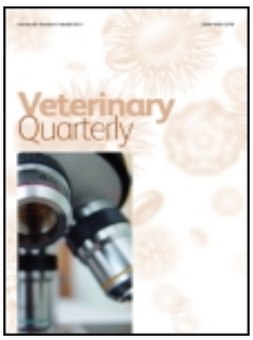

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ORIGINAL ARTICLE

Effects of weaning on total and free iodothyronines in lambs

E. Fazio*, A. Ferlazzo, C. Cravana and P. Medica

Department of Veterinary Sciences, University of Messina, Messina, Italy

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Background: Weaning is a crucial period in the management of lambs, resulting in physiological and mental challenges, that may have prolonged effects on lamb's health and welfare.
Hypothesis/Objectives: To evaluate the effect of weaning on total and free triiodothyronine (T_3, fT_3) and thyroxine (T_4, fT_4) concentrations in serum of lambs by enzyme immunoassay.
Animal and methods: The study was performed on two groups of 17 clinically healthy Comisana cross-bred lambs (7 males and 10 females) with or without weaning at 10 weeks of age. Serum samples were collected at the age of 8 weeks, 24 h and 2 weeks after weaning in the experimental group and at similar times in the non-weaned control group. Enzyme immunoassay was performed. Statistical analysis was done by one-way analysis of variance.
Results: Compared to control animals, weaned animals showed significantly decreased T_3 and elevated T_4 concentrations two weeks after weaning with higher concentrations in both males and females in contrast to 24 h after weaning. Body weight (BW) was significantly restored in both females (11% increase) and males (6%) two weeks after weaning as compared to 24 h after weaning. No gender effects were shown for total and free iodothyronine changes. Significant positive correlations were observed between BW and T_4 concentrations in both females ($r = 0.692$) and males ($r = 0.856$), fT_3 concentrations in males ($r = 0.968$) and fT_4 concentrations in females ($r = 0.862$).
Conclusion and clinical importance: Total iodothyronines could represent an indicator of their different metabolic activity with their magnitude altered two weeks following weaning.

Keywords: lamb; total iodothyronines; free iodothyronines; T4; T3; weaning

1. Introduction

The endocrine responses to feeding and growth are regulated by adequate dietary intake and developmental age in animals. In literature, there are many data available on the effect of feeding on whole-body energy metabolism and carcass characteristics in relationship with plasma concentrations of hormones, including T_3, T_4 and thyroid stimulating hormone (TSH) of lambs (Symonds et al. 1989; Wu et al. 2006; Boland et al. 2008; Nisa et al. 2012; Eckerman et al. 2013), steers (Cassar-Malek et al. 2001) and beef calves (Szabó et al. 2006). Circulating thyroid hormones seem better correlated with feed intake than adiposity status (Caldeira et al. 2007a, b). Growth of lambs was affected positively by fan treatment with live weight increased by 15%, and cooled lambs had the highest thyroxin levels (Koluman & Daskiran 2011). In general, plasma total T_3 and total T_3/T_4 ratio were correlated negatively, whereas total T_4 was correlated positively with size at birth. In addition, absolute growth rates of weight and crown-rump length were correlated positively with total T_3 and T_4 in young lamb between days 15 and 35 postnatally (De Blasio et al. 2006). Recently, Segar et al. (2013) determined the effect of ovine fetal thyroidectomy on cardiac growth and growth-related gene expression, suggesting that in the late-gestation fetal heart, thyroid hormones have important cellular growth functions in both physiological and pathophysiological states. Nevertheless, the effects of weaning of lambs on hormonal changes are not that well defined in detail as that of other animal species, including calves (Hickey et al. 2003) and foals (Fazio et al. 2007; 2009). Little research has been conducted about the metabolic role of thyroid hormones in small ruminants (Heasman et al. 2000; Todini 2007), and how their secretion is affected by lambs' weaning age (Wells et al. 2003; Abdel-Fattah et al. 2013).

Weaning time is a crucial period in the management of ewes and lambs, resulting in a number of physiological and mental challenges, including deprivation of maternal care, confinement, often social mixing and a sudden transition in the source of nutrients that may have prolonged effects on lamb's welfare and related behavioural display. The short- and long-term effects of weaning on adrenocortical and functional response of lambs, with significant decreases of cortisol concentrations and respiratory rates after weaning were recently described (Fazio et al. 2014).

The intensity of the stress response to weaning may be modulated by the duration of this procedure and the type of environment in which weaning occurs, even if the development of diurnal rhythms of thyroid hormones in ewes was not univocal, depending on the season (Ashutosh Dhanda & Kundu 2001).

The main objective of this study was to evaluate the effect of weaning associated with maternal separation on circulating total and free iodothyronine concentrations after the first 24 h weaning period and 2 weeks later in Comisana cross-bred lambs. In addition, we investigated

*Corresponding author. Email: esterina.fazio@unime.it

the alteration of circulating concentrations of thyroid hormones in the different genders.

2. Material and methods

2.1. Animals and their management

The study was conducted on a private farm located in Sicily (Italy), at 37°59′00″N latitude and 13°42′00″E longitude and 450 m above sea level, in October 2013. During the study, average daily minimum and maximum ambient temperature ranged from 15.7 to 16.5 °C and 18.6 to 19.8 °C, respectively. Relative humidity ranged from 33.5% to 34.5%. These were monitored using a Hygrothermograph ST-50 (Sekonic Corporation, Tokyo, Japan).

Care of animals and procedures used were approved by the University of Messina Institutional Board for the Care and Use of Animals and were in accordance with the guidelines of the Italian minister of health for the care and use of animals (D.L. 4/3/2014 n. 26) and European Union (EU) (Directive 2010/63).

Two groups of 17 clinically healthy cross-bred (Comisana × Merinolandschaf) lambs (7 males and 10 females), sired by two rams and born from secondiparous ewes as singles within five days of each other were used as control and experimental group. Before lambing, the dams were handled in the same paddock (pen), together a flock of more than 100 sheep, and maintained under the same conditions: grazing on improved pastures during the day and housing at night. After giving birth, the mothers were placed indoors on a separate pen for 60 days and bedded on straw with their lambs (1.5 m^2/ewe and lambs). Lambs suckled until 60 days of age. Nevertheless, since 30 days of age they were also manually fed with alfalfa hay and commercial concentrate (barley, corn and soya). The nutritional composition of the commercial concentrate consumed by all lambs was 18% protein, 2.5% fat, 4% cellulose and 6.5% ash. Following weaning, started at 10 weeks of age, lambs had free access to their ration and water until slaughter at 120 days of age. Feeders were replenished each day to ensure that the lambs had access to feed at all times. Weaning was done by abrupt visual and acoustical separation of the ewe and lamb. At the time of weaning (at approximately 8 am), the experimental lambs were separated from their mothers by moving them to a different barn with pen (1.0 m^2/lamb), located more than 500 m of distance, preventing visual, chemical or acoustic communication with the ewes. Both groups were studied at the age of 8 weeks in baseline conditions with the experimental animals weaned two weeks later. No anthelmintic and cocciodiostatic treatments were administered to lambs during the study. During the observational period, individual live body weights (BW) were recorded using large animal scales following blood sampling. Mean BW was equal to 16.30 ± 0.24 (SD) kg in males and 15.20 ± 0.11 kg in females at baseline conditions; 15.28 ± 0.11 kg in males and 13.94 ± 0.07 kg in females 24 h after weaning, and 16.04 ± 0.18 kg in males and 15.50 ± 0.11 kg in females 2 weeks after weaning.

2.2. Hormone analysis

Blood samples (5 ml) were collected by jugular venepuncture using 20 G needles (BD Vacutainer Systems, Plymouth, UK) into 10 ml collection vacuum glass tubes without anticoagulant (Vacutainer, Beckton Dickinson, Franklin Lakes, NJ, USA). Blood sampling was carried out at 8 h to minimise the effect of circadian rhythm on hormone measurements, 2 weeks before weaning (at baseline conditions with the lambs aged 8 weeks), and at 24 h and 2 weeks after weaning and at similar times in the non-weaned control group. Lambs were restrained manually by the same farmer, and blood sampling process was completed approximately within 1 min for each collection in order to avoid excessive stress and it was taken in quiet conditions by the same veterinarian. Immediately after withdrawal, blood samples were placed on ice until centrifugation at 3000 rpm for 10 min at room temperature (24 °C) and serum was harvested and stored in polystyrene tubes at −20 °C until analysed. Serum total and free iodothyronine concentrations were determined in duplicate with enzyme immunoassay (EIA) kits (SEAC-RADIM, Rome, Italy). Limits of detection were 0.24 nmol/L for T_3, 5.79 nmol/L for T_4, 0.15 pmol/L for fT_3 and 1.3 pmol/L for fT_4. Intra- and inter-assay coefficients of variation (CV) were 7.3% and 11.4% for T_3, 2.3% and 5.7% for T_4, 4.2% and 11.9% for fT_3, 6.6% and 9.6% for fT_4, respectively, as based on measurements in three different samples.

2.3. Statistical analysis

Data are presented as mean ± standard deviation (SD). The percentage differences (Δ%) of BW were also calculated. Statistical analysis was done by one-way analysis of variance. Significant differences between baseline and post-weaning values were established using Bonferroni's multiple comparison test. Further changes due to gender and BW of the lambs were assessed by student's unpaired *t*-test. The relations between total and free iodothyronines, thyroid hormones and BW were evaluated by Pearson's correlation and linear regression. The level of significance was set at $P < 0.05$. All calculations were performed using the PRISM package (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Circulating total and free iodothyronine levels in lambs before and after weaning period are presented in Figure 1 (a−d). Weaning effects related to age were shown for T_3 ($F = 17.30$; $P < 0.0001$), fT_3 ($F = 3.75$; $P < 0.0331$), T_4 ($F = 6.43$; $P < 0.0045$) and fT_4 ($F = 9.73$; $P < 0.0005$) in experimental lambs. Compared to baseline values, serum T_3 concentrations (Figure 1 (a)) of lambs decreased 2 weeks later ($P < 0.001$), and serum fT_3 concentrations (Figure 1 (b)) decreased 24 h after weaning ($P < 0.05$). However, compared to control group, T_3 ($P < 0.001$) concentrations showed lower values 2 weeks after weaning only in both males and females. Positive correlations were

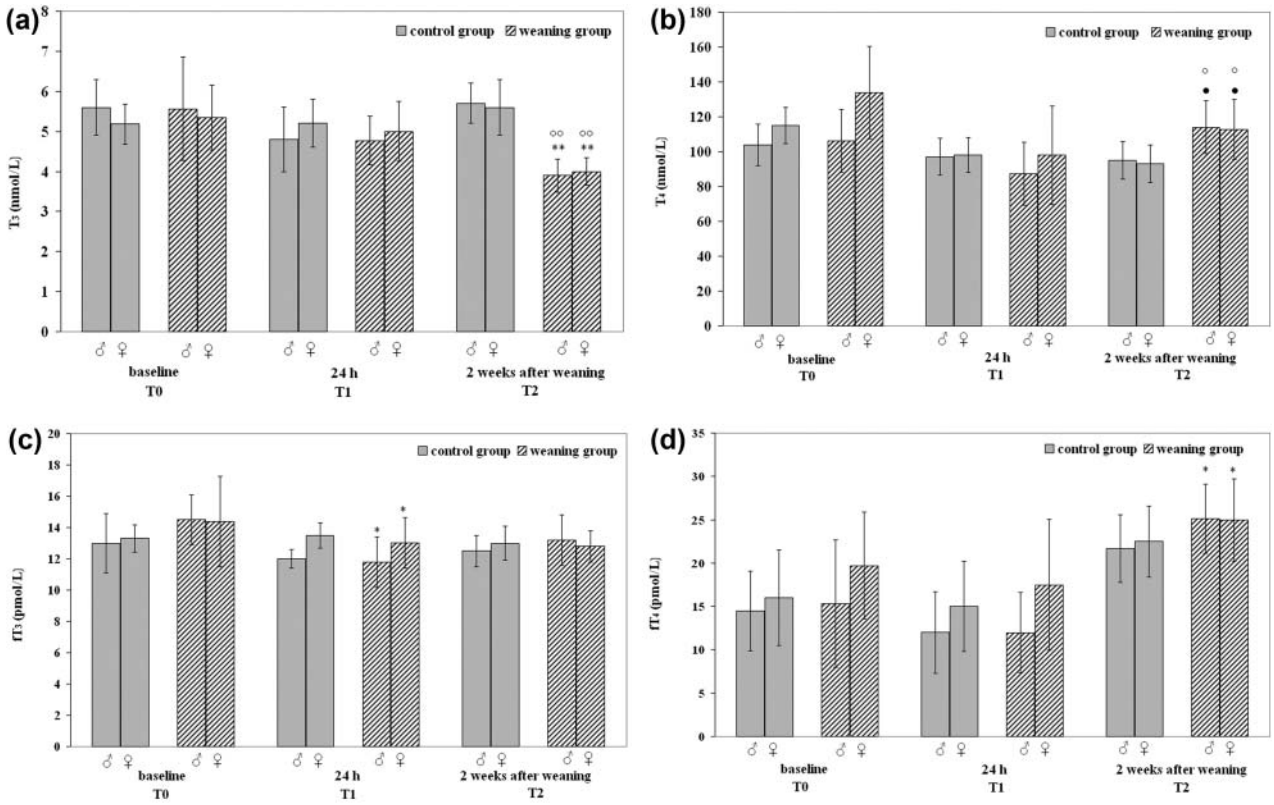

Figure 1. (a)−(d) Circulating total and free iodothyronine concentrations (mean ± SD) of lambs ($n = 17$) before and after weaning period. Asterisks indicate significant ($^{*}P \leq 0.05$; $^{**}P \leq 0.001$) differences in average hormone concentrations versus baseline values. Symbols indicate significant ($^{\bullet}P \leq 0.01$) differences in average hormone concentrations versus 24 h after weaning period and significant ($^{\circ}P \leq 0.01$; $^{\circ\circ}P \leq 0.001$) differences versus control group.

observed between T_3 and fT_3 both at baseline conditions ($r = 0.907$; $P < 0.01$) and 24 h after weaning ($r = 0.668$; $P < 0.01$). Serum T_4 concentrations (Figure 1 (c)) increased 2 weeks later ($P < 0$ .01), compared to 24 h, while serum fT_4 concentrations also (Figure 1 (d)) increased 2 weeks later ($P < 0.05$), compared to baseline values. However, compared to control group, T_4 ($P < 0.01$) concentrations showed higher values 2 weeks after weaning only in both males and females. Positive and significant correlations were observed between T_4 and fT_4 in baseline conditions ($r = 0.518$; $P < 0.05$).

BW (Table 1) in females and males decreased 8% ($P < 0.001$) and 6% ($P < 0.001$), respectively, 24 h after weaning, as compared to baseline values (before 2 weeks). BW was significantly restored in both females (11%; $P < 0.001$) and males (6%; $P < 0.001$) 2 weeks after weaning, as compared to 24 h after weaning.

Table 1. Mean body weight (BW) in female and male lambs before and after 2 weeks of weaning in experimental group.

	BW (± SD) kg	
Time	Females ($n = 10$)	Males ($n = 5$)
Before	15.20 ± 0.11	16.30 ± 0.24
24 h after	13.94 ± 0.07[a]	15.28 ± 0.11[a]
After 2 weeks	15.50 ± 0.15[b]	16.04 ± 0.18[b]

Note: Different superscripts show significant differences two weeks after [a]$P < 0.001$ and 24 h after [b] $P < 0.001$ weaning compared to basal values.

Males and females (Figure 1 (a−d)) showed the typical trend of total and free iodothyronine concentrations observed in the study population as a whole. However, no differences ($P > 0.05$) between males and females in T_3, fT_3, T_4 and fT_4 concentrations were observed between both groups.

Significant positive correlations (Table 2) were observed between BW and T_4 concentrations in both females ($r = 0.692$; $P < 0.05$) and males ($r = 0.856$; $P < 0.01$), between BW and fT_3 concentrations only in males ($r = 0.968$; $P < 0.01$) and between BW and fT_4 concentrations only in females ($r = 0.862$; $P < 0.01$).

Table 2. Pearson's correlation and linear regression between thyroid hormones concentrations and body weight (BW) in female and male lambs in experimental group.

		BW (kg)	
Thyroid hormones		r	P
T_3	♀	−0.460	n.s.
	♂	0.218	n.s.
T_4	♀	0.692	0.05
	♂	0.856	0.01
fT_3	♀	0.207	n.s.
	♂	0.968	0.01
fT_4	♀	0.862	0.01
	♂	0.500	n.s.

n.s., not significant

4. Discussion

Many laboratories have established reliable reference values for thyroid hormone concentrations in blood of healthy animals. In fact, many endogenous and exogenous factors that affect thyroid function may lead to misinterpretation of test results when values for individual subjects are compared with physiological range values. Comparison of our data, related to total and free iodothyronines, with published data for ovine species (T_3: 0.96 − 2.30 nmol/L; T_4: 37.96 − 79.15 nmol/L [Reap et al. 1978]; T_3: 1.50 ± 0.01 nmol/L; T_4: 100.38 ± 5.01 nmol/L; fT_3: 3.99 ± 0.36 pmol/L; fT_4: 24.71 ± 0.78 pmol/L [Anderson et al. 1988]) and growing lambs (T_4: 93.95 ± 57.52 nmol/L; [Kallfelz & Erali 1973]) revealed some discrepancies, although T_4 concentrations were in agreement with data observed in 8-week-old lambs, stabled in paddock and stalls (T_4: 90 − 114 nmol/L [Bowers et al. 1993]) and with data observed in does (T_3: 1.19 − 2.07 nmol/L; T_4: 78.50 − 104.89 nmol/L [Todini 2007]). Nevertheless, slight variation might be ascribed to differences in methods and are the subject of speculation. In addition, some differences may also be explained by nutritional, physiologic, environmental or geographic variables, related to different experimental conditions.

The low concentration of T_3 2 weeks after weaning could be associated with increased use of T_4 and fT_4 concentrations or with changes in the rate of monodeiodination of either T_4 or T_3. Hence, the metabolic effects of total and free iodothyronines could be modulated by their thyroidal or extrathyroidal metabolism, according to physiological requirements, by taking into account also the circannual variation in thyroid hormone deiodinases and related gene expression, recently described in sheep (Foroughi & Dehghani 2013; Sáenz de Miera et al. 2013). This decrease in T_3 would be expected, under situations of undernutrition, such as observed in lambs 24 h after weaning as reported in wild deer (Willard et al., 1998) and Boer goat bucks, aged 6−8 months (Almeida et al. 2002) under food shortage, probably because of the negative energy balance. This was explained as an attempt by the organism to increase its lipid and protein catabolism (Almeida et al. 2002). On the contrary, in adequately fed animals, such as in lambs 2 weeks after weaning, higher concentrations of T_4, but not of T_3, would be expected to increase absorption of intestinal glucose, essential amino acids and lipoproteins.

Data obtained showed that energy deprivation, occurring probably 24 h after weaning, might decrease T_3 and fT_3 concentrations, and confirmed previous data observed in sheep (Ekpe & Christopherson 2000; Abecia et al. 2001; Rae et al. 2002). Conversely, subsequently the nutritive contents of the ration, occurring 2 weeks after weaning, increased only T_4 concentrations. Our data confirmed that animals that have reduced their feed intake probably due to weaning stress showed an earlier and more marked decline in circulating thyroid hormones during the late summer/early autumn, compared to ad libitum fed animals (Rhind et al. 2000).

The gender-dependent differences for total and free iodothyronines were less pronounced and not significant in weaning lambs; these data confirmed that in young animals, there were no sex-dependent differences in blood thyroid hormone concentrations (Celi et al. 2003; Todini 2007).

The interesting finding in this study was the time courses of T_4 and T_3 variations that were different during the weaning period.

Changes in serum fT_3 and fT_4 concentrations generally followed those for T_3 and T_4, respectively, as observed in growing Thoroughbred foals (Fazio et al. 2007). This assumption was confirmed by the existence of significant and positive correlations between both T_3 and fT_3 and T_4 and fT_4 concentrations.

The significant changes of total iodothyronines in weaned lambs confirm data previously observed in foals during the first day after weaning (Fazio et al. 2007), and the presence of co-specifics probably does not reduce psychological stress in this phase. In addition, the period of maternal post-weaning independence can be energetically and physiologically demanding, with energy expenditure to prepare weaned animals for nutritional independence from mothers' milk, as suggested by lower T_3 and higher T_4 concentration 2 weeks after weaning than control group values. Hence, the thyroid responses may, therefore, be the physiological consequence of post-weaning adaptations and may be both the cause and/or the consequence of growth programming, confirming previous results obtained in growing foals (Fazio et al. 2007).

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Disclosure statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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