Regulation of Metabolic Rate and Substrate Utilization by Zinc Deficiency

Stephanie A. Evans, J. Michael Overton, Almamoun Alshingiti, and Cathy W. Levenson

The trace metal zinc (Zn) is essential for the catalytic activity of many enzymes involved in energy nutrient metabolism and appears to regulate hormones, such as insulin, leptin, and thyroid hormone that play key roles in metabolism. Thus, this study used the continuous monitoring of oxygen consumption, carbon dioxide production, locomotion, and food intake to determine the effect of dietary Zn restriction on metabolic rate (MR), basal metabolic rate (BMR), and respiratory quotient (RQ). Rats were fed a Zn-adequate (ZA, 28 ppm) or Zn-deficient (ZD, <1 ppm) diet for 8 days, followed by a 4-day refeeding period. To control for reductions in food intake that characteristically occur in ZD rats, an additional group was pair-fed (PF) the same amount ZA food eaten by ZD rats. The mean caloric intake of ZD rats was significantly lower than ZA rats by day 3. By day 8, ZD and PF rats weighed 64% and 67% of ZA rats, respectively, ($P < .01$). Pair feeding resulted in increased locomotor activity, such that the distance traveled for PF rats (316 ± 43 m) was 6 times that of ZA (53 ± 6 m). Despite the fact that PF and ZD rats had the same food intake, there was no increase in locomotor activity in ZD rats suggesting that the mechanisms responsible for increased physical activity in food restricted animals may be Zn dependent. Furthermore, differences in activity between PF and ZD animals were not reflected in differences in MR. Both ZD and PF significantly reduced MR compared with ZA rats beginning on day 4. There was a significant relationship between RQ and caloric intake ($r = 0.708$, $P < .01$), but no specific effect of Zn status. Thus, while there may be an effect of Zn on locomotion and the energetic cost of activity, it appears that the most profound effect of Zn status on MR and substrate utilization is the result of Zn deficiency–induced anorexia.

DIETARY ZINC (Zn) deficiency has been closely associated with a reduction in food intake in both humans and laboratory rats. Several studies have examined the dietary intake patterns of patients suffering from anorexia and have shown that many anorexics do not meet the current recommendations for Zn intake.[1,2] It has also been shown that anorexics have significantly depressed urinary Zn levels compared with healthy controls. Zn supplementation not only corrected the urinary Zn excretion levels,[1] but also significantly enhanced weight gain compared with placebo controls.[3,4] Thus, Zn supplementation has been recommended as part of the treatment for anorexia nervosa.[4]

In laboratory rats, food intake is significantly reduced 3 to 5 days after the initiation of a Zn-restricted diet. The reduction in food intake is also characterized by a feeding cycle that results in a measurable increase in food intake approximately every 4 days.[5-9] In most studies, Zn deficiency results in an overall 40% to 50% decrease in food intake.[5,6] However, in severe Zn deficiency, food intake can be reduced by as much as 70%.[7,8] Zn deficiency–induced anorexia appears to be the result of a loss of appetite for carbohydrate. When given a choice between carbohydrate, fat, and protein, Zn-deficient (ZD) rats maintained fat and protein intake while essentially eliminating carbohydrate consumption.[6]

While the effect of Zn on food intake has been well characterized, the effect of Zn deficiency on macronutrient metabolism and metabolic rate (MR) is not known. An early study used a pair-feeding regimen to show that ZD rats weighed less than rats that were consuming the same amount of Zn-adequate (ZA) food.[8] This observation was recently confirmed,[5] suggesting that Zn deficiency has an impact on MR that is independent of food intake.

The hypothesis that Zn status regulates MR is supported by the fact that Zn is essential for the catalytic activity of a variety of enzymes needed for macronutrient metabolism, including many dehydrogenases in the glycolytic and tricarboxylic acid pathways.[10] Zn deficiency downregulates the synthesis of pyruvate kinase, a prominent regulator of hepatic glycolysis.[11] Zn status also appears to regulate several key hormonal regulators of metabolism. For example, Zn deficiency reduces growth hormone-releasing factor that is responsible for the release of growth hormone from the pituitary.[12] Furthermore, Zn is a normal constituent of insulin. Thus, Zn deficiency results in impaired cellular glucose uptake.[13] There is also evidence that compared with pair-fed (PF) rats, ZD rats have lower circulating levels of triiodothyronine (T_3)[14] and have an impaired ability to synthesize and secrete leptin,[15] a hormone that among other functions has been shown to regulate substrate utilization and MR.[16]

Given the roles of Zn in macronutrient metabolism, this study was designed to fully characterize the effect of Zn deficiency on MR and substrate utilization by the continuous (23 hours) monitoring of MR, feeding behavior, and water intake in ZD, ZA, and PF rats. Because any measurement of MR must also take into account physical activity, locomotion was measured continuously in every rat. Furthermore, the use of continuous indirect calorimetry allowed us to calculate the respiratory quotient (RQ) and track substrate utilization during the development and maintenance of Zn deficiency.

MATERIALS AND METHODS

Animals and Diets

Weanling male Sprague-Dawley rats were individually housed in shoebox cages (18 × 9.5 × 5 inches, 13 L vol) in custom-constructed environmental chambers that provided computer control of temperature

From the Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL.

Submitted July 25, 2003; accepted January 15, 2004.

Address reprint requests to Cathy W. Levenson, PhD, Florida State University, 237 Biomedical Research Facility, Tallahassee, FL 32306-4340.

0026-0495/04/5306-0022$30.00/0
doi:10.1016/j.metabol.2004.01.009

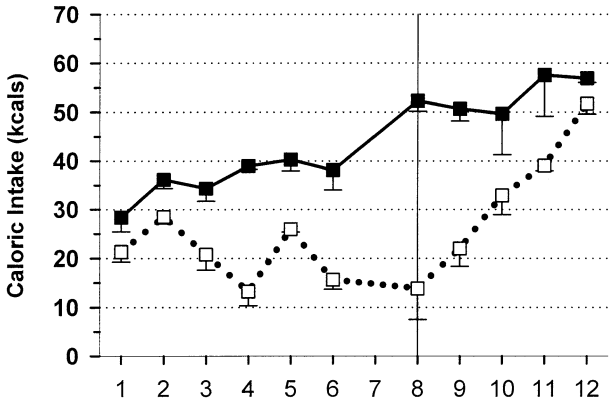

Fig 1. Effect of Zn deficiency on caloric intake. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet for 8 days. Deficient rats were then fed the ZA diet for an additional 4 days. Symbols represent mean ± SEM.

(T_a = 23 °C) and a 12-hour light-dark schedule (lights on at 7 AM).[17] Rats were provided ad libitum access to deionized water and commercially prepared, powdered, egg white-based ZA (28 ppm; n = 6) or ZD (<1 ppm; n = 9) diets. Because Zn deficiency causes anorexia, the reduction in food intake was controlled for by the inclusion of PF rats (n = 9). PF rats were provided the weighed amount of ZA food eaten by the ZD rats on the previous day. After 9 days, ZD animals were refed the ZA diet. Body weights and food and water consumption were measured daily during the 12th hour of the light phase. The Animal Care and Use Committee (ACUC) at Florida State University approved all animal protocols.

Ingestive Behavior

Feeding behavior was monitored by a photo beam sensor across the entrance to the feeder. The duration of photo beam breakage (50 msec resolution) was monitored by computer and accumulated in 30- second bins. The water bottle was positioned in a lick block modified to measure contact at the bottle's spout. Drinking behavior was computer monitored by the number of licks, which was accumulated every 30 seconds.[17] Averages were calculated for data during 12 hours of dark-phase and 11 hours of light-phase ingestive behaviors.

Substrate Utilization and MR

The shoebox cages were fitted with a custom-made polycarbonate lid providing a near air-tight seal for continuous determination of oxygen consumption (V_{O_2};mL/min) and carbon dioxide production (V_{CO_2}; mL/min) using indirect calorimetry. A constant flow rate of fresh air into the chamber was set at 0.85 L/min. Mixed cage air was sampled for 30 seconds every 4 minutes and dried and compressed prior to reaching gas analyzers.[24] V_{O_2} and V_{CO_2} were determined by open circuit respirometry to isolate successive samples.[24] MR is described as V_{O_2} normalized to body weight (mL/min/kg$^{0.75}$). RQ was determined by dividing V_{CO_2} by V_{O_2}. Basal MR (BMR) was calculated by averaging the lowest 15 four-minute V_{O_2} bins. Averages were calculated for data during 12 hours of dark-phase and 11 hours of light-phase activity.

Locomotor Activity

Locomotor activity was measured using a custom-designed force platform with a pivot under its center. The shoebox cage was positioned on this platform to obtain quantification of locomotor activity. Stiff strain-gauge load-beam transducers attached under 2 adjacent corners of the platform prevented the platform from swaying on the pivot. The transducers measured changes in the chamber's center of gravity, allowing localization of the animal's position in 2 dimensions. The cumulated distance of locomotor activity in meters was saved every 30 seconds.[17] Averages were calculated for data during 12 hours of dark-phase and 11 hours of light-phase activity.

The effect of Zn deficiency on voluntary wheel running was also examined. Male weanling rats fed the previously described ZA, ZD, and PF diets were individually housed in stainless steel cages with running wheels. Wheel running was monitored during both the light and dark phases. Running (m) was recorded for the light and dark phases.

Statistical Analysis

Data are presented as mean ± SEM. Each experiment was analyzed with a 2-way analysis of variance (ANOVA) and repeated measures analysis to measure the time by group interaction using SPSS 11.0 software (SPSS, Chicago, IL). Significant main effects were analyzed for differences between groups using Tukey's post hoc test with significance set at $P < .05$. Correlations were also run using SPSS 11.0 software between caloric intake and fluid intake, RQ dark and RQ light, P values less than .05 were considered statistically significant.

RESULTS

Food Intake

At the beginning of the experiment, caloric intake (kcal) was similar for all groups. Feeding behavior (time spent in food bins and number of entries into the bin) was not different between the 3 groups (data not shown). As expected, food intake increased in the ZA group with time (Fig 1). Rats fed the ZD diet failed to increase their food intake. Thus, the mean caloric intake of ZD rats was significantly lower than ZA rats by day 3 and remained reduced until the end of the deficiency period when intake of the ZD rats was 27% of ZA ($P < .01$). ZD rats displayed a feeding cycle that was characterized by increased food intake approximately every 4 days. Refeeding of ZD rats with the ZA diet resulted in food intakes that increased steadily to control levels over the 5-day refeeding period (Fig 1). By experimental design, the caloric intake of the PF rats was the same as the ZD rats. Water intake followed the same pattern as

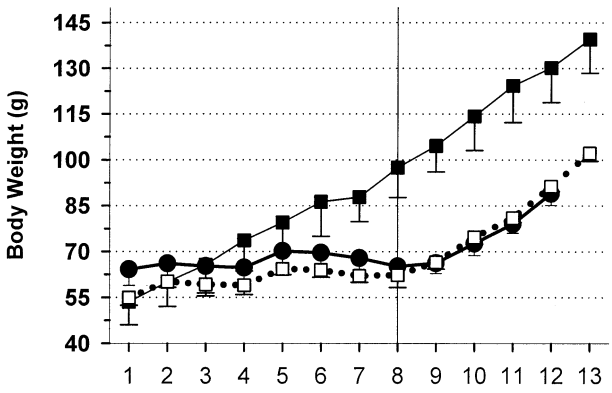

Fig 2. Effect of Zn deficiency on body weight. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). After 8 days, deficient rats were then fed the ZA diet for an additional 4 days. Body weights, represented as mean ± SEM, were recorded daily in grams.

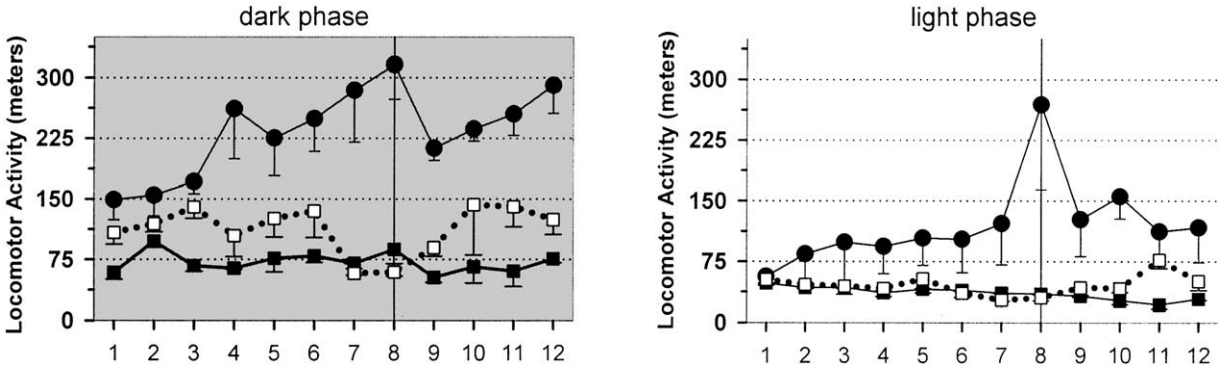

Fig 3. Effect of Zn deficiency on locomotor activity. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). After 8 days, deficient rats were then fed the ZA diet for an additional 4 days. Locomotor activity, expressed in meters (mean ± SEM) was recorded during the light and dark phases.

food intake in each of the groups (data not shown) with a correlation of $r^2 = 0.742$.

Body Weight

As expected, there was a significant increase in body weight over time in the ZA rats. Furthermore, the reduction in food intake by ZD and PF rats led to significant reductions in body weight gain compared with ZA control rats such that by day 8 ZD rats weighed 64% and PF rats weighed 67% of ZA rats ($P < .01$; Fig 2). Body weights of ZD and PF animals were not statistically different at any point during the deficiency or refeeding periods. While Zn refeeding restored caloric intake, body weights of ZD and PF rats remained significantly lower to the end of the study ($P < .01$).

Physical Activity

There were no differences in locomotor activity between groups at the beginning of the study (Fig 3). Dark-phase activity was significantly higher in PF rats beginning on day 4 ($P < .05$). On day 8, PF dark phase locomotor activity (316 ± 43 m) was 6 times that of ZA (53 ± 6 m) and ZD (59 ± 5 m). Light phase locomotor activity was significantly lower in all animals compared with dark phase. However, by day 4, the activity of the PF animals (93.5 ± 33.9 m) was >2-fold higher than ZA (40.6 ± 4.4 m) or ZD (41.8 ± 3.3 m) rats, with a significant increase in locomotor activity on the last day of the deficiency period in PF rats (day 8, $P < .05$). Most significantly, ZD animals were less active in both the light ($P < .01$) and dark phases ($P < .001$). Voluntary wheel running during the dark phase increased with time in ZA, ZD, and PF animals, with no differences between the groups. In the light phase, only PF rats significantly increased their wheel running over baseline ($P < .01$). There were no differences between light phase wheel running of ZA and ZD rats (Fig 4).

MR

An examination of oxygen consumption (V_{O_2})as a measure of MR showed that Zn deficiency and pair feeding significantly reduced MR compared with ZA rats beginning on day 4 (Fig 5). These differences were maintained throughout the refeeding period. However, when the data were normalized for differences in body weight (V_{O_2}/mL/min/kg$^{0.75}$), the MR of ZA and ZD rats were different during the deficiency phase, but were corrected by the second day of refeeding (Fig 5). Interestingly, there were no

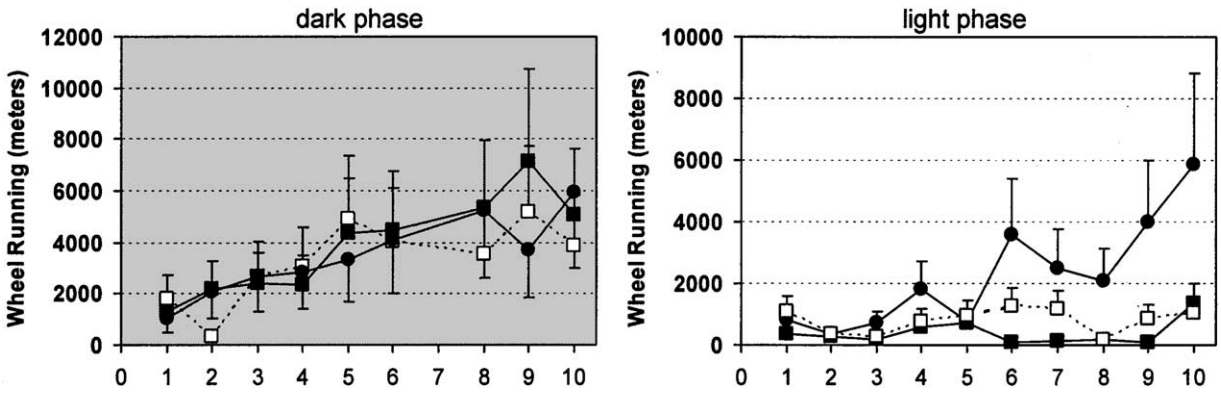

Fig 4. Effect of Zn deficiency on voluntary wheel running. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). Wheel running, expressed in meters (mean ± SEM) was recorded during the light and dark phases.

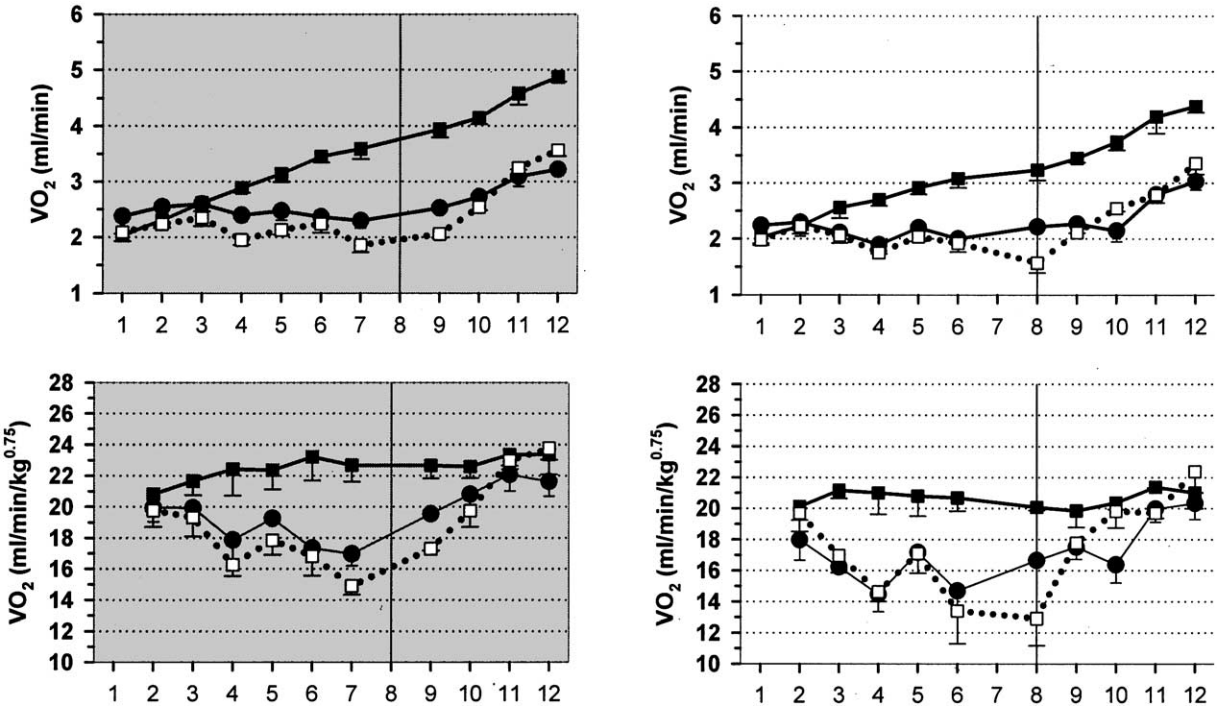

Fig 5. Effect of Zn deficiency on MR. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). MR as determined by oxygen consumption (Vo_2, mL/min) or as oxygen consumption expressed as a function of body weight (Vo_2/mL/min/kg) was recorded during the dark and light phases of each experimental day. Symbols represent mean ± SEM.

differences in MR of ZD and PF rats on any day. Furthermore, BMR increased as expected in ZA rats with time (Fig 6). While ZD and PF rats had lower BMR than ZA, there were no significant differences in the BMR of ZD and PF (Fig 6).

RQ

There was a significant relationship between RQ and caloric intake ($r^2 = 0.708$, $P < .01$) such that as caloric intake decreased in ZD and PF rats, there was decrease in RQ. Decreases in RQ were more pronounced, particularly in PF rats, during the light phase where the RQ of ZD rats was higher than PF rats ($P < .01$; Fig 7).

DISCUSSION

This study was designed to test the hypothesis that Zn deficiency leads to alterations in substrate utilization and MR. This hypothesis was based on the fact that Zn is needed for a wide variety of enzymes and hormones known to participate in the regulation of MR and energy nutrient oxidation. Further-

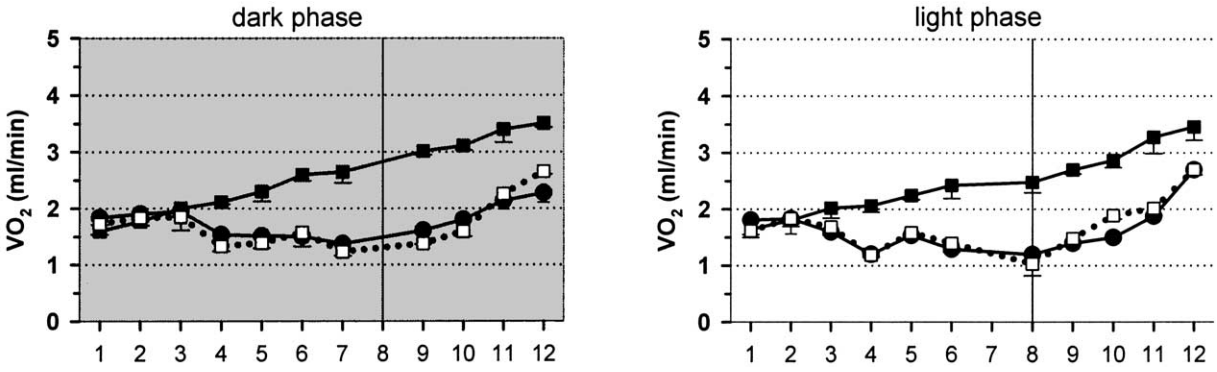

Fig 6. Effect of Zn deficiency on basal MR. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). BMR (mean ± SEM) was calculated by averaging the lowest 15 four-minute Vo_2 bins.

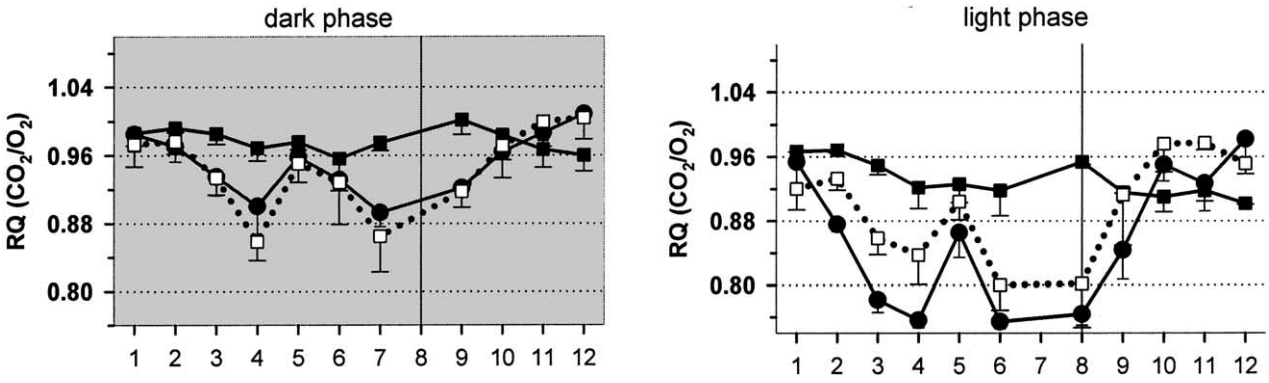

Fig 7. Effect of Zn deficiency on RQ. Young male rats were fed a ZA (28 ppm, ■) or ZD (1 ppm, □) diet. An additional group was pair-fed the weighed amount of food eaten by each deficient rat (●). RQ was determined by dividing V_{CO_2} by V_{O_2} and expressed as mean ± SEM during the light and dark phases of each day.

more, there was recently a report showing that 10 days of Zn restriction resulted in a significantly lower MR compared with both ZA and PF rats.[5] However, the difference between ZD and PF rats was not apparent after 20 or 30 days of Zn restriction.[5] The inconclusive nature of this report may be due to the fact that MR was measured for only 40 minutes during the light cycle on days 10, 20, and 30. Furthermore, locomotion, which would be expected to have an effect on energy expenditure and thus oxygen consumption, was measured only during the dark cycle.

We have now measured feeding behavior, MR, locomotion, and RQ in ZD, ZA, and PF rats. These continuous measurements include data collected during the initial phases of Zn deficiency and during the 4-day feeding cycle, as well as during the refeeding phase. The results show that MR (as measured by oxygen consumption) of ZD rats was not different from PF rats. Thus, it appears that the alterations in MR seen in ZD rats can be explained by the reduction in food intake that occurs with Zn deficiency rather than a specific effect of Zn on metabolism. Furthermore, despite previous reports of differences in body weights between ZD and PF rats, the current study failed to find significant differences. However, it should be noted that there was indeed a trend toward higher body weights in the PF animals during the deficiency period. After refeeding (day 9), this trend was abolished and body weights of ZD and PF rats were nearly identical. Because others have reported differences, it is not known if the trend toward lower body weights in the ZD is a physiologically significant one. Regardless, it appears that any differences in body weight can be minimalized, if not entirely eliminated, by the rigorous pair-feeding regimen used in this study.

RQ is used to estimate substrate utilization. A ratio of CO_2 production to O_2 consumption near 1.0 indicates the predominance of glucose oxidation either directly from dietary sources or from glycogen stores. Reductions in RQ are the result of a shift toward fat and protein oxidation, with pure fat oxidation producing an RQ of 0.703.[18] The RQ data reported here show that when rats were fed the ZD diet there is a rapid shift from carbohydrate utilization to fat oxidation. However, like MR, changes in RQ can be largely attributed to caloric intake, rather than Zn status. In fact, RQ is so tightly controlled by food intake that it closely reflects the 4-day feeding cycle that characterizes Zn deficiency. The finding that the RQ in PF rats is lowest during the light phase is also a result of food intake patterns. PF rats were supplied food at the beginning of the dark phase. Because the food restriction was significant, the PF rats ate all of their food at the beginning of the dark period, and were thus without food during the remainder of the dark period and throughout the light period.

Despite the lack of Zn effect on MR or RQ, both locomotor activity and voluntary wheel running were increased in PF rats due to food restriction, an effect not seen with Zn deficiency. After this surprising observation in rats studied in home cages, we examined the possibility that this finding was a nonspecific result, perhaps due to lethargy or muscle dysfunction. However, in separate groups of rats allowed free access to running wheels, strikingly similar ZD, PF, and ZA distances were observed in the dark phase, where most of the activity normally occurs in the rat. Therefore, the lack of increase in light phase activity appears to be a specific outcome of diminished Zn status. This finding is interesting in light of previous work showing that Zn status may influence child and infant activity patterns, and that Zn supplementation may increase activity following supplementation in ZD subjects.[19,20] Furthermore, it has repeatedly been shown that caloric restriction increases light phase activity,[21-24] an effect commonly referred to as activity-based anorexia (ABA). It has been suggested that ABA is a good model for human anorexia because patients with this disorder frequently display elevated physical activity patterns despite reduced food intake and severe weight loss.[21] Moreover, it has been suggested that a physiologic purpose of caloric restriction-induced hyperactivity may be thermoregulation.[21-24] While there are likely to be a variety of factors, including distorted body image and a desire for excessive weight loss, that contribute to increased physical activity in patients with anorexia nervosa, this raises the interesting possibility that the neurobiologic or hormonal mechanisms responsible for anorexia-induced hyperactivity are dependent on the trace metal Zn. Furthermore, it is curious to note that while PF animals

displayed consistently more locomotor activity than ZD rats, there were no significant differences in MR between these 2 groups. This observation cannot be explained by a higher BMR in ZD rats, as there were no differences found in BMR between ZD and PF rats. Rather, it suggests the possibility that there is an increased metabolic cost of activity in ZD rats.

In conclusion, it appears that the most profound effect of Zn status on MR and substrate utilization is the result of Zn deficiency–induced anorexia. However, there may be specific effects of Zn on the mechanisms governing anorexia-induced changes in locomotor activity, voluntary wheel running, and thermoregulation, in addition to the energetic cost of activity.

REFERENCES

1. McClain CJ, Stuart MA, Vivian B, et al: Zinc status before and after zinc supplementation of eating disorder patients. J Am Coll Nutr 11:694-700, 1992
2. Hadigan CM, Anderson EJ, Miller KK, et al: Assessment of macronutrient and micronutrient intake in women with anorexia nervosa. Int J Eat Disord 28:284-292, 2000
3. Birmingham CL, Goldner EM, Bakan R: Controlled trial of zinc supplementation in anorexia nervosa. Int J Eat Disord 5:251-255, 1994
4. Su JC, Birmingham CL: Zinc supplementation in the treatment of anorexia nervosa. Eat Weight Disord 7:20-22, 2002
5. Gaetke LM, Frederich RC, Oz HS, et al: Decreased food intake rather than zinc deficiency is associated with changes in plasma leptin, metabolic rate, and activity levels in zinc deficient rats. J Nutr Biochem 13:237-244, 2002
6. Rains TM, Hedrick S, Randall AC, et al: Food intake patterns are altered during long-term zinc deficiency in rats. Physiol Behav 65:473-478, 1998
7. Kasarskis EJ, Sparks DL, Slevin JT: Changes in hypothalamic noradrenergic systems during the anorexia of zinc deficiency. Biol Trace Elem Res 9:25-35, 1986
8. Essatara MB, Levine AS, Morley JE, et al: Zinc deficiency and anorexia in rats: Normal feeding patterns and stress induced feeding. Physiol Behav 32:469-474, 1984
9. Gordon EF, Bond JT, Gordon RC, et al: Zinc deficiency and behavior: A development perspective. Physiol Behav 28:893-897, 1982
10. McCall KA, Huang C, Fierke CA: Function and mechanism of zinc metalloenzymes. J Nutr 130:1437S-1446S, 2000
11. Kennedy KJ, Rains TM, Shay NF: Zinc deficiency changes preferred macronutrient intake in subpopulations of Sprague-Dawley outbred rats and reduces hepatic pyruvate kinase gene expression. J Nutr 128:43-49, 1998
12. Rains TM, Mangian HF, Liang T, et al: Growth hormone-releasing factor affects macronutrient intake during the anabolic phase of zinc repletion: Total hypothalamic growth hormone-releasing factor content and growth hormone-releasing factor immunoneutralization during zinc repletion. Nutr Neurosci 4:283-293, 2001
13. Faure P, Roussel A, Coudray C, et al: Zinc and insulin sensitivity. Biol Trace Elem Res 32:305-310, 1992
14. Morley JE, Gordon J, Hershman JM: Zinc deficiency, chronic starvation, and hypothalamic-pituitary-thyroid function. Am J Clin Nutr 33:1767-1770, 1980
15. Ott ES, Shay NF: Zinc deficiency reduces leptin gene expression and leptin secretion in rat adipocytes. Exp Biol Med 226:841-846, 2001
16. Reidy SP, Weber JM: Accelerated substrate cycling: A new energy-wasting role for leptin in vivo. Am J Physiol Endocrinol Metab 282:E312-317, 2002
17. Williams TD, Chambers JB, May OL, et al: Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. Am J Physiol 278:R255-R262, 2000
18. Flatt JP: Body composition, respiratory quotient, and weight maintenance. Am J Clin Nutr 62:1107S-1117S, 1995
19. Bentley ME, Caulfield LE, Ram M, et al: Effect of zinc supplementation on observed activity patterns of rural Guatemalan infants. J Nutr 127:1333-1338, 1997
20. Sazawal S, Bentley M, Black RE, et al: Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. Pediatrics 98:1132-1137, 1996
21. Hebebrand J, Exner C, Hebebrand K, et al: Hyperactivity in patients with anorexia nervosa and in semistarved rats: Evidence for a pivotal role of hypoleptinemia. Physiol Behav 79:25-37, 2003
22. Morse AD, Russell JC, Hunt TW, et al: Diurnal variation of intensive running in food-deprived rats. Can J Physiol Pharmacol 73:1519-1523, 1995
23. Burden VR, White BD, Dean RG, et al: Activity of the hypothalamic-pituitary-adrenal axis is elevated in rats with activity-based anorexia. J Nutr 123:1217-1225, 1993
24. Williams TD, Chambers JB, Henderson RP, et al: Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. Am J Physiol 282:R1459-1467, 2002