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Research Article

Serum Leptin and Ghrelin Levels and Their Relationship with Serum Cortisol, Thyroid Hormones, Lipids, Homocysteine and Folic Acid in Dogs with Compulsive Tail Chasing

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Abstract

The aim of this study was to investigate serum leptin and ghrelin levels and their relations with circulating cortisol, thyroid hormones, lipids, homocysteine (Hcy) and folic acid in dogs with compulsive tail chasing (CTC). The material of this study consists of fifteen dogs with CTC and 15 healthy controls of various weights, breeds, ages of both sexes were enrolled in the study. CTC was diagnosed on the basis of the dog's behavioral history, clinical signs, and results of other medical assessments. None of the dogs were considered to have concurrent medical disease that would account for CTC. Dogs with CTC had a higher leptin (8.3±0.9 ng/mL vs 1.7±0.2 ng/mL, P<0.001) and lower ghrelin levels (74±7 pg/mL vs 144±41 pg/mL, P<0.05) than those of healthy controls. Serum cortisol, lipids (cholesterol, phospholipids and NEFA) and Hcy levels increased (P<0.05), whereas serum folic acid decreased (P<0.001) in dogs with CTC as compared with controls. Serum ghrelin correlated negatively with cholesterol (P<0.05), but serum leptin correlated positively with cholesterol, fT4, and phospholipids (P<0.05). These results suggest that serum leptin and ghrelin levels may bring up a new perspective on our understanding of the pathophysiological mechanisms associated with CTC. Serum levels of both hormones may be associated with serum levels of lipids and free T4.

Keywords: *Leptin, Ghrelin, Thyroid hormones, Lipids, Tail chasing, Dog*

Kompulsif Kuyruk Isıran Köpeklerin Serum Leptin ve Ghrelin Seviyeleri ve Serum Kortizol, Tiroid Hormonları, Lipidler, Homosistein ve Folik Asit İle İlişkileri

Özet

Bu çalışmanın amacı, kompulsif kuyruk ısıran köpeklerde serum leptin ve ghrelin seviyeleri ve sirküle eden kortizol, tiroid hormonları, lipidler, homosistein (Hcy) ve folik asit arasındaki ilişkiyi araştırmaktır. Çeşitli ağırlık, ırk, yaş ve her iki cinsiyetten 15 kuyruk ısıran ve kontrol grubu olarak 15 sağlıklı köpek çalışmaya dahil edildi. Kuyruk ısırma tanısı, davranış anamnez formu, klinik bulgular ve diğer medikal değerlendirmelerin sonuçlarına göre konuldu. Kompulsif kuyruk ısıran köpeklerin hiçbirinde eşlik eden başka bir medikal hastalık bulunmamaktaydı. Kompulsif kuyruk ısıran köpekler, kontrol grubundaki köpeklere göre yüksek leptin (8.3±0.9 ng/mL ve 1.7±0.2 ng/mL, P<0.001) ve düşük ghrelin (74±7 pg/mL ve 144±41 pg/mL, P<0.05) seviyesine sahipti. Kontrol grubu ile karşılaştırıldığında kompulsif kuyruk ısıran köpeklerin serum kortizol, lipid (kolesterol, fosfolipidler ve NEFA) ve Hcy seviyesi artmış (P<0.05), bunun aksine serum folik asid seviyesi (P<0.001) azalmıştır. Serum ghrelin seviyesi, kolesterol (P<0.05) ile negatif korelasyon gösterirken, serum leptin seviyesi, kolesterol, fT4 ve fosfolipidler (P<0.05) ile pozitif korelasyon göstermekteydi. Sonuçlar değerlendirildiğinde, serum leptin ve ghrelin seviyelerindeki değişikliklerin kompulsif kuyruk ısırmanın patofizyolojik mekanizmasını anlamak için yeni bir perspektif getireceği düşünülmektedir. Her iki hormon da serum lipid seviyeleri ve serbest T4 düzeyi ile ilişkili olabilir.

Anahtar sözcükler: *Leptin, Ghrelin, Tiroid hormonları, Lipidler, Kompulsif kuyruk ısırma, Köpek*

INTRODUCTION

Obsessive compulsive disorder (OCD) is a neuropsychiatric disorder in humans and animals. Canine compulsive disorder includes excessive tail chasing, light/shadow chasing and flank sucking [1-3]. Clinical and neurobiological similarities between dogs and humans with OCD were reported [3,4]. Thus, canine compulsive behaviors

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such as compulsive tail chasing (CTC) have been suggested as a promising model for human OCD [3].

Human and canine OCD have been associated with a biochemical disturbance at the level of neurotransmitter systems [1] and activation of hypothalamic-pituitary-adrenal (HPA) system [5,6]. Compulsive disorders are also known to be stress responsive and compulsive symptoms increase at times of stress [6,7]. Serum cortisol level as an indicator of activated HPA system is used to describe the presence of stress in humans [8], dogs [9], and cats [10].

Leptin and ghrelin are two hormones related with energy balance. Leptin, an anorexigenic hormone, is a mediator of long-term regulation of energy balance, suppressing food intake and thereby resulting in weight loss. Ghrelin, an orexigenic hormone, is playing a role in meal initiation [11]. Recently, leptin and ghrelin hormones have also been correlated in the pathophysiology of stress [12]. Leptin inhibits and ghrelin facilitates neuroendocrine stress responses in rats [12]. Hypercortisolemia increases serum levels of leptin and decreases serum levels of ghrelin in dogs. As serum levels of leptin and ghrelin are affected by cortisol [13], it is possible to hypothesize that these hormones might play a role in the regulation of stress response in dogs with OCD.

Current literature shows that some psychiatric disorders in humans might be related to the serum levels of folate and homocysteine (Hcy) [14-16], lipids [17,18] and thyroid function [19]. Thyroid status is considered as an important determinant of the serum level of total Hcy [20] and lipids (cholesterol, lipoproteins, etc.) in humans [17,18,21,22]. There is also one study indicating a relationship between serum level of lipid (serum cholesterol elevations) and OCD in dogs [23]. Based on the accumulated evidence, we hypothesized that circulating leptin, ghrelin, folate, Hcy, lipids and thyroid hormones might have a role in dogs with OCD. Thus, in this study, to understand of pathophysiological mechanism of OCD, we investigated serum leptin and ghrelin levels and their relations with circulating thyroid hormones, folate, Hcy, lipids, and cortisol in dogs with CTC.

MATERIAL and METHODS

Dogs

This study was performed on 15 healthy dogs and 15 dogs with CTC that were referred to the Small Animal Clinics Internal Medicine Department, Faculty of Veterinary Medicine, Uludag University, Bursa, Turkey in January-July 2016. Dogs with CTC were 10-35 kg (mean 27.2 kg±1.5 kg), and of various breeds (3 Anatolian shepherd dogs, 3 German shepherd dogs, 3 Golden retrievers, 3 mixed breeds, 1 Terriers, 1 Doberman, 1 Labrador retriever), sex (11 males, 4 females) and age (8 months-9 years, mean 3.3±0.7 years) were evaluated. Dogs (n=15), which were referred for vaccination purposes, were also enrolled in the study as control after the consent of the owners and on the basis of normal physical examination results and complete blood count. Control dogs were of various weights (23.5±1.5 kg), mixed breeds of either sex (10 males, 5 females), and ranged from 12-96 months (34±2.5 months) in age. No significant differences were observed between the two groups regarding the aforementioned parameters. The body condition score for each dog was evaluated by using a 5-point scale (1: thin, 2: underweight, 3: ideal, 4: overweight and 5: obese) [24]. All experiments conducted by us in this study were performed in line with ethical approval from the ethical committee of University (16/1-5).

Diagnostic Procedures

A behavioural diagnosis was made for each dog on the basis of the dog's behavioural history, clinical signs, and results of other medical assessments, as published in the previous studies [23,25]. Dogs were assessed for seizure disorder, opioid-mediated stereotypy, local vasculitis or neuritis, anal sac diseases and pruritus. None of the dogs were considered to have concurrent medical disease (such as dermatological disease, vector–borne diseases, and renal diseases) that would account for CTC. Behavioral history included age at onset, frequency and duration of bouts since onset, general history, and current or previous medical conditions. All owners reported that their dogs commonly whined, barked, or growled during tail chasing. In this study, affected dogs had to have tail chasing bouts for a minimum of 60 s/bout at least 3 times/d during the previous two months in order to be included into the study.

Sample Collection and Measurements

Venous blood samples were collected, after a fasting period of 12-16 h, from cephalic veins into vacutainer tubes with or without EDTA (Becton Dickinson, Temse**,** Belgium) for complete blood count (Cell Dyne 3500R, Abbott, Germany) and serum biochemistry panel (Aeroset, Abbott), respectively. All dogs were screened for common vector-borne diseases (anaplasmosis, borrelliosis, dirofilariosis, ehrlichiosis, and leishmaniosis) by speed tests (Bionote, Anigen, South Korea), and dogs sero-positive for vector-borne pathogens were excluded from the study.

Serum leptin and ghrelin levels were measured by radioimmunoassay (RIA) using a commercially available kit (Multispecies leptin RIA kit and active ghrelin RIA kit, Linco Research, St. Charles, MO). Validity and reliability of these RIA kits for measuring serum leptin and ghrelin levels in dog were determined in our previous studies [13,26]. Since active ghrelin is too unstable to be measured in stored samples [27] and acidification of plasma prevents rapid desacylation of ghrelin [28], 1 N hydrogen chloride was added to serum samples before freezing[13]. Serum cortisol was measured by a solid-phase chemiluminescent enzyme immunoassay system (Immulite 2000, BioDPC, Los

Angees, CA) as reported earlier [26].

Serum samples were tested for total thyroxin (T_4), free T_4 (fT_4), triiodothyronine (T_3), and free T_3 (fT_3) concentrations. Hormone analysis was performed by RIA techniques (Advia Centaur™) as reported earlier [29].

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C) and triglycerides levels were measured by an automated clinical chemistry analyzer (Architect *ci*8200; Abbott GmbH Co KG, Wiesbaden, Germany) using commercially available assay kits (Abbott GmbH Co KG). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the formula:

LDL-C(in milligrams per deciliter) = total cholesterol - (HDL – C + triglyceride/5).

Serum non-esterified fatty acid (NEFA) concentration was measured using a commercially available enzymatic colorimetric assay kit (Wako Chemicals, Neuss, Germany) [30]. Serum phospholipids were measured by an enzymatic colorimetric method using a commercially available assay kit (Wako Chemicals), which have been reported as choline-containing phospholipids (in milligrams per deciliter) [30].

Statistical Analysis

Data were analysed statistically by two-group comparison student t test (SigmaStat GmbH, Erkrath) and expressed as mean±SEM. Pearson's correlation analysis was used to determine a relationship between the mean levels of serum leptin, ghrelin, cortisol, Hcy, folate, lipids, and thyroid hormones. A *P* value of <0.05 was considered significant.

RESULTS

Routine clinical and hematological findings were within reference limits in all healthy dogs (data not shown). Dogs with CTC had a higher mean body condition score (4.2±0.6; P<0.001) than controls (3.2±0.4).

Dogs with CTC had a higher leptin (8.3±0.9 ng/mL *vs* 1.7±0.2 ng/mL, P<0.001) and lower ghrelin levels (74±7 pg/mL *vs* 144±41 pg/mL, P<0.05) than those of healthy controls *(Table 1)*.

Serum cortisol, lipids (cholesterol, phospholipids and NEFA) and Hcy levels increased (P<0.05), whereas serum folic acid decreased (P<0.001) in dogs with CTC as compared with controls. There were no statistically significant differences on serum thyroid hormones (except fT3) between the groups studied. Serum fT3 level in dogs with CTC was higher (P<0.05) than that of controls *(Table 2)*.

Serum ghrelin correlated negatively with cholesterol (P<0.05) and serum leptin correlated positively with cholesterol, fT_4, and phospholipids (P<0.05). There was a negative correlation (P<0.05) between changes in serum ghrelin and leptin levels in healthy controls.

Table 1. *Serum levels of leptin, ghrelin, lipids, thyroid hormones, homocysteine and folic acid in healthy dogs and dogs with compulsive tail chasing (CTC)*

Parameters	CTC Mean±SEM	Healthy Controls Mean±SEM	P Value
Leptin ng/mL	8.36±0.90	1.70±0.26	=<0.001
Ghrelin pg/mL	74.94±7.29	144.11±41.41	= 0.029
T. Cholesterol mg/dL	203.42±16.03	140.40±10.85	NS
HDL mg/dL	73.00±11.09	89.88±7.26	NS
LDL mg/dL	128.11±11.99	32.44±3.25	=<0.001
VLDL mg/dL	15.94±1.87	12.50±1.28	NS
Tg mg/dL	79.63±9.42	58.75±5.28	NS
Phospholipid mg/dL	338.16±17.05	276.11±14.60	=0.027
Nefa mg/dL	1.74±0.17	0.85±0.15	=0.002
TT_4 µg/dL	1.79±0.09	1.91±0.14	NS
fT_4 ng/dL	0.72±0.10	0.48±0.02	=0.072
TT_3 ng/mL	0.44±0.03	0.34±0.03	NS
fT_3 pg/mL	1.87±0.15	1.34±0.10	=0.03
Hcy µmol/L	11.52±0.84	6.29±0.23	=<0.001
Folic acide ng/mL	3.25±0.32	6.41±0.56	=<0.001

DISCUSSION

This study showed that serum leptin levels were higher and ghrelin levels were lower in CTC dogs when compared with the healthy counterparts [26]. Increased serum leptin and decreased ghrelin levels and their correlation with circulating thyroid hormones and lipids in dogs with CTC provide further information to understand the pathophysiology of OCD, and to develop new treatment strategies for these patients.

In the present study, CTC, one of the most common forms of OCD in dogs, was diagnosed as reported earlier [23,25]. Serum leptin concentrations (1.7±0.2 ng/mL), measured by RIA in healthy dogs, were slightly lower than those of healthy dogs in the previous studies: 2.5±0.1 ng/mL [13], 2.4±0.1 ng/mL [26], and 2.3±0.5 ng/mL [31]. This difference may be explained by diurnal rhythms of circulating leptin as well as using different measurement methods (RIA or ELISA) and kits (multi-species or canine specific leptin) [31]. In this study, no noticeable influence of age, gender, and breed on serum leptin levels was observed as reported by Ishioka et al.[32].

In this study, dogs with CTC had higher leptin (8.3±0.9 ng/mL) and lower ghrelin levels (74±7 pg/mL) than those of healthy controls, indicating a possible association between leptin and ghrelin systems and psychogenic disorders

Table 2. *Serum levels of leptin and ghreline and their relations with serum lipids, thyroid hormones*

Parameters	T. Cholesterol	HDL	LDL	fT4	Phospholipids
Leptin	r: 0.600 P<0.05	NS	NS	r: 0.634 P<0.05	r: 0.643 P<0.05
Ghrelin	r: -0.477 P<0.05	r: -0.512 P<0.05	r: - 0.462 P<0.05	NS	NS

as well as a good inverse correlation between them. The results of serum leptin in this study showed similarity to those of a human study [33], in which serum leptin level was slightly higher (but not statistically significant) in the OCD group than in the healthy control group. High body condition score, observed in dogs with CTC, may enhance the serum leptin concentration by increasing leptin secretion from adipose tissue [24,32,34].

Since active form of ghrelin as compared with total ghrelin is essential in particular for biological [28] and endocrine activities [35], and thus, is physiologically more crucial in terms of OCD [36], active ghrelin measurements were chosen in the present study. Observed serum ghrelin level (144±41 pg/mL) in healthy dogs was in good accordance with the levels of 172±17 pg/mL and 117±42 pg/mL reported for healthy beagle dogs [37] and humans [38], respectively. As compared to healthy controls, serum ghrelin levels in dogs with CTC were found lower in this study, whereas Atmaca et al.[39] and Emül et al.[33] reported a trend of higher ghrelin levels in patients with OCD. These differences of serum ghrelin between the studies might have resulted from the presence of depressive disorders in patients with OCD [33,39].

In this study, elevated levels of serum cortisol, as compared to control dogs, were thought to be associated with the HPA axis activation in dogs with CTC, in concordance with the results in patients with OCD [6]. In the previous studies, in addition to serum cortisol elevation, hyperactivity of HPA axis, the main mammalian system of stress response [6], was confirmed by increased corticotropin-releasing hormone levels in cerebrospinal fluid [40] and increased urinary cortisol levels in patients with OCD [41]. Elevated serum cortisol has been accepted as a physiological marker of stress and behavior abnormalities, particularly for dogs in animal shelter, as well [42].

Our observations on serum lipid profile confirmed and expanded the findings of the previous studies [23,43] reporting that dogs with CTC had significantly higher total cholesterol and LDL-cholesterol compared with control dogs, by demonstrating the increasing serum phospholipids and NEFA in dogs studied. The findings of this study were very similar to those of a previous study [23]; VLDL-cholesterol and triglyceride levels did not differ significantly between the groups. Similar to studies of dogs, elevated cholesterol [44], LDL- and VLDL-cholesterols and triglyceride levels were observed in human OCD patients compared with the control subjects [18]. In agreement with earlier studies in dogs [23,43] and humans [17,18], our observations confirm that serum lipid profile might be changed in dogs suffering from CTC.

On the other hand, a significant increase in serum phospholipid and NEFA brings up a new perspective which may help explain, at least in part, pathophysiological mechanisms associated with OCDs, such as CTC in dogs. Because phosphatidylcholine, as a primary phospholipid, is found at higher levels in myelin, cell membranes and brain parenchyma [45], it may have a crucial role for development of neuropsychiatric disorders in dogs as in humans [45]. In addition, excessive serum levels of NEFA, as observed in the present study, were reported to enhance oxidative stress [46] leading to several neuropsychiatric diseases including OCD [47]. Brain tissue has a high percentage of phospholipids that can easily be peroxidized. Recently, markers of oxidative stress and free radical induced injury to the brain tissues in OCD patients were reported, as well.

In this study, of thyroid hormones, only serum fT3 levels was found to be significantly higher in dogs with CTC than in controls, in accordance with euthyroid syndrome, most probably due to increased tissue metabolic demands in response to CTC as a non-thyroidal illness in dogs studied. One previous study [48] showed that basal values of thyroid hormones and thyroid stimulating hormone were normal in patients with OCD, and another study [19] reported that higher rates of panic disorder, OCD, and major depressive disorder were observed in thyroid patients than in the general population. Aizenberg et al.[49] underlined that dysregulation of the hypothalamic-pituitary-thyroid axis in OCD patients. Our results did not confirm the results arising from human studies indicating that altered levels of thyroid hormones might be associated with pathophysiology or maintenance of OCD.

Since serum Hcy has been considered as a sensitive marker for folate deficiency and they have important roles in carbon transfer metabolism (methylation) [16,39], in this study serum Hcy and folate levels were evaluated together. Our results of serum Hcy (6.2±0.2 µmol/L) and folate levels (6.4±0.5 ng/mL) in healthy dogs were in good accordance with those of the previous studies in dogs (5.1-10.9 µmol/L and 4.2-7.5 µg/L, respectively) [50] and humans (8.1±2.2 µmol/L and 7.5±1.9 ng/mL, respectively) [16]. In this study, serum Hcy levels increased (11±0.8 µmol/L), and serum folic acid decreased (3.2±0.3 ng/mL) in sick dogs, indicating the presence of hyperhomocysteinemia and serum folate deficiency in dogs with CTC. These results were very similar to the human studies on OCD [16,39]. It is well known that elevated serum Hcy and decreased folate

are associated with poor cognitive function and some psychiatric disorders. These results can be explained by the importance for the production of serotonin as well as for other monoamine neurotransmitters and catecholamines. Observations on the antidepressant effects of folate supplementation may support the importance of these nutrients in psychopathology [16].

In the present study, based on the correlation studies, it may be speculated that serum leptin and ghrelin might have a role to regulate circulating lipids (total cholesterol, HDL-C, LDL-C and phospholipids) in dogs with CTC. Serum leptin correlated positively only with serum fT_3 levels amongst thyroid hormones measured in this study. Similarly, in a previous study [51], circulating thyroid hormones were reported not to play a major role in the regulation of leptin synthesis and secretion. In contrast to previous studies reporting an inverse correlation between serum leptin and cortisol [39,52], in this study, there was no relationship between the two variables in dogs.

The data presented here should be interpreted with caution owing to some limitations. First, a relatively small sample size might not be representative of the dogs with CTC. However, more comprehensive and detailed studies are needed to determine the exact role of leptin and ghrelin, as well as the interactions between them in dogs with CTC.

In conclusion, our study results suggest that serum leptin and ghrelin levels bring up a new perspective which may contribute to clarify pathophysiological mechanisms associated with CTC. Increased serum Hcy and decreased serum folate levels should be taken into consideration at diagnostic and therapeutic approaches in dogs with CTC. Increased serum levels of fT_3 should be interpreted with caution during the diagnostic work-up in dogs suffering from CTC, due to euthyroid sick syndrome.

REFERENCES

1. Hewson CJ, Luescher UA: Compulsive disorders in dogs. **In,** Voith VL, Borchelet PL (Eds): Readings in Companion Animal Behavior. 153-158, Veterinary Learning Systems. Trenton, NJ, 1996.

2. Hewson CJ, Luescher UA, Ball RO: The use of chance corrected agreement to diagnose canine compulsive disorder: An approach to behavioral diagnosis in the absence of a 'gold standard'. *Can J Vet Res*, 63, 201-206, 1999.

3. Tiira K, Hakosalo O, Kareinen L, Thomas A, Hielm-Björkman A, Escriou C, Arnold P, Lohi H: Environmental effects on compulsive tail chasing in dogs. *PLoS One,* 7 (7): e41684, 2012. DOI: 10.1371/journal.pone.0041684

4. Goldberger E, Rapoport JL: Canine acral lick dermatitis: Response to the antiobsessional drug clomipramine. *J Am Vet Med Assoc*, 27, 179-182, 1990.

5. De Kloet E, Joels M, Holsboer F: Stress and the brain: From adaptation to disease. *Nat Rev Neurosci,* 6, 463-475, 2005. DOI: 10.1038/nm1683

6. Kluge M, Schüssler P, Künzel HE, Dresler M, Yassouridis A, Steiger A: Increased nocturnal secretion of ACTH and cortisol in obsessive-compulsive disorder. *J Psyciatr Res,* 4, 928-933, 2007. DOI: 10.1016/j.jpsychires.2006.08.005

7. Findley DB, Leckman JF, Katsovich L, Haiqun L, Zhang H, Grantz H, Otka J, Lombroso PJ, King RA: Development of the Yale Children's Global Stress Index (YCGSI) and its application in children and adolescents with Tourette's syndrome and obsessive-compulsive disorder. *J Am Acad Child Adolesc Psychiatry,* 42, 450-457, 2003. DOI: 10.1097/01.CHI.0000046816.95464.EF

8. Baig A, Siddiqui I, Naqvi H, Sabir S, Jabbar J, Shahid M: Correlation of serum cortisol levels and stress among medical doctors working in emergency departments. *J Col Physicians Surg Pak*, 16, 576-80, 2006.

9. Perego R, Proverbio D, Spada E: Increases in heart rate and serum cortisol concentrations in healthy dogs are positively correlated with an indoor waiting-room environment. *Vet Clin Pathol*, 43, 67-71, 2014. DOI: 10.1111/vcp.12118

10. Nibblett BM, Ketzis JK, Grigg EK: Comparison of stress experienced by cats examined in a clinic versus a home setting. *App Anim Beh Sci*, 173, 68-75, 2015. DOI: 10.1016/j.applanim.2014.10.005

11. Klok MD, Jakobsdottir S, Drent ML: The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obes Rev*, 8, 21-34, 2007. DOI: 10.1111/j.1467-789X.2006.00270.x

12. Kawakami A, Okada N, Rokkaku K, Honda K, Ishibashi S, Onaka T: Leptin inhibits and ghrelin augments hypothalamic noradrenaline release after stress. *Stress,* 11, 363-369, 2008. DOI: 10.1080/10253890701820257

13. Yilmaz Z, Ilcol YO, Golcu E: Serum leptin and ghrelin levels in response to methylprednisolone injection in healthy dogs. *Res Vet Sci*, 82, 187-194, 2007. DOI: 10.1016/j.rvsc.2006.07.005

14. Sumi-Ichinose C, Urano F, Kuroda R, Ohye T, Kojima M, Tazawa M, Shiraishi H, Hagino Y, Nagatsu Y, Nomura T, Ichinose H: Catecholamines and serotonin are differently regulated by tetrahydrobiopterin. A study from 6-pyruvoyltetrahydropterin synthase knockout mice. *J Biol Chem,* 276, 41150-41160, 2001. DOI: 10.1074/jbc.M102237200

15. Blom HJ, Smulders Y: Overview of homocysteine and folate metabolism with special references to cardiovascular disease and neural tube defects. *J Inherited Metab Dis,* 34, 75-81, 2011. DOI: 10.1007/s10545-010-9177-4

16. Turksoy N, Bilici R, Yalciner A, Özdemir YO, Ornek I, Tufan AE, Kara A: Vitamin B_{12}, folate, and homocysteine levels in patients with obsessive-compulsive disorder. *Neuropsychiat Dis Treat,* 10, 1671-1675, 2014. DOI: 10.2147/NDT.S67668

17. Peter H, Tabrizian S, Hand I: Serum cholesterol in patients with obsessive compulsive disorder during treatment with behavior therapy and SSRI or placebo. *Int J Psychiatr Med,* 30, 27-39, 2000. DOI: 10.2190/APWF-N1XU-Y7A0-TCBW

18. Agargun, MY, Dulger H, Inci R, Kara H, Ozer EA, Sekeroglu MR, Besiroglu L: Serum lipid concentrations in obsessive-compulsive disorder patients with and without panic attacks. *Can J Psychiatry*, 49, 776-778, 2004.

19. Placidi GP, Boldrini M, Patronelli A, Fioere E, Chiovato L, Perugi G, Marazatti D: Prevalence of psychiatric disorders in thyroid diseased patients. *Neuropsychobiology,* 38, 222-225, 1998. DOI: 10.1159/000026545

20. Catargi B, Parrot-Roulaud F, Cochet C, Ducassou D, Roger P, Tabarin A: Homocysteine, hypothyroidism, and effect of thyroid hormone replacement. *Thyroid*, 9, 1163-1166, 1999. DOI: 10.1089/thy.1999.9.1163

21. Iqbal A, Jorde R, Figenschau Y: Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: The Tromsø study. *J Int Med*, 260, 53-61, 2006. DOI: 10.1111/j.1365-2796.2006.01652.x

22. Rizos CV, Elisaf MS, Liberopoulos EN: Effects of thyroid dysfunction on lipid profile. *Open Cardiovasc Med J,* 5, 76-84, 2011. DOI: 10.2174/1874192401105010076

23. Yalcin E, Ilcol YO, Batmaz H: Serum lipid concentrations in dogs with tail chasing. *J Small Anim Pract*, 50, 133-135, 2009. DOI: 10.1111/j.1748-5827.2008.00704.x

24. Usui, S, Yasuda H, Koketsu Y: Lipoprotein cholesterol and triglyceride concentrations associated with dog body condition score: Effect of recommended fasting duration on sample concentrations in Japanese private clinics. *J Vet Med Sci*, 77, 1063-1069, 2015. DOI: 10.1292/jvms.15-0032

25. Overall KL, Dunham AE: Clinical features and outcome in dogs and cats with obsessive-compulsive disorder: 126 cases (1989-2000). *J Am Vet*

Med Assoc, 221, 1445-1452, 2002. DOI: 10.2460/javma.2002.221.1445

26. Yilmaz Z, Ozarda Ilcol Y, Ulus IH: Endotoxin increases plasma leptin and ghrelin levels in dogs. *Crit Care Med*, 36, 828-833, 2008. DOI: 10.1097/01.CCM.0B013E3181611F5AA

27. Hosoda H, Kojima M, Matsuo H, Kangawa K: Ghrelin and des-acyl ghrelin: Two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochemical Biophys Res Commun*, 279, 909-913, 2000. DOI: 10.1006/bbrc.2000.4039

28. Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, Akamizu T, Kangawa G: Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *Eur J Endocrinol*, 149, R1-R3, 2003. DOI: 10.1530/eje.0.149R001

29. Yilmaz Z, Batmaz H: Short term effects of endotoxin on thyroid hormone concentrations in dogs. *Indian Vet J*, 79, 780-781, 2002.

30. Ilcol YO, Yilmaz Z, Cansev M, Ulus IH: Choline or CDP-choline alters serum lipid responses to endotoxin in dogs and rats: Involvement of the peripheral nicotinic acetylcholine receptors. *Shock*, 32, 286-294, 2009. DOI: 10.1097/SHK.0b013e3181971b02

31. Ishioka K, Hatai H, Komabayashi K, Soliman MM, Shibata H, Honjoh T, Kimura K, Saito M: Diurnal variations of serum leptin in dogs: Effects of fasting and re-feeding. *Vet J,* 169, 85-90, 2005. DOI: 10.1016/j.tvjl.2004.01.003

32. Ishioka K, Hosoya K, Kitagawa H, Shibata H, Kimura K, Saito M: Plasma leptin concentration in dogs: Effects of body condition score, age, gender and breeds. *Res Vet Sci*, 82, 11-15, 2007. DOI: 10.1016/j.rvsc.2006.06.002

33. Emul HM, Serteser M, Kurt E, Ozbulut O, Guler O, Gecici O: Ghrelin and leptin levels in patients with obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*, 31, 1270-1274, 2007. DOI: 10.1016/j.pnpbp.2007.05.007

34. Park HJ, Lee SE, Oh JH, Seo KW, Song KH: Leptin, adiponectin and serotonin levels in lean and obese dogs. *BMC Vet Res*, 13, 113, 2014. DOI: 10.1186/1746-6148-10-113

35. De Ambrogi M, Volpe S, Tamanini C: Ghrelin: Central and peripheral effects of a novel peptidyl hormone. *Med Sci Monit*, 9, 217-224, 2003.

36. Aydin S, Karatas F, Geckil H: Simultaneous quantification of acylated and desacylated ghrelin in biological fluids. *Biomed Chromatogr,* 22, 1354-1359, 2008. DOI: 10.1002/bmc.1065

37. McKnight LL, Eyre R, Gooding MA, Davenport GM, Shoveller AK: Dietary mannoheptulose increases fasting serum glucagon like peptide-1 and post-prandial serum ghrelin concentrations in adult beagle dogs. *Animals,* 16, 442-454, 2015. DOI: 10.3390/ani5020365

38. Macedo DM, Diez-Garcia RW: Sweet craving and ghrelin and leptin levels in women during stress. *Appetite,* 80, 264-270, 2014. DOI: 10.1016/j.appet.2014.05.031

39. Atmaca M, Tezcan E., Kuloglu M, Kırtas Ö, Ustundag B: Serum folate and homocysteine levels in patients with obsessive-compulsive disorder. *Psychiatry Clin Neurosci,* 59, 616-20, 2005. DOI: 10.1111/j.1440-1819.2005.01425.x

40. Altemus M, Cizza G, Gold PW: Chronic fluoxetine treatment reduces hypothalamic vasopressin secretion *in vitro. Brain Res,* 593, 311-313, 1992. DOI: 10.1016/0006-8993(92)91326-A

41. Gehris TL, Kathol RG, Black DW, Noyes RJ: Urinary free cortisol levels in obsessive-compulsive disorder. *Psychiatry Res*, 32, 151-158, 1990. DOI: 10.1016/0165-1781(90)90081-F

42. Hennessy MB, Davis HN, Williams MT, Mellott C, Douglas CW: Plasma cortisol levels of dogs at a county animal shelter. *Physiol Behav*, 62, 485-490, 1997. DOI: 10.1016/S0031-9384(97)80328-9

43. Dodman NH, Knowles KE, Shuster I, Moon-Fanelli AA, Tidwell AS, Keen CL: Behavioural changes associated with suspected complex partial seizures in bull terriers. *J Am Vet Med Assoc*, 208, 688-691, 1996.

44. Peter H, Hand I, Hohagen F, Koenig A, Mindermann O, Oeder F, Wittich M: Serum cholesterol level comparison: Control subjects, anxiety disorder patients, and obsessive-compulsive disorder patients. *Can J Psychiatry,* 47, 557-561, 2002.

45. Maddock RJ, Buonocore MH: MR spectroscopic studies of the brain in psychiatric disorders. *Curr Top Behav Neurosci*, 11, 199-251, 2012. DOI: 10.1007/7854_2011_197

46. Barazzoni R, Zanetti M, Gortan Cappellari G, Semolic A, Boschelle M, Codarin E, Pirulli A, Cattin L, Guarnieri G: Fatty acids acutely enhance insulin-induced oxidative stress and cause insulin resistance by increasing mitochondrial reactive oxygen species (ROS) generation and nuclear factor-κB inhibitor (IκB)-nuclear factor-κB (NFκB) activation in rat muscle, in the absence of mitochondrial dysfunction. *Diabetologia*, 55, 773-782, 2012. DOI: 10.1007/s00125-011-2396-x

47. Chakraborty S, Singh OM, Dasgupta Mandal N, Nath Das E: Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive-compulsive disorder. *Prog in Neuro-Psychopharm*, 33, 363-366, 2009. DOI: 10.1016/j.pnpbp.2009.01.001

48. Hantouche E, Piketty ML, Poirier MF, Brochier T, Olie JP: Obsessive-compulsive disorder and the study of thyroid function. *Encephale*, 17, 493-496, 1991.

49. Aizenberg D, Hermesh H, Gilad I, Munitz H, Tyano S, Laron Z, Weizman A: TRH stimulation test in obsessive-compulsive patients. *Psychiatry Res,* 38, 21-26, 1991. DOI: 10.1016/0165-1781(91)90049-U

50. Patterson BE, Barr JW, Fosgate GT, Berghoff N, Steiner JM, Suchodolski JS, Black DM: Homocysteine in dogs with systemic inflammatory response syndrome. *J Small Anim Pract*, 54, 620-624, 2013. DOI: 10.1111/jsap.12144

51. Corbetta S, Englaro P, Giambona S, Persani L, Blum WF, Beck-Peccoz P: Lack of effects of circulating thyroid hormone levels on serum leptin concentrations. *Eur J Endocrinol,* 137, 659-663, 1997. DOI: 10.1530/eje.0.1370659

52. Liao SC, Lee MB, Huang TS: The counterbalance between leptin and cortisol may be associated with comorbid depression and anxiety. *Psychiatry Clin Neurosci*, 60, 120, 2006. DOI: 10.1111/j.1440-1819.2006.01472.x