Effects of Calcium Plus Copper and Silicon on Bone Marker Values in Young Adult Women Starting Resistance Training Exercise

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation with distinction in Human Nutrition in the College of Human Ecology at The Ohio State University

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June 2006

Project Advisor: Robert DiSilvestro, Ph.D, Professor Department of Human Nutrition
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It is well-accepted that calcium supplementation in combination with resistance exercise contribute to net bone formation. However, this fact has been supported with limited research. This study seeks to reinforce this accepted knowledge. In addition, copper and/or silicon supplementation in addition to calcium supplementation may enhance the effects of calcium supplementation alone. This effect may be through improved absorption or function and has yet to be proven. Silicon, in fact, is not presently considered to be an essential nutrient, although it may become one. This study also seeks to find evidence that one or both of these supplements may improve the effects of calcium on net bone formation. Such data would be useful in the treatment and prevention of osteoporosis.
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Hypothesis: This project attempts to demonstrate that copper and silicon enhance the effects of calcium supplementation and resistance exercises on biochemical markers of bone metabolism.

Background: The ability of calcium supplementation and resistance training to improve bone mass is widely published. The most common problem that results from poor bone health is osteoporosis. Osteoporosis (Latin for "porous bones") is a disease characterized by defects in bone remodeling and loss of normally mineralized bone, resulting in bone fragility and increased susceptibility to fractures.

The main emphasis on diet and osteoporosis has been for calcium, but there are other minerals that also can be involved in bone health. Minerals such as copper and silicon can influence bones via multiple mechanisms that compliment, but don’t overlap calcium. It may be especially useful to study copper and silicon together since silicon may affect bone health in part by enhancing copper function.

Major health problems can arise as a result of low bone mass. However, osteoporosis is the predominant concern. Women are at higher risk for osteoporosis than men. This research is intended to provide women with new tools and recommendations with which to prevent osteoporosis. (Prevention is very desirable since treatment of the disease is relatively limited to slowing its progression.)
Bone metabolism is a dynamic process characterized by constant breakdown (resorption) and formation. In a normal, healthy adult the two processes should be in balance. If a person is losing bone mass, they are forming less than they are breaking down. In young people still increasing bone mass, formation should outweigh breakdown. This process is especially important for the women in this study because peak bone mass is typically attained by thirty years of age. The women in the study were in their late teens and early twenties, a time during which gaining bone mass is of great importance. If peak bone mass is not attained while young, osteoporosis is much more likely in later life.

**Design:** Subjects were recruited via word of mouth, class announcements, and fliers. Interested participants filled out a survey and submitted it via email to the researchers. In order to be approved for participation in the study, subjects were required to be females between the ages of 18 and 24. A total of 59 subjects were included.

Subjects were also asked to disclose any medications or nutritional supplements that they were currently taking. Subjects were excluded from the study if they were taking a medication or supplement that could have had an affect on bone metabolism or markers of bone metabolism. In addition, the potential subjects disclosed their current consumption levels of dairy products to ensure that they were not consuming abnormally high or low levels through their diet. Coffee, soda, and tea consumption was disclosed to ensure that subjects were not consuming inordinate amounts of caffeine in the diet. High caffeine consumption can lead to increased calcium loss due to the diuretic effect. Subjects disclosed their prior exercise experience. Women who were already involved in
regular weight training were excluded from the study because well-trained people do not experience fitness gains as quickly, and it was less likely that a difference would have been observed over the eight week period than in untrained subjects. Finally, subjects were asked to disclose their height and weight data so that the study could be limited to women with normal range BMI values.

Once the final subject approvals had been completed, blood and urine samples were taken from the participants on day one of the study. Samples were taken early in the morning before the subjects had eaten in order to standardize the effects of food consumption on bone metabolism markers across subjects.

Subjects were then divided into one of four groups based on the type of supplements that they would receive. They were either placebo, Bone-Up, Bone-Up + Silicon, or Silicon. The Bone-Up supplement was a predominantly calcium containing supplement (1000 mg of Calcium phosphate) with several additional vitamins and minerals including Magnesium (600 mg Magnesium oxide), Zinc (10 mg as Monomethionine) Manganese (5 mg as Manganese citrate), Copper (1 mg as copper citrate), Boron (3 mg as Sodium borate), Vitamin C (200 mg Ascorbic acid), Vitamin D3 (400 IU cholecalciferol), and Vitamin K1 (100 mcg phylloquinone) to enhance the absorption and function of the calcium. Five mg of silicon was given as orthosilicic acid to the Silicon (Bio-Sil) group. Supplements were taken daily for eight weeks.
Over the course of the same eight week period, subjects were required to perform a series of eight resistance training exercises three times per week. The following exercises were used: bicep curls, shoulder press, bench press, seated row, calf raises, leg extensions, leg curls, and crunches. The workout was designed to provide weight resistance exercise that would work all of the major muscle groups. Subjects were instructed to complete three sets of 8-12 repetitions of all exercises except the calf raises and crunches. (For these they performed one long set of as many reps as necessary to exhaustion.) They were instructed to use enough weight so that they could just finish their sets and should feel that they could not perform more repetitions when done. When their present level became easy enough, they were instructed first to increase the number of repetitions of each exercise per set. When they reached twelve repetitions, they were instructed to increase the amount of weight used and decrease their repetitions if necessary, gradually working their way back up and repeating the process. They recorded their exercise in a log for each session.

At the completion of eight weeks, the subjects’ blood and urine samples were taken again. They were taken in the morning before breakfast for consistency with the original draw and with one another.

To analyze the data, several assays were run in the lab. These have included to present, urinary deoxypyridinoline (DPD), plasma bone-specific alkaline phosphatase, (BAP), and plasma PPD. Vitamin D and Calcium status were also measured by means of 25-OH-vitamin D and parathyroid hormone.
Results: Urinary DPD is a measure of bone breakdown. Increased levels of this substance in the blood indicate increased bone breakdown. DPD helps to form the cross-links between the principal building blocks of bone, mature type I collagen, a triple helical protein that is the predominant organic matter in bone. When the bone is broken down, the DPD is released into circulation and excreted in the urine. The level was measured relative to urinary creatinine to adjust for concentration resulting from hydration status. In this study, the Bone-Up group saw a significant decrease in urinary DPD relative to the placebo group. However, the Bone-Up + Silicon and the Silicon groups did not show additional decreases. This means that the Bone-Up group experienced a significant decrease in bone breakdown.

Plasma BAP is a measure of bone formation. This substance is used in osteoblasts during bone formation. (Osteoblasts are the type of bone cells that work to form bone.) When osteoblasts are engaged in bone formation, they require BAP from the plasma. Hence, a decrease in plasma BAP indicates an increase in bone formation. In this study, the Bone-Up group saw significantly decreased levels of plasma BAP relative to the placebo group, indicating an increase in bone formation. Again, the Bone-Up + Silicon and the Silicon groups did not show significantly greater increases in bone formation.

Plasma PPD reflects plasma ceruloplasmin concentration. Plasma ceruloplasmin is a marker of copper status. We measured copper status to ensure that the Bone-Up group did not receive significant increases in copper status from the trivial amount included in
the supplement. If the copper status had changed as a result of Bone-Up concentration, it would have been necessary to consider whether the copper in the supplement had affected bone metabolism. The ceruloplasmin concentrations did not significantly change in the Bone-Up group, indicating that copper status did not have a role in the improvements made in bone metabolism. When PPD tests are performed with a better absorbed form of copper in higher quantities, the results demonstrate that plasma copper status does actually increase. See the attached graph in the appendix to observe how copper status changed with supplementation of 2 mg of copper glycinate. This data is included to prove the effectiveness of the test itself.

Finally, parathyroid hormone levels were measured to determine calcium and Vitamin D status. Parathyroid hormone levels decrease when calcium levels are adequate. It was determined that Bone-Up did significantly increase calcium and Vitamin D status according to this marker.

**Statistical Analysis:** For each of the assessments, in each treatment group, pre-treatment values were compared to post-treatment values by paired Student's t-test (significance at p < 0.05). These comparisons will indicate whether or not each treatment had any significant effects. Anytime more than one treatment group shows a significant change in an assessment, further analysis will be done. This analysis tested the hypothesis that the degree of change differs between treatments. Here, the first step is to express the values for each of the four treatment groups as changes caused by each treatment (i.e. subtract the initial reading from the final reading for each subject). The changes for each
treatment group are then compared to the changes in the other groups by ANOVA + Tukey comparison (p < 0.05 considered significant).

**Conclusion:** Additional factors would be helpful in determining the meaning of these results. For example, the amount of silicon actually absorbed by the participants would be a valuable piece of information. If a larger dose of silicon had been administered or if it would be absorbed better in another form, there is a chance that significant results would have been observed in both of the previously mentioned assays.

The following can be concluded: As expected, the group taking calcium supplements and performing resistance exercise improved their net bone formation by increasing formation and decreasing resorption. The placebo group, relying on resistance exercise alone, did not improve, perhaps because of the short time frame of the study. The Bone-Up + Silicon and the Silicon groups also saw no increases in net bone formation other than those due to the Bone-Up alone as observed in the Bone-Up + Silicon group.
References

Urinary DPD, A Marker of Bone Breakdown

PLACEBO

PRE

POST

* p<0.05, paired t-test

BONEUP

PRE

POST

*p<0.05, paired t-test
Plasma BAP, A Measure Of Bone Synthesis

*P<0.01, paired t test
Plasma Ceruloplasmin, A Marker of Copper Status

Part of a different study, shown for comparison purposes, $P<0.05$, paired t test
Plasma BAP, A Marker of Bone Synthesis

![Graph showing plasma BAP levels for PLACEBO, SILICON, and SILICON+BONEUP. PLACEBO shows no significant effects.]

- PLACEBO: No Significant Effects
- SILICON: No Significant Effects
- SILICON+BONEUP: Positive effects
Urinary DPD, A Marker Of Bone Breakdown

PLACEBO  
PRE  POST

SILICON  
PRE  POST
No Significant Effects

SILICON+BONEUP  
PRE  POST