THYROID HORMONES AND TESTOSTERONE IN SHEEP AGE RELATED PROFILES OF SERUM THYROXINE, TRI-IODOTHYRONINE AND TESTOSTERONE IN KAGHANI, RAMBOUILLET AND KAGHANI × RAMBOUILLET SHEEP

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Summary

Secretory patterns of serum tri-iodothyronine, thyroxin and testosterone were analysed by specific radioimmunoassays in Kaghani (n = 42), Rambouillet (n = 42) and Kaghani × Rambouillet (n = 42) male sheep to determine age and breed related changes. Animals were examined at 1-2 wk, 8, 10, 12, 20, 22 and 24 months of age (n = 6/age group). All animals were maintained under standard conditions of feeding and management. T_3 and T_4 concentrations were maximal ($p < 0.01$) in 1-2 wk old lambs of all breeds studied (207.4 ± 14.9 − 276.8 ± 20.4 ng/dl and 5.76 ± 0.3 − 7.23 ± 0.9 μg/dl, respectively). For all the breeds, concentrations of T_3 declined markedly ($p < 0.01$) at 8 month of age and then varied in a narrow range (44.5 ± 14.0 − 87.0 ± 4.8 ng/dl) up to the age of 24 month. While, serum T_4 concentrations decreased ($p < 0.01$) to nadir (0.97 ± 0.1 − 1.43 ± 0.3 μg/dl) at 10 months of age in all the 3 breeds. Afterwards, T_4 levels increased gradually and attained a small peak at 20 months before declining again at 22 and 24 months of age. Serum T_3 and T_4 concentrations were correlated significantly ($p < 0.05$) and their ratio was constant at all ages in the different breeds. Age significantly ($p < 0.01$) influenced the secretion of testosterone in the breeds examined but the breed differences were comparable. Mean serum concentrations of testosterone were low from 1-2 wk to 10 months of age in the Kaghani, Rambouillet and Kaghani × Rambouillet animals. Peak testosterone levels were noted in the 12 months old Kaghani (1.75 ± 0.4 ng/ml), Rambouillet (1.30 ± 0.2 ng/ml) and their F_3 cross animals (1.16 ± 0.08 ng/ml). Mean testosterone levels, thereafter, decreased at 20 months and then at 22 and 24 months remained variable but higher than observed at 1-2 wk −10 months of age. The findings of the present study indicate a significant influence of age but not of breed on the peripheral concentrations of thyroid hormones and testosterone. Serum concentrations of T_3 and T_4 were not related to testoscrone levels. High thyroid secretory activity observed at prenatal phase of development may play an imporatnt role in preparing the lambs to extrauterine environments.

(**Key Words** : T_3, T_4, Testosterone, Breed, Sheep)

Introduction

Thyroid secretions have diverse physiological implications in many of the life processes important to animal production. The ontogeny of thyroid hormones secretion has been determined in man (Gregerman and Bierman, 1981), buffalo (Agarwal et al., 1983; Sharma et al., 1985), sheep (Nathanielsz, 1969; Wallace et al., 1979), pig (Nowak, 1983) and dog (Book, 1977). Changes in plasma T_3 and T_4 have been intensively studied in many exotic breeds of sheep and the major feature observed is large increase in the secretion of thyroid hormones at birth (Nathanielsz et al., 1973; Fisher et al., 1977). Iodinated hormones which regulate body temperature (Fisher et al., 1977), maturation of lung surfactant (Erenberg et al., 1979) and intestinal epithelium (Moog, 1979) during the first hours of postnatal life, play a major role in determining survival of new born lambs (Davicco et al., 1980). In view of the important role of thyroid hormones in the production physiology of sheep, this study was designed to describe age related changes of T_3 and T_4 in a major Pakistani breed of sheep, an exotic breed kept under local conditions and their F_3 cross animals. Due to a possible role of thyroid hormones in sexual maturation (Niswender et al., 1974; Kahl et al., 1977; Dwaraknath et

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al., 1982) an attempt has also been made in this study to correlate T_3 and T_4 changes with peripheral androgen concentrations.

Materials and Methods

Secretory patterns of serum thyroid hormones and testosterone were determined in a Pakistani breed of sheep, an exotic breed and their F_3 cross. Male animals of Kaghani, Rambouillet and Rambouillet × Khaghani (F_3) were examined at 1-2 week, 8, 10, 12 months and then at 20, 22 and 24 months of age in a semilongitudinal study. Animals selected at 8 and 20 months of age were followed longitudinally up to 12 and 24 months of age, respectively. All animals were maintained at Livestock Experiment Station sheep farm, Jaba. Lambs were fed colostrum for first three days after birth and afterwards, were allowed to suckle ewes from 2 P.M. to 8 A.M. each day till weaned. From 8-24 months of age, animals were given 250 g/head concentrate (crude protein 13%, TDN 60-60%) in the morning and grazed from 9 A.M. to 3 P.M. in pasture, daily. In the evening corn silage was available *ad libitum*. Animals had accessed to fresh water at all times. Animals below 1 year and 2 years of age were housed separately in semicovered sheds.

Animals were bled by jugular venepuncture and blood samples (3 ml) were obtained between 10 A.M. and 11 A.M. in Oct./Nov. or Jan./Feb. Blood samples were kept at 4℃ overnight and the clotted blood was centrifuged at 4000 rpm for 15 min to separate serum. All sera were stored at −20℃ till hormonal analysis. Serum concentrations of T_3 and T_4 were determined using Coat-A-Count RIA kits (Diagnostic Products Corporation, Los Angeles, CA, USA). All determinations were made in duplicate. Minimum detectable amounts of T_3 and T_4 by these systems were respectively, 7 ng/dl and 0.3 μg/dl. Intraassay and interassay coefficents of variation of the RIA's for T_3 and T_4 were 5% and 7%, and 5% and 10%, respectively. Serum testosterone was determined by a highly specific RIA. The testosterone antiserum had the following crossreactivities: testosterone 100%; 5 DHT 14%; 5 androstenediol 4%; 5 Androstenediol 2.1% and 4 androstenediol 0.8%. Tritiated testosterone (1-2-6-7 ^{3}H) was purchased from Amersham, Buckinghamshire, U.K. Aliquots of serum (100 ml) were extracted with 5 ml diethyl ether prior to assay. All determinations were made in duplicate. Sensitivity of the assay was 0.05 ng/ml. Extraction recovery of labelled or unlabelled testosterone was > 90%. Intra- and interassay coefficients of variation were 10% and 16%, respectively.

Effects of age and breed on the thyroid hormones and testosterone concentrations were estimated by ANOVA and the students *t test*. Associations between the hormones and age were calculated by regression analysis procedures (Steel and Torrie, 1960).

Results

Serum concentrations of total T_3 at different ages in Kaghani, Rambouillet and Kaghani × Rambouillet animals are presented in figure 1. T_3 serum concentrations varied significantly ($p < 0.01$) with age in each breed. However, age related changes in T_3 levels were similar in the three breeds. In Kaghani, Rambouillet and their F_3 cross animals highest T_3 serum concentrations (207.4 ± 14.9, 237.6 ± 10.3 and 276.8 ± 20.4 ng/dl, respectively) were observed at 1-2 week of age (figure 1). T_3 concentrations declined markedly ($p < 0.01$) after 1-2 week of age and reached nadir (62.7 ± 5.4, 49.7 ± 3.1 and 53.9±1

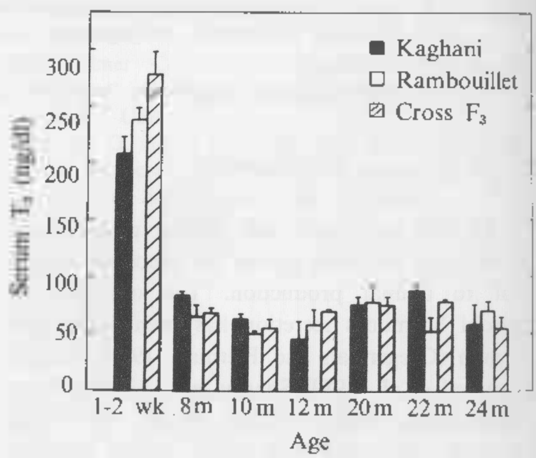

Figure 1. Mean (±SEM) concentrations of serum tri-iodothyronine at various ages in Kaghani, Rambouillet and their corss F_3 sheep. Vertical lines above the bars show ±SEM (n=6 at each age). Age significantly ($p < 0.01$) affected T_3 concentrations in all the breeds.

ng/dl, respectively for Kaghani, Rambouillet and Kaghani × Rambouillet animals) values at 10 month of age in all the breeds. Thereafter, T_3 serum concentrations in the Kaghani, Rambouillet and their F_3 cross animals increased slightly ($p > 0.01$) at 12, 20 and 22 months of age, before declining ($p > 0.01$) once again (figure 1).

Age associated profiles of serum T_4 in the Kaghani, Rambouillet and Kaghani × Rambouillet F_3 animals are shown in figure 2. Although age influenced T_4 secretion significantly in the three breeds, age related T_4 profiles were essentially similar in the different breeds studied (figure 2). Serum T_4 concentrations were maximal ($p < 0.01$) at the age of 1-2 week in Kaghani (7.23 ± 0.9 μg/dl), Rambouillet (7.13 ± 0.3 μg/dl) and Kaghani × Rambouillet F_3 cross animals (5.76 ± 0.3 μg/dl). T_4 levels declined gradually but significantly ($p < 0.05$), thereafter at 8 month of age and lowest T_4 levels were observed at the age of 10 month in the Kaghani, Rambouillet and their F_3 cross animals (1.40 ± 0.4, 0.97 ± 0.1 and 1.43 ± 0.3 μg/dl, respectively). T_4 concentrations in the serum then showed an increasing trend at 12 months and a small peak ($p < 0.05$) was observed at the age of 20 month in Kaghani, Rambouillet and Kaghani × Rambouillet F_3 animals (4.81 ± 0.1, 3.20 ± 0.2 and 3.49 ± 0.1 μg/dl, respectively). Subsequently in all the 3 breeds studied, T_4 serum concentrations decreased gradually at 22 and 24 months of age (figure 2).

Both the thyroid hormones exhibited marked ($p < 0.01$) positive correlations in the Kaghani ($r = 0.79$), Rambouillet ($r = 0.96$) and Kaghani × Rambouillet animals ($r = 0.84$; figure 3). Ratio of serum T_3 to T_4 did not differ significantly at various ages in any breed (figure 4).

Age associated shifts ($p < 0.01$) in the serum concentrations of testosterone were observed for all the breeds studied (figure 4). However, pattern of age related changes was similar in the different breeds examined. Mean serum testosterone concentrations were low form 1-2 week to 10 months of age and ranged from 0.1 ± 0.02 to 0.27 ± 0.11 ng/ml in Kaghani, Rambouillet and Kaghani × Rambouillet sheep (figure 5). At the age of 12 months, mean testosterone levels increased significantly ($p < 0.01$) to maximal values of 1.75 ± 0.4, 1.30 ± 0.2 and 1.16 ± 0.08 μg/ml in the Kaghani, Rambouillet and their F_3 cross

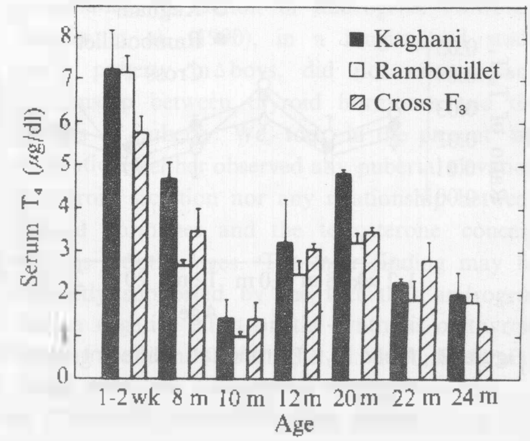

Figure 2. Concentrations of mean serum thyroxine at various ages in Kaghani, Rambouillet and their cross F_3 sheep. Vertical lines above the bars show ± SEM (n=6 at each age). Age markedly ($p < 0.01$) influenced T_4 concentrations in all the breeds.

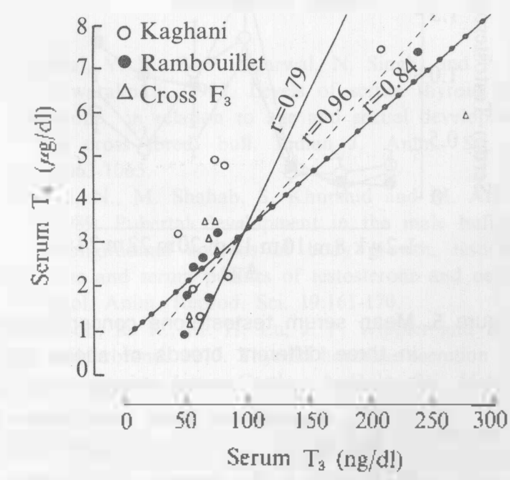

Figure 3. Correlation of serum thyroxine and triiodothyronine at various ages in different breeds of sheep. A significant ($p < 0.01$) positive correlation is demonstrated in Kaghani ($Y=9.76+21.41X$), Rambouillet ($Y=-5.6+32.12X$) and their cross F_3 ($Y=39.18+44.88X$) animals between thyroid hormones.

animals, respectively (figure 5). Thereafter, in each breed, testosterone concentrations decreased at 20-22 months of age but started to rise again at 22-24 months of age (figure 5).

Serum concentrations of T_3 and T_4 were negatively related to testosterone concentrations

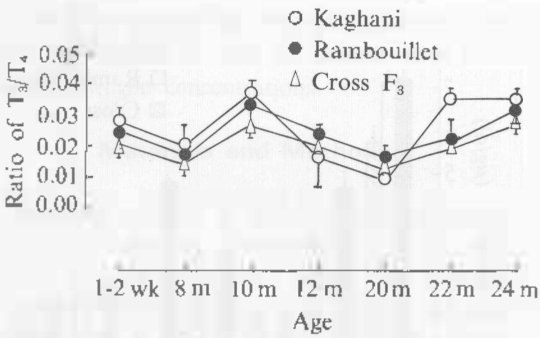

Figure 4. Mean T_3/T_4 ratio at different ages in Kaghani, Rambouillet and their cross F_3 sheep. Bars indicate $\pm$SEM. T_3/T_4 ratio did not differ with age in any breed examined.

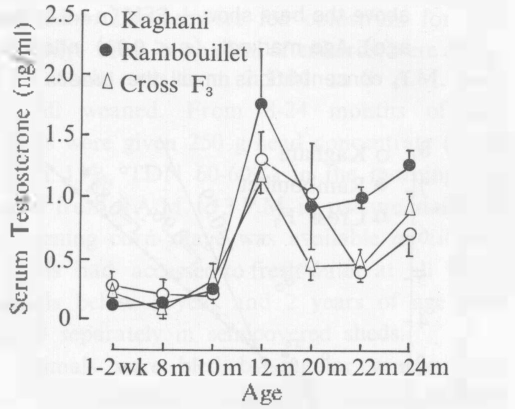

Figure 5. Mean serum testosterone concentrations in three different breeds of sheep from 1-2 week to 24 months of age. Peak ($p < 0.01$) testosterone levels were observed at the age of 12 months in each breed. Each point is the average of 6 hormone determinations.

at various ages in the breeds studied but the correlations were nonsignificant.

Discussion

This study demonstrates significant age dependent variations in the secretion of total serum T_3 and T_4 from birth to 2 years of age in the Kaghani and Rambouillet breeds of sheep and their F_3 cross animals, though no breed differences in the secretion of these hormones were evident. Age has been shown to alter thyroid secretory function not only in sheep (Wallace, 1979; Davicco et al., 1980) but also in several other species including man (Flamboe and Reineke, 1959), cattle (Agarwal et al., 1983), buffalo (Sharma et al., 1985) and dwarf goat (Anwar-ul-Islam et al., 1988) and has been attributed to changing release of TSH and the metabolic malieu.

A negative correlation of T_3 and T_4 with age, observed in this study supports the previous such results presented for goats (Anwar-ul-Islam et al., 1988) and buffaloes (Sharma et al., 1985). In sheep, too, crossectional data indicate that basal T_3 and T_4 levels are significantly low in adult rams as compared to lambs (Lincoln et al., 1982; Nazki et al., 1986).

The major finding of the present study that peak thyroid hormones prevail in circulation at 1-2 week of age in Kaghani, Rambouillet and their cross F_3 animals, is in close agreement with provious findings which indicate higher T_3 and T_4 secretion in sheep at birth (Nathanielsz, 1969; Davicco et al., 1980; Cabello and Wrutniak, 1986; Wrutniak et al., 1987). A neonatal high thyroidal secretory activity has also been shown in man (Fisher et al., 1962), cattle (Kahl et al., 1977), buffalo (Sharma et al., 1985), dog (Book, 1977) and pig (Nowak, 1983). Most of the above mentioned studies demonstrate that high secretory rate of T_3 and T_4 is maintained after birth ranging in time from 2-48 hours to upto 2 months, respectively. Our results, however, show that in Kaghani and Rambouillet breeds and their F_3 cross animals this hypersecretion of thyroid hormones may extend upto 2 weeks of age.

The exact significance of high levels of thyroid hormones around birth is not clear. Higher thyroid hormone secretion could be related to high plasma iodide concentration and its maximum trapping by the thyroid gland (Davicco et al., 1980) or a surge of TSH (Fisher et al., 1977) at birth. Lewis and Ralston (1953) suggested that neonates remain under considerable stress for some time after birth and that the higher levels of thyroid hormone may be one of the mechanisms to combat stress by elevating basal metabolic rate. Anderson et al. (1973) have also advocated that higher thyroid secretory rate in neonatal calves may help to adopt the new born to the extrauterine environments.

In the present study we observed that the ratio of T_3/T_4 in circulation did not vary with age in any breed investigated. This indicates that the thyroidal or extrathyroidal conversion of T_4 into T_3 is relatively constant and the secretion and removal of T_4 and T_3 are more or less proportional from 1-2 week to 24 months of age. Similar results have been shown for buffalo where ratio of T_3/T_4 at various ages was comparable (Dwaraknath et al., 1982). The present work also demonstrates a high positive correlation between serum concentrations of T_4 and T_3 at different ages in Kaghani, Rambouillet and their F_3 cross sheep. This could be explained since the ratio of T_3/T_4 was not affected by age, therefore, similar T_3/T_4 ratio from 1-2 week to 24 months of age results in identical relative changes in the serum concentrations of both the thyroid hormones. Thyroid hormones have also been shown to be positively related with each other in the dwarf goat (Anwar-ul-Islam et al., 1988) and buffalo (Dwaraknath et al., 1982; Sharma et al., 1985).

A significant drop in the serum concentrations of T_3 and T_4 after 1-2 week of age and then more or less uniform secretion upto 24 months as observed in this study is similar to findings in dwarf goats (Anwar-ul-Islam et al., 1988) and buffaloes (Sharma et al., 1985). In these animals too the thyroid hormone levels decreased markedly some time after birth and then remained low up to adulthood.

Peripheral androgens have been reported to increase at or before puberty (Sharma et al., 1985; Ahmad et al., 1989). Therefore, a marked increase in testosterone concentrations observed at 12 months of age in Kaghani, Rambouillet and their cross F_3 rams indicates that puberty is attained around this age in these breeds. Lower concentrations of thyroid hormones at the age of one year when testosterone levels were elevated, suggest that thyroid hormones do not play a role in the sexual development of these breeds of sheep. Controversial reports appear in the literature regarding the involvement of thyroid hormones in the reproductive development. Some workers have found prepubertal and pubertal rises in the bull and the buffalo male (Kahl et al., 1977; Agarwal et al., 1983). It has been hypothesized that higher prepubertal thyroid hormones may interact with rising levels of testosterone and modulate the onset of puberty, possibly by potentiating the action of androgens. However, Dunger et al. (1990), in a longitudinal study about puberty in boys, did not observe any relationship between thyroid hormones and the process of puberty. We, too, in the present investigation, neither observed any pubertal elevation in thyroid secretion nor any relationship between thyroid hormones and the testosterone concentrations at other ages. The later finding may be indirectly supported by the fact that androgens have a negative effect on the synthesis of thyroid binding globulins (Dowling et al., 1956; Federman et al., 1958).

Taken together the present data indicate that as observed in several species, thyroid secretory activity is maximum around birth in Kaghani and Rambouillet sheep and their F_3 cross animals but show no evidence of the involvement of thyroid hormones in the pubertal processes.

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