EFFECT OF DIETARY FISH OIL AND KRILL OIL SUPPLEMENTATION ON PLASMA PARAOXONASE ACTIVITY IN RABBITS WITH EXPERIMENTALLY INDUCED OBESITY

Zhenya Ivanova*, Tanya Tacheva, Tatyana Vlaykova**, Nataliya Grigorova,* Kjetil Berge***, Bodil Bjorndal****, Anton Rousenov*****, Rolf Berge[****], Vladimir Petrov******, Teodora Mircheva*, Svetla Georgieva*******, Ivan Penchev Georgiev***

[*]*Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora*

[**]*Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, 6000 Stara Zagora*

[***]*Aker Biomarine, Oslo, Norway*

[*****]*Insitute of Medecine, University of Bergen, N 5021, Bergen, Norway*

*****Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora*

[******]*Department of Veterinary Microbiology, Infection and Parasatic Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora*

*******Department of Genetics, Breeding and Reproduction, Agriculture Faculty, Trakia University, 6000 Stara Zagora*

ABSTRACT

The purpose of this study was to investigate the effect of dietary fish oil and krill oil supplementation on paraoxonase activity (PON1) in a rabbit model of obesity.

In this experiment 42 male New Zealand white rabbits were used. The animals were divided into 7 groups of 6 rabbits each: GrKO50 – castrated, 50% restricted diet fed, treated with krill oil; GrKO100 – castrated, full diet fed, treated with krill oil; GrFO50 – castrated, 50% restricted diet fed, treated with fish oil; GrFO100 – castrated, full diet fed, treated with fish oil; GrC100 – castrated, full diet fed; GrC50 – castrated, 50% restricted diet fed; GrNC – non-castrated, full diet fed. Krill oil and fish oil were given as gelatinous capsules at a dose rate of 600 mg omega-3 PUFA. The experimental period lasted 60 days. The levels of PON1 activity in rabbis were measured spectrophotometrically.

We observed significant difference in the levels of PON between GrKO50 and GrC50 (413.23±53.80 U/l vs. 221.64±25.89 U/l, p=0.022), GrKO50 and GrFO50 (413.23±53.80 vs. 215.75±26.49 U/l, p=0.027), GrKO100 and GrFO100 groups (452.37±77.57 vs. 212.15±27.01 U/l, p=0.008)

The results of the present study showing higher levels of plasma paraoxonase activity in rabbits treated with krill oil suggest that the krill oil supplementation affects positively antioxidant defense mechanisms.

Key words: rabbits, PON 1, obesity, krill oil, fish oil

INTRODUCTION

Paraoxonase (PON 1) is calcium dependent esterase closely associated with high-density lipoproteins (HDL) and it has antioxidant properties (Senti et al., 2003, Ferretti et al., 2005, Mackness et al., 2006; Vlaykova et al., 2013). An important feature of PON1 is to protect lipoprotein complexes (LDL и HDL) and the membranes from oxidation and that is why it has preventive effect against atherosclerotic lesions (La Du et al., 1999, Watson et al., 1995; Durrington et al., 2001, Mackness et al., 2006). It is considered that antioxidant properties of HDL are due to the PON1 (Ferretti et al., 2005). In humans, there are significant variations in the activity of the enzyme (Doneva-Basheva et al., 2013). It has been estimated that individuals with a lower PON1 activity are more prone to cardiovascular diseases as compared to those with high activity of paraoxonase (Ferretti et al., 2005).

During the last years, it was observed increased numbers of people with so-called metabolic syndrome. Metabolic syndrome is characterized by a constellation of disorders of lipid and carbohydrate metabolism as dyslipidemia, hyperglycemia, elevated blood pressure, etc. which often result in the development of cardiovascular disease and/or type 2 diabetes mellitus (Grandy et al., 2004). The basis of these disorders is obesity and associated insulin resistance.

Many features of lipid metabolism in rabbits are similar to humans - so-called LDL mammals, but differ from the most widely used experimental animals – rats and mice, which are predominantly HDL animals (Fan et al., 2001, Kawai et al., 2006). Therefore, recently, rabbits have been increasingly used as suitable models for the study of various obesity-related disorders in humans.

The purpose of this study was to investigate the effect of the exogenous supplementation of fish oil and krill oil on plasma activity of paraoxonase (PON1) in rabbits with experimentally induced obesity.

MATERIAL AND METHODS

Experimental animals and trial design

In this study were included 42 male New Zealand White rabbits. Castration was used to induce obesity. It was performed under general anesthesia with Anaket.

At the beginning of the experiment rabbits were 3-3,5 months old and were housed in individual metal cages (80 x 60 x 40) in a temperature controlled room. They had free access to water and were fed with standard chow diet for adult rabbits.

Animals were divided into 7 groups of 6 rabbits each

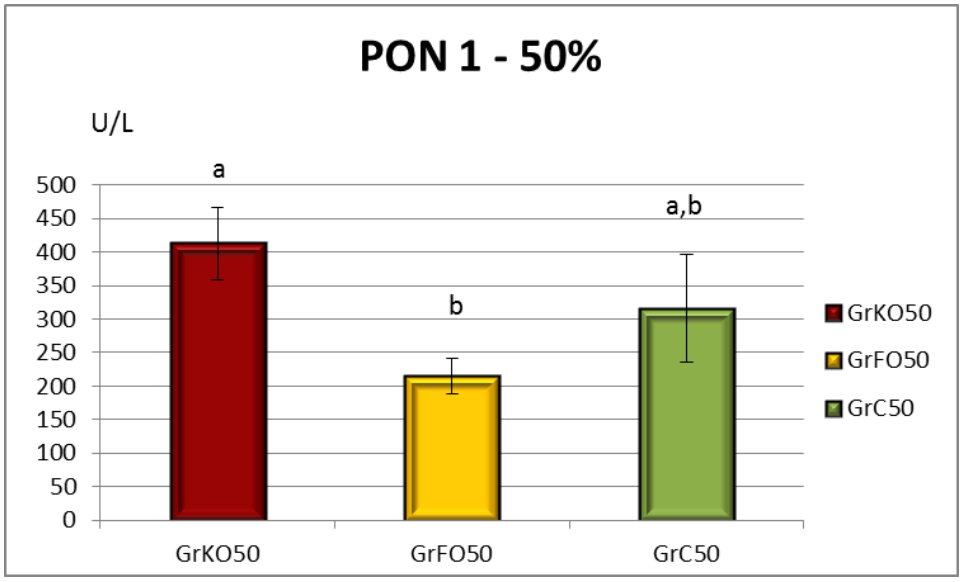
1. GrKO50 – castrated, 50% restricted diet fed, treated with krill oil
2. GrKO100 – castrated, full diet fed, treated with krill oil
3. GrFO50 – castrated, 50% restricted diet fed, treated with fish oil
4. GrFO100 – castrated, full diet fed, treated with fish oil
5. GrC100 – castrated, full diet fed
6. GrC50 – castrated, 50% restricted diet fed
7. GrNC – non-castrated, full diet fed

Statistical analyses

Statistical analysis was performed with the program SPSS 16.0 for Windows (SPSS, Inc., 1989-2007). The effect of the groups on the plasma paraoxonase activity was estimated by ANOVA, and the differences between the independent groups were assessed by LSD test. The accepted level of significance was set at $p<0.05$.

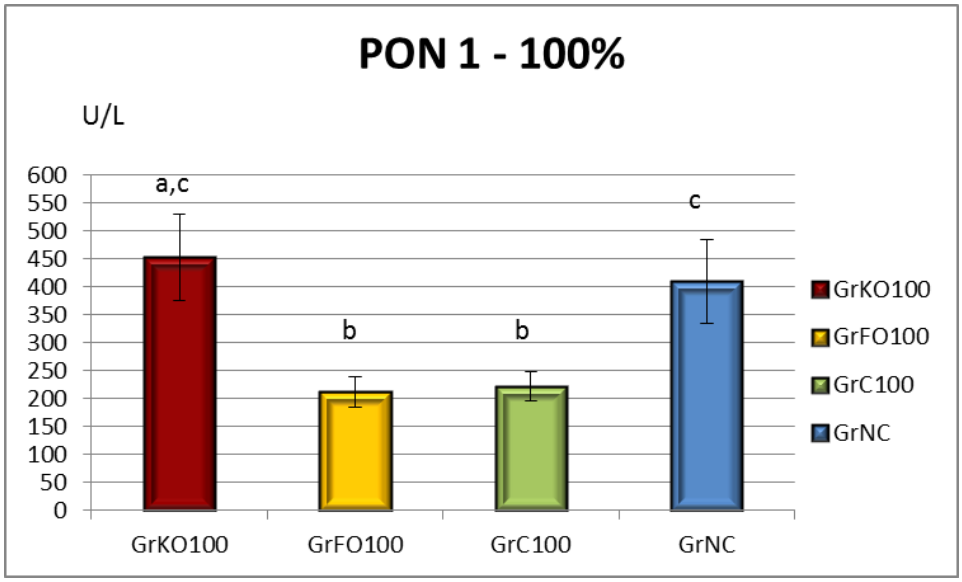
RESULTS AND DISCUSSION

The plasma PON1 activity in different groups of experimental animals is presented in figures 1 and figure 2. The results show that the levels of PON1 in GrKO50 are signficantly higher than in GrFO50 (413.22±53.79 vs. 215.75±26.49, p=0.0296). In full diet fed rabbits, PON1 activity in GrKO100 is significantly higher than in GrFO100 and GrC100 (452.37±77.57 vs. 212.15±27.01 and 452.37±77.57 vs. 221.63±25.89, p=0.011; p=0.009) and is similar to that in GrNC (452.37±77.57 vs. 408.93±75.55, p=0.603).



Different letters mean significant difference between groups at $p<0.05$

Figure 1. Plasma PON 1 activity in restricted diet fed rabbits and treated with krill oil and fish oil.



Different letters mean significant difference between groups at $p<0.05$

Figure 2. PON 1 activity in palsma of rabbits, full diet fed and treated with krill and fish oil. ** p<0,01 - significance of differences between GrKO 100 и GrFO 100

The observed higher plasma paraoxonase activity in rabbits treated with krill oil suggests that it may contribute for increasing antioxidant defense, as its effect was seen both in full diet (100%) and in restrictive diet (50%) fed rabbits. Vlaykova et al., 2013 have reported earlier that *Staphylococcus aureus* inflammation in rabbits led to considerably decreased PON1 activity and suggested this enzyme to be considered as negative acute-phase protein. Low PON1 activity is found in different pathological conditions accompanied with oxidative stress such as dislipidemia, metabolic syndrome, diabetes type 2, etc. (Senti et al., 2003).

The results found in the current study showed that krill oil could be applied for overcoming of unfavorable effects of oxidative stress in aforementioned pathological conditions, which usually are results from obesity. Although rabbits are suitable animal models for investigation of disorders associated with obesity, the results of our study should be carefully interpreted before being

suggested and approximated to humans. Additional investigations are warranted to prove the possible favorable effects of krill oil on the obesity-associated disorders and for increasing the antioxidant defense.

CONCLUSIONS

The results of our study describing an increased plasma paraoxonase activity in rabbits treated with krill oil suggest that the supplementation with krill oil may have a positive effect on the antioxidant defense and may protect the individuals from LDL oxidation and development of atherosclerosis and further from cardiovascular diseases.

Acknowledgments

This study was supported by grant from the Ministry of Education and Science of Bulgaria and Trakia University Science Foundation, Stara Zagora, Bulgaria (grant no. 06/2012) in collaboration with Aker Biomarine, Oslo, Norway and Insitute of Medecine, University of Bergen, Norway

REFERENCES

1. Buri, L., K. Berge, K. Wibrand, R. Berge, J. Barger, 2011. Differential effects of krill oil and fish oil on the hepatic transcriptome in mice, Frontiers in Genetics, 2, 1-8
2. Burri, L., N. Hoem, S. Banni, K. Berge, 2012. Marine omega-3 phospholipids: metabolic and biological activities, International Journal of Molecular Sciences, 13, 15401-15419.
3. Doneva-Basheva K., Anastasov A, Postadzhyan A, Kamenova Z, Vlaykova, T. 2013. Serum paraoxonase and arylesterase activity of pon1 in acute coronary syndrome. Trakia Journal of Science, 1, 39-49,
4. Durrington, P., B. Macknes, M. Macknes, 2001. Paraoxonase and atherosclerosis, Arteriosclerosis Thrombosis Vascular Biology, 2, 473-480
5. Fan, J., H. Unoki, N. Kojima., H. Sun, H. Shimoyamada, H. Deng, M. Okazaki, H. Shikama, N. Yamada, T. Watanabe, 2001. Overexpression of lipoprotein lipase in transgenic rabbits inhibits diet-induced hypercholesterolemia and atherosclerosis, The Journal of Biological Chemistry, 276, 40071-40079.
6. Ferramoskca, A., A. Conte, L. Burri, K. Berge, F. De Nuccio, A. Giudetti. V. Zara, 2012. A krill oil supplemented diet suppresses hepatic steatosis in high-fat fed rats, PLoS ONE 7 (6) e 38797 doi:10.1371/journal.pone.00387971-14
7. Ferretti, G., T. Bachetti, C. Moroni, S. Savino, A. Liuzzi, F. Balzola, V. Bicchiega, 2005. Paraoxonase activity in high-density lipoproteins: A comparison between healthy and obese females, The Journal of Clinical Endocrinology and Metabolism, 90, 1728-1733
8. Grundy, M. S., H. Brewe, I. James, S. Smith,C. Lenfant,, 2004. Difinition of metabolic syndrome: Report of the National Heart, Lung and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition, Circulation, 109, 433-438
9. Kawai, T., T. Ito, K. Ohwada, Y. Mera, M. Matsushita, T. Hitonobu, 2006. Hereditary postprandial hypertriglyceridemic rabbit exhibits insulin resistance and central obesity: A novel model of metabolic syndrome, Arteriosclerosis, Thrombosis, and Vascular Biology, 26; 2752-2757
10. La Du, B., M. Aviram, S. Billecke, M. Navab, S. Primo-primo, R. Sorenson, T. Standiford, 1999. On the physiological role(s) of paraoxonase, Chem. Biol. Interact., 119-120, 379-388
11. Macknes, B., R. Quark, W. Verreth, M. Mackness, P. Holvoet, 2006. Human paraoxonase-1 overexpression inhibits atherosclerosis in a mouse model of metabolic syndrome. Arteriosclerosis Thrombosis Vascular Biology, 26, 1545-150
12. Senti, M., M. Tomas, M. Fito, T. Weinbrenner, M. Covas, J. Sala, R. Masia, J. Marrugat, 2003. Antioxidant paraoxonase 1 activity in the metabolic syndrome, The Journal of Clinical Endocrinology and Metabolism, 88, 5422-5426

13. Tandy, S., R. Chung, E. Wat, A. Kamili, K. Berge, M. Griinari, J. Cohn, 2009. Dietary krill oil supplementation reduces hepatic steatosis, glycemia and hypercholesterolemia in high-fat-fed mice, J. Agric. Food. Chem., 57, 9339-9345

14. Vlaykova, T., T. Georgieva, E Dishlyanova, N. Bozakova, I.P. Georgiev, 2013. Effects of acute Staphylococcus aureus infection on paraoxonase activity, thiol concentrations and ferric reducing ability of plasma in rabbits, Revue de Medecine Veterinaire, 164, 125-131.

15. Watson, A., J. Berliner, S. Hama, B. La Du, K. Faul, A. Fogelman, M. Navab, 1995. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. Journal of Clinical Investigation, 96, 2882-2891