Nitrogen Distribution in Human Milk from 2 to 16 Weeks Postpartum

S. A. ROSS and R. M. CLARK
Department of Nutritional Sciences, U-17
University of Connecticut
Storrs 06268

ABSTRACT
The objective of this study was to determine how the major nitrogen components in human milk vary as total nitrogen in the milk changes. Milk samples were collected from 10 mothers at 2, 6, 12, and 16 wk postpartum. The major nitrogen components compared were total, nonprotein, urea, protein, casein, and whey nitrogens. Total nitrogen decreased from 2.18 mg/ml at 2 wk to 1.76 mg/ml at 16 wk. The percentage of nonprotein nitrogen was 19% and remained constant with time postpartum. Urea nitrogen averaged .14 mg/ml and also remained constant. Protein nitrogen decreased from 1.77 mg/ml at 2 wk to 1.42 mg/ml at 16 wk. As the total nitrogen in milk changed, the percentages of nonprotein nitrogen, whey nitrogen, and casein nitrogen remained relatively constant.

INTRODUCTION
Our knowledge of nitrogen and protein components in breast milk is limited, and the data available are mainly from pooled milk samples or milk samples that were not collected under standardized conditions. Most of these studies were descriptive in nature with little information on the variability of the components. In this report, we present information on total, nonprotein, urea, protein, casein, and whey nitrogens and their variability in mature human milk collected from mothers during 14 wk.

Data in the literature suggest that as colostrum changes to mature milk the decrease in total protein as lactation progresses is primarily due to a decrease in the concentration of whey proteins (7, 8, 11). Therefore, as colostrum changed to mature milk so did the ratio of casein to whey protein. Because of these changes just after parturition, it was of interest to see how the major nitrogen components in breast milk vary relative to each other in milk produced later in lactation. In this report we provide data that shows that, unlike the major shift in nitrogen components in milk during the initial stages of lactation, proportions of nitrogen components in mature milk remain relatively constant as total nitrogen changes.

MATERIALS AND METHODS
Milk samples in this study were donated by 10 mothers whose milk was the only source of calories for their infants. The samples were collected at home using an electric breast pump (Egnell, Inc., Cary, IL). Milk was collected at 2, 6, 12 and 16 wk postpartum. On the day of collection, total milk from one breast was taken during a morning nursing (0930 to 1130 h) and again during an afternoon nursing (1330 to 1530 h). The milk was placed on dry ice and at the end of the day transported in a styrofoam container to the laboratory for storage at -70°C.

On the day of analysis, milk was thawed to 37°C and equal volumes of a.m. and p.m. milk were pooled for analysis. Nitrogen measurements were made on three fractions of the milk by the micro-Kjeldahl method (1).

The analysis of total nitrogen required 80 μl of milk. Protein nitrogen was determined in milk that had undergone dialysis. Milk was placed in dialysis tubing with a molecular weight cutoff of 1000 and dialyzed against distilled water at 4°C. The ratio of water to milk was greater than 1000 to 1. Water was changed after 3, 6, and 9-h of dialysis. After 12 h of dialysis, the milk was transferred to a volumetric flask and diluted to 5.0 ml with .01 N sodium hydroxide. Longer dialysis or more
frequent changes of water did not cause further reduction of nondialyzable nitrogen.

The nondialyzable nitrogen (protein nitrogen) of the milk was subtracted from total nitrogen to calculate the concentration of nonprotein nitrogen. A major component of nonprotein nitrogen, urea nitrogen, was determined colorimetrically by the method of Crocker (2).

With minor modifications, precipitation of casein was according to Nagasawa et al. (13). One milliliter of milk was diluted with 4.0 ml of distilled water and adjusted to a pH of 4.6 with .01 N HCl. The milk was left to stand for approximately 2 h and casein precipitated by centrifugation at 35,000 × g for 30 min. After centrifugation, the supernatant was removed for nitrogen analysis. The supernatant contained the whey nitrogen and nonprotein nitrogen. Whey nitrogen was determined by subtracting the concentration of nonprotein nitrogen from the nitrogen concentration of the supernatant. Casein nitrogen was calculated by subtracting whey nitrogen from protein nitrogen.

Statistical analysis of the nitrogen components was based on a complete block design with repeated measurements as described by Gill (4). The assumptions of equal variances and correlations for univariate analysis were tested. When the compound symmetry assumption could not be met, multivariate analysis was used (4).

RESULTS AND DISCUSSION

Concentrations of nitrogen components in human milk from 2 to 16 wk postpartum are given in Table 1. The values in Table 1 are in agreement with concentrations reported in the literature (9, 10). Total nitrogen, protein nitrogen, casein nitrogen, and whey nitrogen all significantly (<.05) decreased with time postpartum. Nonprotein nitrogen and urea nitrogen did not change as lactation progressed.

Total nitrogen decreased from 2.18 mg/ml at 2 wk to 1.66 mg/ml at 12-wk postpartum. The total nitrogen concentration ranged from 1.28 to 2.50 mg/ml.

Nonprotein nitrogen in breast milk had an average concentration of .36 mg/ml and a range of .17 to .72 mg/ml. Of the total nitrogen, percentage of nonprotein nitrogen did not change significantly. Others also have reported that nonprotein concentrations remained relatively constant in comparison with total nitrogen (3, 11).

Urea nitrogen is one of the major components of nonprotein nitrogen and it averaged .14 mg/ml of milk. The concentration of urea in the milk did not change with time postpartum and is probably controlled by the concentration of urea in blood. Urea seems to diffuse readily between blood and milk (5).

Protein nitrogen ranged from .94 to 2.11 mg/ml. The protein nitrogen decreased from 1.77 mg/ml at 2 wk to 1.42 mg/ml at 16 wk. This decrease in protein nitrogen with time postpartum has been observed by others (3, 7, 8, 11).

The major protein classes in milk, casein, and whey were determined. The separation of casein from human milk by acid precipitation is more difficult than from bovine milk.

<table>
<thead>
<tr>
<th>Nitrogen (N) component</th>
<th>Stage of lactation, wk postpartum</th>
<th>CV²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein N</td>
<td>1.77</td>
<td>1.59</td>
</tr>
<tr>
<td>Nonprotein N</td>
<td>.41</td>
<td>.35</td>
</tr>
<tr>
<td>Whey N</td>
<td>1.01</td>
<td>.99</td>
</tr>
<tr>
<td>Casein N</td>
<td>.75</td>
<td>.61</td>
</tr>
</tbody>
</table>

¹ Mean of 10 observations.

² Coefficient of variation = \( \frac{(error mean square)^{1/2}}{\bar{X}} \times 100 \).
Toyoda and Yamauchi classified human milk for acid coagulability (14). They observed that casein coagulated distinctly in approximately 40% of their milk samples when the pH was adjusted to 4.6. In our study, approximately 35% of the samples were of the acid-coagulable type. However, we did not observe any relationship between acid coagulability and final casein nitrogen concentration. Another difficulty with the acid precipitation procedure is that lactoferrin, a major whey protein in human milk, is known to coprecipitate with casein. It has been estimated that 3 to 10% of the total lactoferrin coprecipitates with casein (12). As a consequence, use of acid precipitation to separate casein and whey in human milk results in a value for casein nitrogen that is too great. Despite these problems, most published values for casein were derived from the use of acid precipitation. Recently, Lonnerdal and Forsum proposed other methods for estimating the casein content of human milk (6). The utility of these methods requires further research.

In our study, nitrogen from the casein fraction ranged from .31 to .88 mg/ml. Casein nitrogen was .75 mg/ml at 2 wk and decreased approximately 20% as lactation progressed. Casein nitrogen was 40% of the total nitrogen and this percentage was constant throughout the study. This value is in agreement with the results of others, who used acid precipitation for separation of casein (3, 9, 10, 11).

The percentage of whey nitrogen was 60 and did not change with time postpartum. Whey nitrogen ranged from .50 to 1.30 mg/ml. The whey nitrogen concentration at 2 wk averaged 1.01 mg/ml and decreased about 16% by 16 wk postpartum.

In previous work, comparing nitrogen distribution in colostrum to mature milk most of the decrease in total nitrogen with time postpartum was due to a decrease in whey nitrogen (7, 8, 11). In our study, in milk produced between 2 and 16 wk postpartum the percentages of nonprotein nitrogen, casein nitrogen, and whey nitrogen were not altered by a change in total nitrogen. From these results we concluded that the decrease in total nitrogen during the first few days of lactation is accompanied by a shift in nitrogen components but that changes in milk produced after 2 wk postpartum do not change the distribution of major nitrogen components in milk.

ACKNOWLEDGMENT
This study was supported in part by federal funds made available through the provision of the Hatch Act.

REFERENCES