Zinc and Health: Current Status and Future Directions

Neurobiology of Zinc-Influenced Eating Behavior[1,2]

Neil F. Shay[3] and Heather F. Mangian

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

ABSTRACT Zinc is an essential nutrient that is required in humans and animals for many physiological functions, including immune and antioxidant function, growth and reproduction. Many aspects of zinc deficiency-induced anorexia have been well studied in experimental animals, most notably the laboratory rat. There is evidence that suggests zinc deficiency may be intimately involved with anorexia in humans: if not as an initiating cause, then as an accelerating or exacerbating factor that may deepen the pathology of the anorexia. The present review describes recent research investigating the relationship between zinc deficiency and the regulation of food intake, along with advances in the understanding of the food intake and body weight regulation systems. For more comprehensive reviews of zinc nutrition and zinc deficiency, readers are referred to the other reviews in this volume and the review text of Mills (1989). An excellent review focused solely on zinc status and food intake has been presented by O'Dell and Reeves (1989). J. Nutr. 130: 1493S—1499S, 2000.

KEY WORDS: • *zinc deficiency* • *anorexia* • *food intake* • *neuropeptide Y* • *galanin* • *leptin*

Zinc deficiency

Zinc deficiency was initially discovered in humans and reported by Prasad et al. (1963). Symptoms reported to accompany zinc deficiency included dwarfism, hypogonadism and poor appetite. In these studies, similarities were noted between zinc-deficient human subjects and known characteristics of zinc-deficient animals. Although zinc deficiency has been studied in many different species, including rats, pigs, chicks, lambs, monkeys and guinea pigs, studies in the rat have provided most of the experimental data regarding zinc deficiency and changes in food intake. The young growing rat is very responsive to the consumption of a zinc-deficient diet. Within 3–5 d, food intake is first observed to decrease. This decrease in appetite is the first visible sign of zinc deficiency, and it occurs well in advance of any other symptoms associated with zinc deficiency. The reduction in growth associated with zinc deficiency is largely caused by the reduction in intake due to this deficiency. This is demonstrated as pair-fed control rats that are provided a reduced amount of zinc-adequate diet equivalent to the amount consumed by similar zinc-deficient rats reduce or cease growth in an essentially similar fashion, as do the deficient rats. Although there are usually slight differences in food efficiency, with pair-fed rats having a slightly greater efficiency compared with zinc-deficient rats, this difference is usually very slight.

Among many, there are two classic studies we mention that provide an excellent summary of the early findings regarding the effect of zinc deficiency on food intake (Chesters and Quarterman, 1970, Chesters and Will, 1973). These findings include the reduction in intake during zinc deficiency, identification of a 3–4 d cycle of variable intake (described in detail by Tamaki et al. 1995), force-feeding zinc-deficient rats is detrimental to their health, reduction in the protein content (or content of essential amino acids) in the experimental diet can affect levels of intake, zinc supplementation rapidly restores normal levels of intake in rats and zinc-deficient rats eat fewer times during the day, but when they do eat, they consume similar-sized meals compared with control rats. A connection between zinc deficiency and dietary protein or amino acid levels may exist; however, a complete theory describing how zinc deficiency and protein metabolism are related is still lacking.

Anorexia and zinc deficiency in humans. Although it is clear that zinc deficiency produces a specific and profound anorexia in experimental animals, the connection between zinc deficiency and human anorexia is less certain. This connection is difficult to advance as a predictive or initiating factor in the development of human anorexias such as anorexia nervosa (AN)[4] typically observed in teenagers and in the anorexia associated with failure-to-thrive syndrome of older people. However, in human populations characterized as

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[3] To whom correspondence and reprint requests should be addressed.

[4] Abbreviations used: AN, anorexia nervosa; NPY, neuropeptide Y, PVN, paraventricular nucleus.

zinc deficient, such as the Middle Eastern populations characterized by Prasad et al. (1963) and populations of American children identified by Hambidge et al. (1972), a correlation has been established among zinc status, growth and appetite. It has been suggested that zinc deficiency contributes to the symptoms of AN. Many of the features of zinc deficiency are observed in AN patients, including the anorexia, poor growth or weight loss, skin abnormalities, amenorrhea and depression. The use of zinc supplementation in the treatment of AN was advocated by Bakan (1979, 1984). Clinical studies indicated that approximately half of all AN patients tested are zinc deficient (Casper et al. 1980, Humphries et al. 1989, Nishi 1980) and that absorption of dietary zinc is diminished during AN (Dinsmore et al. 1985). The dietary (Bakan et al. 1993) and activity (Casper et al. 1991) patterns of AN patients may increase their susceptibility to zinc deficiency. During recovery from AN, sufficient zinc must be available to support growth if normal body weight is to be restored. In open trials, zinc supplementation has been shown to improve weight gain in AN patients (Bryce-Smith and Simpson 1984, Esca et al. 1979, Humphries et al. 1990, Safai-Kutti 1990, Yamaguchi et al. 1992). In a randomized, double-blind, placebo-controlled trial, a daily supplement of 14 mg zinc from zinc gluconate was found to double the rate of body mass increase ($P < 0.03$) compared with patients receiving the placebo control (Birmingham et al. 1994). AN is a complex disorder, with contributions coming from psychological aspects, nutritional deficiencies and genetic predisposition perhaps due to defective gene products involved in the appetite regulation system. Two points are important to recognize with respect to anorexia in humans. First, the contribution to anorexia from a variety of nutrient deficiencies is likely an accelerating or exacerbating factor. Although zinc deficiency is very likely involved, we suggest that other nutrient deficiencies may be playing an important role in the development of or intensification of anorexia. Most notably, thiamin may be implicated. Similar to zinc, thiamin deficiency produces a specific and intense anorexia in experimental animals (see, e.g., Rains et al. 1997). An extended period of voluntary food restriction for any reason must increase the risk of developing marginal or substantial deficiencies for zinc, thiamin and other nutrients. Second, it appears that the connection between zinc deficiency and anorexia in humans may be largely unappreciated or underestimated. Although many clinicians with an interest in nutrition appreciate the connection between nutrient deficiencies and anorexia (Bakan 1984, Birmingham et al. 1994, Humphries et al. 1989, 1990, McClain et al. 1992), many others may not consider nutrient deficiencies as a important factor in eating disorders. A recent review highlighted recent progress in the understanding and treatment of AN and bulimia nervosa (Walsh and Devlin 1998). Although Walsh and Devlin (1998) discussed the possibility that there may be a genetic component to AN, we disagree that there is a lack of an appropriate model to study anorexia. Rodent models using exercise-induced anorexia (Ararich et al. 1995, Dwyer and Boakes 1997) are an option in addition to the nutrient deficiency model described in this review. The recent explosion in the identification of molecular factors involved in obesity and body weight regulation will, we hope, provide insight pertinent to anorexia. Taken together, the contributions of nutrient deficiencies and genetics to the initiation and progression of anorexia in humans will be better understood because of these discoveries.

Appetite regulation. The appetite regulation system consists of both peripheral and central systems. Feedback from the periphery to the brain involves neural feedback, e.g., by the vagus nerve, as well as blood-borne factors, including both metabolites and hormones, which can affect brain function. We have chosen to focus on the central effects of zinc deficiency within the appetite regulation system. This focus is not intended to discount the contribution of the periphery to zinc deficiency–induced anorexia. Indeed, many investigators have shown the effects of zinc deficiency on the physiology of the intestine, adipose and other tissues that may ultimately provide a signal to the brain that is transduced to anorexia (see, e.g., Taneja and Arya 1992). However, a broad hypothesis guiding our research is that regardless of whether the primary lesions of zinc deficiency are peripheral or central, the signals to reduce intake are ultimately integrated or coordinated by central mechanisms. This has been the rationale for the research from our laboratory that focuses on central effects during zinc deficiency.

In the 1970s, it was determined by Wurtman, Fernstrom and colleagues that dietary concentrations of tyrosine and tryptophan could affect the synthesis and concentrations of the neurotransmitters norepinephrine and serotonin. In turn, the diet-affected central concentrations of these neurotransmitters could affect the relative appetite/satiety state of an individual (Fernstrom et al. 1973, Fernstrom and Wurtman 1972, 1974, Gibson and Wurtman 1976, Wurtman et al. 1974, 1978). These findings spurred nutrition researchers to connect zinc deficiency, dietary amino acid intake and anorexia (discussed later). In 1980 it was reported that norepinephrine had a profound influence on feeding behavior within specific sites in the hypothalamus. Leibowitz and Brown (1980) reported that the predominantly inhibitory neurotransmitter norepinephrine had a strong stimulatory effect on food intake. When exogenous norepinephrine was delivered to the paraventricular nucleus (PVN) of the hypothalamus, short-term food intake increased.

Later in the 1980s, neuropeptides were also discovered to have a profound impact on feeding behavior. In 1982, neuropeptide Y (NPY) was first isolated from neural tissue within the porcine intestine (Tatemoto et al. 1982). Soon after, NPY was found to have significant stimulatory effect on food intake (Clark et al. 1984). Although NPY may be synthesized by all neurons within the body, it is synthesized at very high levels within cell bodies derived in the arcuate nucleus of the hypothalamus. A high percentage of these neurons project to the PVN of the hypothalamus. Within the PVN, exogenously administered NPY has been demonstrated to stimulate appetite to a greater degree than any other agent yet tested, when considered on a molar basis (Paez and Myers 1991). Interestingly, it was also found that the administration of NPY to the PVN specifically stimulated carbohydrate intake when rats were allowed to freely select from a three-choice macronutrient diet system (Stanley et al. 1985). Some investigators have also suggested that the results demonstrating an effect of NPY on macronutrient preference may be influenced by the past history or dietary preferences of rats chosen for study (Welch et al. 1994). Even specifics of the diet ingredients used in macronutrient choice studies may influence the results obtained (Glass et al. 1997). Because of its very potent effect on food intake, NPY has been investigated very vigorously at many laboratories. Targets of research have included the effects of NPY, the development of agonists and antagonists of NPY and the identification and study of NPY receptors. The development of an NPY antagonist with an appetite-modulating activity is of interest to pharmaceutical concerns. Consistent with the complex nature of the appetite regulation system, NPY has proved to be a difficult target to study. First, it has been found that there are a family of NPY receptors, and

it is still unclear whether a single NPY receptor or a subset of a few receptors mediate the appetite-generating effect of NPY (Gerald et al. 1996). Second, the NPY knockout mouse regulates food intake in a relatively normal fashion (Erickson et al. 1996a). This has led some to suggest that the large set of physiological studies investigating the effect of NPY on food intake may need to be reconsidered (Palmiter 1998). A possible explanation for normal appetite in the NPY knockout mouse is that NPY action may be accommodated for by other neuropeptides during development. The paradox between physiological data and the NPY knockout results is of great interest and is likely to be further investigated.

Other hypothalamic neuropeptides with a stimulatory effect on appetite have been studied; these peptides include galanin and β-endorphin, which produce their stimulatory effect on appetite within the PVN of the hypothalamus. In addition, galanin has been of interest because of a proposed role in stimulating the preference for the consumption of dietary fat (Temple et al. 1988). There are a growing number of factors to consider in the hypothalamic regulation of food intake; most recently, these new factors include cocaine- and amphetamine-related transcript (Kristensen et al. 1998) and orexin-A and -B (Sakarai et al. 1998). These newly identified factors are regulated with food intake, have been shown to regulate food intake when exogenously administered to the hypothalamus and appear to have a site of action within the lateral hypothalamus. This site of action contrasts with NPY, galanin, β-endorphin and pro-opiomelanocortin, which have a site of action within the PVN. Other important hypothalamic neuropeptides recognized as a part of the hypothalamic regulatory system include corticotropin-releasing hormone, which may be an important antagonist of NPY in the PVN, as well as melanocyte-stimulating hormone (α-MSH) and melanocortin receptors such as MC4-R. Readers are referred to the minireview of Flier and Maratos-Flier (1998) for an excellent overview that integrates how the most recently discovered factors fit into a proposed hypothalamic regulatory system.

Along with the sizable numbers of hypothalamic factors identified in recent years, the discovery of leptin (Zhang et al. 1994) from the *obese* (*Ob*) mutant mouse (Ingalls et al. 1950) has proved to be of importance if appetite regulation in the hypothalamus is to be completely understood. Leptin is a peptide that is produced by adipose tissue and is involved in appetite regulation and body weight maintenance via the regulation of hypothalamic factors. Apparently, leptin travels through the bloodstream, is transported across the blood-brain barrier and produces effects in the brain after binding to specific leptin receptors located in the hypothalamus (Tartaglia et al. 1995). Leptin was found to change NPY levels in the hypothalamus. High levels of leptin, presumably reflecting high or adequate levels of body fat, were found to down-regulate hypothalamic NPY mRNA and NPY levels, which in turn suggests a decrease in appetite in response to the signal of adequate body energy reserves (Erickson et al. 1996b). Similarly, low levels of leptin relate to higher NPY levels, likely stimulating intake to restore energy reserves. However, as the NPY knockout mouse responds to leptin injections with satiety, there must be other targets for leptin in the hypothalamus in addition to NPY. It was subsequently shown that galanin, melanocyte-concentrating hormone, neurotensin and pro-opiomelanocortin are regulated by leptin in addition to NPY (Sahu 1998). Dysregulation of leptin during zinc deficiency has the potential to affect both central and peripheral physiology, in that leptin receptors have been identified within reproductive tissues of the body (Zamorano et al. 1997). This connection may ultimately help us to understand the role of zinc deficiency in reproductive dysfunction.

Zinc deficiency and the regulation of food intake. Beginning in the early 1970s, a large number of studies investigated a possible link between dietary protein or amino acid intake and the anorexia that accompanies zinc deficiency. In early studies (Chesters and Quarterman 1970, Griffith and Alexander 1972), it was shown that lower protein diets appeared to reduce the magnitude of cycling associated with zinc deficiency, mainly by increasing intake during the day of minimal intake within the cycle. However, Griffith and Alexander (1972) also reported that the patterns of plasma amino acid concentrations are changed by zinc deficiency, regardless of dietary protein level. It was later reported that concentrations of norepinephrine in an extract derived from whole brain were higher in extracts prepared from zinc-deficient than in those from zinc-adequate rats (Wallwork et al. 1982). Others (Reeves and O'Dell, 1981a, 1981b) reported no differences in catecholamine levels in whole brain, although samples representing only the hypothalamus were reported as different. Within the brain, Reeves and O'Dell (1981a, 1981b) found no differences in brain levels of tyrosine, tryptophan, catecholamines or serotonin. In contrast, Wallwork and Sandstead (1983) reported an inverse relationship between tyrosine levels and food intake in zinc-deficient rats. In their study, they found no correlation between tryptophan levels and food intake. In their report of 1984, Reeves and O'Dell proposed a relationship between zinc deficiency and catecholamines that suppresses food intake. Kasarkis et al. (1986) also reported increases in norepinephrine concentrations in rats fed a zinc-deficient diet for 10 d but not after 4 d of diet treatment. It is unclear from their report whether food intake was already different at day 4, because food intake was reported cumulatively from days 0–4 and 5–10. Kasarkis et al. (1986) also presented data that inversely correlated concentrations of plasma zinc with changes in food intake. In their review of 1989, O'Dell and Reeves concluded that changes in intake occurred either as a direct result of changing plasma zinc concentrations or indirectly through other agents, including catecholamines, peptides and amino acids.

One important limitation to many of the studies mentioned was that concentrations of brain metabolites, such as amino acids or catecholamines, were determined as a concentration obtained from a homogenate of excised tissue obtained after animals were killed. When considering the effect of catecholamines, for example, it is important to consider active secretion from tissue in addition to total tissue content. If a cellular defect due to zinc deficiency resulted in an impairment of the secretory process, for example, the methods previously described would be unable to detect this result. In fact, if the process of secretion was impaired, it is possible that neurons might attempt to compensate for a reduced effect of catecholamine secretion by increasing the cellular synthesis of these factors. Tissue homogenate would then incorrectly reflect catecholamine effect during zinc deficiency. In these earlier studies, the contribution of receptors to cellular physiology is largely ignored.

An important series of studies was published in 1984 (Essatara et al. 1984a, 1984b, 1984c) in which techniques more appropriate to neurobiological studies were used. Although one of these reports is a more traditional, descriptive study characterizing food intake (Essatara et al. 1984c), the two reports used live rats, and their response to exogenously administered compounds was determined. In Essatara et al. (1984a), the effect of exogenously administered dynorphin was tested in zinc-deficient and zinc-adequate rats. Dynorphin is a

opioid peptide that induces spontaneous feeding in the rat after intracerebroventricular infusion. Zinc-adequate rats responded with a dose-dependent increase in spontaneous feeding after the infusion of 1 and 10 μg of dynorphin into the right ventricle of the rat brain. Zinc-deficient rats were unable to respond to the infusion of 1 μg of dynorphin and responded to the infusion of 10 μg dynorphin less robustly than zinc-adequate rats. As a measure of opiate peptide receptor sites, naloxone binding was quantified. It was found there was a higher binding capability for naloxone in membranes isolated from brain tissue derived from zinc-deficient rats. In Essatara et al. (1984b), the effect of central administration of norepinephrine, the γ-aminobutyric acid agonist muscimol and dopamine agonist bromergocryptine was evaluated in zinc-deficient and control rats. All three compounds were either less effective or unable to produce any stimulatory effect on feeding in zinc-deficient rats. From both studies, Essatara and colleagues suggested that the diminished response of zinc-deficient rats may be due to reduced responsiveness of receptors in the brains of zinc-deficient rats. Although only a very general mechanism is proposed to explain their results, these studies are very important because they demonstrate that mechanistic studies can be performed on animals in vivo to investigate the effects of a nutrient deficiency on normal physiology.

Our laboratory's initial report investigating zinc deficiency and food intake was a macronutrient selection study (Rains and Shay 1995). This simple food intake study was motivated by the macronutrient studies conducted to investigate the effect of centrally administered factors on food intake. Many studies have used the three-choice system, which allows rats to freely choose to eat from three different food cups, each containing essentially pure carbohydrate, protein or fat. The rat, by consuming a number of different meals throughout the day, provides itself with a mixture of carbohydrate, protein and fat that allows it to maintain health and to grow at normal rates. Our Sprague-Dawley rats, when offered three-choice diets containing adequate levels of zinc, choose a very typical pattern of intake consisting of ~70% carbohydrate, ~15% protein and ~15% fat. When similar rats were provided the three-macronutrient diets formulated to provide a deficient level of zinc, the average intake of the group of zinc-deficient rats decreased as expected. Unanticipated results showed that essentially 100% of the reduction in intake was due to reduced consumption of carbohydrate. When zinc-deficient rats were *repleted* in zinc, rats transiently *increased* protein intake for ~2–4 d after zinc repletion. The change in carbohydrate rather than protein intake during zinc deficiency contrasts with the results of Reeves and O'Dell (1981a), who reported that rats fed a zinc-deficient diet reduced their intake of protein when tested in a two-choice system. In our three-choice studies, we found no evidence of changes in protein intake during zinc deficiency; in fact, protein was the most consistently consumed macronutrient during the deficiency period compared with carbohydrate and fat. Because the central administration of NPY has been shown to increase the intake of carbohydrate, we hypothesized that the effect of NPY may be diminished by zinc deficiency. We subsequently chose to study the regulation of NPY during zinc deficiency.

Recently, we investigated food intake behavior of zinc-deficient rats using a two-choice system similar to that described by Reeves and O'Dell (1981). We modified their design to use egg white protein and mixtures of purified amino acids rather than the soy protein used in their study. Interestingly, we found two major differences between our studies and their prior work. First, we were unable, under any conditions, to have Sprague-Dawley outbred rats choose to eat a measurable amount of diet containing 50% protein. Thus, after a series of pilot studies, we prepared two-choice diets containing 10 or 30% protein (or amino acids) for rats to choose from. Second, in four separate and complete trials, two each using egg white protein and complete amino acid mixtures, we found no evidence that rats can selectively decrease protein intake during zinc deficiency (Shay et al. 1998).

Another study from our laboratory (Kennedy et al. 1998) reported the macronutrient preferences of individual rats. In Rains and Shay (1995), we expressed intakes as average values of the zinc-deficient and zinc-adequate groups of rats. However, during those studies, we noticed that some individual zinc-deficient rats, but not zinc-adequate rats, developed a very unusual preference for the consumption of fat. The studies reported by Kennedy et al. (1998) were dedicated to an investigation of these changes in macronutrient selection patterns. The preference for fat, at > 50% of total energy intake, was observed in a subset (~25%) of zinc-deficient rats, and fat preference was never observed in any zinc-adequate rat. However, when this change in preference did occur, there were very significant changes in food intake selection patterns, with some fat-preferring zinc-deficient rats observed to consume > 90% of their total calories from dietary fat. We subsequently identified a set of zinc-deficient fat-preferring rats and assessed their macronutrient intake patterns after normalizing their zinc status. We observed that 50% of the rats reversed their fat-preferring phenotype, but the remaining rats continued preferring dietary fat for 5 wk after zinc repletion, at which time the study was terminated. We speculated that the results observed in these rats might suggest that periods of nutrient deficiencies may cause permanent changes in food intake behaviors, either due to learned responses or because of damage to neurons caused by the nutritional deficiency itself. Whether this speculation may be extrapolated to humans experiencing nutritional deficiencies during a prolonged case of anorexia remains to be demonstrated. We also noted in these studies that a few rats switched from a carbohydrate- to a fat-preferring phenotype as soon as 1–2 d after beginning a zinc-deficiency trial. This switch in macronutrient preference actually preceded the decrease in intake caused by zinc deficiency, which suggests that the change in macronutrient preference may not be linked to anorexia. These results suggest that unusual food cravings or aversions observed in humans might in fact be related to nutrient deficiencies. Because of the relationship suggested by some to exist between preference for fat and hypothalamic galanin, in the report of Kennedy et al. (1998) we chose to measure hypothalamic galanin concentrations in zinc-adequate rats and fat- and carbohydrate-preferring zinc-deficient rats (discussed later).

We characterized NPY levels and responsiveness to NPY during zinc deficiency (Lee et al. 1998). Another report (Selvais et al. 1997) has also provided a great deal of information regarding both NPY and galanin levels during zinc deficiency. Hypothalamic galanin concentrations during zinc deficiency were also reported in Kennedy et al. (1998). Taken together, these three studies provide a consistent description regarding the regulation of NPY and galanin during zinc deficiency–induced anorexia. With respect to NPY, Selvais et al. (1997) reported higher levels of NPY mRNA ($P < 0.01$) but not of NPY peptide levels in the hypothalamus of the zinc-deficient rat. In the report from Lee et al. (1998), NPY mRNA levels were ~100% higher ($P < 0.05$) and NPY peptide levels were ~50% higher ($P < 0.01$) during zinc deficiency. Neither study reported decreases in NPY levels, which might be hypothesized to account for the reduced intake observed during zinc deficiency. Differences in the strain of rat, diet formulation,

length of study and tissue dissection procedures may account for the reported NPY peptide levels. Taken together, these two reports suggest that an NPY "paradox" or "resistance" may exist during zinc deficiency, in that NPY and NPY mRNA levels are elevated yet a physiological situation characterized by low food intake exists. There are possible several explanations for this apparent resistance, such as impairments in the processing of pro-NPY into active NPY, reduced secretion of NPY from neurons and an attenuation of NPY signal transduction. There is some evidence that peptide processing is zinc dependent (Pekary et al. 1991). In Lee et al. (1998), NPY-mediated food intake during zinc deficiency was examined in vivo by delivering exogenous NPY to the PVN of cannulated zinc-deficient and zinc-adequate rats. At doses of 0, 80 and 160 pmol NPY administered bilaterally to the PVN, we could not detect differences in 1-h food intakes after the delivery of NPY to zinc-deficient and zinc-adequate rats. However, in our pilot testing, we observed that zinc-deficient rats were sensitive to infusions of NPY of $>$ 160 pmol. Although zinc-deficient rats did not consume a greater amount of diet compared with zinc-adequate controls at these higher doses, the deficient rats appeared to be highly stressed by these doses of NPY. In light of our results from these pilot tests, we restricted our doses of NPY to $\leq$ 160 pmol, whereas control rats would easily tolerate doses of NPY up to 1000 pmol or more. In some ways, this stress response appears to resemble the results reported by Chesters and Quarterman (1970), who discovered that zinc-deficient rats fed normal levels of diet by gavage did not tolerate this treatment well. The administration of NPY and the stimulation of short-term food intake in the zinc-deficient rat may be equivalent to the delivery of diet by gavage.

Selvais et al. (1997) also reported lower levels of hypothalamic galanin mRNA ($P < 0.001$) but no differences in galanin concentrations during zinc deficiency. In the report of Kennedy et al. (1998), galanin concentrations in the PVN were ~120% higher ($P < 0.05$) in zinc-adequate than in zinc-deficient rats. Galanin mRNA levels were not measured. Again, differences in the design of these two different reports may account for the differences in galanin concentrations reported; however, it appears that there is a consistent trend between galanin and galanin mRNA levels and appetite in these two studies. These data support the premise that galanin is normally regulated during zinc deficiency, in that galanin concentrations reflect the levels of intake of zinc-deficient and zinc-adequate rats. In contrast, NPY levels may be dysregulated by zinc deficiency. These results suggest that other hypothalamic factors such as corticotropin-releasing factor, which is an important counterregulatory factor of NPY, may be playing an important role in the hypothalamus during zinc deficiency.

Zinc deficiency and leptin. Recent studies have investigated the regulation of leptin levels during zinc deficiency. These studies provide useful information in both the rat (Mangian et al., 1998, Ott and Shay, 1998) and humans, (Mantzoros et al. 1998, Ryan et al. 1998). The results of all of these studies are entirely consistent. Circulating leptin concentrations are reduced during zinc deficiency in the rat (Mangian et al. 1998) and in humans (Mantzoros et al. 1998, Ryan et al. 1998). This reduction appears to be due to both a decrease in the amount of body fat present during zinc deficiency (Mangian et al. 1998) and a decrease in the amount of leptin produced per gram of adipose tissue (Ott and Shay, 1998). Reduced levels of leptin as a result of zinc deficiency support the reports of Lee et al. (1998) and Selvais et al. (1997), which show increases in hypothalamic NPY. Circulating leptin levels and hypothalamic NPY are recognized to be inversely related (Schwartz et al. 1996). Reductions in leptin during zinc deficiency likely provide the basis for a new hypothesis relating zinc deficiency to reproductive dysfunction. With the discovery of leptin receptors in reproductive tissues (Barash et al. 1996), a reduction in leptin levels due to zinc deficiency may help explain why certain specific reproductive parameters, such as serum testosterone concentrations (Om and Chung, 1996), are reduced by zinc deficiency. The results of Mantzoros et al. (1998) and Ryan et al. (1998) both demonstrate that zinc deficiency reduces leptin concentrations in humans, suggesting that this finding will be clinically important.

Because the secretion of leptin from adipose tissue is reduced by zinc deficiency (Ott and Shay, 1998) and insulin action is a major factor stimulating the synthesis and secretion of leptin (Barr et al. 1997), we hypothesize that a reduction in the effect of insulin due to zinc deficiency may be partially responsible for the reductions in leptin. From our studies, we obtained other data suggesting that insulin action is reduced during zinc deficiency (Kennedy et al. 1998). This hypothesis is not particularly novel: many studies from the 1970s investigated the link between zinc deficiency and insulin resistance. Recent evidence in humans (Song et al. 1998) and in rats (Tobia et al. 1998) suggests that connection may be important and worth revisiting. If found, a relationship between zinc deficiency and insulin signal transduction pathways, or signal transduction in general, may ultimately help explain many of the physiological pathologies associated with zinc deficiency.

This hypothesis may relate to recent work reported by O'Dell, MacDonald and colleagues (Browning and O'Dell 1995, 1998, MacDonald et al. 1998). Broadly speaking, one of the hypotheses advanced by this research team has been that zinc deficiency may produce a primary defect that is related to growth and that growth failure due to zinc deficiency may be producing a signal ultimately transduced into anorexia. The administration of progestins (Browning et al. 1998) and contributions of zinc deficiency to defects in *N*-methyl-D-aspartate and insulin-like growth factor-I signaling pathways (Browning and O'Dell 1995, MacDonald et al. 1998) have been investigated. It is still unclear how growth impairment feeds back to the hypothalamus. This might be occurring via vagal or a blood-borne message. It also appears that it has not been clearly demonstrated whether growth impairment is occurring before or after anorexia begins. The questions asked by this research group are important, and continued research will provide important answers.

Future directions and priorities for research

Understanding the mechanisms that cause anorexia is a daunting challenge. Interactions among genetic factors, metabolism and neural factors will have to be examined to fully understand this complex pathology. In vivo physiological studies are essential to help understand the contribution of alteration of neuropeptides and neurochemistry to anorexia. The use of agonists and antagonists to neural factors is essential. Within the hypothalamus, it appears that galanin is regulated normally with the reduced intake of zinc deficiency–induced anorexia, whereas NPY is regulated in opposition to decreased intake. A resistance to NPY may exist during zinc deficiency; this possibility must be resolved. Possible avenues of exploration include examination of cellular secretion, signal transduction, peptide processing and regulation of a large number of appetite-regulating neuropeptides during zinc deficiency.

The fact that anorexia nervosa is relatively rare makes the study of readily available populations of patients difficult at

best. If progress is to be made in understanding this disorder, it should be a priority to support the identification and study of human populations, both young and old. In addition, support for the further development of animal models of anorexia, including nutrient deficiency–induced anorexia, should provide additional insight into the etiology of this poorly understood condition.

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