonomous breast-feeding were also excluded, as well those who did not breast-feed their infants exclusively during all study phases.

Sample Collection

Breast milk samples of lactating mothers were obtained before mother discharge on the fourth day after birth, and thereafter on the 10th and 30th postpartum days, close to expression of the colostrum, transitional, and mature milk production phases, respectively (9). All breast milk samples were collected between 6 AM and 8 AM after an overnight bed rest period.

Women were enrolled in the study until there were 14 in all study groups.

All mothers initially breast fed their babies in rooming-in nursing regimens, starting from the first postpartum day. Mothers breast fed ad libitum and were able to provide milk at each sampling point. On the study day, they were asked to modify their routine; before beginning the morning's first feeding, they partly emptied one or both breasts manually by pump into sterile containers to obtain an aliquot of 4 mL to 5 mL of breast milk, 1 mL of which was required for β -EP RIA analysis.

Sample Analysis

All samples were frozen immediately and temporarily stored at -20°C until assayed. Freezing does not significantly modify β -EP concentration. For the determination of β -endorphin-like immunoreactivity, a commercially available β -Endorphin ^{125}I RIA kit (INCSTAR Corporation, Stillwater, MN) was used, according to the modified method of Guillemin et al. (10). After thawing, β -EP was extracted from milk samples by rabbit anti- β -endorphin coated Sepharose particles. Phase separation was achieved by a second antibody method. The assay showed less than 5% cross-reactivity with β -lipotropin. The minimum detectable amount was less than 2 pmol/L. Values reported are the mean of two separate aliquots of sample.

Statistical Analysis

Data are presented as mean \pm standard deviation. Statistical comparison of β -EP concentration among the three lactating mother groups was performed using the Kruskal-Wallis nonparametric test. In addition, to test the hypothesis of a trend toward smaller values with time of β -EP, we computed within each mother group a *P* value per trend (Kruskal-Wallis test) of β -EP concentration averages on the 4th, 10th, and 30th days, respectively. Student *t* test for independent samples was used for the analysis of the other data. The 0.05 significance level was used in the statistical analysis. All computations were made by Digital-Alpha 255 computer (Compaq, Houston, TX).

Ethical Considerations

This study was performed after approval from the Human Ethics Committee of the hospital, and with informed consent from the mothers.

RESULTS

Maternal and neonatal characteristics are listed in Table 1.

Levels of β -EP in the colostrum, transitional, and mature milk of mothers of healthy full-term and preterm newborn infants who planned to breast-feed their newborn infants after vaginal delivery at term (group 1), preterm vaginal delivery (group 2), and at-term elective cesarean section (group 3), respectively, are shown in Figure 1.

TABLE 1. Maternal and neonatal characteristics

Mothers	Premature vaginal delivery	Term vaginal delivery	Elective cesarean section
Number	14	14	14
Nulliparous (%)	8 (19)	4 (9.5)*	8 (19)
Age (y)	30.1 ± 1.0	31.2 ± 1.1	31.1 ± 0.9
Neonates			
Gestational age (wk)	35.6 ± 0.3*	40.2 ± 0.3	39.0 ± 0.3
Apgar score			
1 min	8.6 ± 0.1	8.8 ± 0.4	9.2 ± 0.1
5 min	9.5 ± 0.2	9.5 ± 0.1	9.8 ± 0.1
Weight (kg)			
At birth	2.49 ± 0.08*	3.48 ± 0.09	3.32 ± 0.14
3rd day % decrease	-10.4	-10.5	-10.9
30th day	3.56 ± 0.15	4.55 ± 0.16	4.24 ± 0.18

Mean ± SD.

* P < 0.01 (Poisson statistics).

Colostrum β-EP concentrations on the fourth postpartum day of group 1 and group 2 mothers who were delivered vaginally of a neonate, at-term, or prematurely were significantly higher ($P < 0.01$) than colostrum levels of group 3 mothers, who underwent cesarean section. Moreover, group 2 mothers who were delivered vaginally of a neonate and prematurely presented lasting higher β-EP concentrations until transitional milk phase ($P < 0.05$). No significant differences were instead found across all three groups of lactating mothers in mature milk (30th day) β-EP concentrations. In addition, the β-EP trend toward smaller values with time within each of the three groups from the 4th to the 10th to the 30th day was statistically significant ($P < 0.01$ per trend).

DISCUSSION

The regulatory mechanisms and the biologic effects on the neonate of elevated β-EP concentrations available in human colostrum, approximately twofold higher than in plasma of breast-feeding women (9), and in transitional and mature milk still remain unclear. Passive diffusion from maternal plasma to milk, active concentration of plasmatic peptides in the mammary gland acini, and mammary endogenous productions are indicated as responsible, concentrating mechanisms for the elevated levels in colostrum (2,3), in addition to suckling, for stimulus per se to be able to induce β-EP release in nursing humans (11). Given that the neonatal gastrointestinal tract exhibits less proteolytic activity and is more permeable to proteins than older individuals, these along with milk-borne opioid peptides may be absorbed intact, playing important roles in the related developing functions of breast-fed newborn infants (4–6). A significant amount of colostral opioids may thus reach the central nervous system by circulating levels, and we can assume, bearing in mind this extrapituitary source of the peptide, that mammalian levels could reflect partially their central and peripheral effects on related developing functions (7,8).

In the current study, we found that β-EP concentrations in colostrum of lactating women were higher after vaginal at term and premature delivery, when compared with colostrum levels of mothers after at-term elective cesarean section. The increased opioid levels in colostrum after vaginal delivery are in line with the reported increase in circulating β-EP concentrations in mothers

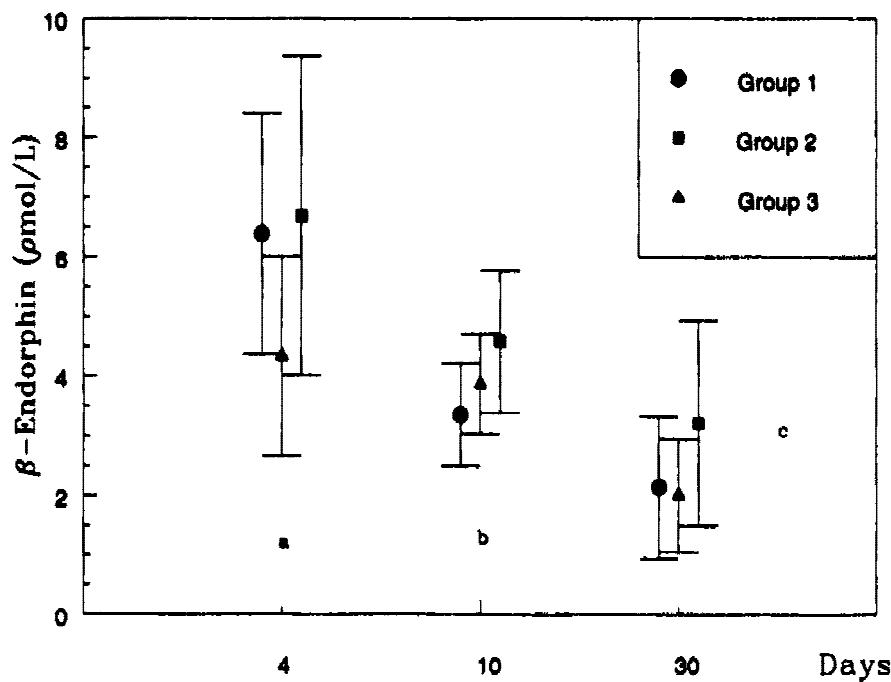


FIG. 1. β-Endorphin concentrations in colostrum (4th day), transitional (10th day), and mature human milk (30th day) of breast-feeding mothers after at-term vaginal delivery of a neonate (group 1), premature delivery of a neonate (group 2), and elective at-term cesarean section (group 3), respectively. Colostrum β-endorphin concentrations of group 1 and group 2 mothers are significantly higher ($P < 0.01$). Transitional milk β-endorphin concentrations of group 2 mothers are significantly higher ($P < 0.05$). The β-endorphin trend toward smaller values with time, within each of the three groups, is statistically significant (P per trend < 0.01).

after the pain experience of natural labor and vaginal parturition (12) and with previous studies that demonstrated that plasma opioid concentrations increase during pregnancy (13), with a significant further increase in the first and second stages of labor (14).

Labor pain, a central aspect of vaginal labor and delivery, has been hypothesized to play a role in stimulating maternal hormonal, vascular, and physiologic systems, which, in concert, contribute to postnatal fetal adaptation (15,16). The possibility that vaginal delivery and the first related hours of extrauterine life may represent a stressful situation for the neonate is supported by the three times higher than normal β -EP levels present in cord plasma and during the first 24 hours of life and by higher plasma cortisol levels found in newborn babies vaginally delivered when compared with neonates delivered by elective cesarean section, in whom the stimulation of the adrenal gland and the stressful peripartal events are reduced (13,17). As a consequence, different β -EP availability in colostral milk seems to favor a mammalian-concentrating finalized effect rather than simply being concomitant with the elevated protein concentrations present in colostral milk (18,19).

Although the physiologic significance of different β -EP levels in colostrum of lactating women may reflect the conceivable roles of β -EP in reducing by central and peripheral action the level of pain the fetus must endure during labor (20), the hypothetical link between the elevated levels of β -EP measured at 4 and 10 days after birth, and birth stress remains unknown, as does the physiologic significance for later postnatal life of maternal milk as a potential enteral source of β -EP. Nevertheless, the milk analyzed in this study reflects what would be received by newborn infants, in particular during the first days when breast milk emptying would be complete. Furthermore, the presence in the neonatal circulation, up to the fourth day of life, of elevated β -EP values, similar to those found in cord plasma (21), seems to indicate that the neonates are capable of sustaining high levels during the first days of postnatal life and that milk β -EP supply could play a contributing role early after delivery (22) and in the postnatal development of several related biologic functions of newborn infants, including the endocrine and the immune response, the sleep-wake patterns (7,8), imprinting, the learning process, feeding patterns, and behavior (21,23).

Our results are limited because we did not evaluate the effect of β -EP availability, related to delivery methods, parity, and individual or diurnal variations, on behavior and development of related biologic function of breast-fed newborn infants. Nevertheless, lasting high β -EP levels in colostrum of mothers of vaginally delivered at-term and preterm newborns suggest a functional role for human milk opioids above that of postnatal adaptation, which may be related to birth stress. Thus, neonates vaginally delivered and those born by cesarean section

performed after prolonged labor may be at a disadvantage in β -EP availability if their mothers have agalactia or difficulty in expressing reasonable amounts of colostrum, transitional, and mature milk.

In conclusion, milk β -EP concentrations of lactating mothers are modified in the colostrum by method of delivery and the related presence or absence of uterine contractions and labor pain, but not by moderate near-term prematurity. If we consider the different needs of growing newborn infants, it is possible that human milk, satisfying a number of important criteria and specific requests for the human neonate, develops and changes in composition and in β -EP content to guarantee adequate supply from the first hours of life. Future studies should be extended to the evaluation of the impact of β -EP milk changes on the behavioral well being of healthy and sick neonates.

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