

Seasonal Changes of Serum Leptin, Triiodothyronine, and Thyroxine Levels in Arabian Mares^[1]

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Summary

The objective of study was to determine seasonal changes of leptin, triiodothyronine, and thyroxine and to determine whether an effect of thyroid hormones on leptin levels in Arabian mare. Blood samples were collected via jugular venipuncture from 16 Arabian mares during breeding and non-breeding season. Hormone concentrations were measured by radioimmunoassay. Mean concentrations of leptin were greater in breeding season (2.84 ± 0.19 ng/mL) than non-breeding season (1.91 ± 0.16 ng/mL). Mean concentrations of triiodothyronine were greater in non-breeding season (221 ± 31.61 ng/dL) than breeding season (105 ± 5.91 ng/dL). No differences in thyroxine levels were observed between breeding (30.30 ± 1.83 nmol/L) and non-breeding season (36.34 ± 4.28 nmol/L). A significant correlation between leptin and triiodothyronine existed for breeding season ($r = 0.860$, $P < 0.01$) but not for non-breeding season ($r = 0.356$, $P > 0.05$). In non-breeding season, there was a significant correlation between triiodothyronine and thyroxine ($r = 0.791$, $P < 0.01$). These seasonal differences and relationships between leptin, triiodothyronine, and thyroxine suggest that secretion of leptin may be affected by thyroid function in Arabian mares.

Keywords: Arabian mare, Leptin, Triiodothyronine, Thyroxine, Season

Arap Kısraklarında Serum Leptin, Triiodotronin ve Tiroksin Seviyelerinin Mevsimsel Değişimleri

Özet

Bu çalışmanın amacı Arap atlarında serum leptin, triiodotronin ve tiroksin seviyelerinin mevsimsel olarak değişimleri ile troid hormonlarının leptin seviyeleri üzerine etkisinin olup olmadığını belirlemektir. Kan örnekleri üreme döneminde ve üremenin olmadığı dönemde 16 Arap kısrağından vena jugularis yolu ile toplandı. Hormon seviyeleri radioimmunoassay yolu ile ölçüldü. Ortalama leptin seviyeleri üreme döneminde (2.84 ± 0.19 ng/mL) üremenin olmadığı dönemden (1.91 ± 0.16 ng/mL) daha yüksekti. Triiodotronin seviyeleri üremenin olmadığı dönemde (221 ± 31.61 ng/dL) üreme döneminden (105 ± 5.91 ng/dL) daha yüksekti. Üreme döneminde (30.30 ± 1.83 nmol/L) ve üremenin olmadığı dönemde (36.34 ± 4.28 nmol/L) tiroksin seviyelerinde fark gözlemlenmedi. Üreme döneminde leptin ile triiodotronin arasında ilişki olmasına rağmen ($r = 0.860$, $P < 0.01$) üremenin olmadığı dönemde böyle bir ilişki bulunamamıştır ($r = 0.356$, $P > 0.05$). Üremenin olmadığı dönemde triiodotronin ve tiroksin arasında anlamlı bir ilişki vardı ($r = 0.791$, $P < 0.01$). Leptin, triiodotronin ve tiroksin hormonlarının mevsimsel değişimleri ve aralarındaki ilişki, Arap atlarında leptin salınımının tiroid fonksiyonu tarafından etkilendiğini göstermektedir.

Anahtar sözcükler: Arap kısrağı, Leptin, Triiodotronin, Tiroksin, Mevsim



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INTRODUCTION

Leptin, a 16 kDa protein hormone, is mainly synthesized and secreted from adipocytes, is involved in regulating many important physiological phenomena such as feed intake, reproduction, and thermogenesis^{1,2}. Leptin is thought to be the hormonal signal to the brain for the nutritional and energy status of the body³. Gentry⁴ showed that body condition scores (BCS) based on the system described by Henneke et al.⁵, ultrasound measurements of fat thickness, and circulating concentrations of leptin were highly correlated in mares. Buff et al.⁶ identified and localized leptin and leptin receptor in the horse. Leptin receptor is expressed in many tissues of the horse including lungs, liver, gonads, brain, and subcutaneous adipose tissue, and leptin blood concentration reflects a significant influence of body fat⁶.

Seasonal variations in leptin secretion have been reported in horse. Leptin tend to decrease during from autumn to winter. Conversely, secretion of leptin is highest in the summer period. However, seasonal or photoperiodic cues such as melatonin, food availability, temperature do not seem to be directly affected on leptin levels in mares^{7,8,9}. The studies have shown that melatonin treatment does not alter concentrations of leptin^{8,10}, suggesting evidence that seasonal changes in concentrations of leptin may be partially regulated by photoperiodic or seasonal signals in horses. Therefore, other hormonal systems or factors have been considered to affect plasma leptin concentrations. Growth hormone, insulin, and triiodothyronine (T₃) may affect in alterations of leptin levels in the horse^{11,12}.

Thyroid hormones [T₃ and its precursor thyroxine (T₄)] play an important role in development and physiology of vertebrate organisms, and altered T₃/T₄ levels manifest themselves in pleiotropic defects such as in energy metabolism, thermogenesis, body growth, neuronal development and in the cardiovascular system^{13,14}. Both thyroid function and leptin are a key player in metabolic homeostasis and feed intake. A relationship has been reported to exist between leptin and thyroid function¹⁵. Production of leptin is directly stimulated by thyrotropin in horses treated with propylthiouracil¹⁶. Furthermore, treatment of T₃ and T₄ caused a reduction circulating concentrations of leptin in human¹⁷ and rat¹⁸.

The purpose of current study was to characterize seasonal changes of leptin, T₃, and T₄ secretion in Arabian mares and to determine if any correlation existed between leptin and thyroid hormones.

MATERIAL and METHODS

Animals

A group of 16 Arabian mares, ranged from 4 to 8 year of age, were used during breeding season (n=8, June 2006) and non-breeding season (n=8, December 2006). The horses were housed on a 15:9 and 9:15 light/dark cycle during breeding and non-breeding season, respectively. The horses were fed with the standard adult horse diet and housed in the stable and kept separately according to gender at Anatolia Agricultural Experiment Station/Eskisehir (General Directorate of Agricultural Enterprises-TIGEM) in Turkey. The station is 890 meters above sea level with a longitude of 30:32 E and a latitude of 39:46 N in northern hemisphere. Mares were weighed and their BCS was performed by using a 1 to 9 scale, according to Henneke et al.⁵. All mares were healthy and treatments for parasite were performed periodically. The experiment was conducted under natural photoperiod in this station. The study protocol was approved by the Ethical Committee of the Ankara University (B.30.2.ANK.0.06.00.01/1385).

Experimental procedures

Peripheral blood samples (10 ml) were collected via jugular venipuncture from each mare into vacuum collection tubes (Vacuette, Greiner Labortechnik) in early morning (at 07:00 am) during breeding and non-breeding seasons. Blood samples were centrifuged at 1500 g for 10 min. Sera were stored at -70°C until endocrine analysis.

Endocrine analysis

Leptin assays were performed using a commercial kit (Multispecies Leptin RIA Kit, Linco Research Incorporated) that has been validated¹⁶ for measuring leptin in equine blood. The intra-assay coefficient variation (CV) was <9% and the sensitivity was 1 ng/mL when using a 100 µL sample size. Triiodothyronine and T₄ levels were measured by radioimmunoassay (RIA) using commercial kits (Triiodothyronine RIA and Thyroxine RIA,

studies. There are two reasons for these conflicting results: a) Studies were conducted different animal, age, weight, sex, and different diet and measurements of leptin. b) Many different parts such as thyroid, adrenal and adipose tissue function and growth hormone related to regulation of leptin secretion are existed. On the other hand, it is likely that there is an inverse relationship between leptin and the thyroid gland ¹⁹.

Rousseau et al.²⁰ reported that hypothalamic response to leptin was regulated by photoperiod in hamster. Photoperiodic or seasonal changes of plasma leptin levels decrease during winter or short day period compared to summer or long day period in Brandt's vole ²¹, gerbil ²², reindeer ²³, and horse ⁷⁻¹⁰. However, no photoperiodic or seasonal effect on leptin levels was found in collared lemming ²⁴ and dairy cow ²⁵. Buff et al.⁹ and Fitzgerald and McManus ¹⁰ reported concentrations of leptin were greater in summer than winter without changes in BW in mares. Gentry et al.⁷ reported concentrations of leptin were lower in mid-winter than early-fall and reported that there was a wide variation in concentrations of leptin in mares with high BCS, indicating that other factors may determine leptin levels in horse. In the present study, we found that concentrations of leptin were greater in summer (2.84 ± 0.19 ng/mL) than winter (1.91 ± 0.16 ng/mL) and not to be affected by BW and BCS. Besides, there was no correlation between leptin and BW or BCS in both breeding and non-breeding season ($P > 0.05$). These findings suggest that seasonal changes of leptin secretion in Arabian mares may be regulated by photoperiodic effect. But, other hormonal factors may also play a role in this regulation.

It has been reported that a seasonal cyclicality in thyroid hormones was found in horse ^{9,26,27}. Buff et al.⁹ observed that plasma concentrations of T₄ in mares were greater in non-breeding season than breeding season (20.3 ± 0.4 and 18.2 ± 0.4 ng/mL, respectively). Johnson ²⁶ has been reported that highest concentrations of T₄ were found during October and November and concentrations of T₃ were highest during December through May and lowest from July to October. In the present study, mean concentrations of T₃ and T₄ were greater in non-breeding season (T₃: 221 ± 31.61 ng/dL and T₄: 36.34 ± 4.28 nmol/L) than breeding season (T₃: 105 ± 5.91 ng/dL and T₄: 30.30 ± 1.83 nmol/L) in Arabian mares. But, statistically significant

difference was found in only T₃ levels. This suggests that thyroid hormones were affected by season and that there is a seasonal cyclicality in concentrations of thyroid hormones in Arabian horse. However, it is more likely that other factors such as leptin may also play a role in regulation of thyroid function ⁹. Additionally, a correlation between T₃ and T₄ was observed during non-breeding season but not breeding season, suggesting relationship between T₃ and T₄ were more prominent in non-breeding season. Thyroxine is considered a plasma reservoir for T₃, which is the biologically active form of thyroid hormone. Pharmacological elevation of T₄ should therefore provide increased concentrations of T₃ as well ^{12,28}.

Cartmill et al.²⁹ discussed that leptin concentrations were different insulin characteristics and daily GH and T₃ concentrations. Insulin insensitivity contributes high resting leptin concentration in horse. The direct effect of thyroid hormone on regulation of leptin synthesis has been reported. Treatment in vitro with T₃ causes an inhibition of leptin secretion on human and rat adipocytes, suggesting that T₃ have opposite effects on leptin secretion from adipocytes and indicating a possible mechanism exists to regulate leptin secretion by thyroid gland ¹⁵. Moreover, it has been reported that an elevation in concentrations of leptin after hypothyroidism in rat ³⁰ and human ³¹. Similar physiological mechanisms have been discussed in horse studies ^{9,12,16}. Cartmill et al.¹⁶ reported that horses treated with propylthiouracil (an inducer hypothyroidism) results an increasing concentration of leptin whereas lower concentration of T₃ and T₄. But, these findings do not seem consistent with Cartmill's other two experiments ^{12,29}. Buff et al.⁹ observed a negative correlation between thyrotropin and leptin levels during winter but not summer in horse. In the same study, plasma concentrations of T₄ were greater in winter than summer. However, relationship between leptin and T₄ secretion were not discussed in that study. In the present study, a correlation was found between leptin and T₃ in breeding season ($r = 0.860$, $P < 0.01$) but not non-breeding season ($r = 0.356$, $P > 0.05$). This finding suggested a possible interaction between production of leptin and T₃. But, more study is needed to investigate the interaction between leptin and thyroid hormones, under natural

conditions in horse.

In conclusion, the current study indicates that seasonal variation was existed in concentration of leptin and thyroid hormones. The body weight and BCS were not to be affected leptin levels in both seasons. A possible relationship was existed between leptin and thyroid hormones in horses. But, it is apparent that more research is needed to clarify the thyroid-leptin interactions in horse.

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