

Applied nutritional investigation

β -Endorphin concentration in colostrums of Burkinabe and Sicilian women

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Abstract

Objective: Endorphins (EPs) present in human colostrum may be relevant for immediate postnatal fetal adaptation because this compound is involved in stress response and adaptation mechanisms. Endorphin levels in human colostrum are two-fold greater than corresponding maternal plasma levels; however, the high endorphin levels in human milk decrease as lactation continues. The aim of this study was to determine the β -EP concentration in colostrums of women residing in Burkina Faso and Sicily. In addition, we investigated the source of potential differences in β -EP levels between these populations, especially ethnic sources of these deviations.

Methods: The concentration of β -EP was determined in the colostrum from the first 3 d subsequent to delivery by an enzyme immunoassay as immunoreactive material (IRM).

Results: The production of β -EP in the colostrum was significantly higher in Burkinabe mothers (0.83 ± 0.04 ng/mL) than in Sicilian mothers (0.31 ± 0.02 ng/mL) at 24 h after delivery. Colostrum levels of β -EP declined progressively during the first 3 d after delivery in both populations (0.64 ± 0.1 and 0.28 ± 0.015 ng/mL, respectively, at 72 h). The level of β -EP-IRM correlated significantly with pain and psychological involvement during and after delivery. In addition, the correlation between β -EP-IRM and length of stage II of labor was significant ($P < 0.0001$) in the colostrums of Sicilian mothers who received ergot derivatives, episiorrhaphy, and child birth preparation. The correlation between β -EP-IRM and length of stage II was less significant ($P < 0.001$) in the colostrums of Burkinabe mothers who received neither ergot derivatives nor child birth preparation.

Conclusion: During the first 3 d after labor the β -EP-IRM concentration in the colostrums of Burkinabe mothers differs from that of Sicilians. In addition, because Burkinabe women produce a larger volume of colostrum, their newborns receive, during the first days of life, a larger absolute amount of β -EP-IRM, likely resulting in better postnatal fetal adaptation. © 2008 Elsevier Inc. All rights reserved.

Keywords: β -Endorphin; Colostrum; Burkinabe women; Sicilian women

Introduction

Human milk contains a multitude of substances that augment the adaptation of newborns to extrauterine life and

whose concentrations are regulated by a biological watch modulated by neuroendocrine and immune factors [1–3].

β -Endorphin (β -EP), a pro-opiomelanocortin fragment released from the pituitary gland, is involved in stress response and adaptation mechanisms [4]. It likely participates in the adaptation of the immune system, which is relevant for tissue reconstitution after injury (e.g., “wound healing”) [5]. The role of β -EP in the relation between mother and

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newborn is complex and continues after delivery. β -EP is released in relatively large amounts into the maternal and fetal circulations during labor in response to stress and pain [6].

The β -EP concentration begins to increase in maternal plasma at the 28th week of pregnancy, reaching a peak during labor and delivery [7]. Moreover, β -EP concentrations are highest during the expulsion phase of delivery and may help women to tolerate acute pain [8]. The concentration of β -EP in maternal plasma decreases drastically immediately after childbirth and the puerperium phase [9].

The β -EP in newborn plasma is important for the development of several adaptive functions such as analgesia, steroid genesis, and cardiovascular and endocrine functions [7,8,10] and in the adaptation to extrauterine stress [11]. In fact, mean fetal β -EP concentrations are significantly lower than values measured in newborns but significantly higher than mean maternal values. Although the fetal pituitary gland is likely the primary source of circulating fetal β -EP, a maternal or placental contribution has not been excluded [12].

Moreover, β -EP represents a physiologic agonist of μ - and δ -opioid receptors by modulating the regulation of endogenous Ca^{2+} levels [13,14] and contributing to adaptive function through analgesia.

After delivery, when lactation begins, β -EP is secreted in human milk at a level twice that of maternal plasma by an unknown mechanism [15]. Although the adaptive and nutritional values of human milk have been thoroughly studied [1–3], few studies have addressed β -EP levels and function in human milk [15,16].

Zanardo et al. [15,16] in 2001 reported that β -EP levels progressively decrease during the maturation phases of lactation, suggesting that this peptide has its maximum effect on neonates during the first days of lactation. However, the volume of milk ingested by infants increases during the early phase of lactation, supporting the hypothesis that total daily transfer of β -EPs could remain constant during lactation.

This study aimed to determine β -EP concentration in human colostrum produced during the first 3 d postpartum and to correlate β -EP levels with anthropologic characteristics of the mothers, the modality and pain of deliveries, and the psychological involvement by Burkinabe and Sicilian mothers.

Material and methods

Study area

Colostrum samples from 53 Burkinabe women were collected between July and October 2002 at the Maternity Centre Medical Saint Camille (CMSC) in Ouagadougou (Burkina Faso), where 25–30 deliveries occur daily. Burkina Faso (formerly Upper Volta) was once a French colony but it gained its independence in 1960 and is currently one of the poorest countries in West Africa. The

population of 11–12 million people consists of several ethnic groups including the Mossi, Peuhul, Gurunsi, and Bobo. They are primarily shepherds or non-nomadic farmers and live in sod-and-thatch huts in small, rural villages. Their socioeconomic status is poor and their hygienic/sanitary conditions are defective with a bad water supply.

Colostrum samples from 30 Sicilian women were collected between October and November 2002 at the Maternity Ward of St. Bambino Hospital of Catania, which is located in east Sicily, Italy.

All samples were from mothers who delivered vaginally at term. Ethical approval for the study was received by the institutional review board at the CMSC and St. Bambino Hospital.

Subjects

Data on anthropologic characteristics of mothers including age, number of deliveries, gestational age, child birth preparation, assistance to delivery, length of delivery periods, obstetric complications such as episiorrhaphy, infections, requirement of antibiotics, and pain or psychological involvement were collected from all participants in the study. Information regarding socioeconomic status (home conditions) was also collected on admission to the maternity wards in Ouagadougou and Catania. Sicilian mothers attended child birth preparation sessions, usually with the help of a midwife who also attended the delivery. No child birth preparation was made by Burkinabe women. Sicilian mothers received a single injection of an ergot derivative (0.2 mg), a powerful vasoconstrictor, immediately after delivery.

The exclusion criteria included human immunodeficiency viral infection, sexually transmitted diseases, and mastitis.

All individuals participating in the study signed informed consent forms.

No epidurals or other types of analgesia were given to mothers of either country.

The psychological involvement in childbirth was quantified by a binary variable within the first 24 h after delivery defined as follows.

Category 1

Category 1 corresponds to moderately severe, mechanical low back pain, which was accepted by mothers as a natural consequence of delivery.

Category 2

Category 2 corresponds to pain in all parts of the abdomen, which was considered a psychological ailment of the mother.

Maternal nutrition was in accord with the traditional habits of their respective countries.

Milk sample collection

Donation of colostrums by mothers occurred in their respective maternity wards. Samples were collected at 24, 48, and 72 h postpartum by the same teams in Italy and in Burkina Faso using a standardized procedure: before breastfeeding their babies, breast milk was collected for 10 min by hand squeezing and transferred into a sterile polystyrene tube in 2-mL fractions.

This procedure was repeated for 3 consecutive days at 24-h intervals.

Colostrum samples after collection were immediately refrigerated at 4°C, transported on ice to the local laboratory, and stored at –20°C. Subsequently, the samples were transferred on dry ice to the Laboratory of Institute of Food Science, National Research Council, Avellino, Italy. After thawing, colostrum samples were first centrifuged at 680g for 10 min at 4°C. The liquid component was removed and recentrifuged at 10 000g for 30 min at 4°C. The floating lipid layer and cellular sediments were removed. After separation, the milk serum fraction of colostrum samples was stored in 1.5-mL polypropylene tubes and frozen at –20°C for subsequent use in our assays.

β -EP assay

The β -EP concentrations in colostrums were assayed by enzyme immunoassay with a commercially available kit from Peninsula Laboratories Inc., San Carlos, CA, USA (catalog no. S-1134). Samples were read using the EL-312e microplate (Bio-Tek Instruments Inc., Highland Park Winooski, VT, USA). Documentation for this kit states that only β -EP immunoreactive material (IRM) is measured with this assay. The intra-assay coefficient of variation (repeatability) was $\pm 4.8\%$, the interassay coefficient of variation (reproducibility) was $<14\%$, and the smallest single value distinguishable from zero with 95% confidence (sensitivity) was 0.03 ng/mL. A standard sample was added to each plate; the reported results were the mean of two determinations.

Statistical analysis

Colostrum β -EP-IRM levels are presented as mean \pm standard deviation. Statistical comparison of β -EP-IRM concentrations between samples collected over 3 consecutive days were performed using non-parametric Wilcoxon rank test for paired and unpaired samples. The correlation between β -EP-IRM and length of stage I (starting after cervical dilation to 10 cm until delivery of the infant) was made through linear regression curve analysis and calculation of r^2 .

The power of tests conducted at an α level of 0.05 with Statmate 2 for Windows (GraphPad Prism 4, USA) was $>60\%$. $P < 0.05$ was selected for significance in all statistical tests.

Results

The characteristics of Burkinabe and Sicilian mothers are summarized in Table 1.

Burkinabe mothers lived in very precarious social and economical conditions ($P < 0.0001$) and had a larger number of deliveries compared with Sicilian mothers (4 versus 2, $P < 0.0001$). The gestational age was comparable and mothers in both populations delivered vaginally. Only Sicilian women received child birth preparation, but all Sicilian and Burkinabe women were assisted by a midwife during delivery. The duration of labor in Burkinabe women was medially 7 h (5–8 h) and the average length of stage II was 30 min (15–60 min). The duration of labor for Sicilian women was longer (6–9 h, median 8 h) and that of stage II was 45 min (20–70 min). Episiorrhaphy was performed on all Sicilian women who also received immediately postpartum an ergot derivative injection.

Results of β -EP-IRM determinations (nanograms per milliliter) are reported in Table 2. Colostrum volumes collected from Burkinabe women were about two to three times larger than those from Sicilians (Table 2).

The mean β -EP-IRM concentration in the colostrums of Burkinabe women was 0.83 ± 0.04 ng/mL in the first day and progressively decreased in the second (0.69 ± 0.04 ng/mL) and third (0.64 ± 0.1 ng/mL) days. β -EP-IRM concentration in the colostrums of Sicilian women was lower (0.31 ± 0.02 ng/mL) than that observed in Burkinabe women during the first day and remained stable in the

Table 1
Anthropometric, obstetric, and delivery characteristics and socioeconomic status of mothers*

	Sicilians (n = 30)	Burkinabe (n = 53)	P
Maternal age (y)	27.0 \pm 6.8	26.6 \pm 7.0	NS
Height (m)	1.61 \pm 0.06	1.62 \pm 0.05	NS
BMI (kgm ²)	22.3 \pm 4.5	23.0 \pm 4.0	NS
Deliveries (n)	2 (1/3)	4 (1/9)	0.0001
Child birth preparation	30	0	0.0001
Gestational age (wk)	40.0 (39–41)	39.5 (38–40)	NS
Vaginal delivery	30	53	NS
Assistance to delivery	30	53	NS
Episiorrhaphy	30	0	0.0001
Length of stage II (min)	45 (20–70)	30 (15–60)	
	45.3 \pm 11.53	29.66 \pm 10.6	0.0001
Ergotamine injection	30	0	0.0001
Obstetric complications (infection, antibiotic use)	0	0	NS
Homes (%) with			
No electricity	0	68.2	0.0001
No refrigerator or freezer	1	82.1	0.0001
No private water supply	0	73.2	0.0001
No private toilets	1	87.0	0.0001
Radio set	100	88.2	NS
Television set	99.0	26.7	0.0001

BMI, body mass index; NS, not significant

* Mean \pm SD or median (range).

Table 2
 β -EP-IRM in colostrums of Burkinabe and Sicilian women

Samples	Parameter	β -EP-IRM (ng/mL) and volume (mL/10 min)		
		1° d	2° d	3° d
Burkinabe (<i>n</i> = 53)	β -EP-IRM (ng/mL)	0.83 ± 0.04*‡	0.69 ± 0.04†‡	0.64 ± 0.1‡
	Volume (mL/10 min)	6.0 ± 0.5*‡	8.0 ± 0.5†‡	10 ± 0.5‡
Sicilian (<i>n</i> = 30)	β -EP-IRM (ng/mL)	0.31 ± 0.02	0.31 ± 0.017†	0.28 ± 0.015
	Volume (mL/10 min)	2.0 ± 0.1*	4.0 ± 0.1†	6.0 ± 0.1

β -EP-IRM, β -endorphin immunoreactive material

* $P < 0.0001$, 1° versus 2° d.

† $P < 0.0001$, 2° versus 3° d.

‡ $P < 0.0001$, Burkinabe versus Sicilian.

second day after delivery before a decrease in the third day (0.28 ± 0.015 ng/mL; Table 2).

No correlation was found between the level of β -EP-IRM and the age of the mothers, or the number of pregnancy, in either group.

The level of β -EP-IRM was significantly higher in the colostrums of 10 Sicilian and 16 Burkinabe mothers who exhibited pain and psychological involvement during and after delivery (Fig. 1a,b). In the colostrums of Sicilian mothers who received ergot derivatives, episiorrhaphy, and child birth preparation, the correlation between β -EP-IRM and length of stage II was significant ($r^2 = 0.53$, $P < 0.0001$). This correlation was present at a less significant level ($r^2 = 0.24$, $P < 0.001$) in Burkinabe mothers who received neither ergot derivatives nor child birth preparation (Fig. 2a,b).

Burkinabe women delivered without complications and came back home after 3 d. The Sicilian mothers did not show severe delivery complications.

Discussion

The biological effects of elevated β -EP content in human colostrum have not been fully elucidated in newborns. We report the colostrum β -EP-IRM levels in Burkinabe and

Sicilian women after full-term delivery, showing that β -EP-IRM levels in Burkinabe women are significantly higher than those found in Sicilian women during the first 3 d of lactation. The socioeconomic factors may influence β -EP-IRM colostrum levels, but we do not know the exact mechanism. The child birth preparation and delivery assistance by a known midwife in Sicilian mothers, which decreased labor pain and psychological stress, decreased β -EP-IRM level in Sicilian colostrums. In contrast, in the absence of these facilities the Burkinabe mothers, who delivered without preparation, showed greater labor stress and correspondingly higher β -EP-IRM colostrum levels.

The β -EP content in human colostrum may be important in overcoming birth stress and in postnatal fetal adaptation. In fact, longer delivery periods with more intense pain and higher stress increase the risk of neonatal tissue injury, which would subsequently require “stress adaptation,” a potential role for β -EP.

The effect of β -EP is not limited to nervous cells, but also to cells of the immune system [17–19]. In particular, several studies have reported the presence of β -EP receptors on immune cells. Moreover, β -EP was found to interact specifically with T lymphocytes in several “in vitro” experiments [20,21]. Other studies have shown that β -EP enhanced in vitro mitogen-induced T-cell proliferation and augmented human natural killer cell cytotoxic activity [22].

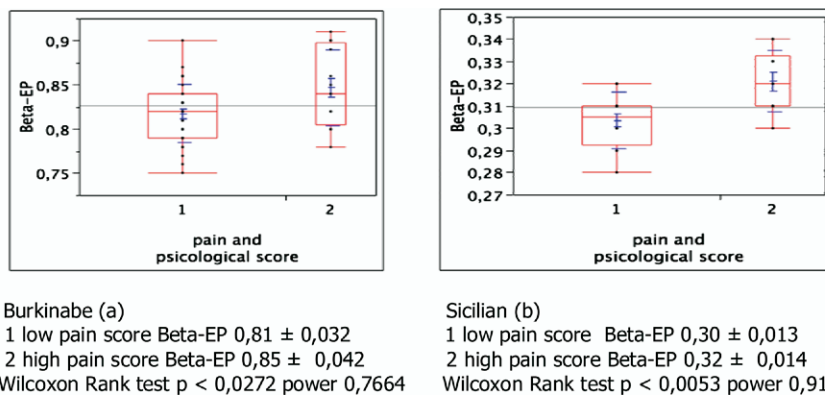


Fig. 1. β -EP-IRM level (nanograms per milliliter) in colostrums of Burkinabe (a) and Sicilian (b) women in the first day of delivery. β -EP-IRM, β -endorphin immunoreactive material.

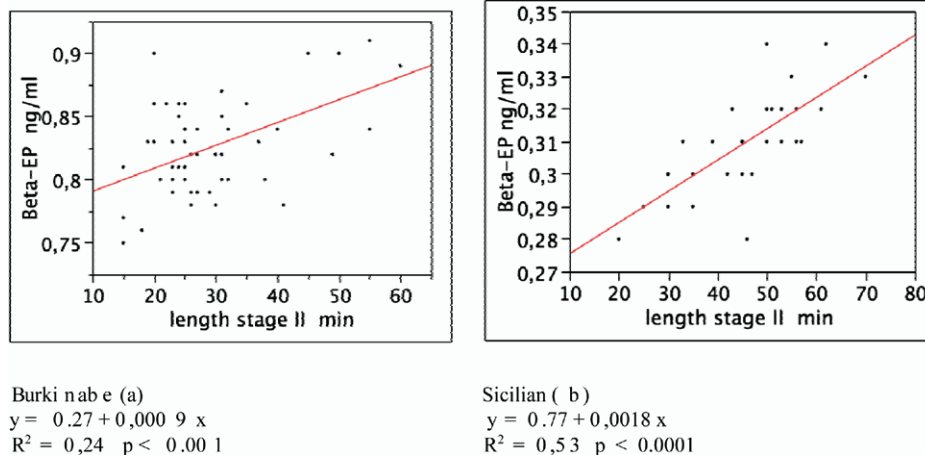


Fig. 2. Correlation between β -EP-IRM levels (nanograms per milliliter) in colostrums of Burkinabe (a) and Sicilian (b) mothers and length of stage II of labor. β -EP-IRM, β -endorphin immunoreactive material.

β -EP also has been reported to enhance interleukin-2 production in mitogen-stimulated murine splenocytes and in cloned T-cell lines [23]. β -EP-derived peptides also were found to stimulate macrophage digestion of virulent micro-organisms, to enhance adhesion and spreading of murine peritoneal macrophages by 20–30%, and to inhibit β -EP binding specifically to non-opioid receptors [24]. Therefore, these peptides may be considered selective agonists of non-opioid receptors for β -EP, the effect of which is particularly relevant for neonates born in African countries, ensuring them a better adaptation to extrauterine life. Immune effects of β -EP may be especially relevant in Burkina Faso where substandard environmental conditions, deficits in proper hygiene, and limited obstetric assistance are the norm.

The higher β -EP-IRM levels measured in the colostrums of Burkinabe women after full-term delivery decreased by approximately 25% from day 1 to day 3, whereas the initial lower levels found in Sicilian women showed little reduction in levels.

We cannot exclude that ergot derivatives injected immediately after delivery influenced the colostrum levels of Beta-EP-IRM. However, the dramatic reduction in colostrum β -EP levels on the first day, due to ergot administration, should recover on the second and third days, resulting in large deviations in levels across the 3 d measured. We observed no such deviations, thus casting doubt on this hypothesis to explain the differences in β -EP levels.

The importance of child birth preparation in the pathogenesis of stress is shown by the lower β -EP-IRM content in Sicilian colostrums and by the significant correlation between β -EP-IRM and length of stage II. This was less significant in Burkinabe mothers because child birth preparation represents an important determinant in the pathogenesis of labor stress.

However, the potential effects of β -EP colostrum concentration are confounded by the fact that Burkinabe women

produce more colostrum (two to three times) than Sicilians. Therefore, the differences observed between Sicilian and Burkinabe mothers may be due to the concentration or absolute amount of β -EP. In Sicilian mothers, the volume of colostrums drawn could be smaller because they received an ergot derivative injection after delivery. An ergot derivative is a dopamine receptor agonist that inhibits prolactin secretion, milk production, and the initiation of breast-feeding by 2–3 d [25].

However, prolactin levels present in colostrums of Sicilian and Burkinabe mothers remained stable in the following 3 d, whereas the content of insulin-like growth factor-1 in colostrum decreased progressively, thus underlining the essential role of prolactin at the beginning of lactation [26]. In this study the effect of the ergot derivative injected only to Sicilian mothers did not seem to influence the prolactin concentration in colostrum, thus supporting the hypothesis of a racial difference in lactogenesis between these two sets of women.

Ethnic factors may affect the timing of stage II lactogenesis and it is possible that the Burkinabe mothers secreted milk sooner than the European women [27,28].

These could be convincing explanations for the volume difference and β -EP-IRM content between Burkinabe and Sicilian colostrums.

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