

## CLINICAL SCIENCE

# Growth of very low birth weight infants fed with milk from a human milk bank selected according to the caloric and protein value

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**OBJECTIVE:** To describe growth and clinical evolution of very low birth weight infants fed during hospital stay with milk from a human milk bank according to the caloric-protein value.

**METHOD:** Forty very low birth weight infants were included: 10 were fed milk from their own mothers (GI), and 30 (GII) were fed human milk bank > 700 cal/L and 2 g/dL of protein. Growth curves were adjusted using nonlinear regression to the measured growth parameters.

**RESULTS:** full enteral diet was reached in 6.3 days by GI and in 10.8 by GII; a weight of 2 kg was reached in 7.3 weeks for GI and in 7.8 for GII. In GI, 3/10 (33.3%) and in GII, 7/30 (23.3%) developed sepsis. Necrotizing enterocolitis did not occur in GI, but in 3/30 (10.0%) in GII. GI presented with urinary calcium > 4 mg/L in 1/10 (10.0%), urinary phosphorus ( $P_u$ ) < 1 mg/L in 10/10 (100%), and Ca/Cr > 0.6 ratio in 1/10 (10.0%) of the cases; in GII, no children presented alterations of the urinary calcium or the Ca and Cr ratio, and  $P_u$  was < 1 mg/L in 19/30 (63.3%). In terms of growth the 50<sup>th</sup> percentile for GI was a weight gain of 12.1 g/day (GI) vs. 15.8 g/day (GII), a length gain of 0.75 cm/week (GI) vs. 1.02 cm/week (GII), and a head circumference gain of 0.74 cm/week (GI) vs. 0.76 cm/week (GII).

**CONCLUSIONS:** Human milk bank allowed a satisfactory growth and good clinical evolution for very low birth weight infants.

**KEYWORDS:** Premature infants; Human milk; Human milk bank; Infant nutrition; Enteral nutrition.

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## INTRODUCTION

Maternal milk (HM) is considered the gold standard to nourish very low birth weight infants (VLBWIs).

VLBWIs benefit from the immunological and nutritional composition of HM, which, during the period of four to six weeks post-birth, has a higher concentration of proteins, lipids, calories, sodium and IgA and smaller concentration of lactose than the mature milk making it adapted for premature needs. The more premature, the higher the protein and lipid contents of the HM.<sup>1</sup>

With the evolution of lactation, there is a reduction of the immunologic factors, caloric-protein and mineral concentration in HM, especially calcium and phosphorus,<sup>2</sup> a fact that makes some researchers question its indication for VLBWIs, especially for those with birth weights lower than 1500 g.<sup>3-5</sup>

With the aim of obtaining benefits from HM for VLBWIs feeding, various studies were developed using HM supplemented with heterologous protein, energy and minerals, especially calcium, phosphorus and sodium, confirming higher weight, linear growth and head circumference (HC) gains as well as a lesser incidence of osseous metabolic disease.<sup>5-8</sup> However, HM with additives and the use of formulas designed for preterm infants are not exempt from complications such as necrotizing enterocolitis (NEC) nor, in the long run, from the accelerated growth especially in the first weeks of life that can cause obesity in childhood and future metabolic syndromes.<sup>9</sup>

The institution of human milk banks has created a new perspective for VLBWI nutrition. With the lack of mother's milk, the donor's milk can be a good alternative for VLBWIs, and donor's milk has advantages in comparison to the use of formulas, especially in terms of a smaller risk of infection, NEC, metabolic disorders and better feeding progression.<sup>10,11</sup> However, there are few studies in the literature on VLBWIs fed with donated milk.

Due to the variations in each donated milk sample and in the nutritional needs of each VLBWI, we studied the use of

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milk from a human milk bank that was selected according to the caloric and protein value (HMB) for VLBWI feeding. Herein, we describe the postnatal growth of these children until they reach two kilograms of weight during their hospital stay and their clinical-laboratorial evolution during internment.

## MATERIALS AND METHODS

Studied cases included 40 VLBWIs (birth weight  $\leq$  1500 g), without intrauterine growth restriction according to the Kramer criteria.<sup>12</sup> VLBWIs using medications that alter protein metabolism, corticosteroids and/or diuretic medication for more than two weeks, those not able to receive enteral feeding for more than a week, and those with major malformations in multiple organs or system dysfunctions were excluded from the study.

The VLBWIs were classified into two study groups: Group I (GI) included 10 VLBWIs who received milk from their own mothers, either drawn and raw or pasteurized. Group II (GII) included 30 VLBWIs with a birth weight  $\leq$  1500 g, without intrauterine growth restriction, who received human milk from a bank previously classified for containing more than 60% protein and calories of the total ingested value. The division of the study groups occurred naturally according to the mother's milk production and the need for complementation with HMB. The study was approved by the ethical Board of São Paulo University Clinics Hospital and University Hospital of São Bernardo do Campo (HMU). The milk used in this research study to GI was the milk from the infants' own mothers, raw or pasteurized, and to GII was mainly transition or mature donated milk from the HMB with a caloric value  $>$  700 kcal/L and a protein value  $>$  2.0 g/dL.

For those VLBWIs that received minimal enteral feeding, the selected milk as preferably the raw colostrums or pasteurized milk from their mothers. In the lack of mother's milk, pasteurized colostrum from the milk up to 600 kcal/L and with protein contents varying from 2 to 3 g/dL, were offered.

In terms of collection, storage, pasteurization and quality control, the milk used in this protocol complies with Brazilian legislation, i.e., the administrative rules of the Ministry of Health regulating the implementation and operation of HMB.<sup>13</sup>

The HMB caloric value was determined through the crematocrit technique,<sup>14</sup> and the protein value was carried out through the Biuret technique.<sup>15</sup>

In the study period, 15,283 samples of HM were analyzed, out of which 1370 (8.9%) were selected for protein concentration dosing for having more than 700 kcal/L; out of these, 623 (45.4%) had more than 2 g/dL of proteins.

The milk samples were selected with the aim of offering the VLBWIs 120 kcal/kg/day and 2.5 to 3.5 g/kg/day protein. This proposal is based on the Polberger study,<sup>6</sup> which considers that an offer above this caloric-protein density has no growth advantage and only causes higher fat deposition. The referred values are close to those proposed by Tsang et al.<sup>16</sup> and by the Nutrition Committee of the European Society of Pediatrics, Gastroenterology and Nutrition.<sup>17</sup> The initial offered volume ranged from 10 to 20 mL/kg/day and was increased according to the tolerance of each child.

During the hospital stay, feeding progression and clinical events such as incidence of sepsis, NEC and bronchopulmonary dysplasia were analyzed. Additionally, calcium and

phosphorus (serum and urine), urea, creatinine, hemoglobin (HB) and hematocrit (Hct) were dosed.<sup>18-21</sup>

## Statistical analysis

The type of study was prospective, observational and sequential with VLBWIs cut type outlining during the hospital stay.

Parametric and nonparametric tests were utilized when the obtained sample was relatively small or presented data variability. The Shapiro-Wilk test was utilized to verify the normality and the Mann-Witney test to compare the nonparametric variables medians.

## Elaboration of growth curves

From data regarding weight, HC and length, nonlinear regression equations that were best adjusted to the individual growth parameters for each infant were established.

Utilizing the CurvExpert® 1.3 software, various models were selected to mimic the growth tendency that each child presented and to obtain a high correlation coefficient allied to a smaller formula standard error. In this manner, the following equations were selected:

- Third-degree polynomial:  $Y = a+bx+cx^2+ dx^3$
- Fourth-degree polynomial:  $y = a+bx+cx^2+ dx^3+ ex^4$
- Fifth-degree polynomial:  $y = a+bx+cx^2+dx^3+ex^4+fx^5$
- Heat Capacity Model:  $Y = a+bx+c/x^2$
- Sinusoidal:  $Y = a+bcos(cx+d)$
- Count =  $a+bx+clogx$
- Hyperbolic:  $y = a+b/x$
- Exponential:  $y = ae^{b/x}$

From the equation defined for each VLBWI, through interpolation, the values of weight, length and HC were estimated at weekly intervals, from 28 until 39 exact weeks of corrected GA. Utilizing these data, for each exact age (weeks), each parameter's mean value was calculated. Based on the mean values, regression curves that were better adjusted to the VLBWI group growth as a whole were established by utilizing the same software and the same described criteria. This is able to smooth out the erratic character that is usually observed in curves based on actual values.

The best adjustment curve for the three parameters in the different ages was a third-degree polynomial.

## Results

In the study period, 11,342 children were born in the hospital HMU, and 1051 of them with GA less than 37 weeks, out of which 195 weighed  $<$  1500 g; of these, 57 (29.2%) were characterized as not having intrauterine growth restriction. Seventeen VLBWI were excluded from the study according to the established exclusion criteria.

Table 1 shows the GI and GII distribution according to birth weight. The groups were similar in terms of birth weight range (1251 g to 1500 g); however, 42.5% of infants in GII weighed less than 1250 g, while this was true for only 20.0% of infants in GI.

The enteral nutrition progress regarding feeding progression, beginning of enteral feeding and full volume to meet the preconized nutritional needs was satisfactory and very quick. At the end of the first week, few children were on

**Table 1 - Group I and Group II studied cases in regards to weight, enteral progression and clinical - laboratory data.**

|                                   | GI                 | GII                  | p           |
|-----------------------------------|--------------------|----------------------|-------------|
| Infants (n)                       | 10                 | 30                   |             |
| Gestational age (weeks)           | 30.3 ± 1.1         | 29.6 ± 2.6           | 0,4165 (NS) |
| Newborn weight (g)                | 1305 ± 197         | 1198 ± 231           | 0,1975 (NS) |
| Full enteral diet (days)          | 6.3 ± 4.3          | 10.8 ± 6.4           | 0,1383 (NS) |
| Recovery to newborn weight (days) | 7.8 ± 5.8          | 10.6 ± 4.0           | 0,0960 (NS) |
| Weeks to reach 2 Kg               | 7.3 ± 2.0          | 7.8 ± 1.9            | 0,4810 (NS) |
| Necrotizing enterocolitis         | -                  | 3 (10.0%) (2IA/1IB)* | 0,5597 (NS) |
| Sepsis incidence                  | 3 (33.3%) (2E/1La) | 7 (23.3%) (3E/4La)** | 0,6893 (NS) |
| Bronchopulmonary dysplasia        | 3 (33.3%) (L)      | 9 (30.0%) (8L/1M)*** | 1,000 (NS)  |
| Hemoglobin (g/dL)                 | 14.1 ± 3.6         | 12.7 ± 4.9           | 0,4123 (NS) |
| Hematocrit (%)                    | 40.6 ± 11.1        | 36.2 ± 11.4          | 0,2942 (NS) |
| Urea (mg/dL)                      | 12.0 ± 5.3         | 19.5 ± 9.6           | 0,0246 (S)  |
| Creatinine (mg/dL)                | 0.2 ± 0.11         | 0.3 ± 0.16           | 0,751 (NS)  |

Normal laboratory values:<sup>30</sup>

Hemoglobin: 9.0–14.0 g/dL

Hematocrit: 28–42%

Urea: 5–18mg/dL

Creatinine: 0.2–0.4mg/dl

\*Necrotizing enterocolitis (NEC) - Bell criterion modified by Walsh and Kliegmann, 1986<sup>19</sup>

\*\*Sepsis – Bone et al. criteria, 1991<sup>18</sup>

\*\*\*Bronchopulmonary dysplasia - Ehrenkranz, 2005<sup>20</sup>

E – early; La – late; L – light; and M – moderate

NS= no significant, S= significant

parenteral nutrition, and most of them had a good caloric-protein ingestion (Table 1).

As shown in Table 1, there were few complications and no differences related to oral feeding such as NEC and other complications associated with prematurity. Otherwise, the incidence of sepsis was low in both groups.

Laboratory assessment (Table 1) of hemoglobin and hematocrit showed no correlation between the use of human milk and the evolution of anemia in the studied cases. Serum urea and creatinine were higher and significant (p=0,0246) in GII which could indicate a greater amount of ingested protein.

The study also monitored possible laboratory phosphorous deficiency<sup>21</sup> and when necessary, corrected it through the use of a calcium and phosphorous manipulated formula. As shown in Table 2, there was no evidence of Osseous Metabolic Disease, but we did observe phosphorus deficiency.

The values obtained in the VLBWI growth parameters of both groups in regard to weight, length and HC expressed in percentiles, for each GA, are shown in Tables 3 (GI) and 4 (GII). The graphs corresponding to these tables for the 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles are shown for weight, length and HC in Figures 1, 2 and 3, respectively.

Tables 3 and 4 show values obtained in length and HC of both groups (GI and GII) are virtually the same for all percentiles. In regards to weight, the same tables show that

the median weight achieved in the corresponding age of 39 weeks of gestation is almost the same; however, the GII had a wider range of values between the median and the extreme percentiles than were observed in GI.

Nevertheless, when considering growth during the study period, Figures 1, 2 and 3 show that both groups presented equal trends and that the gain in weight (p=0,0002) and length (p=0,0038) was higher and significant in GII than GI.

## DISCUSSION

Currently, the consensus is that HM is the better nutrient for VLBWIs. However, there are doubts about its adequacy after the 3<sup>rd</sup> week from birth, especially for those with birth weights below 1250 g.<sup>8,22</sup>

The nutritional strategies currently utilized are formulated to increase human milk caloric-protein, mineral and vitamins value, or complement the human milk with formulas appropriate for premature infants. With the lack of HM, or when its complementation is necessary, HMB can be a good option,<sup>23</sup> however, according to some authors,<sup>24,25</sup> this product might not be nutritionally appropriate. This was shown by studies utilizing HMB during the mature milk stage that came from full-term newborn mothers, which displayed protein and mineral contents insufficient to allow a growth similar to the intrauterine growth in VLBWI.<sup>26</sup>

However, it is worth mentioning that these studies developed with donor HM did not detail the quality control of samples, such as the cold chain and titratable acidity, which are important in the reduction in loss of immunological components and in the maintenance of nutrients. Moreover, these studies did not analyze the protein and caloric contents of the offered HM. It should also be considered that donated HM is obtained through milk-drawing that is done according to the donor need: some use relief milk-drawing, i.e., after milking, due to large milk production, breasts are emptied to avoid ingurgitation. This procedure favors the collection of more caloric milk that is mainly composed of the emulsion fraction; on the other

**Table 2 - Serum and urinary values of Ca, P, FA, and Ca/Cr ratio in GI (n = 10) and in GII (n=30).**

| Variable | Group I                  |    | Group II |    | p    |            |
|----------|--------------------------|----|----------|----|------|------------|
|          | n                        | %  | n        | %  |      |            |
| Blood    | Ps<3.6 mg/dL             | 3  | 30.0     | 16 | 53.3 | 0,2812(NS) |
|          | FA > 900 UL              | 7  | 70.0     | 17 | 56.6 | 0,7110(NS) |
| Urine    | *Ca <sub>u</sub> >4 mg/L | 1  | 10.0     | -  | -    | 0,2500(NS) |
|          | *P <sub>u</sub> <1 mg/L  | 10 | 100.0    | 19 | 63.3 | 0,0381(S)  |
|          | *Ca/Cr>0.6               | 1  | 10.0     | -  | -    | 0,2500(NS) |

**Table 3 - Evolution of Weight (w), Length (l) and Head Circumference (HC) percentiles (P3 to 97), according to the corrected gestational age, estimated through interpolation from the equation defined for each VLBWI of GI (n = 10).**

| Weeks          |     | 28 | 29 | 30      | 31      | 32      | 33      | 34      | 35      | 36      | 37      | 38      | 39      |
|----------------|-----|----|----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Weight (g)     | P3  |    |    | 736.63  | 895.29  | 1043.20 | 1060.99 | 1167.87 | 1334.80 | 1544.25 | 1701.24 | 1719.47 | 1995.29 |
|                | P5  |    |    | 800.32  | 943.00  | 1085.24 | 1113.88 | 1223.89 | 1388.30 | 1577.23 | 1726.03 | 1756.98 | 2002.45 |
|                | P10 |    |    | 898.28  | 1016.37 | 1149.90 | 1195.25 | 1310.06 | 1470.60 | 1627.96 | 1764.15 | 1814.68 | 2013.47 |
|                | P50 |    |    | 1280.00 | 1295.00 | 1372.75 | 1458.73 | 1520.00 | 1691.99 | 1838.81 | 1914.30 | 2005.00 | 2047.11 |
|                | P90 |    |    | 1590.23 | 1534.66 | 1606.59 | 1769.94 | 1918.68 | 2051.91 | 1986.27 | 2033.45 | 2222.22 | 2091.27 |
|                | P95 |    |    | 1688.19 | 1608.03 | 1671.25 | 1851.31 | 2004.85 | 2134.21 | 2037.00 | 2071.57 | 2279.92 | 2102.29 |
|                | P97 |    |    | 1751.88 | 1655.74 | 1713.28 | 1904.20 | 2060.87 | 2187.71 | 2069.98 | 2096.36 | 2317.43 | 2109.45 |
| Length P3 (cm) |     | 28 | 29 | 30      | 31      | 32      | 33      | 34      | 35      | 36      | 37      | 38      | 39      |
|                | P5  |    |    | 34.30   | 35.97   | 36.94   | 37.82   | 38.77   | 39.93   | 41.03   | 41.11   | 42.63   | 42.04   |
|                | P10 |    |    | 34.70   | 36.29   | 37.28   | 38.15   | 39.09   | 40.21   | 41.24   | 41.38   | 42.85   | 42.41   |
|                | P50 |    |    | 35.31   | 36.79   | 37.79   | 38.66   | 39.59   | 40.64   | 41.56   | 41.80   | 43.19   | 42.98   |
|                | P90 |    |    | 38.00   | 38.60   | 39.70   | 40.30   | 41.10   | 42.00   | 42.65   | 43.50   | 44.40   | 44.50   |
|                | P95 |    |    | 39.65   | 40.30   | 41.43   | 42.26   | 43.07   | 43.66   | 43.82   | 44.75   | 45.58   | 46.96   |
|                | P97 |    |    | 40.26   | 40.80   | 41.94   | 42.77   | 43.57   | 44.09   | 44.14   | 45.17   | 45.92   | 47.52   |
| HC (cm) P3     |     | 28 | 29 | 30      | 31      | 32      | 33      | 34      | 35      | 36      | 37      | 38      | 39      |
|                | P5  |    |    | 24.46   | 25.43   | 26.61   | 27.36   | 27.90   | 28.28   | 29.69   | 30.78   | 31.68   | 32.16   |
|                | P10 |    |    | 24.79   | 25.70   | 26.83   | 27.60   | 28.19   | 28.64   | 29.90   | 30.93   | 31.83   | 32.28   |
|                | P50 |    |    | 25.30   | 26.11   | 27.18   | 27.96   | 28.65   | 29.20   | 30.22   | 31.17   | 32.06   | 32.46   |
|                | P90 |    |    | 26.40   | 27.50   | 28.30   | 29.25   | 30.00   | 30.75   | 31.05   | 31.80   | 32.60   | 33.10   |
|                | P95 |    |    | 28.90   | 29.02   | 29.59   | 30.56   | 31.89   | 33.12   | 32.50   | 32.83   | 33.68   | 33.74   |
|                | P97 |    |    | 29.41   | 29.43   | 29.93   | 30.92   | 32.35   | 33.68   | 32.83   | 33.07   | 33.91   | 33.92   |

hand, when the donor draws milk between milking or uses dripping milk, the caloric value is lower because this is the solution fraction.<sup>27</sup> Therefore, the nutritional and immunological composition of the donated HM can be modified due to various factors including period of lactation.

HMB samples were selected as described to meet the medical prescription according to the needs of the VLBWIs and their evolution period. The initial offered volume ranged from 10 to 20 mL/kg/day and was increased according to the child's tolerance. When possible, the mother's own HM was utilized. In this manner, the group

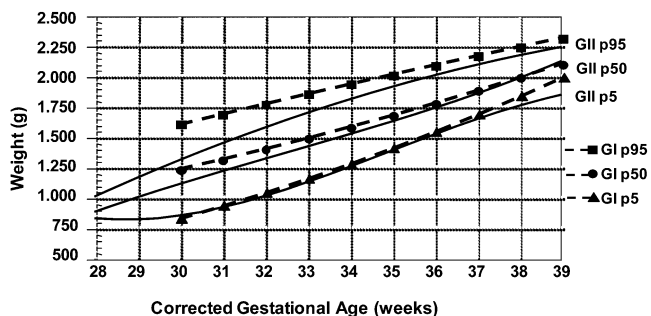
division naturally occurred according to the need for supplementation with HMB.

Enteral feeding had an early start, and the time to reach full enteral feeding in the stable growth phase in GI was  $6.3 \pm 4.3$  days and was  $10.8 \pm 6.4$  days in GII (Table 01). A relevant comparison was the difference found by Schanler et al.<sup>28</sup> who observed an elapsed time of  $28 \pm 7$  days to reach full enteral feeding utilizing supplemented HM and  $36 \pm 17$  days in children fed with formula for premature infants.

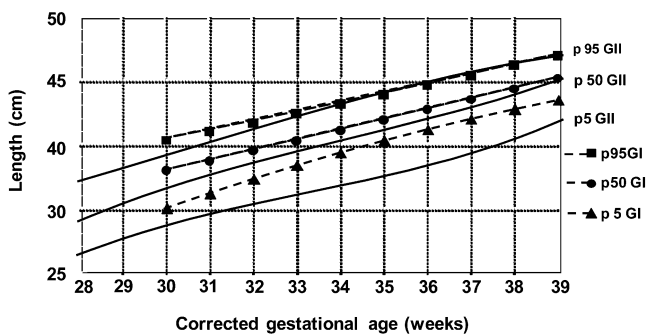
When comparing the birth weight recovery time in GI and GII, we observed, on average,  $7.8 \pm 5.8$  days in GI and

**Table 4 - Evolution of Weight (w), Length (l) and Head Circumference (HC) percentiles (P3 to P97), according to the corrected gestational age, estimated through interpolation from the equation defined for each VLBWI of GII (n = 30).**

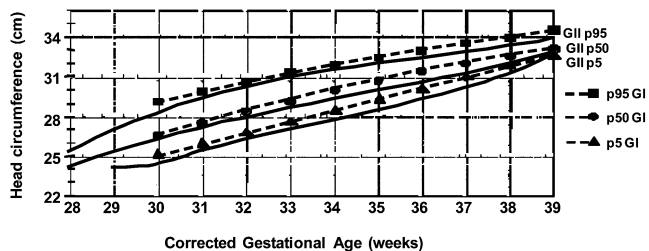
| Weeks       |     | 28     | 29      | 30      | 31      | 32      | 33      | 34      | 35      | 36      | 37      | 38      | 39      |
|-------------|-----|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Weight (g)  | p3  | 837.28 | 745.19  | 815.65  | 894.21  | 1008.68 | 1079.76 | 1185.11 | 1312.30 | 1441.05 | 1625.25 | 1799.29 | 1756.75 |
|             | p5  | 843.23 | 771.65  | 859.17  | 931.70  | 1045.57 | 1118.34 | 1224.50 | 1350.36 | 1478.73 | 1656.02 | 1821.34 | 1791.76 |
|             | p10 | 852.40 | 812.35  | 926.13  | 989.38  | 1102.31 | 1177.68 | 1285.09 | 1408.92 | 1536.69 | 1703.35 | 1855.26 | 1845.62 |
|             | p50 | 887.50 | 940.50  | 1145.00 | 1193.70 | 1330.50 | 1395.00 | 1505.00 | 1609.30 | 1728.80 | 1849.40 | 1987.20 | 2106.70 |
|             | p90 | 917.10 | 1099.85 | 1399.03 | 1396.74 | 1503.10 | 1596.85 | 1713.09 | 1822.52 | 1946.06 | 2037.69 | 2094.83 | 2226.02 |
|             | p95 | 926.27 | 1140.55 | 1465.98 | 1454.41 | 1559.84 | 1656.20 | 1773.68 | 1881.07 | 2004.02 | 2085.02 | 2128.75 | 2279.88 |
|             | p97 | 932.22 | 1167.01 | 1509.51 | 1491.91 | 1596.74 | 1694.78 | 1813.07 | 1919.14 | 2041.70 | 2115.79 | 2150.80 | 2314.89 |
| Length (cm) | p3  | 31.58  | 33.25   | 34.28   | 34.72   | 35.84   | 36.65   | 37.64   | 38.33   | 39.17   | 39.80   | 41.25   | 43.03   |
|             | p5  | 31.94  | 33.55   | 34.65   | 35.10   | 36.18   | 37.00   | 37.98   | 38.68   | 39.56   | 40.23   | 41.56   | 43.26   |
|             | p10 | 32.48  | 34.01   | 35.22   | 35.68   | 36.72   | 37.53   | 38.49   | 39.22   | 40.16   | 40.89   | 42.04   | 43.61   |
|             | p50 | 33.80  | 35.25   | 37.00   | 37.40   | 38.50   | 39.50   | 40.20   | 41.00   | 42.25   | 43.20   | 44.10   | 45.10   |
|             | p90 | 36.36  | 37.26   | 39.26   | 39.80   | 40.47   | 41.31   | 42.14   | 43.07   | 44.38   | 45.57   | 45.42   | 46.07   |
|             | p95 | 36.90  | 37.72   | 39.83   | 40.38   | 41.01   | 41.84   | 42.65   | 43.61   | 44.97   | 46.23   | 45.89   | 46.42   |
|             | p97 | 37.26  | 38.01   | 40.21   | 40.76   | 41.35   | 42.19   | 42.99   | 43.97   | 45.36   | 46.66   | 46.20   | 46.65   |
| HC (cm)     | p3  | 23.50  | 22.34   | 23.83   | 24.87   | 25.46   | 26.13   | 26.94   | 27.83   | 28.71   | 29.58   | 30.63   | 32.00   |
|             | p5  | 23.58  | 22.67   | 24.16   | 25.15   | 25.75   | 26.41   | 27.22   | 28.10   | 28.97   | 29.82   | 30.81   | 32.09   |
|             | p10 | 23.70  | 23.17   | 24.67   | 25.58   | 26.19   | 26.84   | 27.64   | 28.51   | 29.36   | 30.17   | 31.08   | 32.24   |
|             | p50 | 24.15  | 24.60   | 27.00   | 27.10   | 27.65   | 28.30   | 28.95   | 29.80   | 30.80   | 31.20   | 31.90   | 32.60   |
|             | p90 | 24.55  | 26.76   | 28.31   | 28.62   | 29.32   | 29.89   | 30.65   | 31.44   | 32.16   | 32.68   | 32.99   | 33.29   |
|             | p95 | 24.67  | 27.27   | 28.83   | 29.05   | 29.76   | 30.33   | 31.08   | 31.86   | 32.56   | 33.04   | 33.26   | 33.44   |
|             | p97 | 24.75  | 27.60   | 29.16   | 29.33   | 30.04   | 30.61   | 31.35   | 32.13   | 32.81   | 33.27   | 33.44   | 33.54   |



**Figure 1** - Estimated values of 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of GI and GII VLBWIs (in g).



**Figure 2** - Estimated values of 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of GI and GII VLBWI length (in cm).



**Figure 3** - Estimated values of 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of GI and GII VLBWI Head Circumference (HC in cm).

10.6 ± 4.0 days in GII (Table 01). Gross et al.<sup>26</sup> compared the weight gain of the three VLBWI groups with GA between 27–33 weeks and birth weight < 1500 g. One group was fed with the first week’s milk (colostra and transition milk) obtained from VLBWI mothers, another group was fed with mature milk from full-term newborn mothers, and a third group was fed with lacteal formula. Those fed with lacteal formula recovered their birth weight in 10.3 ± 0.8 days, those fed with VLBWI mother’s milk recovered in 11.4 ± 0.8 days, and those using mature milk recovered in 18.8 ± 1.7 days. In this study, the authors utilized a pool of human milk from various donors stored until a year after pasteurization, a procedure that is not utilized in Brazil.<sup>13</sup> It should be noted that the components of HMB obtained through the pool are medianly distributed, and therefore the children receiving it do not benefit from the changes in composition of drawn milk.

In regard to the time elapsed to reach 2 kg, there was no difference between groups. On average, GI took 7.3 ± 2 weeks and GII took 7.8 ± 1.9 weeks (Table 1).

These results are similar to the results from a study by Schanler et al.<sup>28</sup>, which compared VLBWIs fed with supplemented HM and preterm formula. They found an average time to reach 2 kg of 8.4 ± 1.8 weeks with the use of supplemented HM and 7.2 ± 1.7 weeks with formula for VLBWIs. The results suggest that the early introduction of pasteurized HM or HMB allows for a good enteral feeding progression and a very appropriate average time to reach 2 kg.

As for clinical events, in GII, 3/30 (10.0%) children had suspect light NEC with good clinical evolution, and there were no cases of NEC in GI. In regard to sepsis, there were 3/10 (33.3%) cases in GI and 7/30 (23.3%) cases in GII (Table 1). Schanler et al.<sup>29</sup> found a 6% incidence of NEC in the group of children fed with donor HM, 11% in the group of preterm formula, and 6% in the HM group; sepsis incidence was 29% in the group of children fed with donor milk, 23% in the maternal milk group, and 30% in the group using formula.

The incidence of sepsis in the group using HMB was the same found in the group using HM, similar to what was previously described by Schanler et al.<sup>28</sup>

Bronchopulmonary dysplasia (BD), occurred in 3/10 (30.0%) cases in GI and GII 9/30 (30.0%) cases in: 1/30 (3.3%) presented moderate and 8/30 (26.6%) presented light BD (Table 01).

In the studied cases, there was no significant difference of clinical complications when comparing HMB and the mother’s own milk probably due to the preservation of donated HMB protection factors, resulting from quality control techniques used in the National HMB Network.<sup>13</sup>

This study monitored the phosphorous deficiency<sup>21</sup> and corrected it when necessary through the use of a calcium and phosphorous manipulated formula (Table 2). GI presented urinary calcium > 4 mg/L in 1/10 (10.0%), urinary phosphorus <1 mg/L in 10/10 (100%), and Ca/Cr ratio > 0.6 in 1/10 (10.0%) of the cases; in GII, none of the children presented alterations in urinary calcium values, although 19/30 (63.3%) presented urinary phosphorus <1 mg/L. This made us conclude that the urinary excretion of phosphorus was more prolonged and intense in GI than in GII (p = 0,0381), which evolved more adequately, showing economy in phosphorous excretion.

These results suggest that HMB with higher caloric value mostly composed of the suspension and emulsion fraction than the solution fraction has more casein in the colloidal solution with calcium and phosphorus, which causes a higher concentration of these ions. In spite of this, the phosphorous serum level was found to be low in 3/10 (30.0%) of the GI children and in 16/30 (53.3%) of group II, and the alkaline phosphatase was > 900 UL in 7/10 (70.0%) of GI children and 17/30 (56.6%) of GII children (Table 2). These results show the need for continuous Osseous Metabolic Disease monitoring through the control of serum and urinary calcium and phosphorus when indicated by laboratorial parameters.

The growth analysis shows that for GI, the anthropometric data for the construction of curves were computed from the value that each child presented in the 30<sup>th</sup> week of CGA; when analyzed between the 30<sup>th</sup> and 39<sup>th</sup> week of CGA, we verified an average weight gain of 12.1 g/day, length of 0.75 cm/week and HC of 0.74 cm/week in the 50<sup>th</sup> percentile (p50) of the curve (Table 3; Figures 1, 2 and 3).

As for GII, from the 28<sup>th</sup> to the 39<sup>th</sup> week of CGA, in regard to p50, the average weight gain was 15.8 g per day;

however, if we stratify this period, we verified that the weight gain was 14.5 g/day between the 28<sup>th</sup> and the 31<sup>st</sup> week of CGA, 11.6 g/day between the 32<sup>nd</sup> and the 34<sup>th</sup> week, and 17.7 g/day between the 35<sup>th</sup> and 39<sup>th</sup> week (Table 4 and Figure 1). The phase of lesser weight gain between the 32<sup>nd</sup> and 34<sup>th</sup> week coincides with the moment when non-nutritive breast milk suction started, which might have caused an energetic expenditure above that when the nursing bottle or little cup was used.

In regards to the length of GIL, the increase was 1.02 cm/week, and the HC growth was 0.76 cm/week (Table 4 and Figure 2,3).

In 2005, Schanler et al.<sup>29</sup> observed a weight gain of 17.1 ± 5.0 g/kg/day, length of 1.2 ± 0.8 cm/week and HC of 0.9 ± 0.9 cm/week in the group fed milk from donor mothers. In the group fed preterm formula, the weight gain was 20.1 ± 6.7 g/kg/day, length was 1.0 ± 1.0 cm/week and HC was 0.9 ± 0.8 cm/week. The maternal milk group gained 18.8 ± 5.8 g/kg/day in weight, length of 0.6 ± 0.4 cm/week and CP of 0.8 ± 0.5 cm/week. In another study,<sup>28</sup> by utilizing HM with additives, the obtained weight gain was 22 ± 7 g/kg/day, length was 0.79 ± 0.27 cm/day and HC was 0.88 ± 0.26 cm/day. Compared with the studied cases, the weight gain was superior in all groups; however, children in the present study took 7 weeks on average to reach 2 kg in weight and had very similar gains in length and HC, which means that the smaller weight losses soon after birth and the faster recovery of birth weight compensated for the smaller weight gain later on.

An important aspect observed in this study are the growth curves with proportionality in the three studied parameters (weight, length and HC), showing the adequacy of the offered diet (Figures 1, 2 and 3).

Studies with donated HMB in general utilize HM pools, which are extremely vulnerable products with few controlled studies of its physical-chemical quality and caloric and protein value, which are indispensable aspects. Without this information, there are great possibilities of offering a product that does not meet the VLBWI nutritional needs.<sup>7,13,29</sup>

By determining the properties of each milk sample, despite the period of lactation (colostra, transition or mature milk), we have the possibility to individualize the VLBWI needs and offer a more appropriate HMB in regard to its caloric-protein composition for each phase of the child's growth. Quality control of donated human milk established by the Brazilian laws governing human milk bank<sup>13</sup> allows for a better quality product with minimal immunologic and nutritional loss. The usual determination of crematocrit plus that of protein makes the HMB choice economically feasible, for the crematocrit cost is insignificant and the protein dosage is about US\$0.50/sample.

We conclude that HMB utilization is an important strategy in the nutrition of VLBWIs to promote a satisfactory growth with good clinical evolution and should be considered in neonatology services structured with a human milk bank.

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