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Association between vitamin D and malignant mammary tumors in obese female dogs

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Abstract

Overweight and obesity are known risk factors that are involved in the development and aggressiveness of breast tumors in women. In situations of obesity, local and systemic inflammation may worsen the prognosis of oncological patients. Moreover, hypovitaminosis D increases the risk of breast tumors in women. In female dogs, low vitamin D levels have been found in cases of lymphoma, osteosarcoma and mast cell tumor. The present study aimed to make correlations between body fat composition and serum 25(OH)D concentration in female dogs with mammary tumors. Two experimental groups were formed: healthy female dogs (n = 12; control group) and female dogs with mammary tumors (n = 11). An analysis on body composition was performed using dual-energy X-ray absorptiometry (DXA) equipment. In the tumor group, multicentric nodules were most prevalent (63,6%), with diameters of up to 8.2 cm. The most frequent histopathological diagnosis was tubular carcinoma (45.5%), and 9.1% presented metastases in axillary lymph nodes. The mean 25(OH)D concentration in the female dogs with mammary tumors (37.6 ng mL-1) was lower than the level in the control group (65.4 ng mL-1). Multivariate statistical analysis showed that elderly female dogs with malignant mammary tumors and high body fat levels presented lower 25(OH)D concentrations than those of the control group. It was concluded that female dogs with higher body adiposity presented mammary tumors of higher aggressivity, and that the higher the fat percentage was in the female dogs with malignant mammary tumors, the lower their vitamin D concentration was.

Key words: female dogs; hypovitaminosis D; body fat; mammary carcinomas.

Introduction

Obesity is frequently observed within the clinical routine of attendance for pets, especially dogs (50, 7), and this clinical condition is seen more among female dogs (38). It comprises a clinical state of excess adipose tissue in the body, with a body weight at least 20 to 25% higher than the ideal (30). This situation provides a chronic inflammatory microenvironment that favors development of tumor cells (20).

Sedentarism and access to an unbalanced diet due to anthropomorphism intensify the state of canine obesity (42). Consumption of fatty foods by female dogs before the age of one year (52) and presentation of obesity (2) are factors that contribute towards occurrences of mammary tumors.

Obese dogs present higher triglyceride levels after meals than those of non-obese dogs (57). Hypercholesterolemia and hypertriglyceridemia are found in obese spayed adult female dogs (6, 16). However, there is a lack of studies correlating obesity among dogs with the development of diseases relating to obesity (10).

Among women, studies correlating the harmful effects of obesity with a variety of diseases have been conducted. Among these effects, inflammatory states in obese individuals have been correlated with high risk of occurrence of breast tumors (19). Moreover, obesity diminishes 25(OH)D concentration in women, because it is sequestered in adipose tissue (31). Adipocytes retain the metabolites of vitamin D and consequently there is greater usage of this vitamin in obese individuals, which gives rise to lower levels in the blood (46). However, 25(OH)D is considered to be an antineoplastic factor, due to its antimetastatic and antiapoptotic action on neoplastic cells (12).

The antitumor action of vitamin D is partly due to the nuclear receptors of vitamin D, which provide support for this vitamin to regulate the expression of proteins that diminish intracellular energy and thus cause tumor cell death through apoptosis (1). Vitamin D is also known to inhibit angiogenesis, such that it regulates exacerbated proliferation of tumor cells (43).

In obese dogs, tumors are the third most frequent disease, losing only to oral and skin diseases (35). Among these tumors, mammary tumors have a major impact within veterinary medicine because most of them are malignant (24), and these occur more frequently in female dogs (8, 45). Mammary tumors are commonly observed in middle-aged or elderly female dogs (3) that were spayed late or not at all, those with recurrent false pregnancy and those that had received progestogens (53). The observed consequences of obesity mean that routine weight control becomes important, with the aim of preventing this disease (5).

EThe active form of vitamin D, also known as calcitriol, is a secosteroid that is essential in the diet of dogs (23). The serum levels of this vitamin vary widely and directly reflect the quantity of this vitamin that is supplemented through the diet (51). This is because unlike herbivores and omnivores, when dogs are exposed to the action of ultraviolet type B rays, they do not convert the cholesterol derivative 7-dehydrocholesterol in their skin into cholecalciferol (vitamin D3), efficiently or sufficiently. They are therefore dependent on dietary intake (23).

Although dogs can obtain their vitamin D mainly through their diet (58), their vitamin D levels can also be diminished, just like humans' vitamin D levels, in the presence of certain tumors such as lymphoma, osteosarcoma and mast cell tumor (59). Vitamin D supplementation is indicated for women as a protective factor against breast cancer (13). However, in a search of the literature, we did not find any studies corelating vitamin D levels with mammary tumors in obese female dogs, or on the importance of use of vitamin D as a protective factor against these tumors. It is known that vitamin D supplementation in mice gives rise to higher baseline levels of autophagy in normal mammary gland cells (54). Moreover, these authors highlighted the potential of vitamin D as a tumor protection factor.

Large number of cases of obese female dogs with mammary tumors are observed within routine clinical attendance. Furthermore, an inverse relationship between obesity and 25(OH)D levels in women has been described (31). Therefore, the aim of the present study was to elucidate the correlation between serum 25(OH)D levels and obesity in female dogs with malignant mammary tumors.

Material and methods

Selection of animals and epidemiological evaluation

Female dogs were selected without preference for breed or age at the Laudo Natel Veterinary Hospital, School of Agrarian and Veterinary Sciences, UNESP, Jaboticabal Campus, São Paulo, Brazil. For this, the dogs' owners were informed about the procedures that were to be performed and upon giving their authorization, they signed a consent statement. The study was assessed and approved by the Ethics Committee for Animal Welfare of the School of Agrarian and Veterinary Sciences, UNESP, Jaboticabal, state of São Paulo, Brazil, under the protocol number 010092/17.

An epidemiological evaluation was undertaken through interviews with the dogs' owners. The following information was obtained: age, breed and size of the animal; diet (commercial feed, homemade food or a mixture of these two); whether food was being provided in accordance with parameters set by a veterinarian or those of the feed pack label, or whether it was being provided in an arbitrary manner; whether the dog was being offered manufactured snacks or cooked meat; whether the dog was taken for walks and the frequency of these walks. The reproductive profile of the female dogs was also investigated, including their use of contraceptives, occurrences of false pregnancies, spaying, weight gain after spaying and development of sedentary behavior after ovariohysterectomy.

Experimental groups

The female dogs were divided into a control group (CG) comprising 12 animals without mammary tumors and a tumor group (TG) of 11 animals presenting malignant mammary tumors. Clinical examinations, blood tests, electrocardiographic examinations and investigations on diseases that would interfere with body composition or would cause a risk relating to the anesthetic procedure were performed in both groups. In the TG, in addition to these procedures, radiographic examinations were performed on the thorax and ultrasonographic examinations on the abdomen, to investigate metastases. In the absence alterations, the animal was included in the study.

Serum analyses on 25-hydroxyvitamin D and cholesterol

The female dogs were firstly subjected to fasting for 12 hours. Blood samples were then collected under aseptic conditions by means of jugular vein puncture. These samples were kept in vacuum collection tubes (Vacutainer®) with a capacity of 5 mL, without Röschlau anticoagulant. The tubes were centrifuged at 1736.11 G for 10 minutes, to obtain serum. This was stored in 1.5 mL polypropylene microtubes (Eppendorf®) and frozen (-20 °C), for subsequent analysis on the levels of 25(OH)D, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol. The assays on 25(OH)D were performed using the chemiluminescence method, with the reagent Access 25(OH) Vitamin D Total in DXi (Beckman Coulter) (51). The cholesterol and triglyceride assays were performed using enzymatic methods; HDL assays were done using direct colorimetry (48); and these assays were performed in the Mindray BS-200E equipment. The reagents used were enzymatic AA liquid (Wiener Lab) for cholesterol assays; Color GPO/PAP AA liquid (Wiener Lab) for triglyceride assays; and Labtest for HDL assays.

Body composition evaluation

Body composition analyses were performed in the Laboratory for Animal Body Composition and Bone Evaluation Studies, Department of Animal Morphology and Physiology, School of Agrarian and Veterinary Sciences, UNESP, Jaboticabal Campus.

The female dogs were weighed on an analytical electronic balance (Filizola, Personal Line®) and were classified according to their body condition score, from one to nine (29). Their lengths wee measured from the snout to the tail.

The Discovery Si device (Hologic Delphi QDR, Hologic Discovery QDR, Bedford, MA, USA) was calibrated with a phantom.

The female dogs were subjected to pre-anesthesia medication with chlorpromazine at a dose of 1 mg/kg and anesthesia was induced using propofol at a dose of 5 mg/kg (Claris Injectables Limited, India), both intravenously. After the postural reflex had ceased, the patients were positioned in sternal decubitus, close to the Hologic Discovery Si device, which was compatible with the animals' size. Three consecutive sweeps were performed, without any repositioning, using the small animal software supplied by the equipment manufacturer. The following were then determined: percentage body fat (%), quantity of body fat (g), bone mineral content (g), bone mineral density (BMD) (g/cm2), total lean mass, total bone mineral content (g) and percentage lean mass (%) (33).

After the body composition analysis, the female dogs in the mammary tumors group underwent unilateral radical mastectomy in accordance with routine procedures at the Laudo Natel Veterinary Hospital. Material was collected for histological analysis, independent of the nodule size.

Sample processing and histological classification

After surgical removal, the mammary chain with the mammary tumor and axillary and inguinal lymph nodes from the same side, were prepared for histopathological examination. The tissue samples were fixed in 10% formalin solution buffered with monohydrated sodium phosphate acid and anhydrous disodium phosphate (pH 7.4), diced, dehydrated in an increasing series of ethanol concentrations, cleared in xylol and impregnated and embedded in paraffin. Semi-serial sections of thickness 3 micrometers were laid out on histological slides for staining with hematoxylin and eosin, and were then evaluated under an optical microscope.

The tumors were histologically classified as prescribed by the World Health Organization (40) and the guidelines of the second consensus for diagnosing canine mammary tumors (9). In the cases in which there was more than one tumor, the one that presented histological characteristics of greater malignancy was analyzed in this study.

Statistical analysis

In the statistical analysis, the data were subjected to multivariate analyses, including principal component analysis (PCA) and factor analysis. The PCA took into consideration the serum vitamin D and LDL levels, percentage fat, age, bone mineral density, weight, body condition score, physical activity practiced and type of food supplied.

The multivariate structure of the initial dataset was analyzed by a PCA, that condenses relevant information into a smaller set of orthogonal variables (eigenvectors), that are generated by a linear combination of the original variables. The first principal component extracted from the covariance matrix is a linear combination of the original variables, and it accounts for as much of the variation in the samples as possible. The second component is the second linear function of the original variables, and it accounts for the majority of the remaining variability. The coefficients of the linear functions defining the factors are used to interpret their meaning. The sign and relative size of the coefficients are indications of the weights to be used for each variable, and the selected components and factors on PCA, we considered only the variables associated with these components with coefficients higher than |0.50| (22, 32). A bidimensional representation known as a biplot, was created for the principle components. This enabled visualization of the structure of the studied variables and explained the maximum variability of the entire set of variables studied. The first two principal components, PC1 and PC2, were considered, and their eigenvalues were greater than unity (27).

The non-parametric test took into consideration Body fat, age, body weight, 25(OH)D and BMD.A level of significance was considered ($p < 0.05$) (37).

Table 1. Profile of the female dogs in the control group (CG) and mammary tumor group (TG), from information obtained through interviews with the dog owners

Characteristics	**Frequency (%) in CG**			**Frequency (%) in TG**		
DIETARY ITEMS						
	Yes	**No**	-	**Yes**	**No**	-
Snack consumption	50.0	50.0	-	46.0	54.0	-
Meat consumption	41.6	58.3	-	46.0	54.0	-
	Commercial feed	**Commercial feed and homemade food**	-	**Commercial feed**	**Commercial feed and homemade food**	-
Type of food	58.33	41.66	-	36.4	63.6	-
	Feed label	**Veterinarian**	**Dog owner**	**Feed label**	**Veterinarian**	**Dog owner**
Quantity of feed according to the label	25.0	25.0	50.0	27.3	0.0	72.7
PHYSICAL ACTIVITY						
Walks	50.0	50.0	-	18.2	81.8	
REPRODUCTIVE INFORMATION						
	Yes	**No**	-	**Yes**	**No**	-
Spaying	91.6	8.3	-	18.2	81.8	-
	Yes	**No**	**Not spayed**	**Yes**	**No**	**Not spayed**
Weight gain after spaying	8.3	75.0	1.7	9.1	90.9	81.8
Sedentarism after spaying	0.0	91.6	8.3	9.1	90.9	81.8
Contraceptives	0.0	100.0	0.0	9.1	90.9	81.8
False pregnancy	0.0	100.0	0.0	18.3	71.7	-

Results

For the means of the variables age and serum 25(OH)D concentration, the control group and the tumor group had a significant difference between them with the 5% significance level.

The mean age of the female dogs in the control group was four years and most of them were of mixed breed. Regarding reproductive status (Table 1), one of these dogs (8.3%) had not been spayed. Among the spayed dogs, nine (75%) had undergone this procedure between the ages of 2 and 14 months; one had been spayed at the age of six years; and the age at which this was done was unknown in one case. Six of the control group dogs were walked: one (8.3%) every day; two (16.7%) three times a week, one (8.3%) twice a week, one (8.3%) once a week and one (8.3%) only sporadically.

In the tumor group, the mean age of the female dogs was 9.5 years and 40% were seven years old; most of them were of mixed breed (63.3%). Only two (18.2%) of these dogs had been spayed (Table 1): one between the ages of two and three years; and the other at the age of six years. False pregnancies had been observed in 18.2%. Only two of these dogs were walked: one, daily; and the other, three times a week.

The 25(OH)D concentration in the control group ranged from 21.4 ng mL-1 to 104.8 ng mL-1, with a mean of 65.4 ng mL-1 (± 25.77), in the tumor group, it ranged from 6.3 ng mL-1 to 56.4 ng mL-1, with a mean of 37.6 ng mL-1 (± 18.5). Thus, the mean 25(OH)D concentration was higher in the control group than in the tumor group. These values are shown in Table 2, in decreasing order of percentage fat.

The serum LDL concentration in the control group ranged from 10 mg dL-1 to 185 mg dL-1, with a mean of 50.8 mg dL-1; while in the tumor group, it ranged from 16 mg dL-1 to 188 mg dL-1, with a mean of 65.8 mg dL-1, i.e. higher than that of the control group.

The mean body weight of the female dogs in the control group was 21 kg, while it was 14 kg in the tumor group. The body weights ranged from 4.3 kg to 40 kg.

The control group presented morbid obesity in 58.33% of the bitches; severe obesity was verified in 16.67%, obesity in 8.33% and different from the tumor group, 25.0% overweight. The mean percentage of body fat was 33.98 (± 7.81).

The percentage body fat results from the tumor group, evaluated by means of DXA, demonstrated that 54.5% of these animals were morbidly obese, such that 37.9% to 57.9% of their body composition was fat. Severe

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Table 2. Body fat (%), body weight (kg), age (years), bone mineral density (g cm-2) and 25(OH)D concentration (ng mL-1) of the female dogs in the control group (CG) and mammary tumor group (TG), in relation to the histopathological diagnoses of the mammary tumors

Breed		Body fat		Age		Body weight		25(OH)D		BMD		Histopathological diagnosis
CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	TG
Basset	NDB	47.7	57.9	9.0	9.0	4.3	16.6	50.8	28.2	0.5760	0.5900	Mammary osteosarcoma
NDB	NDB	40.4	45.1	3.0	14.0	37.2	7.5	70.4	33.5	0.9673	10.596	Grade I carcinoma in mixed tumor
NDB	Rottweiler	39.7	41.1	7.0	7.0	6.2	40.0	56.3	6.3	0.5876	0.9710	Mammary squamous cell carcinoma
NDB	NDB	38.8	39.4	5.0	10.0	5.8	8.3	21.4	33.9	0.5670	0.6760	Grade II tubular carcinoma
NDB	NDB	38.5	38.2	2.5	12.0	33.4	4.3	52.2	36.8	10.410	0.4570	Grade I tubular carcinoma
Labrador	Poodle	36.4	37.9	2.92	9.0	28.0	6.4	51.5	68.1	0.9370	0.2863	Grade II tubular carcinoma
Golden Retriever	Pitbull	34.7	37.0	7.0	11.0	13.1	28.8	104.8	65.3	0.7906	0.8890	Grade I carcinoma in mixed tumor
NDB	NDB	33.3	34.2	5.0	7.0	13.9	13.0	56.2	31.8	0.7226	0.7420	Grade III papillary carcinoma
NDB	NDB	26.0	30.7	5.0	7.0	14.5	4.5	90.9	56.4	0.8195	0.600	Grade I carcinoma in mixed tumor
NDB	NDB	24.8	28.7	0.92	11.0	23.9	7.6	39.9	26.1	0.8946	0.522	Grade II papillary carcinoma
NDB	NDB	24.7	22.6	1.17	7.4	13.9	16.6	95.3	27.7	0.7243	0.603	Grade I tubular carcinoma
NDB	-	22.8	-	1.3	-	5.3	-	94.5	-	0.5983	-	-
Means		34.0	37.5	4.0b	9.5a	21.0	14.0	65.4a	37.6b	0.7688	0.672	-

“a” and “b” - according to a non parametric test (37).

obesity was seen in 18.2% and another 18.2% were classified as obese.Only 9.1% presented a percentage body fat for which the body composition score was 7, which indicates overweight. These values are shown in Table 2. Mean body fat percentage was 37.5 (± 9.2). There was no statistical difference between groups considering the mean percentage of body fat.

Among the 11 female dogs in the tumor group, there was a total of 16 nodules. Seven animals (63.6%) presented multicentric tumors, while four (36.3%) had single nodules.

The size of the nodules was up to 3 cm in 45.4% of the female dogs, and these were diagnosed as follows: squamous cell carcinoma, grade II tubular carcinoma, grade I tubular carcinoma and one case of grade I tubular carcinoma in which the mammary gland did not present any macroscopic alterations.

Nodules of diameter 3 to 5 cm were present in 36.4% of the cases, and these were diagnosed as follows: grade I carcinoma in mixed tumor, grade III papillary carcinoma and grade II papillary carcinoma.

Nodules larger than 5 cm accounted for 18.2%. Two nodules measuring 5.0 and 6.8 cm were diagnosed as grade I carcinoma in mixed tumor; and one nodule of 8.2 cm was diagnosed as mammary osteosarcoma. The female dog that presented the nodule of largest size also presented the highest percentage fat (57.9%) among the animals in this study.

The cranial and caudal mammary glands were the ones most affected, since malignant tumors were observed in 27.27% of the cases at each of these locations, in 18.2% of the cases at the thoracic caudal mammary gland and in 9.1% of the cases at each of the abdominal cranial, abdominal caudal and inguinal mammary glands. There was also one case (9.1%) in which the inguinal mammary gland only presented a perceptible tumor upon microscopic examination of the sample.

The mammary tumors most frequently diagnosed (Table 2) (Fig. 1) were the following: tubular carcinoma (45.5%), carcinoma in mixed tumor (27.8%), papillary carcinoma (9.1%), squamous cell carcinoma (9.1%) and osteosarcoma (9.1%). More than one histological type of tumor was diagnosed in 63.3% of the female dogs; and 9.1% of the animals had benign and malignant tumors in the same mammary chain.

Among the 11 female dogs with mammary tumors, seven (63.6%) only had mammary tumors, while four (36.4%) also presented other tumors: uterine leiomyoma, lipoma and poorly keratinized squamous cell carcinoma in the skin. Moreover, one female dog presented skin nodules that were diagnosed as squamous cell carcinoma and hemangiosarcoma, along with splenic hemangiosarcoma.

The female dog with mammary osteosarcoma died 40 days after the diagnosis had been made. The dog diagnosed with grade III papillary carcinoma presented metastasis in the axillary lymph nodes upon histopathological examination.

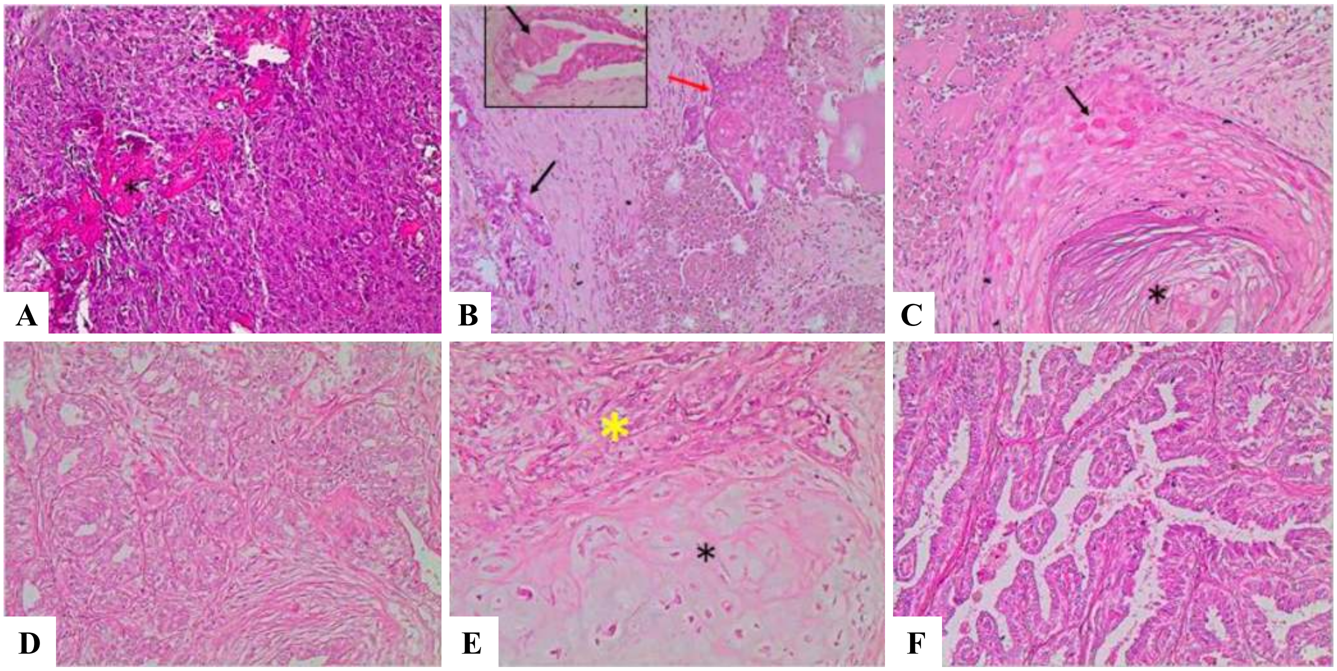

Figure 1. Photomicrographs of the main canine mammary carcinomas histologic subtypes. **A.** Mammary osteosarcoma. Note focus with rudimentary bone trabeculae (*) surrounded by neoplastic osteoblasts. Obj. 40x). **B.** Mammary squamous cell carcinoma. Note a focus of tubular proliferation (black arrow) adjacent to focus of squamous metaplasia of the mammary epithelium (red arrow). (Obj. 10x). Note the alteration of the tubular epithelium in the detail in B (arrow). **C.** Mammary squamous cell carcinoma with keratin pearls formation (*) and individual keratinization foci of tumor cells (arrow, Obj. 20x). **D.** Tubular carcinoma (Obj. 20x). **E.** Carcinoma in mixed tumor (Obj. 20x). Note area of tubular proliferation (yellow *) adjacent to chondroid matrix focus (black *). **F.** Papillary carcinoma (Obj. 20x). Hematoxilin and Eosin.

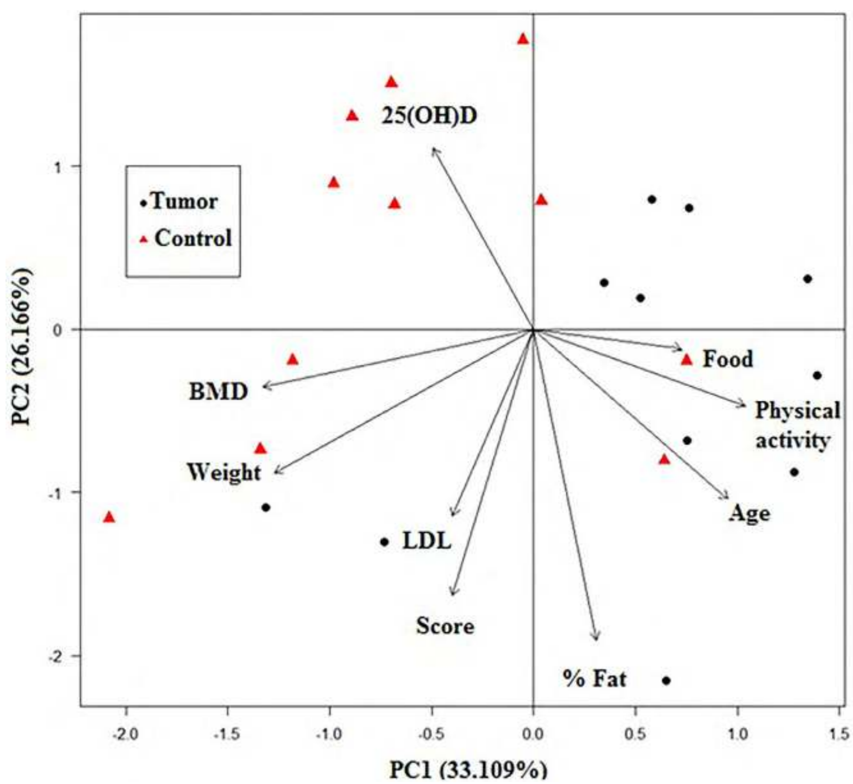

Figure 2. Principal component analysis on the variables relating to the control group and mammary tumor group. The variables considered were the serum vitamin D and LDL levels, percentage fat, age, bone mineral density (BMD), weight, body condition score, physical activity practice and type of food supplied.

Multivariate analysis

The first two main components were evaluated, which together obtained 59.275% of the accumulated explained variance.

Principal component 1 explained 33.109% of the total variance of the standardized variable matrix. Main component 2 explained 26.166% of the information variability. This information illustrates the importance of the analyzed variables, which were responsible for separating the animals from the tumor group in relation to the control group (Fig. 2).

Then, the factor analysis was performed to explain which are the most important variables, capable of separating the groups and how they are related (Table 3).

Factor 1 explained 33.109% and demonstrated that weight and BMD expressed negative values and above 0.5, so they are factors that vary inversely to the variables age and physical activity, which showed positive results and above of 0.5. In other words, older animals that practice physical activity had lower BMD values and lower body weight.

Factor 2 showed significant positive correlations in relation to the variable serum vitamin D level and was negatively correlated with percentage of fat, body condition score and LDL. Thus, bitches with high percentages of fat, high body score and high serum LDL levels tend to have lower serum levels of vitamin D.

The three bitches in the control group located in the upper levels of the 4th quadrant, in Figure 2, presented the highest levels of vitamin D, which corresponded to 104.8, 95.3 and 94.5 ng mL-1 respectively.

Discussion

Statistical difference was found ($p < 0.05$) in the mean age between the groups, with the control group having an average of 4 years and the tumor group, 9. Our result agrees with those observed by other authors who observed an increase in mammary tumors in bitches between 6 and 10 years old, with a mean age of 10 years (44). In addition to old age being considered a risk factor for the incidence of mammary tumors (56), it is also associated with the diagnosis of tumors with a higher degree of malignancy (45). Therefore, the advanced age found in our study should be considered an important variable in the presence of mammary tumors.

Elderly animals do less physical activity (18) and present lower baseline metabolic rates. This predisposes them to weight gain (35). In study conducted in China revealed that 68.6% of the dogs over the age of 11 years were obese (38). The obese female dogs in the present study were the older animals (34).

Among female dogs, each additional year of life carries a 23% increase in the risk of mammary tumors (14). This has been seen through the observation that elderly adult female dogs are more likely to present these tumors (21, 3).

Mammary tumors were observed more frequently in elderly adult dogs, especially those that had not been spayed. This may have been due to changes to the concentrations of circulating hormones, such as estrogen (9). This was also seen in the present study, in which the mammary tumor group was formed by female dogs of mean age 9.5 years that mostly had not been spayed. When female dogs are not spayed before their first heat, their propensity towards mammary tumors is greater because of their higher exposure to hormones (28).

In a previous study, dogs without any defined breed represented 28% of the obese population, followed by animals of the breeds cocker spaniel (10.3%), poodle (10.3%), dachshund (6.5%) and Labrador (5.6%) (26). In the present study, female dogs of no defined breed with mammary tumors were highly prevalent (63.3%) (Table 2). This was also observed by other authors (3), while other papers reported that this occurred most frequently in poodles (36, 14).

Table 3. Results from factor analysis on the variables among the female dogs in this study.

Variable	Factor 1	Factor 2
Body condition score	-0.270	-0.753*
Vitamin D level	-0.333	0.513*
LDL level	-0.268	-0.528*
Weight	-0.858*	-0.407
Percentage body fat	0.208	-0.879*
Age	0.642*	-0.481
Type of food	0.489	-0.055
Physical activity	0.699*	-0.216
Bone mineral density (BMD)	-0.897*	-0.164
Proportion of variance explained	33109%	26166%

*, r > |0.5| - values taken into consideration in interpreting the factors.

In another study conducted in Brazil, 35% of their female dogs had been spayed before the tumor was diagnosed, 9% were receiving contraceptives, 31.1% had presented false pregnancies, 50% were only given commercial feed and 34.4% were given both commercial feed and homemade food (36). These results corroborate the percentage of female dogs were receiving contraceptives in our study. However, we observed that only 18.2% had been spayed, 18.2% had had false pregnancies and 36% were exclusively given commercial feed, while the remainder were given both commercial feed and homemade food. It can be suggested that the profile of these female dogs influenced the differentiation between the tumor group and the control group.

High serum LDL concentration and high expression of Low density lipoprotein receptor (LDLR) in tumor cells are important for the growth of mammary tumors (15). This may have been reflected in the present study, in which the tumor group presented higher serum LDL concentrations and higher mean LDL values than the control group.

The body weight of healthy female dogs has been found to range from 5 kg to 74 kg (61). There was higher incidence of mammary tumors in obese female dogs (47). In the present study, the weights in the control group ranged from 4.3 kg to 37.2 kg and in the tumor group from 4.3 to 40 kg. It was inferred that the body weight was similar between the groups and that the diversity of breeds and sizes of the animals in the present study explained the weight variation.

The percentage fat can be estimated from the body condition score (39). In the present study, there was an association between occurrences of mammary tumors, percentage body fat and body condition score. It is known that the rate of occurrence of breast cancer is 30% to 50% higher among obese women (17). In our study, we observed an association between the percentage body fat and occurrences of mammary tumors, this result is similar to previously reported findings in which 89% of the female dogs with mammary tumors were obese (47). Healthy female dogs presented percentage fat of between 20.4% and 65.2% (61). In the present study, this percentage was between 22.8% and 47.7% in the control group and between 22.6% and 57.9% in the tumor group.

We observed that the four female dogs in the tumor group that had the highest percentage fat presented some of the lowest serum vitamin D levels, which did not exceed 34 ng mL-1. It is known that in dogs with chronic enteral diseases, the serum vitamin D levels are inversely related to systemic inflammation markers (55). Thus, from analysis on the results from the present study, it can be suggested that the greatest percentage body fat found in the tumor group may be correlated with increased quantities of inflammatory proteins, which are associated with presence of mammary tumors and with obesity (25).

The size of the tumor is related to the malignancy of the tumors (42, 9). This was also seen in the present study, in which tumors of more aggressive behavior were prevent in 54.6% of the female dogs with nodules of diameter greater than 3 cm. It can be emphasized that microscopic evaluation of all mammary glands should be a routine protocol, including for those without apparent nodulation, given that we diagnosed one case (9.1%) of grade I tubular carcinoma in an inguinal mammary gland that did not present macroscopic alterations.

Most mammary tumors in female dogs are not malignant (14, 24), but carcinomas are the most common histotype (3, 4). The most prevalent subtypes have varied between studies; for example, solid carcinoma (32.2%) (14) or papillary carcinoma (50.5%) (11). In the present study, tubular carcinoma was most prevalent (45.5%), followed by carcinoma in mixed tumor (27,8%).

In the present study, the highest percentage fat was found in a female dog with mammary osteosarcoma. Occurrences of appendicular osteosarcoma in dogs were unrelated to disease progression or to the survival of obese dogs (49). On the other hand, it is known that although mammary osteosarcoma is uncommon, it is frequently associated with higher rates of metastasis and lower survival among women with breast cancer (60).

More aggressive tumors (66.7%) were observed in female dogs with lower levels of vitamin D. It can be highlighted that the female dog that presented the lowest serum vitamin D level was diagnosed as presenting squamous cell carcinoma, which is one of the tumors with worse prognosis, and also presented 41.1% body fat. Our results were similar to those of other authors (34), who found that the proportion of poorly differentiated tumors was higher in female dogs that were overweight or obese.

Higher 25(OH)D levels are associate with lower risk of breast cancer in women (62), thus demonstrating that vitamin D has a protective effect against this type of tumor. Although there were no measurements of 25(OH)D concentration in the present study before the disease was diagnosed, the serum concentrations found in the tumor group were lower than those in the control group. In this context, other authors confirmed that there was a relationship between tumor presence and altered vitamin D metabolism in dogs with osteosarcoma, lymphoma and mastocytoma, which corroborates our results relating to mammary tumors (59).

Synthesis of vitamin precursors in the skin of dogs takes place in inadequate quantities, such that dogs are dependent on their food sources to supply their vitamin D needs (58). Serum vitamin D levels are influenced by a variety of factors, and they may vary according to the dietary supplementation, quality of feed and factors inherent to the animal, such as breed, sex and reproductive status (51). Considering the good serum results among dogs that receive vitamin D supplementation and the importance of this vitamin for a healthy life, use of vitamin D supplementation in female dogs with mammary tumors should be investigated, especially among elderly and obese animals (51).

Conclusions

There is a relationship between body adiposity and vitamin D status among female dogs with mammary tumors.

Serum 25(OH)D concentration should be investigated within the routine veterinary procedures for obese female dogs with mammary tumors, with the objective of assessing whether there is any need for dietary vitamin D supplementation. Moreover, the results from the present study suggest that vitamin D levels should be investigated frequently among obese elderly female dogs and that this should be inserted into the hospital routine for prevention of mammary tumors of greater aggressivity.

Our study demonstrates that there is a need for the body condition score to be maintained at ideal levels in order to prevent occurrences of more aggressive mammary tumors and, consequently, to prevent early death among female dogs with these mammary tumors.

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Conflict of Interest Statement

The authors declare that there were no conflicts of interest in conducting this study.

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