

# Behavioral and hematologic consequences of marginal iron-zinc nutrition in adolescent monkeys and the effect of a powdered beef supplement<sup>1-3</sup>

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## ABSTRACT

**Background:** The adolescent growth spurt and menarche increase iron and zinc needs and could precipitate functional deficiencies if dietary sources are inadequate.

**Objective:** The effects of mild, combined zinc and iron deprivation during the growth spurt and the ability of meat as a common dietary source of zinc and iron to reverse these effects was studied.

**Design:** Pubertal female rhesus monkeys were fed control diets ( $n = 8$ ) or diets marginally deficient in zinc ( $2 \mu\text{g/g}$  diet;  $n = 8$ ) and iron ( $10 \mu\text{g/g}$  diet;  $n = 8$ ) for 3 mo. A powdered beef supplement ( $104 \mu\text{g Zn/g}$  and  $43 \mu\text{g Fe/g}$ ,  $11 \pm 2 \text{ g/d}$ ) was then fed daily to half of the deprived group for 3 additional months.

**Results:** Growth and hematology were not affected significantly by iron-zinc deprivation, but plasma zinc and iron were somewhat lower in the deprived group than in the control group after 3 mo. The deprived monkeys reduced their participation in behavioral testing, responded more slowly and less frequently to test stimuli, and were less active. The beef supplement increased participation in testing and stabilized activity levels, but response times remained depressed. Plasma ferritin was lower in the nonsupplemented deprived monkeys than in the controls by the end of the experiment. Four of 8 of the deprived monkeys had iron deficiency anemia compared with none of the controls and 1 of 8 who received the beef supplement.

**Conclusions:** Marginal zinc and iron deprivation in early adolescence can lead to behavioral and hematologic dysfunction in nonhuman primates and dietary beef supplements can prevent and reverse some of these effects. *Am J Clin Nutr* 1999;70:1059-68.

**KEY WORDS** Zinc deficiency, iron deficiency, diet, female rhesus monkeys, adolescence, puberty, cognition, attention, learning, motor activity, behavior, beef

## INTRODUCTION

Adolescence is a time of nutritional stress that could precipitate latent dietary inadequacies. Girls in early adolescence are at particular risk for iron and zinc deficiency. The need for both iron and zinc peaks during the adolescent growth spurt and iron requirements continue to be high in females because of the onset of menses. Surveys of adolescents in Shanghai indicated that the incidence of iron deficiency anemia was higher in girls (62%) than

in boys (47%) (1). Furthermore, a sex difference in iron stores appears in adolescence (2); a higher percentage of well-nourished Swedish girls (15%) than boys (5%) have low serum ferritin concentrations (3). The Swedish study also showed that girls had an iron intake that was 44% lower than that of boys, relative to the recommended dietary allowance (RDA). In the United States in 1987-1988, 83% of adolescent girls, compared with 32% of adolescent boys, consumed less than the RDA for iron and 81% of adolescent girls consumed less than the RDA for zinc and 59% consumed <77% of the RDA (4). Although inadequate zinc intake has been documented in adolescent girls, reliable indexes of zinc deficiency are not available and zinc deficiency has not been studied extensively in teenagers. Cherry et al (5) found that a zinc supplement improved some pregnancy outcome measures of pregnant teenagers, depending on their weight and parity.

It could be argued that indications of iron and zinc insufficiency are normal in adolescence if growth and general health are adequate; however, this perspective does not take functional competence into account (3). Both zinc and iron are known to be important in brain function and hematopoiesis. This study examined hematologic and behavioral measures in female adolescent monkeys deprived of both iron and zinc.

Nonhuman primates provide a valuable model for human adolescence because, as do girls, adolescent female rhesus monkeys show a growth spurt, followed by menarche, then a period of continuing growth and intense bone mineralization culminating in full sexual maturity, epiphyseal closure, and cessation of linear growth (6, 7). In girls, the time course extends from 11 to 16 y of age and in rhesus monkeys from 24 to 48 mo of age. The purpose of this study was to determine in adolescent female monkeys the effects of mild, combined zinc and iron deprivation during the growth spurt, and the ability of meat, a common dietary source of zinc and iron, to reverse these effects.

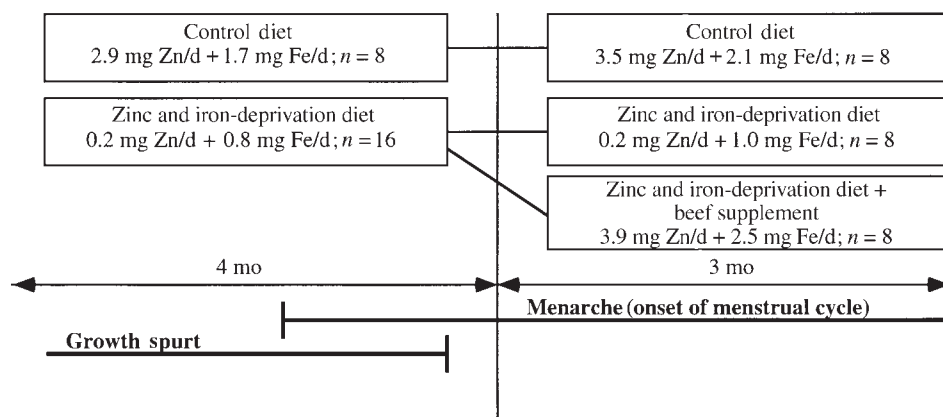
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**FIGURE 1.** Study design. Estimates of daily zinc and iron intakes were based on trace element analysis of the diet and beef supplement and on dietary records.

## METHODS

### Design

An overview of the study design is presented in **Figure 1**. The diet deficient in iron and zinc (deprivation diet) was initiated after the adolescent growth spurt of female rhesus monkeys began (8) and at about the time of the onset of menses. It was anticipated that zinc and iron deficiency would be greatest at this developmental period. The powdered beef supplement was given for 3 mo to allow repletion of zinc and iron stores.

### Subjects and evaluation schedule

Healthy, prepubertal female rhesus monkeys (*Macaca mulatta*) were obtained from the colony at the California Regional Primate Research Center (CRPRC) and selected for the study at 23 mo of age (based on a 30-d month). Because monkeys breed annually, a group of animals in the colony reaches this age each year in May and June. This allowed all animals to enter and complete the study as a single cohort. Because sexual maturation is synchronized with the annual breeding season, the experiment could be planned to coincide with the growth spurt and menarche. Before the study was initiated, monkeys were fed a commercial nonhuman primate diet (Ralston Purina, St Louis). The experiment began when the average age of the monkeys was 29.6 mo.

Monkeys were assigned to treatment groups to balance age and size (body weight) and cage of origin; the monkeys were born outside in 8 cages (2023 m<sup>2</sup>). Monkeys were housed in pairs in light- and temperature-regulated indoor colony rooms (48 animals/room). A double cage with a partition (120 × 65 × 79 cm) allowed separation of pairs for food intake determinations and behavioral testing while permitting socialization at other times. Over the 3 mo before the dietary interventions were initiated, monkeys were acclimated to caging and purified diets and received behavioral training. A daily health check was conducted throughout the study to identify menses and common health problems, which were recorded by using standardized codes.

All procedures used at the CRPRC conformed to the guidelines of the Animal Welfare Act and to the guidelines for the care and use of laboratory animals of the National Research Council. The CRPRC is accredited by the International Association for Accred-

itation of Laboratory Animal Care. The protocol for this experiment was approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis.

### Diet and diet administration

The protein source of the basal diet was sprayed egg white and the diet contained amounts of macro- and micronutrients that had been used previously in studies of adolescent monkeys (8–11). Vitamin C (500 mg/wk) was provided separately from the diet to minimize enhancement of iron absorption. Preweighed 75-g portions of the pelleted diet (Dyets, Inc, Bethlehem, PA) were provided twice daily. Deionized water was available ad libitum via an automatic watering system.

The control diet contained 30 μg Zn/g diet as zinc carbonate and 100 μg Fe/g diet as iron chloride. The zinc content of the zinc-iron deprivation diet (2 μg/g diet) was selected on the basis of the diet used in our previous studies in rhesus monkeys, whereas the iron content (10 μg/g diet) was selected on the basis of the diet used in a study of long-term dietary iron deficiency in developing rhesus monkeys (12). The diet was intended to produce marginal-to-moderate deficiencies only. The protocol included provisions for zinc or iron supplementation if overt clinical malnutrition occurred. However, this was not necessary.

### Dietary meat protein supplement

Lean ground beef (<2% fat) was baked, frozen at –20°C, and lyophilized with a high-capacity freeze dryer (Vertis Company, Gardener, NY). The freeze-dried meat was ground into powder and incorporated into a 4-g tablet commercially (PJ Noyes Co, Lancaster, NH). Each tablet contained 104 μg Zn/g and 43 μg Fe/g. The amount of supplement provided to the monkeys was based on their food intake during the presupplement period and monkeys received an average of 4.0 ± 0.5 tablets/d. Monkeys consumed an average of 80% of the supplement provided. The tablets were spread with commercial marshmallow creme or grape jelly to motivate consumption if necessary.

### Weights and food intake

Body weight was measured monthly to the nearest 0.01 kg. Food intake was recorded once per week (Wednesday) by count-

ing the number of pellets remaining at the end of the day. The percentage of partially eaten pellets remaining was estimated. Food intake was summarized monthly and expressed as g/d and as  $\text{g} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$  on the basis of body weight at the beginning of the month. Supplement intake was estimated by counting the number of discarded or partially eaten tablets 30 min after the tablets were fed.

### Growth and maturation

A physical exam was conducted 3 times after the monkeys were anesthetized with ketamine (10 mg/g): before dietary deprivation, before initiation of the supplement, and at the end of the experiment. Body weight, crown-rump length, arm and thigh circumferences, and anogenital distance were measured as described previously (13). Nipple length and width were also measured and used to compute a nipple volume measure (10, 14). Menses was checked for during the daily health check and was considered present if blood of perineal origin was evident.

### Hematology

Blood samples for hematologic determinations were obtained at the time the monkeys were selected for the study, before the dietary interventions were initiated, at the end of the growth spurt, and at monthly intervals during the supplement period. Complete blood counts were performed at the CRPRC clinical laboratory.

### Zinc and iron status

Blood samples for trace metal determinations were obtained at monthly intervals throughout the study. Plasma and red blood cell (RBC) zinc and iron concentrations were determined by inductively coupled plasma atomic emission spectroscopy after digestion with ultrapure nitric acid (1 mol/L). In addition, serum transferrin was measured by using human antibody, and plasma ferritin was measured with a commercial kit (Magic Fer; Chiron, Walpole, MA).

### Behavioral training

Monkeys were trained in their home cage to use a computer-controlled video touch screen (Carroll Touch, Round Rock, TX) to obtain a food reward (45-mg fruit-flavored sugar pellets; PJ Noyes Co). Monkeys were not food deprived for training, but the daily ration of food was withheld until after the completion of training. A series of successive approximations of the final task was used. At the end of the training, monkeys reliably touched squares of different colors in different screen locations to obtain a sugar pellet.

### The continuous performance test

We evaluated both participation in testing and level of performance. For the continuous performance test (CPT), colored rectangles ( $7.8 \times 10.5$  cm) were presented to the monkeys for 3 s at 2-s intervals on the touch screen. Three colors (white, red, and green) appeared in a semirandom sequence such that each color appeared 24 times in each 10-min period. Responses to the white target were correct and were rewarded with a sugar pellet, whereas responses to the green or red target were incorrect and resulted in a delay (5 s) before presentation of the next target. This task provides a measure of the vigilance aspect of attention by recording the number of "hits" (response to the white target: number of correct responses to the white target/number of presentations of the correct target). It also measures response inhibition by recording the number of correct rejections (the number of

failures to respond to incorrect stimuli, ie, red or green targets/number of incorrect stimuli). Because the task requires that the monkeys be attentive to the screen, it was preceded by a session-initiation requirement. The monkey had to press a white target that remained on the screen 5 times within 30 s. If the session was not initiated during the first 30-s period, a second 30-s period was initiated. Because the sessions had a fixed length, monkeys sometimes stopped responding before the end of the session. Thus, only sessions with  $\geq 25\%$  "hits" were included in the data analysis. After 2 incomplete session-initiation periods, testing was discontinued. Testing was conducted on 4 weekdays per week.

### The delayed nonmatch-to-sample test

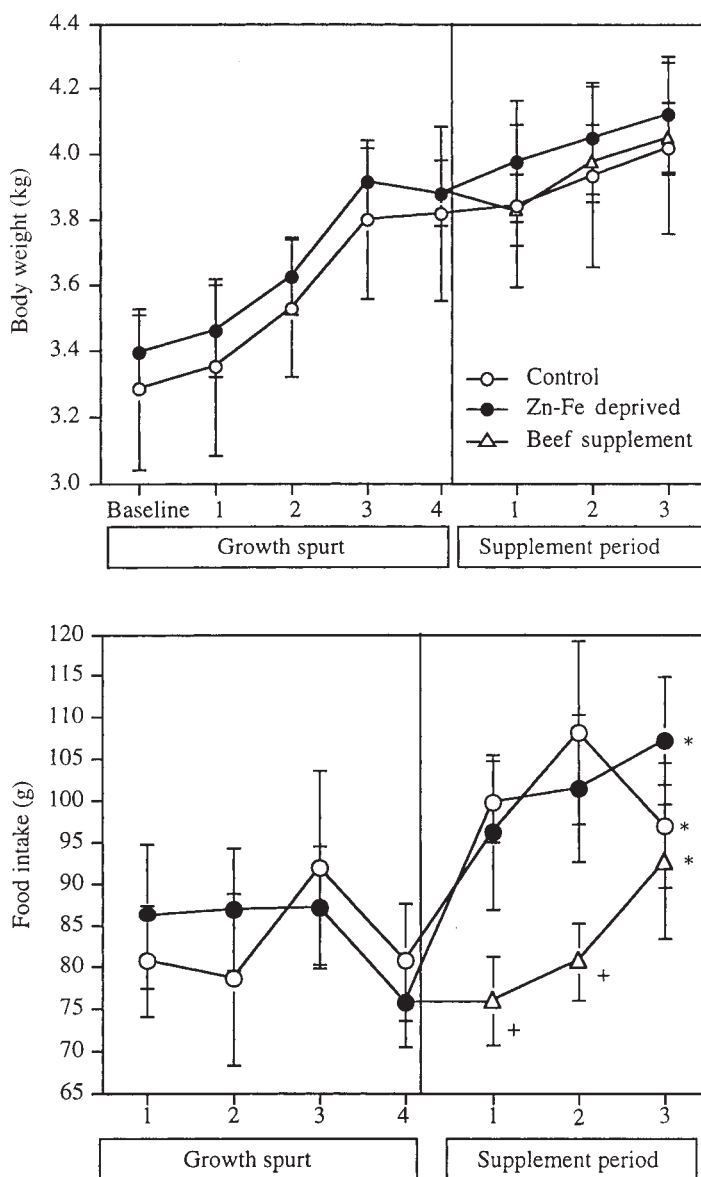
This test is part of the Cambridge Neuropsychological Test Automated Batteries (CANTAB) test program (CeNeS, Cambridge, United Kingdom). Participation in this task was measured as the number of sessions with  $\geq 25\%$  completed trials. A rectangle ( $2.5 \times 7$  cm) containing a complex colored pattern is displayed on the touch screen. The monkey touches this sample pattern to indicate readiness to perform the task. The sample disappears and is immediately replaced by 2 rectangles, one of which is identical to the sample. A correct response consists of choosing the stimulus that does not match the previously displayed sample and is rewarded with a sugar pellet. The incorrect response results in a 5-s delay before the next trial. The maximum duration of display for the sample and choices is 20 s, with a 2-s intertrial interval. Each session consisted of 120 trials, each with a trial-unique sample, or a maximum time of 15 min. In addition to recording the monkeys' choice, latency between display of the sample or the choices and the response was recorded. Sessions were conducted on 4 weekdays per week.

### Assessment of activity level

Activity was measured once monthly over a continuous 48-h period (weekend). A small (29 g) Personal Activity Monitor (Individual Monitoring Systems, Baltimore) designed for use in children was placed in a pouch attached to a harness worn by the monkey. The assembly resembles a small backpack. The monitor measures movement of the trunk in 2 directions by closures of a mercury switch, which are recorded by a microchip embedded in the monitor. When removed from the pouch and attached to a computer, the monitor provides a minute-by-minute record of the number of movements during the 48-h period in spreadsheet or graphic form as well as a daily average. This monitoring system was shown previously to record reduced activity levels in zinc-deprived rhesus monkeys (11).

### Statistical analysis

The statistical analysis emphasized within-animal comparisons at time points before and after the growth spurt, before and after the deprivation diets were initiated, before and after the beef supplement was initiated, and from the beginning to the end of the study. Heterogeneity of variance between different groups and at different ages reduced the value of testing overall between- and within-group effects in the same analysis (repeated-measure analysis of variance). The most common approach used was paired *t* testing. SAS software (version 6.0; SAS Institute Inc, Cary, NC) and STATVIEW (version 4.5; Abacus Concepts, Inc, Berkeley, CA) were used for the analyses.



**FIGURE 2.** Mean ( $\pm$ SEM) body weights and food intakes of the 3 diet groups ( $n = 8$ /group) throughout the study. At the end of the growth spurt, half of the deprived group began receiving a daily supplement of 4 g powdered beef. \*Significantly different from value for the same group at the beginning of the period,  $P < 0.05$  +Significantly different from the control group at the same time point,  $P < 0.05$ .

## RESULTS

### Food and supplement intakes

Monthly food intake did not increase during the growth-spurt period in the deprived group, but did increase significantly after the growth spurt in all 3 groups (control and beef-supplement groups,  $P < 0.01$ ; deprived group,  $P < 0.001$ ; **Figure 2**). After the beef supplement was initiated, food intake in the beef-supplement group was lower than that in the control and deprived groups, being significantly different from the control group at 1 ( $P = 0.02$ ) and 2 ( $P = 0.03$ ) mo but not at later time points when overall food intake was greater in all groups. The average intake of the beef supplement during the supplement period was

$11 \pm 2$  g/d, or about one-third of the total protein intake of the beef-supplement group.

### Growth and sexual maturation

Monthly body weights are charted in **Figure 2**. Group means and changes in morphometric indexes during the growth spurt and supplement periods are shown in **Table 1**. Weight increased at a rapid rate (6.7% of baseline body weight/mo) during the growth spurt in controls. Linear growth was also apparent, although the increase in crown-rump length was not significant in the control group. After the growth spurt, a small weight gain occurred in controls (1.8% body weight/mo). Arm and thigh circumferences did not change significantly during the growth

**TABLE 1**Measures of growth and sexual maturation in the control, zinc- and iron- (Zn-Fe) deprived, and beef-supplement groups<sup>f</sup>

|                                  | Growth spurt           |                            | Supplement period        |                           |                            |
|----------------------------------|------------------------|----------------------------|--------------------------|---------------------------|----------------------------|
|                                  | Control<br>(n = 8)     | Zn-Fe deprived<br>(n = 16) | Control<br>(n = 8)       | Zn-Fe deprived<br>(n = 8) | Beef supplement<br>(n = 8) |
| Body weight (kg)                 |                        |                            |                          |                           |                            |
| Beginning                        | 3.3 ± 0.3              | 3.5 ± 0.1                  | 3.8 ± 0.2                | 3.9 ± 0.2                 | 3.9 ± 0.2                  |
| End                              | 3.8 ± 0.2 <sup>2</sup> | 3.9 ± 0.1 <sup>2</sup>     | 3.9 ± 0.3 <sup>3,4</sup> | 4.0 ± 0.2 <sup>4</sup>    | 4.0 ± 0.1 <sup>4</sup>     |
| Crown-rump length (cm)           |                        |                            |                          |                           |                            |
| Beginning                        | 424 ± 7                | 425 ± 3                    | 432 ± 7                  | 436 ± 5                   | 427 ± 3                    |
| End                              | 432 ± 7                | 432 ± 3 <sup>2</sup>       | 440 ± 10 <sup>4</sup>    | 447 ± 4 <sup>3,4</sup>    | 437 ± 5 <sup>3,4</sup>     |
| Thigh circumference (cm)         |                        |                            |                          |                           |                            |
| Beginning                        | 17 ± 1                 | 18 ± 0.2                   | 18 ± 0.3                 | 18 ± 0.3                  | 17 ± 0.3                   |
| End                              | 18 ± 0.3               | 18 ± 0.2                   | 18 ± 0.3                 | 19 ± 0.4 <sup>3</sup>     | 19 ± 0.3 <sup>3,5</sup>    |
| Nipple volume (mm <sup>3</sup> ) |                        |                            |                          |                           |                            |
| Beginning                        | 46 ± 10                | 45 ± 3                     | 94 ± 26                  | 88 ± 9                    | 117 ± 10                   |
| End                              | 94 ± 26 <sup>3</sup>   | 103 ± 7 <sup>2</sup>       | 144 ± 19 <sup>4</sup>    | 128 ± 13 <sup>4</sup>     | 129 ± 18 <sup>4</sup>      |
| Anogenital distance (mm)         |                        |                            |                          |                           |                            |
| Beginning                        | 36 ± 2                 | 32 ± 1                     | 18 ± 2 <sup>3</sup>      | 20 ± 2                    | 17 ± 2                     |
| End                              | 18 ± 2 <sup>2</sup>    | 18 ± 1 <sup>2</sup>        | 15 ± 1 <sup>4</sup>      | 14 ± 1 <sup>3,4</sup>     | 14 ± 1 <sup>4</sup>        |

<sup>f</sup> $\bar{x} \pm \text{SEM}$ .<sup>2,3</sup>Significantly different from the beginning of the period (paired *t* test): <sup>2</sup>*P* < 0.01, <sup>3</sup>*P* < 0.05.<sup>4,5</sup>Significantly different from the beginning of the experiment (paired *t* test): <sup>4</sup>*P* < 0.01, <sup>5</sup>*P* < 0.05.

spurt, but thigh circumference increased during the period after the growth spurt, with values being significantly different between the deprived and supplement groups. The deprivation diet did not result in significant changes in body weight or weight gain during the growth spurt. After the growth spurt, during the supplement period, crown-rump length and thigh circumference increased significantly in the deprived and beef-supplement groups, whereas body weight increased significantly in the controls. These results might suggest that different growth patterns related to different diet compositions during the supplement period.

Nipple volume increased and anogenital distance decreased during the growth spurt, reflecting sexual maturation around the time of menarche. This rate of change did not persist after the growth spurt. The deprivation diet and the beef supplement did not influence these indexes or the pattern of changes. There was no significant difference between groups in the age at menarche: controls, 31.9 ± 0.8 mo; deprived group, 30.6 ± 0.5 mo; beef-supplement group, 31.1 ± 0.3 mo. No menses was recorded for one control monkey during the study.

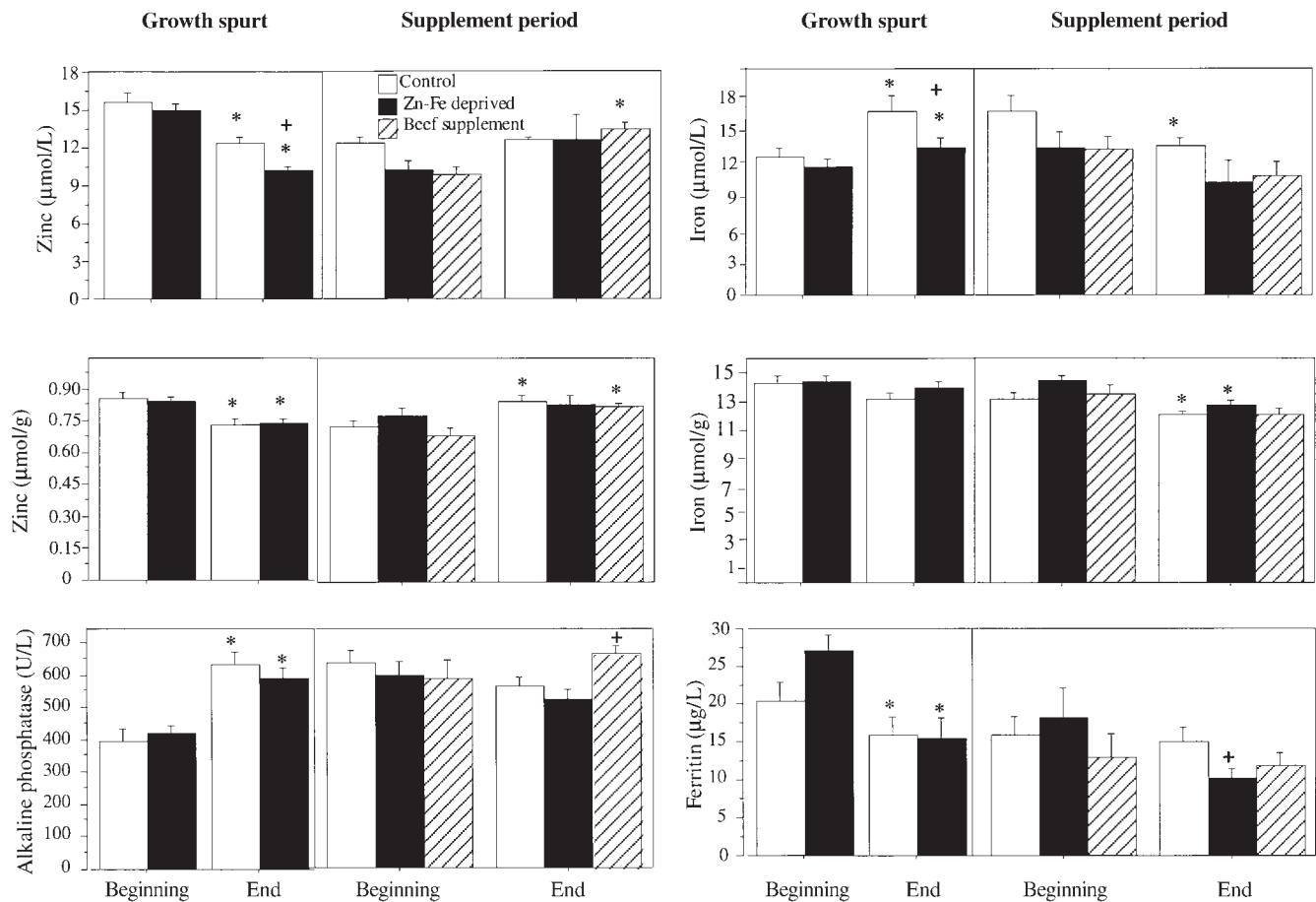
### Zinc and iron status

Plasma and RBC zinc and iron indexes are plotted in **Figure 3**. Plasma zinc declined during the growth spurt in both the control and deprived groups. The decline was greater in the deprived group, with values being significantly lower than those of the controls at the last time point. From the beginning to the end of the supplement period, plasma zinc rose significantly only in the beef-supplement group; there was no significant change in the control and deprived groups. In contrast with zinc, plasma iron increased, rather than decreased, during the growth spurt in the control group and also showed a marginally significant increase in the deprived group. The increase was smaller in the deprived group, with values being significantly lower than those of controls at the last time point. During the supplement period, plasma iron declined significantly in the

control group only. Plasma iron concentrations in the deprived group, already low, did not decline further and were not significantly different from those of the controls at the end of the supplement period. Plasma copper was also measured (data not shown) and it declined significantly during the growth spurt in both the control and deprived groups, but did not change significantly from the beginning to the end of the supplement period. There were no significant effects of the dietary interventions on plasma copper.

Zinc and iron concentrations were also measured in RBCs (**Figure 3**). RBC zinc declined significantly in the control and deprived groups during the growth spurt, but increased during the supplement period, with concentrations in the control and beef-supplement groups being significantly higher at the end of the supplement period than concentrations at the beginning of the supplement period. In contrast, RBC iron did not change significantly during the growth spurt, but decreased significantly in the control and deprived groups during the supplement period. RBC iron decreased in the beef-supplement group during the supplement period, but not significantly so.

Alkaline phosphatase was measured as an index of zinc status, and ferritin and transferrin (total-iron-binding capacity; TIBC) were measured as indexes of iron status. Alkaline phosphatase increased substantially (~50%) during the growth spurt in both the control and deprived groups (**Figure 3**). This increase, in light of declining plasma zinc concentrations, suggests possible enhanced bone metabolism rather than a change in zinc status. A smaller, nonsignificant decline in alkaline phosphatase was seen in the deprived and control groups after the growth spurt, whereas a nonsignificant increase in alkaline phosphatase was seen in the beef-supplement group during the supplement period. Plasma ferritin decreased during the growth spurt in both the control and deprived groups; no significant changes were identified in any group during the supplement period. However, at the end of the experiment, ferritin was significantly lower in the deprived group than in the control



**FIGURE 3.** Mean ( $\pm$ SEM) plasma concentrations of zinc- and iron-status indexes for the 3 diet groups ( $n = 8$ /group) at the beginning and end of the growth-spurt period and supplement periods. \*Significantly different from the value for the same group at the beginning of the period,  $P < 0.05$ . +Significantly different from the control group at the same time point,  $P < 0.05$ .

group ( $P = 0.048$ ). TIBC (data not shown) also declined during the growth spurt, although the decrease in the deprived group was not significant ( $P = 0.06$ ). During the supplement period, TIBC increased significantly in the beef-supplement group only, reaching values seen before the growth spurt.

### Hematologic status

Three hematologic measures—RBCs, hematocrit, and hemoglobin—showed a similar pattern of effects. These indexes decreased substantially during the growth spurt, with no apparent recovery during the supplement period and no apparent changes as a result of the deprived diet or the beef supplement (Table 2). Plasma protein concentrations suggested that the pattern of change was not secondary to expanded plasma volume; plasma protein was  $68.8 \pm 1.2$ ,  $68.8 \pm 1.4$ , and  $68.2 \pm 0.7$  kg/L in controls at the beginning of the growth spurt, the end of the growth spurt, and the end of the supplement period, respectively.

The mean cell hemoglobin content (MCH; hemoglobin/RBC) was the only hematologic index influenced by dietary deficiency during the growth spurt, decreasing in the deprived group. Thus, it was not possible to conclude that the deprived group was clinically iron deficient during the growth spurt.

Mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC; MCH/MCV) were sensitive to the dietary interventions during the supplement period. MCHC, like hemoglobin, declined markedly during the growth spurt, but, unlike hemoglobin, rose again after the growth spurt. This rise was seen in controls but not in the deprived or beef-supplement groups. In contrast with other hematologic indexes, MCV did not decline significantly during the growth spurt or supplement period in any of the individual groups.

In addition to evaluating group differences in individual hematologic indexes, we identified whether iron deficiency anemia was present in individual animals at the end of the study on the basis of the following criteria: hematocrit  $< 0.30$ , hemoglobin  $< 100$  g/L, MCV  $< 70$  fL, and ferritin  $< 10$  µg/L. These criteria were met by 4 of the 8 continuously deprived monkeys, 1 of the 8 monkeys in the beef-supplement group, and none of the 8 monkeys in the control group. The difference between the deprived and control groups was significant ( $P = 0.04$ , Fisher's exact test).

### Activity

Spontaneous motor activity, measured as the average hourly number of switch closures in the activity monitor over 48 h, was generally lower in the deprived group than in the control group

**TABLE 2**  
Hematological measures in the control, zinc- and iron- (Zn-Fe) deprived, and beef-supplement groups<sup>1</sup>

|  | Growth spurt                   |                                | Supplement period              |                              |                                |
|--|--------------------------------|--------------------------------|--------------------------------|------------------------------|--------------------------------|
|  | Control<br>(n = 8)             | Zn-Fe deprived<br>(n = 16)     | Control<br>(n = 8)             | Zn-Fe deprived<br>(n = 8)    | Beef supplement<br>(n = 8)     |
| Red blood cells ( $\times 10^{12}/L$ ) |                                |                                |                                |                              |                                |
| Beginning                              | 4.99 $\pm$ 0.13                | 5.17 $\pm$ 0.08                | 4.47 $\pm$ 0.15                | 4.77 $\pm$ 0.10              | 4.52 $\pm$ 0.13                |
| End                                    | 4.47 $\pm$ 0.15 <sup>2</sup>   | 4.65 $\pm$ 0.08 <sup>2</sup>   | 4.37 $\pm$ 0.14 <sup>3</sup>   | 4.62 $\pm$ 0.08 <sup>3</sup> | 4.60 $\pm$ 0.16 <sup>3</sup>   |
| Hemoglobin (g/L)                       |                                |                                |                                |                              |                                |
| Beginning                              | 118 $\pm$ 1                    | 121 $\pm$ 1                    | 103 $\pm$ 2                    | 107 $\pm$ 2                  | 104 $\pm$ 3                    |
| End                                    | 103 $\pm$ 2 <sup>2</sup>       | 106 $\pm$ 2 <sup>2</sup>       | 104 $\pm$ 3 <sup>3</sup>       | 101 $\pm$ 4 <sup>3</sup>     | 101 $\pm$ 3 <sup>3</sup>       |
| Hematocrit                             |                                |                                |                                |                              |                                |
| Beginning                              | 0.345 $\pm$ 0.007              | 0.354 $\pm$ 0.004              | 0.315 $\pm$ 0.009              | 0.327 $\pm$ 0.007            | 0.317 $\pm$ 0.009              |
| End                                    | 0.315 $\pm$ 0.009 <sup>2</sup> | 0.322 $\pm$ 0.005 <sup>2</sup> | 0.308 $\pm$ 0.009 <sup>3</sup> | 0.317 $\pm$ 1.0 <sup>3</sup> | 0.307 $\pm$ 0.008 <sup>3</sup> |
| Mean cell volume (fL)                  |                                |                                |                                |                              |                                |
| Beginning                              | 69 $\pm$ 1                     | 69 $\pm$ 1                     | 71 $\pm$ 1                     | 69 $\pm$ 1                   | 70 $\pm$ 1                     |
| End                                    | 71 $\pm$ 1 <sup>2</sup>        | 70 $\pm$ 1 <sup>2</sup>        | 70 $\pm$ 1 <sup>3</sup>        | 66 $\pm$ 2                   | 67 $\pm$ 2                     |
| Mean cell hemoglobin (g/L)             |                                |                                |                                |                              |                                |
| Beginning                              | 23.6 $\pm$ 0.3                 | 23.5 $\pm$ 0.3                 | 23.2 $\pm$ 0.4                 | 21.8 $\pm$ 0.9               | 23.1 $\pm$ 0.4                 |
| End                                    | 23.2 $\pm$ 0.4                 | 22.8 $\pm$ 0.3 <sup>2</sup>    | 23.8 $\pm$ 0.4 <sup>4</sup>    | 22.9 $\pm$ 0.9               | 22.2 $\pm$ 0.8                 |

<sup>1</sup> $\bar{x} \pm$  SEM.

<sup>2,4</sup>Significantly different from the beginning of the period (paired *t* test): <sup>2</sup> $P < 0.01$ , <sup>4</sup> $P < 0.05$ .

<sup>3</sup>Significantly different from the beginning of the experiment,  $P < 0.01$ .

during the growth spurt, but differences were not significant ( $P = 0.06$ ; **Figure 4**). During the supplement period, the control and beef-supplement groups maintained their activity levels while the activity of the continuously zinc and iron deprived group declined ( $P = 0.041$ ). The average activity of the continuously deprived group was lower than that of the control group during the supplement period ( $P = 0.021$ ). Another measure, the maximum length of inactivity on each day, did not change significantly during the experiment and there were no effects of the dietary interventions.

### Continuous performance test

For participation in testing, in the deprived group but not in the control group, the number of days when they did not initiate CPT sessions during the growth spurt increased, so that by the end of the growth spurt their failure to initiate sessions each month was greater than that of controls (**Figure 5**). There were no significant differences between groups during the supplement period. The data on the number of sessions that were  $\geq 25\%$  complete suggested that zinc and iron deprivation also adversely affected participation in testing beginning in the growth-spurt period, but there were no significant differences between groups.

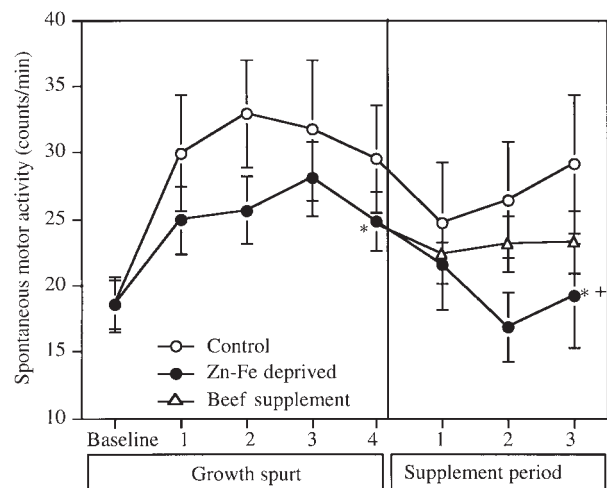
For the CPT sessions that were  $\geq 25\%$  complete (**Figure 5**, left, middle panel), the control group significantly improved its attention performance in terms of the percentage of hits from the beginning to the end of the growth spurt. Although the percentage of hits for the deprived group tended to be greater at the end than at the beginning of the growth spurt, the difference was not significant. None of the groups showed a significant improvement from the beginning to the end of the supplement period; however, the beef-supplement group continued to improve their score so that by the end of the experiment, they were performing significantly better than they had at the beginning of the experiment, as was the control group.

In contrast with the results for the number of hits, the number of correct rejections improved significantly in the deprived group during the growth spurt and also improved in the control

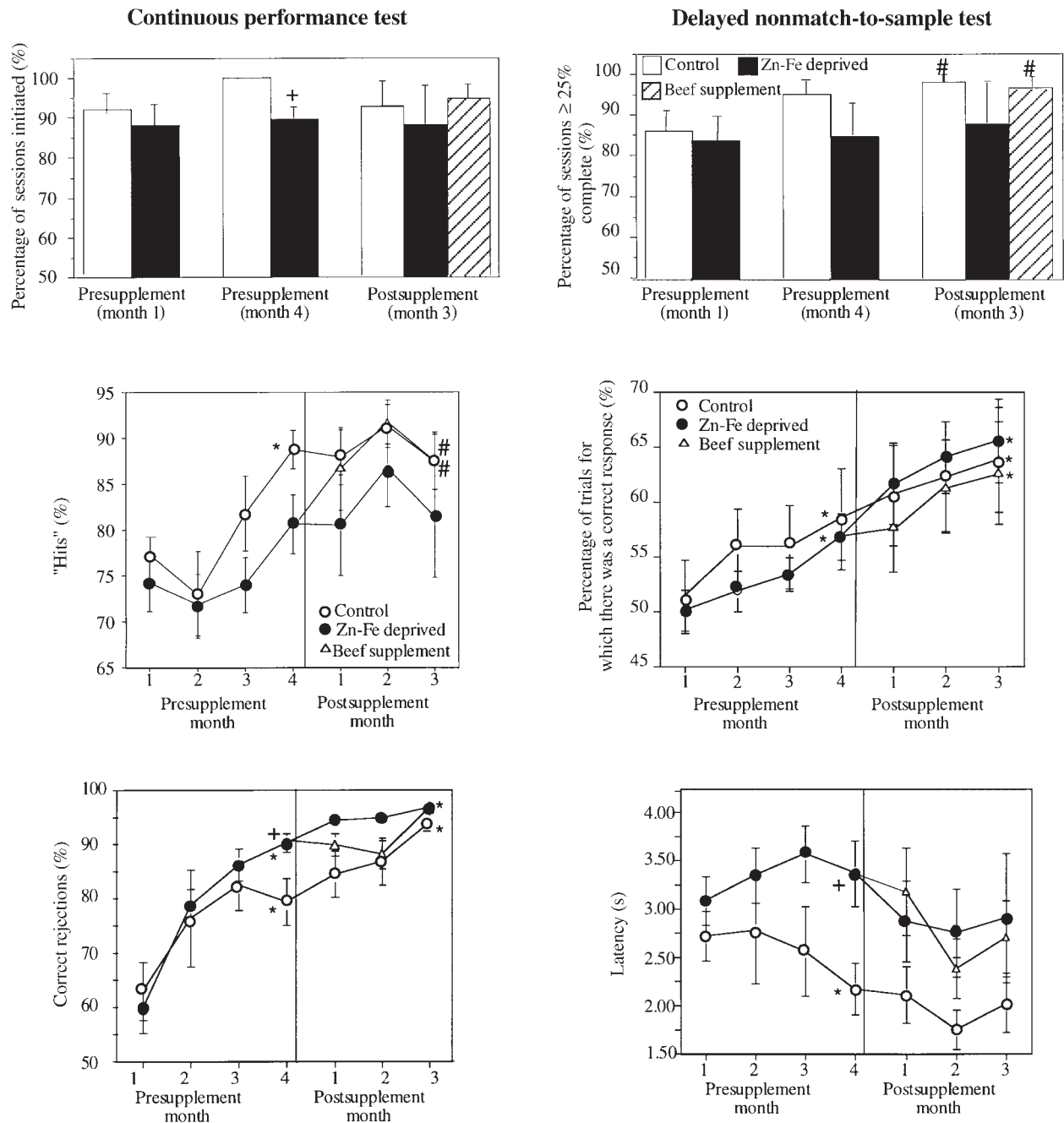
group, but not significantly so. During the supplement period, this measure improved significantly in both the control and the beef-supplement groups so that their performance was no longer significantly different from that of the deprived group. A high percentage of correct rejections and a low percentage of hits indicated a lack of response to the targets by the monkeys and not specific deficits in attention or response inhibition.

### The delayed nonmatch-to-sample test

Participation did not change significantly in either the control or deprived group during the growth spurt, although the number of average sessions initiated declined in the deprived group. During the supplement period, the number of sessions during which



**FIGURE 4.** Mean ( $\pm$ SEM) spontaneous motor activity of the 3 diet groups ( $n = 8$ /group) as determined by actimeter readings over 48 h. \*Significantly different from the value of the same group at the beginning of the period,  $P < 0.05$ . †Significantly different from the control group at the same time point,  $P < 0.05$ .



**FIGURE 5.** Mean ( $\pm$ SEM) behavioral performance measures of the 3 diet groups ( $n = 8/\text{group}$ ) during the growth spurt and supplement period. \*Significantly different from the value for the same group at the beginning of the period,  $P < 0.05$ . #Significantly different from the value for the same group at the beginning of the study,  $P < 0.05$ . +Significantly different from the control group at the same time point,  $P < 0.05$ .

the monkeys completed  $\geq 25\%$  of the available trials increased in both the control and beef-supplement groups. By the end of the experiment, participation had increased significantly from the beginning of the experiment in both the control and beef-supplement groups. The actual number of trials completed increased significantly in the control group (data not shown;  $P = 0.005$ ), but not in the deprived group during the growth spurt. At the end of

the growth spurt, the deprived group completed significantly fewer trials than the control group ( $P = 0.03$ ). There was no significant effect of the beef supplement during the supplement period. The percentage of correct responses increased in all groups during the growth spurt and the supplement period (Figure 5, right, middle panel). There was no significant effect of the dietary interventions on the number of correct responses. Posi-

tion perseveration (choosing the same side repeatedly) declined during the course of the study, but there were no significant group differences (data not shown).

Sample and choice latencies (the latency between the appearance of the display and trial initiation or choice) were sensitive to the deprivation diet, but not to the beef supplement. During the growth spurt, latencies decreased in the control group, as anticipated, but increased (NS) in the deprived group. At the end of the growth spurt, latencies were longer in the deprived group than in the control group. There were no further changes in latency in any group during the supplement period.

## DISCUSSION

The data indicate that the deprived diet produced hypozincemia during the growth spurt that was reversed by consumption of the powdered beef supplement. This finding was evidenced by lower plasma zinc concentrations in the deprived group than in the control group at the end of the growth spurt and by an increase in both plasma and RBC zinc concentrations as well as in alkaline phosphatase activity in the beef-supplement group during the supplement period but not in the deprived group. Iron deficiency was not clearly shown in the deprived group during the growth spurt. However, plasma iron was lower in the deprived group than in control group at the end of the growth-spurt period and plasma ferritin was marginally lower in the continuously deprived group than in the control group at the end of the study.

In addition to diet-induced changes in iron and zinc status, changes associated with the adolescent growth spurt also occurred, as seen in the controls. Zinc status was negatively affected during the growth spurt and indexes of iron status declined after the growth spurt. The growth spurt led to a decrease in plasma and RBC zinc concentrations in the control group. RBC zinc concentrations recovered to former values after the growth spurt in controls, whereas plasma zinc concentrations did not recover. The immediate effect of the growth spurt on iron status was less striking; plasma iron increased somewhat in controls during the growth spurt, but RBC iron did not change significantly.

There were signs of the development of iron deficiency anemia in response to the deprived diet. By the end of the growth spurt, the amount of hemoglobin per RBC (MCH) had decreased in the deprived group, whereas it remained stable in controls. After the growth spurt, the MCV and MCHC of the previously deprived monkeys (continuously deprived and beef-supplement group) fell below those of the controls. Microcytic, hypochromic anemia with low ferritin appeared in 4 of the 8 monkeys of the continuously deprived group at the end of the study. The beef supplement may have counteracted this hematologic effect of zinc-iron deprivation because only 1 of the 8 monkeys receiving the powdered beef supplement had this syndrome. Also, the average ferritin concentration was marginally lower in the continuously deprived group, but not in the beef-supplement group, than in controls at the end of the study.

There were also changes in behavior in response to zinc-iron deprivation, characterized by lower activity levels, slower response times, and less engagement in behavioral tasks. Specifically, the deprived group exhibited less spontaneous activity, responded more slowly during the delayed nonmatch-to-sample test (response latency was not measured in the continuous performance task), and completed fewer trials per session, com-

pleted fewer sessions that were  $\geq 25\%$  complete, and had fewer responses to both correct and incorrect targets in the continuous performance task. This syndrome of slowness, apathy, and reduced activity developed during the first few months of the experiment. The beef supplement appeared to improve participation in testing and to prevent a further decline in spontaneous motor activity. However, response latencies remained depressed throughout the supplement period.

Lack of recovery of response latencies may have been due to an effect of the deprived diet on the maturational decline in processing time in the controls. Several experiments in children have shown a decline in response latency as well as in the latency of event-related potentials during late childhood and adolescence, presumably because of faster central processing times resulting from completion of central nervous system myelination (15, 16). We found that the maturational time course of response latency in monkeys resembles that reported in teenagers (MS Golub, unpublished observations, 1995). It is possible that the deprivation diet interfered with this maturational process and led to a long-term or permanent slowing of central processing time. Another explanation might be that plasma iron concentrations did not recover in response to the supplement, and complete recovery of iron status after a longer period of supplementation would eventually result in lower response latencies.


We previously described behavioral effects of dietary zinc deficiency in adolescent rhesus monkeys. We are aware of no previous studies of iron deficiency or of combined iron-zinc deficiency in adolescent rhesus monkeys. Our previous studies showed that a moderately zinc-deficient diet, when fed for 15 wk during the adolescent growth spurt, could produce changes in performance of attention and memory tasks and in the amount of time spent active, and that these changes could be reversed by adding zinc carbonate to the diet (9). In human adolescents, there have been studies of iron deficiency and cognitive function. An abstract published in 1995 reported slower response times and a failure to decrease response times during a tachistoscopic task by iron-deficient 14–19-y-old mothers tested within 6 wk of delivery (17). Slower response times were also found in the present experiment. Ballin et al (18) found that an iron supplement improved lassitude, concentration in school, and mood in 16–17-y-old iron-deficient girls, but did not improve measures of physical performance. An early study found lower scores on a standardized achievement test in 11–14-y-old boys and girls with iron deficiency anemia than in a control group (19). More recently, Bruner et al (20) showed that iron supplementation improved verbal learning performance of nonanemic, iron-deficient 13–18-y-old girls, although several tests of attention were not affected. Additionally, studies of iron deficiency and brain chemistry in rats indicated that dopamine reuptake is impaired by iron deficiency (21). The findings of these rat studies were considered relevant to human adolescents because they were done at the pubertal stage of maturation.

A possible hypothesis concerning the basis of the behavioral findings is that regulatory centers in the brain respond to lack of zinc and iron by initiating an altered level of behavioral arousal that conserves nutrients for basic life processes. It is also possible that there are more specific effects on learning, memory, attention, sensory processing time, and other discrete cognitive functions. No indication of these specific effects were found in the present study; however, we did not test many individual cognitive functions. Brain mechanisms that might detect and respond to reduced nutrient availability and the neural circuits that might coordinate

and execute the change in arousal level are not known. Progress in understanding such a mechanism could provide an integrating framework for interpreting studies of malnutrition on behavior.

How relevant are the results of this study to teenage girls? Rhesus monkeys are a good model for adolescent girls because the pubertal growth spurt and onset of menses occur in conjunction with pubertal maturation. There are also many differences between girls and female rhesus monkeys that are worth noting. Sex-related differences in body composition associated with sexual maturation are much less marked in monkeys than in girls. Also, sexual maturation is a seasonal event, coordinated with the annual breeding cycle, in monkeys but not in girls. Thus, the growth spurt was confounded by the time of year in monkeys. Finally, not all maturational events are synchronized in the same way with puberty; for example, adrenarche (production of steroid hormones by the adrenal) occurs well after menarche in monkeys (22), whereas it occurs before menarche in girls.

Adolescent humans and monkeys both show dramatic changes in iron stores (ferritin) around the time of puberty. In the present study, we found a 37% decrease in plasma ferritin during the growth spurt in female monkeys. In a longitudinal study of 12–13-y-old boys, serum ferritin also decreased by 37% over 18 mo (23); girls were not studied. We also found a smaller (11–12%) decrease in RBC count and hematocrit during the growth spurt. No parallel reports were found for human teenagers; however, these changes were small and may not be detectable in a less controlled setting. Changes during the growth spurt in hematologic measures are accompanied by parallel changes in zinc and iron status. Better understanding of zinc and iron nutrition during adolescence may clarify the origin of hematologic changes and their health-related significance. Although changes during the growth spurt in controls indicated effects of the growth spurt in our experiment, note that the timing of the growth spurt was confounded by the time of year and also with time from initiation of the study.

The levels of zinc and iron deprivation and beef consumption used in the present experiment were within the range experienced by teenagers. Although the deprivation diet had a statistically significant influence on some zinc- and iron-status indexes, values did not fall into a clinically deficient range. The beef supplement was equivalent in terms of grams protein to 51% of the protein obtained by the monkeys from the zinc-iron deprivation diet during the supplement period, or  $\approx$ 33% of total protein consumed during that period. One standard (100 g) serving of lean ground beef provides the equivalent of 61% of the RDA for protein for 11–14-y-old girls. Both rhesus monkeys and humans are omnivores and the natural diet for either species is not well defined. Rhesus monkeys have been observed to consume only small amounts of animal products in the wild (24). Aiello and Wheeler (25) hypothesized recently that inclusion of animal products in the diet played a role in the brain evolution of primate species. 

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