

Influence of Environmental Temperature on Thyroid Hormone Production in Sheep

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Abstract

The effect of high (28°C) and low (14°C) temperature on thyroid hormone production were studied in five male sheep since the activity of thyroid releasing hormone (TRH) cell in the hypothalamus that influence the thyroid stimulating hormone (TSH) production and release can be affected through the temperature regulatory center. The aim of this study were to measure the iodine (the core of thyroid hormone) needed in the different temperatures, T₄ (thyroxine) production, T₄ and T₃ (triiodotironine) concentration. Two experiments were performed in both condition to investigate the thyroidal ¹²⁵I uptake and to measure T₄ production. The sheep were shorn at the beginning of each adaptation period (22°C). The first treatment was high temperature and the second treatment was low temperature. The experiments were done at least five weeks of each temperature. The mean of rectal temperatures during the warm period were significantly higher than that in the cold period. The feed intake during the warm period tend to decrease significantly, but there was no increase during the cold period. The absolute iodine uptake (AIU) over 24 hours was markedly increased during the second period (7.94±1.37 µg/24 hr) compare to the first period (1.65±0.13 µg/24 hr). It means that cold exposure has a potential to stimulate the thyroid gland in order to uptake more iodide. The mean of T₄ concentration was 51.6±2.1 ng/ml during the warm and 61.2±5.5 ng/ml four weeks after shift to the cold period and did not differ, but four weeks later the concentration was significantly increased (77.4±1.47)(33%). The same pattern occurred to the T₃ concentration that the four weeks after shifting the level of T₃ was not significantly increased from value of 0.39 ng/ml to value of 0.51 ng/ml, eight weeks after shifting the T₃ value (0.65 ng/ml) was significantly rose. This finding provides convincing evidence that the T₄ and T₃ concentration indeed increase during the cold period. The conclusion that the production of thyroid hormones were influenced by temperature. T₄ and T₃ concentration were increase during the cold period. The AIU was absolutely higher during the cold period, that means the amount of iodine needed in high temperature is less than in low temperature.

Key Words: Sheep, Iodine, T₄ (Thyroxine), T₃ (Triiodotironine)

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Abstrak

Pengaruh suhu tinggi (28°C) dan suhu rendah (14°C) terhadap produksi hormone tiroid diselidiki pada 5 ekor domba jantan karena aktivitas sel thyroid releasing hormone (TRH) dalam hipotalamus yang mempengaruhi produksi dan pelepasan thyroid stimulating hormone (TSH) dapat dipengaruhi melalui pusat pengaturan suhu. Tujuan dari penelitian ini adalah untuk mengukur kebutuhan yodium (inti dari hormone tiroid) dalam suhu berbeda, produksi T₄ (tiroksin) dan konsentrasi T₄ dan T₃ (triiodotironin). Dua penelitian dilakukan dalam kedua kondisi suhu untuk menyelidiki penyerapan ¹²⁵I oleh kelenjar tiroid dan untuk mengukur produksi T₄. Domba dicukur pada permulaan setiap periode adaptasi (22°C). Penelitian pertama dilakukan pada suhu tinggi dan kedua pada suhu rendah. Penelitian dilakukan paling sedikit lima minggu untuk setiap kondisi suhu. Rataan suhu rectal selama periode panas sangat nyata lebih tinggi daripada periode dingin. Feed intake selama periode panas cenderung menurun secara nyata, tetapi tidak terjadi peningkatan selama periode dingin. Penyerapan yodium absolute selama 24 jam meningkat dengan cepat selama periode dingin (7.94±1.37 µg/24 jam) dibandingkan dengan periode panas (1.65±0.13 µg/24 jam). Hal ini berarti bahwa suhu rendah mempunyai potensi untuk merangsang kelenjar tiroid untuk menyerap lebih banyak yodium. Rataan konsentrasi T₄ adalah 51.6±2.1 ng/ml selama periode panas dan 61.2±5.5 ng/ml empat minggu setelah masuk periode dingin dan tidak berbeda, tetapi empat minggu berikutnya konsentrasinya meningkat secara nyata (77.4±1.47)(33%). Pola yang sama terjadi pada konsentrasi T₃ bahwa empat minggu setelah pindah ke periode dingin level T₃ tidak meningkat secara nyata dari nilai 0,39 ng/ml ke 0,51 ng/ml, empat minggu berikutnya nilai T₃ meningkat secara nyata (0.65 ng/ml). Penemuan ini memberikan bukti yang menyakinkan bahwa konsentrasi T₄ dan T₃ sungguh meningkat selama periode dingin. Kesimpulan bahwa produksi hormone tiroid dipengaruhi oleh suhu dan konsentrasi T₄ dan T₃ meningkat selama periode dingin. Penyerapan yodium oleh kelenjar tiroid lebih tinggi selama periode dingin yang berarti jumlah yodium yang dibutuhkan selama suhu tinggi adalah lebih sedikit dari pada suhu rendah.

Kata Kunci: Domba, Yodium, T₄ (Tiroksin), T₃ (Triiodotironin)

Introduction

Thyroid hormone is extremely important for fetal and postnatal development due to its effects on metabolism, promote normal growth, development and maturation of the nervous system. Production of this hormone by the thyroid gland is regulated by the

release of thyroid stimulating hormone (TSH) from the anterior pituitary gland. TSH binding to its receptor on the thyroid cell results in an increase in cAMP (second messenger) that lead to the synthesis of thyroglobulin, for which dietary iodine is needed (Fox, 1997).

TSH production and release is under the influence of thyrotropin releasing hormone (TRH) from the hypothalamus. The activity of TRH cell can be affected by the temperature regulatory center that integrates the environmental temperature. The effect of exposure to high environmental temperatures and emotional stress on blood thyroid hormone concentrations have been subject of numerous reviews and articles. An increase in thyroid hormone binding capacity in sheep increased thyroid hormone concentration during the initial phases of exposure to the hot-humid environment, but in the third week of exposure to the same environment it depressed thyroxine level by 23%, while the thyroid hormone level did not change during hot-dry period compared with a cool dry (Guerrini dan Bertchinger, 1983). Lambs receiving bromocriptine during heat stress (43.3-43.8°C) demonstrated significantly higher thyroxine (T4):T3 ratio than that of control (Alshaikh, 1997).

Rearing at cool ambient temperature stimulated metabolic rate and resulted in higher plasma concentrations of thyroid hormones (Symonds dkk, 1995) and exhibits improved thermoregulation compared with lambs at warm ambient temperature

without any detectable differences in brown adipose tissue (BAT) function (Symonds dkk, 1996). These studies, however, have not examined how much iodine, the essential element of thyroid synthesis, should be given to the animal in the different temperatures in order to achieve a certain goal of production.

Since the thyroid hormone secretion increases in cold and decreases in warm environment (Falconer , 1976), it is plausible to assume that thyroid hormone production is higher in cold condition than that in warm condition. On this basis, it is possible to measure the iodine needed at different temperatures.

Materials and Methods

Five adult lambs (66-78 kg body weight) were housed in individual metabolism crates in a controlled-environment room and were trained to stand quietly during experimentation and give free access to feed and water. The drinking water container was filled twice daily with fresh tap water. No attempt was made to maintain the drinking water at a constant temperature throughout the day. The sheep were shorn 2 weeks before starting the experiment.

During experiment, sheep were placed first at a high temperature (28°C) and the second

period were at a low temperature (14°C). The warm period lasted for 54 days and the cold period lasted for 60 days. Feed intake data for cold period was recorded until 41 days. Before each of two periods, the lambs were adapted for 14 days at 22°C. The temperature was recorded continuously by using maximum and minimum mercury-in-glass thermometer at 9.00 and 17.00 every day. Humidity was recorded continuously on a 2% error margin. Temperature and humidity were not allowed to vary by more than 2% during any 24-h period. The length of the daylight was 12 hrs, which was the same as for length of dark period.

The feed used in this experiment was pelleted grass and was offered twice daily *ad libitum*. The chemical contents of feed used based on dry matter are 147.9g/kg ash, 168 g/kg crude protein, 33 g/kg crude fat, 248 g/kg crude fiber and 403 g/kg NFE. Each morning the unconsumed feed of the last 24 hr was removed and weighed.

Design of the study

Two experiments were performed during each environmental temperature period: one was to measure ¹²⁵I uptake by the thyroid and the second experiment was to measure T₄ production by a pulse-dose kinetics. Inserting a

semi permanent PVC cannula ("Rubber", Hilversum) into the right jugular vein (DiStefano dkk, 1982; Roelfsema dkk, 1980) was done one day before kinetics experiment.

During warm period, the ¹²⁵I uptake study was done after 37-46 days and 24-29 days for the cold period. The blood samples for T₄ and T₃ measurements were obtained at 36 days exposure during warm period, while during cold period at 23 and 60 days exposed.

Analytical Procedure

Determination of iodothyronine

To determine the iodothyronine concentration, the plasma (100-500 µl in duplicate) was used for counting the total ¹²⁵I activity. The plasma samples were extracted with ethanol/25% ammonia (197:3 vol/vol) with 0.1 mM propylthiuracil (PTU). The dried extracts were dissolved in 0.1 ml 0.2 M ammonia containing carrier T₄, T₃ and potassium iodide (1 mg/10 ml) and subjected to high-performance liquid chromatography (HPLC) to separate the iodothyronines. The analyses were performed with HPLC using a reverse phase C18 1x0.6 cm column (Mallinckrodt Baker B.V. Deventer, The Netherlands). The column was activated by 3x0.5 ml methanol followed by 3x0.5 ml 0.3 M

ammonium acetate. Then the sample was applied followed by 3x0.5 ml 0.3 M ammonium acetate and there after 3x0.5 ml water. The fractions and column were counted in a γ -counter, and their radioactivity was expressed as a percentage of the daily dose per ml plasma (Schroder-van der elst dan van der Heide, 1990).

Radio immunoassay for measuring T₄ and T₃

The endogenous concentrations of T₄ and T₃ in plasma were measured by means of a specific RIA in samples drawn directly.

Thyroidal iodide uptake

Unanesthetized sheep remained in the cage which the head positioned outside, in order to make it as easy as possible to place the scintillation probe [type 42A, NaI unalterably 23 mm, 1 mm thick, connected to mini-assay type 6-20 (mini instruments Lts, Essex, UK)] as close as possible to the thyroid region.

To measure the thyroidal iodide uptake in the rams, a 5 ml bolus of saline containing 10 μ Ci carrier free Na ¹²⁵I (Amersham, Aylesbury, UK) was injected into the right jugular vein. The radioactivity taken up by the thyroid was measured at several time points until maximum was

reached and decline after maximum and calculated as a percentage of the injected dose. The percentage dose of iodide was fitted to the equation $y_t = y_m (t/t_m)^c e^{c(1-t/t_m)}$, by using non-linear regression program, where y_t present the percentage of dose taken up by the thyroid, y_m and t_m are easily recognizable parameter. The parameters c is the rate of increase before maximum and the decrease after maximum. The c determines the steepness of the curve.

Absolute iodide uptake

During 24-h after injection of the [¹²⁵I]T₄ the urine was collected. Radioactivity measured (thyroid, urine) was expressed as percentage of the injected dose. The assumption was that during a steady state situation the specific activity of iodine (ratio of ¹²⁵I) in urine is the same as that in the plasma from which it originated (Versloot dkk, 1997). The absolute iodide uptake (AIU) and the plasma inorganic iodide (PII) can be calculated after determination of the specific activity in urine and the iodine concentration in urine.

$$AIU = \frac{\% \text{ dose after 24 hrs}}{\% \text{ dose urine} / \mu\text{M iodide urine}} (= \mu\text{M} / 24 \text{ hr})$$

$$PII = \frac{\% \text{ dose} / \text{ml plasma}}{\% \text{ dose urine} / \mu\text{M iodide urine}} (= \mu\text{M} / \text{ml})$$

Determination of iodide in urine

The iodine in urine was measured according to the method of Human and Animal Physiology (Human and Animal Physiology, 1992). Some of the volumes mentioned were changed to be able to measure a low iodine concentration. The reaction of iodide catalyses is the following:

Data Analyses

Cold and warm period are concerned as a treatment and sheep are the experimental units. Data were analyzed by method of paired t-test by using SPSS Program (SPSS, 1998) to compare the average of the differences of the individual animals. The model for each variable is:

$$d_i = \alpha + e_i$$

where:

d_i is the difference of the same variable at temperature 28°C and 14°C for sheep i .

α is the average difference.

e_i is the random error for the difference of d .

Results and Discussion

Room and Rectal Temperature

The relative humidity during both periods was lower than 65%. Therefore the room condition was considered as a hot-dry environment during the warm period and as a cold-dry environment during the cold

period (Guerrini dan Bertchinger, 1983).

The a.m. and p.m. rectal temperatures were not different ($P>0.05$) in both period indicating that the temperature was relatively constant over the day. The a.m. and p.m. mean rectal temperatures over the day agreed closely with the means of several sheep breed reported earlier (Guerrini dan Bertchinger, 1983). During the warm period the rectal temperature was significantly higher ($P<0.01$) than during the cold period for both the morning and the afternoon. It may be assumed that this is caused by the higher environmental temperature, although there was no direct indication for this.

Body weight of total sheep was increased ($P<0.01$) but there was no significant difference ($P>0.05$) in body weight gain during the warm and cold period. The average feed intake in term of gram/day during the cold period was slightly higher. However, expressed per MW feed intake was higher during the warm period. The regression analysis of feed intake based on g/day/MW in both condition showed the following result: $Y_w=134-1.16X$ and $Y_c=98-0.14X$, indicating that during warm period feed intake will decrease larger and did not increase during the cold period and

this finding was different from reported earlier. The faster decrease could be affected by the high temperature because adaptation to a higher heat loss after shearing in warm period. A higher mean feed intake was found during cold exposure for sheep (Guerrini dan Bertchinger, 1983).

T₄, T₃, T₄:T₃ Ratio

The T₄ concentration was 51±2.1 ng/ml at the end of the warm period. Four weeks after shifting to the cold period, the T₄ concentration was not significantly increased to a value of 61.2±5.5 ng/ml. Eight weeks after shifting the concentration was significantly increased to a value of 77.4±1.5 ng/ml (P<0.01). The augmentation of the T₄ concentration eight weeks after shifting to low temperature was about 33%. The result of the present study provides convincing evidence that T₄ increases in the course of cold condition as long as four weeks gradually. This indicates that it took more than four weeks of exposure to cold temperature for the thyroid to increase the T₄ concentration significantly. Secretion of thyroid hormone increases in cold and decreases in warm condition (Fregly, 1989) and rearing at cool ambient temperature stimulates the hypothalamic-thyroid axis and increases the hypothalamic to release of TRH and resulted in

higher plasma thyroid hormone concentration (Symonds dkk, 1996; Macari, 1984).

The same pattern occurred to T₃ concentration. Four weeks after shifting to cold period the level of T₃ was not significantly increased from a value of 0.39±0.03 ng/ml to 0.51±0.07 ng/ml. Significantly increase of T₃ level (0.65±0.02 ng/ml) was found eight weeks later to low temperature. The increase in T₃ concentration during cold period presumably improved thermoregulation and control of body temperature. Cold placed lambs were able to maintain higher plasma thyroid hormone levels and showed improved thermoregulation compared with warm placed lambs' (Falconer, 1976).

The T₄:T₃ ratio was not changed neither four weeks nor eight weeks after moving to the cold period. These phenomena suggesting that during this length of cold period, T₃ production remain largely depend on the production from the thyroid gland.

Iodide Concentration and ¹²⁵I Uptake

There was no difference in the mean of iodide excretion in the cold period (4.28±0.43 μM) compared to the warm period (4.63±0.51 μM). Table 1 shows specific activity of the urine.

Table 1. Urinary Specific Activity, Absolute Iodine Uptake by Thyroid and Plasma Inorganic Iodide At 24 Hr.

Item	Warm	Cold
Urine:		
1. % dose in urine/24 hr	30.59□□□□□□	16.8±3.05**
2. I-content/24 hr (μM)	4.28±0.43	4.63±0.51ns
3. Urinary specific activity (% dose/μM I in urine)	7.14±0.25	3.63±0.44**
% dose in thyroid/24 hr	11.69±0.77	26.71±1.62**
AIU, μg/24 hr	1.65±0.13	7.94±1.27**
% dose I/L plasma	0.63±0.18	0.091±0.036*
PII, μM/L plasma	0.16±0.023	0.023±0.012*

Values are means □ *SE. ns=not significant; *:P<0.05,significant, **:P<0.01:highly significant*

Another method of measuring thyroid function is to measure how much iodine is taken up by the thyroid gland from a tracer dose of radioactive iodine or excreted in the urine over the next few hours. The result show that the absolute iodide uptake (AIU) over 24 hours was markedly augmented caused by lower temperature nearly fivefold. That means that cold exposure has a potential to stimulate the thyroid gland in order to uptake more of iodide. It could be argued the TSH level released from anterior pituitary by TRH is augmented, as a consequence the amount of iodine taken up is elevated to produce more thyroid hormones. This may have contributed to the utilization of colloid within the thyroid gland in course of cold exposure. Cold temperature activates the hypothalamic-pituitary-thyroid axis and

increases the hypothalamic release of TRH in rats that had been acclimatized to 30°C (Macari, 1984). Thyroidal iodide uptake as well as synthesis and release of thyroid hormone are under the control of TSH (Rapoport dan Spaulding, 1996). Further evidence that urinary activity was reduced as well as the radio iodide activity in plasma, which in turn the value of PII was significantly lower.

The radioactive iodine is absorb into the blood and concentrate in thyroid gland. The remaining radioactive iodine is excreted in the urine. The result in the present study demonstrated that the amount of radioactive excreted in the urine of sheep exposed to cold was not change compare to warm-exposed sheep. It might be there are T₄ partitioning into the urine without filtering by

the kidney. Concentration of radioactive T_4 and T_3 liver and kidney were reduced significantly in cold-exposed animals, suggesting an increased metabolism of the hormone by tissues (Wrutniak dan Cabello, 1989).

Conclusion

1. Feed intake did not increase during cold period but metabolism and production of thyroid hormone were influenced by ambient temperature.
2. The absolute iodide uptake during the cold period was absolutely higher ($7.94\mu\text{g}/24$ hr) compared to in course of warm period ($1.65\mu\text{g}/24$ hr). That means the amount of iodine needed in warm ambient temperature is less than in cold ambient temperature.
3. Exposed to the hot-dry environment was stressful and as consequence the T_4 and T_3 concentration are lower.

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