Relationships Between Thyroid Hormones, Serum Trace Elements and Erythrocyte Antioxidant Enzymes in Goats

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ABSTRACT
Thyroid hormones might be able to regulate the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) enzymes. The role of thyroid hormones in metabolic pathways and antioxidant enzyme activities are well known in many species. Nevertheless, there is no report describing the probable relationship between thyroid hormones status, erythrocyte antioxidant enzymes and serum profiles of trace elements. This study was undertaken to investigate the relationship between these parameters in Iranian native goats. Blood samples were taken from the jugular vein of 50 clinically healthy Iranian native goats under aseptic conditions during 6 consecutive days of summer. The serum was analyzed for serum profile of thyroid hormones, trace elements, SOD and GPX activity. There were no significant differences in serum thyroid hormones, serum levels of zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), selenium (Se) and antioxidant enzymes on different days (P>0.05). There were significant correlations between triiodothyronine (T3) and GPX (P<0.05; r=0.203) and thyroxine (T4) and GPX (P<0.05; r=0.312). There was no significant correlation between other parameters.

INTRODUCTION
The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body (Das and Chainy, 2001). Thyroid hormones seem to regulate the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) enzymes 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). Serum levels of thyroid hormones are mainly affected by 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).

It is well known that steroid hormones, electrolytes and trace elements all play an important role in controlling the reproductive functions in animals (Al-Qarawi et al., 2000). In camels, plasma testosterone concentrations have been found to be correlated significantly with the contents of Na+, K+, Ca++ and Mg++ in all genital organs but only with epididymal contents of phosphorus and iron (Zia-ur-Rahman et al., 2007). But the serum cholesterol level generally varies inversely with thyroid activity (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 the best of our knowledge, there is no report describing the probable relationship between thyroid hormone status, erythrocyte antioxidant enzymes and serum profiles of trace elements. Therefore, this study was undertaken to investigate the relationship between these parameters in Iranian native non pregnant goats.

MATERIALS AND METHODS
Experimental animals
The investigation was carried out on 50 non pregnant uniparous Iranian native goats which were reared mainly in South of Iran (Fars province). All the animals were clinically healthy and free from internal and external parasites. All the goats were treated with fenbendazol (10 mg/Kg) 30 days before the start of the study.
Blood sampling

Blood samples (10 ml) were taken from the jugular vein of experimental goats under aseptic conditions. The samples were taken at 8 a.m. during 6 consecutive days in summer, 2009 with a mean temperature of 38°C. For the determination of hemoglobin, superoxide dismutase (SOD) and glutathione peroxidase (GPX), blood samples were collected by jugular venepuncture into vacutainers containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. For determination of serum thyroid hormones and trace elements, blood samples were collected into vacutainers, serum was separated by centrifugation at 750g for 15 min and stored at -20°C. The samples showing hemolysis were discarded.

Measurements

Serum triiodothyronine (T3) and thyroxine (T4) were measured by radioimmunoassay (RIA) method (kits available from Immunotech Company, IMMUNOTECH-Radiova-Prague-Czech Republic) in Namazi Research Center, Shiraz, Iran. The areas of validation for T3 and T4 assays included limits of detection and precision in standard curve following sample dilution, inter- and intra-assay coefficients of variation. The analytical sensitivity of T3 and T4 were 0.3 and <12.6 nmol/L, respectively. Intra- and inter-assays coefficients of variation for T3 and T4 were below 6.2 and 8.6%, and 3.3 and 8.6%, respectively.

Haemoglobin concentration was measured by cyanmethemoglobin 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 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 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 catalyzes 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. For the measurement of serum trace elements, digestion of serum was performed by a mixture of perchloric and nitric acid (3:7 ratio), respectively. Manganese, copper, iron, selenium and zinc were measured using an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan).

Statistical analysis

The data were expressed in international units (SI) and analyzed by a repeated measure ANOVA and the Bonferroni multiple comparison test using SPSS/PC software (Norusis, 1993). Pearson's correlation coefficient was calculated for determination of the relationship between different biochemical markers during the consecutive days. All values were expressed as mean ± standard error (SE) and P<0.05 was considered as statistically significant.

RESULTS

Diurnal variations of serum thyroid hormones (T3 and T4), antioxidant enzymes (SOD and GPX), and serum levels of trace elements (Zn, Cu, Fe, Mn and Se) in Iranian native goats during 6 consecutive days in summer are presented in Table 1. There were no significant differences in serum thyroid hormones, serum level of zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), selenium and antioxidant enzymes among different days (P>0.05). There were significant correlations between T3 and GPX (P<0.05; r=0.203) and T4 and GPX (P<0.05; r=0.312). There was no significant correlation between other parameters.

DISCUSSION

In the present study, significant correlations between triiodothyronine (T3) and GPX (P<0.05; r=0.203) and thyroxine (T4) and GPX (P<0.05; r=0.312) were observed. There was no significant correlation between other parameters. The significant correlation between T3 and GPX and T4 and GPX are probably due to important role of thyroid hormones in lipid metabolism and antioxidant function of GPX in lipid peroxidation. The T3 markedly affects lipid peroxidation and antioxidant enzyme activities in rat liver (Varghese et al., 2001). 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 (Shinhoara et al., 2000; Sawant et al., 2003). Thyroid hormones may increase the activity of SOD and decrease GPX (Pereira et al., 1995). It seems as if the T3:T4 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).

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 (T4) into the more active triiodothyronine (T3) via the enzyme type 4 deiodinase (Awadeh et al., 1998). Additionally, selenoperoxidases and thioredoxin reductase protect the thyroid gland from peroxides produced during the synthesis of hormones (Author and Beckett, 1999). However, there are some other trace elements such as iron, zinc and copper that their role in the thyroid is less well defined but sub-or super optimal dietary intakes of these elements can adversely affect thyroid hormone metabolism (Author...
and Beckett, 1999). Nevertheless, we couldn’t find any significant correlation between trace elements, thyroid hormones and antioxidant enzymes. Interrelationships among copper and iodine and thyroid hormones were studied in rats by Author et al. (1996). Kececi and Keskin (2002) reported a significant negative correlation between zinc concentrations of erythrocytes and serum thyroid hormones in healthy male Herino lambs and Angora goats. Copper deficient rats showed a decrease in the content of organic iodine was observed. In fact, copper deficiency enhances the effect of hypothyroidism (Kececi and Russanov, 1988) found a positive correlation between plasma concentration of copper and SOD activity of erythrocytes. However, we could not show any correlation between copper concentration and SOD activity in Iranian native goats. Inversely, there was a negative correlation between these parameters in human as previously stated (Tungtrongchit et al., 2003). We could not find any correlation between zinc concentration and SOD activity either, but it is known that zinc is an essential element that controls the balance in oxidant-antioxidant system (Brighthope, 2004).

It is not possible to explain fully results of the present study at this moment of time. Further investigations are needed to interpret these changes.

### REFERENCES


Awadeh FT, RL Kincaid and KA Johnson, 1998. Effect of level and source of dietary selenium on

### Table 1: Variation in the concentrations of serum thyroid hormones, trace elements and antioxidant enzymes in Iranian native goats during 6 days (n = 50)

<table>
<thead>
<tr>
<th>Days</th>
<th>T₃</th>
<th>T₄</th>
<th>Zinc</th>
<th>Copper</th>
<th>Iron</th>
<th>Manganese</th>
<th>Selenium</th>
<th>SOD</th>
<th>GPX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(µmol/L)</td>
<td>(U/g Hb)</td>
<td>(µmol/L)</td>
</tr>
<tr>
<td>1st</td>
<td>± 0.13</td>
<td>0.94</td>
<td>± 2.38</td>
<td>± 2.55</td>
<td>± 0.67</td>
<td>± 1.07</td>
<td>± 0.004</td>
<td>± 0.02</td>
<td>± 77.79</td>
</tr>
<tr>
<td>2nd</td>
<td>± 0.11</td>
<td>0.94</td>
<td>± 2.72</td>
<td>± 2.93</td>
<td>± 0.93</td>
<td>± 1.17</td>
<td>± 0.005</td>
<td>± 0.03</td>
<td>± 81.24</td>
</tr>
<tr>
<td>3rd</td>
<td>± 0.14</td>
<td>0.94</td>
<td>± 2.65</td>
<td>± 2.38</td>
<td>± 0.89</td>
<td>± 1.36</td>
<td>± 0.006</td>
<td>± 0.04</td>
<td>± 71.37</td>
</tr>
<tr>
<td>4th</td>
<td>± 0.13</td>
<td>0.94</td>
<td>± 2.11</td>
<td>± 2.87</td>
<td>± 1.09</td>
<td>± 1.02</td>
<td>± 0.007</td>
<td>± 0.03</td>
<td>± 85.26</td>
</tr>
<tr>
<td>5th</td>
<td>± 0.17</td>
<td>0.94</td>
<td>± 2.88</td>
<td>± 2.63</td>
<td>± 1.07</td>
<td>± 1.19</td>
<td>± 0.006</td>
<td>± 0.04</td>
<td>± 78.87</td>
</tr>
<tr>
<td>6th</td>
<td>± 0.15</td>
<td>0.94</td>
<td>± 2.56</td>
<td>± 2.89</td>
<td>± 1.13</td>
<td>± 1.12</td>
<td>± 0.006</td>
<td>± 0.05</td>
<td>± 83.93</td>
</tr>
</tbody>
</table>

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

and and Beckett, 1999). Nevertheless, we couldn’t find any significant correlation between trace elements, thyroid hormones and antioxidant enzymes. Interrelationships among copper and iodine and thyroid hormones were studied in rats by Author et al. (1996). Kececi and Keskin (2002) reported a significant negative correlation between zinc concentrations of erythrocytes and serum thyroid hormones in healthy male Herino lambs and Angora goats. Copper deficient rats showed a decrease in the content of organic iodine was observed. In fact, copper deficiency enhances the effect of hypothyroidism (Kececi and Russanov, 1988) found a positive correlation between plasma concentration of copper and SOD activity of erythrocytes. However, we could not show any correlation between copper concentration and SOD activity in Iranian native goats. Inversely, there was a negative correlation between these parameters in human as previously stated (Tungtrongchit et al., 2003). We could not find any correlation between zinc concentration and SOD activity either, but it is known that zinc is an essential element that controls the balance in oxidant-antioxidant system (Brighthope, 2004).

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