J. Elementol. 2011, 16(1): 21–30

ANALYSIS OF SERUM COPPER AND ZINC CONCENTRATION AMONG EXCESS BODY MASS PERSONS DUE TO THEIR AGE

Sylwia Dzięgielewska[1], Ewa Wysocka[1], Szczepan Cofta[2], Lech Torliński[1]

[1]Department of Clinical Biochemistry and Laboratory Medicine
[2]Department of Pulmonology, Alergology and Respiratory Oncology
Poznan University of Medical Sciences

Abstract

Metabolic alterations in physiological aging may depend on oxidative-antioxidative balance and biomineral status. The aim was to analyze concentrations of serum copper (Cu) and zinc (Zn) among excess body mass persons due to their age. Material: 72 healthy Caucasians, divided into 3 groups: AGE1, AGE2, AGE3 (30-45, 46-60, 61-75 years old respectively), with body mass index (BMI) ≥25 kg m^{-2} were qualified for oral glucose tolerance test (OGTT) and fasting (G0') and 2-hours (G120') glycemia were determined (enzymatically). Type 2 diabetes mellitus was excluded. Concentration of serum Cu and Zn (AAS), insulin (ELISA) and plasma lipids: total cholesterol, high density lipoproteins cholesterol, triacyloglyceroles (enzymatically) were measured in fasting samples. Low density lipoproteins cholesterol was obtained using Friedewald formula. Insulin Resistance ratios and Cu/Zn ratio were calculated. Results: No differences concerning BMI, waist, diastolic blood pressure, lipids and insulin concentrations and insulin resistance ratios were observed. Increasing G0', G120' and systolic blood pressure from group 1 to 3 (p=0.01, p=0.01, p=0.04 respectively) were found. AGE2 group had the lowest Zn concentration 10.67±3.37 μmol dm^{-3}, (p=0.002) and the highest Cu/Zn ratio 1.73±0.64 (p=0.0003). We calculated negative correlations Zn and SBP (R=-0.45, p=0.04), Zn and DBP (R=-0.46, p=0.04), Zn and G 120' (R=-0.45, p=0.03) in the oldest group. Conclusions: Different zinc concentration while aging may occur, and may imply different metabolic usage of the biominerals, especially in obese patients otherwise healthy subjects.

Key words: aging, trace elements, zinc, cooper.

dr n. med. Sylwia Dzięgielewska, Department of Clinical Biochemistry and Laboratory Medicine, Poznan University of Medical Sciences, Grunwaldzka 6, 61-780 Poznań, e-mail: syladzie@hotmail.com

ANALIZA STĘŻEŃ CYNKU I MIEDZI W SUROWICY OSÓB ZE ZWIĘKSZONĄ MASĄ CIAŁA W ZALEŻNOŚCI OD WIEKU

Abstrakt

W procesie starzenia obserwuje się rozwój zaburzeń metabolicznych, w których dyskutuje się udział stanu równowagi oksydacyjno-antyoksydacyjnej oraz biopierwiastków: cynku i miedzi. Celem badań była ocena stężenia cynku i miedzi w surowicy osób o zwiększonej masie ciała w zależności od ich wieku. W badanich uczestniczyły 72 osoby rasy białej o zwiększonym BMI $^{3}25$ kg m^{-2}; przeprowadzono test doustnego obciążenia glukozą (OGTT), mierząc glikemię na czczo (G0') i po 2 h (G120') enzymatycznie. Wykluczono osoby z cukrzycą typu 2. Na czczo oznaczono stężenie cynku i miedzi (AAS), insuliny (ELISA) w surowicy oraz stężenie parametrów lipidowych: cholesterolu całkowitego, cholesterolu frakcji HDL, triacylogliceroli (enzymatycznie). Cholesterol frakcji LDL wyznaczono wzorem Friedewalda. Obliczono wskaźniki insulinooporności oraz stosunek Cu/Zn. Badano grupy w zależności od wieku: AGE1, AGE2, AGE3 (odpowiednio: 30-45, 46-60, 61-75 lat). Stwierdzono, że grupy nie różniły się BMI, obwodem pasa, rozkurczowym ciśnieniem tętniczym, profilem lipidowym, stężeniem insuliny oraz wskaźników insulionooporności. Analiza grup 1-3 wykazała wzrastające stężenie G0', G120' i ciśnienie skurczowe (SBP), odpowiednio p=0,01; p=0,01; p=0,04 oraz różnice w stężeniu Zn (p=0,002) i Cu/Zn (p=0,0003), z najniższymi wartościami Zn 10,67±3,37 µmol dm^{-3} i najwyższym Cu/Zn 1,73±0,64 – u AGE2; u AGE3 zaobserwowano ujemne korelacje dla Zn i SBP (R=-0,45; p=0,04), Zn i DBP (R=-0,46; p=0,04), Zn i G120' (R=-0,45; p=0,03). Dysproporcja stężeń Zn między grupą w średnim a podeszłym wieku może być tłumaczona różnymi sposobami wykorzystania Zn w obliczu zaburzeń metabolicznych.

Słowa kluczowe: starzenie się, pierwiastki śladowe, miedź, cynk.

INTRODUCTION

Owing to modern medicine, the population of elderly people has been growing. Increased body mass resulting in overweight or obesity and different metabolic alterations may occur while aging. Studies concerning obesity, hypertension, dyslipidemia and hyperglycemia are currently discussed (Cerello 2008, Hsueh et al. 2010, Su et al. 2008).

The oxidative-antioxidative status may play an important role in the development of obesity-related disorders when we age. Copper and zinc are essential cofactors involved in it. There are necessary for normal functions of antioxidant, hematological, vascular, and neurological systems. It is known that overweight and obese individuals are predisposed to lower blood concentrations of vitamins and minerals compared to people with normal body weight (Singh et al. 1998, Marreiro et al. 2006).

Elderly people are more prone to insulin-resistance than middle-aged individuals. Starting from the age of 30, plasma glucose concentration rises: fasting glycemia increase about 1-2 mg cm^{-3} and postprandial glycemia about 2-4 mg cm^{-3} in every 10 years (Winger, Hornick 1996).

There is very little knowledge about trace elements such as zinc or copper or biomechanisms causing aging and finally death. Studies on longevity and metabolic factors contributing to healthy aging are observed. Alterations in the bioavailability of essential trace elements i.e. zinc and copper, which are components of certain enzymes and nucleoproteins, may lead to some tissues and systems dysregulations. During physiological aging, the zinc status declines (Mocchegiani et al. 2006) while plasma copper increases (Milne, Johnson 1993).

It is difficult to assess whether the changes in the trace element status are associated with chronic disease or aging. Studies on the biomineral status in humans and the balance of trace elements may combine all pathological mechanisms involved and explain obesity-related problems while aging.

The aim of this study has been to analyze concentration of serum copper (Cu) and zinc (Zn) among obese persons due to their age.

MATERIAL AND METHODS

The study was performed in the Department of Clinical Biochemistry and Laboratory Medicine, Chair of Chemistry and Clinical Biochemistry of Poznan University of Medical Sciences, under the permission from the local ethics group in accordance with the Declaration of Helsinki of 1975 for Human Research, and the study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences in Poznan, Poland.

Subjects and Settings: Healthy Caucasians, 72 persons from the Poznan metropolitan area, with no acute disease or severe chronic disorder, were assessed. The following exclusion criteria were complied: coronary arterial disease, history of diabetes, neoplastic diseases, inflammatory diseases, previous therapy, use of antioxidant drugs, alcohol use, smoking and electrocardiograph findings specific for myocardial ischaemia. Subjects were divided into 3 age-groups, such as AGE1: 0-45 years old (n=24, males=12, females=12), AGE2: 46-60 years old (n=24, males=12, females=12) and AGE3: 61-75 years old (n=24, males=12, females=12). *Blood sampling and biochemical analysis*: Blood was collected by venous arm puncture. All studied persons were qualified for an oral glucose tolerance test (OGTT) according the WHO recommendation (World Health Organization, 1999). The newly diagnosed type 2 diabetic patients were excluded. *Glucose and lipids assay:* The concentration of plasma glucose at 0 minutes (fasting) and 120 minutes (postprandial) of the 75-g OGTT, and plasma lipids: total cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), triacyloglyceroles (TAG) were evaluated enzymatically using a bioMerieux reagent kit (France) and a UV-160A Shimadzu spectrophotometer (Japan). Low density lipoprotein cholesterol (LDL-C) was obtained using Friedewald formula. *Insulin assay:* Fasting se-

rum insulin was measured by the ELISA method (BioSource, Belgium) on a microplater reader (Sunrise Tecan, Switzerland). Insulin resistance, IR ratio – IR = {Ins/G0' (mg cm^{-3})} and Homeostatic Model Assessment for Insulin Resistance, HOMA-IR = {G0' (mmol dm^{-3})*Ins}/22,5 were calculated. *Trace elements assay:* Serum copper and zinc concentrations were determined in duplicate by flame atomic absorption spectrometry (Zeiss AAS-3, Germany). The reference sera level 1 and level 2 (Randox, United Kingdom) were used for monitoring the accuracy of the determinations. The Cu/Zn ratio was established.

Statistical analysis: Statistica (version 6.0) for Windows was used for statistical analysis. The normality of value distribution was checked by the Shapiro-Wilk test. The non-parametric Kruskal Wallis followed by the Man-Whitney U test were applied to assess the significance of differences between the groups. In order to determine the relation between Cu or Zn and the other factors, Pearson linear correlation was used. A $p<0.05$ was considered statistically significant. The results are expressed as a mean +/- standard deviations (SD) and a median, given in round brackets.

RESULTS

Among the 72 studied persons, 24 were young adults aged 39 ± 4 (41), 24 were middle-aged 52 ± 4 (51) years old and 24 were elderly people aged 65 ± 6 (64). The baseline characteristics and clinical parameters of the age-groups 1-3 are presented in Table 1. There were no differences concerning the BMI, waist, diastolic blood pressure, lipids and insulin concentrations as well as IR and HOMA-IR ratios in investigated groups. The Kruskal-Wallis test followed by the Man-Whitney U test showed increasing fasting (G0') and postprandial (G120') glycemia and systolic blood pressure (SBP) from group 1 to 3 (p=0.01, p=0.01, p=0.04, respectively). In the youngest group (AGE1), positive correlation between age and G0' (R=0.43, p=0.034) was observed. In the middle-aged group (AGE2), positive correlation between age and BMI (R=0.37, p=0.036), age and SBP (R=0.35, p=0.046) and negative correlation between Zn and waist (R=-0.36, p=0.05) were calculated. In the last group of elderly patients (AGE 3) negative correlations between Zn and SBP (R=-0.45, p=0.044), Zn and DBP (R=-0.46, p=0.042), Zn and G120' (R=-0.45, p=0.026) were observed (all relationships in Table 2). We did not observe any differences in cooper concentration among the age-groups. In AGE2 group, the lowest zinc concentration:10.67±3,37 µmol dm^{-3} (p=0.002) and the highest Cu/Zn ratio: 1.73±0.64 (p=0.0003) were noticed (Figures 1 and 2).

Table 1

Baseline characteristics and clinical parameters of the age subgroups

Parameters	AGE1 n=24	AGE2 n=24	AGE3 n=24	Mann-Whitney U test
Age (years)	39.0 ± 4.0 (41.0)	52.0 ± 4.0 (51.0)	65.0 ± 6.0 (64.0)	
BMI (kg m^{-2})	28.7 ± 4.0 (28.6)	30.1 ± 4.6 (29.8)	28.0 ± 4.0 (28.0)	
Waist (cm)	97.4 ± 12.9 (99.0)	99.7 ± 15.5 (99.0)	98.4 ± 13.0 (98.0)	
SBP (mm Hg)	123.0 ± 18.0 (120.0)*	135.0 ± 14.0 (135.0)*	142.0 ± 25.0 (140.0)	* p=0.022
DBP (mm Hg)	80.0 ± 12.0 (80.0)	84.0 ± 10.0 (81.0)	88.0 ± 15.0 (88.0)	
G0' (mmol dm^{-3})	5.374 ± 0.676 (5.252)		5.885 ± 0.605 (5.830)	
G120' (mmol dm^{-3})	6.545 ± 1.793 (6.352)	6.325 ± 1.815 (5.995)#	7.865 ± 1.925 (7.920)#	# p=0.004
Ins (mU dm^{-3})	10.6 ± 5.4 (10.0)	13.0 ± 10.6 (10.0)	13.5 ± 12.7 (8.7)	
IR	0.106 ± 0.045 (0.099)	0.128 ± 0.105 (0.097)	0.125 ± 0.114 (0.075)	
HOMA-IR	2.67 ± 1.58 (2.31)	3.29 ± 2.73 (2.44)	3.63 ± 3.57 (2.20)	
T-C (mmol dm^{-3})	5.772 ± 0.988 (5.382)	6.188 ± 1.326 (6.084)	5.980 ± 1.118 (6.032)	
TAG (mmol dm^{-3})	1.727 ± 1.072 (1.399)	1.908 ± 1.479 (1.387)	1.614 ± 0.768 (1.434)	
HDL-C (mmol dm^{-3})	1.349 ± 0.372 (1.313)	1.295 ± 0.455 (1.131)	1.347 ± 0.416 (1.326)	
LDL-C (mmol dm^{-3})	3.614 ± 0.728 (3.640)	4.004 ± 0.936 (3.952)	3.900 ± 0.910 (3.770)	
Cu^{2+} (µmol dm^{-3})	16.36 ± 2.42 (16.13)	16.70 ± 1.86 (16.66)	16.46 ± 2.35 (16.53)	
Zn^{2+} (µmol dm^{-3})	13.52 ± 2.56 (13.42)*	10.67 ± 3.37 (10.40)*#	12.73 ± 2.58 (12.66)#	* p=0.0023 # p=0.025
Cu/Zn	1.28 ± 0.47 (1.19)*	1.74 ± 0.64 (1.55)*#	1.34 ± 0.30 (1.32)#	* p=0.0004 # p=0.006

– mark which parameters/groups differ significantly

Table 2

Correlations in 1-3 age groups

Correlations	Age 1	Age 2	Age 3
Age and G0'	R=0.43, p=0.034	NS	NS
Age and BMI	NS	R=0.37, p=0.036	NS
Age and SBP	NS	R=0.35, p=0.046	NS
Zn and waist	NS	R=-0.36, p=0.05	NS
Zn and SBP	NS	NS	R=-0.45, p=0.044
Zn and DBP	NS	NS	R=-0.46, p=0.042
Zn and G120'	NS	NS	R=-0.45, p=0.026

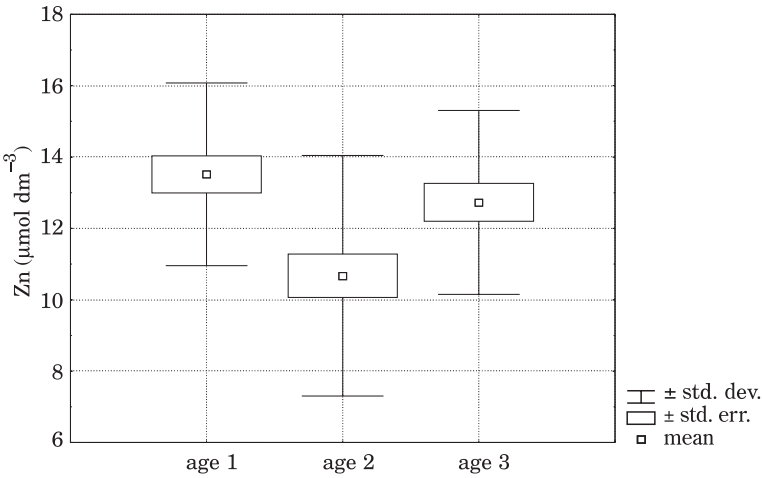

Fig. 1. Comparison of the groups – Zn concentration. Kruskal-Wallis ANOVA test, p=0.02

DISCUSSION

The age-related changes in body mass depend on reduction in muscle mass and water volume (30-40%) alongside increased fat mass. Additionally, biochemical imbalance such as glucose and lipid concentrations, is observed. Therefore, it is difficult to decide whether obesity could be attributed to aging processes or to metabolic disorders, including changes in trace elements.

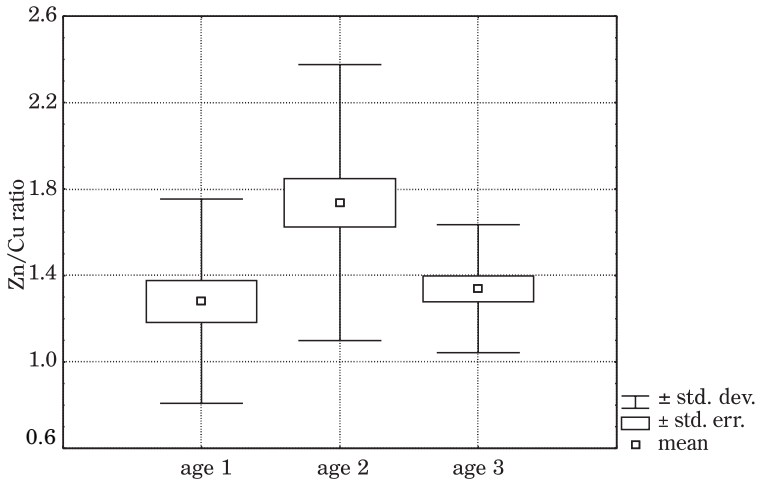

Fig. 2. Comparison of the groups – Cu/Zn ratio. Kruskal-Wallis ANOVA test, p=0.0003

The status of zinc and cooper is broadly discussed in the context of aging (Hotz et al. 2003, Morris 2006, Belbraouet 2007, Lam 2008). In most of these studies, zinc deficiency is defined as a serum concentration below 10.7 μmol dm^{-3}. Holtz et al. (2003) observed that serum zinc concentration tends to increase into the third decade of age and decline afterwards. Ravaglia et al. (2000) investigated the oldest-old (octogenarians) and found that serum zinc significantly decreased in this population group compared to elderly (over 60 years old) and younger populations. Our study on an obese population showed the lowest zinc level and the highest Cu/Zn ratio in the middle-aged group (10.67±3.37 μmol dm^{-3} and 1.73±0.64, respectively) while in the younger subjects and the elderly group, zinc concentration was higher than the deficiency status.

Ghayour-Mobarhan et al. (2008) showed significant differences in the copper and zinc status in patients with dyslipidemia, with or without established coronary arterial disease, compared with control subjects, arguing that differences in the serum zinc decrease and copper increase may be related to inflammation. The groups we examined Our did not differ in the lipid profile, but the total cholesterol, LDL-cholesterol and triacylglyceroles were the highest in the middle-aged group. Thus, the zinc deficiency in observed AGE2 group could be associated with disturbed lipids.

In a study run by Belbraouet et al. (2007), elderly hospitalized patients were at a higher risk of zinc than copper. We investigated obese but otherwise healthy subjects and confirmed Belbraouet's observations that the zinc status decreases and copper does not change in obese patients while they age.

CONCLUSIONS

Essential trace elements, zinc and cooper, are involved in many biochemical processes and their plasma concentrations may depends on the dietary, age and health status. While we age, changes in the plasma zinc concentration could imply different metabolic usage of the biominerals, especially in obese but otherwise healthy subjects. Thus, investigations on trace elements may help to prevent and treat diseases, thus extending people's lifespan.

Acnowledgement

This work was supported in part by the Poznan City Grant for the Poznan Academic Scientists 2010 (RoM. III-3420+53-10 Fn 2626-10) (screening for the glucose metabolism problems and Cu, Zn concentration in elderly patients).

No conflict of interest was declared with relation to this work.

REFERENCES

Belbraouet S., Biaudet H., Tébi A., Chau N., Gray-Donald K., Debry G. 2007. *Serum zinc and cooper status in hospitalized vs. health elderly subjects.* J. Am. Coll. Nutr., 12: 26(6): 650-654.

Ceriello A. 2008. *Possible role of oxidative stress in the pathogenesis of hypertension*. Diabetes Care, 31: 181-184.

Ghayour-Mobarhan M., Taylor A., Kazemi-Bajestani S.M., Lanham-New S., Lamb D.J., Vaidya N., Livingstone C., Wang T., Ferns G.A. 2008. *Serum zinc and copper status in dyslipidaemic patients with and without established coronary artery disease.* Clin Lab., 54(9-10): 321-329.

Hotz C., Person JM., Bron K.H. 2003. *Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976-1980).* Am. J. Clin. Nutr., 10: 78(4): 756-764.

Hsueh W.A., Orloski L., Wyne K. 2010. *Prediabetes: the importance of early identification and prevention*. Postgrad. Med., 122: 129-143.

Lam P.K., Kritz-Silverstein D., Barrett Connor E., Milne D., Nielsen F., Gamst A., Morton D., Wingard D. 2008. *Plasma trace elements and cognitive function in older men and women: the Rancho Bernardo study*. J. Nutr. Health Aging., 1: 12(1): 22-27.

Marreiro D.N, Geloneze B., Tambascia M.A., Lerário A.C., Halpern A., Cozzolino S.M. 2006. *Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women.* Biol Trace Elem Res., 112: 109-118.

Milne D.B., Johnson P.E. 1993. *Assessment of cooper status: effect of age and gender on reference range in healthy adults.* Clin Chem., 5: 39(5): 883-887.

Mocchegiani E., Costarelli L., Giacconi R., Cipriano C., Muti E., Tesei S., Malavolta M. 2006. *Nutrient-gene interaction in ageing and successful ageing. A single nutrient (zinc) and some target genes related to inflammatory/immune response*. Mech. Ageing Dev., 127: 517-525.

Morris M.C., Evans D.A., Tangney C.C., Bienias J.L., Schneider J.A., Wilson R.S., Scherr P.A. 2006. *Dietary copper and high saturated and trans fat intakes associated with cognitive decline.* Arch. Neurol., 8: 63(8): 1085-1088.

RAVAGLIA G., FORTI P., MAIOLI F., NESI B., PRATELLI L., SAVARINO L., CUCINOTTA D., CAVALLI G. 2000. *Blood micronutrient and thyroid hormone concentrations in the oldest-old*. J. Clin. Endocrinol. Metab., 1: 85(6): 2260-2265.

SINGH R.B., BEEGOM R., RASTOGI S.S., GAOLI Z., SHOUMIN Z. 1998. *Association of low plasma concentrations of antioxidant vitamins, magnesium and zinc with high body fat per cent measured by bioelectrical impedance analysis in Indian men*. Magnes Res., 11: 3-10.

SU Y., LIU X.M., JIN H.B., FU R., WANG Y.Y., WU Y., LUAN Y. 2008. *The relationship between endothelial dysfunction and oxidative stress in diabetes and prediabetes*. Int. J. Clin. Pract., 62: 877-882

World Health Organization 1999. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation*. Part 1. *Diagnosis and Classification of Diabetes Mellitus*. Geneva, WHO Department of Noncommunicable Disease Surveillance, 1-59; http://www.who.int

WINGER J.M., HORNICK T. 1996. *Age-associated changes in the endocrine system*. Nurs. Clin. North Am., 10; 31(4): 827-844. (review)