Moderate Zinc-Iron Deprivation Influences Behavior but Not Growth in Adolescent Rhesus Monkeys

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ABSTRACT Primate species demonstrate a prolonged period of development before reproductive maturity that includes distinctive periods of rapid growth in the late fetal, late infancy and early adolescent stages. Rhesus monkeys resemble humans in this discontinuous pattern of growth and also in its relationship to brain development. Studies of zinc deprivation in rhesus monkeys have suggested an important relationship among growth rate, nutrient status and behavioral performance in infancy as well as adolescence. Recently, moderate combined zinc and iron deprivation (intake 0.2 mg Zn and 0.8 mg Fe/d, compared with control intake of 2.9 mg Zn and 1.7 mg Fe/d) during the adolescent growth spurt (29–32 mo of age) of female rhesus monkeys (n = 8/group) was shown to influence behavior without affecting growth. Behavioral assessments included the Continuous Performance Test, the Delayed Nonmatch to Sample Test and activity (measured with an actimeter). The behavioral syndrome was characterized by reduced activity, reduced participation in behavioral testing and slower response. These changes could be reversed or prevented to some extent by altering the diet to include tablets of powdered beef (adding ~1.7 mg Zn and 0.7 mg Fe to daily intake). The study suggests that behavior may be sensitive to the quality of the diet available during the period of rapid adolescent growth and development. J. Nutr. 130: 354S–357S, 2000.

KEY WORDS: activity • behavior • monkeys • diet

Patterning of growth during development is one of many ways in which primates have been distinguished from other mammalian species (Watts 1986). In primates, a period of rapid growth commences in utero after embryogenesis and is completed postnatally, corresponding to the end of lactation. Growth continues at a slower pace through a prolonged juvenile phase (childhood) that is infrequently encountered in nonprimate species. Then, before puberty, a striking growth spurt occurs, reproductive maturity is gradually established and linear growth ceases as a result of epiphyseal closure at the end of adolescence. This discontinuous pattern of growth is not present in common laboratory animals used to study the effects of malnutrition. However, it is important to note that there are also further differences within primate species in growth and maturational staging.

We have been studying the effects of developmental zinc deprivation for a number of years in rhesus monkeys. Purified diets were used in these studies to allow precise control of trace element composition, and the zinc deprivation was characterized as marginal or moderate (Keen et al. 1993) depending on whether the diet contained 2 or 4 µg Zn/g. During these studies, we noted that clinically detectable zinc deficiencies appeared only toward the end of periods of rapid growth, i.e., the third trimester, late infancy and early adolescence. Behavioral measures were included in a number of our studies, as well as in other studies of zinc deficiency in rodents and monkeys (Golub et al. 1995).

More recently, we have turned from infancy to adolescence in our studies of developmental zinc deprivation (Golub et al. 1995). The adolescent growth spurt in rhesus monkeys is a convenient and valuable model system for studying effects of malnutrition on growth and behavior. Monkeys are seasonal breeders; puberty is timed to the breeding season, with menstrual occurring in the breeding season of y 3 of life (30 mo of age) and the growth spurt occurring just before this. Growth is approximately twice as fast during the 5-mo peak in growth rate (age 28–33 mo) than in the previous or succeeding months (Blackwelder and Golub 1996). Seasonal breeding also means that monkeys are born within a few months of each other each spring and will go through the growth spurt in synchrony.

With this information, it was possible to plan dietary in-
tervations during the growth spurt in female monkeys; our most recent experiment combined iron and zinc deprivation in female rhesus (Golub et al. 1999). Iron and zinc co-occur in high protein food items frequently missing from poor quality diets, and these nutritional deficiencies are often seen together in malnourished children. In the U.S., studies indicate that 83% of adolescent girls consume less than the RDA for iron and 81% consume less than the RDA for zinc (Johnson et al. 1994). Thus, an isolated zinc deficiency is not likely during this time. Also, in girls, the onset of menstruation at the peak of the growth exacerbates dietary deficiencies of iron.

The effect of diet on the rapid growth of adolescents is of concern, but it is also appropriate to consider the effect of nutrient deficiency on brain function during development (Beard 1995, Sandstead et al. 1998). Adolescent brain development has several distinct characteristics. Cognitive development is marked by onset of the ability to manipulate symbols and conceptual representations. Brain electrical activity demonstrates increased coherence and speed, with less energy use. Anatomically, myelination in brain commissures is completed, allowing faster transmission of information. There is a decrease in gray matter in some cortical areas, presumably due to completion of synaptic “pruning.” In the hypothalamus, intricate networks of growth factors, neurotransmitters and hormone releasing factors are established for controlling reproductive cycles. Changes in neurotransmitter receptor expression and sensitivity to agonists and antagonists appear throughout the brain.

In addition to studying iron-zinc deprivation, a component of the study examined the effect of meat as a common dietary source of zinc and iron. At the end of the anticipated period of most rapid growth, we introduced a supplemental food in the form of 4-g tablets of baked, lyophilized beef. The zinc content of the control diet (35 µg/g) and deprived diet (2 µg/g) were based on our previous experience with marginal and moderate zinc deprivation. The iron deprivation content (10 µg/g) was adopted from a study of developmental iron deficiency in rhesus infants (Munro 1987); controls were fed 20 µg Fe/g diet. We intended the supplement to be equivalent to a 100-g serving of beef for an 11- to 14-year-old girl in terms of the percentage of daily protein intake, and to restore zinc and iron intake to near normal levels. However, it should be noted that, by using beef, rather than adding zinc and iron to the basal diet, we cannot be sure whether the additional zinc and iron were responsible for the effects of the beef tablets.

Figure 1 shows that the short-term zinc-iron deprivation did not decrease growth rate during the growth spurt or later during the supplement period. Group differences in plasma zinc and iron were seen by the end of the growth spurt, although the deprived group could not be said to be in a state of clinical zinc or iron deficiency. (Plasma zinc and iron may not be good indicators of zinc and iron status in cross sections of children, but they are more reliable with the controlled environments and well-defined diets and ages of the monkeys in this experiment.) Further indication of the effect of the deprived diet was seen in hematological parameters at the end of the study. Half of the continuously deprived group (4 of 8) met the criteria for iron-deficiency anemia [hematocrit < 30%; hemoglobin < 10 gm/dl; mean cell volume (MCV) < 70 fL; ferritin < 10 ng/mL] by the end of the experiment, but only 1 of 8 in the beef-supplement group and 0 of the 8 controls met these criteria. Thus, a detectable but mild deficiency was suggested in plasma trace element and hematological parameters.

Two behavioral tests that are also used to evaluate cognition in children (Overman 1990, Roberts and Sahakian 1993, Rutschman et al. 1986) were conducted four times weekly throughout the growth spurt and supplement period. The Continuous Performance Test assesses the vigilance aspect of attention; the Delayed Nonmatch to Sample Test assesses visual discrimination and short-term memory. For testing, a video screen displayed colored stimuli and monkeys responded by touching the screen. Selection of the correct stimulus
resulted in a reward, a small fruit-flavored sugar pellet. The monkeys were not food deprived for testing. In addition to assessing performance in terms of the percentage of correct responses, we also assessed the time taken to respond, i.e., the response latency. A third type of measure was termed participation and was measured as the number of sessions that the monkey started, or during which it completed at least 25% of the possible responses.

Figure 2 shows the influence of the deprived diet and the supplement on performance of these tasks. In the Continuous Performance test, colored squares flashed on the video screen, and the monkey had to touch the white square when it appeared (a correct "hit") and avoid touching the red and green squares (correct rejection). The percentage of correct "hits" was lower for the deprived group than controls (middle left panel). However, the percentage of correct rejections (bottom left panel) was higher, indicating that the monkeys were generally responding less to the stimuli. Diet did not influence performance on the Delayed Nonmatch to Sample Test (middle right panel). The data also indicated that the monkeys were less willing to participate in the behavioral testing (top panel) and were slower to respond to the stimuli (bottom right panel). The performance of the beef-supplement group tended to return toward that of the control group, with the exception of response latencies.

Thus, the deprived monkeys could be characterized as lethargic and apathetic. Further indication of this syndrome came from measures of spontaneous motor activity (data not shown) taken at intervals during the study (Golub et al. 1999). The continuously deprived group was generally less active after the onset of the experiment and significantly less active by the end of the study. The activity of the beef-supplement group stabilized after introduction of the supplement. There was considerable individual variability in these data, especially in controls, but the general pattern agrees with the performance measures from the behavioral tasks.

In this study, no effects on growth and only marginal effects on iron and zinc status were recorded along with a behavioral syndrome of lethargy and apathy. Interindividual variability was seen throughout the data base, so that many of the patterns of changes were not significant group differences.

Table 1 shows correlations between growth rate, activity level and hematocrit in zinc-iron-deprived adolescent monkeys. The positive correlations suggest that the monkeys whose behavior was most affected also had the lowest growth rates and hematocrit values. The finding that only behavioral vari-
End of experiment

Anemia (Bruner et al. 1996). Understanding of these relations have demonstrated effects on behavior in the absence of growth and hematological parameters were not, suggests that behavior was the most sensitive measure.

Lethargy and apathy seen in malnourished populations have often been considered an adaptation that allows nutrients to be used for vital life processes (Spurr 1990, Waterlow 1986). Interestingly, energy restriction (undernutrition) without changes in diet quality (malnutrition) has not been found to lead to reduced activity in nonhuman primate and rodent studies; in fact, increased activity levels are reported (Weed et al. 1997). Thus, micronutrients such as zinc and iron may be the determiners of the behavioral lethargy/apathy syndrome. During adolescence, reduced activity could help conserve these nutrients for continued growth before the onset of growth retardation, as has been previously suggested in preschoolers (Spurr 1990). Subsequently, stunting of growth could be considered an adaptation that allows continued supply of micronutrients needed for behavioral adaptation. Certainly under conditions of severe deprivation, both types of endpoints could be affected. But under mild or moderate deprivations, there may be a dissociation of effects on growth and behavior. In the case of iron deficiency, studies in girls have demonstrated effects on behavior in the absence of anemia (Bruner et al. 1996). Understanding of these relationships could contribute to identification and remediation of diet-induced growth and behavioral function deficits in malnourished teenagers.

### Literature Cited


### Table 1

**Correlations between growth, hematocrit and activity level in zinc-iron deprived female adolescent monkeys**

<table>
<thead>
<tr>
<th>Hematocrit vs. activity</th>
<th>Weight gain vs. activity</th>
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<tbody>
<tr>
<td>End of growth spurt1</td>
<td>$r = +0.51$, $P = 0.04$</td>
</tr>
<tr>
<td>End of experiment2</td>
<td>$r = +0.84$, $P = 0.005$</td>
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1 Includes 16 monkeys deprived of iron and zinc from the beginning of the experiment to the end of the growth spurt.

2 Includes 8 monkeys deprived of iron and zinc from the beginning to the end of the experiment.