Nutritional supplementation and the outcome of pregnancy. II. Visual habituation at 15 days[1-3]

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ABSTRACT Colombian women at risk of mild-to-moderate malnutrition were enrolled in a health care program and randomly assigned into nutritional supplementation and control groups at the beginning of the third trimester of pregnancy. One hundred unsupplemented and 144 supplemented infants were tested at 15 days of age: a 2 × 2 checkerboard was presented eight times followed by a single presentation of a 6 × 6 checkerboard. Unsupplemented infants showed less initial attention followed by slower habituation and higher levels of movement than the supplemented infants. The observed effect of maternal nutritional supplementation during the last trimester of pregnancy on new-born attention was interpreted to be maturational in nature. *Am. J. Clin. Nutr.* 32: 463–469, 1979.

It has been suggested that the quality of maternal nutrition during pregnancy affects the infant's perceptual-cognitive development (1–3). Specifically, it has been hypothesized that development would be most vulnerable to the effects of malnutrition if it occurs during the period of rapid brain growth which in humans begins in the second half of pregnancy and continues through the first 2 or 3 years of life.

Evidence for an association between mild-to-moderate protein-calorie malnutrition and mental development comes from studies with older infants and preschool children (4–6). In these studies, however, the effects of prenatal nutrition have not been separated from those of postnatal nutrition. The present study examines the effect of maternal food supplementation beginning during the last trimester of pregnancy, upon 15-day old infants' ability to respond to and process information, as measured by the orienting response and habituation of attention (7–9). Individual differences in these attentional processes can be reliably measured in very young infants, and appear sensitive to the effects of various pre- and perinatal risk variables (10–12) and perhaps nutrition (13).

At present, empirical evidence for an attentional deficit associated with malnutrition comes from a study in which 1-year-old malnourished and well-nourished infants were compared in cardiac habituation to auditory stimuli (14). The malnourished infants showed little or no orienting response to stimulus onset, and neither habituation to repeated presentations nor dishabituation to qualitative changes in the stimulus. In the present study, attention to visual stimuli was selected because it is more reliably associated with overt responses than attention to stimulation through other modalities (14, 15) and can be reliably measured under field conditions.

Present research is part of a longitudinal field experiment based on random assignment of pregnant women at risk of malnutrition to nutritional treatment and control groups. Comparing a nutritional treatment group with a true experimental control group eliminates the problem of confounding environmental variables (16–18) and studying infants in the first few weeks of life minimizes

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differences in experience which covary with malnutrition and mental development (19, 20).

Methods

Sample and design

The sample was selected from a densely populated slum district of Bogotá, Colombia, an area typical of urban poverty in Latin American cities (16). In order to identify families at risk of malnutrition, women were selected who were less than 6 months pregnant and who had children under 5 years of age, the majority of whom were malnourished. Malnutrition was defined as 85% or less in weight-for-age with respect to Colombian standards (21).

The families who agreed to participate in the study were enrolled in a free health care program and randomly assigned to either a group which received nutritional supplements for the whole family, or to an unsupplemented control group. In order to locate families who fulfilled the above criteria, four surveys were conducted of the study area at 6-month intervals (22). Because of logistic reasons the sample of the study herein reported consisted of the 319 families enrolled in the last three surveys. Between the time of enrollment and measurement at 15 days, 23 subjects were lost due to: stillbirths (8), perinatal mortality (11), changing address (4), and refusal (4). Fifty-two more subjects were not tested because of the baby falling asleep during testing, being ill at the time, or mother failing to bring him for testing. Thus, the available sample consisted of 144 infants in the supplemented group and 100 in the unsupplemented group. Sex distribution in the groups was similar; 50 boys and 50 girls in the unsupplemented group, and 75 boys and 69 girls in the supplemented one.

Family income, mother's education and age, type of dwelling, and type of neighborhood did not differ significantly between the two groups (Table 1). Nor were there differences in these variables between the sample families and those who could not be tested.

Treatment

Free medical care was provided for all mothers and children under 7 years old. The program was staffed by one half-time obstetrician and two part-time pediatricians. Services included pre- and postnatal care, attendance during delivery, emergency hospitalization, ongoing pediatric services, and free provision of all prescribed medications.

Supplementation began at 6 months of pregnancy. The supplement, which consisted of dry skim milk, protein-enriched bread, and cooking oil, was delivered weekly for take-home at a center much like a neighborhood store. There was no control for the intrafamily distribution of the supplement, which provided 623 cal and 30 g of protein per capita per day to each family member over one year of age. Pregnant and lactating women were allotted 856 cal and 38.4 g of protein, and in addition to the food supplement, they received 6024 IU of vitamin A and 18 mg of iron in capsules.

Procedure

Pregnancy and anthropometric variables. As measures of newborn attention have appeared sensitive to effects of a number of nonnutritional variables, data was collected on length of gestation, maternal medication during labor and delivery, and physical development of the newborn.

Length of gestation was measured in weeks since last menstruation, as reported by the mother during her first visit to the study obstetrician. A 24-hr recall dietary survey was carried out in the 6th and 8th month of pregnancy to assess maternal caloric and protein consumption. Information on medication during labor and delivery was obtained by direct observation in the hospital (66%), or from hospital records and mother's report at 15-day postnatal check-up when direct observation was not possible. Apgar scores at 1 min were available only for those babies who were born in the study hospital. Physical development was measured by anthropometric indices. Weight in grams and length in millimeters were measured concurrently with visual attention.

Visual attention. Infants were tested on the 15th day after birth (mean = 14.8, SD = 1.6) in the study laboratory, located at the district health center. The infant's state of arousal was observed for 60 sec before testing and rated on a 6-point scale ranging from quiet-inactive to agitated distress (23). Testing was initiated only if rating was 1 or 2. If rating was 3 or more, or if the baby fell asleep, testing was postponed until the next weekday. There were no group differences in the initial state of arousal. Babies were rated alert if they visually explored the interior of the testing enclosure, or non-alert if they did not.

TABLE 1
Sample characteristics by sex and supplementation

Characteristic	Boys				Girls			
	Unsupplemented		Supplemented		Unsupplemented		Supplemented	
	mean	*SD*	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*
Mother's age	26.9	5.9	25.8	5.9	27.2	6.3	25.8	4.9
Mother's education (yr of school)	2.8	1.9	3.1	1.7	3.0	2.1	3.2	1.7
Family income per mo in pesos[a]	1526	677	1378	500	1372	660	1553	850
Calories at 6 months	1458	521	1589	604	1506	556	1573	632
Proteins (g) at 6 months	30.1	15.1	36.6	21.3	35.2	19.7	34.1	18.4

[a] A peso = 0.03 American dollar.

The baby was placed on a bed surrounded by a light-blue enclosure lying on his side, facing the stimulus at a distance of approximately 19 cm. A pillow was set on the side of the exposed cheek to discourage head movement. The stimuli were presented through a slot on the side of the enclosure. On each side of the slot, there was a 1-cm peephole used to observe the baby during stimulus presentations. The standard stimulus, a 2 × 2 black and white checkerboard, which measured 15 × 15 cm, was presented for eight 60-sec trials separated by 10-sec intervals. The habituation trials were followed by one 60-sec presentation of the discrepant stimulus, a 6 × 6 checkerboard (24). Trial timing was indicated to the observers by pretaped instructions through earphones.

Total fixation time, number of fixations, fret/cries, and gross limb movements per trial were recorded by two observers blind to the infant's treatment group. One examiner recorded visual fixation defined as the reflection of the stimulus in the pupil of the baby's eye. The duration and number of fixations were recorded using a counter and timer that registered in tenths of a minute. The other examiner, who presented the stimuli, recorded the number of fret/cries and movements.

Fixation data were analyzed as percent of total trial time. Habituation was defined as a decrement in fixation time between trial 1 (T1) and trial 8 (T8), and habituation rate was measured as the fraction of fixation time at T1 persisting at T8 (T1 - T8/T1). Negative rates were counted as 0, and the scores thus ranged from 0 to 1.0 (25). Dishabituation and dishabituation rate were similarly defined, (T9 - T8/T9). The fixation data were also analyzed using a supplementation by sex by trial analysis of variance, in which factors were assumed fixed and repeated measures were over the last factor (26).

Intertester reliability was high. Two independent observers reached 87% agreement for fixation time, 97% for fret/cries, and 95% for movements. Moreover, the same two examiners observed 81% of the sample.

Results

Table 2 presents descriptive statistics by group and sex on gestational age, dietary and anthropometric variables. Supplemented mothers of both boy and girl babies consumed significantly more protein than the unsupplemented mothers. Calorie intake was significantly affected by supplementation only among mothers of boys. These differences may be unreliable due to missing data in the second dietary survey. The effect of supplementation on height and weight at 15 days was significant only for boys.

Distributions of maternal medication, Apgar scores and breast-feeding are presented in Table 3. Low Apgar scores were more frequent among unsupplemented boys than among supplemented ones, $z = 2.67$, $P < 0.01$. No mean or distribution differences between groups were observed in length of ges-

TABLE 2
Descriptive statistics by sex and supplementation on length of supplementation, length of gestation, dietary and anthropometric variables

Variable	Boys								Girls							
	Unsupplemented			Supplemented		Significant contrasts			Unsupplemented			Supplemented		Significant contrasts		
	n	*mean*	*SD*	*n*	*mean*	*SD*	*t*	*p*	*n*	*mean*	*SD*	*n*	*mean*	*SD*	*t*	*p*
Wk of supplementation				75	13.1	4.1						69	13.1	40		
Wk of gestation	49	39.8	2.1	75	39.6	2.1						69	39.5	2.2		
Calories at 8 mo	39	1349	512	65	1871	572	−4.68	*a*	41	1542	491	57	1672	484		
Proteins (g) at 8 mo	39	29.4	13.5	65	61.0	20.7	−8.50	*a*	41	33.9	17.3	57	52.9	18.9	−5.08	*b*
Weight at 15 days (g)	50	3143	449	75	3286	471	−1.69	*c*	50	3070	381	67	3080	443		
Length at 15 days (cm)	50	49.3	1.8	75	49.9	2.0	−1.71	*c*	50	48.8	1.8	67	49.0	1.9		

[a] One-tail $P < 0.001$. [b] One-tail $P < 0.01$. [c] One-tail $P < 0.05$.

TABLE 3
Distributions by sex and supplementation on breast-feeding, Apgar-scores, and maternal medication during labor

Variable	Boys				Girls			
	Unsupplemented		Supplemented		Unsupplemented		Supplemented	
	%	n	%	n	%	n	%	n
Breast-fed	96.0	50	94.7	75	94.0	50	95.5	69
Apgar score								
10	72.2	36	91.7	48	92.3	26	90.7	43
8–9	19.4	36	2.3	48	7.7	26	9.3	43
7 or less	8.3	36	6.2	48	0.0	26	0.0	43
Medication during labor								
None	20.4	49	24.3	74	36.7	49	35.3	68
Analgesics, anesthetics	20.4	49	21.6	74	8.2	49	16.1	68
Oxytocin	59.2	49	54.1	74	55.1	49	48.6	68

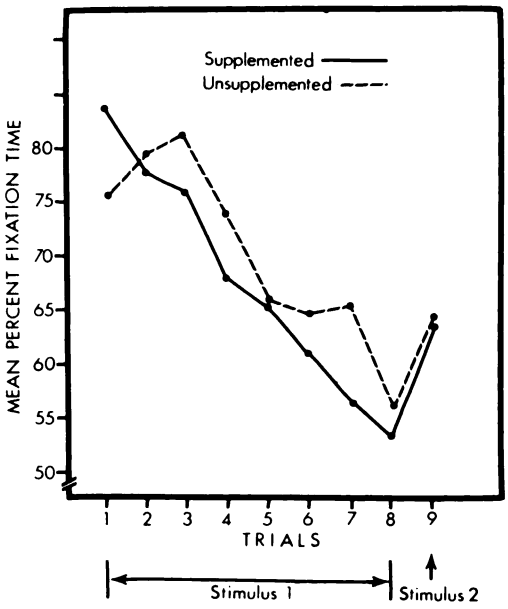

FIG. 1. Mean percent fixation time per trial, supplemented versus unsupplemented male infants. Stimulus 1 = 2 × 2 checkerboard; stimulus 2 = 6 × 6 checkerboard.

tation, maternal medication or frequency of breast-feeding.

Figures 1 and 2 show percent fixation time per trial by group for boys and girls respectively. More supplemented babies (70%) displayed habituation ($T_1 > T_8$) when compared with the unsupplemented ones (56%), $t(242) = 2.31$, $P < 0.05$. Habituation rate was significantly higher in supplemented girls (mean = 0.44, SD = 0.42) than in unsupplemented ones (mean = 0.27, SD = 0.35), $t(117) = 2.33$, $P < 0.01$. This difference was not significant in the boys.

The analysis of variance on habituation revealed a main effect for trials, $F(7,1680) = 35.44$, $P < 0.001$, a supplementation by trials interaction, $F(7,1680) = 3.60$, $P < 0.001$, and a supplementation by sex by trials interaction, $F(7,1680) = 1.99$, $P = 0.05$. The supplementation by trials interaction was due to group differences mainly at T_1 and T_7. In comparison with the supplemented babies, the unsupplemented group displayed a shorter fixation time at T_1, $F(1,239) = 14.62$, $P < 0.001$, but a longer one at T_7, $F(1,239) = 17.82$, $P < 0.001$. Overall, trials accounted for 20% of the variance in the supplemented group and for 6.2% in the unsupplemented one.

Dishabituation of attention was displayed by similar proportions of unsupplemented (42%) and supplemented babies (38.2%). Dishabituation rate did not differ by group and the analysis of variance on dishabituation revealed only a main effect for trials, $F(1,242) = 25.86$, $P < 0.001$.

In both groups the average number of movements as well as the number of infants moving increased with trials. The unsupplemented babies moved more during the habituation trials (mean = 11.80, SD = 6.85) than did the supplemented ones (mean = 10.19, SD = 5.53), $t(242) = 2.03$, $P < 0.05$.

Evidence for less moving during the dishabituation trial (mean = 1.32, SD = 1.08) in comparison with the last habituation trial (mean = 1.70, SD = 1.15) appeared only among the unsupplemented girls, $t(48) = 2.03$, $P < 0.05$.

There were no initial group or sex differences in fussing, which in both groups increased with trials. However, supplemented

girls grew fussier than the unsupplemented ones and cried significantly more during the habituation trials (mean = 2.26, SD = 3.65), $t(117) = 2.42$, $P < 0.01$. Level of fussing during the dishabituation trial did not differ significantly from that observed during the last habituation trial.

Intercorrelations by group between the various measures of attention are presented in Table 4. Visual altertness prior to stimulus presentations was associated with higher number of fixations, shorter fixation times at T_1, T_8, and T_9, less total attention, as well as with higher habituation rates. Cries and movements were positively interrelated, but both were negatively associated with total attention. Habituation and dishabituation rates were moderately positively correlated, but there was a strong negative relationship between habituation rate and fixation time to the dishabituation stimulus. The pattern and level of intercorrelations were similar in the two groups, except for moving that was related to fixation times and habituation only in the supplemented group.

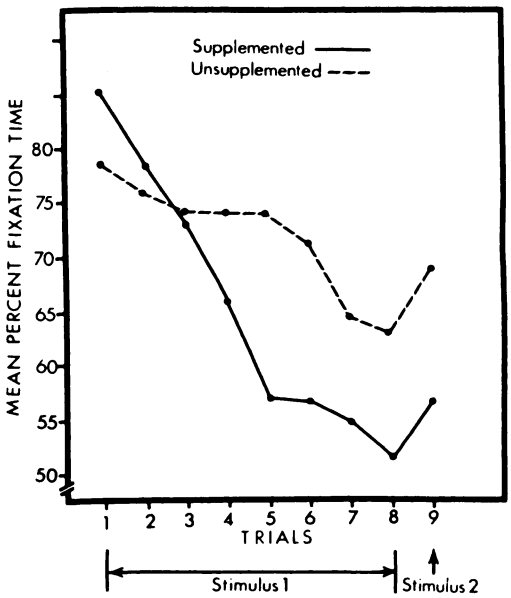

FIG. 2. Mean percent fixation time per trial, supplemented versus unsupplemented female infants. Stimulus 1 = 2 × 2 checkerboard; stimulus 2 = 6 × 6 checkerboard.

Discussion

The findings clearly show that food supplementation of pregnant women at risk of malnutrition had an effect on the neonate's ability to respond to and process visual information. In comparison with a randomly assigned unsupplemented control group, the babies of supplemented mothers displayed stronger response to stimulus onset, as indicated by longer initial fixation, and a more rapid habituation to stimulus repetitions. These findings are consistent with previous research on effects of pre- and perinatal risk variables upon newborn attention (12, 15, 27) and on differences in cardiac orienting response and habituation between 1-year-old malnourished and wellnourished infants (14).

Differences in attention between supplemented and unsupplemented infants may be maturational in nature. Sigman et al. (12)

TABLE 4
Intercorrelations between measures of attention[a]

	Visual alertness	Fixation time			Habituation rate	Dishabituation rate	Total no. of fixations	Total attention	Cries	Movements
		T1	T8	T9						
Visual alertness	1.00	0.19	0.44	0.25	−0.36	−0.30	−0.37	0.39		
Fixation time										
T1	0.27	1.00	0.34	0.32				0.64	−0.27	
T8	0.51	0.35	1.00	0.79	−0.93	−0.54	−0.28	0.85	−0.51	
T9	0.49	0.36	0.85	1.00	−0.73			0.77	−0.58	
Habituation rate	−0.47	−0.20	−0.97	−0.82	1.00	0.50	0.19	−0.73	0.47	
Dishabituation rate			−0.33		0.32	1.00	0.42	−0.30		
Total no. of fixations	−0.19						1.00			0.21
Total attention	0.59	0.47	0.85	0.82	−0.79			1.00	−0.56	−0.19
Cries	−0.23		−0.55	−0.59	0.58				1.00	0.39
Movements			−0.23	−0.18	0.27			−0.22	0.55	1.00

[a] Data on supplemented infants are in the lower triangle and on unsupplemented infants in the upper triangle of the matrix. Only correlations with P values lower than 0.05 are displayed. $r = 0.20$, $P < 0.05$. $r = 0.26$, $P < 0.01$.

found that orienting response magnitude was the most sensitive indicator of attentional differences between neonates varying in levels of neurological maturation. Present data on lower Apgar scores and lower levels of initial response among unsupplemented boys are consistent with such previous findings. Furthermore, conditioning which is related to orienting response (28, 29) shows age differences in the rate of acquisition, but not in the rate of extinction of a conditioned response (30, 31). Likewise, age-related differences have been found in habituation, but not in dishabituation (32). The observed differences between supplemented and unsupplemented infants in orienting response and habituation and the absence of a difference in dishabituation are consistent with a maturational effect.

Supplementation effects were found to be sex related. Habituation rate, moving, and fussing showed supplementation differences only among girls, while physical growth was affected by supplementation among boys. In the neonatal period girls lead boys by 4 to 6 weeks in central nervous system maturity (33). Supplementation differences in habituation among girls may thus be interpreted in terms of the greater susceptibility of the organism maturing at a faster rate. Similarly, the sex-specific supplementation effect on physical growth may be related to the faster rate of weight gain among boys during the last trimester of pregnancy (34) and in the first few weeks of life (35).

Both groups moved and fussed more with stimulus repetitions which is consistent with previous findings of arousal behaviors as they accompany habituation (24, 36). It also appeared that supplemented infants responded in a more integrated fashion, as moving was consistently associated with fixation times and habituation only in the supplemented group.

In summary, less initial response to stimulus onset, slower habituation to stimulus repetitions, and higher levels of movement were characteristic of unsupplemented infants' visual attention when compared with supplemented infants. This pattern represents a less mature response to visual stimuli. Although an effect of supplementation upon maternal behavior toward the newborn is possible, the observed differences between groups most likely represent an effect of pre- and perinatal nutritional intake, independent of social variables.

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