

## A survey on the relationship between body condition score with lipid profiles and serum testosterone concentration in dog

Sajad Chegini<sup>1</sup>, Bahman Mosallanejad<sup>2\*</sup>, Mohammad Razi Jalali<sup>2</sup> and Saad Gooraninejad<sup>2</sup>

<sup>1</sup> DVM Graduated, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

<sup>2</sup> Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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### Abstract

Several factors play a role in the development of obesity in animals, which is the most important of them lack of exercise, excessive feeding, and hormonal disorders. Testosterone is responsible for many of the physical characteristics specific to males. Body condition score (BCS) is used to determine the body status. Body weight increases significantly with castration in dogs. The purpose of the present study was to investigate the relationship between body condition score with lipid profiles and serum testosterone concentration in obese, normal and thin dogs. This survey was accomplished on one hundred and fifty intact male dogs referred to Veterinary Hospital of Shahid Chamran University of Ahvaz. The owned dogs were divided into three equal groups: group A (obese), group B (normal) and group C (thin). There were fifty dogs in each group. Body condition score system was used from number one to nine. The dogs were selected from large breeds (German shepherd and Doberman Pinscher), male and in the age range 1-7 years-old. Serum lipid profiles (triglyceride, total cholesterol, HDL-C, LDL-C and VLDL-C concentrations) were measured using spectrophotometric method. Testosterone concentration was also determined by ELISA method. Body condition score and testosterone concentration had an effect on lipid profile levels, so that the increase of BCS caused a significant increase of triglyceride ( $122.06 \pm 67.99$  mg/dl), total cholesterol ( $241.08 \pm 67.25$ ), LDL-C ( $134.18 \pm 63.10$ ) and VLDL-C ( $23.96 \pm 13.42$ ) in obese dogs (A) and a significant decrease of HDL-C ( $82.98 \pm 26.86$ ) and testosterone ( $3.36 \pm 0.57$ ). It was also identified that the decrease of BCS in thin dogs (C) caused a significant decrease of serum triglyceride ( $76.24 \pm 29.67$  mg/dl), total cholesterol ( $203.54 \pm 59.89$ ), LDL-C ( $89.99 \pm 55.99$ ) and VLDL-C ( $15.25 \pm 5.93$ ) and a significant increase of HDL-C ( $97.64 \pm 35.65$ ) and testosterone ( $9.00 \pm 0.82$ ). Means of testosterone concentrations were obtained  $3.36 \pm 0.57$ ,  $5.71 \pm 0.55$  and  $9.00 \pm 0.82$  ng/ml in the obese, normal and thin dogs, respectively. The results showed that serum testosterone concentration decreased with obesity (increase of BCS), and increased with emaciation (decrease of BCS). Further investigations are necessary to be determined the better understand of relationship between obesity and hormonal changes.

**Key words:** Body condition score, Dog, Lipid profiles, Testosterone

### Introduction

Obesity and weight gain have always been considered as one of the most common nutritional disorders in pets. Dogs have overweight when their body weight is more than 15% their ideal weight, and they are

considered obese, when their weight exceeds 30% of optimal. Prevalence of canine obesity is estimated to be around 5-20% and up to 30-40% when all overweight

\* **Corresponding Author:** Bahman Mosallanejad, Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran  
E-mail: [bmosallanejad@scu.ac.ir](mailto:bmosallanejad@scu.ac.ir)



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dogs are considered in the statistical review (Porsani et al, 2019).

Numerous factors play a role in the development of obesity in animals, which the most important of them are lack of exercise, excessive feeding, and hormonal disorders. Genetic factors such as breed, administration of drugs, and physiological factors including castration or ovariectomy are associated with an increased risk of obesity. Obesity is associated with various diseases, like cardiovascular complications. Weight loss and muscle loss (emaciation) can be attributed to physiological changes, dietary inadequacies, presence of the different diseases and increasing age (Courcier et al, 2010).

To determine the physical condition of the dogs, body condition score (BCS) is used from number one to nine. In most studies, BCS is divided into three groups: thin (BCS=1-3), normal (BCS=4-5) and obese (BCS=6-9) by measurement of body physical situation. The condition of the ribs, spine, lumbar region, and abdominal dislocation play an important role in this assessment (Ettinger et al, 2017). It was suggested that the measurement of chest and abdominal girths would be an effective method to estimate BCS scores in dogs that helps non-professionals to manage their own dog's nutritional condition (Chun et al, 2019). It was suggested that BCS at 7 months of age can be used as predictive factors in overweight adult dogs (Leclerc et al, 2017). Body weight increases with elevation of age and castration in dogs (Bond et al, 2016; Ettinger et al, 2017).

Medical studies on the relationship between sexual hormones and obesity, show conflicting results; nevertheless, most studies show that there is a negative correlation between BCS and serum testosterone especially in human (Shamim et al, 2015; Biabangard et al, 2015; Malik et al, 2019). The aim of the present study was to investigate the relationship between BCS with lipid profiles (triglycerides, total

cholesterol, HDL-C, LDL-C and VLDL-C) and serum testosterone concentration in obese, normal and thin dogs. It was hypothesized that there is an inverse relationship between testosterone levels and BCS in obese dogs and a direct relationship between serum testosterone and BCS in thin dogs.

## **Materials and Methods**

### **Animals and sample size**

The present investigation was performed on one hundred and fifty intact male dogs (clinically healthy) and in age range 1-7 years-old (mean:  $4.15 \pm 1.87$ ). In the present study, all owned dogs were referred to Veterinary Hospital of Shahid Chamran University of Ahvaz, during seven months (May 2016 to November 2017). The dogs were selected from large breeds (German shepherd and Doberman Pinscher). This survey was approved by the Animal Care and Research Committee of Shahid Chamran University of Ahvaz. It was conducted based on the Guidelines for Animal Care and Use (Ethical code: 97581225). Dogs were divided into three equal groups: group A (obese), group B (normal) and group C (thin). There were fifty dogs in each group. To determine the body physical condition of animals, BCS system was used from number one to nine. In group A, BCS was between 6-9. In group B, the dogs had good physical condition (BCS=4-5) and in group C, BCS was between number 1-3. After recording the animal weight, the physical status was evaluated by observing the dogs from the angles of sides, top, front and back, as well as by touching the thorax and abdomen carefully. The position of the lumbar deep and abdomen dislocation were important factors. Assessment of body condition score was done based on sources (Ettinger et al., 2017). All dogs with hyperlipidemia (increased triglycerides, cholesterol or both), were investigated for complications such as fat accumulation in the anterior chamber of the eye or joint deformity.

### Collection of Samples

After obtaining the consent of the owners, fasting blood samples (at least 3 ml) were collected from the cephalic or external saphenous veins of dogs. After separation, serum samples were stored at -80° C. They were used for the analysis of biochemical parameters.

### Measurement of lipid profiles

The levels of serum triglycerides, total cholesterol and LDL-C were measured using an autoanalyzer (Biotecnica, BT-1500, Italy) and with commercial diagnostic kits of Pars Azmoon Company (Tehran, Iran), based on enzymatic calorimetric and photometric method. HDL-C was quantitatively measured using Pishtaz-Teb kit (Tehran, Iran). VLDL-C values were obtained by subtracting the total HDL-C and LDL-C from total cholesterol. Normal levels of biochemical indices have been reported for triglycerides (29-291 mg/dl), total cholesterol (92-324), HDL-C (80-120), LDL-C (20-60) and VLDL-C (6-58) concentrations in healthy dogs, although these values vary slightly from one source to another reference (Warren et al, 2011).

### Testosterone measurement

Testosterone concentration was measured using ELISA method by commercial testosterone kits (Atieh Perfect Diagnostic Co., Iran) and then the results were interpreted by ELISA reader (Tisa Teb Novin Azma, Tehran, Iran). The basis of competitive ELISA is the amount of antibody bound to the antigen adhering to the bottom of the plate well. The hormones were measured according to the manufacturer's instructions. Normal levels of testosterone are observed between 0.5-9 ng/dl (Warren et al, 2011).

### Statistical analysis

All data are expressed as mean±SE in different groups (A, B and C). Statistical comparisons were accomplished among groups using repeated measures ANOVA, one way analysis of variance and followed

by Kolmogorov-Smirnov and Kruskal Wallis test. Statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., IL, Chicago, USA). Differences were considered significant when  $P \leq 0.05$ .

### Results

The obtained results showed that the distribution of variables were normal for blood cholesterol, LDL-C and HDL-C concentrations, but the distribution for testosterone, triglyceride and VLDL-C levels were abnormal; therefore, they were analyzed using non-parametric Kruskal Wallis test. The mean±SD of cholesterol, triglyceride, LDL-C, VLDL-C, HDL-C and testosterone concentrations are shown in each group of obese, normal and thin dogs (Table 1). It was found a significant difference in cholesterol levels between the different groups ( $P < 0.05$ ). These values were statistically significant between obese and thin dogs ( $P < 0.05$ ); but it was not significant, between the normal group with the other two groups ( $P > 0.05$ ), as well as, the difference was significant for triglyceride levels, between the three groups and these values were statistically different between the obese and normal groups, and also between the obese and thin groups ( $P < 0.05$ ). The difference was significant for LDL-C concentration between the obese and thin dogs ( $P < 0.05$ ), but it was not significant between the normal group with the other two groups ( $P > 0.05$ ). In the following, the difference was significant for VLDL-C concentration between dogs in group A with groups B and C also ( $P < 0.05$ ); however, it was no significant between groups B and C ( $P > 0.05$ ). It was shown that the difference was not significant for HDL-C levels between the three groups ( $P > 0.05$ ). Also, it was detected that the difference was significant for testosterone concentration between the three groups ( $P < 0.05$ ). The mean±SD and statistical relationship of the above parameters are marked for all three groups in Table 1.

**Table 1: Mean±SD of lipid profiles (mg/dl) and testosterone concentration (ng/dl) in dogs of groups A, B and C**

Animal condition (group)	Obese (A)	Normal (B)	Thin (C)
Testosterone (ng/dl)	3.36±0.57 <sup>a</sup>	5.71±0.55 <sup>b</sup>	9.00±0.82 <sup>c</sup>
Cholesterol (mg/dl)	241.08±67.25 <sup>a</sup>	211.42±69.55 <sup>ab</sup>	203.54±59.89 <sup>b</sup>
Triglyceride (mg/dl)	122.06±67.99 <sup>a</sup>	85.40±21.31 <sup>b</sup>	76.24±29.67 <sup>b</sup>
HDL-C (mg/dl)	82.98±26.86 <sup>a</sup>	87.16±32.73 <sup>a</sup>	97.64±35.65 <sup>a</sup>
LDL-C (mg/dl)	134.18±63.10 <sup>a</sup>	107.17±53.85 <sup>ab</sup>	89.99±55.99 <sup>b</sup>
VLDL-C (mg/dl)	23.96±13.42 <sup>a</sup>	17.09±4.27 <sup>b</sup>	15.25±5.93 <sup>b</sup>

\*Different lower-case letters show significant differences between groups (P<0.05).

## Discussion

The present study showed that body condition score and serum testosterone concentration had an effect on lipid profiles, so that the increase of BCS in obese dogs led to a significant increase in triglyceride, total cholesterol, LDL-C, VLDL-C and a significant decrease in serum HDL-C and testosterone levels. It was also found that a decrease in BCS in thin dogs caused a significant reduction in the levels of triglycerides, total cholesterol, LDL-C, VLDL-C and a significant increase in serum HDL-C and testosterone concentrations. Testosterone inhibits lipid uptake and lipoprotein-lipase activity in adipocytes, and stimulates lipolysis by increasing of lipolytic adrenergic receptors (Ettinger et al, 2017).

The blood testosterone concentration decreases with gain weight, especially in men with abdominal obesity. When Leydig interstitial cells are stimulated by luteinizing hormone from the anterior pituitary gland, testosterone is secreted of these cells. In addition, a number of non-hormonal factors play an important role in the secretion of this hormone, such as insulin-like growth factor I and III, leptin and ghrelin (Etienne, 2020).

There are many environmental and genetic factors that influence on this balance. Obesity is also associated with various endocrine disorders. Obesity may lead to insulin resistance and there is a close relationship between increased abdominal fat accumulation and the degree of insulin

resistance (Xenoulis & Steiner, 2010; Porsani et al, 2019).

According to the sources, most of the articles on the relationship between testosterone levels with BCS and lipid profiles were concentrated in human. In most of these articles, there was an inverse relationship between serum testosterone levels and lipid profiles. In a research, it was specified the relationship between sex hormones (testosterone), leptin and BCS in men. The results showed that the obese people had lower testosterone concentration than normal weight men, as well as, there was an inverse relationship between serum testosterone concentration and BCS (Malik et al, 2019). The results of the present study were completely consistent with the latest studies, so that with the increase of BCS in obese dogs, testosterone concentration decreased and testosterone levels increased with the reduction of BCS in thin dogs. The obtained results indicate the possible importance of testosterone measurement and its administration in obese dogs for weight loss; of course, it should not be forgotten the importance of other hormones, including thyroid hormones, as well as, the effect of diet control and physical activity (Bauer, 2004; Etienne, 2020).

In another study, it was determined the relationship between serum testosterone, age and serum lipoproteins with BCS in men in Golestan province. Their results showed that there was a significant inverse relationship between BCS and testosterone

levels. In their survey, the increase of BCS was also associated with an increase in LDL-C and age. Furthermore, an inverse relationship was observed between HDL-C concentration and BCS (Biabangard et al, 2015). In the present study, many similarities were found with the results of the above researchers, however, no significant relationship was specified in related of age, which may be due to the age limited of dogs between 1-7 years-old. Other researchers stated the relationship between obesity and lipid profiles, by measurement of BCS around the waist that there was a positive and a significant correlation between the above indices and triglyceride, total cholesterol and LDL-C concentrations (Kimiagar et al, 2011).

In an experimental study, it was conducted the relationship between serum adiponectin levels and testosterone, cortisol and lipid profiles in inactive men. Their results showed that low concentrations of adiponectin and testosterone in inactive people can be associated with a disorder in the control of lipid profiles, and this may increase the risk of cardiovascular diseases (Moradi et al, 2014). The results of the latest study showed that testosterone had a lower concentration in inactive individuals that was consistent with the present study; however, because of a series of limitations, it was not possible to assess the association between testosterone concentration with adiponectin and cortisol. Also, the evaluation of sperm was not performed (especially for motility); therefore, it is proposed that in future studies, semen parameters are considered in dogs.

It was evaluated the relationship between testosterone concentration and BCS. Their results showed that men with low testosterone concentration were more obese than people with normal testosterone (Shamim et al, 2015). In another retrospective study, it was determined the effect of testosterone concentration on lipid profiles. The researchers stated that the total testosterone had a negative and linear

correlation with triglyceride, total cholesterol and LDL-C levels and a positive and linear correlation with HDL-C concentration. Testosterone low concentration may have harmful effects on lipid profile, indicating a risk factor for hypercholesterolemia, hypertriglyceridemia, increase of LDL-C and decrease of HDL-C concentration (Zhang et al, 2014). It was conducted a study on sixty obese men and examined the relationship between testosterone levels and obesity. They found that with increase of BCS, increased serum leptin levels, triglyceride, insulin, LDL-C to HDL-C ratio, while decreased insulin sensitivity and serum testosterone concentration (Goncharov et al, 2009). In the present study, it was shown that with increase of BCS (in obese dogs), serum testosterone and HDL-C levels decreased, but LDL-C increased, indicating that dogs are at risk.

There is a correlation between hyperlipidemia and animal gender, age and castration in very obese dogs (BCS= 8-9) compared with the control group (BCS=5) (Brunetti et al, 2011). In the present study, there was a significant association between hyperlipidemia and obesity, however, since all dogs were intact male and were not operated (including castration), it was not possible to check the effect of gender and castration. In another survey on Beagle dogs, it was determined that there was a relationship between obesity and hyperlipidemia, so that chronic obesity caused to a significant increase in concentrations of triglyceride, cholesterol and plasma leptin (Jeusette et al, 2005). It has been emphasized the measurement of lipid profiles to confirm obesity, in addition to consider of BCS (Etienne, 2020).

There was an inverse relationship between BCS and the number of daily walks in dogs (Warren et al, 2011). Although in the present study, it was not investigated in different groups of the dogs, but it is clear that with increase of physical activity, BCS decreases. In another survey

on dogs in China, it was reported an overall prevalence of obesity about 44.4%. Risk factors in obese dogs were related to type of food, castration or ovariectomy, number of feedings per day and type of animal activity and gender. The prevalence of obesity was higher in breeds of Pug, Cocker spaniel, Pekingese, Pomeranian and Golden retriever (Mao et al, 2013). It was also announced the prevalence of obesity about 34.1% in dogs over one years-old. Castrated animals and those were fed mostly with semi-wet commercial foods were more affected (Perry et al, 2020). In the present study, the studied dogs were German shepherd or Doberman Pinscher, male and in the age range 1 to 7 years-old.

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### **Conflict of interest**

The authors declare that they have no conflict of interest.

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## بررسی ارتباط بین شاخص امتیاز بدنی با پروفایل‌های لیپیدی و غلظت تستوسترون سرم در سگ

سجاد چگینی<sup>۱</sup>، بهمن مصلی‌نژاد<sup>۲\*</sup>، محمد راضی‌جلالی<sup>۲</sup> و سعد گورانی‌نژاد<sup>۲</sup>

<sup>۱</sup> دانش‌آموخته دکتری عمومی دامپزشکی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

<sup>۲</sup> استاد گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

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### چکیده

فاکتورهای متعددی، در ایجاد چاقی در حیوانات نقش دارند، که از مهم‌ترین آن‌ها می‌توان فقدان تمرین، تغذیه‌ی بیش از حد و اختلالات هورمونی را نام برد. تستوسترون، مسوول بسیاری از مشخصات فیزیکی در حیوانات نر است. شاخص امتیاز بدنی (BCS)، جهت مشخص نمودن وضعیت بدن به کار برده می‌شود. وزن بدن، با اخته کردن، به شکل معنی‌داری در سگ‌ها افزایش می‌یابد. هدف از انجام تحقیق حاضر، بررسی ارتباط بین شاخص امتیاز بدنی با پروفایل‌های لیپیدی و غلظت سرمی تستوسترون در سگ‌های چاق، نر مال و لاغر بود. این مطالعه بر روی ۱۵۰ قلابه سگ نر سالم، ارجاعی به بیمارستان دامپزشکی دانشگاه شهید چمران اهواز، انجام شد. سگ‌های صاحب‌دار، به سه گروه مساوی تقسیم شده بودند: گروه A (چاق)، گروه B (نرمال) و گروه C (لاغر). ۵۰ قلابه سگ در هر گروه وجود داشت. از سیستم BCS، شماره ۱ تا ۹، استفاده شده بود. سگ‌ها از بین نژادهای بزرگ (ژرمن شفرد و دوبرمن پینچر)، جنس نر و در محدوده‌ی سنی ۱ تا ۷ سال انتخاب شدند. فاکتورهای لیپیدی سرم (غلظت تری‌گلیسرید، کلسترول تام، HDL-C، LDL-C و VLDL-C) با استفاده از روش اسپکتروفتومتری اندازه‌گیری شدند. غلظت تستوسترون نیز به روش الیزا، تعیین گردید. شاخص توده‌ی بدنی و غلظت تستوسترون، بر میزان پروفایل‌های لیپیدی تأثیر داشتند، به نحوی که افزایش شاخص توده‌ی بدنی در سگ‌های چاق (A)، منجر به افزایش معنی‌دار در سطح تری‌گلیسرید (۱۲۲/۰۶±۶۷/۹۹ میلی‌گرم/دسی‌لیتر)، کلسترول تام (۲۴۱/۰۸±۶۷/۲۵)، LDL-C (۱۳۴/۱۸±۶۳/۱۰) و VLDL-C (۲۲/۹۶±۱۳/۴۲) و کاهش معنی‌دار HDL-C (۸۲/۹۸±۲۶/۸۶) و تستوسترون (۳/۳۶±۰/۵۷) گردید. همچنین مشخص گردید که کاهش شاخص توده‌ی بدنی در سگ‌های لاغر (C)، باعث کاهش معنی‌دار در سطح تری‌گلیسرید (۷۶/۲۴±۲۹/۶۷ میلی‌گرم/دسی‌لیتر)، کلسترول تام (۲۰۲/۵۴±۵۹/۸۹) LDL-C (۸۹/۹۹±۵۵/۹۹) و VLDL-C (۱۵/۲۵±۵/۹۳) و افزایش معنی‌دار HDL-C (۹۷/۶۴±۳۵/۶۵) و تستوسترون (۹/۰۰±۰/۸۲) شد. میانگین غلظت تستوسترون در سگ‌های چاق، نرمال و لاغر به ترتیب ۳/۳۶±۰/۵۷، ۵/۷۱±۰/۵۵ و ۹/۰۰±۰/۸۲ نانوگرم/اسی‌سی به دست آمد. نتایج نشان داد که غلظت تستوسترون، با چاقی (افزایش BCS)، کاهش و با لاغری (کاهش BCS)، افزایش می‌یابد. تحقیقات بیشتر لازم است تا درک بهتری از ارتباط بین چاقی و تغییرات هورمونی، مشخص گردد.

**کلمات کلیدی:** شاخص توده‌ی بدنی، سگ، پروفایل‌های لیپیدی، تستوسترون

\* نویسنده مسئول: بهمن مصلی‌نژاد، استاد، گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: bmosallanejad@scu.ac.ir



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