Serum Level of Thyroid Stimulating Hormone, Triiodothyronine, Thyroxin and Weight Rate in Caucasian Pit Viper (Viperidae: Gloydius Halys Caucasicus) of Iran during Different Seasons

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Authors contribution

This work was carried out in collaboration between all authors. Author SF managed the literature searches, wrote the protocol, performed the methods and statistical analysis and wrote the first draft of the manuscript. Author QHV guided the writing of the protocol and managed the analyses of the study. Author FT managed the analyses of the study, guided the writing of the protocol, managed the literature searches and rewritten the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

In this research, concentration of thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxin (T4) and their relationship with body weight in Caucasian viper have been studied. The female Caucasian pitviper (n=26) were collected from Lar in Tehran province, the animal's body weight and length, and serum TSH, T3 and T4 levels were measured by electrochemiluminescence method during autumn, winter and summer in year 2014. The results showed T3 hormone concentration in autumn was 0.74± 0.06 nmol/Lt that was higher than its level in winter, 0.46± 0.8

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nmol/Lt (P≤0.05) and no significant different with its concentration in summer 0.76 ± 0.05 nmol/Lt. The concentration of T4 hormone in autumn, 0.84±0.28 nmol/Lt was higher than winter 0.76±0.033 nmol/Lt (P≤0.19) and also summer 0.64 ±0.018 nmol/Lt (P≤0.001). TSH concentration was 0.005 nmol/Lt in autumn; reach to 0.013±0.008 nmol/Lt in winter and again 0.005 nmol/Lt in summer that shows relative increase in winter (P≤0.05). Mean body length and weight of female Caucasian vipers were 47.50±0.69 cm and 50.80±2.53 gram in autumn and 34.33±2.33 cm, 43.33±2.40 gram in winter and 51.64±1.26 cm and 64.78±3.47 gram in summer, respectively. This is indicated that Caucasian vipers' body weight is decreased in winter could be because of body fat consumption, inaccessibility to food and beginning of reduction of nutritional activity at the end of autumn compare to summer that may result to lower body weight in winter. Concentration of T3 and TSH hormone is decreased in summer and increased in winter. It seems by decreasing temperature in winter serum concentration of TSH is increased and viper`s body weight reduced (Fat consumption) to provide required energy and heat for Iranian Caucasian viper survival. So, seasonal and temperature variation has significant effect on Iran Caucasian viper`s thyroid hormones that is important for animal maintenance. Although further study is needed.

1. INTRODUCTION

In reptiles thyroid activity appears to influence processes such as shedding [1], growth, development [2] reproduction [3], metabolic rate [4], nutrient assimilation and activity. Many factors also influence thyroid values including age, sex, diurnal changes, seasonal changes, day length, shedding, illness, stress and breeding [5]. In general reptiles have plasma concentrations of thyroid hormones that are far less than mammals [6]. T4 and T3 values in reptiles are roughly 20 and 25%, respectively, of the average values in mammals [6].

Seasonal variation in both activity of the thyroid gland and secretion of T4 and T3 has been documented in many species, including reptiles [7,8], amphibians [9,10], and mammals [11,12,13]. The seasonal variation in the thyroid gland and its hormones may be involved in both energy investment required for reproduction and the mobilization of energy stores during breeding [14,10]. TSH secretion, and its sensitivity to TRH stimulation, is affected by renal failure, starvation, sleep deprivation, depression, and hormones, including cortisol, growth hormone, and sex steroids [15,16]. It is well established that thyroid hormone status correlates with body weight and energy expenditure [17,18,19]. Castilla and Bauwens [20] mentioned that fat body weight is correlated with reproductive activity in most reptiles where it decreased significantly following mating and increased again before getting into winter hibernation. In viviparous viper and Cortalid in vitellogenesis, nutritional recession (hibernation) and during pregnancy [21] body fat is reduced, likewise is reported in *stejnegeri stejnegeri Trimeresurse* snakes [22].

The thyroid gland is subjected to a great number of exogenous and endogenous factors, imposed by the external environment, chemicals, drugs, and a variety of diseases processes. Changes in environmental temperature may cause alterations in TSH secretion and in the serum concentration of thyroid hormones and their metabolism. Prolonged exposure to cold generally results in maintenance of the total T4 (TT4) and free T4 (fT4) levels with maintenance of a normal or decreased total T3 (TT3) and free T3 (fT3) levels [23], however, others have shown prolonged arctic residence leads the increase in TSH to be associated with an increase in, thyroglobulin and T3 [24]. These alterations may be partly consequence of a direct effect of temperature on the rate and pathways of thyroid hormone metabolism with more rapid production and clearance of T3. However, the variation demonstrated in several studies [25,26] show that T4 and T3 values are higher during the colder months.

In general, an increase in ambient temperature has produced effects opposite to those observed during cold exposure, thyroid hormone levels in serum tend to be lower during the summer months. A decrease in the serum T3 concentration, with reciprocal changes in the levels of rT3, have been observed in normal subjects acutely exposed to heat and during febrile illnesses (increased body temperature but uncertain cause) [27,28]. A decrease in the elevated serum TSH level associated with

primary hypothyroidism has been induced by increases in body temperature [29].

Alterations in light also cause some changes in the thyroid gland. The diurnal variation in hypothalamic TRH content, reflecting both rhythmic synthesis and secretion, is, however, blunted in the absence of cyclical light changes. The normal TSH rhythm can be reset by a pulse of light [30]. Since thyroid hormone plays a central role in the regulation of total body metabolism, so nutritional factors may profoundly alter the regulation, supply, and disposal of this thermogenic hormone. Study on snake shows secretion of thyroid hormones is little in winter but reaches to its maximum in summer. Also, in sexual activity season and ecdysis/ shedding time, thyroid hormone is increased in snakes [31].

In reptiles, including snakes of temperate-zone there is a seasonal cycle specified in thyroid function. Wu & Huang [32] in a study analyze changes of thyroxine (T4), Triiodothyronine (T3), and serum cortisol in cobra snake (Naja Naja Linnaeus) before hibernation, hibernate, and after their winter hibernation. These changes were on the metabolism indices, such as oxygen consumption, serum glucose, triglyceride, liver glycogen content, and the content of triglycerides from fat body, at three periods. Before hibernation to hibernate, showed that metabolic rate tends to decrease associated with low levels of T3, T4 hormones in serum in winter and snake sleep at low temperature. Low temperature during hibernation have limited three-hormonal activities that rising in hibernation. After hibernation, the temperature rise leads to significant increase in serum levels of three hormones, and it gradually stimulates the metabolism. Wu& Huang [32] in a study also stated that, hibernation in the ectotherms is an adaptive strategy to protect against the cold in winter. Apparently metabolic rate during reptilian hibernation is different compare to those who are active, and counts a physiological adaptation. There is a significant body fat determines the phase before hibernation [33] and during hibernation, snakes body weight is reduced because of fat use, but after sleeping, weight increases because of the resumption of feeding [34]. In viviparous viper and Cortalid in vitellogenesis, nutritional recession (hibernation) and during pregnancy [21] body fat is reduced, it is also reported in *stejnegeri stejnegeri Trimeresurse* snakes [22]. The function of thyroid hormone in reptile is less documented. However

the thyroids hormone assessment in Iranian snakes is not reported and in this research we evaluate the concentration of TSH, T3, T4 and rate of body weight in Caucasian pit viper of Iran during different season for the first time in the country.

2. MATERIALS AND METHODS

2.1 Lar Geographical Conditions and Specifications

Lar is a protected region in western domain of Damavand Mount in Mazandaran and Tehran provinces and is located in 32´ -51° to 32´ -52° eastern length and 52 ´-35° to 50´- 36° northern width and it is confined in north side to Noor mount, from west to Bargah Xatoon and Garmabedar, from south side to Lavasanat heights and from east to Damavand mount and Plour region, by area 27.788 hectares, it is counted as mountainous area. This park is consisted of wet and cold heights and too much downfall as snow and has particular and various animal and plant species.

2.2 Animals Sampling

Twenty six Caucasian vipers were collected from Lar region (Damavand), from Tehran province in June, August, November of year 2014, and transmitted to venomous animals department of Razi vaccine and serum production institute. Samples were maintained in a vivarium under conventional conditions. Snakes were weighed by point scale and body length was measured by meter (calibrated). Blood samples were collected from ventral tail vein of snakes into tubes containing EDTA. The samples were immediately centrifuged (in 1500 rpm, at 4℃ for 15 min), and the serum was stored at −20℃ until assay. The hormones assay was carried out by a method of competition immunological type in heterogeneous phase or electrochemiluminesence to measure TSH, T4, T3 hormones.

3. RESULTS

3.1 Body Weight of Female Caucasian Vipers

The body weight of female Caucasian viper (C. viper) was highest in summer 64.78± 3.47 gram and lowest in winter, 43.33±2.40 gram and in autumn was 50.80± 2.53 gram (Fig. 1). In winter, mean body weight of snake was less than the summer and autumn seasons and differences

were respectively significant at P≤ 0.05 and P≤ 0.001.

3.2 Hormones Concentration

3.2.1 Thyroid-stimulating hormone (TSH) serum concentration

Serum concentration of TSH hormone in female C. vipers at both seasons of summer and autumn was 0.005 nmol/Lt and in winter, 0.013 ± 0.008 nmol/Lt that shows comparatively increased in hormone level of winter and differences were significant at P≤ 0.001 (Fig. 2).

It seems there is a relationship between temperature variation with weight rate in female Caucasian viper and thyroid gland activity.

3.2.2 Triiodothyronine (T3) serum concentration

Serum concentration of T3 hormone was highest in summer, 0.76± 0.05 nmol/Lt, and had decreased in autumn 0.74± 0.06 nmol/Lt and winter, 0.46± 0.08 nmol/Lt. Concentration of this hormone in variance analysis results shows there were no significant differences between concentrations of T3 hormone in female Caucasian viper in three seasons (Fig. 3).

3.2.3 Thyroxin (T4) serum concentration

Concentration of serum T4 hormone in female C. vipers was 0.84 ± 0.28 nmol/Lt in autumn that was higher than winter, 0.76±0.033 nmol/L(P ≥0.19) (Fig. 4). The reduction in the hormone level have continued and reached to 0.64 ± 0.018 nmol/Lt in summer (P ≥0.001).
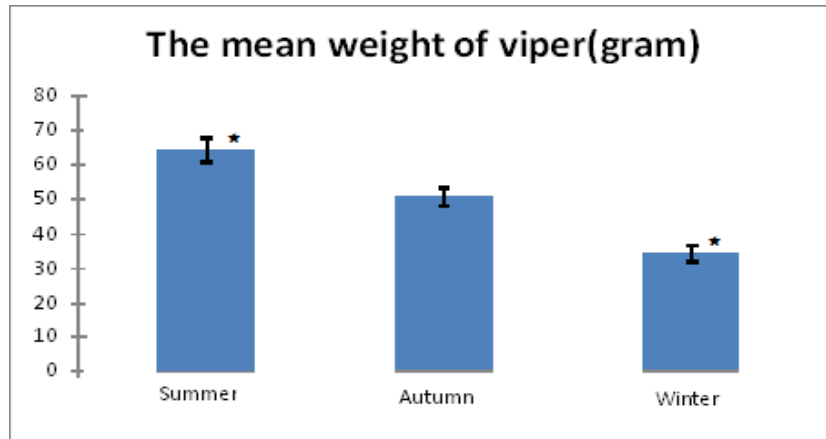

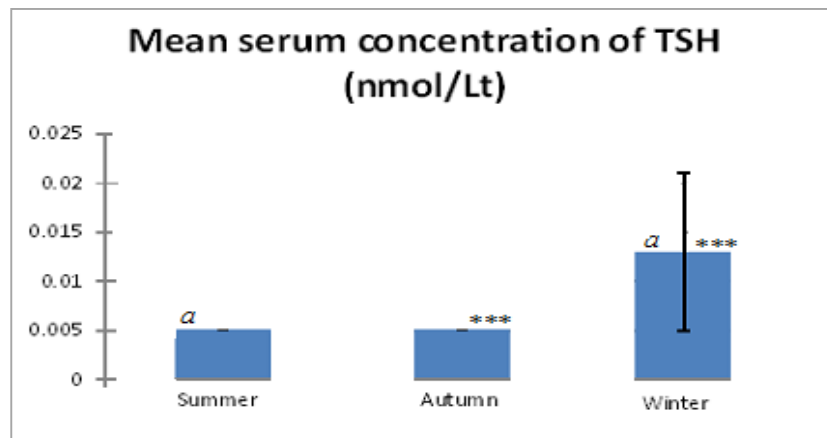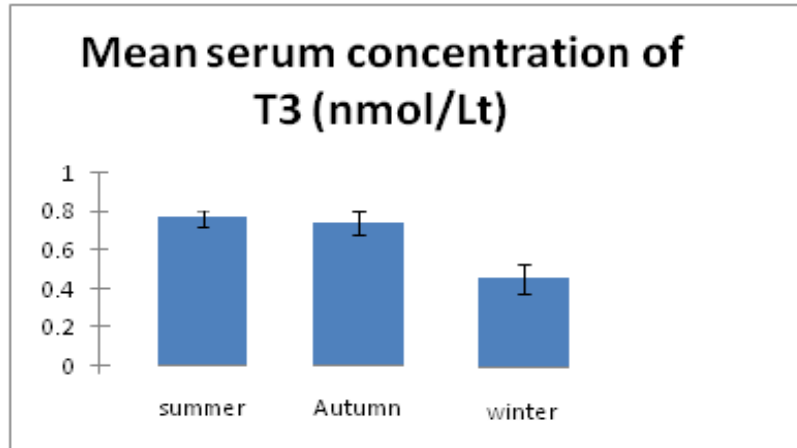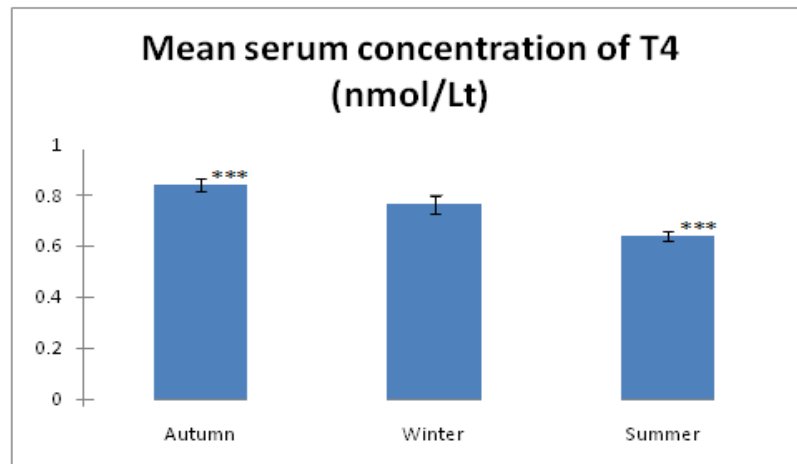**Fig. 1. Mean body weight of female Caucasian viper in different seasons (±SEM, * indicates significant differences)**

Fig. 2. Mean serum concentration of TSH hormone in three seasons in female Caucasian viper (± SEM, * and a, indicate significant differences)**

Fig. 3. Mean serum concentration of T3 hormone in three seasons in female Caucasian viper (±SEM)

Fig. 4. Mean serum concentration of T4 hormone in three seasons in female Caucasian viper (±SEM, * indicate significant differences)**

4. DISCUSSION

Secretions of thyroid gland have significant effect on weight, growth and survival of animals. There is considerable relation between thyroid gland function and seasonal variation and energy investment during reproduction and energy store mobilization in breeding snakes [3]. Beyond seasonal and thyroid gland influences, body weight in snakes is also influenced by reproductive aspects of the individual, because high energy demand is associated with reproductive activity of animal [5,21]. Many snakes use a capital breeding strategy rather than one of income breeding [35]. In capital breeder's snakes, reproductive activity is based on energy reserves more than current food intake [36]. The relationship between body condition and reproductive activity is well-established in snakes; however, little is known about the thyroid hormonal regulating this relationship in snakes. At the present study, body weight of female *Caucasian viper* (*C. viper*) in summer was more than autumn and winter (P≤ 0.05) that may be because of snake activity in suitable temperature [37] and food availability [34] in summer that is similar to body fat increment in viviparous vipers according to Afsharzadeh, et al. [38], following mating, the body weight of snake have decreased in autumn. Our previous studies have shown male C. vipers have summer/ aestival spermatogenesis [37] and mating with female may occur at the time of ovulation in summer [39,40]. It is reported that fat body weight is correlated with reproductive activity in most reptiles where it decreased

significantly following mating [41]. We have seen also a decrease in body weight of female C. viper in winter (Fig. 1). In the cold region because of the temperature reduction, thyroid gland activity decreases in snakes [31] following that body metabolism is reduced that leads to snakes weight reduction in winter. By temperature and seasonal variation, body weight of female Caucasian viper is changed as well. The lowest average is related to winter, during this time, the snakes are preparing for reproduction and vitellogenesis [40,42] and energy provision needs stored lipids consumption. So to provide this energy, devoted stored energy to reproduction should reach to enough amounts in body before beginning of reproduction in female viper [43]. In viviparous viper and Cortalid body fat is reduced in vitellogenesis, during pregnancy [21], nutritional recession and body fat use (in winter) [33] and likewise is also reported in *stejnegeri stejnegeri Trimeresurse* snakes [22] and body weight increases with the resumption of feeding at active season [34].

In reptiles, including temperate-zone snakes there is a seasonal cycle specified in thyroid function. Changes in environmental temperature may cause alterations in TSH secretion and in the serum concentration of thyroid hormones and their metabolism. Temperature, light and reproduction have big effects on thyroid physiology. Continuous exposure to light in *Testudo horsfieldi* caused thyroid production to peak at five days with a gradual decline to inactivity by 35 days [1]. Reports indicate in animals, cold exposure causes a prompt increase in TSH secretion, which gives rise to thyroid hormone release and leads to thyroid gland hyperplasia. Part of this effect is due to an apparent increase in the need for thyroid hormone by peripheral tissues and to an excessive rate of hormone degradation and excretion [31]. In this study the serum concentration of TSH hormone of female C. vipers at summer and autumn was same but an increase was observed in the hormone level in winter that was significantly different at $p \leq 0.001$. When thyroid hormone levels drop too low, the hypothalamus secretes thyroid releasing hormone (TRH), which alerts the pituitary to produce thyroid stimulating hormone (TSH). Exposure to a cold environment triggers TRH secretion, leading to enhanced thyroid hormone release [44] *Pseudemys scripta* kept under continuous light and constant temperature (28℃) for 4.5 months showed a pronounced depression of plasma T4 (2.6 +/- 1.1 ng/ml) compared to

animals raised under variable conditions (T4 = 69.6 +/- 22 ng/ml) for the last 2 months [45]. In soft-shelled turtles, *Lissemys punctata punctata*, thirty days exposure of short photoperiod (2 hours light, 22 hours dark) stimulated thyroid activity and long photoperiod (22 hours dark, 2 hours light) inhibited thyroid activity [46]. Plasma T4 levels in captive green sea turtles, *Chelonia mydas*, with little environmental annual and seasonal temperature variation (Cayman Turtle Farm, Grand Cayman Islands) remained uniform [7]. Gravid reptiles (Lacerta) may have higher thyroid values because they are basking more which raises body temperature [1]. In a wide variety of temperate reptiles (*Sceloporus, Lacerta, Dipsosaurus, Natrix, Vipera, Gopherus, Chrysemys, Trachemys, Pseudemys*) thyroid activity is high during summer / higher temperatures and low during winter and lower temperatures [47,4] even in some non-hibernating species (Xantusia, Leilopisma). But the picture is generally never so simple. For example, in Sceloporus thyroid levels peak with female folliculogenesis and ovulation then decrease from July through September during some of the hottest months when the lizards aestivate [1]. In many species reproductive activity can cause spikes in thyroid values. T4 levels in *Gopherus agassizii* 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 second peak of T4 in July and August coincident with male-male combat and spermatogenesis [4]. 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 [48]. Data reported by Chiu, et al. [48] show that there are two peaks of thyroid activity during the year in the cobra snake. The first is in May just after the animal emerges from hibernation, the second is during October-November just before the animal enters hibernation. This conclusion agrees with that of St. Girons et al. [49] who reported on two species of Vipera. As Vipera mates twice a year, in spring when the animal emerges from hibernation, and prior to hibernation in autumn, St. Girons, et al. [49] concluded that the seasonal changes in gland activity are associated with mating in the male, and ovulation in the female, rather than with environmental temperature. In fact, in both sexes of *V. aspis* and *V. berus*, low thyroid activity was noted during the period of highest environmental temperature, July and August. The male cobra shows spermiogenesis and mating after

emergence from hibernation in April-May, as this coincides with high thyroid activity [49].

However, as cobra only mates once a year, the second peak of thyroid activity in the autumn cannot be related to sexual activity as has been concluded for Vipera [49]. Data relating thyroid activity to spermatogenesis in squamates is paradoxical. 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 [1]. Exposure to heat has an opposite effect, although of lesser magnitude. A small seasonal variation in serum thyroid hormone levels that follow this general pattern has been reported. Starvation has also profound effect on thyroid function, causing a decrease in serum T3 concentration and a reciprocal increase in rT3 level. These alterations in thyroid function are believed to reduce the catabolic activity of the organism and thus to conserve energy in the face of decreased calorie intake. Nutrition can also influence thyroid levels. Withholding food for 14 days significantly decreased T4 levels in Gopherus agassizii, once fed, T4 levels rose within 36 hours [4].

The thyroid gland secretion is low in winter season but it reaches to its maximum in summer during sexual activity and ecdysis in snakes [31]. Study about T4 and reproduction in snakes by Bona-Gallo et al. [50] represented in females, at the time of peak activity of ovary (May month) T4 concentration was reached to its peak and thyroid gland activity increased as well. But TSH hormone concentration level is in very low level, because there is a reverse relationship between concentration of TSH and T4 and T3 level. So it is natural by increasing T3 and T4, we subject with TSH reduction. The basal serum TSH level during calorie deprivation is either normal or low, the response to TRH is blunted and the normal nocturnal rise in TSH is blunted. These changes are quite surprising given the consistent and profound decrease in serum T3 levels. In our study by reducing temperature in winter there was reduction of serum T3 level and increase in the concentrations of TSH and T4 in Caucasian viper. But by warming weather in summer, there was an increase in T3 level and reduction in TSH and T4 concentration level and also there was variation in snake body weight with seasonal changes. We conclude that increase in thyroid activity in snakes is associated with the animal's activity, both behavioral and metabolic. Thus the thyroid activity and body weight rate could be

associated with a variety of behavioral activities, e.g., food-finding, territorial establishment and also mating. Intensive food-searching (leading to a storage of fat utilized during winter) in the autumnal period, would possibly represent a total increase in general activity.

5. CONCLUSION

Seasonal variation and reproductive status seems to be important factors in variation of thyroid hormones concentration and body weight of female Caucasian viper of Lar region in Tehran province.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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