Trakia Journal of Sciences, Vol. 8, Suppl. 2, pp 61-65, 2010

Available online at:
http://www.uni-sz.bg

**ISSN 1313-7050 (print)**
**ISSN 1313-3551 (online)**

# EFFECTS OF ZINC SUPPLEMENTATION ON SOME HEMATOLOGICAL PARAMETERS OF SPONTANEOUSLY HYPERTENSIVE RATS

**A. Dimitrova[1]*, A. Russeva[2], M. Atanasova[3], D. Strashimirov[1]**

[1] Department of Pathophysiology, Medical University, Pleven, Bulgaria
[2] University Hospital, Medical Diagnostic Clinical Laboratory, Pleven, Bulgaria
[3] Department of Biology, Medical University, Pleven, Bulgaria

**ABSTRACT**

The present study was undertaken to determine influence of dietary supplements of Zn containing diet (50 or 155 or 250 mg Zn/kg lab chow) on the hematological parameters of spontaneously hypertensive rats (SHR). Male SHR (n=92) and their normotensive ancestors, Wistar-Kyoto rats (WKY, n=89), were divided into 6 groups on different diet: WKY – G1-50 mg Zn/kg laboratory chow (control), G2-155 mg Zn/kg, G3-250 mg Zn/kg and SHR – G4-50 mg Zn/kg (control), G5-155 mg Zn/kg, G6-250 mg Zn/kg. The diets started at the beginning of the development of hypertension (2 months after birth) and the animals were fed for 8 weeks. At the end of the study, blood sample was taken from each animal in all groups and a complete blood count was performed. There are no statistically significant differences in the studied parameters between rats of the WKY and SHR lines. RBC and Hb were lower ($p<0.05$) in groups with zinc supplementation – G3 and G6, however, MCV, MCH and MCHC were higher ($p<0.05$) in this groups. A decreased hematocrit was found in supplemented groups ($p<0.05$) and the platelets count increased significantly (p=0.001). Our findings suggest that zinc supplementation is a factor, decreasing erythrocytes and hematocrit, and increasing the platelets count in the both rat's strains.

**Key words:** WKY, zinc diets, hypertension, blood count

## INTRODUCTION

The blood is one of the major homeostatic systems of the body in humans and animals, supporting normal viability, integrity, and adaptive responses. The functional state of the blood systems changes dynamically according to the nature, strength, and duration of exposure to external factors.

The use of megadoses of vitamin and mineral supplements has become common. Zinc is a often used supplement and is widely available as a standard component of many over-the-counter products but excessive zinc intake can have toxic effects. A number of reports have identified an association between increased zinc intake and severe cytopenia (1). High intakes of zinc relative to copper can lead to copper deficiency (2, 3, 4). When a zinc-induced hypocupremia developed, the result is anemia, leukopenia, and neutropenia (5). The serum zinc levels of zinc have a negative effect on anemia by blocking the utilization of iron in the iron reserves of anemic subjects (6). Limited data suggest that sustained hyperzincaemia predisposes individuals to thrombogenesis (7).

The purpose of our study was to determine influence of dietary supplements of Zn containing diet (50 or 155 or 250 mg Zn/kg lab chow) on the hematological parameters of spontaneously hypertensive rats (SHR) and their normotensive ancestors Wistar-Kyoto rats.

## MATERIALS AND METHODS

Male Wistar-Kyoto rats (WKY, n=89), (190-210 g) and male spontaneously hypertensive rats (SHR, n=92), (180-205 g) were used. The animals were housed in groups of 5 per cage and kept under a normal 12 h light/dark cycle

***Correspondence to:** *Dr. Aneliya A. Dimitrova Department of Pathophysiology, Medical University 1 St. Kliment Ohridski Str., 5800 Pleven, Bulgaria, Tel: +359 64 884 273*
*E-mail: anelija.dimitrova@gmail.com*

and 22 °± 2°C. Rats had free access to food and water. They were divided in accordance to the amount of Zn in the diet as follows:

Group 1 (G1) – n = 40, with 50 mg/kg diet Zn - WKY
Group 2 (G2) – n = 29, with 155 mg/kg diet Zn - WKY
Group 3 (G3) – n = 20, with 250 mg/kg diet Zn - WKY
Group 4 (G4) – n = 43, with 50 mg/kg diet Zn - SHR
Group 5 (G5) – n = 29, with 155 mg/kg diet Zn - SHR
Group 6 (G6) – n = 20, with 250 mg/kg diet Zn - SHR

The diets were introduces at the beginning of the development of hypertension (2 months after birth) and the animals were fed for 8 weeks. The food consumption was monitored daily over the dietary conditioning. The quantity of diet ingested was comparable in all groups of rats. In addition, rat body weight was measured immediately before the start of dietary treatment and at the end of dietary manipulation.

All experiments described in the present study were carried out in accordance with the guidelines of the Animal Care and Use Committee of the Medical University - Pleven. At the end of the treatment period, following overnight fasting, the abdominal cavity of rats was opened under pentobarbitone sodium anesthesia (26 mg/kg body weight, i.p.). Blood was collected from the bifurcation of the aorta for the measurements of hematocrit (HCT), red blood cells (RBC), leukocytes (WBC), hemoglobin (Hg), mean corpuscular volume (MCV(fl)), mean cell hemoglobin (MCH (pg)), mean corpuscular hemoglobin concentration (MCHC (g/dl)), platelets (PLT).
Zinc content in the laboratory chow and serum concentration of zinc and copper were analyzed by a direct flame atomic-absorption spectrophotometry (NAS) using Perkin-Elmer, Model-Analyst 300 apparatus.

**RESULTS**

The results of hematological parameters of WKY are shown in **Table 1**. There was a significant decrease in the number of RBC in the group with zinc supplemental diet (G3) compared to G2 (p=0.020) and G1 (p=0.025), **(Figure 1)**, and white blood cell (WBC) (p=0.003) counts decreased significantly in G3 in comparison with other groups. The hematocrit showed a significant decrease in the groups G3 (p=0.022) and G2 (p=0.005) as compared to the control, while all measured RBC indices, i.e. MCV, MCH, and MCHC had a significant increase. The platelets count was significantly increased in G3 compared to G1 (p=0.0001) and G2 (p=0.005).

***Table 1.*** *Hematological parameters in WKY on supplemental diets. Values are given as mean ± SEM.*

| **Groups →** <br> **Parameters ↓** | **Group 1** <br> **G1-control** <br> **WKY** | **Group 2** <br> **G2** <br> **WKY** | **Group 3** <br> **G3** <br> **WKY** |
|---|---|---|---|
| **WBC x10$^9$/l** | 14.14±0.55 | 15.36±0.67 | 11.92±0.72 $^{\#\#}$ |
| **RBCx10$^{12}$/l** | 9.26±0.06 | 9.30±0.08 | 8.89±0.16 ****,** $^{\#\#}$ |
| **Hb (g/l)** | 159.28±0.83 | 161.96±2.02 | 160.94±2.11 |
| **HCT** | 0.44±0.005 | 0.45±0.006 | 0.42±0.009 ***,** $^{\#}$ |
| **MCV (fl)** | 51.17±0.38 | 49.58±0.40 | 52.57±0.41***,** $^{\#\#}$ |
| **MCH (pg)** | 18.52±0.33 | 17.86±0.27 | 20.15±0.19 ***,** $^{\#\#}$ |
| **MCHC (g/dl)** | 360.64±4.71 | 359.86±4.33 | 383.42±4.07 ***,** $^{\#}$ |
| **PLT X10$^9$/l** | 815.41±23.45 | 803.35±24.21 | 961.26±25.92****,** $^{\#\#}$ |
| **NUMBERS (N)** | 40 | 29 | 20 |

Significant change compared to control group ***p<0.05; ****p<0.001* and compared to G2 $^{\#}$*p<0.05;* $^{\#\#}$*p<0.001*.

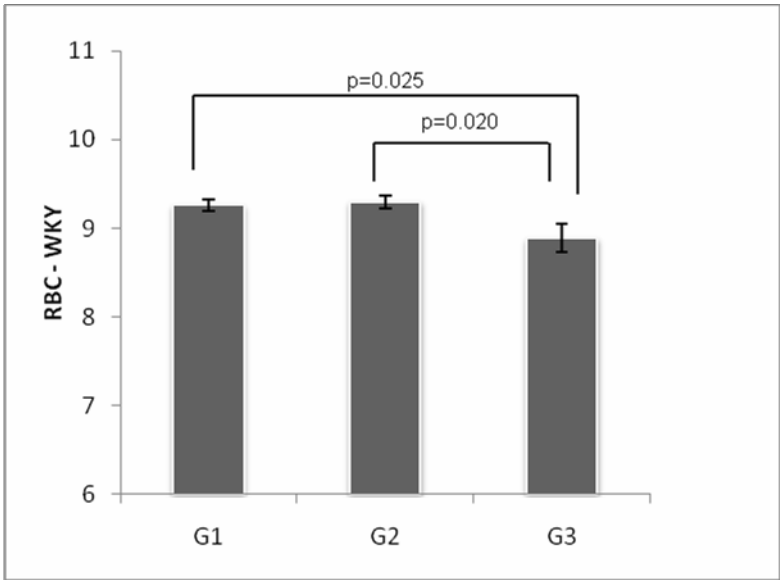


**Figure 1.** Erythrocytes in WKY on zinc supplementation. Data are present as means ± SEM.

The results of hematological parameters of are shown in **Table 2.** The RBC count **(Figure 2)** decreased significantly (p=0.033) in G6 compared to G4. The hematocrit was significantly decreased in the groups G6 (p=0.005) and G5 (p=0.027) compared to the control group-G4, while all measured RBC indices, i.e. MCH, and MCHC had a significant increase. The platelets count was significantly increased in G6 compared to G5 (p=0.001).

***Table 2.*** *Hematological parameters in SHR on supplemental diets. Values are given as mean ± SEM.*

| **Groups →** <br> **Parameters ↓** | **Group 4** <br> **G4-control** <br> **SHR** | **Group 5** <br> **G5** <br> **SHR** | **Group 6** <br> **G6** <br> **SHR** |
|---|---|---|---|
| **WBC x10$^9$/l** | 15.04±0.87 | 14.11±0.81 | 13.75±0.43 |
| **RBCx10$^{12}$/l** | 8.96±0.16 | 8.93±0.15 | 8.31±0.18***** |
| **Hb (g/l)** | 157.76±1.14 | 158.00±3.75 | 159.05±1.07 |
| **HCT** | 0.44±0.006 | 0.46±0.008 | 0.40±0.005 ******,$^{\#\#}$ |
| **MCV(fl)** | 50.9±0.30 | 51.00±0.31 | 50.15±0.31 |
| **MCH (pg)** | 18.55±0.24 | 17.99±0.26 | 19.99±0.17****** |
| **MCHC (g/dl)** | 365.00±4.07 | 352.24±4.12 | 398.85±4.34 ******,$^{\#\#}$ |
| **PLTx10$^9$/l** | 858.55±15.66 | 775.58±22.25 | 895.25±21.60 $^{\#\#}$ |
| **NUMBER (N)** | 43 | 29 | 20 |

Significant change compared to control group *****p<0.05; ******p<0.001 and compared to G5 $^{\#}$p<0.05; $^{\#\#}$p<0.001.

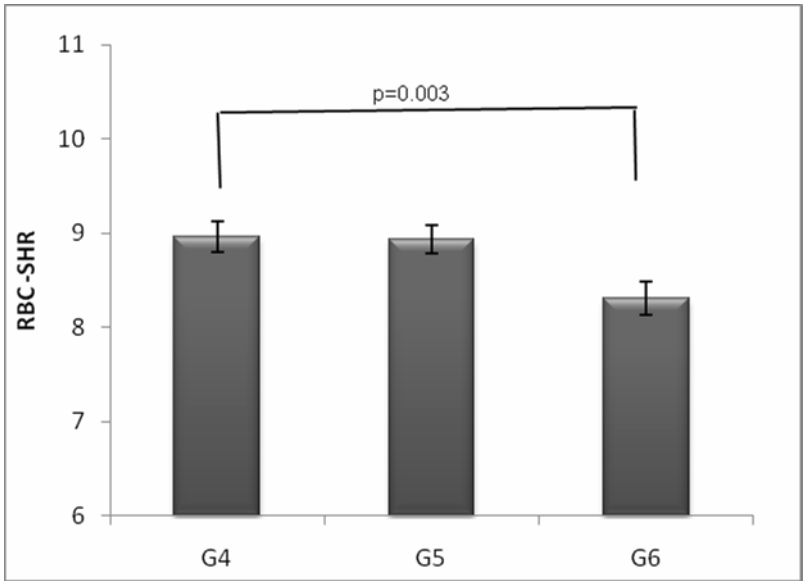


**Figure 2.** Erythrocytes in SHR on zinc supplementation. Data are present as means ± SEM.

## DISCUSSION

It is well known that the zinc supplementation to the diet is a reason for decrease the bioavailability of the copper (3). The zinc absorption is sin strong connection with the copper absorption, because of their competition for the carrier (8). The decreased bioavailability of the copper could express as anemia (9) that is why we investigated the hematological parameters of the rats on zinc diets.

The alterations in hematological parameters are investigated in humans and animals in receiving of zinc in different dosage and duration of the diet. The results in humans are controversial. **Yadrick M.** et al. (10) established that zinc supplementation (50 mg/day, for 10 weeks) decrease hematocrit, without changing the hemoglobin. In contrast **Bonham M.** et al. (11) stated that 40 mg/day Zn does not influence the blood profile/parameters. Zn supplementation as zinc sulphate 2 mg/kg body weight results in development of anemia in human subjects (12). In another investigation in 2–month old rats, zinc chloride 12 mg/kg daily dosage, for 4 weeks, decreases the hemoglobin 85% compared to the control animals (13). In new born rats, undergoing diet with zinc sulphate, for 6 weeks, decrease serum levels of the transport protein of copper ceruloplasmin (14). Our results are similar to those of **Maita K.** et al. (15) that established that the long time zinc supplementation decreases the hematocrit, erythrocytes and leucocytes.

## CONCLUSIONS

In the groups with zinc supplementation G3 and G6 we established significant decrease ($p<0.05$) of erythrocytes compared to the other groups. The zinc supplementation decreases the number of erythrocytes, but increases the quantity of the hemoglobin in the erythrocytes (MCV, MCH, MCHC are increased in GГ3 - WKY and MCH, MCHC при G6 - SHR).
The hematocrit decreases significantly ($p<0.05$) in WKY and SHR and this influence the blood rheology. The number of platelets increases significantly ($p<0.05$) in the groups with zinc supplementation.

## REFERENCES

1. Irving, J., Mattman, A., Lockitch, G., Farrell, K. and Wadsworth, L., Element of caution: a case of reversible cytopenias associated with excessive zinc supplementation. *CMAJ*, 169(2):129-131, 2003.
2. Maret, W. and Sandstead, HH., Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Bio*, 20(1):3-18, 2006.
3. Willis, M.S., Monaghan, S.A., Miller, M.L., McKenna, R.W., Perkins, W.D., Levinson, B.S., Bhushan, V. and Kroft, S.H., Zinc-induced copper deficiency: a report of three cases initially recognized on bone marrow examination. *Am J Clin Pathol*, 123(1):125-131, 2005.

4. O'Dell, B., Role of zinc in plasma membrane function. *J Nutr,* 130(5S Suppl):1432S-1436S, 2000.
5. Salzman, M.B., Smith, E.M. and Koo, C., Excessive oral zinc supplementation. *J Pediatr Hematol Oncol*, 24(7):582-584, 2002.
6. Choi, J.W. and Kim, S. K., Relationships of Lead, Copper, Zinc, and Cadmium Levels versus Hematopoiesis and Iron Parameters in Healthy Adolescents. *Ann Clin Lab Sci,* 35:428-434, 2005.
7. Hughes S. and Samman S., The Effect of Zinc Supplementation in Humans on Plasma Lipids, Antioxidant Status and Thrombogenesis. *J Amer Coll Nutr*, 25(4):285-291, 2006.
8. Reeves, P.G. and Chaney, R., Marginal untritional status of zinc, iron, and calcium increases cadmium retention in the duodenum and other organs of rats fed a rice-based diet. *Environ Res,* 96:311-322, 2004.
9. Field, M., Lewis, C.G. and Lure, M.D., Antioxidant defense system in lung of male and female rats: interaction with alcohol, copper, and type of dietary carbohydrate. *Metabolism,* 45(1):49-56, 1996.
10. Yadrick, M.K., Kenney, M.A. and Winterfeldt, E.A., Iron, copper, and zinc status: response to supplementation with zinc or zinc and iron in adult females. *Am J Clin Nutr,* 49:145-150, 1989.
11. Bonham, M., O'Connor, J.M., Alexander, H.D., Coulter, J., Walsh, P.M., McAnena, L.B., Downes, C.S., Hannigan, B.M. and Strain, J.J., Zinc supplementation has no effect on circulating levels of peripheral blood leucocytes and lymphocyte subsets in healthy adult men. *Br J Nutr,* 89(5):695-703, 2003.
12. Broun, E.R., Greist, A., Tricot, G. and Hoffman, R., Excessive zinc ingestion. A reversible case of sideroblastic anemia and bone marrow depression. *J Am Med Assoc,* 264:1441-1443, 1990.
13. Zaporowska, H. and Wasilewski, W., Combined effect of vanadium and zinc on certain selected haematological indices in rats. *Comp Biochem Physiol C,* 103(1):143–147, 1992.
14. L'Abbe, M.R. and Fischer, P.W.F., The effects of high dietary zinc and copper deficiency on the activity of copper-requiring metalloenzymes in the growing rat. *J Nutr,* 114:813-822, 1984.
15. Maita, K., Hirano, M. and Mitsumori, K., Subacute toxicity with zinc sulfate in mice and rats. *J Pest Sci,* 6:327-336, 1981.