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SERUM LEVELS OF TSH, T4 AND T3 HORMONES IRAN'S CAUCASIAN MALE PITVIPERIN ACTIVE SEASONS

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ABSTRACT

The present study providing data on circulating TSH, T3 and T4 concentration of Caucasian snake in the active seasons· The parameters of body length and weight were measured in the Caucasian male vipers (n=24) from Lar area in Tehran province· The serum levels of the TSH, T_4 and T3 hormones were measured in the October- November, of 2014, by ELISA in the wave length of 450 nm· The results show that the mean values of the body length and weight of the male Caucasian vipers were 43·2 ± 2.3cm and 427·5± 45 g respectively· The circulating TSH, T4 and T3 concentration were higher during per-hibernating time in fall and lower in summer p(≤ 0·001) during breeding season· Therefore a seasonal variation was observed in C· Vipers, thyroid function appears to be influenced by temperature, that may directly alter the hypothalamic-hypophysis-thyroid axis, and by reproductive activity, supporting a role for thyroid hormones in energy-demanding activities of animal.

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INTRODUCTION

Seasonal changes in the thyroid gland related to temperature have been observed in number of reptiles. Thyroid gland plays a major role in maintaining physiologic homeostasis in all vertebrates (Taylorand De-Nardo, 2010). Reptile thyroid levels are well below that of mammals (Hulbert, 2007), with snakes being the lowest and chelonians the highest, crocodilians are similar to birds (Greenacre, 2001). Thyroid physiology influences many processes such as shedding, growth, development (Rupik, 2011), reproduction (Fani *et al*. 2017), metabolic rate, nutrient assimilation and activity. Many factors also influence thyroid values including age, sex, diurnal changes, seasonal changes, day length, shedding, illness(Boyer 2011), stress and breeding (Greenacer, 2001). As seasonal changes in the thyroid gland activity of reptiles are well established (Lynn, 1970), it was considered essential that any attempt to study the nature of thyroidal secretion in these animals should be done on a seasonal basis.

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In fact the thyroid of most cold-blooded animals show evidences of a marked decrease in activity during the winter, but the seasonal changes in the thyroid have sometimes been regarded as the causal factors in the seasonal cycles of activity in these animals. There is evidence (Stein and Carpenter, 1943) that differences in illumination in summer and winter may also be a factor in the annual thyroid cycle in Triturus. Reptiles living in temperate regions show a seasonal change in the thyroid activity (Eggert, 1936; Evans and Hegre, 1940), but there is an indication, for at least one form, that the thyroid undergoes no period of marked inactivity in animals inhabiting warmer regions (Evans and Hegre, 1938). Thyroid hormone warrants specific attention as a potential regulator between temperature and reproduction. There is a direct relationship between temperature and iodine uptake by the thyroid), and cold temperatures can also reduce target tissue response to thyroid hormones (Turner & Tipton, 1972). Thyroxin (Capital T) thyroxin (T4) increases tissue metabolism in snakes (Thapliyal, Kumar, and Oommen, 1975), but it may also have a direct effect on reproduction. In the most thorough study of T4 and reproduction in snakes, Bona-Gallo *et al.* (1980) demonstrated a pronounced annual T4 cycle with plasma concentrations lowest in winter and substantially elevated during late spring. In males, plasma T4 concentrations elevates when testes mass and plasma T concentrations peaks in May (when females were vitellogenic), but plasma T4 concentrations do not actually peak until June, which led to suggest that T4 may play a role in testis regression. In females, T4 concentrations peak at the height of ovarian activity (May). Walker (1973) observes that, in *S. cyanogenys,* thyroxin at low temperatures can be stocked in the plasma by liaison of bound proteins and the assimilation of the hormone by target tissues; but on the other hand, TSH secretion is photosensitive. Incoming light releases TSH secretion after long periods of cold and it then presides over the physiological awakening of the reptile. In the *V. aspis*, thyroxin values are low throughout the winter except for 2 to 3 weeks before emergence. It is also possible that, if hibernation causes endocrine structures to function slowly, it does not stop then, and a gradual accumulation of intraglandular plasma thyroxin within is feasible; by the end of hibernation it could reach a sufficient value to reactivate metabolism and await the signal for release of TSH, the mode of functioning is not known precisely. The gross morphology of the thyroid gland in reptiles closely approaches that found in higher vertebrates. It has referred to the reptilianthyroid, simply as a single, lobulate, follicular structure, which contains colloid, and lies close to the trachea (Lynn, 1960). Beginning with the work of Weigmann (1932) there has been considerable interest in seasonal changes in the activity of the reptilian thyroid.

Weigmann found significant seasonal changes in epithelial height in the thyroid of Lacerta vivipara which indicated a much greater functional activity in summer than in winter. Eggert (1935) found that the thyroid exhibits signs of maximal activity during June and July. At this time all follicles contain thin, freshly formed colloid with many peripheral chromophobe droplets. The epithelium is high and many cells exhibit mitotic figures. Epithelial height and mitotic rate gradually decrease during late August and September and there is an increase in stored colloid. The gland is most inactive during the winter months when the lizards are in deep hibernation. Experiments on the effects of high and low temperature led to the conclusion that the seasonal changes are largely dependent upon environmental temperature (Eggert, 1935; Igbok weand Ezeasor, 2015). Eggert (1935) found no evidence of changes in histology correlated with the breeding season in Lacerta. Wilhoft (1966) also demonstrated that prolonged exposure to temperatures which are optimal for normal activity (34 - 350C) causes death in some animals, a greater frequency of molting in some, and a rise in heart rate and in thyroid epithelial height in all. In non-hibernating lizards (**Anoliscarolinensis** from Louisiana) thyroid histology indicates high secretory activity even during the coldest months and the lowest epithelial heights are found during spring and early summer (Evans and Hegre, 1938). Furthermore, to determine the concentration of thyroid activity and whether it is related to seasonal events is assessed in this study.

MATERIAL AND METHODS

Twenty four Caucasian male vipers (*Gloydiushalyscaucasicus*) were collected from Lar region (Damavand mountain ,32´ -51° to 32´ -52° eastern length and 52 ´ -35° to 50´- 36° northern width) from Tehran province in June, August, November of year 2014. Samples were maintained in a vivarium under conventional conditions in the venomous animals department of Razi vaccine and serum production institute. Snakes were weighed by point scale and body length was measured by meter (calibrated). Blood serum samples were collected from ventral tail vein bleeding of snakes and stored at −20°C until assay. The TSH, T4, T3 hormones were assayed by ELISA(Commercial kits, AccuBind ELISA Micro wells, Monobind, Inc. Lake Forest, CA, USA).

RESULTS

Concentration of TSH, T4 and T3

Serum level of Thyroid stimulating hormone (TSH), Triiodothyronine (T3), Thyroxin (T4) are illustrated in figure1.

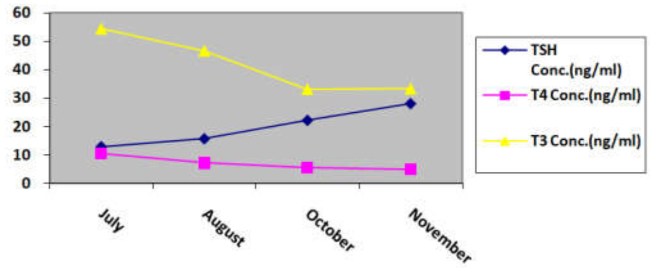

Fig 1. Concentration of TSH, T3 and T4 in male Caucasian viper during different month of year

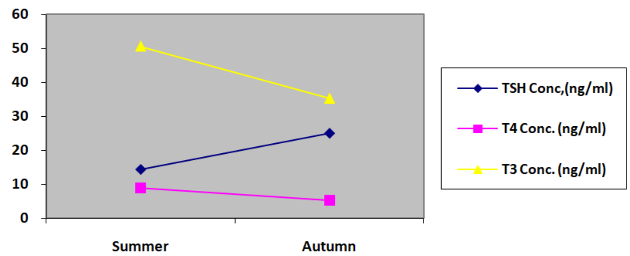

Fig 2. Comparison of thyroidal activity in C. viper in prehibernation and at active season

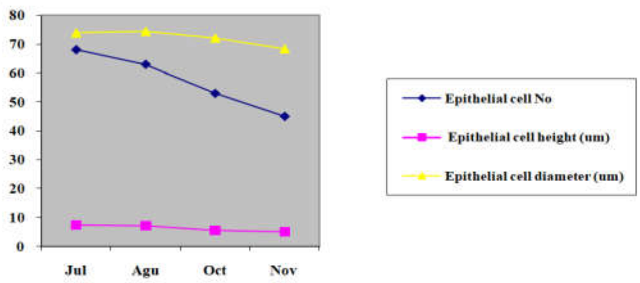

Fig. 3. Variation in Thyroid epithelial cells profiles in Caucasian viper

Circulating TSH, T3 and T4 show seasonal variation (Figure2). Higher concentration in October/November, prehibernating period and lower concentration during breeding season in summer (Shakoori, *et al.,* 2014; Bahri, *et al.,* 2012).

Thyroid histology during autumn and summer seasons

The thyroid is an unpaired gland, spherical, or ovoid shape in snake, located just anterior to the heart and close to the trachea. The gland is simply as a single, lobulate, follicular structure, which contains colloid.

Histology of thyroid gland in male Caucasian viper shows that there is a gradual decrease in epithelial cells number from July to October. Although no significant changes were seen in height and diameter of these cells (Figure 3) but height and diameter of the thyroid epithelium were lower during the autumn (October to November).

DISCUSSION

The circulating TSH, T4 and T3 concentration relating to seasonal events in male pit vipers of Lar are presented in this paper. Serum concentrations of T4 and T3 decreased from July to August in summer, reaching lower levels in November (fall), therefore circulating T3 and T4 in summer were higher than fall. Sciarrillo, *et al*. (2000) reported, the T3 and T4 levels in lizard of Naples are seen to be high in early spring and summer, while low contents were observed in November-January. This monthly variation in the thyroid content of these hormones is paralleled by variations in the thyroid gland morphology, as demonstrated by histological observations (Boyer, 2011; Sciarrillo, *et al.,* 2000). In fact, in the specimens captured in the field we observed an annual cycle of the thyroid gland histology with a reduction of activity in November, low follicular epithelium and compact colloid and devoid of reabsorption vacuoles, a better secretory activity in July- August, very high follicular epithelium and retracted colloid, with clear signs of reabsorption. While TSH concentration was lower in July and gradually increased from August to October and higher in November. It is noteworthy that T4 concentrations increase with increase levels of plasma TSH, but in our experiment when a greater TSH releases (November) was not paralleled by high serum T4 concentrations.

It can be concluded that the thyroid becomes virtually refractory to stimulation with TSH. In mammals (Hulbert, 2007) and amphibians (Kohel, *et al.,* 2001), several cases have been described in which endocrine glands evolve in a state of refractoriness or desensitization following previous contacts with physiological or high concentrations of their homologous hormones. These observations, therefore, support the hypothesis that, in C. viper, thyroid desensitization might have occurred, when a high concentration of TSH is unable to induce a greater T4 release. Higher level of T3, and T4, in July/August could be related with enhanced food consumption, this supports the hypothesis that thyroid hormone production is promoted during metabolic activation that is similar to the results of Virgilio, *et al.,* (2004). In adult male Egyptian cobras *Najahajehaje*, triiodothyronine (T3) level decreased significantly during prehibernation and deep hibernation (24.54% and 39.77%, respectively). Reactivation of the thyroid occurred at the end of hibernation after the emergence of cobras in the spring as the T3 level increased, but it remained lower than when cobras were active. Thyroxin (T4) levels exhibited a highly significant decrease during pre-hibernation and hibernation (26.73%, 40.11%, respectively). Animals in deep hibernation were found to exhibit a maximum decrease, whereas arousing animals nearly restored their active T4 level (El Deib, 2005). In male European adders (V. berus), circulating concentrations of T4 are greatest during the period of sexual activity (Garstka *et al.,* 1982) that support our study that male C. viper also is sexually active in summer and has an eastival spermatogenesis (Mozafari, *et al.,* 2012). Interestingly, thyroid-stimulating hormone (TSH) treatment of hypophysectomized male glossy snakes (Arizona elegans) restored spermatogenesis (Chiu & Lynn, 1971), but the mechanism of this effect was not determined and the effect may simply be a result of cross-reactivity of TSH with GTH receptors. In many species reproductive activity can cause spikes in thyroid values. T4 levels in *Gopherusagassizii* were lowest just before and during hibernation, rose during early emergence and peaked in early spring in females then declined from May through August. Males showed a peak in T4 level in July and August coincident with male-male combat and spermatogenesis (Kohel, 2001). Some reptiles (*Natrix*, *Naja*, *Vipera*) don't conform to this pattern having lower thyroid activity in the summer and peaks associated with mating or forging behavior (Chui *et al.,* 1969).

The highest seasonal values in *Cnemidophorus* were found during hibernation (Sellers, et al, 1982), these were significantly higher than during warmer summer months. Tropical species, such as *Anolis*, may have higher thyroid values in the cooler months and decreased values in the warmer months when they are often less active (Lynn, 1970). Similar alternation is reported by El- Deib (2005) the metabolic hormones, T3 and T4, correlated with food intake, displayed maximum declines in hibernating cobras. This demonstrated that the cold stress might inhibit triiodothyronine and thyroxine synthesis which might be taken as an indication of reduced thyroid activity. Seasonal changes in histology both in relation to temperature and breeding season are of particular interest. The low thyroidal activity observed in winter (low plasma thyroxin level and low glandular activity) has been observed in other reptiles also; Vivien-Roels (1969) has shown in *Pseudemysscriptaelegans*, a low glandular activity in winter followed by a distinct stimulation in spring. This has been shown also by Saint Girons and Duguy (1993) for *V. aspis* and *Natrixmaura*, though less clearly in the latter species. In C. viper by July the gland is active with higher peripheral follicles containing stored colloid. The epithelial height decreases gradually during the pre-hibernating period (October to November). In females the thyroid indicates a rapid decrease in activity shortly after ovulation in the early summer (Fani, *et al.,* 2017). In males there is a more gradual decrease beginning somewhat later. It appears that plasma thyroxin reflects the secretory activity of the gland. Indeed, in winter Saint Girons and Duguy (1993) observed relative inactivity by the gland; it recommenced activity as soon as the animals first emerged, reached a maximum 1 month later followed by a semi rest stage until the summer, lower activity period occurred in autumn pre hibernating time. Virgilio (2004) reported that morphologically, the thyroid gland in *C. ocellatus* exhibit distinct cycles, being lowest during the winter and rising in the summer. In fact, they have noticed an annual cycle of activity of the morphology in the thyroid gland with a reduction of activity in November, a complete blockade in the period December-January with low follicular epithelium and colloid compact and devoid of reabsorption vacuoles. It has shown that thyroxin is involved in the behavioral phenomenon in which snakes thermo regulate with a “chosen” body temperature, and that reptiles in general use behavioral, moving in search of a warm place (Lutterschmidt and Hutchison, 2003) rather than metabolic processes to maintain their temperature while active. However study of Wong and Chiu (1974) on the thyroid gland of the male cobra (*Najanaja L.*) show the epithelial height was greatest during May and October-November, lowest in August. Variations in the thyroid gland activity were associated with epidermal sloughing but not great enough to alter the overall pattern of seasonal variation. They suggested that the annual cyclic changes in the thyroid gland activity are probably related to the general activity of the male animal throughout the year and are not associated with fluctuations in environmental temperature or, as in the female; with any specific aspect of the reproductive cycle. These paradoxical findings may be because of differences in species and geographical conditions. In conclusion, in C. Vipers, thyroid function appears to be influenced by temperature, that may directly alter the hypothalamic-hypophysis-thyroid axis, and by reproductive activity, supporting a role for thyroid hormones in energy-demanding activities of animal.

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