Evaluation of lactational performance of Navajo women[1, 2]

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ABSTRACT The effect of suboptimal maternal nutrition on lactational performance of 23 Navajo women was studied in terms of milk volume, milk composition, and infant growth. The mean milk volume produced by 10 Navajo women was 634 ± 113 ml/24 h after approximately 1 month of lactation. The content of protein, lactose, and lipid were within normal limits. Retinol and carotene content were 32.9 ± 15.7 and 19.7 ± 6.3 μg/dl, respectively. Milk folacin averaged 56.4 ± 23.9 ng/ml. The mean contents of zinc, iron, and copper were 2.8 ± 1.1, 0.8 ± 0.6, and 0.3 ± 0.2 mg/l, respectively. Despite evidence of suboptimal nutriture among these Navajo women, lactational performance was adequate in terms of infant growth, milk volume, and milk composition with the exception of vitamin A which was lower than normal. *Am. J. Clin. Nutr.* 34: 2210–2215, 1981.

KEY WORDS Navajo, lactation, milk composition, milk volume, infant growth

Introduction

Maternal nutrition is one of the many factors that interplay in the production of human milk. It was once thought that lactation was exempt from nutritional insult, but there is increasing evidence that suboptimal maternal nutrition adversely affects lactational performance evaluated in terms of milk volume, milk composition, and infant growth.

It is difficult to compare studies of human milk production due to differences in methodology and experimental design. Some investigators have estimated milk yield by the expression technique (1, 2), but most have used the test-weighing method (3–7). Although the number of studies on well-nourished women is limited, investigations by Wallgren (8) and Lönnerdal et al. (3) provide reliable standards of milk quantity for the first 6 months of lactation. Milk production may vary with the stage of lactation, with the time of day and with environmental-psychological stresses (9).

Milk composition may be influenced by several factors: the stage of lactation, the time of day, the stage of feeding as well as maternal food intake. Extensive data on milk composition have been published (9), but the interactions with maternal nutrition have not been thoroughly studied.

Lactational adequacy is also reflected in the growth and development of the suckling child. Infant growth as an indicator of lactational performance should be evaluated in conjunction with modifying determinants such as genetic potential, health history, and supplemental feedings. Criteria for lactational adequacy ought to include maternal nutritional status; lactation cannot be considered satisfactory if maternal health status is deteriorating. Too often investigators have been more interested in milk production and infant well-being than in the health of the reproductive female.

The nutritional status of the Navajo women who participated in this study has been discussed in a previous paper (10). In this paper, lactational performance will be evaluated by the criteria of milk volume and composition and infant growth.

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Methods

Subjects

Twenty-three Navajo women, ages 16 to 23 yr, participated in the study after consenting to the protocol which was approved by the Navajo Tribal Health Council, Indian Health Service Research and Publications Committee, and the Committee for the Protection of Human Subjects of the University of California, Berkeley. All the women were in full lactation on the day of assessment, which varied from day 19 to 62 of lactation. Experimental procedures were performed in the woman's home unless the woman agreed to the optional test-weighing session in which case the investigations were conducted in a residence provided by the hospital.

Determination of 24-h milk volume

Ten mothers agreed to the 24-h test weighing session. Before and after each feeding the infant was weighed by the investigator on a beam balance accurate to ±15 g. Disposable, plastic-covered diapers were provided to prevent loss of urine during the feeding period. No schedule was imposed upon the mothers who breast-fed on demand. Weighings were performed at all feedings irrespective of the duration of a feed.

Collection of milk samples

Milk samples (n = 23) were expressed manually or extracted with a manual pump between 12:00 and 4:00 PM. Many times it was necessary to allow the infant to suck to initiate flow. An effort was made to avoid collection of fore- and hind-milk. The milk samples were immediately placed on ice and later frozen at −20°C until analysis.

Milk analysis

Total nitrogen was determined by a modified Kjeldahl method (11). Protein was calculated from nitrogen [corrected for nonprotein nitrogen (NPN)] by the conversion factor 6.25. NPN was assayed after precipitation of proteins with 24% (v:v) and 12% trichloroacetic acid.

Lactose was determined by high-pressure liquid chromatography. After centrifugation the aqueous phase was filtered through a Millex disposable filter unit and then injected into the chromatograph. Fat content was based upon an enzymatic determination of triglycerides (Worthington Triglycerides Reagent Set, Worthington Biochemicals Corporation, Freehold, NJ).

Milk retinol and carotene content were assayed by the Carr Price reaction utilizing trifluoracetic acid (12). After a 6-h incubation (37°C) with chicken pancreas conjugase, milk folacin was determined by microbiological assay using *Lactobacillus casei* (ATCC 7469) (13).

After digestion by nitric acid plus perchloric acid, milk samples were diluted and aspirated into an atomic absorption spectrophotometer for determination of iron, copper, and zinc. Analysis performed at the Human Nutrition Laboratory, USDA/SEA, P.O. Box 7166 University Station, Grand Forks, ND 58201.

Total solids were estimated after drying for 3 h in a vacuum oven (98 to 100°C).

Infant anthropometric measurements

The infant was weighed nude on a beam balance especially adapted with an infant tray. Supine length was marked with an infant measuring board. Circumferences of the head (fronto-occipital), chest (at the level of the areaolae), and arm (mid-upper) were determined with a plastic measuring tape. Skinfold thicknesses were measured with a Lange caliper using the technique described by McGowan et al. (14) at the following sites: flank, subscapular, triceps, and quadriceps. Except for birth weight which was extracted from the medical records, the above measurements were made by the investigator in the hospital after delivery and in the woman's home or testing house at approximately 1 month postpartum.

Interview

Maternal and infant health since hospital discharge were reviewed by a questionnaire.

Statistical analysis

The data were analyzed by SPSS programs on a Control Data 6400 computer. The Pearson correlation coefficient and the paired t statistic were used to study relationships and differences between variables. Growth data were analyzed by linear regression.

Results

Milk volume and composition

The mean (± SD) milk volume (**Table 1**) produced by the 10 Navajo mothers was 634 ± 113 ml/24 h (range 460 to 870). An average of 11 feedings was included in the yield. Relationships between milk yield and various indices pertaining to the infant were examined. No statistically significant correlations were found for the following variables (correlation coefficients shown in parentheses): infant weight at the test-weighing (0.19); birth weight (−0.23); infant weight in terms of grams gained per day (0.54); days postpartum (0.27); and number of feedings (0.10).

The proximate analysis of the milk produced by the 23 Navajo women is presented in **Table 2**. The mean contents of protein, lactose, and lipid were 1.4, 6.1, and 4.0 g/dl, respectively. The energy value of the milk, 66 kcal/dl, was calculated using the respective conversion factors 4, 4, and 9 kcal/g of protein, lactose, and lipid. The protein value has been corrected for NPN which was 0.28 mg nitrogen/ml milk or 12% of the total nitrogen. Milk protein was inversely related to the lactose content (r = 0.840, p = 0.001).

The milk content of selected vitamins and minerals is shown in Table 2. The mean

TABLE 1
Milk volume produced by the 10 Navajo women (test-weighing procedure)

Subject no.	Days postpartum	No. feedings per day	Volume
			ml/24 h
1	29	14	707
2	30	8	604
3	51	9	634
4	30	14	570
5	21	11	614
6	31	6	600
7	27	12	870
8	19	12	460
11	46	10	730
19	31	11	548
$\bar{X}$	31.5	11	634
SD	9.9	2.5	113

TABLE 2
Milk composition of the 23 Navajo women (1 month postpartum)

Variable	Mean (SD)	Range	Reference standard (9)
Protein (g/dl)	1.4 (0.3)	1.0–2.3	1.1
NPN (mg/ml)	0.3 (0.1)	0.2–0.4	0.4
Lactose (g/dl)	6.1 (0.6)	4.4–7.3	6.8
Lipid (g/dl)	4.0 (1.4)	1.8–6.8	4.5
Energy (kcal/dl)	66 (12)	45–91	75
Total solids (%)	13.5 (2.1)	10.2–17.5	12.9
Vitamin A (μg/dl)	32.9 (15.7)	10.7–64.7	56.9
Carotene (μg/dl)	19.7 (6.3)	11.1–32.3	
Folacin (ng/ml)	56.4 (23.9)	34.0–135.8	52.0
Zinc (mg/l)	2.8 (1.1)	0.7–4.6	3.0–5.0
Iron (mg/l)	0.8 (0.6)	0.01–2.22	0.5
Copper (mg/l)	0.3 (0.2)	0.06–0.71	0.4

retinol and carotene content were 32.9 ± 15.7 and 19.7 ± 6.3 μg/dl, respectively. Milk retinol and carotene were strongly related ($r = 0.317$, $p = 0.015$). Milk fat was positively correlated with milk retinol content ($r = 0.317$); however, this relationship did not attain statistical significance. In contrast, the positive correlation between milk fat and milk carotene did achieve significance ($r = 0.73$, $p = 0.001$).

Milk folacin averaged 56.4 ± 23.9 ng/ml. The mean (± SD) content of zinc, iron and copper was 2.8 ± 1.1, 0.8 ± 0.6, and 0.3 ± 0.2 mg/l, respectively. No significant correlations were seen between the trace minerals.

Milk samples were collected from a subsample of seven women at 3 months postpartum. A significant decline was observed in NPN from 0.29 to 0.24 mg/ml ($p < 0.002$) and in zinc from 3.0 to 1.9 mg/l ($p < 0.03$). Conversely, folacin significantly increased from 50.0 to 77.7 mg/dl ($p < 0.04$).

Infant growth

Infant growth was described as a linear regression of weight on age. The mean rate of growth as indicated by the slope was 35.9 g/day (31.8 g/day for females and 39.8 g/day for males). The rate of weight gain between birth and 1 month of age corresponded to the 75th percentile of the Iowa growth standards for females and to the 90th percentile for males (15).

The NCHS growth standards (16) were utilized to rank weight, height, and head circumference by percentiles. Examination of weight revealed no failure to thrive as defined as 2 SD below the mean. Equally important in evaluating infant growth is progress along the infant's percentile projectile; 39% of the infants stayed within the same percentile, 39% demonstrated a slight shift upward, and 22% a slight shift downward. Such fluctuations are well within the realm of normal growth. Height and head circumference values (**Table 3**) fell within or above 2 SD of the reference mean, indicating adequate skeletal development.

One infant exceeded the 95th percentile for weight by height which would be indicative of obesity. By the definition of obesity suggested by Fomon (15), four of the infants would be classified as obese at one month of age. Their triceps and subscapular (Table 3) skinfold thickness exceeded 2 SD above the mean.

All the mothers reported their infants to be

TABLE 3
Anthropometric indices of the Navajo infants*

Variable	At birth	At approximately 1 mo postpartum
Height (cm)	51.1 ± 1.9	55.3 ± 2.3
Weight (g)	3514 ± 448	4667 ± 698
Circumferences (cm)		
Head	34.4 ± 1.2	37.8 ± 1.2
Chest	34.1 ± 1.7	37.6 ± 1.8
Arm	11.1 ± 0.8	12.4 ± 1.6
Skinfold thickness (mm)		
Flank	4.9 ± 1.7	9.8 ± 2.4
Subscapular	4.6 ± 1.1	8.0 ± 2.2
Triceps	5.3 ± 1.3	7.9 ± 1.3
Quadriceps	6.7 ± 1.3	11.6 ± 2.3

* Mean ± SD.

generally healthy. There were a few episodes of fever, diarrhea, and colds. The 1st month of life presented no grave problems for the infants. Basically, the infants were solely breast-fed, but milk substitutes were occasionally given. Seven mothers mentioned infrequent usage of proprietary formulas. Lack of reliable recall prevented quantification of the caloric contribution by the formula. About 70% of the infants were given water regularly. Introduction of solid foods was rare. One infant had been given yellow corn meal and another had been offered eggs, bacon, and cereal.

Discussion

By NCHS standards (17) the growth of the Navajo infants was satisfactory during the 1st month of life. Based on skinfold measurements some of the infants were classified as obese. There is evidence that breast-fed infants have a greater increase in subcutaneous fat than formula-fed infants during the first 6 wk of life despite similar weight gains in the two groups (17). It would seem inappropriate, therefore, to evaluate breast-fed infants with standards derived mainly from formula-fed infants. A more reliable index for this study might be weight-for-height. By this criterion only one infant was classified as obese.

The quantity of milk produced at 1 month postpartum (634 ml/24 h) was comparable to reports in the literature on Swedish well-nourished women (3, 8) who produced between 558 and 610 ml/day and on Akamba women who averaged 675 ml/day (18). Despite concordance among investigators, it is well recognized that the test-weighing procedure may interfere with the let-down reflex of lactation and thus result in an underestimation of milk production.

The true protein content of the Navajo human milk (1.4 g/dl) was higher than the 0.9 g/dl reported in the literature (19). NPN accounted for only 12% of the total nitrogen in the Navajo milk; NPN was 0.28 mg/ml in contrast to 0.40 mg/ml detected by Lönnerdal et al. (20). The difference could be methodological or may reflect differences in protein intake between the Navajo and Swedish women. A recent study (19) demonstrated that true protein and NPN content of human milk may be altered by protein intake. The NPN is primarily derived from urea. The concentration of urea in the plasma and milk were found to be similar, indicating that urea is passively distributed between these body fluids.

The lactose content of the Navajo human milk (6.1 g/dl) fell below the normal range (21) reported in the literature (6.9 to 7.2 g/dl). Routine methodology based on total reducing sugars overestimates the lactose content. The high-pressure liquid chromatograph used in this study detected only lactose and not the other sugars present which explains the lower result.

Although within normal limits, the mean milk fat content was below the reference standard, accounting for the low caloric content. Milk fat has been shown to vary not only among women, but also among samples from the same woman. In one study (22), fat ranged from 0.20 to 10.4 g/dl. Therefore, the slightly lower value determined for our Navajo samples seems trivial.

The retinol content in the breast milk of the Navajo women (32.9 ± 15.7 μg/dl) was lower than that reported for healthy Swedish mothers (47.8 ± 16.2 μg/dl) and midway between levels reported for privileged Ethiopian mothers (36.2 ± 9.5 μg/dl) and for non-privileged Ethiopian mothers (29.0 ± 9.5 μg/dl) (23). The output of retinol in breast milk is influenced by maternal intake of retinol. Supplementation trials have demonstrated significant increases in the level of breast milk retinol (24, 25). Dietary and serum levels of retinol of these Navajo women were presented in a previous paper (10). Maternal serum retinol was found to be highly correlated with the level of retinol in the breast milk ($r = 0.526$, $p < 0.009$) which would indicate that the better fed Navajo women produced breast milk of higher retinol content. The carotene content of the Navajo milk agreed with Swedish values. Although carotene is readily excreted by the mammary gland, no significant correlation was seen between serum and milk carotene levels.

The total folacin content of the Navajo milk as determined by *L. casei* activity after conjugase treatment was comparable to the reported range of 52 to 64 ng/ml (26), but less than the range of 62 to 280 ng/ml detected in the milk of Japanese women (27). No significant correlations were found, however, between milk folacin and serum or red blood cell folacin in these Navajo women (10).

Although statistical significance was not demonstrated, there was a tendency for maternal folacin status to deteriorate between pregnancy term and one month of lactation. The mean serum folacin for 20 of the Navajo women declined from 16.8 to 14.0 ng/ml after 1 month of lactation. Furthermore, a subsample of seven mothers was reexamined after 3 months of lactation. The level of folacin in their breast milk increased from 50.0 to 77.7 ng/ml in the presence of declining serum and red blood cell folacin levels. The mean serum folacin decreased from 11.4 to 8.7 ng/ml and red blood cell folacin declined from 212.5 to 166.5 ng/ml.

There appears to be a regulatory mechanism which preferentially maintains folacin levels in human milk notwithstanding possible detriment to the maternal hemopoietic system (27, 28). A folacin binder in breast milk is not present in the serum which partially explains the distribution advantage. In cases of megaloblastic anemia, oral doses of folic acid resulted in increased levels of folacin in breast milk before hematological responses in the mothers (28). Although no hematological changes were observed in these Navajo women, the diminishing blood levels of folacin indicate that lactation may drain maternal folacin stores.

The concentration of iron, copper, and zinc in the Navajo breast milk samples fell within ranges cited in the literature. Reported mean values (22) vary for iron from 0.44 to 5.0 μg/ml; for copper from 0.22 to 1.5 μg/ml; and for zinc from 1.3 to 12.4 μg/ml. However, the mean value of iron was higher than that reported by others (22, 29–31). Due to the limited amount of sample, analysis was not repeated to verify these results. Elevated iron values seem inconsistent with what is known about iron levels in human milk. No differences were found in the iron content of breast milk from mothers with serum iron levels varying from deficient to overload levels (30). Iron supplementation did not augment the milk content of iron (31).

In this study, no significant correlations were found between the trace mineral content of breast milk and the corresponding dietary or serum levels of the mothers (10). Yuori et al. (31) reported dietary intakes of iron, copper, and zinc which were very similar to those estimated for the Navajo women and concluded that the intake level had no effect on the concentration in human milk.

In conclusion, despite evidence of suboptimal nutriture (10) among these Navajo women, lactational performance was adequate in terms of infant growth and milk production. By NCHS standards infant growth demonstrated satisfactory progress. The amount of milk produced after approximately 1 month of lactation was consistent with the quantity reported for well-nourished mothers. The milk composition was in reasonable concordance with literature data with the exception of retinol which was lower than normal.

The authors thank the Navajo women for their willing participation in the study. Special thanks is due to Mrs. Elsie Jim Yazzie and Ms. Bernice Willeto for their active participation and guidance on the reservation. The laboratory analyses provided by Dr. H. H. Sandstead, Dr. D. B. Milne, Dr. A. Olson, and Ms. Sandra Gallagher are gratefully acknowledged.

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