Poor nutrition during pregnancy and lactation negatively affects neurodevelopment of the offspring: evidence from a translational primate model

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ABSTRACT

Background: Studies of the effects of prenatal nutrition on neurodevelopment in humans are complicated because poor nutrition occurs in the context of psychosocial stressors and other risk factors associated with poor developmental outcomes.

Objective: Under controlled experimental conditions, we tested an effect of prenatal nutrition on neurodevelopmental outcomes in the nonhuman primate.

Design: Juvenile offspring of 19 female baboons, whose diets were either restricted [maternal nutrition restriction (MNR)] or who were fed ad libitum (control), were administered the progressive ratio task from the Cambridge Neuropsychological Test Automated Battery. Activity, persistence, attention, and emotional arousal were coded from videotapes. These established, reliable methods were consistent with those used to assess individual differences in the behaviors of school-age children.

Results: MNR offspring (3 female and 4 male offspring) had significantly fewer responses and received fewer reinforcements on the progressive ratio task than did control offspring (8 female and 4 male offspring). MNR offspring showed a more variable activity level and less emotional arousal than did control offspring. Female MNR offspring showed more variable and lower levels of persistence and attention than did female control offspring. Thus, under controlled experimental conditions, data support a main effect of prenatal nutrition on highly translatable neurodevelopmental outcomes.

Conclusions: Nutritional interventions during pregnancy have been successfully used to target neurodevelopmental problems, such as increasing folic acid intake during pregnancy to decrease the incidence of neural tube defects. Results from the current study can be used to support the testing of nutritional preventive interventions for the most-common childhood behavior problems. Am J Clin Nutr 2013;98:396–402.

INTRODUCTION

Poor nutrition during pregnancy is a major public health problem in the United States and other countries. There is compelling evidence from epidemiologic studies that poor nutrition during pregnancy, in the form of insufficient intake, low protein, or deficiencies in micronutrients, is associated with poor developmental outcomes in children including lower cognitive functioning, deficits in attention, and disruptive behavior problems. However, in all human studies, poor nutrition occurs in the context of psychosocial stressors and genetic risk factors that also increase risk of poor developmental outcomes independent of prenatal nutrition. Because prenatal nutrition is potentially more modifiable than many environmental and genetic risk factors for suboptimal neurodevelopment, an exploration of how much variance in developmental outcomes is explained by nutrition is extremely important. A controlled study in a well-established, nonhuman primate model of maternal undernutrition allowed us to establish whether the variance in developmental outcomes is explained by prenatal nutrition.

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a touch-screen testing system used to assess human cognitive function and neurodevelopment. We recently adapted the CANTAB system for use in baboons. The assessment of cognitive performance by using the CANTAB and observing attention, persistence, and activity during testing are standard methods for the assessment of individual differences in school-age children. Thus, developmental outcomes measured in juvenile baboons by using this approach are highly translatable to humans.

We hypothesized that a significant main effect of nutrition during pregnancy on behavioral and cognitive functioning in the offspring would be observed, with higher levels of activity and lower levels of persistence, attention, and motivation during the CANTAB shown in offspring of female baboons whose diets were restricted than in offspring of female baboons who were fed ad libitum.

MATERIALS AND METHODS

All animal procedures were performed in accordance with accepted standards of humane animal care approved by the Texas...
Biomedical Research Institute and University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee and conducted in facilities approved by Association for Assessment and Accreditation of Laboratory Animal Care International Inc. The 19 baboons (Papio spp.) studied were born at the Southwest National Primate Research Center to mothers selected before pregnancy who were housed in outdoor cages in groups of up to 16 with a fertile male. Females were of similar age (mean ± SEM: 11.5 ± 0.51 y) and morphometric phenotype. Animals were trained before pregnancy to feed in individual cages as described previously (8). Each baboon’s weight was obtained while crossing an electronic scale (GSE 665; GSE Scale Systems. Water was continuously available in feeding cages, and animals were fed a commercial nonpurified diet (Purina Monkey Diet 5038; Purina). Females of similar morphometric phenotype were allocated to one of 2 cages until both cages contained the required number of females to form a harem group (n = 16). One cage was randomly selected for ad libitum feeding on normal primate feed pellets (control diet: 12% energy from fat, 18% from protein, and 69% from carbohydrate that consisted of 0.29% glucose and 0.32% fructose) and one cage for 70% of the feed required number of females to form a harem group (n = 16). One cage was randomly selected for ad libitum feeding on normal primate feed pellets (control diet: 12% energy from fat, 18% from protein, and 69% from carbohydrate that consisted of 0.29% glucose and 0.32% fructose) and one cage for 70% of the feed eaten by control females on a weight-adjusted basis from the time of diagnosis of pregnancy (~30 d gestation) for the rest of pregnancy and through lactation (9). By the time the study commenced, 12 females were pregnant in the control cage, and 7 females were pregnant in the cage assigned to nutrition restriction.

Mothers delivered spontaneously at full term, and offspring were reared, with their mothers, in group-based housing until early adolescence (9 mo of age). The juvenile offspring were transferred to the University of Texas Health Science Center at San Antonio in cohorts of 5–7 subjects over a 9-mo period and housed individually in the visual and auditory presence of ≥6 other peers in the Laboratory Animal Resources facility. Subjects were behaviorally tested at 3.3 ± 0.2 y of age and within a normal weight range (10). The range of ages was relatively broad (2.6–5.1 y; Table 1), and thus, age was included as a covariate. We previously reported sex differences in performance on the CANTAB (11). Therefore, we tested the effect of sex on behavior during cognitive testing. For ease of explication, female and male offspring of control mothers are referred to as control females and control males, respectively, and female and male offspring of mothers in the maternal nutrition restriction (MNR) group are referred to as MNR females and MNR males, respectively.

Our use of the CANTAB system for baboon behavioral testing has been described in detail (4). The progressive ratio task used to measure the motivation to work for reward was based on that developed for rhesus and marmosets (12, 13) but modified for the baboon. Subjects were required to touch a single, large (10-cm square) purple stimulus presented in the center of the screen. Response requirements to obtain a successive reinforcement progressively increased: beginning with one and increasing by one for the first 8 reinforcements. Thereafter, the increment doubled after the eighth reinforcement. Each session lasted ≤30 min but was terminated early if the subject did not respond within a 3-min period. Two reinforcement pellets were delivered automatically when the number of responses required had been reached. Daily feeding rations were calculated before training on the CANTAB by administering food ad libitum over a course of 2 wk and measuring consumption. Each subject was fed one-half this amount 2 times/d over the course of the study. Subjects were tested for 13 progressive ratio sessions. For the first 3 training sessions, subjects were not fed in the 4 h before the task to facilitate engagement. Subjects were fed 2 h before the task for the following 10 sessions. Data from the latter 10 sessions were included in the current analyses. Dependent variables measured were the number of responses made, rewards earned, and breakpoint (time of session termination because of inactivity).

Raters who were unaware of the prenatal nutritional status coded each of the 10 training sessions in three 7-min segments. Smaller segments were used to prevent rater fatigue and halo effects. Behavioral ratings were averaged over the 3 segments. According to the work of Willcutt et al (7), 4 ratings (persistence, attention, activity, and arousal) were made for each segment for each session on 7-point Likert interval scales with higher levels of

### TABLE 1

Descriptive statistics for morphologic and testing data of offspring

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 19)</th>
<th>MNR (n = 7)</th>
<th>Control (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphologic data for offspring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female [n (%)]</td>
<td>11 (57.90)</td>
<td>3 (42.90)</td>
<td>8 (66.60)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>0.83 ± 0.03a</td>
<td>0.74 ± 0.05</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>Age at testing (y)</td>
<td>3.23 ± 0.15</td>
<td>2.89 ± 0.22</td>
<td>3.43 ± 0.17</td>
</tr>
<tr>
<td><strong>Testing data for offspring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakpoint</td>
<td>1035.90 ± 83.32</td>
<td>970.37 ± 152.15</td>
<td>1074.12 ± 101.30</td>
</tr>
<tr>
<td>No. of responses</td>
<td>642.87 ± 99.92</td>
<td>504.99 ± 109.80</td>
<td>723.30 ± 142.95</td>
</tr>
<tr>
<td>No. of reinforcements</td>
<td>23.06 ± 1.32</td>
<td>21.26 ± 2.10</td>
<td>24.12 ± 1.69</td>
</tr>
<tr>
<td>Level of activity</td>
<td>3.47 ± 0.14</td>
<td>3.55 ± 0.20</td>
<td>3.42 ± 0.19</td>
</tr>
<tr>
<td>Level of persistence</td>
<td>3.72 ± 0.14</td>
<td>3.60 ± 0.14</td>
<td>3.80 ± 0.21</td>
</tr>
<tr>
<td>Level of attention</td>
<td>3.21 ± 0.15</td>
<td>3.09 ± 0.14</td>
<td>3.28 ± 0.22</td>
</tr>
<tr>
<td>Arousal behaviors [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>6 (31.60)</td>
<td>4 (57.10)</td>
<td>2 (16.70)</td>
</tr>
<tr>
<td>2–9</td>
<td>8 (42.10)</td>
<td>3 (42.90)</td>
<td>5 (41.70)</td>
</tr>
<tr>
<td>≥4</td>
<td>5 (26.30)</td>
<td>0 (00.00)</td>
<td>5 (41.70)</td>
</tr>
</tbody>
</table>

*Testing data are based on averages over the 10 sessions; arousal behaviors are a sum across the 10 sessions.

1 MNR, maternal nutrition restriction.

2 Mean ± SE (all such values).
the behavior reflected by higher scores. Persistence was operationalized as the time spent in front of the testing screen engaged with the task. Attention was coded in terms of the level of focus when the animal was in front of the screen. An example of low attention was when an animal looked away while touching the screen. Ratings of attention included both the frequency and intensity of the movement. Arousal was operationalized as grimaces, fur rising on the back, startled responses with hyperalertness to surroundings, ears back, or behavior that seemed skittish and jerky. Base rates for arousal were low. Thus, scores for arousal were dichotomized as the presence or absence of arousal behaviors. Interrater reliabilities, which were based on the recoding of 10% of sessions, were all in the acceptable range: Intraclass correlations were 0.64 for activity, 0.88 for persistence, 0.71 for attention, and 0.84 for arousal and were consistent with the level of reliability achieved in humans (7, 14). Analyses were conducted with IBM SPSS Statistics 20.0 (IBM Corp). Descriptive data for testing variables are presented in Table 1.

RESULTS

With the exception of arousal, effects of the nutrition group on each dependent measure was tested via repeated-measures ANOVA for which between-group effects, effects of sex, effects of group × sex, and effects of group × time were included in each model controlled for age. Significant effects of the nutrition group on the number of responses ($F_{[1,14]} = 5.20, P = 0.039$, partial $\eta^2 = 0.27$) and number of reinforcements earned ($F_{[1,14]} = 5.92, P = 0.029$, partial $\eta^2 = 0.42$) were observed; no significant effect was observed for the breakpoint. Rodriguez et al (11) reported effects of the nutrition group on a variety of CANTAB tests including the progressive ratio task in this same sample. In that set of analyses, age was not controlled for, and the difference between MNR and control animals on the number of responses and reinforcements was reported as marginally significant ($P = 0.06$). In the current study, we conducted all analyses controlling for age, as would be the case in a study of human children.

With the effect of age controlled for, a significant effect of the nutrition group on the performance of the progressive ratio task was observed. There were no significant effects of sex, and there were no nutrition group or sex effects on the pattern of response over time. Differences in the level of response and number of reinforcements received are shown in Figures 1 and 2; control animals made a higher number of responses and received a higher number of reinforcements averaged across the 10 sessions.

There was a significant effect of the nutrition group on the activity level over time ($F_{[9,126]} = 2.41, P = 0.015$, partial $\eta^2 = 0.17$) but no effect of sex or sex by nutrition group, which may have been due in part to the lower reliability of this measure. As shown in Figure 3, MNR offspring showed more variability in the level of activity across sessions (range: 2.6–4.0) than did control offspring (range: 3.2–3.8).

The observed persistence over time was associated with the interaction of the nutrition group and sex ($F_{[9,126]} = 2.19 P = 0.027$, partial $\eta^2 = 0.16$). This effect is shown in Figure 4 for females. When the level of persistence was averaged across the 10 sessions, no group or group × sex differences were observed (data on females is shown in Figure 4A). When the level of persistence was examined over time, control females evidenced increasing levels of persistence, whereas the level of persistence in MNR females decreased over time (Figure 4B). Levels of persistence over time for MNR and control males were similar (data not shown).

The observed attention also varied over time as a function of the interaction of nutrition group and sex ($F_{[9,126]} = 3.55, P = 0.001$, partial $\eta^2 = 0.25$). As shown in Figure 5, there were no differences between control and MNR females in the average level of attention across the 10 sessions (Figure 5A); however, as was the case with persistence, control females evidenced increasing levels of attention over time, whereas the level of persistence in MNR females decreased over time (Figure 5B). The level of attention over time between MNR and control males was similar (data not shown).

We tested the effect of nutrition on emotional arousal by computing an ordinal logistic regression with the total number of observed indicators of arousal across the 10 sessions grouped into 3 levels of 0–1, 2–3, and 4+; because of the small number of observations, testing the interaction with sex was not feasible. However, there were no sex effects on the occurrence of arousal...
behaviors (Kendall’s τ-b = 0.79, P = 0.719). A significant effect of the nutrition group on the occurrence of arousal behaviors was observed (Wald’s chi-square = 4.91, P = 0.027). As shown in Figure 6, slightly more than one-half of the MNR animals showed low levels of arousal during testing (0–1 occurrences), whereas <20% of control animals showed low levels of arousal. In addition, although the observation of ≥4 instances of arousal was common in control animals, none of the MNR animals reached that level of arousal during testing.

DISCUSSION

Adequate prenatal nutrition is a critical component of healthy growth and development, and brain development is particularly sensitive to adequate proteins and lipids (15). A number of studies have showed associations between nutritional intake during pregnancy and behavioral and emotional functioning in offspring in humans (1). However, such studies have been limited because, although better nutrition can be experimentally manipulated, poor nutritional intake cannot be experimentally manipulated. Because correlates of poor nutrition such as low-income status, substance use, and maternal physical and mental health problems are also associated with poor developmental outcomes for the offspring, confirming or refuting the role of prenatal nutrition in neurodevelopment in the human has been challenging. The current translational value of this nonhuman primate study was that it helped to overcome the limitations of conducting studies in humans without significantly sacrificing the translational effect by using a standardized assessment of cognitive performance and observed ratings of behavior and emotional arousal that are consistent with those used to probe individual differences in the same domains in children.

The results showed that, in addition to a poorer performance on the progressive ratio scale by using the CANTAB, which reflected decreased motivation, the testing behavior varied significantly as a function of suboptimal nutrition during pregnancy and lactation. The activity level and arousal significantly differed as a function of the nutrition group, with no additional moderation by sex. The pattern was one of greater variability in the activity level over the 10 testing sessions and lower levels of arousal in

FIGURE 2. Number of responses by nutrition group averaged across sessions (A) and for each session (B). Significant between–nutrition-group effect on the number of responses with age and sex controlled for (ANOVA): F{sub}_{1,14} = 5.20, P = 0.039, partial η² = 0.27. There were no significant effects of sex and no nutrition group or sex effects on the pattern of response over time. CTL, control; MNR, maternal nutrition restriction.

FIGURE 3. Activity level by nutrition group averaged across sessions (A) and for each session (B). Effect of nutrition on the activity level over time (repeated-measures ANOVA): F{sub}_{9,126} = 2.41, P = 0.015, partial η² = 0.17. CTL, control; MNR, maternal nutrition restriction.
offspring of mothers whose diets were restricted than in offspring of controls who were fed ad libitum. In addition, in MNR females, the persistence and attention across the 10 sessions decreased compared with in control females. In studies of humans, the combination of low arousal, poor attention, and persistence and a difficulty modulating activity is particularly problematic in that the combination of behaviors is associated with significant morbidity and mortality (16).

Our finding that nutrition was associated with attention and behavior problems in the baboon was highly consistent with the existing literature in humans. Significant associations between indicators of poor nutrition and childhood behavior problems and cognitive functioning have been reported. For example, Liu et al (17) assessed 4 indexes of early malnutrition at age 3 y including anemia, cracking in the lips, hair dyspigmentation, and sparse hair. These factors were related in a dose-dependent way to aggression, hyperactivity, and delinquency assessed at ages 8, 11, and 17 y. Although Liu et al (17) reported that such findings were maintained after psychosocial adversity was controlled for, other investigators have reported that effects of prenatal nutrition on later disruptive behavioral problems are largely mediated by psychosocial factors (18). Without an adequate distribution of poor nutrition across levels of socioeconomic status, it is difficult to test for such effects in humans. The results of the current study indicated that poor nutrition accounts for the unique variance in disruptive behavioral problems.

The data also suggested that females may be more susceptible to the effect of prenatal nutrition than males. Halas et al (19) reported a similar finding in the rat, whereby prenatal undernutrition was associated with higher levels of aggression in offspring but only in female offspring. However, in other animal models, males appeared to be the more vulnerable sex, particularly when a stressor was encountered early in gestation (20). Studies in humans regarding sex differences in the susceptibility

FIGURE 4. Persistence level for females by nutrition group averaged across sessions (A) and for each session (B). A: With the use of ANOVA, there were no significant effects of group or group by sex on the level of persistence averaged across sessions; data shown for females. B: With the use of repeated-measures ANOVA, there was an interaction effect of nutrition group and sex on persistence over time ($F_{[9,126]} = 2.19, P = 0.027, \eta^2 = 0.16$); data shown for females. CTL, control; MNR, maternal nutrition restriction.

FIGURE 5. Attention level for females by nutrition group averaged across sessions (A) and for each session (B). A: With the use of ANOVA, there were no significant effects of group or group by sex on level of persistence averaged across sessions; data shown for females. B: With the use of repeated-measures ANOVA, there was an interaction effect of nutrition group and sex on attention over time ($F_{[9,126]} = 3.55, P = 0.001, \eta^2 = 0.25$); data shown for females. CTL, control; MNR, maternal nutrition restriction.
to prenatal stress have been equally mixed and depended in part on the range of behavioral phenotypes (21). In the current study, a relatively narrow behavioral repertoire was measured in a single context. The assessment anxiety and depression behaviors in a novelty or learned helplessness task would be useful to elucidate sex-specific effects of prenatal nutrition on psychopathology more broadly. Moreover, interaction effects with small sample sizes, such as those in the current study, require replication. Larger samples are also needed to adequately model covariates such as age, weight, sleep, and other factors that may affect individual differences in performance on and behavior during neurodevelopmental tasks.

Finally, it is plausible that the association between poor nutrition and performance on the CANTAB was in part a result of poor testing behavior, particularly because the assessment of cognitive capacity requires modulated activity, persistence, and attention. The administration of methylphenidate to children diagnosed with attention-deficit hyperactivity disorder has been associated with improvements in reaction time, attention, and accuracy during testing (22). The current study did not have a large-enough sample size to model mediating effects, but the testing of such effects will be helpful to narrow the possible mechanisms by which prenatal nutrition affects fetal neurodevelopment.

In conclusion, our data support a main effect of prenatal nutrition on neurodevelopmental outcomes in the nonhuman primate under controlled experimental conditions. These outcomes are highly translatable to the human primate. Nutritional interventions during pregnancy have been successfully used in the past to target neurodevelopmental problems, such as increasing folic acid intake during pregnancy to decrease the incidence of neural tube defects (23). To our knowledge, results from the current study are among the first to support testing the hypothesis that nutritional preventive interventions could be effective in reducing risk of childhood attention and behavior problems. These are the most-common and persistent forms of psychopathology in children (24) and are associated with significant morbidity and mortality (25). Efforts directed at developing preventive interventions that target modifiable factors, such as nutrition, could lead to significant reductions in the burden that results from these disorders.

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REFERENCES


FIGURE 6. Observed arousal behaviors across all testing sessions by nutrition group. With the use of ordinal logistic regression, there was an effect of nutrition group on total number of observed arousal behaviors summed across session (Wald’s chi-square = 4.79, P = 0.029). MNR, maternal nutrition restriction.