

Transcutaneous Bilirubin in Exclusively Breastfed Healthy Term Newborns Up to 12 Days of Life

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KEY WORDS

bilirubin, breastfeeding, hyperbilirubinemia, jaundice, infant, newborn

ABBREVIATIONS

SB—serum bilirubin

TcB—transcutaneous bilirubin

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WHAT'S KNOWN ON THIS SUBJECT

Transcutaneous bilirubin levels increase up to the fourth to sixth day of life in healthy term newborns, but it is not known how high these levels can be.



WHAT THIS STUDY ADDS:

In this study, the 95th percentile transcutaneous bilirubin level at 24-hour level of 4.8 mg/dL on the sixth day of life, and 8.5 mg/dL were still seen in healthy term newborns.

abstract

OBJECTIVE: To determine the natural history of transcutaneous bilirubin in exclusively breastfed term neonates up to 12 days of life.

METHODS: In a prospective cohort study, we performed a 12-day evaluation of 223 healthy, exclusively breastfed, appropriate-for-gestational-age neonates who roomed-in for at least 48 hours. Each newborn had forehead transcutaneous bilirubin and body weight measured at the end of 1, 2, 3, 4, 5, 6, 8, 10, and 12 days. Regression analysis was used with bilirubin as a third-degree polynomial function of time. The 25th, 50th, 75th, 90th, and 95th percentile curves were constructed by using the residual mean square for each day.

RESULTS: Patients were 46% white, 34% mixed race, and 20% black, the mean birth weight was 3260 g (range: 2560–4090 g), the mean gestational age was 39.4 weeks (range: 37.0–41.9 weeks), 51% were male, 74% were born by vaginal delivery, and 66% had been breastfed since delivery. The mean highest weight loss was 4.7% (range: 1%–12%) at the second or third day, and in most infants the weight returned to the birth weight at the fifth day. With 2007 total bilirubin measurements, bilirubin concentrations reached the 50th percentile level (5.6 mg/dL) at the third and fourth days and returned to the 24-hour level (4.8 mg/dL) at the sixth day. The 95th percentile bilirubin level was 8.2 mg/dL at 24 hours of life, reached 12.2 mg/dL on the fourth day, and declined to 8.5 mg/dL on the 12th day.

CONCLUSIONS: The transcutaneous bilirubin curve represents the natural history of bilirubinemia in exclusively breastfed healthy term newborns in the first 12 days of life. *Pediatrics* 2011;128:e565–e571

Approximately 70% of term newborns present with jaundice in the first week of life, and in most cases this jaundice it is a transient and benign phenomenon. However, the serum bilirubin level that is considered physiologically normal is not well known and depends mainly on the ethnic and racial distribution of the population and on the type of feeding provided in the first days of life, in addition to other epidemiological factors.

The temporal evolution of neonatal bilirubinemia was initially investigated in the United States in a longitudinal study with 29 term newborns. The results of this study showed that the bilirubin level has a mean peak at day 3 and drops on days 4 and 5 to a level similar to that of the first day of life.² Later, results of studies performed with term and near-term neonates in Canada,³ Nigeria,⁴ Japan,⁵ Israel,⁶ Turkey,⁷ Thailand,⁸ Italy,⁹ and Greece¹⁰ verified that the serum bilirubin (SB) or transcutaneous bilirubin (TcB) could be elevated even at the fifth or sixth day of life during hospitalization, with the mean and maximum peaks occurring between the third and sixth days of life. It was not possible, however, to determine the magnitude and time of the decrease in bilirubin levels. De Luca et al¹¹ compared 4 TcB nomograms and showed that the bilirubin rates of increase tended to plateau at ~96 hours of life, with some differences between populations. Maisels and Kring¹² studied the natural course of neonatal bilirubinemia in the first 96 hours of life in newborns of 35 or more weeks of gestation and demonstrated the progression of the mean TcB to 8 mg/dL at the end of the study period; nonetheless, these investigators observed that the mean peak was not reached in the first 96 hours of life. Engle et al¹³ followed Hispanic newborns of 35 or more weeks of gestation for up to 72 hours of life and found a

progressive increase in TcB, with the 95th percentile of 12.4 mg/dL occurring at 72 hours of life.

Currently, there is much concern about the association of hyperbilirubinemia levels above 25 mg/dL and breastfeeding.¹⁴ In the United States in 2005, 72% of children had any breastfeeding in the first 14 days of life,¹⁵ whereas in Brazil in 2008, 67% of newborns were exclusively breastfed for up to 15 days of life.¹⁶ In this context, it is important to understand the behavior of physiological bilirubin values to educate mothers about hyperbilirubinemia during their hospital stay and after their hospital discharge in a follow-up program.

Given the good correlation between SB and TcB in healthy newborn infants of diverse races and ethnicities,¹⁷ as well as lack of knowledge about bilirubinemia in the second week of life, this study was conducted to measure TcB levels and construct a curve to demonstrate the behavior of TcB levels during the first 12 days of life in healthy, exclusively breastfed term neonates who did not need phototherapy.

PATIENTS AND METHODS

The present study was designed as a prospective cohort study conducted at a public secondary maternity hospital with 4800 births per year in the state of São Paulo, Brazil. The project was approved by the research ethics committee of the *Universidade Federal de São Paulo*, and written informed consent was obtained from parents of the enrolled infants.

Patients who met the inclusion criteria for the study were singleton infants born after a gestation of 37 to 41 weeks who were appropriate for gestational age,¹⁸ born at the hospital with an Apgar score of 7 or above at 5 minutes, exclusively breastfed,¹⁹ roomed-in for at least 48 hours, and followed up at home for up to 12 days

of life. All patients were born on Thursdays or Fridays between 8 AM and 6 PM, in the period between August 2000 and February 2001, to allow for the same researcher (Dr Draque) to make a longitudinal evaluation of each newborn.

The exclusion criteria for neonates were positive indirect Coombs test in the maternal serum or positive direct Coombs test in the umbilical cord blood, presence of cephalohematoma or ecchymosis, and having received phototherapy. The direct Coombs test is part of routine care at the institution. Glucose-6-phosphate dehydrogenase was measured in all patients, and those with a deficiency of this enzyme were excluded.

The maternal and neonatal characteristics that may have affected the course of bilirubinemia were recorded. Patients were weighed every day by the rooming-in nursing team, and after hospital discharge, the weight was measured by the researcher (Dr Draque) at home, daily from the third to the sixth days, and at the eighth, 10th and 12th days. Weight loss was calculated as the daily weight minus the birth weight divided by the birth weight. The infants were weighed on a digital balance (Filizola, São Paulo, Brazil) in the hospital and on another digital balance from the same manufacturer in the newborn's house.

TcB was measured in each neonate at days 1, 2, 3, 4, 5, 6, 8, 10, and 12 of life. The first 2 or 3 measurements were obtained during the rooming-in, and the others at the newborn's home. TcB was measured on the forehead of each patient by use of the BiliCheck spectrophotometric method (SpectRx Inc, Norcross, GA) with individual BiliCal calibration tips (SpectRx Inc, Norcross, GA). When the TcB level obtained was ≥ 15 mg/dL, the SB was measured by use of the Jendrassik Grof method, according to Bhutani et al.¹⁷

If the result of ≥ 15 mg/dL for the TcB level was confirmed, the infant was submitted to a diagnostic evaluation of hyperbilirubinemia and to phototherapy, and was excluded from the study. After the 12th day of life, if the TcB level was >6 mg/dL, the infant was evaluated every 3 days until the TcB level was <5 mg/dL.

Blood samples were drawn from the heel at 48 to 72 hours to determine the correlation between capillary bilirubin levels and TcB levels with the Pearson's coefficient at the same time that hematocrit was determined.

The clinical data have been analyzed with the χ^2 test, Fisher's exact test, and the Student's *t* test, as appropriate.

In theory, the function that represents the change in bilirubin levels in neonates over time should be nonlinear.¹¹ This curve should show an increase during the first days of life and then decrease to a stable level (an asymptote). Multiple curve-estimation procedures were performed to find the best-fitting mathematical model, and a third-degree polynomial function of time was used to describe trends in the bilirubin levels over time for data that included bilirubin levels obtained for each individual neonate on 9 occasions $TcB = 3.9027 + [1.1518 \times d] - [0.229 \times d^2] + [0.0107 \times d^3]$; $R^2 = 0.6475$, where *d* is days. The data were analyzed with SAS 9.2 (SAS Institute, Inc, Cary, NC) for Windows.

RESULTS

During the 7-month data collection period, 253 newborns fulfilled the inclusion criteria up to 48 to 72 hours of life, and 30 were not able to be followed up at home. Seventeen patients had incorrect addresses, 9 received phototherapy during the hospital stay (SB: 14.5–19.0 mg/dL at 48 hours of life), 2 patients received phototherapy during home follow-up visits at days 4 and 5 of life (SB: 16.0 and 18.3 mg/dL, respec-

TABLE 1 Maternal and Neonatal Characteristics of 223 Patients Included in the Study

Characteristic	Value
Maternal age, mean \pm SD, y	24 \pm 6
Race, <i>n</i> (%)	
White	102 (46)
Mixed race	77 (34)
Black	44 (20)
Years of school, mean \pm SD, y	7 \pm 3
First gestation, <i>n</i> (%)	91 (41)
Prenatal care, <i>n</i> (%)	220 (99)
Maternal hypertension, <i>n</i> (%)	17 (8)
Maternal diabetes, <i>n</i> (%)	2 (1)
Previous newborn with jaundice, <i>n</i> (%)	21 (16)
Use of tobacco, <i>n</i> (%)	45 (20)
Oxytocin, <i>n</i> (%)	129 (58)
Anesthesia with bupivacaine, <i>n</i> (%)	63 (28)
Vertex presentation, <i>n</i> (%)	217 (97)
Meconium-stained amniotic fluid, <i>n</i> (%)	36 (16)
Vaginal delivery, <i>n</i> (%)	166 (74)
Birth weight, mean \pm SD, g	3262 \pm 329
Gestational age, mean \pm SD, wk	39.4 \pm 1.1
Male gender, <i>n</i> (%)	114 (51)
Apgar score at 1 min, mean \pm SD	8.0 \pm 1.0
Apgar score at 5 min, mean \pm SD	9.0 \pm 0.3
ABO incompatibility, <i>n</i> (%)	27 (12)
Rooming-in at first hour of life, <i>n</i> (%)	158 (71)
Breastfeeding in first hour of life, <i>n</i> (%)	148 (66)
Passage of first stool, mean \pm SD, h	6 \pm 7

tively), 1 neonate was fed with formula from the fourth day of life, and 1 had a deficiency of glucose-6-phosphate dehydrogenase. The maternal and neonatal characteristics of the 223 included patients and of the 30 excluded patients were similar.

Table 1 displays the maternal and neonatal characteristics of 223 patients included in the study. The mothers of 148 neonates (66%) breastfed in the first hour after birth, and 62 (44%) started breastfeeding at the third to fourth hour of life. The 223 newborns remained exclusively breastfed and were discharged at 52 ± 6 hours of life. The maximum weight loss in relation to birth weight was $4.7\% \pm 2.0\%$ (range: 1%–12%) between the second and third days of life, and 132 patients (59%) recovered their birth weight by the fifth day of life. The 223 newborns gained 43 ± 15 g per day (range: 10–85 g) from the lowest weight recorded up to the 12th day of life, and 13 (6%) did not return to their birth

weight during the period of the study (Fig 1).

The TcB and SB levels were highly correlated: the Pearson's correlation coefficient was 0.96 (95% confidence interval: 0.95–0.97). The mean hematocrit at 48 to 72 hours of life was 61.9% (95% confidence interval: 61.1%–62.7%).

On the basis of the 2007 TcB measurements in the 223 patients who were followed up to 12 days of life, a 50th-percentile TcB curve was constructed as a function of time with the 25th, 75th, 90th, and 95th percentiles (Table 2 and Fig 2). The highest 50th percentile TcB level was 5.6 mg/dL, which was observed between the third and fourth days of life. The highest 95th percentile value was 12.2 mg/dL, which was observed on the fourth day of life.

On the sixth day of life, the 50th percentile of the TcB level dropped to the value of the first day of life (4.8 mg/dL). The 95th percentile value obtained up

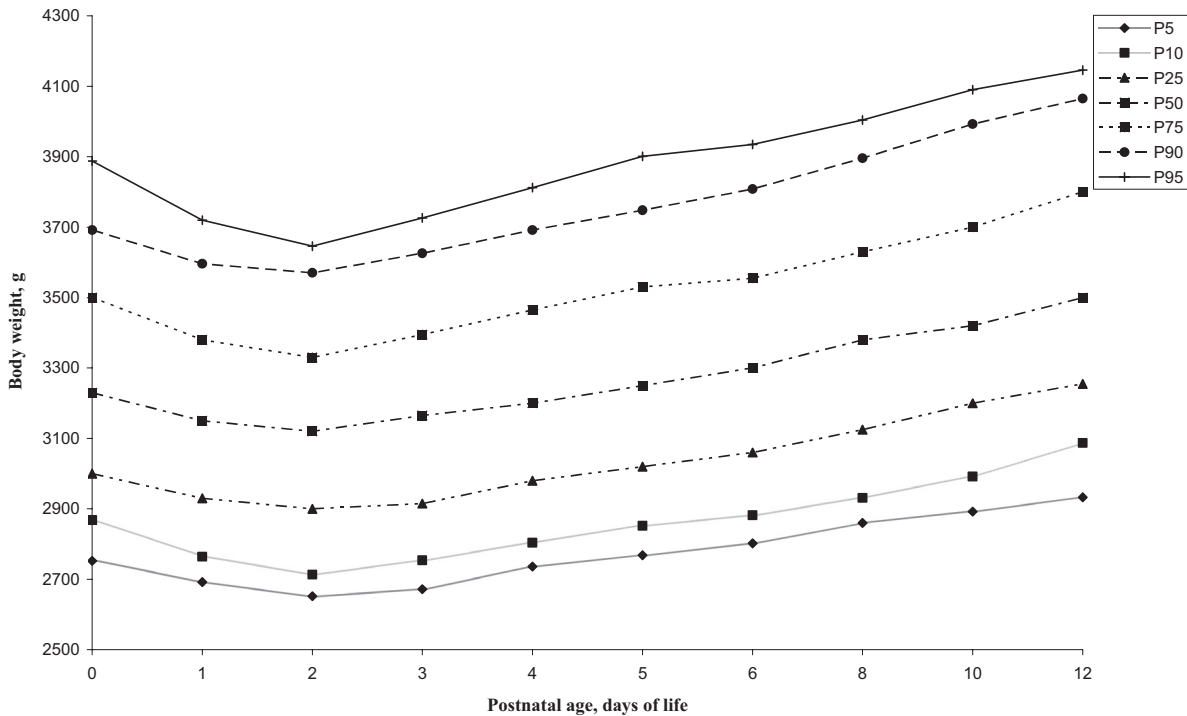


FIGURE 1 Body weight percentiles (P) in the 223 exclusively breastfed healthy term newborns in the first 12 days of life.

to the 12th day of life remained higher than the level at the first day of life.

On the 12th day of life, 41 newborns (18%) presented with a TcB level >6 mg/dL, which reached lower levels, on average, at 21 days of life (range: 15–42 days). At 30 days of life, 3 infants (1.3%) were breastfed and still jaundiced, with a mean TcB level of 8.8 mg/dL (measured levels for each infant: 8.3, 8.7, and 9.3 mg/dL).

DISCUSSION

The results of this study show the rise and the fall of TcB levels in healthy

term newborns who were exclusively breastfed, most of whom recovered birth weight on the fifth day of life. The unique features of this study were that data collection was longitudinal for the same infants rather than cross-sectional and that TcB levels were measured until the infants were 12 days old. To our knowledge, there have been no studies on jaundice in neonates who were exclusively breastfed and whose weight changes were monitored for up to 12 days of life.

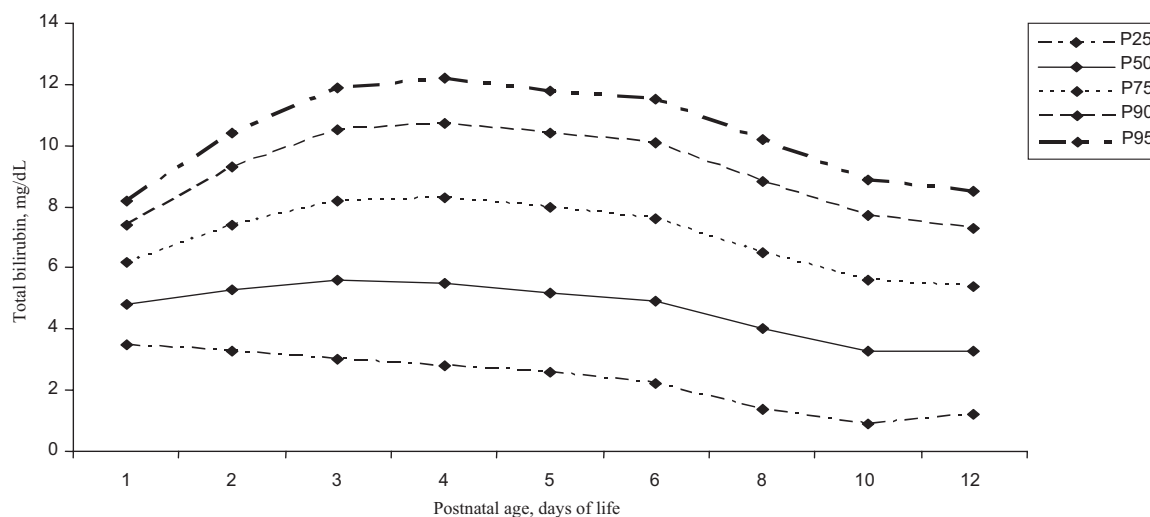
In some studies conducted in other countries, investigators have considered a maximum of 96 hours of life during the hospital stay and showed a rise in total bilirubin levels, but did not detect the drop.^{8,9,12,13} In 2 reported studies^{7,10} nomograms of TcB and SB levels for the first 5 to 7 days of life showed that TcB levels decreased as of the fourth day of life in term neonates.

Previously reported studies have included nomograms based on measurements of TcB levels that were obtained from term neonates during the first 3 to 4 days of life,⁸ healthy infants aged ≥ 35 weeks during the first 96 hours,¹² term neonates for up to the first 96 hours,⁹ term neonates for the first 12 to 120 hours,¹⁰ and Hispanic neonates aged ≥ 35 weeks during the first 72 hours of life.¹³

In the present study of 223 term newborns, 54% of them were mixed race and black, but none were of Asian ethnicity. The highest mean value (50th percentile) of the total bilirubin level

TABLE 2 Total Bilirubin Percentiles in the 223 Exclusively Breastfed Healthy Term Newborns in the First 12 Days of Life

Postnatal Age, d	Postnatal Age, Mean (95% Confidence Interval), h	Total Bilirubin Percentiles, mg/dL				
		25th	50th	75th	90th	95th
1	26.7 (26.0–27.4)	3.5	4.8	6.2	7.4	8.2
2	50.1 (49.4–50.8)	3.3	5.3	7.4	9.3	10.4
3	75.4 (74.7–76.1)	3.0	5.6	8.2	10.5	11.9
4	99.3 (98.6–100)	2.8	5.5	8.3	10.7	12.2
5	123.4 (122.7–124.1)	2.6	5.2	8.0	10.4	11.8
6	147.4 (146.7–148.1)	2.2	4.9	7.6	10.1	11.5
8	195.4 (194.7–196.1)	1.4	4.0	6.5	8.8	10.2
10	243.3 (242.6–244.0)	0.9	3.3	5.6	7.7	8.9
12	291.0 (290.3–291.7)	1.2	3.3	5.4	7.3	8.5

**FIGURE 2**

TcB percentiles (P) in the 223 exclusively breastfed healthy term newborns in the first 12 days of life.

was 5.6 mg/dL and occurred between the third and fourth days of life. This value was close to that reported for formula-fed newborns, which was roughly 6 mg/dL.^{2,3} Some investigations conducted in newborns in North America, Africa, Asia, and Europe showed mean total bilirubin-level peaks that ranged from 7.5 to 12.0 mg/dL between the third and sixth days of life. Some studies included newborns who were exclusively breastfed, some who were on formula, and others on mixed feeding.^{3-5,7-10,12}

In the present study, the 95th percentile of the TcB level reached 12.2 mg/dL on the fourth day of life, which is a value similar to that obtained by the National Collaborative Perinatal Project of 12.9 mg/dL, in a study conducted when the use of formula for feeding infants predominated,²⁰ and by 2 other studies, 1 in which 67%¹² and another in which 32% of patients breastfed.¹³ However, 95th percentile values of 15 mg/dL have been reported in European investigations.^{9,10} The lowest total bilirubin levels found in the present study could be associated with appropriate breastfeeding practices, as demonstrated by the mean maximum weight loss of 4.7% ± 2.0% between the sec-

ond and third days of life and by the recovery to the birth weight at 5 days in most newborns. Among the 223 patients, 66% initiated breastfeeding in the delivery room, and all were breastfed in the rooming-in period for at least 48 to 72 hours of life; with breastfeeding during the rooming-in period, which is recommended by the World Health Organization, mothers could better understand the technique, and this led to successful breastfeeding.²¹ The volume of milk produced by the nursing mother is closely related to when breastfeeding begins, breastfeeding frequency, complete emptying of the breast, and ability of the newborn to effectively suck. Hence, initiation of breastfeeding in the first hour of life, followed by nursing 10 to 12 times per day in the first week, with no other supplementation of water or other foods, together with appropriate breastfeeding techniques that ensure effective provision of milk to the neonate, minimize weight loss to <7%, and keep bilirubin levels low.^{22,23,24} Although the number of times of breastfeeding was not recorded in this study, the weight recovery in the fifth day of life and the maximum weight loss of

12% in 1 infant could be indicative of appropriate breastfeeding.

On the sixth day of life, the mean total bilirubin level returned to 4.8 mg/dL, which was the value detected on the end of the first day. Few authors have investigated the decrease in bilirubinemia during the neonatal period. In 2 studies, 1 with 29 patients and another with 39 North American formula-fed newborns, bilirubinemia of 3 mg/dL occurred on the sixth day, a level similar to that of the first day, and bilirubinemia of 1 mg/dL occurred at 11 days of life.^{2,25} Studies in breastfed newborns revealed mean estimated bilirubin levels of 5 to 7 mg/dL at the end of the first week of life and of 3 to 5 mg/dL at the end of the first month.²⁵ In a sample of 336 Japanese newborns, the mean values were 12.3 mg/dL at 7 days and 7.6 mg/dL at 30 days of life, values that did not reach the levels of the first day of life,⁵ and the 95th percentile values remained higher as of the 12th day compared with those as of the first day of life.

In the present study, 41 patients (18%) had bilirubinemia levels of >6 mg/dL at 12 days of life and took, on average, 21 days (range: 15–42 days) to achieve

bilirubinemia lower than 6 mg/dL, which confirmed the finding that 20% to 30% of newborns remained clinically icteric for 2 to 3 weeks.^{25–27} This slower drop in bilirubin levels has been attributed to breastfeeding, but the exact mechanism that causes the prolonged hyperbilirubinemia still needs to be identified. The existence of a substance in maternal milk is speculated, which would inhibit glucuronyl transferase or increase the enterohepatic circulation of bilirubin.²⁶ Gourley et al²⁸ reported that transcutaneous bilirubin in breastfed term newborns remained higher (8 mg/dL) than the initial values for up to 20 days of life. In patients fed with whey-protein formulas, values similar to the initial levels of 5 mg/dL were measured at 20 days; moreover, in a third group fed with casein, the initial bilirubin level was achieved at 10 days of life. The authors speculated that this faster drop could be explained by the fact that casein-formulas contain a β -glucuronidase inhibitor, which decreases the enterohepatic circulation of bilirubin. In addition to this possibility, in a recent Taiwan study investigators demonstrated the association between prolonged indirect hyperbilirubinemia in breastfed newborns and a mutation in the uridine diphosphate-glucuronosyltransferase (*UGT1A1*) gene.²⁹

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The main limitation of this study was its application to other populations and ethnicities. It is necessary to confirm whether low total bilirubin levels can be found in areas where exclusive breastfeeding is a predominant practice in the first month of life. Furthermore, in the present investigation, near-term newborns of 35 to 36 weeks of gestation were not evaluated, and patients requiring phototherapy were excluded. One study that included measurement of TcB levels until initiation of phototherapy showed 95th percentile values of 15 mg/dL,¹¹ but studies without patients treated with phototherapy revealed 90th to 95th percentile values from 12 to 13 mg/dL,^{12,13} which were similar to our findings. With the use of the values of bilirubin recommended by the American Academy of Pediatrics (2004)²⁴ as indicators to initiate phototherapy, it was only in 2 patients in this study that the treatment was not indicated. This small number should not have materially affected the outcome. Furthermore, patients with some major risk factors for the development of severe hyperbilirubinemia, such as hemolytic disease because of blood group incompatibility with positive direct Coombs, glucose-6-phosphate dehydrogenase deficiency, cephalohematoma, or significant bruising, were excluded. These

stringent inclusion criteria may have biased our results.

CONCLUSIONS

On the basis of the results of this study, we conclude that in healthy, term, exclusively breastfed neonates born predominantly by vaginal delivery with good health conditions at birth who stayed in rooming-in for at least 48 hours, the weights returned to the birth weights at 5 days of life. We observed mean bilirubin levels of 5.6 mg/dL, and the 95th percentile of 12.2 mg/dL between the third and fourth days of life showed a progressive decline, achieving the values found at the end of the first 24 hours on the sixth day of life. These data are useful for clinicians who educate parents about the natural history of bilirubinemia and the importance of appropriate breastfeeding in term newborns.

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Cecilia Maria Draque, Adriana Sañudo, Clovis de Araujo Peres and Maria Fernanda Branco de Almeida

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