

THYROID HORMONES (TH) AND 5'-MONODEIODINASE (5'-MD) ACTIVITY IN GOAT'S MILK FROM THE EARLY, MID- AND LATE LACTATION PERIOD

A. B. ŚLEBODZIŃSKI^{1*} and J. TWARDON²

¹ENDO-CANIS, Endocrine Research and Diagnostics, ul. Grunwaldzka 250, 60-166 Poznań, Poland; ²Department of Animal Reproduction and Clinic of Obstetrics, Agricultural University in Wrocław, Wrocław, Poland

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The physiological significance of thyroid hormones (TH) present in colostrum and milk is still under consideration. The present study was aimed at determining milk thyroxine (T₄) and triiodothyronine (T₃) levels in three lactation phases (early, mid- and late) of the goat, and to measure activity of the milk 5'-deiodinase (5'-MD) enzyme responsible for the intramammary conversion of pro-hormone T₄ to its metabolically highly active form T₃. Thirty-two milk goats (Polish White breed) fed a standard diet were used for milk sampling. The highest TH levels in mammary secretion were recorded during the first 2–3 days post partum. Then the hormone levels decreased, and by about Day 7 fluctuated around the overall mean for the early-lactation phase (Days 1 to 24 of lactation), recording $0.134 \pm 0.059 \mu\text{g T}_4$ and $150.8 \pm 2.80 \text{ ng T}_3$ in 100 ml of the milk. Such T₄ concentrations appeared to be comparable to those in the rabbit and human, whereas the concentration of T₃ was higher than in the cow, pig and mare's milk. Milk 5'-MD activity was higher ($P < 0.01$) during early and late lactation, compared to the mid-lactation phase. It coincided with low T₄ and high T₃ milk levels during early lactation, and with high milk T₄ and low T₃ concentrations during late lactation. The quantity of T₄ and T₃ available to newborn kids in milk suggests that TH ingested with the colostrum may have a physiological role during the early postnatal life of suckling goats.

Key words: Triiodothyronine, thyroxine, milk, 5'-deiodinase, newborn goat, colostrum

Several hormones and growth factors are supplied from the mother to the neonate via the mammary secretion (milk). Hormonal milk components, jointly with growth promoting factors, affect galactopoiesis and help the newborn adapt to the extrauterine life (Grosvenor et al., 1993).

Among the hormonal substances present in colostrum and milk, insulin and prolactin have been considered to be factors particularly influencing the

*Corresponding author; E-mail: endocrin@rose.man.poznan.pl; Phone/Fax: +48 (61) 868 53 28

newborn. The significance of other hormones, e.g. the thyroid hormones (TH), is less well established (Pearlman, 1991; Nowak et al., 1994). A variety of functions for TH in milk for the maturation process of the intestinal enzyme metabolism (in rodents) or for the absorption of immunolactoglobulins (in calves) have been found (Koldovsky et al., 1974; Ślebodziński et al., 1995).

Hormones contained in colostrum or milk can either be transferred from the peripheral blood or, in addition, may be produced locally within the mammary gland tissue. Regarding thyroid hormones, both thyroxine (T_4) and triiodothyronine (T_3) in milk originate from the blood plasma, and T_3 but not T_4 is produced in the mammary gland by the enzymatic process of T_4 to T_3 conversion (Ślebodziński and Brzezińska-Ślebodzińska, 1991).

The galactopoietic role of TH has long been recognised as resulting from the own action of TH or from the synergistic action with growth hormone factors in the mechanism of homeorhesis controlling the efficiency of nutrient partitioning during lactation (Avecas et al., 1985). In spite of the recognised importance of thyroid hormones for mammary gland function in various species, and their benefit to the offspring, such studies in goats are lacking. Amongst farm animals (cows, horses, pigs and sheep) goats have higher TH blood concentration, and the highest activity of liver 5'-MD (type I), the enzyme which is responsible for the main part of peripheral T_3 production and T_3 blood concentrations (Anderson et al., 1988; Schoenmakers et al., 1992).

In view of the increasing consumption of goat milk with the human diet, the purpose of the present study was to investigate the thyroid hormone (TH) concentrations in the milk. From the endocrinological point of view it became interesting to study the milk TH levels during various phases of lactation in relation to 5'-MD activity in the goat milk, and in other domesticated species (pigs, cows and horses) known to have a relatively low TH in blood. For interspecies comparison of potential effects of milk T_4 for newborns, the quantity of T_4 consumed by the suckling goat per day has been estimated.

Materials and methods

Animals

Thirty-two milk goats (Polish White Annblad breed) during their second and third lactations, of 35–45 kg body weight (BW), were used for the study. The peak milk production (about 3.5 kg/day) for this breed occurs during the third to fourth month of lactation. All the animals were under veterinary control and only clinically healthy ones were included in the trial. Experiments were performed with the approval of the Ethics Committee (II) for Experiments on Animals at the Agricultural University in Wrocław.

The goats (early-lactation group) were kept in separate boxes at the departmental clinics for the convenience of consecutive milk samplings ($n = 7$), from Days 1 to 22 after kidding, and for blood collection ($n = 8$) from Days 1 to 21, in the order shown in the figures. After 8 weeks the goats were transferred to the farm where the number of does was increased up to 28 and the does were divided into two groups. The first ($n = 14$), arbitrarily named mid-lactation group, served for milk and blood collection during the fourth month of lactation. The second group ($n = 14$), named late-lactation group, served as a milk and blood collection group during the last four days of lactation.

Until newborn kids were weaned, their mothers (the early-lactation group) were not hand milked. Later, on the farm, they were milked twice daily. The milking goats of all three groups were fed hay, concentrated feed or green forage with mineral supplements not modified for the purpose of the present study.

Sample collection

In the early-lactation group, the colostrum and milk samples were taken sequentially one day after kidding and then successively up to Day 22. In the groups kept on the farm, the milk samples were collected twice during the mid-lactation, and twice during the late-lactation period. Milk samples, about 10 ml each, obtained with hand milking were cooled in an ice-water bath, transported cold to the laboratory, divided into aliquots and left in a freezer (-20°C) until extraction.

Extraction of TH from milk

The extraction of milk was performed with cold (-20°C) alkaline ethanol, using a procedure validated for animal colostrum and milk (Ślebodziński et al., 1986). The recovery of T_4 added to the goat milk was 92% and that of T_3 was 97–104%.

Radioimmunoassay (RIA) of T_4 and T_3

One day before analysis, the milk extracts were diluted (1:1) with a buffer used for RIA. The TH concentrations in the extracts of milk or colostrum were measured by RIA as in a previous work (Ślebodziński et al., 1998). Intra-assay and inter-assay coefficient of variation for milk quality control extracts in this trial was 7.0 and 10.4% for T_4 and 5.1 and 9.3% for T_3 , respectively.

5'-monodeiodinase determination (5'-MD)

The measurement of 5'-MD activity was based on release of ^{125}I from (^{125}I)-rT3 according to the method of Leonard and Rosenberg (1980) as modified by Jack et al. (1994).

Statistical analysis

The statistical calculations were conducted with the aid of Duncan's new multiple range test and the paired Student's *t*-test where applicable. Differences between means were considered significant when $P < 0.05$. Values are given as mean $\pm$ SEM.

Results

Early-lactation phase

Thyroid hormone in milk. The overall mean T_4 concentration in consecutive milk samples obtained from Day 1 to Day 24 post partum (7 goats) was 0.134 ± 0.059 $\mu\text{g/dl}$ (mean $\pm$ SEM). On the first and the second day after kidding the concentration was 0.199 ± 0.026 and 0.155 ± 0.025 $\mu\text{g/dl}$, respectively. The milk T_4 levels decreased during the first 5 days to a mean value of 0.117 ± 0.05 $\mu\text{g/dl}$ and then fluctuated around 0.13 $\mu\text{g/dl}$ (the overall mean) until the end of observation (Fig. 1A). The T_4 milk content was relatively stable for the individual goats during this early phase of lactation (Fig. 2A). Except for goat No. 1, which had an overall mean below 0.1 μg of T_4/dl (0.087 ± 0.012), the mean values for the rest of the goats were above that level and varied from 0.125 ± 0.016 (goat No. 2) to 0.200 ± 0.028 $\mu\text{g } T_4/\text{dl}$ (goat No. 7).

During the first to third day after kidding, the mean T_3 concentrations in the milk were high. On these days the mean values ranged from 301.0 ± 55.80 to 332.1 ± 57.70 ng T_3/dl . Then the T_3 concentration reached a nadir on Day 11 (119.8 ± 1.35 ng/dl). The relationship between the milk T_3 and days of lactation showed a negative correlation ($R = 0.86$) which could be expressed by the following exponential equation: $Y = 295.3^{-0.134 X} + 89.5$; where Y = concentration of T_3 , X = day of lactation, as shown in Fig. 1B.

Among the 7 goats, 4 does had significantly lower milk triiodothyronine (T_3) concentration of 150.8 ± 12.80 ng/dl (mean $\pm$ SEM) and the other 3 very high concentrations of 372.0 ± 45.08 ng T_3/dl during the early postpartum period (Fig. 2B). The overall mean for T_3 in consecutive milk samples obtained from Day 1 to Day 24 post partum was 250.8 ± 31.4 ng/dl.

Mid- and late-lactation phases

The overall milk T_4 concentrations in mid- and late lactation were 0.074 ± 0.0165 and 0.115 ± 0.0040 $\mu\text{g/dl}$, respectively. The differences between the mean values for the mid-lactation phase and the late- and early-lactation phases were statistically significant at $P < 0.05$ (Fig. 3).

The mean content of T_3 in milk was the highest in the early-lactation phase (colostral and milk period). It decreased to 204.7 ± 18.37 (n = 14) in the mid-lactation and to 165.7 ± 18.14 ng T_3/dl (n = 14) in the late-lactation phase (Fig. 3).

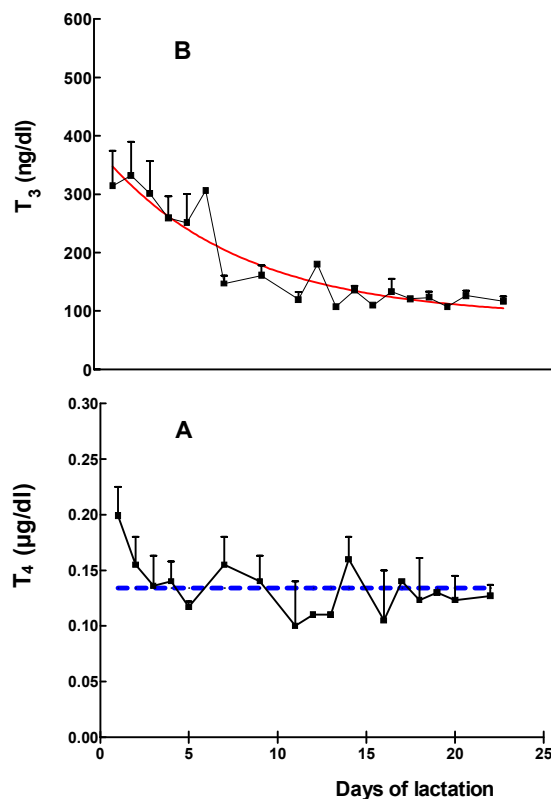


Fig. 1. Changes in goat milk thyroxine (T_4 ; A) and triiodothyronine (T_3 ; B) during the early-lactation phase. The overall mean for T_4 was 0.134 ± 0.059 $\mu\text{g/dl}$ (broken line). The relationship between the milk T_3 and days of lactation shows a negative correlation and can be expressed by an exponential equation (see the text). Values represent means $\pm$ SEM

Activity of thyroxine 5'-monodeiodinase (5'-MD) in milk

The overall mean for milk 5'-MD activity expressed as pmol I^- released/mg protein/min in the early lactation period (Days 1 to 24 post partum) was different for each animal, ranging from the lowest 1.84 ± 0.233 to the highest 2.90 ± 0.616 . The mean activity of 5'-MD in milk from the early-lactation phase and from the late-lactation period was 2.47 ± 0.145 and 3.00 ± 0.345 , respectively, being the lowest (1.43 ± 0.174) in the mid-lactation phase. Differences between the latter and the other two means were statistically significant ($P < 0.01$; Fig. 4).

The pattern of changes in milk 5'-MD activity was similar to changes in the milk thyroxine level in that it was higher in milk collected during the early-lactation phase and during the late-lactation period. No comparable similarity to the pattern of changes in the milk triiodothyronine was noted.

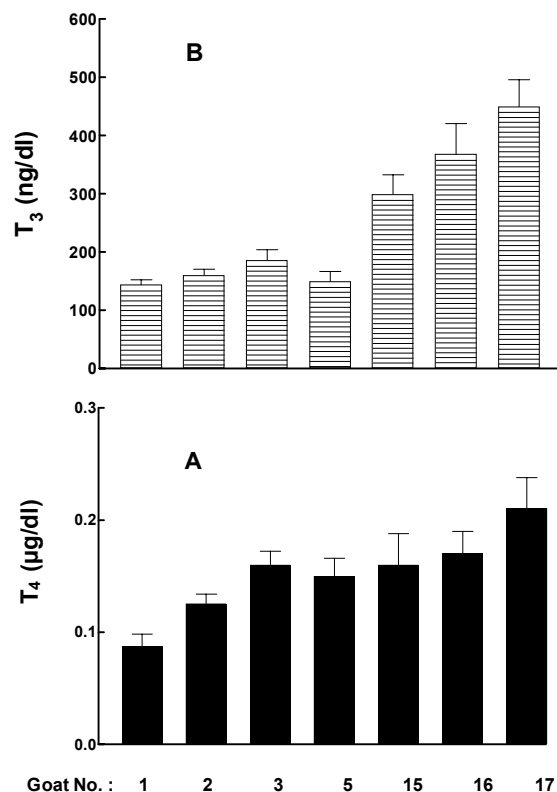


Fig. 2. Mean milk thyroxine (T₄; A) and triiodothyronine (T₃; B) concentrations in individual goats. Milk samples were collected from Day 1 to Day 22 post partum. Values represent means $\pm$ SEM

Concentration of thyroid hormones in blood serum

The concentration of TH in the blood of milking goats in each lactation phase is shown in Fig. 5. The thyroxine level was the lowest in blood collected during the early-lactation phase (3.95 ± 0.064 µg/dl) and it tended to rise till the highest level in the late-lactation phase (8.9 ± 0.914 µg/dl).

The concentration of T₃ was relatively constant but it was lower ($P < 0.01$) in the late-lactation period. No correlation was found between T₃ concentrations in blood serum and in milk in the early or mid-lactation phases. There was also no statistically significant difference either between the mean content of T₃ in blood serum and in milk from individual goats or between the mean content of T₃ in blood serum and in milk for the group of goats in each lactation phase.

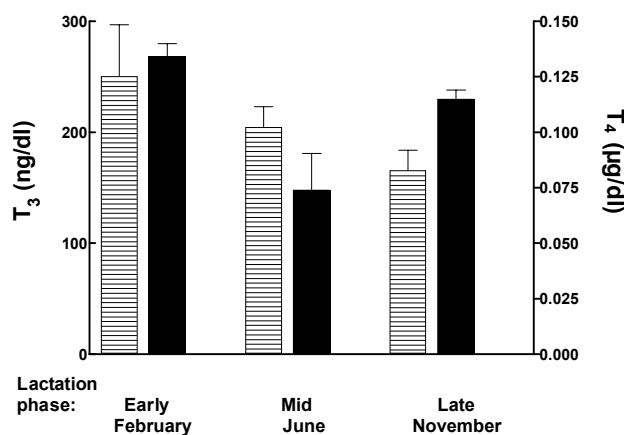


Fig. 3. Comparison of milk thyroxine (T₄) and triiodothyronine (T₃) in goats in various phases of lactation. Differences between means for T₄ concentration in three phases of lactation were statistically significant ($P < 0.01$). Milk concentration was decreasing towards the end of lactation. Only the difference between the early- and the late-lactation phase was significant ($P < 0.01$). Values represent means $\pm$ SEM

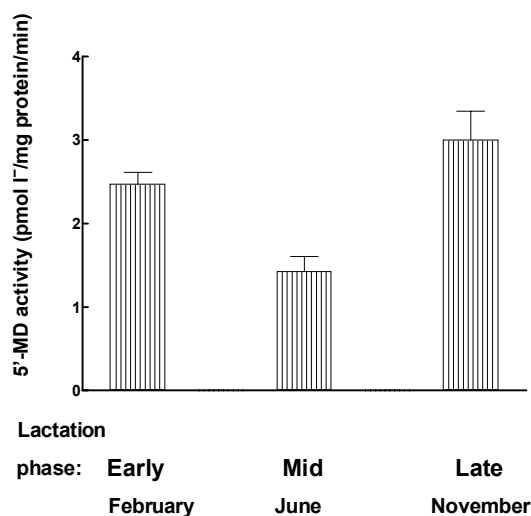


Fig. 4. Milk 5'-MD activity was significantly higher ($P < 0.01$) during early and late lactation when compared with the mid-lactation phase. The unexpected rise at the end of lactation is not readily explained. Values represent means $\pm$ SEM

Thyroid hormones ingested by newborn goats with the milk

The assumed amount of thyroxine ingested with 1 litre of colostrum or milk varied owing to the different thyroxine content of the mammary secretion and relative to the phase of lactation. In early lactation, the average T₄ content in

one litre of milk consumed was $1.8 \pm 0.016 \mu\text{g}$ in the first 72 h after birth, $1.34 \pm 0.010 \mu\text{g}$ on Days 4–7, and $1.29 \pm 0.008 \mu\text{g}$ on Days 8–19. The concentration of T_4 in colostrum and milk progressively declined, irrespective of individual differences in milk T_4 level. Taking into account that the quantity of T_4 consumed by newborn goats depended on lactation phase as well as on whether the mother's milk was primarily lower- or higher-thyroxine milk (Fig. 2A), the estimated amount of T_4 consumed by newborn goat kids in 1 litre of milk may vary from 2.5 to $1.1 \mu\text{g } T_4$ during the first 72 h after birth and from 1.73 to $0.90 \mu\text{g } T_4$ on Days 4–7 of lactation.

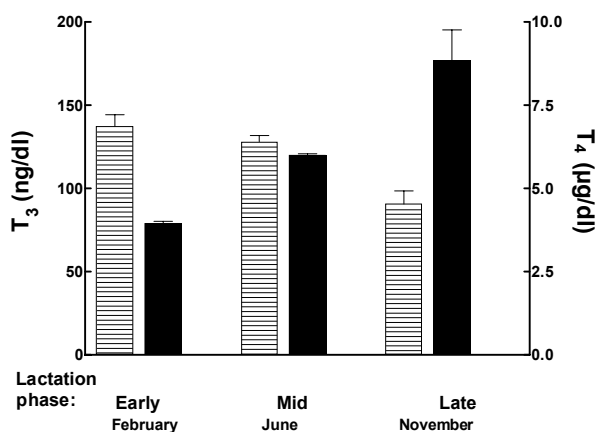


Fig. 5. Serum thyroxine (T_4 ; black bars) and triiodothyronine (T_3 ; shaded bars) in lactating goats in various phases of lactation. Differences between means for T_4 concentration in the three phases of lactation were statistically significant ($P < 0.01$); for T_3 , significant differences were obtained only between the late-lactation phase and the other two phases ($P < 0.01$)

Similarly as for T_4 , the amount of consumed T_3 depended on the phase of lactation and on individual differences in milk T_3 content. Taking into account individual differences in colostrum T_3 level, the quantity of T_3 consumed by a kid during the first 72 h was approximately 1.5 to $5.0 \mu\text{g } T_3/1$ litre of colostrum. On Days 4–7 of lactation that amount was near the average milk T_3 content for the group, that is approximately $2.0 \mu\text{g } T_3$ consumed per litre. During the subsequent days (Days 8–19 of lactation), $1.3 \mu\text{g } T_3$ was likely taken up with 1 litre of milk.

Discussion

The mean T_4 concentration in goat milk was found comparable to that in rabbit and human milk (about 2 nmol/L), whereas the mean T_3 content of milk in the early-lactation phase appeared to be higher in goats (2.3 to 5.7 nmol/L) when

compared with 1 to 2 nmol/L in rabbits, about 1.0 nmol/L in pigs, and 0.8–1.2 nmol/L T_3 in horses (Ślebodziński et al., 1986; Ślebodziński et al., 1998).

In the goat serum, measurements of T_4 and T_3 showed high thyroid hormone levels (Fig. 5), and this result is in full agreement with the earlier findings (Anderson et al., 1988; Davis et al., 1999). The blood T_4 concentration was found to be decreased at the beginning of lactation which is consistent with a similar observation made in dairy cows (Mixner et al., 1962; Hart et al., 1978). The low blood and the high milk T_4 level (Figs 3 and 5) is a phenomenon assumed to be associated with an adaptive decrease in the T_4 utilisation rate to negative energy balance. The latter has been found in early lactation in cows (Mixner et al., 1962; Thilsted, 1985) as well as in goats (Riis and Madsen, 1985), and seems to favour the mammary gland in partition of nutrients (homeorhetic regulation) between mammary and non-mammary body tissues.

The mean T_4 concentration in the goat milk rose concomitantly with the progressive stages of lactation, being the highest around the time of last milking or the beginning of the dry period. Increasing milk T_4 concentration from mid- to late lactation seemed to be a reflection of changes in the blood T_4 levels. As it has already been found by Riis and Madsen (1985), there is an increasing thyroxine secretion rate from early to late lactation in milking goats, suggesting adaptive changes to body energy balance, that is to a negative energy balance at the beginning of lactation and to zero or positive energy balance during late lactation. This suggestion seems to be supported by more recent observations in lactating goats in which bovine somatotropin (bST) treatment, capable of improving milk yield, caused a decrease in thyroxine plasma concentration (Davis et al., 1999).

The relatively high milk T_4 level towards the end of lactation in the goat coincided inversely with decreased T_3 in blood (Figs 3 and 5). The mean triiodothyronine milk/serum ratio during the early and the subsequent lactation phases was in a narrow range from 1.4 to 1.7. On comparative grounds, the milk T_3 to serum T_3 ratio was higher in goats than in rabbits, pigs (Ślebodziński et al., 1986) and horses (Ślebodziński et al., 1998).

Mean 5'-MD activity in milk was higher ($P < 0.01$) during the early-lactation period than during the mid-lactation phase, which may indicate a dependence of colostrum and milk T_3 concentrations on the activity of 5'-MD (Fig. 4). While the mean level of 5'-MD was generally high in the early-lactation phase, no relationship was found between 5'-MD and milk T_3 levels in individual goats (Fig. 7). The increased 5'-MD activity of milk during early lactation has already been observed in lactating women and animals (Ślebodziński and Brzezińska-Ślebodzińska, 1991).

The ultimate T_3 level in colostrum or milk results from T_3 transferred from the blood and from enzymatic generation of T_3 within the mammary gland. The presence of 5'-MD in the mammary gland secures adequate supply of T_3 sup-

porting lactogenesis, and makes T_3 available for mammary gland cells independently of temporal variations in TH in the systemic circulation (Ślebodziński et al., 1999). High serum T_3 during the early-lactation phase observed in milking goats may indicate a concomitant rise in type I 5'-MD activity in the liver and the kidneys, the isoenzyme which is responsible for the blood level of T_3 and which among farm animals shows the highest activity in the goat (Schoenmakers et al., 1992). The involvement of blood T_3 , insulin and glucagon in the homeorhetic adaptation to lactation in goats has been suggested in a recent work (Pereka and Riis, 1993).

The high 5'-MD activity found in the present work at the very beginning of lactation in goats is in good agreement with observations made in other species. By contrast, an increase in milk 5'-MD activity at the very end of lactation has not been observed or published yet. In fact, such a phenomenon was noted by us in a similar kind of study carried out in milking rabbits (unpublished observations). The increase found in 5'-MD activity during the late-lactation phase is not easy to explain. It may be related to the involution of the mammary gland tissue and/or to inhibition of the galactopoietic process.

The approximate intake of TH per newborn kid calculated from the TH concentration of the colostrum of individual goats and from the volume of colostrum ingested by a goat kid per day might vary from 1.25 to 2.0 $\mu\text{g } T_4$ and from 1.5 to 3.72 $\mu\text{g } T_3$ per day. Although the present work is not aimed at determining the physiological role of thyroid hormones present in colostrum or milk, it reveals, particularly during the early stage of lactogenesis, that the quantity of ingested TH may have some physiological implications for the mammary gland itself and for the development, maturation and metabolism of the suckling animal.

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