Zinc Deficiency Changes Preferred Macronutrient Intake in Subpopulations of Sprague-Dawley Outbred Rats and Reduces Hepatic Pyruvate Kinase Gene Expression 1,2,3

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ABSTRACT  Macronutrient selection patterns of male rats were analyzed using a 3-choice macronutrient selection system providing either adequate (+Zn) or deficient (−Zn) levels of zinc (30 or 1 mg Zn/kg). In study 1, rats were provided +Zn and −Zn diets for 28 d. All rats preferred carbohydrate (>50% carbohydrate intake) at the onset, consuming an average of 71% carbohydrate (cho), 17% protein (pro), and 12% fat. By the end of the study, 25% of the −Zn rats switched preference from cho to fat, whereas no +Zn rats changed. In study 2, −Zn rats preferring fat increased their total intake to normal levels, but only 50% reverted to carbohydrate preference after 35 d of zinc repletion. Hypothalamic concentrations of galanin were measured in groups of +Zn and −Zn cho- and fat-prefering rats. Galanin, which may be regulated with fat intake, was not different in −Zn rats preferring fat vs. −Zn rats preferring cho. Galanin concentrations were higher in +Zn than in −Zn rats (P < 0.05) and higher in +Zn rats preferring fat than in +Zn rats preferring cho (P < 0.05). Hepatic pyruvate kinase (PK) mRNA concentrations were related to cho preference, regardless of zinc status. When PK mRNA levels were measured in rats consuming a single AIN-93–based diet, PK mRNA levels were significantly reduced by zinc deficiency (P < 0.05). Because PK is highly regulated by insulin, the effect of insulin may be reduced by zinc deficiency, making it more difficult for −Zn rats to catabolize dietary cho. This may explain why some −Zn rats switched from preferring cho to fat after developing zinc deficiency. J. Nutr. 128: 43–49, 1998.

KEY WORDS:  zinc deficiency  food intake regulation  galanin  pyruvate kinase  rats

Strain, sex and dietary components influence the amount of cho, pro and fat a rat will select when a 3-choice system is used (Li and Anderson 1982, Miller et al. 1994). Some studies have shown rats choosing very high amounts of either cho or fat; other studies have identified rats with similar intakes of all three macronutrients. Because of the disparity of results among laboratories, the best application of the 3-choice system is to compare changes in selection patterns within a single set of experiments, rather than trying to compare results from studies using different diets or animals of different strain or sex. By using a 3-choice system in our own laboratory, we found that >95% of commercially obtained male Sprague-Dawley outbred rats consumed >50% of their energy from carbohydrate, whereas very few rats (<2%) selected fat at >50% or consumed roughly equivalent amounts from the three diets (<3%). It is not known what factors are most important in developing a preference for a certain macronutrient, but peripheral control (Barton et al. 1995), central control (Stanley et al. 1985) and prior experience (Reed et al. 1992) have all been shown to affect the selection of macronutrients and the development of preference for a macronutrient.

Experimental zinc deficiency causes anorexia among other symptoms. The sense of taste may be altered by zinc deficiency (Catalanotto 1978). Carbonic anhydrase IV has been proposed to play a role in the mechanism of taste, and restoration of taste in humans with carbonic anhydrase IV deficiency has

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been achieved by zinc supplementation (Henkin 1996). The strongest hypothesis explaining the anorexia associated with zinc deficiency is related to alterations in amino acid metabolism, which may cause changes in concentrations of amino acid–derived neurotransmitters (O’Dell and Reeves 1989). Very recently, changes in concentrations of appetite-regulating factors such as neuropeptide Y and galanin (Li et al. 1996, Selvais et al. 1997) and leptin (Mangian et al. 1997) have been reported. Although it is unclear whether a single factor or multiple factors are responsible for the loss of appetite associated with zinc deficiency, it may be that several factors contribute to this state. Considering that zinc deficiency–induced anorexia is still not well understood and that a growing body of work has examined how specific peripheral and central factors affect macronutrient selection (Bray 1992), we recently used the 3-choice self-selection scheme to identify changes in macronutrient selection during the development of zinc deficiency (Rains and Shay 1995). During the course of a 28-d administration of +Zn and –Zn 3-choice diets, –Zn rats as a group selectively reduced carbohydrate intake while maintaining relatively constant intakes of protein and fat. This reduction in carbohydrate intake by the –Zn group represented all of the 50% reduction of intake associated with zinc deficiency (Rains and Shay 1995). During the course of that earlier study, we noted that some –Zn rats consumed very high (50–95% of total energy intake) amounts of fat as zinc deficiency developed.

On the basis of the earlier observations, the objectives of the present study were as follows: 1) to examine this phenomenon of switching from cho to fat preference during the development of zinc deficiency, 2) to characterize the selection patterns of fat-prefering –Zn rats during zinc repletion, 3) to measure hypothalamic concentrations of galanin, an appetite-regulating peptide proposed to be involved in the regulation of fat intake (Bray 1992) and 4) to measure expression of the liver pyruvate kinase (PK) gene, which is a key regulator of hepatic glycolysis and is strongly regulated by carbohydrate intake and insulin. Although PK is regulated at transcriptional, posttranscriptional, and posttranslational levels, the level of PK gene expression reflects trends occurring over the course of multiday experiments, in contrast with measurements of hepatic PK enzyme activity, which are perhaps better used to indicate short-term changes reflecting the pre- and postprandial states. Additionally, changes in the expression of this cho-regulated gene may provide insight into the changes in macronutrient intake observed in some –Zn rats.

**MATERIALS AND METHODS**

**Animals and diets.** Outbred male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), were housed individually in suspended, stainless cages maintained on a 12-h light:dark cycle at 21°C. All animal protocols were approved by the University of Illinois Animal Care Advisory Committee. Rats used in studies 1 and 2 were allowed free and simultaneous access to three diets, each containing cho, pro or fat, plus vitamin and mineral supplements. The carbohydrate diet used is a mix of sucrose and cornstarch, the protein source is spray-dried egg white, and the fat diet used is a mix of soybean oil and cellulose. This fat-containing diet was formulated to provide a texture similar to the cho and pro diets. The formulation of these diets has been published previously (Rains and Shay 1995). Energy content was calculated from Atwater values, and spray-dried egg white was considered to be 85% protein. Distilled water was provided in zinc-free plastic bottles. Macronutrient diets were designed to contain either adequate (30 mg/kg) or deficient (1 mg/kg) levels of zinc using zinc–adequate (+Zn) or Zn–deficient (–Zn) AIN-93 mineral mix (Harlan Teklad, Madison, WI). The actual zinc concentration of every batch of diet was determined using atomic absorption spectrophotometry (AAS) as previously described (Rains and Shay 1995). All batches of diet had AAS-measured zinc concentrations equivalent to the nominal value ± 2 mg/kg. Diets were stored in 7.5-cm diameter glass feed jars covered with stainless steel lids with a 3-cm diameter hole in the center. Diet jars were weighed manually on a daily basis or every other day to determine macronutrient intake values. Spillage was collected and weighed. After weighing of diet jars, jars were returned to cages in a randomized orientation to prevent the development of preference based on location within the cage. Accumulation to the 3-choice powdered diets lasted for 1 wk; during that time, the +Zn diets were provided to all rats and intake was measured. Rats with similar intake profiles during the pretest were matched and assigned to the +Zn or –Zn groups. Rats used in study 3 were provided a single pelleted AIN-93G diet (Reeves et al. 1993), which also provided adequate or deficient levels of zinc as described above. Zinc deficiency was confirmed by anorexia and reduced growth. In addition, representative femur samples were collected from groups of rats for zinc content measurements by AAS. Intakes were expressed as kJ consumed/100 g body weight (BW) of each rat to account for the differential growth rates of +Zn and –Zn rats.

**Format of studies.** Study one included a 1-wk pretest followed by a 28-d period during which rats (100–125 g) were provided the +Zn (n = 46) or –Zn (n = 48) diets. Because of the large number of rats tested, this study was completed as four separate trials. Study two used 32 rats (90–100 g) that were tested in one trial. These rats were acclimated to diets for 1 wk as in Study 1; then all 32 rats were provided –Zn, 3-choice diets for 28 d. Rats that developed a preference for fat during zinc deficiency were then maintained on +Zn, 3-choice diets for an additional 35 d. In studies 1 and 2, cumulative 4-d intakes from d 1–4 and 25–28 were compared to contrast intake patterns before and after zinc deficiency–induced anorexia had been well established. Comparing 4-d periods of intake ensured that each rat would be measured during both the high and low portions of the ~3.5-d intake cycle exhibited by –Zn rats.

Rats in study 3 (n = 36 total, 60–75 g) were acclimated to a 4-h meal-feeding period that coincided with the first 4 h of dark. All rats in study 3 were provided access to their diet jars only during this 4-h period. This protocol was used to synchronize the pattern of intake of the –Zn and +Zn groups with that of the food-restricted, pair-fed rats; the latter group rapidly became meal-feeders and consumed their daily allotment of diet in a few hours. After 1 wk of acclimation, rats were placed into three groups (n = 12) and provided free access to either zinc–adequate (AL) or zinc-deficient (–Zn) AIN-93G diets, a third group was pair-fed the reduced amount consumed by the –Zn group (pair-fed, PF). Rats within each group were divided into three subgroups and maintained for either 4 or 8 wk on the experimental diets (n = 4). On the day designated for killing, rats were killed before that day’s meal period.

**Northern analysis.** Northern analysis was performed as described previously (Shay and Cousins 1993). Liver RNA was isolated, and 15 μg of each RNA sample was size-separated by MOPS/formaldehyde electrophoresis and blotted to Hybond-N nylon membranes (Amer sham, Arlington Heights, IL). Blots were hybridized to [32P]-dCTP-labeled cDNA corresponding to the liver PK mRNA and the 28S rRNA. Ethidium bromide staining of gels indicated equivalent loading of ± 10% between samples. After washing of blots, hybridization was visualized first by exposure to Kodak X-AR film and then to a phoshorimaging screen ( Molecular Dynamics, Sunnyvale, CA). Exposed phosphorimaging screens were then quantitated using a phosphorimager, with normalization of PK values to the 28S mRNA. Mean values of PK/28S ratios were compared by setting the mean value of a control group to 1.0.

**Galanin radioimmunoassay.** Coronal slices (2 mm thick) were made by using a rat brain matrix; the slices were centered 1.3 to 2.6 mm from bregma, according to the atlas of Paxinos and Watson (1986). A 2 mm (diameter) × 1 mm (dorsosventral) piece of tissue was microdissected from each slice, and an acetic acid–soluble peptide fraction was prepared from each sample by boiling in 500 μL of 0.5 mol/L acetic acid for 15 min followed by centrifugation at 17,200 × g for 15 min. Supernatants were collected and stored at −80°C until used. Galanin content in the paraventricular nucleus was measured using a rat galanin RIA (Phoenix Pharmaceuticals, Mountain View,
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TABLE 1
Four-day macronutrient energy intakes of rats selecting from separate carbohydrate-, protein-, and fat-containing zinc-adequate or zinc-deficient diets for the first 4 and last 4 d of a 28-d period

<table>
<thead>
<tr>
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<th>First 4 d</th>
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<th>Last 4 d</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cho</td>
<td>Pro</td>
</tr>
<tr>
<td>+Zn</td>
<td>808 ± 12</td>
<td>573 ± 18</td>
<td>134 ± 5.9</td>
</tr>
<tr>
<td>−Zn</td>
<td>758 ± 12</td>
<td>540 ± 19</td>
<td>134 ± 6.3</td>
</tr>
</tbody>
</table>

% of intake/100 g BW

<table>
<thead>
<tr>
<th></th>
<th>First 4 d</th>
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<th>Last 4 d</th>
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<tbody>
<tr>
<td>+Zn</td>
<td>71 ± 1.6</td>
<td>17 ± 0.7</td>
<td>12 ± 1.5</td>
</tr>
<tr>
<td>−Zn</td>
<td>71 ± 1.8</td>
<td>18 ± 0.8</td>
<td>11 ± 1.9</td>
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Values are means ± SEM; n = 46 for +Zn; 48 for −Zn. Intakes of carbohydrate (cho), protein (pro), and fat (fat) are expressed as kJ consumed/100 g body weight (BW) for each 4-d interval. Four-day macronutrient intakes are also expressed as a percentage of the total energy consumed/100 g body weight for each 4-d period.

* Significantly different than the −Zn group, (P < 0.05).

RESULTS

Study one. During the 1-wk pretest of 100 rats with the 3-choice macronutrient system, 94 of the rats tested selected cho as the preferred macronutrient. The remaining 6 rats had various phenotypes, including one rat that consumed >50% of energy as fat. These 6 rats were not eating diet at levels sufficient to support normal growth and were judged to be ill or not adapted to the powdered macronutrient diets; thus they were excluded from further testing. The average energy intake profile for the 94 cho-prefering rats during the acclimation was 71% cho, 17% protein, and 12% fat. In studies 1 and 2, using 3-choice macronutrient diets, +Zn rats consumed significantly greater amounts of diet than did the −Zn rats from d 7 through 28 (P < 0.05). As soon as intake was reduced in −Zn rats consuming macronutrient diets, they began to exhibit the 3.5-d cycle of intake associated with zinc deficiency. In −Zn rats, the preferred macronutrient (a macronutrient consumed at >50% of total energy intake) had no effect on the length of the cycle or magnitude between maximum and minimum levels of intake. Once −Zn rats displayed a fat-prefering phenotype, the fat diet was selected every day as the macronutrient of choice, whether the rat was at a maximum or minimum of the 3.5-d cycle, and −Zn, fat-prefering rats did not revert to carbohydrate preference when provided −Zn diets. Pooled femur zinc concentrations were 1.4 μmol Zn/g bone for −Zn rats and 3.8 μmol Zn/g bone for +Zn rats at d 28 and mean body weights were 154 ± 7 vs. 206 ± 9 g (P < 0.01, −Zn vs. +Zn, respectively).

To contrast changes in selection patterns before and after the onset of zinc deficiency and also to consider intakes from each rat from each part of the 3.5-d cycle of intake exhibited by −Zn rats, intake was compared between the +Zn and −Zn groups during the first 4 and last 4 d of the 28-d feeding period. Daily intakes as a group throughout the 28-d period were entirely consistent with our previous report of macronutrient intakes during the development of zinc deficiency (Rains and Shay 1995) and are not shown here. Energy intake/100 g BW of each macronutrient, total energy intake/100 g BW and percentage of energy consumed from each macronutrient for the first and last 4 d of the 28-d study are presented in Table 1. As reported previously, the only macronutrient consumed differently between groups of rats was carbohydrate. In contrast to the average intakes of the +Zn and −Zn groups shown in Table 1, Figure 1 shows the preferred macronutrient intakes of individual rats within the +Zn and −Zn groups during the first and last 4 d of the 28-d study 1. When considering macronutrient preference of individual rats by categorizing them as preferring cho or fat rather than considering the aver-
age intake of a large population of rats, differences in intake patterns are clearly observed between the +Zn and -Zn rats. Ninety-eight percent (45 of 46) of the +Zn rats preferred cho during the first 4 d (the 46th rat consumed cho at 49% for the first 4 d), and every +Zn rat consumed cho at >50% throughout the rest of the 28-d test period, including the last 4 d. In the -Zn group, 94% (45 of 48) rats preferred cho during the first 4 d of the study, but by the last 4 d of the 28-d period, a significantly lower percentage (67%, or 32 of 48) of the -Zn rats maintained a cho-prefering phenotype (94 vs. 67%; P < 0.05 as analyzed with a z-test). Twenty-five percent (12 of 48) of the -Zn rats preferred fat at >50% of total energy at the end of the study compared with only 2% (1 of 48) that did so during the first 4 d of the study (P < 0.05). Eight percent (4 of 48) of the -Zn rats did not consume any of the three macronutrients diets at 50% or more during the last 4 d, and thus were not placed into any preference group. The 3 of 48 (6%) -Zn rats that did not prefer cho over the first 4 d of the study exhibited an immediate decrease in cho intake and an increase in fat intake during the first 4 d of the feeding period, although these rats consistently preferred cho during the 7 d pretest period and at the onset of the 28-d zinc-depletion period. The other -Zn rats that developed the fat-prefering phenotype most often switched from cho preference during wk 2 or 3 of the study. These results are consistent with results of study 2, described below, in which 10 of 32 rats developed a preference for the fat-containing diet after becoming zinc deficient. In combination, the number of rats from studies 1 and 2 challenged with -Zn, 3-choice diets totals 80, and 22 of these 80 rats (28%) switched to a fat-prefering phenotype within 28 d.

Other tests we have conducted have determined that a vegetable shortening–based, fiber-free diet, when used as the fat-containing diet in a 3-choice system also allows this same fat-prefering phenotype to develop in ~30% of -Zn rats (data not shown); thus it appears that fat and not fiber is the preferred factor in the development of this phenotype. Other studies in our own laboratory using rats of different ages and weights also show that ~30% of -Zn rats switch to fat preference when 3-choice diets are used. In combination, we have challenged more than 200 rats with -Zn 3-choice diets in eight different trials; in every trial performed, ~30% of these rats develop a preference for fat. The fat-prefering phenotype was never displayed by any rat in the +Zn group. We have not observed a single +Zn rat switch from cho to fat preference while being provided a +Zn set of 3-choice diets. In addition, we have never observed a single case of a +Zn or -Zn rat exhibiting a preference (>50%) for protein.

**Study 2.** Because switching to the fat-prefering phenotype was found to be singular to -Zn rats, study 2 was designed to examine the stability of the fat-prefering phenotype. Thirty-two rats were provided -Zn macronutrient diets for 28 d, and 10 became fat preferers. After the 28-d depletion period, only the fat-prefering rats were tested further by providing them zinc-containing macronutrient diets (2 of the 10 -Zn, fat-prefering rats were not tested any further because of advanced secondary signs of zinc deficiency, leaving 8 rats for further testing). All of these zinc-repleted rats increased their intake during the first 1–2 d of repletion, equivalent to +Zn levels. However, only half of the fat-prefering rats reverted to carbohydrate preferrence (Fig. 2). Two of the rats reverted to carbohydrate preference during wk 1, and two more reverted during wk 2 and 3. At the end of the 28-d depletion and 35-d repletion periods, mean body weights were 106 ± 1 and 252 ± 6 g, respectively. After a total of 5 wk of repletion, study 2 was discontinued because it appeared that the selection pattern of the remaining four fat-prefering, zinc-repleted rats was stable.

Considering the results of studies 1 and 2, the following four separate phenotypes of zinc and macronutrient preference were established while zinc content was manipulated in 3-choice diets: 1) +Zn, cho-prefering; 2) -Zn, cho-prefering; 3) +Zn, fat-prefering; and 4) -Zn, fat-prefering. Hypothalamic galanin concentrations and the expression of the pyruvate kinase mRNA were then measured in samples obtained from rats in studies 1 and 2 representing each of the four zinc/macronutrient phenotypes.

Galanin concentrations in the paraventricular nucleus of the hypothalamus did not differ in +Zn and -Zn, cho-prefering rats (3.1 ± 0.3 mmol/L; n = 10 each) and were lower (1.5 ± 0.35 mmol/L; P < 0.05) in -Zn/fat-prefering rats (n = 5). The highest galanin concentrations were in samples derived from the +Zn/fat-prefering rats (n = 4, 6.9 ± 0.41 nmol/L; P < 0.05). When considering main effects of zinc and macronutrient on galanin levels, galanin was higher in +Zn (n = 14) than in -Zn (n = 15) rats (5.0 ± 0.22 vs. 2.3 ± 0.22 mmol/L; P < 0.05) and higher in fat- (n = 9) than in cho-prefering (n = 20) rats (4.2 ± 0.23 vs. 3.1 ± 0.19 nmol/L; P < 0.05). Although galanin concentration was higher in fat- than in cho-prefering rats, there was not a positive correlation between fat consumption and hypothalamic galanin during zinc deficiency.

Rats consuming high carbohydrate displayed higher PK mRNA levels regardless of dietary zinc content when comparing hepatic RNA obtained from rats representing the four different zinc/macronutrient phenotypes (Fig. 3). PK was 100% higher in cho-prefering than in fat-prefering phenotypes (P < 0.05). The influence of cho consumption on PK levels was dominant to any effect of zinc status on the expression of the PK mRNA. Because there were differences in total intake along with differences in zinc status in studies one and two, we decided to measure PK mRNA levels in a situation in which -Zn rats could be compared with PF and AL (both +Zn) groups, which is not possible with the use of 3-choice diets. This motivated the design of study 3.

**Study 3.** Study 3 (Fig. 4, upper panel) allowed PK mRNA levels to be compared during a short-term zinc deficiency, from the point at which zinc deficiency was just beginning to affect intake, and also in later stages of zinc deficiency. Pooled bone zinc concentrations confirmed zinc status; these concentrations were 0.8, 1.5 and 1.4 μmol Zn/g bone for the -Zn, AL and PF groups, respectively, for rats fed diets for 28 d. These bone concentrations were somewhat lower than those measured in studies 1 and 2, consistent with the smaller size of
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PK gene expression was not affected by day of study. At each day of testing, including the earliest measurement at d 4, -Zn rats generally had the lowest PK expression, PF rats had intermediate levels and AL rats had highest PK gene expression, although differences were not significant (Fig. 4, middle panel).

DISCUSSION

The combination of a 3-choice macronutrient selection and manipulation of dietary zinc content was used to evaluate the...
self-selection of macronutrient diets during zinc deficiency. In Sprague-Dawley outbred male rats, 94% of the rats tested displayed a preference for cho when provided adequate dietary zinc. This preference for cho in +Zn rats was found to be stable for a 35-d period. In contrast, nearly 30% of −Zn rats increased fat intake and greatly reduced carbohydrate intake, whereas the remaining −Zn rats maintained stable cho consumption. These percentages are nearly identical to those observed within the −Zn group of an earlier study that tested female rats (Rains and Shay 1995). There was no increase in spillage when rats were switched from +Zn diets to −Zn diets; spillage is considered an indicator of exploration for new food sources. Genetic variability within these outbred rats may be a factor in the development of fat preference during zinc deficiency. Two studies, subsequently completed and presented here, address possible metabolic changes during zinc deficiency that may explain the changes we observed in macronutrient consumption.

Three unusual −Zn rats switched from preferring cho to fat during the first 4 d of study 1. These rats may have been especially sensitive to zinc deficiency, changing their consumption early in the test period, a change made later in the test period by other −Zn rats. Typically, reduced intake is considered the first noticeable indication of zinc deficiency. In this case, these switch to fat preference by these three rats slightly preceded their reduction in intake. No differences in total intake or zinc status indices were found between the cho or fat-prefering −Zn rats, suggesting that the change to fat preference was not a mechanism to conserve or increase body zinc stores or growth, or to allow the consumption of more energy. It may be that the changes observed in macronutrient selection might be due to zinc-dependent alterations in the central control of appetite, perhaps caused by reduced synthesis of mRNAs encoding appetite-regulating or macronutrient-regulating neuropeptides. There may also be changes in the synthesis or processing of these peptides into active form. There is ample evidence that zinc status causes changes in the transcription of specific genes (Hamer 1986), reductions in protein synthesis (Duerr et al. 1977) and peptide processing (Pekary et al. 1991). We chose to measure galanin because it has been correlated, in some studies, to differences in fat intake. Although we found differences in galanin levels in the PVN of +Zn and −Zn rats (+Zn > −Zn, P < 0.05), hypothalamic galanin concentrations did not correlate positively with the −Zn-fat-prefering phenotype, suggesting that galanin may not be the factor causing some −Zn rats to dramatically increase fat consumption. Enterostatin is another factor that has been shown in some studies to relate to fat intake; it may be worthwhile in future studies to examine circulating and central enterostatin levels in −Zn fat- and cho-prefering rats.

A second specific factor examined was the expression of the liver-type pyruvate kinase mRNA. Pyruvate kinase catalyzes one of the three regulated steps of glycolysis and is strongly regulated by carbohydrate intake, acting via action of insulin and glucose on the PK promoter (Thompson and Towle 1991). In studies 1 and 2, cho intake so strongly regulated PK levels that it was difficult to determine if there was any effect of zinc status on the expression of this gene. Study 3 was therefore designed to use a single diet that would not allow the development of macronutrient preferences. Also, a time course element was added to measure PK expression during short-term zinc deficiency, just as intake was being reduced by zinc deficiency; this is in contrast to studies 1 and 2, which were conducted after intake was reduced and macronutrient preferences established. We found PK expression to be significantly reduced in −Zn rats, even in comparison with PF rats that consumed the same amount of energy. This suggests that there may be a reduction in insulin production or effect due to zinc deficiency, and that hepatic glycolysis may be impaired to some degree by zinc deficiency. Perhaps it is more difficult for rats to utilize energy from carbohydrate efficiently, which possibly explains why some −Zn rats switched to a predominantly fat-containing diet: it may be more efficient metabolically for rats with reduced insulin action to obtain energy from fat rather than carbohydrate. One report suggests that oxidation of carbohydrate is not affected by zinc deficiency (Theuer and Hoekstra 1966), but these measurements were whole-body measurements not indicative of hepatic function specifically. Reeves and O’Dell (1983) measured higher amounts of glucose incorporation into glycogen, which is consistent with an impairment in flux through hepatic glycolysis. If glucose is not utilized by glycolysis, it may be more available for glycogenesis. We noted from Northern analyses of the PK mRNA that there was heterogeneity in the expression of this mRNA among individual rats in the same treatment group. Perhaps the −Zn rats with the lowest levels of PK expression are the most likely candidates to switch to the fat-prefering phenotype. There have been many studies examining insulin during zinc deficiency, several with contradictory results. Reeves and O’Dell (1983) concluded that insulin effect, rather than amount, is reduced by zinc deficiency. We have measured insulin levels during zinc deficiency and have not found any reductions in serum insulin concentrations (Mangian et al. 1997). Perhaps insulin binding or the insulin signal transduction pathway is negatively affected by zinc deficiency, reducing PK mRNA levels. Other metabolic measures, including PK enzyme activity, will be helpful to deduce if and how glycolysis is impaired.

In study two, we tested the intake patterns of −Zn, fat-prefering rats during a 5-wk period of zinc repletion. During this repletion period, only 50% of the rats reverted to the original cho-prefering phenotype. The fact that the fat-prefering phenotype is not readily reversed upon zinc repletion suggests at least one possible explanation for the changes observed in selection patterns: the change in intake selection was a learned phenomenon, and this learned behavior is resistant to reversal. It may be that zinc deficiency has caused an irreversible change in the function of some of the neurons that participate in the appetite regulation system. Although we are speculating by extrapolating these results to humans, this study suggests that there might be long-term or perhaps even permanent changes in the function of the appetite regulation system after recovery from a significant zinc deficiency. This could have implications for individuals recovering from severe nutrient deficiencies that might accompany loss of appetite disorders. The results from this study demonstrate that zinc deficiency, along with causing a reduction in appetite, plays a role in alterations in preferences for fat or carbohydrate.

LITERATURE CITED

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