Original Research Communications—surveys

Nutritional assessment of pregnant and lactating Navajo women[1,2]

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ABSTRACT Nutritional parameters of 87 Navajo women were assessed at term and in 23 of these women after 1 month of lactation. Serum levels of zinc, retinol, retinol-binding protein, folacin, protein, Hb, and ferritin and hair zinc content were determined. Twenty-four-hour dietary recalls were recorded for the subsample of 23 women. Median nutrient intakes were less than 60% of the Recommended Dietary Allowance of calcium, magnesium, zinc, copper, vitamins A (lactation only), D, E, and B_6, biotin, and folacin. Serum zinc fell below 50 μg/dl in 68% of the subsample at term and remained below 65 μg/dl in 43% during lactation. Serum retinol was below normal (< 33 μg/dl) in 24% of these women at term and 23% at 1 month. Low serum folacin (< 6 ng/ml) was detected in 9% at term and 24% at 1 month. Anemia was present in 15 to 20% of the women. Serum protein, retinol-binding protein, ferritin, and hair zinc were essentially normal. Biochemical findings confirmed dietary inadequacies among Navajo women and indicate needed nutritional improvement. *Am. J. Clin. Nutr.* 34: 2216–2228, 1981.

KEY WORDS Navajo, nutritional assessment, pregnancy, lactation, retinol, folacin, ferritin, retinol-binding protein, zinc

Introduction

In 1976 to 1977, a total of 59 diagnostic measurements of serum retinol were requested by medical staff of the Indian Health Service Unit at Tuba City, AZ, on the basis of suggestive eye lesions: 13 (22%) were in the deficient range (< 20 μg/dl) and another 20 (34%) were in the low-marginal range (20 to 30 μg/dl). Fourteen cases of xerosis and corneal ulceration, diagnosed as vitamin A deficiency lesions, were treated with therapeutic doses of retinol. Most of the patients responded to the retinol, but some recovered only after the additional administration of zinc supplements.

Since eye lesions and low serum levels constitute advanced manifestations of vitamin A deficiency, it was thought that inadequate nutriture among Navajo could have been more prevalent than recognized. To define the magnitude and severity of this apparently multifactorial problem, it was necessary to examine the nutritional status of the broader population, particularly those that might have been at high risk. At the request of the Indian Health Service, a study was designed to determine the prevalence of specific nutrient deficiencies among vulnerable members of the the Navajo tribe. This paper presents findings from one phase of the study

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which focused on pregnant and lactating Navajo women residing on the reservation.

The Navajo reservation is a semi-arid, predominantly rural area of the Southwest. This study was conducted with the Tuba City Service Unit, which has a land area of 4400 square miles and serves 9766 persons (1977) (1). Traditionally, subsistence on the reservation was organized around the sheep herd with some seasonal farming. Due to increasing population density and an overgrazed land base, the Navajo have been forced to seek alternative livelihoods. Despite measurable progress in education and public services, the economy remains severely depressed. Economic deprivation and poor living conditions are not conducive to adequate nutriture. This research project assessed the nutritional status of women contending with increased nutrient needs of pregnancy and lactation.

Methods

Subjects

Navajo women attending the prenatal clinic of the Tuba City Indian Health Service Hospital were asked to participate in the study. The selected women met the following criteria: age 16 to 35 yr; parity 1 to 4; free from any debilitating disease; and with the intention of breastfeeding. Twenty-three Navajo women, 16 to 23 yr of age, agreed to participate in the study. An additional 64 Navajo women were screened biochemically at pregnancy term in order to determine if the sample of 23 were representative of the child-bearing population in the Tuba City area. The study was conducted during the period April through September, 1979. The women gave their informed consent 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.

Biostatistics pertaining to the 468 births that occurred in the Tuba City Indian Hospital during the period, April to September 1979, were extracted from the medical records. The study sample (n = 23) was representative of the obstetrical population (n = 468) in terms of the variables summarized in **Table 1** (z test statistic). The population mean birth weight was 3407 ± 524 g. Of the 468 newborns, 16 (3%) had birth weights below 2.5 kg; 7 (1%) were premature.

Experimental design and procedures

Nutritional status, including dietary, clinical, anthropometric, biochemical, and socioeconomic parameters, was assessed in the hospital after delivery and in the home after approximately 1 month of lactation. Socioeconomic information was obtained by interview.

Food habits and beliefs were ascertained by means of a questionnaire and food intake by a 24-h dietary recall record. Interview forms were not pretested, but were composed with the advice of local Native Americans. The nutrient content of the diets was analyzed with the MINILIST (2), which is a computerized data bank of 230 foods with 48 nutrients per food devised at the University of California, Berkeley, from USDA Handbook 8 (3) and other publisehd and unpublished analyses. For food items without chemical analysis, nutrient levels have been approximated from similar foods; therefore, there are no missing values.

A complete physical examination was performed postpartum as described by Jelliffe (4); the following anthropometric indices were measured: weight, height; head, arm, and calf circumferences; and triceps, biceps,

TABLE 1
Biostatistical comparison between sample and general Navajo obstetric population*

Variable	Population (n = 468)	Sample (n = 23)
Maternal age (yr)	24.6 ± 6.2	22.4 ± 4.0
Age of menarche (yr)	13.1 ± 1.4	13.5 ± 1.3
Gravidity	2.9 ± 2.2	2.3 ± 1.3
Parity	1.7 ± 2.0	1.1 ± 1.2
Gestational duration (wk)	39.9 ± 1.4	39.7 ± 0.8
Materanl height (cm)	158.9 ± 5.1	157.9 ± 4.4
Prepregnancy weight (kg)	60.2 ± 10.9	59.0 ± 9.4
Term weight (kg)	71.9 ± 11.2	72.4 ± 9.5
Pregnancy weight gain (kg)	11.7 ± 6.6	13.4 ± 3.9
Term weight/ideal body weight	1.3 ± 0.2	1.4 ± 0.2
Birthweight (g)	3407 ± 524	3514 ± 448
Sex	0.48M/0.52F	0.52M/0.48F
Apgar score—1 min (1-10)	7.8 ± 1.5	8.3 ± 0.7
Apgar score—5 min (1-10)	9.0 ± 0.7	9.0 ± 0.4
Maturation rating (wk)	39.0 ± 1.8	39.3 ± 1.7

* Mean ± SD. April to September, 1979.

subscapular, and suprailiac skinfolds. Body fat was estimated from the formula of Durnin and Rahaman (5).

Collection and preparation of samples

Blood samples were drawn at the time of delivery or within 24 h postpartum from 86 Navajo women and at approximately 1 month postpartum from the subsample (n = 23) between the hours 08:00 and 16:00. (Blood sample size was not always sufficient for all determinations so in some instances the stated n is smaller.) Usually, 20 ml venous blood was drawn into a Monovette syringe equipped with 20-gauge stainless steel needle; 3 ml was immediately ejected into a heparinized test tube for a complete blood count. For determination of red blood cell (RBC) folacin, 1 ml of heparinized blood was transferred to another test tube with 5 mg sodium ascorbate. Aliquots of serum were frozen (−20°C) for protein electrophoresis and for analysis of retinol, carotene, retinol-binding protein, zinc, ferritin, and folacin. The latter was protected with 5 mg sodium ascorbate and adjusted to pH 6.0 with concentrated NaOH.

A spot urine specimen was collected in a polyethylene container during the postpartum home visit.

Maternal hair samples were cut close to the scalp in the occipital area using stainless steel scissors. The infant hair sample was taken randomly from all areas of the scalp.

Sample analysis

Total serum protein was measured by a total solids refractometer. Serum proteins were separated by cellulose acetate electrophoresis and quantified by densitometry. A complete blood count was performed by automated counter in the clinical laboratory of the Tuba City Indian Hospital. This included Hb, hematocrit, and red and white cell counts.

Retinol and carotene were determined by the Carr Price reaction utilizing trifluoracetic acid (6). Retinol-binding protein was determined by radial immunodiffusion (M-Partigen Retinol Binding Protein Accupak Kit, Behring Diagnostics, American Hoechst Corp., Somerville, NJ 08876). Serum zinc was determined by direct aspiration of a water-diluted sample into an atomic absorption (AA) spectrophotometer. Serum ferritin was analyzed by radioimmunoassay. (Analyses performed at the Human Nutrition Laboratory, USDA/SEA, P.O. Box 7166 University Station, Grand Forks, ND 58201.)

Serum and red blood cell folacin were determined microbiologically by *Lactobacillus casei* (ATCC 7469) (7).

Urine was analyzed for nitrogen content (micro-Kjeldahl), urea (automated colorimetric determination utilizing diacetylmonoxime), and creatinine (automated alkaline picrate method). Urinary zinc was determined by direct aspiration of water-diluted samples into an AA spectrophotometer. After repeated washings with polar and nonpolar solvents and dodecyl sodium sulfate, the hair samples were ashed in a low temperature asher or muffle furnace and analyzed by AA spectrophotometry.

The data were analyzed by SPSS programs on a Control Data 6400 computer. The significance between means and variances of population and subsample data were determined by z test statistic. Relationships between dietary, anthropometric, and biochemical parameters were analyzed by the Pearson correlation coefficient. The paired t statistic and t statistic for two means were performed to discern differences between sampling periods and groups.

Results

Socioeconomic status

The average household size was five persons consisting of three adults and two children. The mean educational levels attained by the father and mother were 10.7 ± 3.7 and 10.6 ± 2.7 yr, respectively. The fathers were engaged in occupations of coal mining, construction, equipment operation, mechanics, and custodial work. The mean income reported was $130 per capita/month. The median food expenditure was $31 per capita/month.

Geographically, 52% of the families rersided in rural settings and 48% in towns. The type of housing was quite variable: 35% of the families lived in substantial homes or mobile homes; 26% in trailers; 26% in the traditional, one-room hogan, and 13% in one-room houses. Only 48% of the households had running water. Most families had privately owned vehicles.

Dietary intake

Although the 24-h dietary records included a wide variety of food items, there was a preponderance of certain foods which constituted a distinctive meal pattern (as indicated by the frequency with which a given food was mentioned). The Navajo women usually ate three meals a day with occasional midmorning and evening snacks. Breakfast was a substantial meal consisting typically of eggs, bacon or mutton, cereal or potatoes, bread, milk, and orange juice. The lunch and dinner usually included meat, potatoes, bread, and a vegetable. Frequently mentioned beverages were coffee, tea, and soda pop. Alcoholic beverages were not consumed by these women. The most popular meats were mutton, beef, chicken, and pork. White bread, fry bread, or tortillas accompanied most meals. Other than potatoes, fruits and vegetables were not consumed in large quantities. Soft drinks and other snack items were frequently purchased at the trading posts. The frequent use of relatively low nutrient density food items such as white bread, fry bread, soda pop, lard-fried potatoes, and baked goods tended to dilute the nutrient content of the diets.

Home-grown crops contributed seasonally to the food supply in 65% of the households. Corn, squash, beans, melons, peaches, and apricots were among the frequently cultivated crops. About half of the households owned livestock, usually sheep and goats, and occasionally horses and cattle. The sheep and goats were commonly used for food whereas the cattle and horses were reserved for transportation and commerce.

Federal food assistance is available on the Navajo reservation; eligible individuals have the option to elect food stamps or food commodities. Only three households were receiving food commodities and two were receiving food stamps. In contrast, 70% of the women participated in the Supplemental Feeding Program for Women, Infants and Children (WIC).

The women stated that they did not alter their food habits during pregnancy. This is consistent with the Navajo view of pregnancy as a natural experience. The women did not specify any food taboos, although some food avoidances developed after episodes of heartburn, diarrhea or nausea. Starchy, highly seasoned, and greasy foods were commonly reported to be disagreeable during pregnancy.

The women did not recognize any foods as galactagogues, but mentioned the importance of sufficient liquids. Milk, tea, juice, water, and broth were recommended. Chili and other spicy foods were avoided as likely to have adverse effects on the milk.

Nutrient intake

According to the US Recommended Dietary Allowances (RDA) (8), the group energy intake was adequate at term pregnancy, but inadequate during lactation (**Table 2**). The group median energy intake at term pregnancy was 2406 kcal/day and 1911 kcal/day during lactation. The energy requirement for women, 19 to 22 yr of age, is 2400 kcal/day during pregnancy and 2600 kcal/day during lactation. Therefore, the diet provided 100% of the energy requirement during pregnancy and 74% during lactation. Energy intake was not significantly correlated to maternal body weight or change in weight.

The protein intake was 82 g/day (111%

TABLE 2
Daily food-nutrient intake* of 22 Navajo women during pregnancy and lactation

Nutrient	Unit	At pregnancy term				At 1 mo postpartum			
		Median	(% RDA)	Mean	(SD)	Median	(% RDA)	Mean	(SD)
Energy	kcal	2406	(100)	2448	(887)	1911	(74)	2190	(990)
Protein	g	82	(111)	95	(41)	91	(142)	87	(38)
Calcium	mg	668	(56)	897	(515)	578	(48)	718	(458)
Phosphorus	mg	1318	(110)	1421	(583)	1134	(95)	1270	(559)
Magnesium	mg	230	(51)	241	(86)	212	(47)	221	(114)
Zinc	mg	11.4	(57)	12.5	(6.9)	11.7	(47)	12.2	(5.3)
Copper	mg	1.42	(57)	1.57	(0.66)	1.44	(58)	1.63	(0.93)
Iron	mg	16.2	(90)	15.3	(4.9)	14.4	(80)	15.2	(5.9)
Vitamin A	I.U.	3554	(71)	4609	(2972)	2488	(41)	5467	(10,002)
Vitamin D	I.U.	121	(24)	166	(163)	116	(23)	136	(115)
Vitamin E	mg	3.4	(34)	4.2	(2.9)	3.5	(35)	4.5	(3.8)
Vitamin C	mg	112	(140)	138	(130)	97	(97)	150	(165)
Thiamin	mg	1.48	(99)	1.53	(0.52)	1.31	(82)	1.39	(0.69)
Riboflavin	mg	1.58	(99)	1.86	(0.85)	1.48	(82)	1.81	(1.07)
Niacin	mg	18.9	(118)	21.4	(11.7)	19.4	(102)	20.1	(9.1)
Vitamin B_6	mg	1.48	(57)	1.45	(557)	1.33	(53)	1.44	(688)
Vitamin B_{12}	μg	4.62	(116)	5.28	(4.23)	4.40	(110)	7.96	(1.47)
Biotin	μg	54	(36)	67	(43)	46	(31)	54	(35)
Folacin, free	μg	144	(36)†	140	(65)	134	(54)†	169	(139)
Pantothenate	mg	5.11	(93)	5.30	(2164)	5.26	(94)	5.42	(3045)

* Analysis excludes nutrient supplementation taken by some women (see text).
† WHO recommended intake used as reference standard (9).

RDA) at term pregnancy and 91 g/day (142% RDA) during lactation.

The distribution of calories contributed by protein (16%), carbohydrate (47%), and fat (37%) did not differ between periods.

Dietary analysis indicated substandard intakes of several vitamins and essential minerals. The median nutrient intakes that were less than 60% of the RDA were: calcium, magnesium, zinc, copper, vitamin A (lactation only), vitamin D, vitamin E, vitamin B_6, biotin, and folacin. The lower energy intake during lactation was associated with a diminished intake of most nutrients.

A multivitamin-mineral tablet[6] and ferrous sulfate were routinely dispensed in the prenatal clinic. During pregnancy 61% of the 23 women reported to have taken these supplements daily; 17% took them one to three times per week; and 22% not at all. Upon hospital discharge, the postpartum women were given Hexavitamin[7] tablets and ferrous sulfate. At 1 month postpartum, 61% of the women were reportedly compliant; however, of the nutrients at risk, only vitamin A was augmented by the supplement. The intake of the nutrient supplements was not verified. Mean serum levels of self-stated users versus nonusers were tested for differences by the t statistic for two means. Supplementation resulted in no significant differences in serum levels of retinol, retinol-binding protein, folacin, or zinc at term or at 1 month postpartum.

Clinical findings

In the postpartum examination, 30% of the women were classified as pale. Cases of fluorosis (n = 7), worn teeth (n = 4), and carious teeth (n = 1) were recorded. Swollen, red papillae on the gums were noted in two of the women. Observations of slight lingual papillary filiform atrophy (n = 6) and slight papillary fungiform hypertrophy (n = 1) were recorded. A slightly enlarged thyroid was noted in one patient with malar pigmentation, and enlarged parotid glands in another. There was one reported case each of slightly-ridged fingernails and facial hyperpigmentation.

Cardiovascular changes that would be considered pathological in the absence of pregnancy were recorded. Mild pretibial edema was seen in 48% of the women. Systolic blood pressure ranged from 90 to 134 mmHg and diastolic blood pressure from 48 to 84 mmHg. Systolic heart murmurs, common in normal pregnant women (10), were detected in seven of the women. The cardiovascular findings on the Navajo women would be considered normal except in the case of subject number 20 who was diagnosed as preeclampsic.

TABLE 3
Anthropometric characteristics of Navajo women*

Variable	Postpartum	At 1 month lactation
Weight (kg)	66.9 (9.2)	63.8 (9.5)†
Body fat (%)	34 (3)	34 (3)
Circumferences (cm)		
Head	55.8 (1.8)	
Arm	28.2 (2.8)	27.9 (3.1)
Calf	33.3 (3.1)	32.7 (2.7)
Skinfold thickness (mm)		
Triceps	19.6 (6.0)	18.3 (4.4)
Biceps	12.4 (4.2)	11.0 (4.3)
Subscapular	21.0 (5.2)	21.0 (5.8)
Suprailiac	27.8 (4.1)	24.8 (5.8)†

* Mean (SD).

† Paired t test for differences between periods, $p < 0.001$.

Maternal anthropometry

Changes in body composition during pregnancy and lactation were evaluated by anthropometric measurements (**Table 3**). In the persent study, 35% of the women were overweight at the onset of pregnancy. The mean prepregnancy weight was 59.0 ± 9.4 kg (44.1 to 80.0 kg), approximately 12% above ideal weight (11). The mean term weight was 72.4 kg and the postpartum weight, 66.9 kg. Maternal prepregnancy weight ($p < 0.004$), term weight ($p < 0.01$), and postpartum weight ($p < 0.003$) were strongly associated to birth weight.

The average weight gain during pregnancy was 13.4 ± 3.9 kg (4.7 to 20.8 kg). In this series, weight gain did not correlate signifi-

[6] Prenatal multivitamin-mineral tablet contained: 8000 USP units vitamin A (acetate), 400 USP units vitamin D (ergocalciferol), 30 mg vitamin E (D-α-tocopheryl acetate), 90 mg ascorbic acid, 1.7 mg thiamin mononitrate, 20 mg niacin, 8 μg cyanocobalamin, 200 mg calcium, 45 mg iron, 0.8 mg folic acid, 2.0 mg riboflavin, 4.0 mg pyridoxine HCl, 150 μg iodine, and 100 mg magnesium.

[7] Hexavitamin contained: 5000 USP units vitamin A (acetate), 400 USP units vitamin D (ergocalciferol), 75 mg vitamin C, 2.0 mg thiamin mononitrate, 20 mg niacin, and 30 mg riboflavin.

cantly with birth weight. Birth weight was positively correlated to the following maternal parameters: arm ($p < 0.04$) and calf ($p < 0.02$) circumferences; triceps ($p < 0.05$) and subscapsular ($p < 0.01$) skinfolds. At 1 month postpartum there were significant associations between infant weight and the following maternal measurements: weight ($p < 0.001$); head ($p < 0.03$), arm ($p < 0.002$), and calf ($p < 0.01$) circumferences; triceps ($p < 0.03$), subscapular ($p < 0.002$), and suprailiac ($p < 0.01$) skinfold thicknesses.

After delivery, mean maternal body weight was 26% in excess of ideal and body fat was estimated to be 35% of body weight, based on skinfold measurements. After 1 month of lactation there had been an average weight loss of 3.1 ± 2.1 kg, but the mean weight remained 21% above ideal body weight. Six women had returned to their appropriate weight and one had fallen 8% below ideal. The only skinfold thickness which decreased significantly during the 1st month of lactation ($p < 0.001$) was the suprailiac, an area of gestational weight deposition. The percentage body fat (34.5%) did not change significantly over time.

Protein nutriture

The mean serum total protein of the 86 Navajo women screened at pregnancy term was 6.4 ± 0.7 g/dl. Less than acceptable (12) levels (< 6.0 g/dl) were found in 8.4% of the women. Mean serum albumin was 3.2 ± 0.5 g/dl; 3.6% of the values were unacceptably low (< 3.0 g/dl).

In the subsample (n = 23), serum proteins were normal after 1 month of lactation. Serum total protein rose significantly from 6.5 ± 0.7 at term to 7.6 ± 0.6 g/dl at 1 month postpartum ($p < 0.001$). Serum albumin similarly increased from 3.2 ± 0.5 to 4.2 ± 0.3 g/dl ($p < 0.001$). There was a statistically significant decline in α_1-globulin and rise in γ-globulin postpartum. These changes are normal, physiological adjustments expected during the puerperium (13).

The concentration of urinary nitrogen excreted at 1 month post-partum was 8.71 ± 3.84 mg/ml, 83% of which was urea nitrogen. On a normal or high protein diet, approximately 85% of the total nitrogen is urea nitrogen (14, 15); the fraction present as urea declines when protein intake falls. Thus this biochemical marker verifies the adequate protein intake reported at 1 month postpartum.

Hematology

The mean Hb level (n = 20) at pregnancy term was 11.9 ± 1.4 g/dl (**Table 4**). Ten percent of the women had Hb levels below 10 g/dl and 10% had marginal levels between 10 to 10.9 g/dl. Although the mean hematocrit (34.2 ± 5.5%) fell within normal limits, 19% of the values were marginal, and 12% were low (16). The mean RBC count ($10^6/mm^3$) was 3.9 ± 0.6 and the mean corpuscular Hb concentration (%) averaged 33.0 ± 2.0.

The mean Hb value was 13.0 ± 1.3 g/dl after 1 month of lactation. Of the 20 Navajo

TABLE 4
Hematological parameters of Navajo women

Variable	At pregnancy term				At 1 month postpartum		
	Rating		n (%)	Mean ± SD	Rating	n (%)	Mean ± SD
Hb (g/dl)	Acceptable	(>11)	16 (80)	11.9 ± 1.4	(12+)	17 (85)	13.0 ± 1.3
	Marginal	(10.0–10.9)	2 (10)		(10.0–11.9)	3 (15)	
	Low	(40)	2 (10)		(<10)	0	
Hematocrit (%)	Acceptable	(>11)	18 (69)	34.2 ± 5.5	(38+)	15 (71)	39.0 ± 4.1
	Marginal	(30–32)	5 (19)		(31–37)	4 (19)	
	Low	(<30)	3 (12)		(<31)	2 (10)	
RBC ($10^6/mm^3$)			22 (100)	3.9 ± 0.6		20 (100)	4.5 ± 0.4
Mean corpuscular Hb concentration (%)			20 (100)	33.0 ± 2.0		20 (100)	33.4 ± 1.6

women assessed, 15% had Hb levels in the marginal range (10 to 11.9 g/dl). The hematocrit values revealed a slightly higher incidence of mild anemia; 19% were in the marginal range (31 to 37%) and 2% were in the low range (<31%). The mean RBC count ($10^6/mm^3$) was 4.5 ± 0.4 and the average mean corpuscular Hb concentration (%) was 33.4 ± 1.6.

Serum ferritin

Serum ferritin at pregnancy term (n = 83) was 39.2 ± 38.6 ng/dl (range 4.5 to 182.4) (**Table 5**). Eleven women had levels below the norm of 9 ng/dl as established by the reference laboratory. Serum ferritin was positively correlated (r = 0.570, p < 0.003) with Hb.

In the subsample of 23 women (**Table 6**) serum ferritin rose, but not significantly, from 34.1 ± 21.4 at term to 43.3 ± 31.4 ng/dl after one month of lactation. In one subject, serum ferritin fell below the reference norm.

Folacin status

Serum folacin of 79 Navajo women at pregnancy term was 17.8 ± 11.6 ng/ml (range 2.2 to 45.8) (Table 5). The serum level of one subject was in the deficient category (<3.0 ng/ml) while the values of six individuals were low (<6.0 ng/ml). According to these standards (17), 9% of the women presented less than acceptable serum folacin levels.

Serum folacin was positively correlated with Hb (r = 0.565, p < 0.005), hematocrit (r = 0.526, p < 0.003), and ferritin (r = 0.346, p < 0.001). Although the low incidence of anemia in this sample precludes causal analysis, the interrelationships seen here suggest a potential multifaceted etiology of anemia in this population.

In the subsample (Table 6) serum folacin decreased, although not significantly, from a mean of 17.7 ± 12.8 to 13.7 ± 13.1 ng/ml at 1 month postpartum; 24% of the women had unacceptably low serum folacin (< 6.0 ng/ml). RBC folacin is regarded as a more accurate, less variable index of folacin nutriture than serum folic acid. The mean RBC folacin was 246 ± 146 ng/ml. Values were less than acceptable (17) in 37% of the subjects: five individuals were in the deficient range (<140 ng/ml) and two were in the low range (<145 ng/ml).

Zinc nutriture

The mean serum zinc measured at term in the 86 women was 51.2 ± 16.8 μg/dl (27.0 to

TABLE 5
Mean values and distributions of serum biochemical indices for the Navajo women at term pregnancy

Variable	Rating		n (%)	Mean ± SD	Range
Vitamin A (μg/dl)	Acceptable	(>33)	52 (60)	36.6 ± 13.0	6.9–66.0
	Marginal	(20–33)	25 (29)		
	Low	(10–19)	8 (9)		
	Deficient	(<10)	1 (1)		
Carotene (μg/dl)	Acceptable	(≥40)	82 (95)	128.8 ± 58.6	1.6–263.9
	Low	(<40)	4 (5)		
Retinol-binding protein (μg/dl)	Acceptable	(≥27)	72 (86)	43.4 ± 14.2	15.4–81.6
	Low	(<27)	12 (14)		
Zinc (μg/dl)	Acceptable	(≥50)	41 (48)	51.2 ± 16.8	27.0–109.0
	Marginal	(40–49)	22 (26)		
	Low	(30–39)	21 (24)		
	Deficient	(<30)	2 (2)		
Folacin (ng/ml)	Acceptable	(≥6.0)	72 (91)	17.6 ± 11.6	2.2–45.8
	Low	(3.0–5.9)	6 (8)		
	Deficient	(<3.0)	1 (1)		
Ferritin (ng/ml)	Acceptable	(≥9)	72 (87)	39.2 ± 38.6	4.5–182.4
	Low	(<9)	11 (13)		

TABLE 6
Mean values and distributions of serum biochemical indices for the Navajo women at pregnancy term and at 1 mo postpartum

Variable	Rating	At pregnancy term			At 1 mo postpartum			
		n (%)	Mean ± SD	Range	Rating	n (%)	Mean ± SD	Range
Vitamin A (μg/dl)	Acceptable (>33)	16 (76)	41.5 ± 10.7	23.9–62.3		18 (78)	42.2 ± 14.3	22.5–88.1
	Marginal (20–33)	5 (24)				5 (22)		
Carotene (μg/dl)	Acceptable (≥40)	22 (100)	140.5 ± 39.7	65.8–234.2		22 (100)	103.6 ± 39.4*,†	40.1–191.7
	Low (<40)	0						
Retinol-binding protein (μg/dl)	Acceptable (≥27)	21 (95)	47.2 ± 12.6	26.0–69.2		23 (100)	46.4 ± 9.7	29.6–65.8
	Low (<27)	1 (5)						
Zinc (μg/dl)	Acceptable (≥50)	7 (32)	45.3 ± 10.6	31–64	(≥ 65)	13 (57)	66.1 ± 8.4*,‡	50–82
	Marginal (40–49)	8 (36)			(60–64)	4 (17)		
	Low (30–39)	7 (32)			(50–59)	6 (26)		
Folacin (ng/ml)	Acceptable (≥6.0)	19 (86)	17.7 ± 12.8	4.2–45.8		16 (76)	13.7 ± 13.1	2.5–61.4
	Low (3.0–5.9)	3 (14)				3 (14)		
	Deficient (<3.0)					2 (10)		
Ferritin (ng/ml)	Acceptable (≥9)	21 (95)	34.1 ± 21.4	6.4–73.1		22 (96)	43.3 ± 31.4	7.1–106.3
	Low (<9)	1 (5)				1 (4)		

* Paired *t* test for differences between period means, † $p < 0.005$, ‡ $p < 0.001$.

109.0 μg/dl) (Table 5). Over half of the 86 women had values below the provisional lower limit (18) of 50 μg/dl for pregnancy; 26% were in the marginal range (40 to 49 μg/dl); 24% were in the low range (30 to 39 μg/dl); and 2% were in the deficient range (<30 μg/dl). Since plasma zinc circulates loosely bound to albumin, the positive ($r = 0.385$, $p < 0.001$) correlation seen between these parameters was not surprising.

As would be expected with postpartum samples (18), serum zinc (Table 6) increased significantly ($p < 0.001$) from 45.3 ± 10.6 to 66.1 ± 8.4 μg/dl. However, serum levels did not normalize in all cases. Levels were below the nonpregnant reference value of 65 μg/dl in 43% of the women; 17% of the values were in the marginal category (60 to 64 μg/dl) and 26% of the values were in the low range (50 to 59 μg/dl). Serum zinc was no longer positively correlated with serum albumin at 1 month postpartum.

Zinc levels of a control sample of 14 nonpregnant, nonlactating Navajo women, ages 20 to 38 yr, were determined in order to provide a geographically specific comparison. Their mean serum zinc level was 67 ± 13 μg/dl (range 43 to 87 μg/dl).

Maternal (n = 41) and infant (n = 34) hair zinc at delivery were analyzed (**Table 7**). Maternal hair zinc averaged 164 ± 28 ppm (range 109 to 232 ppm). One-third of the values fell below 150 ppm zinc but none was below 100 ppm zinc, which is considered suggestive of inadequate zinc nutriture (18). The mean hair zinc for the neonates was 181 ± 14 ppm (range 152 ± 213 ppm) which was similar to the zinc concentration of 174 ± 8 ppm reported by Hambidge et al. (19) for 25 neonates. No significant correlations were demonstrated in the matrix of the following variables: maternal serum zinc, maternal hair zinc, maternal dietary zinc, cord blood zinc, and infant hair zinc.

Maternal urinary zinc (Table 7) was measured at 1 month postpartum in 24 h samples collected from eight of the lactating women. Their mean 24-h urinary zinc value was 458 ± 233 μg (range 126 to 780 μg). Except for one case, all values fell within normal limits (18). An additional 12 spot specimens were collected in the field. Zinc excretion was expressed in reference to urinary creatinine. The mean zinc/creatinine ratio was 476 ± 203 μg/g (range 155 to 847 μg/g). Four women (20%) had ratios below 200 μg/g suggestive of poor zinc status.

Vitamin A nutriture

Serum retinol of the 86 Navajo women was determined at term and the mean was 36.6 ± 13.0 μg/dl (Table 5). At term 40% of the women fell below the normal limit (<33 μg/dl for the reference laboratory). Of the 86 cases, 25 (29%) were in the marginal range (20 to 33 μg/dl), 8 (9%) were in the low range (10 to 19 μg/dl), and one was in the deficient range (<10 μg/dl) (20). The mean carotene was 128.8 ± 58.6 μg/dl. Four women had abnormally low carotene levels (<40 μg/dl). The mean RBP was 43.4 ± 14.2 μg/dl. Values were low (<27 μg/dl) in 14% of the women. Serum retinol was highly correlated with both carotene ($r = 0.367$, $p < 0.001$) and RBP ($r = 0.694$, $p < 0.001$), but negatively ($r = -0.335$, $p < 0.001$) with serum ferritin.

After 1 month of lactation (Table 6), no significant changes were seen in serum retinol or RBP of the 23 women; however, serum carotene significantly declined ($p < 0.001$) from 140.5 ± 39.7 to 103.6 ± 39.4 μg/dl. The mean serum retinol was 42.2 ± 14.3 μg/dl;

TABLE 7
Zinc parameters of Navajo women and infants

Variable	Rating	n (%)	Mean ± SD	Range
At delivery				
Maternal hair zinc (ppm)	Acceptable (≥150)	28 (68)	164 ± 28	109–232
	Marginal (100–149)	13 (32)		
Infant hair zinc (ppm)	Acceptable (≥150)	34 (100)	181 ± 14	152–213
At 1 mo postpartum				
Urinary zinc (μg/ml)		20	442 ± 191	164–799
Urinary zinc/creatinine (μg/g)	Acceptable (≥300)	16 (80)	476 ± 203	155–847
	Marginal (<300)	4 (20)		

22% of the values fell below the normal limit. Three cases were in the range 30 to 33 μg/dl and two cases in the marginal range (20 to 29 μg/dl). RBP values were within the normal range in all postpartum cases.

A significant ($r = -0.555, < 0.001$) negative correlation was seen between serum retinol and zinc. RBP was negatively ($r = -0.267$, $p < 0.007$) correlated to serum zinc. Thus it would appear that in those cases with low serum retinol, zinc was not the limiting factor.

Discussion

Food intake

The food intake of the Navajo women was nearly adequate in energy and protein, but deficient in several of the vitamins and minerals. The median energy intake was ample at term, but decreased to 1911 kcal/day at 1 month postpartum. Dietary 24-h recalls are notorious for underreporting, which may be the case here. The women could have been fatigued by all the interviewing.

If the recorded intakes were valid, it is unlikely that successful lactation would have been sustained much longer at an energy level of 34 kcal/kg/day. Studies of well-nourished women have found 47 kcal/kg/day to be consistent with successful lactation. Lactating women reportedly increase their energy intake approximately 600 kcal/day above normal, nonlactating levels (21, 22). These Navajo women had considerable body stores to draw upon for milk production, but low energy intakes may prohibit mobilization of adipose tissue for milk production. Whichelow (23) observed that women who conscientiously restricted their food intake in order to lose weight were unable to produce sufficient milk for their infants.

The substandard dietary intakes of calcium, magnesium, zinc, copper, vitamins A, D, E, B_6, and folacin do present a significant risk of deficiency. The intake of these nutrients fell below 60% of the RDA (8). The prenatal vitamin-mineral supplement that was discontinued during lactation provided all of the above nutrients except zinc and copper. The body has a considerably capacity to store vitamins A, D, E, and the minerals calcium and magnesium, but the reserves of vitamin B_6, folacin, zinc, and copper are limited. Depleted reserves not replenished by either diet or supplementation would result eventually in a deficient state. Although supplementation may compensate for some nutrient inadequacies, it cannot provide for all the essential nutrients. Special problems of toxicity and ill-defined requirement levels arise when dealing with trace minerals. In this study, marginal zinc deficiency was identified, but most likely the intake of other trace minerals was suboptimal as well.

Clinical findings

The clinical examinations at term did not detect gross nutrient deficiencies. Clinical findings tend to be nonspecific and nondiagnostic and, therefore, should be interpreted cautiously. A number of cases of slight papillary filiform atrophy and fungiform hypertrophy were noted. This may be caused by a variety of deficiencies: iron, zinc, folacin, B_{12}, B_6, niacin, and riboflavin (24). Fluorosis was observed in several of the women. Parts of the reservation are known to have excess fluoride in the well waters.

Biochemical results

Iron. Serum ferritin levels provide an indirect method to assess body iron stores. A gradual depletion of iron stores results in a progressive decline in serum ferritin. Theoretically, levels below 12 ng/ml represent an exhaustion of iron stores (25). Subsequently, Hb synthesis and transferrin saturation decrease, and free erythrocyte protoporphyrin rises. According to the standards of the reference laboratory, 13% of the women had unacceptable levels (<9 ng/ml) at pregnancy term. By the criteria suggested above (<12 ng/ml), 19% of the women had depleted iron stores. This estimate would corroborate the 20% incidence of anemia based on Hb values. The most probable causes of anemia were iron deficiency and acute blood loss. Although the diets provided a fair amount of iron primarily from animal products and fortified cereals (14 to 16 mg iron/day), this may be insufficient to allow absorption of the required 3.5 mg iron/day during pregnancy (8). These findings would support a recommendation (8) of iron supplementation for 2 to 3 months postpartum in order to replenish depleted reserves.

Folacin. Although the mean serum folacin values were well above normal, 9% of the pregnant Navajo women had levels considered marginal or deficient. Reports in the literature have documented signs of maternal folic acid deficiency without megaloblastic anemia (26). Signs seen late in pregnancy included lowered serum folacin, hypersegmentation of neutrophils, and elevated urinary formiminoglutamic acid after a histidine load. Although the deficit was not severe enough to cause anemia, speculations on possible untoward effects on the fetus have been forthcoming without conclusive results. Some investigators have found abruptio placentae, fetal malformation, and abortion to be more prevalent in patients with folacin deficiency (26). The association between pregnancy wastage and folacin deficiency has yet to be substantiated.

There was a tendency in this study for serum folacin to decrease as lactation progressed. Metz (27) has suggested that lactation is the conditioning factor that precipitates overt megaloblastic anemia in women consuming diets marginal in folic acid. Since serum folacin is more a reflection of recent dietary intake than of tissue reserves, erythrocyte folacin levels were analyzed in 19 lactating women to confirm findings of marginal folacin status. Of these women, 37% had RBC folacin levels in the low-deficient ranges.

In order to meet the increased folacin requirements during pregnancy and lactation, folacin supplementation may be advisable, but should be accompanied by nutrition education on good food sources of folic acid, since dietary records were noticeably low in leafy green vegetables and other fresh vegetables and fruits.

Vitamin A. Interpretation of serum retinol levels during pregnancy and lactation is complicated by inconsistencies in the literature (28). Some investigators have reported a fall during pregnancy, followed by an increase postpartum; however, others contend that this decline is prevented by vitamin supplementation and thus not "physiological." Alternatively, some studies have shown no significant changes in serum retinol during pregnancy. Conflicting reports may be due partially to varying factors such as season of the year, vitamin supplementation, and fetal sex. For interpretation of the Navajo data, the reference standards presume no alterations due to pregnancy or lactation.

Unacceptably low retinol levels were seen in 10% of the population screened at term; an additional 29% of the women had marginal values. Retinol-binding protein was highly associated with serum retinol and was depressed in 14% of the women. In the longitudinal sample, marginal levels were seen at term and persisted after 1 month of lactation.

The etiology of the low serum retinols appeared to be inadequate dietary sources of vitamin A. Identifiable sources were eggs, butter or margarine, and seasonal fruits and vegetables. However, the vegetables consumed regularly were poor sources of vitamin A.

Zinc. Reports in the literature (18) agree that plasma zinc declines during pregnancy, but interpretation of plasma values is confounded by several factors. Since 60 to 70% of plasma zinc circulates loosely bound to albumin, circulating levels in part are determined by serum albumin concentration and affinity of albumin for zinc (29). In hypoalbuminemic conditions such as pregnancy, plasma zinc levels fall. Furthermore, elevated endogenous estrogen has an independent depressing effect on serum zinc. Therefore, it is difficult to delineate to what extent low serum zinc levels during pregnancy are physiological or diet induced. However, it has been estimated that plasma zinc drops approximately 25% during normal pregnancy (18). Plasma zinc normally lies between 80 to 100 $\mu g/dl$. Serum zinc levels are generally 5 to 15% higher than plasma values. Based on these observations a provisional lower limit for plasma zinc during pregnancy has been designated at 45 to 50 $\mu g/dl$.

In the present study, 52% of the Navajo women had serum zinc levels below the provisional lower limit at pregnancy term. The mean serum zinc level was 24% below the corresponding controls. It would appear from the low nonpregnant zinc values (67 ± 13 $\mu g/dl$) that the Navajo women are entering pregnancy with low serum levels. The hypoalbuminemia of pregnancy would account for approximately 70% of the decline. Nevertheless, the low values attained during pregnancy are disconcerting.

The medical records were reviewed for deleterious effects associated with the low serum zinc values. Birth weight tended to increase linearly with serum zinc, but the correlation ($r = 0.171$, $p = 0.066$) did not attain statistical significance. Maternal age, parity, hours of labor, and Apgar scores were not related to maternal serum zinc. Complications at delivery were recorded among the 468 births. The prevalence of postpartum hemorrhage was strikingly high at a rate of 0.130. Preeclampsia, fetal distress, and arrest in progress followed in order of frequency. The small number of cases with complications on which biochemical information was available precluded any statistical analysis.

Maternal and infant hair zinc values did not reflect chronic zinc deprivation. However, the distribution of maternal levels revealed that 32% of the women had levels below 150 ppm zinc which some investigators consider marginal.

The low serum zinc levels observed in 42% of the sample after 1 month of lactation cannot be attributed to hypoalbuminemia. Although serum albumin levels had returned to normal, serum zinc levels remained depressed.

Due to the wide variability obtained with urinary zinc measurements, it had not been considered a very accurate index of zinc status. Nevertheless, urine samples were analyzed after 1 month of lactation for zinc concentration. Depressed urinary excretion of zinc was seen in four of the lactating women. There was no evidence of hyperzincuria.

Marginal zinc status among these Navajo women appeared to be diet related. The women were consuming between 11 and 12 mg zinc/day, derived principally from animal products, eggs, and legumes. The validity of the diet analysis could be questioned, although a recent unpublished study verified calculated intakes based on the MINILIST by analyzing the zinc content of composite diets (K. Todd and D. H. Calloway, unpublished results).

Further confirmation of the dietary zinc intakes follows from the zinc/protein ratio. Zinc has been shown to be more highly related to the protein content of the diet than the energy level. A mixed diet provided approximately 1.5 mg zinc/10 g protein (30). In this study, 1.3 to 1.4 mg zinc/10 g protein were recorded. Also, a highly significant correlation existed between dietary zinc and dietary protein ($r = 0.944$ at term, and $r = 0.923$ at 1 month postpartum).

The diets reported by the Navajo women provided only 57 and 47% of the RDA for zinc during pregnancy and lactation, respectively. Maintaining the same zinc/protein ratio, the women would have to consume 154 and 178 g protein/day during pregnancy and lactation to meet the RDA. Such an increase may not be economically feasible nor consistent with sound nutrition. However, the low serum zinc levels observed in this study indicate that 12 mg zinc/day is insufficient to maintain normal serum levels in pregnant and lactating women. There were no known problems of malabsorption or increased urinary and sweat losses. Therefore zinc supplementation of the diets may be indicated.

Conclusion

These dietary and biochemical findings demonstrated marginal nutrient deficiencies of zinc, vitamin A, folacin, and iron among these Navajo women. The slightest risk to pregnant and lactating women is unacceptable. Stopgap measures can be implemented to ameliorate the situation, but their limitations need to be recognized. Vitamin-mineral supplementation is one such intervention; however, this solution cannot provide for all the essential nutrients. The food patterns in this study revealed frequent use of foods low in nutritive value. Food selection is bound by economic constraints, food availability, and food preferences. On the Navajo reservation other factors affecting food intake were lack of transportation, refrigeration, fuel, and running water. Geographical and weather problems restrict access to food and nutrition services, which at best have an impact on only a portion of the Navajo population. The challenge is formidable, but the only feasible, safe way of assuring ingestion of all nutrients is through improvement of the overall quality of the Navajo diet.

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