

Seasonal changes in gonadal-thyroid functions in the adult male Sundevall's jird (*Meriones crassus*) (Rodentia: Muridae, 1842).

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Abstract

Thyroid hormones play an important role in all tissues of the body, including gonadal function. In Sundevall's jird, *Meriones crassus* (Rodentia, Gerbillinae), caught in its natural biotope in the Algerian Sahara Desert, the testis activity was evaluated by testicular and seminal vesicles weights and plasma testosterone level. The thyroid activity was evaluated by plasma concentrations of total triiodothyronine (T3) and thyroxin (T4) using the radioimmunoassay (RIA) method. The testis activity is characterized by a maximum in spring, a regression during summer, a minimum in autumn, and a lower activity in winter. The plasma concentrations of total T3 and T4 varied non-significantly during the seasons. However, thyroid activity varied in contrast to testicular structure, especially during the breeding season when the testis activity is maximum. In order to specify the implication of thyroid function in testis seasonal variations, we investigated the relationship between both activities. This study could further explain the seasonal regulation of testicular function concerning the environmental cues in this jird.

Keywords: Plasma T3 and T4, Plasma testosterone, Seasonal variations, Sundevall's jird

Introduction

Thyroid hormones play a main role in the metabolism and development of tissues and are implicated in reproductive function. The sexual differences occurring in the structure and function of the rat thyroid gland depend on the male pituitary sensitivity to TRH, and this phenomenon is mediated by testosterone (Chen and Walfish, 1979; Christianson et al. 1981). The relationships of thyroid-gonadal function were

investigated during the development of laboratory rats using thyroidectomy or gonadectomy (Palmero, and Cook, 1992; Panno et al., 1996; Jannini et al. 1999). T3 receptor numbers located in the rat Sertoli cells increase during the fetal and perinatal periods, and then decrease significantly until puberty and virtually disappear in the adult (Fugassa et al., 1987, Palmero et al., 1993; Jannini et al., 2000).

In the Siberian hamster, central or systemic T3 injections over-ride short period induced testicular regression (Billings et al., 2002). Wild animals always developed various endocrine rhythms to adapt to rhythmic environmental cues such as food, water, and photoperiod. In the European Badger (*Meles meles*) and in the *Vulpes vulpes*, the molt began in summer, when the plasma testosterone level decreases and the thyroxin level is high (Maurel et al., 1981, 1987). Similar results were shown in the rabbits (Yosha et al., 1984). However, in the adult male European hedgehogs, captured in the wild field, plasma levels of both testosterone and thyroxin exhibited a marked annual cycle with a minimum in autumn and a maximum during spring and summer. Petterborg et al., (1984) reported the thyroid hormone rates compared to reproductive organ weights during long and short photoperiods in three long day breeders' rodents: the hamster (*Mesocricetus auratus auratus*), in Mongolian gerbil (*Meriones unguiculatus*), the white-footed male mice (*Peromyscus leucopus*) and in the Snell strain house mice (*Mus musculus*). These studies suggest that changes in reproductive weight may not necessarily be accompanied by alteration in circulating levels of thyroid hormones in all four species.

Seasonal variations in the thyroid-gonadal function in desert rodents were still few documented and limited to the work carried out in the sand rat (*Psammomys obesus*) in the Algerian Sahara Desert (Khammar and Brudieux, 1991) and in *Meriones shawi* in Morocco (Benani Kabchi, 1988). Indeed, in the sand rat (*Psammomys obesus*), the total T4 varies according to a seasonal cycle with low values in winter, a significant increase in nonbreeding season (spring), and a decrease in summer (June-July) and a minimum in the breeding season (autumn). T3 follows an opposite profile to that of T4 in *Psammomys obesus*. A difference between T4 and TSH has also been reported in *Meriones shawi* living in Morocco (Benani Kabchi, 1988).

Under various climate conditions, mammals have distinct reproductive and thyroid activities. These examples show that in hot or cold environments, each population of animals seems to develop adaptive strategies to its habitat, climate, and manners. To supplement the comparative endocrinology data and in order to preserve species in the desert hot environment and to well control the adaptive mechanisms of wild animals live in arid and semi-arid areas, the objective of this study is to determine the seasonal profiles of the thyroid and gonadal cycles in the adult male Sundevall's jird (*Meriones crassus*).

Material and methods

Animals

Sundevall's jird (*Meriones crassus*), is a nocturnal desert rodent, with a carnivorous diet (seeds and dates), and is sometimes insectivorous. In the Sahara, this species could be considered, as a desert test, as it is the most desert among other species (Le Berre, 1990). *Meriones crassus* is quite lonely, instead of *Meriones libycus* and *Psammomys obesus*. Animals were provided from their natural biotope in the Béni-Abbès area (30°07'N, 2°10'W) in Algeria. A total of 10 to 15 adult males were captured every season between September 1st and 10th corresponding to the resting season, between 25th December to 25th January and from, 10th March 1st April corresponding to the breeding season, and between 15th June to 10th July. The animals were placed individually in cages (50 cm in length, 35 cm in width, and 30 cm in height) and fed with natural alimentation. The adult reproductive condition was estimated according to their body weight (60-140g) and the genital tract development. Genital status was assessed during the breeding season. All animals used in this study were adult males. This work was conducted in accordance with the directive 2010/63/EU for animal experiments and with that of AASAE: Algerian Association of Sciences of Animal Experimentation (Agreement Number 45/DGLPAG/DVA.SDA.14). Animals were kept for approximately 24h and then euthanized under ketamine anesthesia between 13:00 and 18:00. Blood is immediately collected (0,5ml), transferred into heparinized tubes (20 µl heparin per ml of blood), and centrifuged for 15 min at 3000xg. Plasma was immediately stored at -25°C till hormone assays.

Plasma testosterone assay

Plasma testosterone is measured by radioimmunoassay (RIA), after extraction with the cyclohexane/ethyl acetate (v/v) using a specific antibody manufactured in the laboratory (Darbeida, 1980). The validity tests show that the technique is reliable since it is specific (due to the antibody's specificity), sensitive (the logit-log transformation gives a straight line from 20 to 400pg and the correlation coefficient is 0.96, reproducible (inter-system coefficient of variation generally less than 15%) and exact (variation between the read and expected values is less than 2pg/ mL).

Thyroid hormone-assay

The total triiodothyronine (T3) and thyroxin (T₄) were determined by the RIA method, using commercial kits (CIS kits, BIO international, Essonne, France). For each immunoassay, plasma samples (50 µl) assay was conducted in duplicate. The methods are specific and sensitive (0.1ng T3 and 8ng for T₄). The intra-assay and inter-assay coefficients of variation were 6.5% (n=15) and 7.8% (n = 10), respectively, for triiodothyronine and thyroxine.

Statistical analyses

All data are expressed as mean \pm standard error. Differences between means were evaluated using Student's t-test. GraphPad Prism (version 5; GraphPad Software Inc., San Diego, CA, USA) was used to determine the differences in the various parameters between seasons. A value of $p < 0.05$ was considered to be significant.

Results

Testis activity and weight

The absolute weight of the testis varied between 57 ± 18 mg and 775 ± 50 mg (Table 1). From the low values observed in October, the testicular weight increased significantly from January and reached the maximum in March-April (Table 2). A significant decrease occurred in June-July and reached the maximum in December (Tables 1 and 2). The relative weight of the testis varied between 78 ± 26 and 993 ± 60 mg/100g body weight. The evolution of the relative weight of the testis is narrowly parallel to that of absolute weight and confirms the previous data. The minimum weight recorded in October was followed by a significant increase in March-April and a significant decrease occurred between March-April and December (Table 2).

Table 1. Seasonal changes of body weight and absolute weight of testis and seminal vesicles in the adult male Sundevall's jird.

Period	Body weight (bw) (g)	Number of animals	Testis weight (TW)		Seminal vesicles weight (SVW)	
			Absolute TW (mg)	Relative TW mg/100g of bw	Absolute SVW (mg)	Relative SVW (mg/100g bw)
October 2017	74 ± 05	10	57 ± 18	78 ± 18	46 ± 17	64 ± 25
January 2018	57 ± 05	10	569 ± 15	715 ± 75	128 ± 33	155 ± 50
Marsh-April 2018	77 ± 03	10	764 ± 29	993 ± 60	818 ± 70	833 ± 227
June –July 2018	83 ± 05	10	740 ± 15	886 ± 177	866 ± 177	1057 ± 74
December 2018	75 ± 00	10	84 ± 00	112 ± 00	87 ± 00	115 ± 00
January 2019	97 ± 09	10	775 ± 50	803 ± 24	611 ± 35	507 ± 135

Seminal vesicles weights

The absolute weight of seminal vesicles varied between 46 ± 17 and 1057 ± 74 mg (Table 1). As for testis weight, the low values were observed on October; a significant increase occurs in January, and reached a maximum in March-April (Table 3). The relative weight of seminal vesicles varied between 64 ± 25 mg and 866 ± 177 mg/100g of body weight. The minimum occurs in October was followed by a significant increase in April (Table 3), and a significant reduction between March-April and December (Table 3).

Table 2. Seasonal changes in the testis weight. Differences between means and their statistical significance.

Compared patched	Absolute TW weight (mg)		Relative TW weight (mg/100g bw)	
	Differences %	<i>p-value</i>	Differences %	<i>p-value</i>
January 2018./October. 2017	+898	$p < 0.001$	+817	$p < 0.001$
April 2018/ January. 2018	+34	$p < 0.01$	+39	$0.02 < p < 0.05$
April 2018/ October 2017	+1240	$p < 0.001$	+1173	$p < 0.001$
December 2018/April 2018	-89	$p < 0.001$	-89	$p < 0.01$

Table 3. Seasonal changes in seminal vesicles weight. Differences between means and their statistical significance.

Compared patched	Absolue SVW (mg)		Related SVW mg/100g of bw	
	% differences	<i>p-value</i>	% differences	<i>p-value</i>
January.2018./October. 2017	+178	$0.001 < p < 0.01$	+142	$p < 0.02$
April.2018/ January. 2018	+539	$p < 0.001$	-32	$0.02 < p < 0.05$
April. 2018/ October 2017	+1678	$p < 0.001$	+1552	$p < 0.001$
Décember.2018/April 2018	-89	$p = 0.01$	-89	$0.02 < p < 0.05$

Plasma testosterone concentrations

Plasma testosterone level varies between 106 ± 33 to 2118 ± 363 pg/mL. The low values were observed in October, followed by a non-significant increase in January (Table 4), which reached a peak value in March-April ($p < 0.01$). However, we observed again an important but statistically non-significant increase in January 2019 (Table 5).

Table 4. Seasonal variations in plasma concentrations of testosterone in the adult male Sundevall's jird.

Periods	Number of animals	Plasma testosterone contents (pg/ml)
October 2017	10	106±33
January 2018	10	235±47
Mar.-Apr. 2018	10	2118±363
June –July 2018	10	1414±200
Dec. 2018	01	210±00
January 2019	10	1610±15

Table 5. Seasonal changes in plasma concentration of testosterone. Differences between means and their statistical significance.

Compared patched	Plasma testosterone (pg/mL)	
	Differences %	<i>p-value</i>
January 2018./October 2017	+122	<i>p</i> <0.20
April 2018/ January 2018	+672	<i>p</i> <0.01
April 2018/ October 2017	+1898	<i>p</i> <0.01
Décember 2018/ April 2018	-90	<i>p</i> <0.10
January 2019./October 2018	+ 667	<i>p</i> <0.30

Thyroid activity

Plasma thyroxin (T4) and triiodothyronine (T3) concentrations

The plasma concentration in total T4 varied between 11.72±3.74 and 28.48±2.65 ng/mL and was 40 times higher than the total (T3) which varied between 0.17±0.11 and 0,94±0.11 ng/mL (Table 6). The two profiles are evolving in the same way. Indeed, both hormones showed low concentrations in summer (June) and vary in small proportions but were statistically significant between the two breeding periods (Table 7). Only one significant reduction was observed between April and June for T3, while it was at its significant limit for thyroxin (Table 7).

Table 6. Seasonal variations of thyroid weight, the plasma concentration of Thyroxine (T4) and triiodothyronine (T3) in the adult male Sundevall's jird.

Period	Number of animals	Thyroid weights		Plasma concentrations (ng/mL)	
		Absolute weight (mg)	relative weights (mg/100g bw)	Thyroxine T4	Triiodothyronine T3
January 2018	14	10±01	12±01	27,78±3.71	0.73±0.07
March-April 2018	15	07±01	09±01	23,63±2.88	0,72±0.08
June/July 2018	12	07±01	05±01	11.72±3.74	0,17±0.11
December 2018	01	04±00	03±00	23.71±00	0.48±0,00
January 2019	13	06±02	06±02	28.48±2.65	0,94±0.11

bw*: Body Weight

Table 7. Seasonal changes in plasma concentrations of thyroid hormones. Differences between means and their statistical significance.

Compared patched	Thyroxine (T4)		Triiodothyronine (T3)	
	Differences %	<i>p-value</i>	Differences %	<i>p-value</i>
April 2018/ January 2018	-50	<i>p</i> <0.20	-1	<i>P</i> =0.90
June/April 2018	+102	<i>p</i> <0.01	-76	<i>p</i> <0.01
December 2018/ July 2018	+20	<i>p</i> <0.01	+182	<i>p</i> <0.50
January2019/ December 2018	+ 667	<i>p</i> <0.10	+96	<i>p</i> <0.20

Comparative patterns of plasma concentrations of testosterone and thyroxine

The interrelationship between the thyroid gland and male gonads has been revealed based on plasma testosterone levels (pg/mL) related to plasma thyroxine level (ng/mL). The results showed that thyroid activity decreases between the resumption of sexual activity in winter (January) and the breeding season in spring (April) when sexual activity increases. Conversely, thyroid function increases when sexual activity declines (Fig.1).

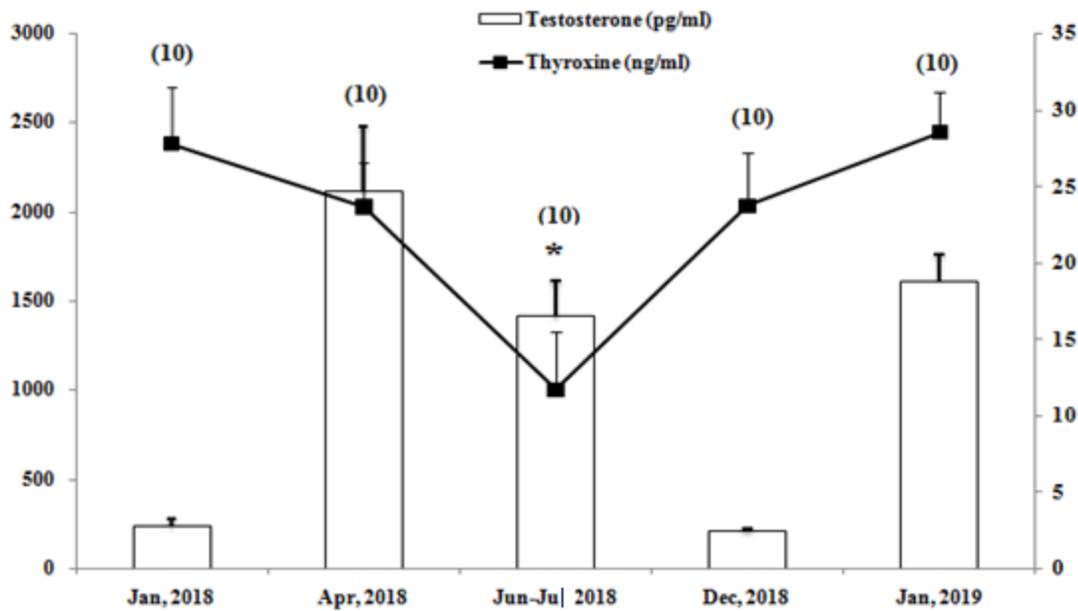


Figure 1. Comparative seasonal patterns of plasma concentration of testosterone (pg/mL) and of Thyroxine (T4) (ng/mL) in the adult male Sundevall's jird (*Meriones crassus*) in the Algerian Sahara desert. Numbers between brackets represent the number of animals and * $p < 0.05$.

Discussion

The adult male Sundevall's jird (*Meriones crassus*) exhibits a seasonal reproductive cycle, with a period of reproductive quiescence during autumn and winter and a period of sexual activity during early spring until early summer. Similar results were obtained in Libyan jird (*Meriones libycus*) living in the same biotope (Belhocine et al., 2007; Boufermes et al., 2014). These results are similar to those observed in other seasonal breeder desert rodents living in arid and semi-arid regions. Indeed, during the active period, the reproductive organs show considerable growth in weight; structure, and ultrastructure are developed and both testicular and plasma testosterone levels increase. The resting season is typically characterized by a reduction in the size of reproductive organs (seminal vesicles and prostate) induced by a marked reduction in the testicular size and the plasma testosterone) (Khammar and Brudieux 1987, Zaim et al., 1992; El Bakry et al., 1998; Gernigon et al., 1994). In the Sahara Desert, several cycles were postponed by Le Berre (1990). In Burkina Faso, in the Sahel, *Arvicanthis niloticus*, a rodent living in a desert biotope, presents the same cycle as the fairy-wren (Sicard, 1987). The same author has highlighted the reproduction period of some female species (*Gerbillus nigrae* and *Taterillus pettri*) desert rodents living in the Sahel. The cyclicity in these species depends on changes in the testicular activity that starts during estivation (April), and decreases at the beginning of the dry season (October

- November). The gerbil, *Gerbillus henleyi*, presents two separate periods of reproduction, in spring and late summer, with a break in July (Shenbort et al., 1994).

We reported for the first time a comparative study of seasonal variations in both thyroid-and gonads activities in the adult male desert rodent *Meriones crassus* a long-day breeder living in the Algerian Sahara Desert. Thyroid activity assessed by hormone levels of T3 and T4, varies in very low proportions over the seasons and the differences between the means are not statistically significant. However, there was a slight decrease in the concentrations of T4 and T3 in the spring and summer corresponding to the breeding season in this species. Seasonal changes in thyroid activity of desert rodents were few documented. The results in *Psammomys obesus* (Khammar and Brudieu, 1991) are similar to *Meriones crassus*. A difference between T4 and TSH was also observed in *Meriones shawi* in Morocco (Benani Kabchi, 1988). In *Meriones shawi* captured in the Tunisian desert, the thyroid is active in summer and more active in winter when T3 and T4 are maximum (Lachiver et al., 1978). Thyroid hormones circulate in the bloodstream mostly bound to a set of plasma proteins that widely differ in their concentration and affinity for the hormones (Robbins et al., 1986). The three major transport proteins are T4-binding globulin (TBG), T4-binding pre-albumin (TBPA), and albumin. TBG carries about 70% of T4 in the circulation and, therefore, represents physiologically the most important T4-binding protein. The decrease in the rate of T3 is due to decreases in basal metabolism (Robbins et al., 1986).

The comparison of thyroid-gonads functions based on plasma testosterone contents data and plasma contents of total T4 in different seasons showed that the testis and thyroid activities seem to evolve in the opposite manner in several species. Indeed, during the breeding season (April), thyroid activity becomes reduced. Conversely, during the non-breeding season (autumn-winter), thyroid function was activated. This allows us to conclude that in *Meriones crassus*, at specific times of the seasonal cycle, either inactive or in the rest of testicular activity, thyroid function has a negative correlation. In desert rodents, the study of the testis-thyroid interrelationships in relation to environmental factors was studied in order to better explain the adaptive mechanisms of these species with regard to the hot climate (Chouacha et al., 1997; Banta and Holcombe, 2002). The adaptation of some desert rodents (*Dipodomys merriami*, *Spermophilus leucurus* and *Neotomalipida*), the thyroid activity in warm (35°C), significantly decreased the rate of T3, on the other hand, there is no or very little effect on T4 and TSH (Youcef et al., 1984). Other exogenous factors such as food and age can influence the testicular and thyroid functions. In the Sahel of Burkina-Faso, in two insectivorous desert rodents (*Gerbillus nigeriae* and *Taterillus petteri*); the interruption of reproduction coincides with the moment when animal change their food intake and become granivorous (Sicard and Fuminier, 1994). Compared to laboratory animals

maintained under a low iodine diet, the thyroid of Sundevall's jird is better adapted to a diet extremely low in iodine (Lachiver and Chapa, 1974). In desert rodents, *Dipodomys and Prognanthis*, secretion of T4 level is lower than that found in rodents living in temperate areas (Hudson and Deaves, 1976). Circulating T4 increases with the level of basal metabolism and it is lowest in desert rodents than in mountainous rodents (Scott and Youcef, 1976). Finally, in woodchucks, a low rate of T4 in the summer would be facilitated by the lack or decrease in food consumption at these times of the year (Young et al., 1986).

To better understand the interrelationships of thyroid-gonads, some authors, attempted either the castrations, in the resting period or active one (Saboureau, 1979, Maurel, 1981) or the thyroidectomies (Khar et al., 1980, Anthony et al., 1995). Other authors have attempted to follow the physiological changes of the testicle-thyroid functions during the development of animals (Fugassa et al., 1987; Jannini et al., 1990, Palmero, and Cook, 1992, Palmero et al., 1993; Panno et al., 1996).

At the molecular level, Martinez et al., (2019), have studied in the mice the effect of premature overexposure to thyroid hormone which causes deep effects on testis growth, spermatogenesis, and male fertility. These authors used genetic mouse models of type 3 deiodinase (DIO3) deficiency to determine the genetic programs affected by premature thyroid hormone action and to define the role of DIO3 in regulating thyroid hormone economy in testicular cells and found that DIO3 protects testicular cells from untimely thyroid hormone signaling and demonstrate that the mechanism of cross-talk between somatic and germ cells in the neonatal testis is involved in the regulation of thyroid hormone availability and action.

Conclusion

The study of thyroid and gonadal functions in *Meriones crassus* shows that the testicular endocrine function seems to have an inhibiting action on that of the thyroid essentially during periods of maximum sexual activity. This phenomenon is probably due to metabolism and environmental factors such as photoperiod and diet. The relationships in thyroid-gonadal function deserve to be examined at hypothalamic-pituitary level even if there is a theoretical possibility that another pathway dependent on TSH but independent of T3 causes seasonal changes in the reproductive axis. The most parsimonious model is that in which the action of T3 on RF-amides neurons links the photoperiodic production of TSH in the part tubularis to the seasonal control of GnRH secretion (Henson et al., 2013). The research of thyroid receptors in the testis's cells or those of androgens in the thyroid cells seems to be another interesting way for further investigation.

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