Preparation of Fat and Protein from Banked Human Milk: Its Use in Feeding Very-Low-Birth-Weight Infants

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Current recommendations, both in Europe and the United States, emphasize breast feeding as the regimen of choice for the healthy term infant (1,2). However, there is still considerable debate in the pediatric literature about the nutritional adequacy of human milk for the preterm infant. Recent studies (3–5) have shown that moderately premature infants (31 to 36 weeks gestational age) attain intrauterine growth rates with adequate intakes of human milk, but the question of the nutritional requirements of the very-low-birth-weight infant still remains unanswered.

There is evidence that the requirements of the very small preterm infant for protein, energy, calcium, and sodium may be greater than can be provided by mature human milk alone. The recent findings that milk from mothers delivering preterm infants contains more protein, calories, and sodium than term milk also suggest this (6,7). However, in addition to being a source of nutrients, human milk also contains important nonnutritional components such as specific antiinfective factors, hormones, enzymes, and growth factors, which may be of great importance for the development of the very-low-birth-weight infant.

In order to initiate a controlled clinical study with the aim of defining the special nutritional requirements of the very-low-birth-
weight infant, we have designed a pilot plant to prepare fat and protein fractions from unpasteurized human donor milk. These fractions can be used for the enrichment of human milk. The overall aim of our studies is not simply to find an optimal nutritional supply for the very-low-birth-weight infant but also to supply the developing organism with the protective factors, growth modulators, enzymes, and hormones that are uniquely present in human breast milk. In this chapter, we describe the method used to prepare the cream and protein fractions and also present some preliminary analytical data on these milk fractions.

PILOT PLANT TECHNIQUE

Fat Separation

After gentle heating to ~50°C, the human milk was separated into skim milk and cream with an Alfa-Laval separator, LAPX 202. The separator was run at a speed of 7,000 rpm. The skim milk had a fat content of less than 0.5%, and the fat phase 50 to 60%. The cream was frozen in 5-g pellets and packed in vacuum.

Ultrafiltration

The equipment used was a DDS (Danish Sugar Industry) Lab module 20–072. The module has a filtration area of 0.72 m². The membrane used is of type GR 61 PP with a cut-off value of 20,000 (molecular weight).

The warm skim milk, 45 to 50°C, was immediately poured into a jacketed stainless steel tank. The tank was cooled by running cold tap water, 12°C, and within 10 min the milk reached room temperature, 15 to 20°C. In order to get sufficient permeate flow, the temperature was kept at 15 to 20°C. Lactose and water-soluble salts were separated from the milk by ultrafiltration. Small amounts of low-molecular-weight proteins were lost with the permeate.

Each cycle started with 15 liters of milk. About 12 to 13 liters of skim milk remained after fat separation. The ultrafiltration process was run for 6 to 8 hr in order to achieve the desired level of
demineralization and protein concentration. The permeate was replaced by cold tap water. A constant volume and viscosity of the recirculating milk kept the permeate flow at a desirable high level. Bacterial analysis of the final protein and fat preparations showed acceptable quality.

**Freeze-Drying**

After ultrafiltration, the concentrate was freeze-dried. Freeze-dried protein powder from 120 liters of human milk was mixed thoroughly and packed into polyethylene-lined aluminum pouches containing ~10 g each. A scheme for the technique used is shown in Fig. 1.

![Diagram](image)

**FIG. 1.** Fractionation of fat and protein from human milk.
Preparation of Supplemented Human Milk

Supplemented milk is prepared for an infant freshly each day in a volume sufficient to last for 24 hr. The mother’s own “raw” milk is preferentially used and is then complemented with banked milk to full volume. In general, the “mother’s own” milk comprises ~30 to 50% of the total volume. When the mixture of “mother’s own” and “pooled” milk is ready, it is supplemented with either protein powder or fat or both in amounts to make a total addition of 1 g protein and 1 g fat per 100 ml milk. This produces a final protein concentration of ~2 g/100 ml and a fat concentration of ~5.5 g/100 ml. The milk is then thoroughly mixed and divided into smaller plastic flasks, each containing the volume of milk needed for one feed. Samples for bacteriology and chemical analysis are taken from the supplemented milk. The criteria of acceptability are those currently employed by the milk bank.

PRELIMINARY ANALYSIS OF THE PROTEIN POWDER AND THE SUPPLEMENTED HUMAN MILK (HUMAN MILK FORMULA)

The crude protein powder contained 51% protein and 22.8% lactose (Table 1). When pooled human milk with a mean protein content of ~1 g/100 ml was supplemented with protein powder equivalent to 1 g of protein, there was a slight increase in the osmolality from 283 mOsm/kg to 290 mOsm/kg. Supplementation of the milk with fat produced no change in the osmolality (Table 2). There was no increase in sodium and potassium concentrations of the reconstituted human milk formula, but the calcium content rose by ~40% (Table 2) when protein was added.

In addition to the above assays, the milk protein powder was studied for the recovery of various whey proteins and enzyme proteins, which could theoretically be of advantage for the immature infant if administered in higher concentration than that present in pooled human milk.

Table 3 shows that the recovery in three separate production batches of α-lactalbumin, lactoferrin, lysozyme, and albumin varied
TABLE 1. *Protein and lactose in human milk protein powder*

<table>
<thead>
<tr>
<th></th>
<th>Protein 51 g/100 g</th>
<th>Lactose 22.8 g/100 g</th>
</tr>
</thead>
</table>

TABLE 2. *Osmolality and electrolytes in human milk preparations*

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Osmolality (mOsm/kg)</th>
<th>Na⁺ (mm)</th>
<th>K⁺ (mm)</th>
<th>Ca²⁺ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human milk</td>
<td>283</td>
<td>7</td>
<td>16.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Human milk + HM protein (1 g/dl)</td>
<td>290</td>
<td>8</td>
<td>16.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Human milk + HM-fat (1 g/dl)</td>
<td>278</td>
<td>7</td>
<td>16.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

between 60 and 100%. Previous studies (8,9) have also shown a substantial recovery of lactoferrin and IgA in human milk protein preparations. The somewhat higher recovery in the present study was probably obtained because the protein powder was not pasteurized. Our human milk protein powder also contains activity of α₁-antitrypsin, amylase, and bile-salt-stimulated lipase (Table 3). Human milk bile-salt-stimulated lipase is readily inactivated when incubated at +50°C (10). In the present study, the low enzyme activity (15%) was apparently a result of the heating of the milk before the separation of the cream. In subsequent protein preparations, heating will be omitted in order to minimize the lipase inactivation. Almost 100% of the enzyme protein was, however, recovered in the human milk powder (preliminary finding).

Clinical tolerance studies in very-low-birth-weight infants (<1,500 g body wt.) have shown that milk supplemented with 1 g of protein and/or 1 g of fat per 100 ml is well tolerated. Further controlled
TABLE 3. *Whey proteins and enzymes in human milk protein powder*

<table>
<thead>
<tr>
<th>Component</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (mg/g)</td>
<td>Calculated recovery (%)</td>
<td>Amount (mg/g)</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>100</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>60</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>19</td>
<td>75</td>
<td>20</td>
</tr>
<tr>
<td>Albumin</td>
<td>19</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>sigA</td>
<td>13.5</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td>0.34</td>
<td>28</td>
<td>0.34</td>
</tr>
<tr>
<td>Antichymotrypsin</td>
<td>0.0025</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>Amylase</td>
<td>160 (U/g)</td>
<td>100</td>
<td>180 (U/g)</td>
</tr>
<tr>
<td>Lipase</td>
<td>135</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Calculated recovery is expected recovery as related to fresh mature human milk.

*Expressed in micromoles of fatty acid released per gram per minute.
clinical studies are needed in order to evaluate the growth and metabolic effects of specially supplemented human milk in order to establish optimal feeding regimens for very-low-birth-weight infants. Such studies are currently in progress.

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REFERENCES