Zinc May Regulate Serum Leptin Concentrations in Humans

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Key words: zinc, leptin, interleukins, tumor necrosis factor, obesity, body fat

Objective: Leptin, the product of the ob gene, plays a key role in a feedback loop that maintains energy balance by signaling the state of energy stores to the brain and by influencing the regulation of appetite and energy metabolism. Zinc also plays an important role in appetite regulation. Thus, we evaluated the relationship between zinc status and the leptin system in humans.

Methods: We studied nine healthy men with marginal zinc deficiency, induced by dietary means, before and after zinc supplementation.

Results: Zinc restriction decreased leptin levels while zinc supplementation of zinc-depleted subjects increased circulating leptin levels. In addition, zinc supplementation increased IL-2 and TNF-α production that could be responsible for the observed increase in leptin concentrations.

Conclusions: Zinc may influence serum leptin levels, possibly by increasing the production of IL-2 and TNF-α.

INTRODUCTION

The regulation of body weight requires a balance between energy intake and energy expenditure. Evidence has accumulated to support the existence of a physiological system that is responsible for the long-term regulation of energy balance and body weight [1,2]. Leptin, the product of the ob gene [2,3] is produced by adipocytes in rodents and humans and its circulating levels reflect energy stores in adipose cells [4,5]. Recent data indicate that administration of leptin increases energy expenditure and decreases appetite [6–9] by decreasing hypothalamic levels of orexigenic neurotransmitters [9,10]. Thus, leptin may not only reflect peripheral adipose tissue mass but may also play a key role in a feedback loop maintaining energy balance by signaling the state of energy stores to the brain and by influencing the regulation of appetite and energy metabolism.

Zinc plays an important role in appetite regulation too [11,12]. In rodents and humans, zinc deficiency decreases appetite, while zinc supplementation increases appetite [12,13]. Furthermore, zinc supplementation of zinc deficient subjects increases their lean body mass while their fat mass either remains stable or decreases, depending on the degree of their baseline zinc deficiency [13,14]. The most widely postulated mechanism for zinc-induced changes in appetite is alteration in hypothalamic neurotransmitter metabolism [12,13,15–20]. Thus, the distinct possibility exists that zinc status could influence the regulation of appetite and metabolism by influencing the leptin system. However, although recent experimental evidence in rodents demonstrates that zinc deficiency decreases, while zinc supplementation increases leptin levels [21] the relationship between zinc status and the leptin system in humans remains unknown.

To assess the possible relationship between zinc and the leptin system we studied nine healthy men with marginal zinc deficiency, induced by dietary means, before and after zinc supplementation.

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MATERIALS AND METHODS

Experimental Design

Study participants were volunteers recruited under the guidelines of the Wayne State University and University of Michigan Human Investigation Committees. Nine normal adult men aged 30±1.6 years (mean±SE) were recruited for induction of mild zinc deficiency by dietary means according to methods previously published [22,23]. All subjects were non-smokers and were deemed healthy on the basis of baseline history, physical and laboratory examination at the University Hospital, University of Michigan, Ann Arbor, MI. All volunteers maintained their routine levels of physical activity throughout the study, but were instructed not to change their exercise patterns during the study. The volunteers ate all their meals at the metabolic Unit of the Clinical Research Center (CRC). Their diets were prepared by the CRC Research Dietitian and were administered under her supervision daily. Subjects received the usual hospital diet for the first month of the study (“run in” phase). The average daily zinc content of this diet (mean±SE) was 12.5±0.08 mg/day. Baseline plasma and cellular zinc concentrations were initially measured 4 weeks after admission to the CRC. Zinc concentrations in plasma, granulocytes, and lymphocytes were found to be in the normal range in six subjects. Three subjects were, however, already marginally zinc deficient at baseline based on cellular zinc criteria (see below).

Experimental Human Zinc Deficiency

In order to induce zinc deficiency, the volunteers were placed on a semipurified diet based on texturized soy products (General Mills Company, Minneapolis, MN). The soy products were washed with 0.5% disodium EDTA, and the EDTA-washed food products were rinsed at least six times with distilled deionized water. Proteins (soy-chicken and soy-hamburgers) used for the low-zinc diet were cooked and prepared with distilled deionized water in bulk and stored in −20°C. The remaining dietary components were purchased in bulk weekly with the exception of vitamin and mineral mixtures. Stored food for each subject was defrosted, weighed and heated in a microwave oven. One g of phytic acid was mixed with lunch and 1 g was mixed with dinner to further decrease the zinc bioavailability [13,22,23]. Addition of phytic acid when mixed with food had no effect on taste and acceptability of food. Vitamins and mineral supplements were provided throughout the study to provide recommended levels as established by the National Research Council for all nutrients except zinc [13,22,23]. The caloric intake of study subjects varied between 2800 to 3680 kcal (mean 3100, SE; 85.53), the distribution of which was 8.5% protein, 31% fat and 60.5% carbohydrate.

The daily dietary intake of zinc during the zinc restricted period ranged from 4.2 to 5.5 mg. The diet supplied all essential nutrients according to RDA except zinc. The average copper intake throughout the study was 2.8 mg/day. Through this technique a deficient state specific for zinc in human volunteers was induced. The goal of the zinc depletion phase was to decrease cellular zinc levels below the previously defined limit for marginal zinc deficiency [22,23]. Briefly, subclinical or marginal zinc deficiency has been defined as the zinc cellular concentrations one SD below the mean for normal young adults. Cellular zinc concentrations were measured every 4 to 6 weeks to document compliance with the diet as reflected by decreasing zinc concentrations. Cellular zinc concentrations at the end of the zinc depletion phase dropped below the previously published limit for marginal zinc deficiency (lymphocytes 50.3 μg/10^10 cells and granulocytes 41.7 μg/10^10 cells). In addition to assessing zinc status (plasma, granulocyte and lymphocyte zinc concentrations) measurement of serum hormone levels was performed at the end of the zinc depletion and repletion phases. Blood was centrifuged immediately and serum was kept at −70°C until the determination of the hormones. The duration of the zinc depletion phase was 4.56±2 months (mean±SD). At the end of the zinc depletion phase, subjects were supplemented with zinc (zinc supplementation phase).

Zinc Supplementation Phase

All subjects participated in the zinc supplementation phase. Subjects received well balanced isocaloric hospital diet during zinc supplementation. Zinc supplementation consisted of 30 mg elemental zinc/day (as zinc acetate) for 12 weeks in five subjects and 60 mg elemental zinc/day (as zinc acetate) in four subjects for 6 to 8 weeks. At the end of supplementation period blood was drawn and zinc concentrations were measured to document adequate zinc repletion, i.e., normalization of their cellular zinc concentrations. One subject was occasionally delinquent in taking his supplement. We have, however, included all subjects for data analysis.

Laboratory Methods

Body Weight and Composition. Body weight was measured and recorded by the Research Dietitian every 4 weeks using standard techniques throughout the study period. Every attempt was made to adjust the caloric need such that the body weight was maintained. Height was also measured and recorded at baseline and the end of the zinc depletion and repletion periods. Body composition was assessed using the bioelectrical impedance technique, a reliable technique that has been used in similar studies in the past [24]. The precision of this test to determine fat mass is within ±3% and its accuracy has consistently been >95% [25]. Additionally, this test has been externally validated against results derived from hydrodensitometry [25].
**Plasma and Cell Zinc Assays.** Zinc was measured in plasma, lymphocytes and granulocytes by previously established techniques with precautions to avoid contamination during collection, preparation and analysis. For determination of zinc in cells we used a Varian spectrAA-40 flameless atomic absorption spectrophotometer equipped with a Zeeman background corrector (Varian Instruments, Palo Alto, CA). The plasma zinc concentration (mean ± SD) for normal subjects of both sexes between the ages of 20 to 50 years in our laboratory is 105.7 ± 8.8 μg/dl. The lymphocyte and granulocyte zinc concentrations for normal subjects of both sexes between the ages of 20 to 50 in our laboratory are 56.0 ± 6.3 μg/10^10 cells and 46.9 ± 5.1 μg/10^10 cells [11,22,23].

**Hormonal Assays.** Early morning venous blood samples were collected from fasted subjects, aliquoted, labeled and centrifuged promptly. Serum was stored at –70°C until determination. Dehydroepiandrosterone sulfate (DHEAS) and insulin were determined in duplicate using commercially available radioimmunoassays (Diagnostic Products Corp., Los Angeles, CA). The sensitivity, intra-assay and interassay coefficients of variation of the assays used were as follows: DHEAS: 1.1 μg/dl, 6.0–9.8%, 4.9–9.5%, and insulin: 5.5 nmol/l, 2.6–5.7% and 4.5–6.3%, respectively. The crossreactivity between the hormones measured has been <0.1%.

Leptin was measured in duplicate by a sensitive and specific radioimmunoassay method using components obtained from Linco (Indianapolis, IN) as described previously [26,27]. The sensitivity of this assay is 0.5 ng/ml and the intra-assay coefficients of variation is 4.4% for low levels (2.9 ng/ml) and 5.7% for high levels (14.1 ng/ml). The interassay coefficients of variation are 6.9% and 9.0%, respectively.

**TNF-α and IL-2 Assays.** Production of TNF-α and IL-2 were assayed by commercially available ELISA kits (Quantikine, R and D systems, Minneapolis, MN) in peripheral blood mononuclear cells (PMNC), isolated by histopaque as previously described [28]. The interassay variation was 7.7 to 9.2% for IL-2 and 2.5 to 5.5% for TNF-α. TNF-α was assayed in all nine subjects. IL-2 assay, however, was done in only six subjects. In three cases the sequential collection of all samples was incomplete.

**Statistical Analysis**

Repeated measures analysis of variance was used for the comparison of baseline data with data from the zinc depletion and repletion phases, with post hoc comparison of data from the zinc depletion with data from the zinc repletion phase. All tests were non-directional (two-tailed probability) and the level of p=0.05 was used as the cut-off point for statistical significance. The statistical package STATVIEW (Berkeley, CA) was employed for the statistical analysis of the data.

**RESULTS**

**Effect of Zinc Depletion and Repletion on Zinc Concentrations and Anthropometric Characteristics**

Zinc concentrations in plasma (baseline: 106.33 ± 5.5 μg/dl), granulocytes (baseline: 43.76 ± 0.06 μg/10^10 cells) and lymphocytes (baseline: 51.99 ± 1.79 μg/10^10 cells) decreased, and achieved their lowest level after 6 months of zinc depletion (88.5 ± 4.44 μg/dl, 40.74 ± 1.12 μg/10^10 cells, and 47.69 ± 0.98 μg/10^10 cells, Table 1). Zinc supplementation resulted in a significant increase of zinc concentrations in plasma (124.36 ± 8.57 μg/dl), granulocytes (46.38 ± 1.71 μg/10^10 cells) and lymphocytes (56.02 ± 1.39 μg/10^10 cells). Fat mass remained unchanged after zinc depletion or repletion.

**Effect of Zinc Depletion and Repletion on TNF-α and IL-2**

The production of both TNF-α and IL-2 were dependent on zinc status. Zinc depletion decreased TNF-α production which was restored towards normal (p<0.01) after zinc supplementation. The production of IL-2 was decreased due to zinc depletion and it increased following zinc supplementation. The

<table>
<thead>
<tr>
<th>Table 1. Mean and SE of Anthropometric Parameters and Zinc at Baseline, After Zinc Depletion and After Zinc Repletion</th>
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</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Age (completed years)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Kg of fat mass</td>
</tr>
<tr>
<td>% Fat mass</td>
</tr>
<tr>
<td>Kg of fat free mass*</td>
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<tr>
<td>Plasma zinc (μg/dl)</td>
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<tr>
<td>Granulocyte zinc (μg/10^10 cells)*</td>
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</tbody>
</table>

Significantly different in all three phases by repeated measures ANOVA, *p<.05, **p<.01.

Significantly different from zinc depletion phase (post-hoc test), *p<.05, **p<.01.

Repeated measures analysis of variance for all parameters except age were done in nine subjects.
statistical significance, however, was borderline due to the small number of study subjects.

**Effect of Zinc Depletion and Repletion on Hormonal Concentrations**

Insulin and DHEAS concentrations did not change with zinc repletion. By contrast, serum leptin concentration changed significantly (p<0.01) in response to zinc depletion and supplementation (Table 2).

**DISCUSSION**

This study investigated the possible interrelationships between two molecules that are involved in the physiologic regulation of energy homeostasis, leptin and zinc. Leptin is a circulating adipocyte-derived molecule [4,5] that stimulates energy expenditure and inhibits appetite through altering neuropeptide concentrations at the level of the hypothalamus [3,5–10].

Marginal zinc deficiency has also been associated with decreased appetite as well as lean body mass that can be restored by zinc administration [12,13]. Although changes of taste acuity and altered membrane fluidity may contribute to zinc’s effect on appetite, the most widely postulated mechanism for zinc-induced changes in appetite is alteration in concentrations of neurotransmitters either in the circulation or locally in the hypothalamus [13,15–17]. To explore a possible effect of zinc status on the leptin system for regulation of hypothalamic neurotransmitters, appetite and body composition in humans, we examined the effect of zinc supplementation on serum leptin concentrations in healthy men in whom a marginal deficiency of zinc was induced by dietary means. Serum leptin concentrations were affected by zinc status significantly. More specifically, leptin levels decreased in response to zinc depletion and increased after zinc supplementation. Importantly, the magnitude of leptin level changes were proportional to the changes of cellular zinc (Table 1).

What could be the underlying mechanism for the increase of leptin concentrations observed in the present study? First, it could be argued that the increased circulating leptin concentrations could simply reflect changes of the energy stores, i.e., fat mass. This is not supported by the data, however, since % fat mass and kg of fat mass of the study subjects remained unchanged. In addition to being proportional to adipocyte triglyceride stores in the fed state, leptin gene expression can also be regulated by glucocorticoids, and insulin [29,30]. Furthermore, cytokines have been recently shown to increase leptin concentrations in rodents [31].

To explore whether endogenous hormones or cytokines might have been responsible for the observed increase of leptin levels, we measured circulating insulin, DHEAS, and production of IL-2 and TNF-α by peripheral blood mononuclear cells. Insulin and DHEAS levels remained unaltered after zinc supplementation indicating that neither increased circulating insulin levels nor an activation of the hypothalamic-adrenal axis could be responsible for the observed increase of leptin concentrations. By contrast, cytokine production rate was significantly increased after zinc supplementation.

Cytokines, including TNF-α and interleukins, have been shown to induce several factors known to regulate appetite, including leptin [31,32]. Cytokines increase expression of the hypothalamic neuropeptides that suppress food intake [32] and in the periphery, cytokines decrease gastric emptying and may regulate glucagon, insulin, CCK and corticosteroid concentrations, all of which influence appetite [31,33]. It is possible that increased TNFα and IL-2 production in response to zinc supplementation may be implicated in the increased circulating leptin concentrations demonstrated in this study. Although in this study changes in IL-2 production due to changes in zinc status were not statistically significant, perhaps due to a small number of subjects, our previous studies have shown that IL-2 production is zinc dependent [13,28]. Zinc has been shown previously to act as a cofactor that regulates the expression of several genes [11,12]. Whether zinc may regulate leptin gene expression directly and/or indirectly, by affecting circulating leptin concentrations through increasing IL-2 and TNF-α levels, remains a matter of speculation at this time.

Irrespective of the precise underlying pathophysiological mechanism, the distinct possibility exists that the increased

**Table 2. Hormonal and Cytokine Concentrations at Baseline, After Zinc Depletion and After Zinc Repletion**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study phase</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Insulin (nmol/l)</td>
<td>5.6 (1.0)</td>
</tr>
<tr>
<td>DHEAS (μg/dl)</td>
<td>364.3 (37.1)</td>
</tr>
<tr>
<td>Interleukin 2α</td>
<td>447.8 (122.8)</td>
</tr>
<tr>
<td>Tumor necrosis factor αRIL</td>
<td>2976.3 (188.8)</td>
</tr>
<tr>
<td>Leptin (ng/ml)αRIL</td>
<td>3.6 (81)</td>
</tr>
</tbody>
</table>

Significantly different in all three phases by repeated measures ANOVA. *p=0.058. **p<0.01.  
Significantly different from zinc depletion phase (post-hoc test) # p=0.08. * p<0.05. ** p<0.01.  
Repeated measures analysis of variance for all parameters was done performed nine subjects except for IL-2 production which was performed including only six subjects due to missing values.
Zinc and Leptin

appetite and energy intake caused by zinc supplementation, in conjunction with simultaneously elevated leptin concentrations, could result in the use of this increased energy intake to build lean body mass and keep fat mass stable and/or decreased. Thus, changes of circulating leptin may contribute pathophysiologically to the body composition changes that have been previously observed after zinc supplementation [13–15]. However, this notion remains to be further elucidated by future, larger studies. Indeed, given the difficulties encountered in performing such a long metabolic study, the number of participating subjects is rather small. However, the relatively small sample size could not have affected the validity of the data presented here. Finally, the findings reported here may have public health implications. Recent reports indicate that mild or marginal zinc deficiency in man is probably widespread and common throughout the world, including the US [34]. A western diet, by providing less than the RDA recommended amount of zinc, may place the whole population in general, and more specifically certain groups in which zinc absorption is also reduced, such as children [35] or the elderly [36] at risk of significant zinc deficiency.

In summary, it appears that zinc supplementation of zinc depleted subjects, in addition to its well known effects on appetite and body composition, may increase circulating leptin levels. The pathophysiological pathways by which zinc and leptin regulate energy intake and body composition need to be further elucidated by future studies.

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