

STUDIES ON THE PHYSIOLOGICAL RELATIONSHIP
BETWEEN THYROID HORMONES, SERUM LIPID PROFILE
AND ERYTHROCYTE ANTIOXIDANT ENZYMES IN
CLINICALLY HEALTHY IRANIAN FAT-TAILED SHEEP

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Summary

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The aim of the present study was to determine whether there was any correlation between serum thyroid hormones, lipids, lipoproteins and the activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) in adult nonpregnant uniparous Iranian pure bred fat-tailed ewes. Blood samples (10 mL) were taken from the jugular vein of 30 clinically healthy Iranian adult non-pregnant uniparous ewes at 8 a.m during 6 consecutive days of summer with a mean ambient temperature of 40°C. The serum was analyzed for thyroxine (T₄), tri-iodothyronine (T₃), free thyroxine (fT₄), free tri-iodothyronine (fT₃), cholesterol, triglyceride, lipoproteins [high density lipoprotein (HDL-cholesterol), low density lipoprotein (LDL-cholesterol) and very low density lipoprotein (VLDL-cholesterol)], SOD and GPX activity. There were no significant differences in serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days (P>0.05). In addition, there were no significant correlations between serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days (P>0.05).

Key words: erythrocyte antioxidant enzymes, Iranian fat-tailed sheep lipids, lipoproteins, thyroid hormones

INTRODUCTION

The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body (Das & 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). Asayama *et al.* (1987) suggested that the enhanced oxidative metabolism and decreased GPX in hyperthyroid rats resulted in an increase in lipid peroxidation. Endothelial cells produce superoxide anions and oxidize low-density lipoprotein

(LDL) *in vitro*, however, the role of superoxide anions in endothelial cells induced LDL oxidation is unclear (Fang *et al.*, 1998). Thyroid hormones raise the activity of SOD and decrease that of GPX (Pereira *et al.*, 1995). T₃ has a profound influence on lipid peroxidation and antioxidant enzyme activities in rat liver (Varghese *et al.*, 2001b). In fish, T₃ and T₄ 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 & 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 between serum thyroid hormones, lipids, lipoproteins and the activities of erythrocyte antioxidant enzymes (SOD and GPX) in sheep. The aim of the present study was to determine whether there was any relationship between serum thyroid hormones, lipids, lipoproteins and the activities of erythrocyte SOD and GPX in nonpregnant uniparous Iranian fat-tailed ewes.

MATERIALS AND METHODS

Experimental animals

This study was performed using blood samples from 30 adult nonpregnant uniparous Iranian pure bred fat-tailed ewes at the Animal Husbandry Unit, College of Agriculture, Shiraz University, located 20 km north of Shiraz, Iran. Animals were fed with hay (mainly alfalfa and grass) from the pasture near the husbandry unit. An experienced veterinarian supervised this unit. All animals were clinically healthy and free from internal and external parasites. They were treated with fenbendazole (Damloran Company, Borujerd, Iran) 10 mg/kg 30 days prior to the study. The prevention of internal and external parasites is a routine practice in this unit. Each ewe had a separate file including all necessary records so that its characteristics, including age, sex, etc., could be determined. Iranian fat-tailed sheep are reared under the climatic conditions of Iran. The physiological importance of the fat tail is to provide energy during drought seasons and conditions of feed deprivation, which are not uncommon under the climatic conditions of Iran.

Blood sampling

Blood samples (10 mL) were taken from the jugular vein of 30 clinically healthy Iranian ewes at 8 a.m during 6 consecutive days of summer with a mean ambient temperature of 40°C. For the determination of haemoglobin, SOD and GPX, blood samples were collected by jugular venepuncture into vacutainers containing EDTA as an anticoagulant. For the determination of serum lipids, lipoproteins and 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.

Studied parameters and methods

Serum T_4 , T_3 , fT_3 and fT_4 were measured by radioimmunoassay 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. The serum was analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis & Ashwood, 1994) and triglycerides by the enzymatic procedure of McGowan *et al.* (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. HDL-cholesterol was measured by the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to serum to aggregate non-HDL lipoproteins, which were sedimented by centrifugation (10000 g for 5 min). The residual cholesterol was then measured by the enzymatic method (Burtis & Ashwood, 1994). LDL-cholesterol was calculated as the difference between cholesterol measured in the precipitate and that in the HDL fraction. VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedewald *et al.*, 1972). Haemoglobin was measured by the 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-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (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 that which caused a 50% inhibition of the rate of reduction of INT under the conditions of the assay. GPX was measured by the method of Paglia & Valentine (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 oxidised 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.

Statistical analysis

The data were expressed in SI units and analyzed by repeated measurement ANOVA and the Bonferroni multiple comparisons test using SPSS/PC software (Norusis, 1993). All values were expressed as mean and standard error (SEM), and $P < 0.05$ was seen as statistically significant.

RESULTS AND DISCUSSION

The concentrations of serum thyroid hormones (T_3 , T_4 , fT_3 and fT_4), lipids (cholesterol and triglyceride), lipoproteins (HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) and antioxidant enzymes (SOD and GPX) in adult nonpregnant uniparous Iranian ewes during 6 consecutive days period in summer are presented in Table 1. There were no significant differences in serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days ($P > 0.05$). There were some findings such as those of Oki & Atkinson (2004) showing that in harbour seals, neither total nor free T_4 or T_3 displayed a diurnal rhythm in summer and

Table 1. Concentrations of serum cholesterol, triglycerides, HDL, LDL, VLDL, SOD, GPX, T₃, T₄, fT₃, fT₄ in adult nonpregnant uniparous Iranian ewes during 6 consecutive days in summer (n=30)

Days	Parameter										
	T ₃ , nmol/L	T ₄ , nmol/L	fT ₃ , pmol/L	fT ₄ , pmol/L	Cholesterol, mmol/L	Triglyceride, mmol/L	HDL cho- lesterol, mmol/L	LDL cholesterol, mmol/L	VLDL cholesterol, mmol/L	SOD, U/g Hb	GPX, U/g Hb
1 st day	1.53 ±0.04	60.01 ±1.20	4.48 ±0.09	20.49 ±0.34	1.65 ±0.03	0.20 ±0.005	0.97 ±0.01	0.57 ±0.03	0.04 ±0.001	1073.91 ±87.92	93.50 ±6.74
2 nd day	1.57 ±0.05	65.90 ±0.53	4.48 ±0.16	20.68 ±0.49	1.64 ±0.03	0.20 ±0.008	0.97 ±0.01	0.58 ±0.03	0.04 ±0.001	1017.44 ±74.31	86.17 ±6.11
3 rd day	1.53 ±0.03	67.36 ±0.39	4.58 ±0.11	21.88 ±0.43	1.67 ±0.03	0.20 ±0.006	0.96 ±0.01	0.62 ±0.03	0.04 ±0.001	966.4 ±66.23	85.59 ±6.03
4 th day	1.56 ±0.04	63.84 ±0.57	4.53 ±0.13	20.70 ±0.48	1.66 ±0.03	0.18 ±0.006	0.96 ±0.01	0.61 ±0.03	0.04 ±0.001	944.06 ±93.47	95.73 ±6.67
5 th day	1.58 ±0.05	61.68 ±0.75	4.62 ±0.17	20.53 ±0.46	1.66 ±0.01	0.21 ±0.002	0.97 ±0.01	0.59 ±0.02	0.04 ±0.0004	944.75 ±82.58	83.60 ±8.00
6 th day	1.56 ±0.04	66.05 ±0.65	4.54 ±0.15	21.15 ±0.37	1.65 ±0.02	0.19 ±0.006	0.95 ±0.01	0.62 ±0.03	0.04 ±0.001	950.37 ±81.12	99.91 ±6.69

Values are presented as mean±SEM. There were no significant differences in serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days (P>0.05).

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. In the present study there were no significant relations between serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in different days ($P>0.05$).

Our results about serum concentrations of SOD and GPX did not allow to confirm the observations of Pereira *et al.* (1994) for increased SOD and reduced GPX activities 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).

The serum cholesterol level generally varies inversely with thyroid activity (Bartley, 1989; Gueorguieva & Gueorguiev, 1997). The net effect of thyroid hormones on cholesterol metabolism is to increase the rate of its catabolism by the liver, thereby lowering the cholesterol (Bartley, 1989). Gueorguieva & Gueorguiev (1997) reported that in dairy cows serum cholesterol was consistently negatively correlated with serum T_4 and T_3 levels. Ibrahim *et al.* (1984) reported that in Nubian goats, hyperthyroidism decreased serum triglyceride, cholesterol and phospholipid concentrations. An increase in VLDL-cholesterol is commonly associated with hypothyroidism (Bartley, 1989). In contrast to the above opinions, 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 were no significant correlations between thyroid hormones and serum cholesterol, triglyceride, total lipids and lipoproteins. The results of the present study agreed well with the results of Wasfi *et al.*, (1987) and Nazifi *et al.*, (2002). Perhaps, in clinically normal animals there was no correlation between serum thyroid hormones, lipids and lipoproteins and antioxidant enzymes. Probably in hypo- and hyperthyroidism however, there should be correlations between the above mentioned parameters. In the present study, the effective factors such as age, sex, breed and pregnancy were omitted and nonpregnant uniparous Iranian ewes were used. However, in the present study there were no significant changes in the levels of studied parameters. In addition, there were no significant correlations between serum thyroid hormones, lipids, lipoproteins and antioxidant enzymes in

different days ($P>0.05$). The explanation for these findings is not possible at this moment in 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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