Zinc Intake and Biochemical Markers of Bone Turnover in Type 1 Diabetes

Raelene E. Maser, PhD1,2; John N. Stabley, MS3; M. James Lenhard, MD2; Phyllis Owusu-Griffin, MD2; Michelle A. Provost-Craig, PhD3; William B. Farquhar, PhD3

1Department of Medical Technology, University of Delaware, Newark, DE
2Diabetes and Metabolic Research Center, Christiana Care Health Services, Newark, DE
3Department of Health, Nutrition, and Exercise Sciences, University of Delaware, Newark, DE

Address correspondence to:
Raelene E. Maser, Ph.D.
E-Mail: rmaser@udel.edu

Submitted 12 June 2008 and accepted 2 September 2008.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Objective – To examine the relationship between zinc nutritive status and biochemical markers of bone turnover in type 1 diabetes.

Research design and methods - Serum osteocalcin, urine N-telopeptides, and dietary intake data, obtained by 3-day food records, were assessed for 66 individuals with type 1 diabetes.

Results - Zinc intake correlated with osteocalcin in the group overall (r=0.48, p<0.001) but not with N-telopeptides. Examined by gender, zinc and osteocalcin correlated for men (r=0.57, p<0.001), but did not reach statistical significance for women (r=0.34, p=0.09). A direct-entry linear regression model with osteocalcin as the dependent variable was performed. Duration, gender, HbA_{1c}, insulin use/kg, total calorie intake and zinc intake were entered as potential independent variables. The model was statistically significant (R^2=0.32, p<0.01). Zinc intake (p<0.001), however, was the only independent correlate of osteocalcin.

Conclusions – This study provides evidence of a positive relationship between zinc intake and osteocalcin in type 1 diabetes.
Zinc is important in bone metabolism (1). Work in cell cultures and animal models have shown stimulation of osteoblasts by zinc (2) while osteoclastic cell formation was inhibited (3). Reduced zinc levels associated with decreased bone mineral content (BMC) have been observed for type 1 diabetic individuals (4). To our knowledge, there have been no studies in type 1 diabetes that examined zinc nutritive status and biochemical markers of bone turnover.

RESEARCH DESIGN AND METHODS
Sixty-six type 1 diabetic participants (age=42±10 years) were evaluated in the Human Performance Laboratory, University of Delaware, Newark, DE. This study had approval of the Institutional Review Board of the University of Delaware. Individuals with possible secondary causes of osteoporosis (e.g., hyperparathyroidism) were excluded although 7 did have borderline decreased vitamin D levels but normal parathyroid hormone levels. Analysis of the data omitting these subjects produced similar results, and thus they were included in the study cohort.

Women who were in menopause were excluded from participation. Menopausal status was based on self-reported frequency of menstrual cycles.

Participants recorded their dietary intake for 3 days. Nutrient content was determined with the Food Processor Nutrition Analysis and Fitness software package (Version 8.0, ESHA Research, Salem, OR).

Blood and urine samples were collected in the morning following an overnight fast. Serum osteocalcin was measured via an immunoradiometric assay. Urine N-telopeptides was determined via a Vitros ECi competitive assay on a spot morning sample.

Univariate analyses included the Student’s t test, chi-square, and Pearson correlations. Linear regression assessed potential independent associations for markers of bone turnover (i.e., dependent variable).

RESULTS
Biomarkers of bone turnover stratified by gender are presented in Table 1. Participants consumed, on average, slightly more zinc than the daily recommended dietary allowance (RDA) (i.e., RDA=11 mg/day for men and 8 mg/day for women). It should be noted, however, that approximately ⅓ of the individuals demonstrated values less than the RDA. Osteocalcin levels were lower for individuals with zinc intake levels below the RDA (15.9±5 (n=21) versus 19.6±6 ng/mL (n=45), p<0.05).

Zinc intake correlated with osteocalcin in the group overall (r=0.48, p<0.001). When examined by gender, zinc intake and osteocalcin levels were highly correlated for men (r=0.57, p<0.001), but did not reach statistical significance for women (r=0.34, p=0.09). No significant correlations were observed for N-telopeptides and zinc.

A direct-entry linear regression model, with osteocalcin as the dependent variable, was performed. With duration of diabetes, gender, HbA1c, insulin use/kg, total calorie intake and zinc entered as potential independent variables, the overall model was significant (R²=0.32, p<0.01). Zinc intake (p<0.001), however, was the only independent correlate of osteocalcin, while gender was borderline statistically significant (p=0.061). A potential interaction between gender and zinc intake was investigated and found not to be significant. In gender specific models, controlling for the other variables, zinc intake (p<0.01) was independently associated with osteocalcin for men (R²=0.34, p<0.05 for the model) but not for women. We also examined the R² for the model when magnesium, phosphorus, or calcium was entered as a potential independent variable replacing zinc. No other micronutrient
produced as strong a $R^2$ for the regression model as did zinc and only phosphorus was independently associated with osteocalcin ($p<0.05$). No independent association was found for zinc when N-telopeptides was used as the dependent variable.

CONCLUSIONS

This study indicates that zinc intake is associated with a marker of bone turnover. While it has been shown that zinc stimulates osteoblasts and the ZENITH study (5) showed some, albeit inconsistent, evidence of a relationship between zinc nutritive status and bone turnover, to our knowledge this is the first study in type 1 diabetes that shows an independent association for zinc intake and osteocalcin. Our results, however, suggest that this relationship may be stronger for men than for women.

Zinc plays several roles in bone metabolism. Zinc stimulates bone protein synthesis and formation in tissue cultures (2). The anabolic effect of insulin-like growth factor I in osteoblasts is enhanced by zinc (6). Zinc deficiency, however, impairs DNA synthesis and protein metabolism, negatively impacting bone formation (1). In type 1 diabetic individuals with poor glycemic control, Arreola et al. (4) showed a significant decrease in BMC and zinc suggesting that zinc deficiency may be a contributory factor to bone loss. Some have suggested that zinc deficiency leads to an increase in free radical production (7). Oxidative stress has been shown to be an independent risk factor for osteoporosis (8).

Why zinc intake appears to be more associated with osteocalcin for men when compared to women is not clear. It may be because the women in our study were not in menopause. Herzberg et al. (9) showed that urinary discharge of zinc is increased in post-menopausal women with osteoporosis. Thus, perhaps the role of zinc in bone metabolism plays a larger role for women once they have reached menopause. It should be noted that there was no significant association of age with markers of bone turnover for either gender.

No association between zinc intake and N-telopeptides was noted. Only one morning urine sample was collected for N-telopeptides which may explain a lack of an association since there is a large intra-individual variability for N-telopeptides.

This study provides evidence of a relationship between zinc and a marker of bone turnover in type 1 diabetes. The cross-sectional nature of the study indicates associations and causality remains to be clarified. A second limitation, for 9 of 25 subjects taking a multivitamin the exact zinc content of their supplement could not be determined. A common multivitamin with an average zinc, calcium, magnesium and phosphorus content was assigned to these subjects. This could have attenuated the associations potentially obscuring one between N-telopeptides and zinc intake. Multivariate analysis omitting these subjects produced similar results.

While the importance of higher levels of osteocalcin with regard to bone health is not clear, dietary factors are modifiable. Given that an inadequate intake of zinc has been reported as a risk factor for fractures in men (10), zinc may be important in reducing this risk in type 1 diabetes.
REFERENCES

Table 1 - Bone biomarkers and zinc intake levels for the study cohort (n=66)

<table>
<thead>
<tr>
<th></th>
<th>Men (n=39)</th>
<th>Women (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>19.9±5.5</td>
<td>16.2±5.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>N-telopeptides (nmol/mmol)*</td>
<td>31.2±12.8</td>
<td>25.1±10.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>15.8±9.0</td>
<td>14.2±7.1</td>
<td>NS</td>
</tr>
<tr>
<td>n (%) of subjects below the RDA for zinc intake per day</td>
<td>15 (38)</td>
<td>6 (22)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SD. NS is non-significant. *Units of measurement for urine N-telopeptides are nmol bone collagen equivalents/nmol creatinine.