Histomorphometry and hormone secretion of the thyroid gland of the dromedary (*Camelus dromedarius*)

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Abstract

This study was carried out on 120 camels (60 males and 60 females). Thyroid glands were recovered during summer and winter seasons. For both sexes the animals were classified into three age groups: less than 3 (Subgroup 1); 3 to 5 (Subgroup 2); and 15 or more years (Subgroup 3). The results showed that the activity of the thyroid gland of the dromedary (*Camelus dromedarius*) is variable according to age, sex and season. The thyroid activity was higher ($P<0.05$) in Subgroup 2 compared with the first and third subgroups. In this respect the smallest follicular surface was observed for animals in Subgroup 2 (4913 ± 467 µm^2) with mean concentrations of 20.41 and 5.70 Pmol/l for T4 and T3 respectively, and 0.25 miU/l for TSH. The highest follicular surface was observed in animals of Subgroup 3 (6874 ± 670 µm ^{2}). For animals in this subgroup T4 and T3 concentrations respectively averaged 12.64 and 3.80 Pmol/l while TSH serum concentration was 0.09 miU/l. Conversely, the highest epithelial cells were observed in animals of Subgroup 2 (11.5 ± 1 µm), while the lowest figure was recorded for animals in Subgroup 3 (8.5 ± 0.7 µm). The height of the thyrocytes in anials of Subgroup 1 was 10 ± 1.1 µm ($P<0.05$). Average follicular surface was greater ($P<0.05$) for females in comparison to males: 6048 ± 986 and 5576 ± 880 µm^2, respectively. The epithelial cells were higher ($P<0.05$) in males (10.5 ± 1.6 µm) than in females (9.5 ± 1.4 µm). T4 and T3 concentrations varied in the same direction, being higher ($P<0.05$) in males than in females. Finally, average follicular surface was greater ($P<0.05$) in summer (6117 ± 882 µm^2) than in winter (5507 ± 943 µm ^{2}) and the thyrocytes were higher ($P<0.05$) in winter (10.7 ± 1.5 µm) than in summer (9.3 ± 1.3 µm), irrespective of sex and age. Inversely, serum concentrations of T4, T3 and TSH were higher in winter than in summer.

Keywords: Camel, thyroid, histomorphometry, thyroid hormones, TSH

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Introduction

In Tunisia the dromedary as a livestock species is gaining economic interest because of its adaptation to harsh climatic conditions and resistance to diseases (Bengoumi, 1992; Faye, 1997; Faye and Bengoumi, 2000). Such adaptation is the basis for the reputation of this species to be uniquely able, among other large mammals, to survive under desert conditions. One of the dromedary's mechanisms of adaptation to its natural habitat is changes in the activity of some endocrine glands, such as the thyroid which is implicated in several vital functions, yet numerous morphological variations of this gland are not well explored. Indeed, the secretion cycle of the follicles is associated to changes in the follicular surface, the colloid surface, the height of the follicular epithelium and blood concentrations of thyroid hormones. Some studies, such as Atoji et al., (1999) and Abdel-Magied et al., (2000), have addressed these aspects but they remain incomplete. Moreover, data on physiological variations of the circulating thyroid hormones are lacking and to our knowledge there are no reported studies linking histomorphometry data of the thyroid gland and the thyroid hormones. This study was therefore carried out in an attempt to simultaneously study morphometric traits of the thyroid gland and changes in serum concentrations of the thyroid hormones and TSH (Thyroid Stimulating Hormone) and how they are affected by the factors age, sex and season. The studied histomorphometric traits were the follicular surface, the intrafollicular colloid surface, the activation index and the height of the follicular epithelium. Serum concentrations of thyroxine (T4), tri-iodothyronine (T3) and TSH were also determined.

Material and methods

Animals

The reported study was carried out using thyroid glands that were recovered from 120 camels (60 males and 60 females) at Tozeur slaughter house (southern Tunisia). Tozeur is located in south-west Tunisia close to the Algerian border. It has a desert type of climate with an average annual temperature of 23 °C, cold winters (average temperature 10.5 °C in January) and high temperatures in summer (42.3 °C in August). Average annual rainfall is a low 100 mm. The age of the animals was determined according to their dental formulae, as described by Faye (1997). Animals were assessed to be clinically healthy after inspection just before blood was sampled and animals sent to slaughter. Jugular blood was collected into 10 ml vacutainer tubes in the morning. Blood was centrifuged at 3000 g for 15 minutes at 4° C immediately after sampling and equal volumes of serum were poured into plastic tubes and stored at -20°C until assayed for T4, T3 and TSH. For both sexes, threes ubgroups were selected according to age and season. Age subgroups were defined according to the classification of Al-Qarawi et al., (2000): Subgroup 1 represented animals aged less than 3 years; Subgroup 2 was composed of animals around puberty aged between 3 and 5 years; while Subgroup 3 represented animals 15 years of age or older (Table 1). Animals aged between 5 and 15 years were not retained in the studied sample because the number of individuals for each sex and season was not sufficient for the purpose of the present study (less than 10). This is mainly due to the law regulating the livestock sector in Tunisia that prohibits slaughtering of females that can potentially reproduce.

Table 1. Distribution of the number of camels used in this study according to sex, age and season

Season	Age	Males	Females
Winter	<3 years	10	10
	Between 3 and 5 years	10	10
	≥15 years	10	10
Summer	<3 years	10	10
	Between 3 and 5 years	10	10
	≥15 years	10	10
Total		60	60

Morphometric study

The entire thyroid glands were fixed in 10% neutral formalin for at least 48 hours. Orientation and embedding of the glands was carried out in paraffin wax. The glands were sectioned sagittally at a thickness of 3 µm and one sagittal section per animal was retained for the morphometric study. Such a section is considered as representative of the thyroid (Denef et al., 1979).

The qualitative description based upon the aspect of the section (homogenous or heterogeneous), and the shape and size of the follicles as well as the height of the follicular epithelium is realised after coloration with hemalun eosin. Morphological examination was carried out after histochemical silver impregnation of the sections using the method of Gordon and Sweets (Ganter and Jolles, 1969) which stain positively in black for basal lamina of the follicles and for reticular fibres. One sagittal section per animal was studied using an automatic image analyzer (Leica Qwin). Considering the length of the camel thyroid gland, each section was divided into three equivalent portions. Measured follicles were those legible on successive fields along the thyroid axis. Follicles located on the periphery were not considered on a strip of approximately 1000 µm because they are in general large, inducing wide standard deviations. Counting of an average of 300 follicles (100 follicles per slide) allowed a complete examination of a sagittal section of the camel thyroid gland. All the measurements were realised with the same calibration using a x 25 magnification (Optical microscope, Olympus®, Germany). Measured traits were:

- thyroid follicle surface area: $A_1, A_2, \ldots, A_{300}$,
- colloid surface area: $B_1, B_2, \ldots, B_{300}$,
- epithelium surface area: $(A_1 - B_1), (A_2 - B_2), \ldots, (A_{300} - B_{300})$.

The epithelial surface area and the colloid surface area are used to estimate the activation index which is the ratio of the mean volumetric percentage of the epithelial cells to the mean volumetric percentage of the colloid substance (Kalisnik, 1972). The height of thyrocytes was estimated using a x 25 magnification by measuring the height of 100 cells per slide (2 cells per follicle), that is 300 cells per sagittal section.

Hormone assays

Serum concentrations of T4, T3 and TSH were assessed by radioimmunoassay using specific kits provided by Immunotech® (Prague, Czech Republic). Assays were realised according to the manufacturer's instruction. The limits of detection were 0.5 pM, 0.5 pM and 0.025 mIU/l respectively for T4, T3 and TSH. For each hormone the samples were included in one single assay; the coefficients of intra assay of variation were 6.1%, 0.6%, and 17% respectively for T4, T3 and TSH.

Statistical analysis

The analysis of the sources of variation on the follicular surface area, the colloid surface area, the activation index, the height of the follicular epithelium and serum concentrations of T4, T3 and TSH were undertaken using the GLM procedure of SAS (SAS, 1994). The sources of variation retained in the model were the sex of the animal (2 levels), the age subgroup (3 levels) and season (2 levels). When the model was significant ($P<0.05$), means were compared using the Duncan test.

Results

Morphometric study

The qualitative descriptive analysis after staining with Hemalun – Eosin showed that for the same histological section, thyroid follicles have a heterogeneous aspect. The size of the thyroid follicles is highly variable; they are larger for adult animals during the summer. Follicles on the periphery are larger than central ones in the same histological section (Plate 1).

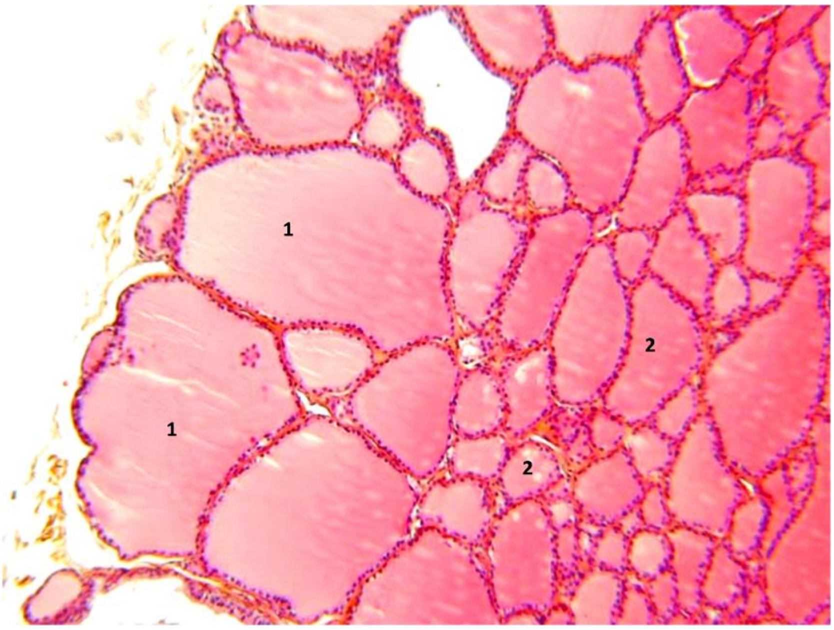

Plate 1. Thyroid of the dromedary (H& E x 250)

1: Peripheral follicles; 2: Central follicles

Nevertheless, for individuals of the same age, sex and during the same season, differences in the size and shape of the follicles persisted. Staining with Hemalun – Eosin didn't allow a precise determination of the external limits of the follicles, and this limitation was overcome by the silver impregnation which allows a clear visualisation of the basal lamina of the thyroid follicles (Plate 2).

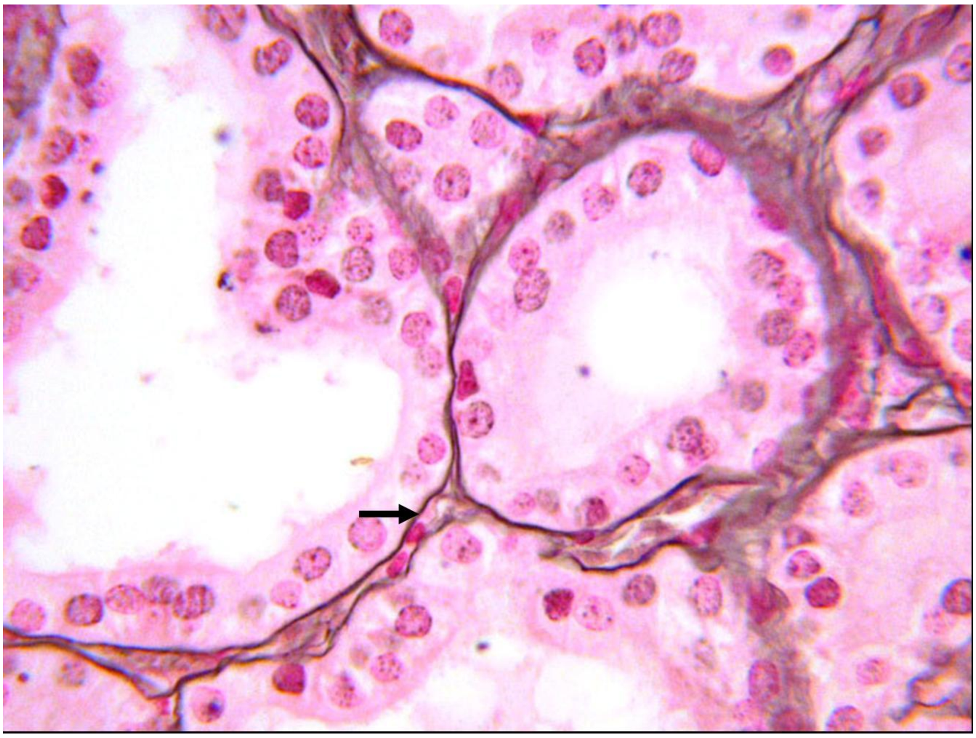

Plate 2. Thyroid of the dromedary; Silver impregnation stain x 400. Visualisation in black of the basal lamina (→) appeared black due to silver staining

All the factors retained in the model had a significant effect on morphometric measurements of the camel thyroid gland, with coefficients of determination of the model varying between 88 and 91.5% (Table 2). Average values for these morphometric measurements are reported in Table 3.

According to age, the largest follicular surface area was recorded for animals of the Subgroup 3 (6873±670 μm^2); the smallest value corresponded to animals in Subgroup 2 (4912±467 μm^2), while animals in Subgroup 1 were intermediate with a mean value of 5650±386 μm^2 ($P<0.05$).

Table 2. Sources of variation of the morphometric characteristics of the dromedary thyroid gland selected parameters

Morphometric characteristic	Number of observations	R^2 (%)	Fixed effect	Significance
Follicular surface area	120	87.93	Age	***
			Sex	***
			Season	***
Colloid surface area	120	88.33	Age	***
			Sex	***
			Season	***
Activation index	120	88	Age	***
			Sex	***
			Season	***
Thyrocyte height	120	91.53	Age	***
			Sex	***
			Season	***

***: P <0.001

The average follicular surface area is larger for females than for males (6048±986 and 5576±880 μm^2 respectively; $P<0.05$). For the season effect, the follicle surface area is larger during summer (6117±882 μm^2) than during winter (5506±943 μm^2) ($P<0.05$).

Table 3. Mean values of the dromedary thyroid morphometric characteristics according to age, sex and season

Factor		**N**	**surface area (µm²)**			**Activation index**	**Thyrocyte height (µm)**
			Follicle	Colloid	Epithelium		
Age	<3 years	40	5650^{a} ±386	2010^{a} ±368	3629^{a}±195	1.8^{a} ±0.4	10^{a} ± 1.1
	3–5 years	40	4912^{b} ±467	1521^{b} ±307	3338^{b}±172	2.2^{b} ±0.5	11.5^{b}± 1
	≥15 years	40	6873^{c} ±670	3015^{c} ±581	3754^{c}±185	1.3^{c}±0.32	8.5^{c} ± 0.7
Sex	Male	60	5576^{a} ±880	2031^{a} ±734	3538^{a}±270	1.9^{a} ±0.6	10.5^{a} ± 1.6
	Female	60	6048^{b} ±986	2333^{b} ±760	3609^{b}±231	1.7^{b}±0.48	9.5^{b} ± 1.4
Season	Winter	60	5506^{a} ±943	1874^{a} ±715	3624^{a}±289	2.1^{a} ±0.56	10.7^{a} ± 1.5
	Summer	60	6117^{b} ±882	2490^{b} ±677	3523^{b}±200	1.5^{b}±0.33	9.3^{b} ± 1.3

Values in the same line with different superscripts (a, b, c) differ significantly ($P < 0.05$).

Similarly to the follicular surface area, the colloid surface area is smallest for animals of Subgroup 2 (1521±307 µm^2), largest for Subgroup 3 (3015±581 µm^2) and intermediate (2010±368 µm^2) for Subgroup 1. For this morphometric trait, females have larger colloid surface area than males (2333±760 vs. 2031±734 µm^2; P<0.05) and it is larger in summer in comparison to winter (2490±677 vs. 1874±715 µm^2; P<0,05).

The activation index is highest for animals of Subgroup 2 (2.2±0.45) and decreases to values of 187±0.39 and 1.3±0.32 for respectively animals in Subgroups 1 and 3. Males have a higher activation index than females (1.94±0.6 versus 1.69±0.48 ; P<0.05) and this parameter is more important during winter than during summer (2.13±0.56 versus 1.5±0.33; P<0.05).

The influence of age on the height of thyrocytes shows that the highest cells are observed for animals in Subgroup 2 (11.5±1 µm) being followed by animals in Subgroup 1

(10±1.1 µm) while animals in Subgroup 3 have the least thyrocyte height being 8.5±0.7 µm (P<0.05). The average thyrocyte height is 10.5±1.6 µm for males being higher than the average figure of 9.5±1.4 µm for females (P<0.05). Thyrocytes are higher during winter than during summer (10.7±1.5 versus 9.3±1.3 µm; P<0.05) irrespectively of sex and age.

Serum concentrations of hormones

All the factors included in the model were significant and coefficients of determination ranged between 28% and 79% (Table 4). Mean values of serum T4, T3 and TSH concentrations according to age, sex and season are reported in Table 5. The highest concentrations of these hormones are observed for animals in Subgroup 2 and they were lowest for those in Subgroup 3 (P<0.05). Highest and lowest serum concentrations were 20.41 and 12.64 Pmol/l (S.E.M. = 0.30), 5.70 and 3.80 Pmol/l (S.E.M. = 0.11) and 0.25 and 0.09 miU/l (S.E.M. = 0.01) for T4, T3 and TSH respectively.

Table 4. Sources of variation of serum concentrations of the dromedary thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH)

Hormone	**N**	**R^2 (%)**	**Fixed effects**	**Degrees of freedom**	**Significance**
T4	120	79.09	Age	2	***
			Sex	1	***
			Season	1	***
T3	120	62.80	Age	2	***
			Sex	1	***
			Season	1	***
TSH	120	28.39	Age	2	***
			Sex	1	*
			Saison	1	*

*: P <0.05; ***: P <0.001

Table 5. Mean serum concentrations of the dromedary thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) according to age, sex and season

Factor of variation		T4 (Pmol/l)	T3 (Pmol/l)	TSH (miU/l)
Age	<3 years	14.59 ^{a} ± 0.30	4.35 ^{a} ± 0.11	0.14 ^{a} ±0.01
	3 to 5 years	20.41 ^{b} ± 0.30	5.70 ^{b} ±0.11	0.25 ^{b} ±0.01
	≥15 years	12.64 ^{c} ± 0.30	3.80 ^{c} ±0.11	0.09 ^{c} ±0.01
Sex	Male	16.86 ^{a} ±0.25	4.94 ^{a} ±0.09	0.18 ^{a} ±0.01
	Female	14.90 ^{b} ±0.25	4.29 ^{b} ±0.09	0.14 ^{b} ±0.01
Season	Winter	17.24 ^{a} ±0.25	5.05 ^{a} ±0.09	0.19 ^{a} ±0.01
	Summer	14.52 ^{b} ±0.25	4.19 ^{b} ±0.09	0.13 ^{b} ±0.01

For each factor of variation, values in the same column with different superscripts (a, b, c) differ significantly ($P < 0.05$).

Discussion

Morphometric study

This study demonstrated that follicular and colloid surface areas are smaller in individuals around puberty (Subgroup 2, 3 to 5 years). They are much larger in animals aged 15 years and above. Animals in Subgroup 1 have intermediate figures. The results also present evidence that the activation index and the epithelium height vary in the same direction. Indeed, the highest cells and the most elevated figures of the activation index are observed for animals in the Subgroup 2 while the lowest levels for both traits are observed for animals in Subgroup 3. These results stand for an important thyroid activity in young animals (< 3 years) which is even higher for animals around puberty and then decline for older animals (≥ 15 years of age). Basal metabolism is usually high around puberty (Sai, 1980; Girod, 1980). In addition, our results showed that both the follicular and the colloid surface areas are larger in females than in males, a finding in line with those of Delverdier et al., (1991) and Malendowicz, (1977) who showed that the follicular surface is reduced in the male rat.

The reduction in the activation index as well as the thyrocyte height concomitantly with an increase in the colloid surface area in animals aged ≥15 years in both males and females is consistent with previous results by Malendowicz and Majchrzak, (1981) in the female rat. Nevertheless, Delverdier et al., (1991) demonstrated that the morphological aspect of the thyroid follicles is related to a constant over time functional activity in the female rat, while this activity is higher in the male but declines progressively with age. According to the same author, decline of the thyroid functional activity in males is coincident with andropause.

This study also showed that the follicular epithelium is higher for males than females. This is probably a sexual dimorphism in the dromedary which is similar to what has been reported for the rat (Delverdier et al., 1991; Malendowicz, 1977). Based on previous results on the morphological variations of the thyroid follicles during their secretion cycle, there seems to be a more intense physiological activity in the dromedary male when compared to females. This conclusion is backed by reports by Bengoumi et al., (1999) that activity of the thyroid gland is more important in male camels.

As to the influence of the season, data from this study indicated that the surface areas of the follicles and colloids are more important during summer, while the height of the thyrocytes is more elevated during winter. This is consistent with results by Abdel-Magid et al., (2000) who reported that thyroid follicles are large and lined with flat epithelium during summer but they are narrow and lined with a simple cuboidal or pyramidal type of epithelium in winter. These conclusions would favour a maximal activity during winter and much less activity during summer. This is strengthened by the increase in the activation index during winter for all age subgroups and both sexes.

Hormone concentrations

Results of this study showed that concentrations of the thyroid hormones are much higher for animals around puberty in comparison to younger or aged animals. This is consistent with the reports by Elrayh et al., (2009) that concentration of thyroid hormones are higher for animals less than 5 years old in comparison to animals older than 10 years. Similarly, Benoit et al. (1989) showed that plasma concentrations of T4 in dogs aged 5 months (35.4 Pmol/l ± 8.6) are significantly higher than concentrations for animals more than 10 years old (12.8

Pmol/l ± 6.9). Moreover, Mickaël et al., (2008) found that primiparous young cows had much higher T4 concentrations than older ones.

The role of sexual hormones, particularly testosterone, in influencing the concentration of the thyroid hormones was proven by Bengoumi et al., (1999) who produced evidence that castration has a significant effect on the reduction of the concentration of thyroid hormones in the dromedary. Al-Qarawi et al., (2000) reported that plasma concentrations of testosterone are higher for dromedaries between 3 and 5 years old (3.2±0.4 ng/ml) than animals younger than 3 years (1.1±0.1 ng/ml) or older than 15 years (2.6±0.3 ng/ml). In this study and similarly to what has been reported by Bengoumi et al., (1999), males had a higher concentration of thyroid hormones than females. However, studies by Elrayah et al., (2009) and Elkasmi et al., (1999) failed to show differences between sexes with regards to the concentration of thyroid hormones.

Similarly to many other reports (Bengoumi et al., 1999; Yagil et al., 1978), it appeared that the thyroid activity as assessed by concentrations of thyroid hormones and TSH is more important during winter than summer. This is in relation to the fact that thyroid gland plays a role in body thermoregulation. Exposure to cold leads to an increased secretion of TSH that stimulates thyroid hormone secretion and acceleration of the metabolism. In contrast, during the hot summer season there is a slowing down of general metabolism, hence of thyroid activity (Bengoumi and Faye, 2002; Djegham and Belhadj, 1985). Diminution of the basal metabolism is correlated to a diminution of the concentrations of circulating thyroid hormones (Yagil et al., 1978; Bengoumi, 1992; Bengoumi, 2003). Such a finding was also reported for cattle by Mickaël et al., (2008). These seasonal variations are indicative of adaptation of the dromedary to its hot environment. In fact, under arid conditions the dromedary accumulates heat which leads to increases in its body temperature. To dissipate heat load the dromedary uses several mechanisms that are mainly a modification of the activity of the thyroid gland. For this species, ambient heat reduces the thyroid activity and the general metabolism (Faye, 1997).

This study revealed large variations in the morphometric characteristics of the dromedary thyroid gland according to age, sex and season in southern Tunisia. These morphometric changes are associated with functional variations. The dromedary can therefore represent a remarkable biological model for physiological adaptation to harsh climatic conditions of the desert. Further studies are required to better explore the relationships between the morphological changes, the thyroid and sexual hormones secretions.

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