Relationship between ghrelin hormone and some thyroid hormones during seasons in Turkish Awassi Rams in Iraq

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Abstract

The present study was designed to investigate the role of seasonality changes on some physiological aspect of Ghrelin and some parameters related to thyroid function in adult rams. Ten adult Turkish Awassi rams (1.2-1.3 years in age) and bodies weights ranged (45-58kg) were used in this study, which lasted from the beginning of January 2016 to the end of October 2016. Body weights of each individual ram were taken. Fasting blood samples were collected every ten days along the experiment (considered as fasting state), and then blood samples was collected one hour after feeding for the same ten adult rams (considered feeding state). The blood was collected from the jugular vein, and divided into two parts, which was centrifuged at (3000 rpm for 20 minutes) to separate the serum which was required for estimation (Ghrelin, TSH, T3 and T4). The environmental temperature was measured by using special thermometer every day along experimental period. The results of this study revealed a significant increase ($p \leq 0.01$) in ghrelin hormone concentrations in Turkish awassi rams serum at fasting state as compared to feeding during spring months. Serum TSH, T3 and T4 levels showed a significant increase at the feeding state as compared to fasting and represent the lowest level during winter months of the year. In conclusion, there is a negative relationship between the ghrelin hormone and thyroid hormones under the effect of seasonality in the Turkish awassi rams. In conclusion, the results of this study was the first in Iraq, give an important document that ghrelin hormone correlates negatively with thyroid hormones during different seasons.

Key word: Ghrelin, TSH, seasonality, Turkish awassi rams

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العلاقة ما بين هرمون الكرلين ومستويات بعض هرمونات الدرقية خلال فصول السنة لدى الكباش العواسية التركية في العراق

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الخلاصة

استهدفت هذه الدراسة معرفة دور التغيرات الموسمية على بعض الجوانب الفسلجية لهرمون الكريلين المتعلقة بوظائف الدرقية في الأغنام العواسي التركية. تم استخدم عشرة كباش بالغة بعمر (1.2-1.3 سنة) وبوزن يتراوح ما بين (45-58 كغم) بدات التجربة من بداية شهر كانون الثاني 2016 واستمرت لغاية نهاية شهر تشرين الأول 2016. تم تصويم الحيوانات عن الأكل فقط لمدة 24 ساعة (اعتبرت مرحلة الصوم) وذلك لتهيئها لعملية سحب الدم التي أجريت كل عشرة أيام وعلى طول فترة التجربة وبعد ذلك تم تقديم العلف للكباش المصومة (اعتبرت مرحله التغذية) وأجريت إعادة سحب الدم بعد ساعة من تعليف الكباش وتم سحب الدم (10 مل) عن طريق الوريد الوداجي استخدم عزل المصل لإجراء الفحوصات الهرمونية والتي تشمل (قياس مستويات كل من الكرلين و،TSH T3 T4). تم قياس أوزان

الكباش العواسي كل عشرة أيام وعلى طول فترة التجربة ولكل كبش على حدا. أظهرت النتائج تفوقا معنويا في كل من مستوى هرمون الكرلين في مصل الدم للكباش العواسية خلال فترة الصيام مقارنة بفترة الغذاء. كذلك أوضحت النتائج تفوق معنويا في تراكيز كل من TSH, T3, T4 في المصل خلال فترة الغذاء مقارنة بالصيام حيث سجلت اوطىء المعدلات خلال اشهر فصل الشتاء. ان مقياس الترابط ما بين المعايير المدروسة في هذه الدراسة قد أوضحت وجود ترابط معنوي سلبي ما بين هرمون الكرلين وتركيز هرمونات الدرقية. مما يؤدي إلى استنتاج علاقة عكسية ما بين الكرلين وهرمونات الدرقية في الكباش العواسية التركية خلال مواسم السنة.

الكلمات المفتاحية: الكرلين، هرمونات الدرقية، الموسمية، الأغنام العواسي التركي.

Introduction

Ghrelin is octanoylated peptide containing a 28 amino acid which is chiefly produced by the stomach, is the natural ligand of the type 1a growth hormone secretagogue receptor (GHS-R1a) as reported by (1). The gastric ghrelin hormone originates from 117 amino acid, and its precursor preproghrelin encoded by the Ghrelin gene (GHRL) found on chromosome 3 (3p25-26) as mentioned by (2). It is well known that ghrelin has been found in several peripheral tissues, such as the gastrointestinal tract, adrenal gland, thyroid, breast, ovary, placenta, fallopian tube, testis, prostate, liver, gallbladder, fat tissue, human lymphocytes, spleen, kidney, lung, skeletal muscle, myocardium, vein, and skin (3). Ghrelin is a participant in regulating the complex process at which both energy has to be adjusted. Energy input by adjusting hunger signals and energy output by adjusting the proportion of energy going to ATP production, glycogen storage, fat storage and short term heat loss. Natural and synthetic Ghrelin system(GHS) stimulate GH release from somatotroph cells *in vitro*, by depolarizing the somatotroph membrane and by increasing the amount of GH secreted per cell. At the hypothalamic level, ghrelin and GHS act via mediation of growth hormone-releasing hormone (GHRH)-secreting neurons in which showed by signal that passive immunization against GHRH, as well as pretreatment with GHRH antagonists, reduces their stimulatory effect on GH secretion (4). The GH-releasing effect of Ghrelin system GHS is marked by age-related variations, which increasing at puberty, reaching a plateau in adulthood, and decreasing during aging. The mechanisms were differ by age. The enhanced GH-releasing effect of GHS at puberty, is due to the progressive influence of increased serum estrogen levels, which increase GHS-R expression (5). In hyperthyroid patients, in which ghrelin levels are also suppressed, according to the degree of thyrotoxicosis and to glucose levels (6), through thyroid hormones which act on decreases ghrelin gene expression in mouse models (7). Concerning hunger, ghrelin levels do not seem to correlate with hunger in the hyperthyroid state, in this case patients were consumed more food than euthyroid patients even at low ghrelin levels (7). Moreover the hyper-insulinemic hyperglycemic state or excess of thyroid hormone (hyperthyroidism) itself is leading to decline the ghrelin level in turn, affecting on carbohydrate metabolism that shift away from energy storage and towards energy use which may have a role in the hyper catabolic state. Ghrelin has an effect on the Hypothalamus–Pituitary–Thyroid (HPT) axis in humans. Thus, the early free thyroxin (fT4) increase was possibly induced by direct ghrelin action on the thyroid gland wherever ghrelin receptors have been well-known. Caminos (8), reported that ghrelin suppressed the thyroid stimulating hormone (TSH) by inhibition at hypothalamic level or through feedback inhibition through fT4, or both, the circulating levels of leptin and ghrelin was increased and associated with activated HPA axis (increases in serum cortisol) and elevated levels of serum thyroid hormones (9), therefore, this experiment was designed tp study the concentration of ghrelin in Awassi rams and their relation to thyroid activity during different seasons of the year.

Materials and Methods

- **Experimental animals**: This study was conducted in Ruminant Researches Station at Abu–Ghraib/ Department of Animal Resource Researches/ Office of Agricultural Researches/ Ministry of Agriculture. It was carried out from 7th January 2016 up to 31st October 2016, to estimate of the total serum ghrelin level, explore the relationship between ghrelin hormone and some parameters related to thyroid gland and study the up regulation of ghrelin receptors type 1 in blood. Ten Turkish Awassi rams. They were of 1.2-1.3 years age and 45-58 Kg body weight were used in this study. All rams were healthy and free of diseases. The drinking water is available to the animals constantly as well as mineral salts templates for the duration of the experiment. All rams were starved every 10 days' intervals throughout the study for 24 hours from the concentrate diet and roughage, while the drinking water was available for the animals constantly, in order to prepare all those animals for body weight and blood collection. Fasting blood samples were collected every ten days along the experiment (considered as fasting state), and then blood samples was collected one hour after feeding for the same ten adult rams (considered feeding state) from the jugular vein after sterilization the site of injection by using disposable sterilized blood collection tubes free from the anticoagulant substance (Vacationer geltubes). Then serum was isolated by centrifugation at (3000 rpm) for 20 minutes. Serum were obtained to estimate the ghrelin hormone, thyroid stimulating hormone TSH, Triiodothyronine (T3), thyroxine hormone (T4).
- **Determination of Ghrelin hormone assay:**

* **Principle:** The coated well immunoenzymatic assay for the quantitative measurement of GHRL utilizes a multiclonal anti-GHRL antibodyand an GHRL-HRP conjugate (BIOLABO/FRANCE). The assay sample and buffer are incubated together with GHRL-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the GHRL concentration since GHRL from samples and GHRL-HRP conjugate compete for the anti-GHRL antibody binding site. Since the number of sites is limited, as more sites are occupied by GHRL from the sample, fewer sites are left to bind GHRL-HRP conjugate. Standards of known GHRL concentrations are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density) to the concentration of GHRL. The GHRL concentration in each sample is interpolated from this standard curve.

- **Determination of Thyroid stimulating hormone TSH(ng/ml)**

* **Principle**: The immunoradiometric assay of thyroid-stimulating hormone (TSH) is a "sandwich" type assay. Mouse monoclonal antibodies directed against two different epitopes of TSH and hence not competing.

- **Determination of Triiodothyronine hormone T3 (ng/ml).**

- **Principle:** This ELISA test is based on the use of specific monoclonal antibody against a distinct antigenic determinant on the intact T3 molecule. Anti-T3 monoclonal antibody with a certain concentration is used for coating the wells (10).

- **Determination of Thyroxine hormone T4 (ng/ml).**

- **Principle**: This ELISA test is based on the use of specific monoclonal antibody against a distinct antigenic determinant on the intact T4 molecule. Anti-T4 monoclonal antibody with a certain concentration is used for coating the wells (11).

- **Statistical analysis:** The Statistical Analysis System- SAS (2012) program was used to effect of difference factors (season and stage) in study parameters. Duncan's (1955) test was used to significant compare between means in this study.

Results

- **Serum Ghrelin concentration (pg/ml):** The effect of season on serum ghrelin concentration in Turkish awassi rams during fasting and feeding status is illustrated in table (1). Although, ghrelin concentration shows at non-significant (p≥0.05) increase during fasting compared with feeding state during all seasons, this level is highly significant (p≤0.01) during Spring months at fasting state, in comparison with feeding state.

Table (1) Effect of seasonality changes and nutritional status on serum Ghrelin concentration (pg/ml) in Turkish Awassi rams

Season	**Nutritional Status**		Level of sig.
	Fasting	Feeding	
Winter	3.06 ± 0.57 A a	2.14 ± 0.28 A a	NS
Spring	2.81 ± 0.23 A a	2.12 ± 0.07 B a	**
Summer	2.32 ± 0.17 A a	2.03 ± 0.06 A a	NS
Autumn	2.25 ± 0.08 A a	2.02 ± 0.11 A a	NS
Level of sig.	NS	NS	----

Values represent mean± SE, n=10, * (P≤0.05), ** (P≤0.01), NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

- **Serum thyroid stimulating hormone (TSH) concentration:** Concerning serum TSH variation of different nutritional states and seasons, table (2) shows a high significant (p≤0.01) increase in TSH concentration in rams serum at feeding state in comparison with fasting state at spring months, while, a non-significant (p≥0.05) increase during other months. At the meantime, TSH concentration decreased significant (p≤0.05) in rams serum during Winter months at both fasting and feeding states as compared to other seasons. Months at both fasting and feeding states as compared to other seasons.

Table (2) Effect of seasonality changes and Nutritional state on serum thyroid stimulating hormones (TSH) concentration (ng/ml) in Turkish Awassi rams

Season	Stage		Level of sig.
	Fasting	Feeding	
Winter	6.04 ± 0.44 A b	6.94 ± 0.37 A c	NS
Spring	7.81 ± 0.14 B a	8.60 ± 0.21 A a	**
Summer	7.98 ± 0.19 A a	8.31 ± 0.20 A a	NS
Autumn	7.58 ± 0.17 A a	7.61 ± 0.27 A b	NS
Level of sig.	**	**	----

Values represent mean ±SE, n=10, * (P≤0.05), ** (P≤0.01), NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

- **Serum Triiodothyronine (T3) concentration (ng/ml):** Table (3) represents the values of serum T3 concentration during different seasons of the year in turkish Awassi rams. It's clear that T3 level is significantly ($p \leq 0.01$) higher at feeding state during Spring, Summer and Autumn seasons as compared to fasting state. Moreover, T3 showed higher significant ($p \leq 0.01$) increase in T3 rams serum at Autumn feeding state, as compared with Winter, Spring, and Summer within same state. The table also reveal a significant ($p \leq 0.01$) decrease in T3 concentration during Winter season at feeding state only In comparison to other seasons.

Table (3) Effect of seasonality changes and nutritional state on serum triiodothyronine hormones (T3) concentration(ng/ml) in Turkish Awassi rams

Season	Nutritional status		Level of sig.
	Fasting	Feeding	
Winter	17.26 ± 0.23 A a	17.39 ± 0.19 A c	NS
Spring	17.18 ± 0.16 B a	17.78 ± 0.13 A b	**
Summer	17.42 ± 0.13 B a	17.95 ± 0.10 A ab	**
Autumn	17.46 ± 0.12 B a	18.18 ± 0.24 A a	**
Level of sig.	NS	**	----

Values represent mean ±SE, n=10, * (P≤0.05), ** (P≤0.01), NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

- **Serum thyroxine hormone (T4) concentration (ng/ml):** The effect of season on serum T4 concentration is represented in table (4). This table shows that T4 is significantly ($p \leq 0.05$) higher at feeding state during Winter and Spring as compared to fasting state. At the meantime, difference become highly significant ($p \leq 0.01$) in rams serum during Autumn months. On the other hand, T4 concentration is highly decreased ($p \leq 0.01$) in rams serum during Winter months as compared with other seasons of the year in both fasting and feeding states.

Table (4) Effect of seasonality changes and feeding state on serum thyroxine hormones (T4) concentration(ng/ml) in Turkish Awassi rams

Season	Nutritional status		Level of sig.
	Fasting	Feeding	
Winter	6.16 ± 0.75 B b	8.83 ± 0.79 A b	*
Spring	11.95± 0.39 B a	13.28 ± 0.40 A a	*
Summer	11.00 ± 0.57 A a	12.29 ± 0.42 A a	NS
Autumn	11.44 ± 0.51 B a	13.32 ± 0.26 A a	**
Level of sig.	**	**	----

Values represent mean ±SE, n=10, *(P≤0.05), **(P≤0.01), NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

Discussion

Our results illustrated that, there is a negative significant correlation between ghrelin hormone and the T3 concentration in ram's serum i.e. increasing ghrelin is associated with decreasing thyroid hormone concentration and vice versa. Previous studies have reported decreased levels of ghrelin in hyperthyroidism (12), and increased levels in hypothyroid patients (13, 14). The acute exogenous TSH administration has a suppressive effect on ghrelin secretion independent from changes in thyroid status (7). This documented a previous study which reported that, thyroid hormones which act on decreases ghrelin gene expression in mouse models (15). And also reported in our study. Some researchers reported that ghrelin suppressed the thyroid stimulating hormone (TSH) by inhibition at hypothalamic level or through feedback inhibition through free thyroxin fT4, or both (8). Hypothyroidism also up-regulated the expression of GHS-R1 in the anterior pituitary, whereas T4 replacement partially suppressed the expression of GHS-R1(9). Thyroid releasing hormone (TRH) stimulate the pituitary to produce thyroid-stimulating hormone (TSH). The TSH, in turn, stimulate the thyroid to produce thyroid hormones (T3 and T4) until levels in the blood return to normal (16). However, thyroid hormones are crucial hormones that primarily regulate the metabolism of the entire body cells. In the present study, there was elevation in the concentration of the active form (T3) at feeding state At the feeding state the TSH concentration, expressed a significant elevation at Summer and Spring as compared with other two seasons, in which at the same nutritional state and seasons the concentration of ghrelin hormone was decreased and this confirm the negative relationship between ghrelin and thyroid hormones. Excess of thyroid hormone (hyperthyroidism) itself is leading to decline the ghrelin level in turn, affecting on carbohydrate metabolism that shift away from energy storage towards energy use which may have a role in the hyper catabolic state. Ghrelin levels increase back into the normal range when hyperthyroidism is treated(17) whether, this represents a direct effect of thyroid hormones on ghrelin secretion or clearance is not clear. The present study reveals a positive correlation between rams body weight and environmental temperature with TSH and T4 concentrations. Moreover, the environmental temperature correlated negatively with ghrelin concentration which could be attributed in the compensatory response for energy metabolism during cold weather. In this case, the circulating levels of lepton and ghrelin was increased and associated with activated HPA axis (increases in serum cortisol) and elevated levels of serum thyroid hormones (9). The increasing or decreasing environmental temperature may act as a stress factor, that resulted in activation of HPA axis, which leading to elevate of cortisol, that has effect on the level of ghrelin hormone. The environmental temperature has a predominant effect on the thyroid gland activity of the White Goats in different physiological periods (18, 19). From these results, it was concluded a negative correlation with thyroid hormones during different seasons of the year.

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