2009 International Nuclear Atlantic Conference - INAC 2009
Rio de Janeiro,RJ, Brazil, September27 to October 2, 2009
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-03-8**

CORRELATION STUDIES BETWEEN SERUM CONCENTRATIONS OF ZINC AND LIPOPROTEINS

Mitiko Saiki[1], Edson R. Alves1[1], Nairo M. Sumita[2], Omar Jaluul[3], M.B.A. Vasconcellos[1] and Wilson Jacob-Filho[3]

[12] Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
mitiko@ipen.br, eralves@ipen.br, mbvascon@ipen.br

[2]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03)
Hospital das Clínicas da Faculdade de Medicina, USP
Rua Dr Eneas de Carvalho Aguiar, 155, Cerqueira César
05403-010, São Paulo, SP
dlc.bioquimica@hcnet.usp.br

[2] Faculdade de Medicina, Universidade de São Paulo
Rua Dr Enéas de Carvalho Aguiar 155, Cerqueira César
05403-010, São Paulo, SP
jaluul@uol.com.br, wiljac@usp.br

ABSTRACT

In this study, serum zinc and lipoprotein concentrations were determined in order to assess the health status of an elderly population residing in São Paulo city, SP, Brazil. This population consisted of elderly considered healthy and participating of a "Successful Ageing" program of the São Paulo University Medical School. Fasting blood samples were collected from 87 elderly individuals (63 females and 24 males) aged 60-91 and mean age of 72 ±7 years. Zn concentrations were determined by neutron activation analysis at the IPEN-CNEN/SP and, the lipoprotein (HDL, LDL and total cholesterol) concentrations were determined using routine analysis methods of the Central Laboratory Division, Hospital das Clínicas, FMUSP. Results obtained for Zn indicated that all the individuals presented this element within the recommended value. For total cholesterol and HDL-cholesterol concentrations, 96 % of elderly presented levels within the desired range but for LDL cholesterol concentrations only about 70.0 % of individuals were in the desired range. Serum concentration of Zn were positively correlated to LDL-cholesterol levels (correlation coefficient $r = 0.21$, $p < 0.06$). Furthermore, the ratios of [HDL-cholesterol] / [LDL-cholesterol] were negatively correlated with Zn concentrations ($r = -0.234$, $p < 0.04$). The positive correlation found between the serum concentrations of Zn and LDL-cholesterol indicates the possible effect of this element in serum lipoprotein profiles. Thus ,these findings suggest that more investigations should be conducted on Zn supplementation in elderly subjects with cardiovascular diseases.

1. INTRODUCTION

Especially in industrialized countries, the trend of low birth rate and low death rate has continued to progress. Also in Brazil, the ageing population has increased rapidly according to the Nation's Official Census (IBGE)[1]. Thus, the knowledge about of health status of elderly individuals is becoming of great interest, especially in order to explore the mechanisms for a healthy life style and, ultimately, to achieve a better quality of life. Among

several biochemical parameters used to examine the health of an individual, lipoprotein determinations are one of the most important parameters and most commonly evaluated.

Zinc is an essential trace element for a variety of biochemical and physiological processes. It plays an important role as a catalytic component of more than 200 enzymes as well as a structural constituent of many proteins and as an antioxidant in preventing free radical formation [2]. As a vital component of several enzymes, zinc is involved in the metabolism of proteins, carbohydrates, lipids and energy, being required for normal development and maintenance of immune function [3].

In the elderly group, zinc is considered an important element, necessary for a healthy ageing due to its impact on immune function, bone mass, cognitive function and oxidative stress [4]; it is has been observed a positive effect of zinc on certain immunological indicators [5]. However, the beneficial effect of zinc is dependent of the dose and length of treatments since it can cause zinc accumulation with subsequent damage on immune efficiency or copper deficiency [6,7].

From all these considerations, zinc and lipoproteins concentrations were evaluated in blood serum, in order to assess the health status of an elderly group and to evaluate if there is a relationship between these two parameters.

2. MATERIALS AND METHODS

2.1. Sampling and Sample Preparation

Procedures for blood sample collection and concerning samples contamination are described in our previous study [8]. The study population consisted of elderly considered healthy and participating of a "Successful Ageing" program of the São Paulo University Medical School. The elderly were selected following the guidelines of the SENIEUR protocol admission criteria [9].This research project was approved by the Ethics Committees of institutions involved. Fasting blood samples were collected from 87 elderly individuals (63 females and 24 males) aged 60-91 and mean age of 72 ±7 years.

Serum Zn concentrations were obtained by neutron activation analysis (NAA). For these analyses, part of blood was centrifuged after completely clotted. Aliquots of 3.0 mL of serum were frozen for transportation to the Neutron Activation Analysis Laboratory, IPEN-CNEN/SP. For NAA, the sera were freeze-dried at - 54 ^{o}C for about 10 hours. The weight loss during this freeze-drying process was about 90.9 ± 0.5 %.

2.2. Biochemical Analysis

Biochemical analyses were performed at the Central Laboratory Division of the Hospital das Clínicas, FMUSP certified by ISO 9001:2000 standards. The total cholesterol and high density lipoprotein (HDL) cholesterol analyses were carried out on Roche/Hitachi MODULAR ANALYTICS PP (Roche Diagnostics GmbH, Mannheim, Germany), using specific kits from Roche Diagnostics, too. The levels of low density lipoprotein (LDL) cholesterol were calculated using the Friedewald equation.

2.3. Neutron Activation Analysis (NAA)

NAA of serum was performed as described in the previous study [8]. Briefly the procedure consists of irradiating aliquots of about 200 mg of serum weighed in polyethylene envelopes in the IEA-R1 nuclear reactor along with the synthetic standard of Zn. The synthetic standard of Zn was prepared by pippeting 50 μL of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. This Zn solution was prepared using certified standard solution provided by Spex Certiprep Chemical, USA. The pippetor and volumetric flask were both calibrated before the use. The filter paper sheets were dried at room temperature inside a desiccator and, then were placed into clean polyethylene envelopes, which were heat sealed. The mass of Zn in this standard was of 35.0 μg.

Sixteen-hour irradiations under a thermal neutron flux of about 5 x 10^{12} n cm^{-2} s^{-1} were performed and after decay times of about one week, the irradiated samples and standard were measured by a hyperpure Ge detector Model GX2020 coupled to a gamma ray spectrometer. The ^{65}Zn radioisotope measured was identified according to its half life and gamma- ray energy. Zn concentration was calculated by a comparative method.

To evaluate the precision and accuracy of the results, certified reference materials NIST 1566b Oyster Tissue and IAEA-A-13 Animal Blood were analyzed. The results showed good precision and accuracy (relative standard deviations and relative errors < 10 %).

3. RESULTS AND DISCUSSION

The serum Zn and lipoprotein concentrations obtained are presented in Table 1 together with the data used as reference intervals in clinical laboratories [10], for comparison.

Table 1. Concentration means and standard deviations (SD) obtained for a group of n individuals and reference intervals of Zn and biochemical data

Parameters	This study				Reference intervals used in clinical laboratory [10].
	n	Mean ± SD[a]	Intervals	Healthy individuals, %	
Zn (μg dL^{-1})	87	98.1 ± 13.6	66.6 – 139.7	100	70 – 120
Total Cholesterol (mg dL^{-1})	81	213 ± 38	129 -328	96.0	< 200 (Recommended); 200-239 (Borderline high); >240 (High)
HDL-Cholesterol (mg dL^{-1})	80	61 ± 14	34 -103	96.2	> 40
LDL-Cholesterol (mg dL^{-1})$^{-}$	80	129 ± 33	52 -208	70.0	<100 (Optimal); 100-129 (Near optimal); 130 – 159 (Borderline high); 160 – 189 (High); ≥ 190 (Very high)

a. Arithmetic mean and standard deviation

Our results indicated that all the individuals of the group presented Zn within the reference intervals and the biochemical tests showed that more than 96 % of the elderly presented concentrations of total cholesterol and HDL-cholesterol within the recommended values. However, only about 70 % presented recommended concentrations of LDL-cholesterol concentrations.

Our biochemical data did not show correlation with the age of the individuals. However, serum Se concentrations from elderly group aged 60-74 years were significantly higher than those found for the group of 75-91 years [11].

Serum Zn concentrations were positively related to LDL-cholesterol (correlation coefficient r = 0.21, p< 0.06) (Fig.1). Furthermore, the ratios of [HDL-cholesterol] / [LDL-cholesterol] as can be seen in Fig 2, were negatively correlated with Zn concentrations (r = - 0.234, p< 0.04). In Fig. 1 and 2, regression lines were drawn through the scatter plots to summarize the relationship between the studied parameters. According to Fasmile [12], ingestion of Zn supplements can cause toxic manifestations. This element can interfere in the utilization of other nutrients, to impair immune functions and negatively affect lipoprotein profile.

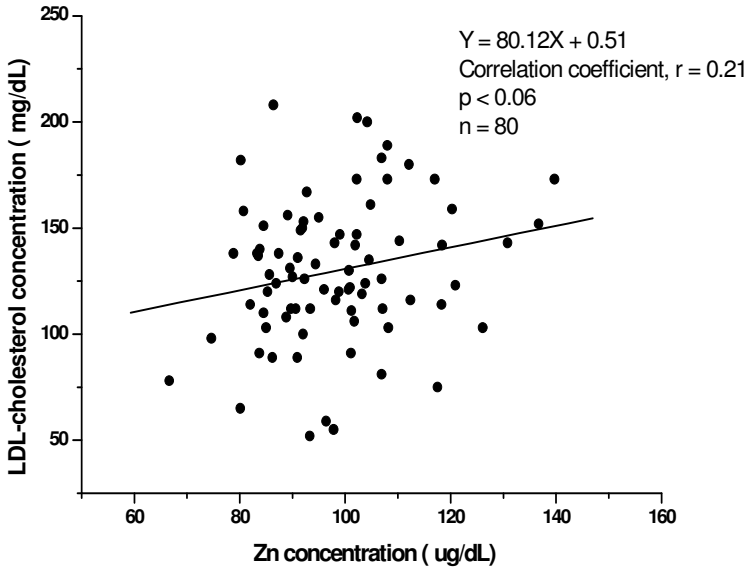

Figure 1. Relationship between serum Zn and LDL cholesterol concentrations

The effect of serum zinc on lipoproteins is a controversial subject. The mechanism of zinc effect on lipoproteins has not been clarified. Besides, serum elemental data for elderly are scarce for comparison. Goodwin et al [13] found an increase of HDL-cholesterol, a decrease

of LDL-cholesterol and an improvement in the HDL-cholesterol and LDL-cholesterol ratios in a healthy elderly population when Zn supplementation was stopped. On the other hand, Back et al [14] suggest that Zn supplements lower serum HDL cholesterol levels.

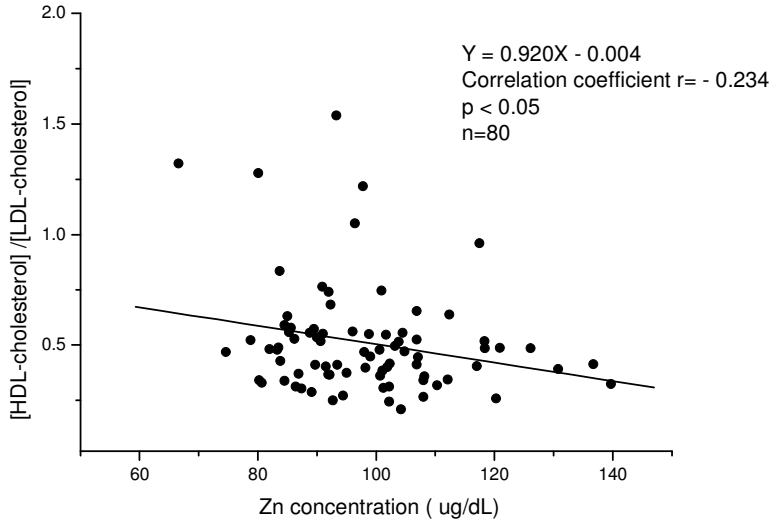

Figure 2. Relationship between serum Zn concentrations and the ratios [HDL-cholesterol] / [LDL-cholesterol].

4. CONCLUSION

As conclusion, Zn concentrations obtained for elderly group of São Paulo city are within the reference intervals established for general population and in use in clinical laboratories. The positive correlation found between the concentrations of serum Zn and LDL-cholesterol indicates the possible effect of this element in serum lipoprotein profiles. Thus these findings suggest more investigations on Zn supplementation in elderly subjects with cardiovascular diseases.

ACKNOWLEDGMENTS

The authors are grateful to FAPESP and CNPq of Brazil for financial support

REFERENCES

1. IBGE, http://www.ibge.gov.br/home/presidencia/noticias/25072002pidoso.shtm, accessed in June, 2008.
2. E. Mocchegiani , M. Muzzioli, C. Cipriano, R. Giacconi, “Zinc, T-cell pathways, aging: role of metallothioneins,” *Mech. Ageing Dev.,* **106**, pp 183-204 (1998).

3. M. Stefanidou, C. Maravelias, A. Dona, C. Spilioupoulou, “Zinc: a multipurpose trace element”, *Arch. Toxicol.*, **80**, pp.1-9 (2006).
4. M. Andriollo-Sanchez, I. Hininger-Favier, N. Meunier, E. Toti, M. Zaccaria, M. Brandolini-Bunlon, A. Polito, J.M. O’Connor, M. Ferry, C. Condray, A.M. Roussel, “Zinc intake and status in middle-aged and older European subjects: the ZENITH study”, *Eur. J. Clin. Nutr.,* **59**, Suppl. 2, pp.S37-S41, (2005).
5. C. Fortes, F. Forastiere, N. Agabiti, V. Fano, R. Pacifi, F. Virgili, G. Piras, L. Guidi, C. Bartoloni, A. Tricerri, P. Zuccaro, S. Ebrahim, C. Perucci, ‘The effect of zinc and vitamim A supplementation on immune response in an older population” *J. Am. Geriatr. Soc.,* **46**, pp. 19-26, (1998).
6. W. Maret, H.H. Sandstead, “Zinc requirements and risks and benefits of zinc supplementation”, *J. Trace Elem. Med. Biol*., **20**, pp. 3-18, (2006).
7. Mocchegiani, E.; Giacconi, R.; Cipriano, C.; Muzzioli, M.; Fattoretti, P.; Bertoni-Freddari, C.; Isani, G.; Zambenedetti, P.; Zatta, P. “Zinc-bound metallothithioeins as potential biological markers of ageing”. Brain Res. Bull., 55, 147-153, 2001
8. M. Saiki, N.M. Sumita, L.F. Sobrerro, W. Jacob-Filho, M.B.A. Vasconcellos, “Establishing a protocol for trace element determinations in serum sample from healthy elderly population in São Paulo city, SP, Brazil”. J. Radioanal. Nucl. Chem., 269, pp. 665-669 (2006).*J. Radional. Nucl. Chem.,* 269,(2006).
9. G.J. Ligthart, J.X. Corberand, C. Fournier, P.Galanaud, W. Hijmans, B. Kennes, H. K. Muller-Hermelink, G.G. Steinmann. Admission criteria for immunogerontological studies in man: The senieur protocol. Mech. Ageing Dev. 28, (1984)47-55..
10. A.H.B. Wu. Tietz Clinical Guide to laboratory tests, Saunders Elsevier, St. Louis, Missouri. 2006
11. M. Saiki, O. Jaluul, N. M. Sumita, M .B. A. Vasconcellos, W. Jacob-Filho, “Trace element contents in serum of healthy elderly population of metropolitan São Paulo area in Brazil”, *J. Trace Elem. Med. Biol.,* **21,** pp 70-73**,** (2007)
12. G.J. Fasmire, “Zinc toxicity”, *Am. J. Clin. Nutr*. **51**, pp. 225-227 (1990).
13. L. J.S. Goodwin, W.C. Hunt, P. Hooper, P.J. Garry, “Relation between zinc intake, physical activity, and blood concentrations of high-density lipoprotein cholesterol in a healthy elderly population”, *Metab.Clin. Exp.,* **34,** pp. 519-523, (1985)
14. M. R. Black, D. M. Medeiros, E. Brunett, R. Welke, ”Zinc supplements and serum lipids in young adult white males”, *Am. J. Clin. Nutr.,* .**47**, pp. 970-975, (1988)