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The Relationship Between Serum Level of Thyroid Hormones and Antioxidant Enzymes in Clinically Healthy Ostriches

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Abstract: Thyroid hormones might be able to regulate the activities of Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GPX). The role of thyroid hormones in metabolic pathways and antioxidant enzyme activities are well known in many species. Nevertheless, there is no report describing probable relationship between thyroid hormones status and erythrocyte antioxidant enzymes. This study was undertaken to investigate the relationship between these parameters in ostriches. Blood samples were taken from the jugular vein of 50 clinically healthy ostriches under aseptic conditions during 6 consecutive days of summer. The serum was analyzed for serum profile of thyroid hormones, SOD and GPX activity. There were no significant differences in serum thyroid hormones and antioxidant enzymes in different days ($p>0.05$). There were significant correlations between SOD and T_3 ($p = 0.021$, $r = 0.421$), also between f T_3 and T_4 ($p = 0.000$, $r = 0.997$). There was no significant correlation between other parameters.

Key words: Antioxidant enzymes, ostrich, thyroid hormones

INTRODUCTION

Thyroid hormones raise the activity of SOD and decrease that of GPX (Pereira *et al*., 1995). Triiodothyronine (T_3) has a profound influence on lipid peroxidation and antioxidant enzyme activities in rat liver (Varghese *et al*., 2001b). In fish, T_3 and thyroxine (T_4) are effective in lipid peroxidation and antioxidant enzyme activities (Varghese *et al*., 2001a).

Mano *et al*. (1995) reported that concentrations of SOD and GPX were increased in hyperthyroid compared with euthyroid rats. Shinohara *et al*. (2000) reported that SOD activity was greater in hyperthyroid than in the euthyroid state. Sawant *et al*. (2003) stated that SOD activity was decreased in both hyper- and hypothyroid rats, but more in hyperthyroid rats. GPX activity was increased while reduced glutathione levels remained unaltered in both hypothyroid and hyperthyroid rats. There are contradictory findings regarding the relation between serum thyroid hormones and cholesterol and triglycerides. The serum cholesterol level generally varies inversely with thyroid activity (Bartley, 1989; Gueorguieva and Gueorguiev, 1997). In contrast, the concentrations of thyroid hormones were not correlated with cholesterol levels in camels and goats (Wasfi *et al*., 1987; Nazifi *et al*., 2002). There is no information about the relation a stressful condition leads to the excessive production of the radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system (Khadija *et al*., 2009). Generation of free radicals is an integral feature of normal cellular function. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Reactive Oxygen Species (ROS) including superoxide radical, hydrogen peroxide and hydroxyl radical have a great impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen (Singh *et al*., 1999). Halliwell and Gutteridge (1999) described several lines of defense against reactive oxygen species in animals. Enzymes with important antioxidant functions include: i) Superoxide Dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) Catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water and iii) Glutathione Peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides. Reduced Glutathione (GSH), a tri-peptide thiol, is an important antioxidant, as well as a co-factor for various antioxidant enzymes (Kidd, 1997). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical (Gonzales *et al*., 1984). The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX. Polyunsaturated fatty acids present in membrane phospholipids are the main target substrates for oxygen radical activity which results in disorganization of cell

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framework and function (Patterson and Leacke, 1998). Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including Malondialdehyde (MDA). The quantization of MDA is widely used as an indicator of lipid peroxidation (Simsek *et al*., 2006). Increased levels of lipid peroxidation products such as MDA have been reported in a variety of diseases like *Dicrocoelium dendriticum* infection in sheep (Simsek *et al*., 2006) and kidney diseases in dogs (Kargin and Fidanci, 2001). The brain injury is reported to be caused by superoxide radical and hydrogen peroxide (Kotos and Wel, 1986). Distomatosis (*Fasciola hepatica*, *Fasciola gigantica* and *Dicroceolium dendriticum* infections) in sheep causes production of reactive oxygen species and lipid peroxidation by significant increase in liver MDA (Deger *et al*., 2008).

Comparative aspects of plasma antioxidant status in sheep and goats and the influence of experimental abomasal nematode infection were investigated by Lightbody *et al*. (2001). Also, Kizil *et al*. (2007) reported oxidative stress and antioxidant status in goats naturally infected with *Mycoplasma agalactiae*. The aim of this study was to present the reference values of oxidative stress parameters in ostrich and investigation of relationship between thyroid hormones and antioxidant enzymes in ostriches. Such information would allow us to make comparisons between normal and abnormal states and provide a better understanding in diseases accompanied by oxidative stress.

The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body (Das and Chainy, 2001). Thyroid hormones might be able to regulate the activities of Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GPX) in the lymphoid organs and skeletal muscles (Pereira *et al*., 1994). The role of thyroid hormones in metabolic pathways and antioxidant enzyme activities are well known in many species such as rat (Asayama *et al*., 1987) and camel (Zia-ur-Rahman *et al*., 2007). The serum levels of thyroid hormones are mainly affected with general body metabolism (Yagil *et al*., 1978), season (Nazifi *et al*., 1999; Abdel-Magied *et al*., 2000) and the water availability (Yagil *et al*., 1978). In camels, serum cholesterol level generally varies inversely with thyroid activity (Bartley 1989; Gueorguieva and Gueorguiev, 1997) and the concentrations of thyroid hormones do not correlate with cholesterol level in camels (Wasfi *et al*., 1987) as in goat (Nazifi *et al*., 2002).

To our best knowledge, there is no report describing probable relationship between thyroid hormones status and erythrocyte antioxidant enzymes. Therefore, this study was undertaken to investigate the relationship between these parameters in ostriches.

MATERIALS AND METHODS

Animals: The investigation was carried out on ostriches which were reared mainly in south of Iran (Fars province). Fifty ostriches, aged < one year old were screened for this study. All the animals were clinically healthy and free from internal and external parasites.

Blood sampling: Blood samples (10 ml) were taken from the jugular vein of 50 clinically healthy ostriches under aseptic conditions. The samples were taken at 8 A.M. during 6 consecutive days of spring with a mean temperature of 32°C. For the determination of haemoglobin, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX), blood samples were collected by jugular venepucture into vacutainers containing Ethylenediamine Tetra-Acetic Acid (EDTA) as an anticoagulant. For determination of serum thyroid hormones, blood samples were collected into vacutainers and serum was separated by centrifugation at 750 g for 15 min and stored at -20°C until use. The samples with haemolysis were thrown away.

Measurements: Serum T_4, T_3, fT_3 and fT_4 were measured by Radioimmunoassay (RIA) kits in the Namazi Research Center, Shiraz, Iran. The areas of validation for T_3, T_4, fT_3 and fT_4 assays including limits of detection and precision in standard curve following sample dilution, inter-and intra-assay coefficients of variation results were considered. Intra-and inter-assays for T_4 and T_3 were found to below 6.2%, 8.6%, 3.3% and 8.6% respectively.

Haemoglobin concentration was measured by Cyanmethaemoglobin method. SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (RANSOD kit, Randox Com United Kingdom). This method employs xanthine and Xanthine Oxidase (XOD) to generate superoxide radicals which react with 2-(4-Prodiodophenyl)-3- (4-nitrophend)-5-phenyltetrazoliumchloride (INT) to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD was considered a 50% inhibition of reduction of INT under the condition of the assay. GPX was measured by the method of Paglia and Valentine (1967) (RANSEL kit, Randox Com, United Kingdom). GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was measured at 340 nm. Digestion of serum was performed by a mixture of perchloric and nitric acid (3:7 ratios respectively).

Table 1: Mean±SE serum concentration of SOD, GPX, T_3, T_4, fT_3 and fT_4 in 50 clinically healthy ostriches

Parameter	SOD U/gHb	GPX U/gHb	T_3 ng/ml	T_4 g/dlμ	fT_3 Pg/ml	fT_4 g/dl
Healthy ostriches	25.4022±0.7345	1.7196±0.0496	1.7333±0.1704	3.1267±0.1987	4.3733±0.3498	3.0033±0.2657

Table 2: The correlation between serum concentrations of SOD, GPX, T_3, T_4, fT_3 and fT_4 in 50 clinically healthy ostriches

Parameter	SOD	GPX	T_3	T_4	fT_4	fT_3
SOD	1	-0.033	0.421*	-0.106	0.115	0.122
GPX	-0.033	1	-0.074	0.243	0.201	0.210
T_3	0.421*	-0.074	1	0.102	0.331	0.351
T_4	-0.106	0.243	0.102	1	0.250	0.234
fT_4	0.115	0.201	0.331	0.250	1	0.997**
fT_3	0.122	0.210	0.351	0.234	0.997**	1

*Significant in $p<0.05$; **Significant in $p<0.01$

Statistical analysis: The data were expressed in SI units and analyzed by repeated measurements ANOVA and the Bonferroni multiple comparisons test using SPSS/PC software (Norusis, 1993). All values were expressed as mean and Standard Error (SE) and $p<0.05$ was seen as statistically significant.

RESULTS

The Mean±SE of concentrations of SOD, GPX, T_3, T_4, fT_3 and fT_4 in healthy ostriches are shown in Table 1. The correlation between serum concentrations of SOD, GPX, T_3, T_4, fT_3 and fT_4 in healthy ostriches are observed in Table 2. Reference values of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) in 50 ostriches < one year old are showed in Table 1. The values were as followed: SOD 24.6677-26.1365 U/gHb, GPX 1.6700-1.7692 U/gHb. There were significant correlations between SOD and T_3 ($p = 0.021$, $r = 0.421$), also between fT_3 and fT_4 ($p = 0.000$, $r = 0.997$). There was no significant correlation between other parameters.

DISCUSSION

There is little information about the oxidative stress parameters (SOD, GPX,) of ostriches. The activity of antioxidant enzymes in<one year old clinically healthy ostriches were very lower than the values reported about sheep (Nazifi *et al.*, 2010; Lightbody *et al.*, 2001; Kizil *et al.*, 2007). A comparison of our results to those reported by Todorova *et al.* (2005) showed that reference values of oxidative stress indices such as SOD in carnivores (dogs and cats) were higher than the values obtained in ostriches. This status may be due to their different diets, in other word, generation of free radicals in carnivores and herbivores is more than that in birds. The oxidative status is variable and can be changed by different factors.

In the present study, all ostriches were young. In normal conditions, age influences greatly free radical generation and consequently, the level of antioxidant defense enzymes. In similar investigations on rats at different ages, decreased plasma levels of antioxidant vitamins C and E, decreased SOD activity and increased CAT activity was observed (De and Durad, 1991). There were significant correlations between SOD and T_3 ($p = 0.021$, $r = 0.421$), also between fT_3 and fT_4 ($p = 0.000$, $r = 0.997$). The significant correlation between T_3 and SOD are probably due to important role of thyroid hormones in lipid metabolism and antioxidant function of SOD in lipid peroxidation. The T_3 markedly affects lipid peroxidation and antioxidant enzyme activities in rat liver (Varghese *et al.*, 2001a,b). It has been demonstrated with more accuracy that thyroid status controls the mitochondrial antioxidant defense system (Das and Chainy, 2001) by regulating the activities of SOD, catalase and GPX. Some studies have highlighted some complex relationships between thyroid status and antioxidant SOD and GPX activities. Asayama *et al.* (1987) suggested that increased lipid peroxidation in hyperthyroid rats was linked to enhanced oxidative metabolism and decreased GPX activity, whereas Mano *et al.* (1995) observed increased SOD and GPX activities in hyperthyroid rats compared to euthyroid animals. It was stated that GPX activity was increased, while glutathione concentrations remained unaltered in both hyperthyroid and hypothyroid rats (Shinohara *et al.*, 2000; Sawant *et al.*, 2003). Nazifi *et al.* (2010) observed, significant correlations between triiodothyronine (T_3) and GPX ($p<0.05$; $r = 0.203$) and thyroxine (T_4) and GPX ($p<0.05$; $r = 0.312$) in goats (Nazifi *et al.*, 2010).

In this study there was no significant correlation between other thyroid hormones and antioxidant enzymes. Nazifi *et al.* (2009a) couldn't find significant correlation between trace elements, thyroid hormones and antioxidant enzymes in dromedary camels, but they revealed that there is a significant negative correlation between Fe and SOD ($p<0.01$, $r = -0.493$). Also there was no significant relation between serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days in Iranian ewes (Nazifi *et al.*, 2009b). Wasfi *et al.* (1987) reported that the concentrations of thyroid hormones were not correlated with cholesterol levels. Furthermore, Nazifi *et al.* (2002) reported that in clinically healthy Iranian male goats there was no significant correlation between thyroid hormones and serum cholesterol, triglyceride, total lipids and lipoproteins.

Our results of serum concentration of SOD is similar that reported by Pereira *et al.* (1994) about increase in SOD activity with hyperthyroidism, but serum concentration of GPX did not allow to confirm the observations of Pereira *et al.* (1994) for reduced GPX activity under the influence of thyroid hormones. At the same time, our findings do not rule out the necessity of additional studies on the basis of conflicting data about SOD and GPX activities in hyperthyroid states: in some communications, SOD (Shinohara *et al.*, 2000) as well as GPX (Sawant *et al.*, 2003) were reported to be elevated whereas in other investigations, SOD was found to be decreased (Sawant *et al.*, 2003) as well as GPX (Asayama *et al.*, 1987).

Humphries *et al.* (1983) revealed that in experimental copper deficiency in calf, plasma concentration of copper and SOD activity of erythrocytes decreased fast and severely. Similar investigation was performed by Konstantinova and Russanov (1988), in which there was a positive correlation between plasma concentration of copper and SOD activity of erythrocytes. It seems as if the T_4: T_3 ratio is more important than the level of individual hormone (Zia-ur-Rahman *et al.*, 2007) and it might be influenced by the season, temperature and effect of seasonal variation in the feed supply (Fay *et al.*, 2003). In this study that sampling was done in spring, the mean concentrations of thyroid hormones in ostriches was very down in comparison pervious research that sampling was done in winter, these differences can be due different dietary. Also, there were not some findings such as those of Oki and Atkinson (2004) showing that in harbor seals, neither total nor free T_4 or T_3 displayed a diurnal rhythm in summer and winter. However, T_4, T_3 and fT_3 levels were significantly higher in the winter than in the summer. In summer the activity of the thyroid gland is minimal and generally, the function of this gland is connected with systemic adaptation to low temperatures. Komosa *et al.* (1990) reported that in mature mares diurnal rhythm was observed in T_3 concentration only in summer months. Freake *et al.* (1989) stated that a diurnal variation was maintained in all thyroid states, with the peak value in the middle of the dark period being 3-fold higher than the nadir. However, Sturgess *et al.* (1989) found that TSH followed a diurnal rhythm with a peak level at 23:30 h and a trough level at 14:30 h. This study showed significant time-related variability in TSH and thyroid hormone levels in treated hypothyroid patients. Flisinska-Bojanowska *et al.* (1991) reported that in mares a diurnal rhythm in T_3 level was found throughout the pregnancy. No diurnal rhythm in the T_4 level was observed. Normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones.

Selenium is required for conversion of thyroxine (T_4) into the more active triiodothyronine (T_3) via the enzyme type deiodinase (Awadeh *et al.*, 1998). Additionally, selenoperoxidases and thioredoxin reductase protect the thyroid gland from peroxides produced during the synthesis of hormones (Aurthor and Beckett, 1999; Aurthor *et al.*, 1992). However there are some other trace elements such as iron, zinc and copper that their role in the thyroid are less well defined but sub-or super optimal dietary intakes of all these elements can adversely affect thyroid hormone metabolism (Aurthor and Beckett, 1999). Interrelationships among copper and iodine and thyroid hormones were studied in rats by Esipenko and Marsakova (1990) and Aurthor *et al.* (1996). Kececi and Keskin (2002) reported a significant negative correlation between zinc concentration of erythrocytes and serum thyroid hormones in healthy male Herino lambs and Angora goats. Copper deficient rats showed a decrease in the value of iodine metabolism in different organs and tissues excluding liver, whereas a sharp increase in the content of organic iodine was observed. In fact copper deficiency enhances the effect of hypothyroidism (Aurthor *et al.*, 1996).

Wichtel *et al.* (1996) showed that the plasma concentration of total thyroxin was increased by selenium treatment and Bik (2003) determined the effect of selenium and iodine oral supplements on the concentration of T_3 and T_4 in the serum of sheep. It is important to note that only when selenium levels decreased by more than 80%, deiodinase activity was markedly decreased (Bates *et al.*, 2000). Bates *et al.* (2000) stated that with the exception of liver, skin and nonpregnant uterus, all of the tissues studied (including cerebrum, thyroid, pituitary, brown adipose tissue, ovary, testes and placenta) maintained substantial deiodinase activity (>50%) during prolonged selenium deficiency. Although the ability of a tissue to maintain deiodinase activity in the face of dietary selenium deprivation was associated in some tissues with a concomitant local preservation of selenium concentration, this was not the case for all tissues. How selenium levels are maintained in specific tissues, whether selenium is sequestered in specific cells of a tissue or organ during dietary selenium deprivation and the precise mechanism which plasma T_3 levels are maintained in selenium deficient animals remain unanswered (Bates *et al.*, 2000). Copper is the main component of SOD that plays a vital role as an antioxidant and protects the testis from oxidative stress (Henkel *et al.*, 2003; Zini and Schlegel, 1997). Humphries *et al.* (1983) showed that in the use of iron enriched diets, there was a negative correlation between Fe and SOD activity.

Conclusion: According to the previous studies, there is no correlation between serum thyroid hormones, specially thyroxin, lipids and lipoproteins and antioxidant enzymes in clinically healthy animals but in hypo-and hyperthyroidism, there may be correlations between these parameters. However, in the present study there were a positive significant correlation between triiodothyronin and SOD, but there wasn't any significant correlation between thyroid hormones and GPX, also between thyroxin and SOD. In addition, the explanation for these findings is not possible at this moment of time. The cause of these findings is unclear and there is no earlier report in this respect. No other explanation for the lack of proportionality among these parameters is actually available and further investigations are needed to clarify this point.

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