7-2012

High Serum Cu and Cu/Zn Ratios Correlate with Impairments in Bone Density, Physical Performance and Overall Health in a Population of Elderly Men With Frailty Characteristics

Eric D. Gaier
University of Connecticut School of Medicine and Dentistry

Alison Kleppinger
University of Connecticut School of Medicine and Dentistry

Richard E. Mains
University of Connecticut School of Medicine and Dentistry

Anne M. Kenny
University of Connecticut School of Medicine and Dentistry

Betty A. Eipper
University of Connecticut School of Medicine and Dentistry

Published in final edited form as:
Exp Gerontol. 2012 July ; 47(7): 491–496. doi:10.1016/j.exger.2012.03.014.

High serum Cu and Cu/Zn ratios correlate with impairments in bone density, physical performance and overall health in a population of elderly men with frailty characteristics

Eric D Gaier[a], **Alison Kleppinger**[b], **Martina Ralle**[c], **Richard E Mains**[a], **Anne M Kenny**[b,*], and **Betty A Eipper**[a]

[a]Department of Neuroscience, University of Connecticut Health Center, Farmington, CT 06030, USA

[b]Center on Aging, University of Connecticut Health Center, Farmington, CT 06030, USA

[c]Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, OR 97239, USA

Abstract

Serum Cu levels rise with age and high Cu/Zn ratios are linked with multiple-cause mortality in the elderly. The relationships of these parameters to measures of musculoskeletal health and frailty have not yet been analyzed. We used inductively coupled mass spectrometry to assess serum levels of Cu and Zn and probed for relationships between serum Cu levels and the Cu/Zn ratio with specific measures of bone, physical and overall health in a cohort of 144 frail elderly men. Subjects were divided into quintiles based on serum metal levels and comparisons for functional measures were made between the reference (middle) group and the low and high groups. Subjects' serum metal values were normally distributed. We found significant correlations between high Cu/Zn ratios and deficits in femoral bone mineral density, measures of speed and strength, muscle mass and hematocrit. High Cu/Zn ratios were also correlated with decreased triglycerides and increased reliance on ADL assistance. This study identifies specific deficits associated with high Cu/Zn ratios that span multiple organ systems and supports earlier studies indicating that serum Cu levels and the Cu/Zn ratio may serve as useful predictive biomarkers for poor health in the elderly.

Keywords

Copper; Zinc; Bone Mineral Density; Muscle Strength; ADL; Hematocrit

1. Introduction

Cu is essential to life; its redox activity contributes to its utility as a co-factor for electron transfer reactions. Cuproenzymes like cytochrome c oxidase, Cu/Zn-dependent superoxide dismutase, lysyl oxidase, tyrosinase, dopamine β-hydroxylase and peptidylglycine α-amidating monooxygenase are essential to the maintenance of multiple organ systems (Uriu-Adams et al. 2005). Many other proteins bind Cu and play a role in Cu transport and homeostasis. Cu deficiency affects the cardiovascular, musculoskeletal, hematopoietic and nervous systems (Uauy et al. 1998; Uriu-Adams et al. 2005). It is now clear that a complex

[*]**Corresponding Author:** Kenny, Anne M Kenny@nso1.uchc.udu 263 Farmington Ave Farmington, CT 06030-5215 Telephone: 860-679-3956 Fax: 860-679-1307.

regulatory system governs Cu homeostasis to keep availability of Cu at optimal levels (Kim et al. 2010).

Cu overload, which can result in oxidative stress and the generation of free-radicals that contribute to chronic/degenerative diseases and carcinogenesis, is also detrimental (Uriu-Adams et al. 2005). High serum Cu concentrations have been linked to advancement of chronic diseases such as diabetes mellitus and Alzheimer's disease (Uriu-Adams et al. 2005; Mezzetti et al. 1998; Arnal et al. 2010; Viktorinova et al. 2009). Previous studies linked high serum Cu and concomitant low serum Zn, another essential element, to mortality in elderly populations (Leone et al. 2006; Malavolta et al. 2010; Reunanen et al. 1996). Zn competes with Cu for uptake in the gut and is thought to serve as a natural antioxidant (Uriu-Adams et al. 2005; Malavolta et al. 2010). In several cases, the Cu/Zn ratio proved to be a better predictor of disease severity and/or mortality than Cu levels (Leone et al. 2006; Malavolta et al. 2010; Reunanen et al. 1996; Mezzetti et al. 1998).

Frailty is a geriatric syndrome characterized by low functional and physiologic reserve resulting in increased vulnerability to stressors and poor health outcomes, such as fractures, hospitalization and death (Fried et al. 2001; Bortz 2002; Lipsitz 2002; Hamerman 1999). The physiologic and clinical findings that characterize frailty are not likely to be due to changes in a single system but to the interaction of several systems resulting in a global decline (Ford et al. 2003). Sensitivity to nutritional and physiological challenges increases with age, as do serum Cu levels (Malavolta et al. 2010; Mezzetti et al. 1998). Serum Cu, Zn and Cu/Zn ratios have been explored as biomarkers (Malavolta et al. 2010) and may well be markers of frailty. We searched for a relationship between Cu, Zn and Cu/Zn ratio and physical and mental measures of well-being in a population of frail elderly men to test the hypothesis that low and/or high Cu levels or abnormal Cu/Zn ratios were associated with poor physiologic performance and could contribute to the frailty syndrome.

2. Methods

2. 1 Study Population

Frail men aged 60 years or older residing in the community or assisted living were recruited to participate in the study. The individuals were screened for potential participation in a previously reported study to assess testosterone effects on bone and frailty in men (Kenny et al. 2010). The data used in this analysis are baseline assessments. Exclusion criteria: [1] Diseases or medications known to affect bone or muscle metabolism (i.e. Paget's disease, osteomalacia, hyperparathyroidism: current use of corticosteroids, calcitonin, heparin, phenytoin, phenobarbital, methotrexane, bisphosphonates, selective estrogen receptor modulator or PTH); [2] Use of estrogen, DHEA or androgen in the preceding year; [3] Metastatic or advanced cancer (other than skin cancer); [4] History of prostate cancer; [5] Active cardiac ischemia by history of angina or myocardial infarction in the preceding 6 months; [6] Elevation of PSA; [7] History of sleep apnea; [8] Polycythemia. All study participants provided written informed consent. The Institutional Review Board at the University of Connecticut Health Center approved the study.

2.2 Independent Variables

Demographic characteristics collected included age, BMI, and dietary intake by 3 day food record. Dietary records were analyzed using Food Processor (version8.1; Salem, OR). Bone assessment included BMD via dual energy absorptiometry (BMD; Lunar DPX-L, Madison, WI; coefficient of variation of BMD measurement at the proximal femur was <1%), bone turnover markers (bone formation [BAP, PINP and OC]; markers of bone resorption [NTX of type I collagen and free DPD], calcium regulating hormone levels [25OHD and PTH],

and sex hormones (testosterone, estradiol and DHEA) (Kenny et al. 2010). Body composition ([LBM (kg)], total fat mass (kg)) were obtained (Lunar DPX-L, Madison, WI). ASM was determined by combining the lean tissue mass of the arms and legs, excluding all other regions from analysis (Wang et al. 1996). We adjusted ASM for height by dividing each by height^2 (m^2). Physical function assessment including frailty evaluation was done as described (Fried et al. 2001): frail = 3-5 characteristics; intermediate frail =1-2 characteristics; non-frail = 0 characteristics. Leg extension strength [1 repetition maximum (Judge et al. 1996); intra- and inter-tester variability <10%] and power were measured on the Keiser Sitting Leg Press. Handgrip strength was measured by Jamar dynamometer. Physical performance was assessed by the SPPB [ability to rise from a chair, static balance and 8 foot walk] (Guralnik et al. 1994), and by the Get-Up-and-Go test (Podsiadlo et al. 1991). Other health measures included Katz ADLs and IADLs (6), cholesterol panel (6), hematocrit (6) and medical history.

2.3 Measurement of serum Cu and Zn levels

Inductively coupled plasma mass spectrometry analysis was performed using an Agilent 7700x equipped with an ASX 250 autosampler at a radio frequency power 1550 W, argon plasma gas flow rate 15 L/min, and Ar carrier gas flow rate of 1.04 L/min. Cu, Fe and Zn were measured in kinetic energy discrimination mode using He gas (4.3 mL/min). For analysis, serum samples were diluted 25-fold into 1% HNO_3 (Fisher Scientific). Data were quantified using a 5-point (0-1000 ppb (ng/g)) calibration curve with external standards. For each sample, data were acquired in triplicate and averaged. An internal standard (Er) introduced with the sample was used to monitor for plasma instabilities. Cp activity was measured using *o*-dianisidine HCl as the substrate (Prohaska 1991). For analysis, subjects were divided into quintiles based on serum Cu and the Cu/Zn ratio, with comparisons made between the lowest or highest quintile and the reference (middle quintile). Quintile ranges were as follows: Serum Cu Low (<836.7 ppb), Reference (906.4-1013.9 ppb), High (>1193.1 ppb); Cu/Zn Ratio Low (<0.93), Reference (1.12-1.27), High (>1.51).

2.4 Statistical Analysis

Baseline and clinical characteristics were reported using percentages, means and standard deviations. All variables were checked for normal distribution and the impact of outliers. Outliers beyond 3 standard deviations were dropped from the analyses (n=3). Correlation coefficients were used to detect preliminary associations of serum Cu with other measures of interest. Quintiles for serum metals and the Cu/Zn ratio were defined proportionately and separate independent t-tests were used to compare the means of the extreme quintiles to the reference quintile. Statistical analyses were performed using SPSS version 18.0 (Chicago, IL).

3. Results

3.1 Cu, Zn and the Cu/Zn Ratio

Baseline information and nutritional data for the study population appear in Table 1. Subjects consumed a mean of 1.21 ± 1.07 mg of Cu and 12.9 ± 10.6 mg of Zn per day. Serum levels of Cu, Zn and the Cu/Zn ratios were normally distributed among subjects; the Cu/Zn ratio data are shown in Fig.1. Dietary intake of Cu and Zn shared no statistical relationship with serum values (not shown). As expected, Cp activity correlated tightly with serum Cu (not shown). Indicators of bone health, fitness and overall health were compared to Cu, Zn and Cu/Zn ratios; comparisons to the Cu/Zn ratio proved to be the most informative.

3.2 Cu/Zn Ratio and Bone Parameters

Mean BMD and factors contributing to BMD were compared across serum Cu/Zn ratios (Table 2). The mean femoral neck BMD t-score was −2.12 ± 0.75 g/cm^2. Bone turnover markers, sex hormone levels (data not shown), 25OHD and PTH were typical of levels found in older men (Kenny et al. 2010). Deficits in BMD were found in individuals in the high Cu/Zn ratio group (Table 2 and Fig.2A). Femoral neck BMD was also lower in subjects in the high Cu quintile (0.747 ± 0.113 g/cm^2 compared to 0.806 ± 0.070 g/cm^2; p=0.031 [not shown]). No significant associations were found with bone sex hormones or turnover markers (not shown). PTH was significantly elevated in the high Cu/Zn ratio group (Table 2 and Fig.2B), though no association was found with 25OHD levels.

3.3 Cu/Zn Ratio and Physical Parameters

Most men in the study group (91%) met the criteria for frailty (18%) or prefrailty (72%). Approximately 50% had ASM/Ht^2 values that met criteria for sarcopenia or low muscle mass associated with aging (Baumgartner et al. 1998). Multiple physical measures, including whole body lean mass, lean mass corrected for height, handgrip strength, lower extremity strength and power, and the SPPB, a composite score of lower extremity function, were impaired in those in the highest quartile of Cu/Zn (Table 3 and Fig.3). Subjects in the high serum Cu quintile displayed slower best walk speeds (0.76 + 0.26 vs. 0.94 + 0.24 m/sec; p=0.015) and longer Get-Up-and-Go times (15.6 + 7.2 vs. 11.2 + 3.4 sec; p=0.008 [not shown]). These relationships did not reach statistical relationships for the Cu/Zn ratio (Table 3).

3.4 Cu/Zn Ratio and Overall Health

Overall, the men in the study group were community dwelling but had some limitation in function, with 20% requiring assistance in IADLs and 12% requiring ADL assistance. High Cu/Zn ratio subjects had an increased need for ADL assistance (Table 4). High Cu/Zn ratio subjects had significantly lower total cholesterol and triglyceride levels (Table 4 and Fig. 4A). Hematocrit values were significantly lower in both the high serum Cu (38.5 + 4.2 vs. 41.5 + 4.2 %; p=0.030 [not shown]) and Cu/Zn ratio (Table 4 and Fig.4B) groups. There was a significant reduction in serum Fe for subjects in the high Cu/Zn ratio group, but not for subjects in the high serum Cu group (values). Subjects in these high metal groups did not have daily dietary Fe intakes different from their respective references (not shown). No significant differences in hematocrit were found for the low Cu (43.5 + 3.2 vs. 41.5 + 5.0%; p= 0.08) or low Cu/Zn ratio groups (Table 4).

4. Discussion

In the present study we found significant relationships between high Cu/Zn ratios with lower BMD, lean mass, strength and power, lower extremity function, cholesterol, hematocrit, and ADLs. The measures represent clinically relevant indicators of health and independence in a cohort of frail elderly men. Several recent studies demonstrated a clear link between increased serum Cu levels and/or Cu/Zn ratios with several progressive, degenerative diseases (Arnal et al. 2010; Viktorinova et al. 2009) and increased risk for all-cause mortality in the elderly (Leone et al. 2006; Malavolta et al. 2010). Consistent with previous reports, we found more consistent and robust associations with serum Cu/Zn ratios than with Cu levels alone. Our results support use of the Cu/Zn ratio as a functional and predictive biomarker for overall health, independence and frailty (Malavolta et al. 2010).

The importance of Cu in bone health has been studied in the context of Cu deficiency and osteoporosis (Palacios 2006; Chaudhri et al. 2009; Lowe et al. 2002). Chaudhri et al. (Chaudhri et al. 2009) found a linear relationship between serum Cu values and bone density

in post-menopausal women. By contrast, the men in our study with low serum Cu levels had BMD similar to those of the reference group. Those with high serum Cu and a high Cu/Zn ratio had lower BMDs, possibly due to elevated PTH and low 25OHD. These conflicting results may reflect sex differences in age-associated bone loss or may be due to our evaluation of an older, more frail population. Nielsen et al. recently found that supplementing postmenopausal women with Cu and Zn resulted in loss of BMD in those consuming adequate dietary Zn, while Zn supplementation to reach 8 mg/d prevented BMD loss. Serum Cu and Zn levels were not reported, precluding comparison to our Cu/Zn ratios. Nevertheless, these results support our findings indicating that high Cu is negatively associated with BMD.

There is a complex interplay of Cu and Zn with Mg (Nielsen et al. 2011; Nielsen et al. 2003). While Mg intake in our study group was below the RDA [320 mg/d; (Food and Nutrition Board and Institute of Medicine 1997)], serum Mg levels were not assessed. Mg deficiency is associated with decreased osteoblast function and increased osteoclast numbers (Rude et al. 2009), increased all-cause mortality (Leone et al. 2006) and increased inflammatory markers (Chacko et al. 2010; Chang et al. 2012). Future studies aimed at assessing interplay between Cu, Zn and Mg should be informative.

The serum Cu/Zn ratio has been studied in the context of several chronic progressive diseases. High ratios predicted mortality in cardiovascular and cancer patients in several prospective studies with 3.5 to 18 year follow-ups (Leone et al. 2006; Malavolta et al. 2010; Reunanen et al. 1996). Our measures of serum Cu and Zn by mass spectrometry were consistent with previous values using similar methods (Easter et al. 2010; Malavolta et al. 2010). We found similarly impaired physical function in those in the highest Cu/Zn ratio group. Older men in the high serum Cu group demonstrated deficits in walking speed and Get-Up-and-Go test, tasks with a strong cardiovascular component. In contrast, men in the high serum Cu/Zn ratio group displayed more impaired muscle strength and mass. This curious divergence likely speaks to distinct interactions between the roles of Cu and Zn in the cardiovascular and musculoskeletal systems. In a cross-sectional study, Mezzeti et al. (Mezzetti et al. 1998) found an association between high serum Cu and LDL and triglycerides that purportedly contributed to the association with cardiovascular disease. We found that a high Cu/Zn ratio predicted lower triglyceride and total cholesterol levels. These conflicting results may reflect a survivor effect in the frail elderly and/or differences in participant selection criteria between studies.

A well-documented symptom of chronic Cu deficiency is anemia that is refractory to Fe supplementation (Uauy et al. 1998; Halfdanarson et al. 2008) to which the elderly are susceptible (Carmel 2008). In contrast, we observed a reduction in hematocrit in the high Cu/Zn ratio group which was accompanied by a reduction in serum Fe levels. This unexpected result likely reflects the complex interconnected pathways that govern Fe and Cu homeostasis (Uriu-Adams et al. 2005; Uauy et al. 1998; Collins et al. 2010; Kim et al. 2010). Subclinical anemia could also contribute to a heightened risk of cardiovascular mortality through long-term increased demand on the heart. Additionally, poor physical performance of elderly men with high serum Cu and Cu/Zn ratios could arise through inadequate delivery of oxygen to muscle. Independent of the cause, poor physical performance by men with high Cu/Zn ratios has major clinical and functional relevance. Strength, speed and stability contribute to an individual's level of independence through his ability to care for himself. Indeed, we found a significantly increased proportion of the men in the high Cu/Zn group required ADL assistance.

The oxidative properties of Cu and the antioxidant properties of Zn are hypothesized to be influential factors in chronic progressive conditions such as cardiovascular disease (Leone et

al. 2006; Reunanen et al. 1996), neurodegenerative disease (Mezzetti et al. 1998; Arnal et al. 2010), and secondary complications of diabetes mellitus (Viktorinova et al. 2009). Many studies consistently report an age-dependent increase in serum Cu, but a concomitant reduction in serum Zn predicts disease in an age-independent manner (Malavolta et al. 2010; Mezzetti et al. 1998). Imbalance of Cu and Zn is thought to result in oxidation of lipids, which in turn compromises the integrity of vascular and neuronal membranes. In combination with other factors, oxidative stress mediated through trace metal imbalances could contribute to the increase in susceptibility to and progression of degenerative diseases with age.

In conclusion, we characterized a set of significant relationships between serum Cu and Zn and clinically relevant measures of bone, physical function and independence in frail elderly men. Our findings highlight the importance and potential clinical utility of trace elements as serological markers in identifying at-risk elderly and raise a major question for future study: Can manipulation of Cu and the Cu/Zn ratio alone improve bone health, physical performance and/or independence? Prospective studies will be needed to test these questions and to determine the predictive power of serum Cu and Cu/Zn ratios in long-term health.

Acknowledgments

This work was supported by a University of Connecticut Incentive Grant and by a grant from the National Institutes of Health (DK 32949).

Abbreviations

25OHD	25-Hydroxy-vitamin D
ADL	Activities of Daily Living
ASM	Appendicular Skeletal Mass
BAP	Bone-specific Alkaline Phosphatase
BMI	Body Mass Index
BMD	Bone Mineral Density
Cp	Ceruloplasmin
Cu	Copper
DHEA	Dehydroepiandrosterone
DPD	Deoxypyridinoline
HDL	High Density Lipoprotein
IADLs	Instrumental Activities of Daily Living
LBM	Lean Body Mass
LDL	Low Density Lipoprotein
NTX	Crosslinked N-Telopeptide
OC	Osteocalcin
PINP	N-terminal type I Procollagen Peptide
PSA	Prostate Specific Antigen
PTH	Parathyroid Hormone

SPPB	Short Physical Performance Battery
Zn	Zinc

Reference List

Arnal N, Cristalli DO, de Alaniz MJ, Marra CA. Clinical utility of copper, ceruloplasmin, and metallothionein plasma determinations in human neurodegenerative patients and their first-degree relatives. Brain Res. 2010; 1319:118–130. [PubMed: 20026314]

Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am.J.Epidemiol. 1998; 147:755–763. [PubMed: 9554417]

Bortz WM. A conceptual framework of frailty: a review. J.Gerontol.A Biol.Sci.Med.Sci. 2002; 57:M283–M288. [PubMed: 11983721]

Carmel R. Nutritional anemias and the elderly. Semin.Hematol. 2008; 45:225–234. [PubMed: 18809092]

Chacko SA, Song Y, Nathan L, Tinker L, de B, I, Tylavsky F, et al. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. Diabetes Care. 2010; 33:304–310. [PubMed: 19903755]

Chang SS, Weiss CO, Xue QL, Fried LP. Association between inflammatory-related disease burden and frailty: results from the Women's Health and Aging Studies (WHAS) I and II. Arch.Gerontol.Geriatr. 2012; 54:9–15. [PubMed: 21763008]

Chaudhri MA, Kemmler W, Harsch I, Watling RJ. Plasma copper and bone mineral density in osteopenia: an indicator of bone mineral density in osteopenic females. Biol.Trace Elem.Res. 2009; 129:94–98. [PubMed: 19139831]

Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. Nutr.Rev. 2010; 68:133–147. [PubMed: 20384844]

Easter RN, Qilin C, Lai B, Ritman EL, Caruso JA, Zhenyu Q. Vascular metallomics: copper in the vasculature. Vasc.Med. 2010; 15:61–69. [PubMed: 19808712]

Food and Nutrition Board and Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. 1997

Ford ES, Mokdad AH. Dietary magnesium intake in a national sample of US adults. J.Nutr. 2003; 133:2879–2882. [PubMed: 12949381]

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J.Gerontol.A Biol.Sci.Med.Sci. 2001; 56:M146–M156. [PubMed: 11253156]

Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J.Gerontol. 1994; 49:M85–M94. [PubMed: 8126356]

Halfdanarson TR, Kumar N, Li CY, Phyliky RL, Hogan WJ. Hematological manifestations of copper deficiency: a retrospective review. Eur.J.Haematol. 2008; 80:523–531. [PubMed: 18284630]

Hamerman D. Toward an understanding of frailty. Ann.Intern.Med. 1999; 130:945–950. [PubMed: 10375351]

Judge JO, Schechtman K, Cress E. The relationship between physical performance measures and independence in instrumental activities of daily living. The FICSIT Group. Frailty and Injury: Cooperative Studies of Intervention Trials. J.Am.Geriatr.Soc. 1996; 44:1332–1341. [PubMed: 8909349]

Kenny AM, Kleppinger A, Annis K, Rathier M, Browner B, Judge JO, et al. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. J.Am.Geriatr.Soc. 2010; 58:1134–1143. [PubMed: 20722847]

Kim BE, Turski ML, Nose Y, Casad M, Rockman HA, Thiele DJ. Cardiac copper deficiency activates a systemic signaling mechanism that communicates with the copper acquisition and storage organs. Cell Metab. 2010; 11:353–363. [PubMed: 20444417]

Leone N, Courbon D, Ducimetiere P, Zureik M. Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. Epidemiology. 2006; 17:308–314. [PubMed: 16570028]

Lipsitz LA. Dynamics of stability: the physiologic basis of functional health and frailty. J.Gerontol.A Biol.Sci.Med.Sci. 2002; 57:B115–B125. [PubMed: 11867648]

Lowe NM, Lowe NM, Fraser WD, Jackson MJ. Is there a potential therapeutic value of copper and zinc for osteoporosis? Proc.Nutr.Soc. 2002; 61:181–185. [PubMed: 12133199]

Malavolta M, Giacconi R, Piacenza F, Santarelli L, Cipriano C, Costarelli L, et al. Plasma copper/zinc ratio: an inflammatory/nutritional biomarker as predictor of all-cause mortality in elderly population. Biogerontology. 2010; 11:309–319. [PubMed: 19821050]

Mezzetti A, Pierdomenico SD, Costantini F, Romano F, De CD, Cuccurullo F, et al. Copper/zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. Free Radic.Biol.Med. 1998; 25:676–681. [PubMed: 9801067]

Nielsen FH, Lukaski HC, Johnson LK, Roughead ZK. Reported zinc, but not copper, intakes influence whole-body bone density, mineral content and T score responses to zinc and copper supplementation in healthy postmenopausal women. Br.J.Nutr. 2011; 106:1872–1879. [PubMed: 21733304]

Nielsen FH, Milne DB. Some magnesium status indicators and oxidative metabolism responses to low-dietary magnesium are affected by dietary copper in postmenopausal women. Nutrition. 2003; 19:617–626. [PubMed: 12831948]

Palacios C. The role of nutrients in bone health, from A to Z. Crit Rev.Food Sci.Nutr. 2006; 46:621–628. [PubMed: 17092827]

Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J.Am.Geriatr.Soc. 1991; 39:142–148. [PubMed: 1991946]

Prohaska JR. Changes in Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase and glutathione transferase activities in copper-deficient mice and rats. J.Nutr. 1991; 121:355–363. [PubMed: 1848285]

Reunanen A, Knekt P, Marniemi J, Maki J, Maatela J, Aromaa A. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. Eur.J.Clin.Nutr. 1996; 50:431–437. [PubMed: 8862478]

Rude RK, Singer FR, Gruber HE. Skeletal and hormonal effects of magnesium deficiency. J.Am.Coll.Nutr. 2009; 28:131–141. [PubMed: 19828898]

Uauy R, Olivares M, Gonzalez M. Essentiality of copper in humans. Am.J.Clin.Nutr. 1998; 67:952S–959S. [PubMed: 9587135]

Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. Mol.Aspects Med. 2005; 26:268–298. [PubMed: 16112185]

Viktorinova A, Toserova E, Krizko M, Durackova Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism. 2009; 58:1477–1482. [PubMed: 19592053]

Wang ZM, Visser M, Ma R, Baumgartner RN, Kotler D, Gallagher D, et al. Skeletal muscle mass: evaluation of neutron activation and dual-energy X-ray absorptiometry methods. J.Appl.Physiol. 1996; 80:824–831. [PubMed: 8964743]

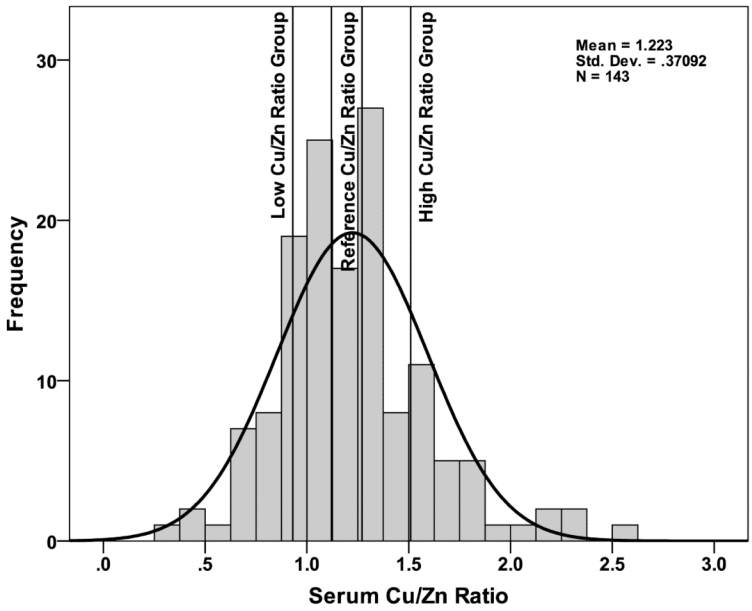

Fig. 1. Serum Metals

Graph depicts frequency histogram for the Cu/Zn ratio. Values for serum Cu and serum Zn were also normally distributed. Vertical lines denote quintile boundaries.

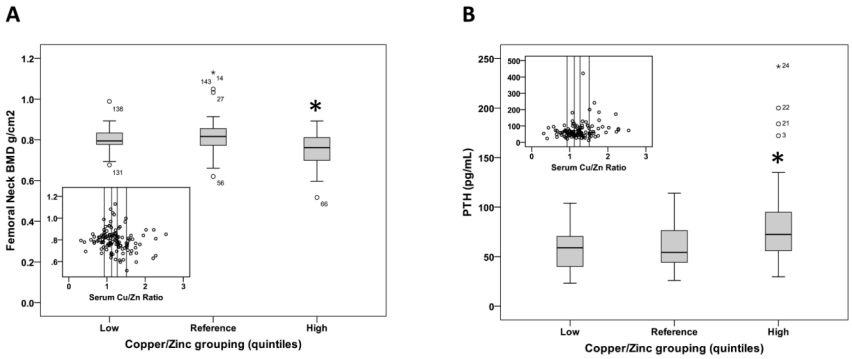

Fig. 2. Bone Mineral Density (BMD) and Parathyroid Hormone (PTH)

(A) BMD was assessed from the femoral neck of each subject. (B) Subjects in the high Cu/Zn ratio group had significantly higher PTH values than their reference group cohorts.

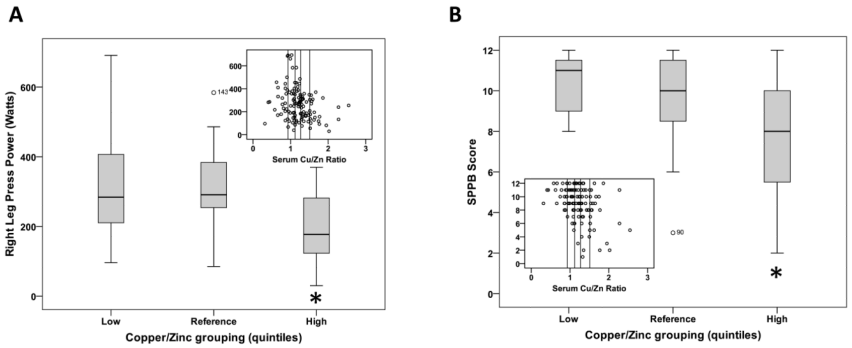

Fig. 3. Muscle Strength and Short Physical Performance Battery (SPPB)

(A) Subjects were tested for muscle strength by measuring power generated by right leg press. Individuals in the high Cu/Zn ratio generated significantly less power than the reference group. (B) Individuals in the high Cu/Zn ratio group achieved significantly lower SPPB scores than individuals in the reference group.

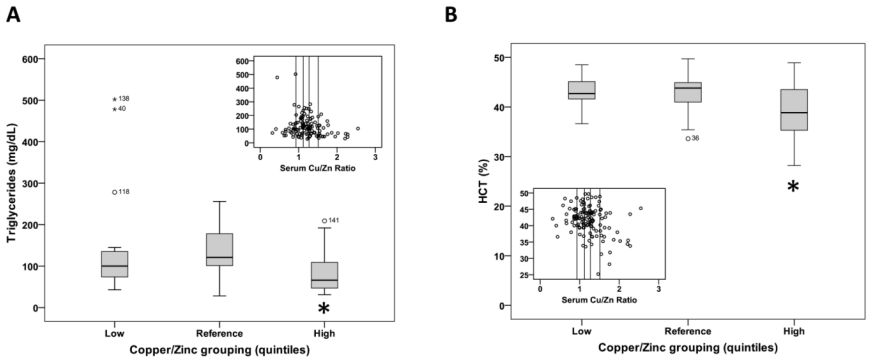

Fig. 4. Lipid Metabolism and Hematocrit

(A) Individuals in the high Cu/Zn group had significantly lower triglyceride levels than the reference group. (B) Individuals in the high Cu/Zn ratio groups had significantly lower hematocrit values than subjects in their respective reference groups.

Table 1

Study Population Characteristics

	Mean ± SD (N=144)
Age (yrs)	77.1 ± 7.6
BMI (kg/m^2)	26.9 ± 4.4
	% (N)
Heart disease	30 (38)
Hypertension	23 (30)
On Ciioiesteroi Medication	28 (36)
On Hypertension Medication	41 (59)
Nutrition	
(per day)	**Mean ± SD**
Calories	2046 ± 1130
Calcium (mg)	898 ± 660
Vitamin D (IU)	209 ± 276
Copper (mg)	1.21 ± 1.07
Protein (g)	94.8 ± 51.5
Magnesium (mg)	285.4 ± 161.9
Zinc (mg)	12.9 ± 10.6
Iron (mg)	19.8 ± 12.9
Serum Levels	
	Mean ± SD
Copper (ppb)	1009 ± 241
Ceruloplasmin (μU/μL)	188.4 ± 46.3
Zinc (ppb)	877 ± 281
Iron (ppb)	970 ± 531
Copper/Zinc Ratio	1.22 ± 0.37

Table 2

Cu/Zn ratio groupings by bone mineral density and Ca regulating hormones

	Group average (n=79)	**Low (n=26)**	**Reference (n=27)**	**High (n=26)**	***P* values Low**	***P* values High**
Femoral Neck BMD (g/cm^2)	0.794 ± 0.098	0.802 ± 0.063	0.823 ± 0.116	0.750 ± 0.090	0.416	**0.014**
Femoral Total BMD (g/cm^2)	0.886 ± 0.113	0.907 ± 0.078	0.921 ± 0.124	0.833 ± 0.122	0.631	**0.012**
Femoral Trochanter BMD (g/cm^2)	0.794 ± 0.121	0.801 ± 0.089	0.837 ± 0.135	0.743 ± 0.133	0.253	**0.013**
Vitamin D (nmol/L)	86.5 ± 36.2	83.9 ± 35.1	78.8 ± 24.6	78.1 ± 37.0	0.545	0.930
iPTH (pg/mL)	69.7 ± 47.9	57.1 ± 21.0	61.1 ± 23.0	89.0 ± 55.4	0.519	**0.020**

Bold represents p values <0 .05.

Table 3

Cu/Zn ratio groupings by frailty and physical performance

	Group average (n=74)	Low (n=24)	Reference (n=27)	High (n=23)	*P* values Low	*P* values High
Nonfrail % (n)	9 (12)	7 (2)	15 (4)	4 (1)	0.413	0.172
Prefrail % (n)	72 (94)	85 (22)	70 (19)	65 (17)	0.215	0.697
Frail % (n)	18 (24)	8 (2)	15 (4)	31 (8)	0.413	0.165
ASM (Kg)	22.5 ± 3.5	22.8 ± 3.6	23.6 ± 2.9	21.0 ± 3.1	0.406	**0.003**
ASM/Ht^2	7.6 ± 1.0	7.7 ± 1.0	7.9 ± 1.0	7.3 ± 0.9	0.590	**0.042**
Whole body lean mass (Kg)	53 ± 7	53.8 ± 7.7	55.5 ± 5.1	50.0 ± 5.8	0.350	**0.001**
Leg Press Strength (Newtons)	666 ± 207	713 ± 228	697 ± 190	564 ± 202	0.779	**0.021**
Leg Press Power (Watts)	254 ± 135	309 ± 163	296 ± 137	195 ± 94	0.754	**0.005**
Handgrip (Kg)	25.3 ± 8.2	24.2 ± 8.1	29.5 ± 8.7	23.0 ± 8.0	**0.029**	**0.009**
Walk best speed (m/sec)	0.89 ± 0.23	0.95 ± 0.16	0.95 ± 0.25	0.81 ± 0.26	0.994	0.064
Get-Up-and-Go (secs)	12.8 ± 8.0	10.6 ± 2.0	11.4 ± 4.4	14.6 ± 7.0	0.433	0.056
SPPB score	9.0 ± 2.6	10.4 ± 1.3	9.7 ± 2.2	7.8 ± 3.0	0.178	**0.014**

Bold represents p values <0.05.

Table 4

Cu/Zn ratio groupings by overall health

	Group average (n=74)	Low (n=24)	Reference (n=27)	High (n=23)	*P* values Low	*P* values High
IADL needs assistance % (n)	20 (24)	4 (1)	11 (3)	32 (7)	0.357	0.074
ADL needs assistance % (n)	12 (15)	4 (2)	0	22 (5)	0.284	**0.011**
Total cholesterol (mg/dL)	188 ± 38	184 ± 40	193 ± 29	173 ± 40	0.333	**0.041**
Triglycerides (mg/dL)	119 ± 75	131 ± 115	138 ± 64	84 ± 49	0.785	**0.001**
HCT (%)	41.6 ± 4.3	43.0 ± 2.9	43.0 ± 3.8	39.0 ± 5.1	0.990	**0.002**

Bold represents p values < 0.05.