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Relation between Habitual Diet and Canine Mammary Tumors in a Case-Control Study

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In the present case–control study several dietary and nutritional factors were investigated to determine if a relationship exists between diet and development of mammary tumors in female dogs. Control female dogs (n = 86) were compared with a case group of dogs (n = 102) with dysplasias or tumors of the mammary gland. A questionnaire providing information on the dog's body conformation and dietary and reproductive histories was answered by the owners. Serum selenium and retinol concentrations and the fatty acid profile in subcutaneous adipose tissue were analyzed as indicators of nutritional status. Obesity at 1 year of age and 1 year before the diagnosis of mammary nodules was found to be significantly related to a higher prevalence of mammary tumors and dysplasias. The intake of homemade meals (compared to that of commercial foods) was also significantly related to a higher incidence of tumors and dysplasias. Other significant risk factors were a high intake of red meat, especially beef and pork, and a low intake of chicken. The subcutaneous fatty acid profile and the serum selenium concentration were not significantly different in the cases and the controls, with the exception of C18:1 fatty acid (oleic acid) content, which was significantly higher in the cases than in healthy controls. Serum retinol concentration was significantly lower in the cases than in the controls. In the multivariate analysis, older age, obesity at 1 year of age, and a high red meat intake were independently and significantly associated with the risk of developing mammary tumor and dysplasias.

Key words: Diet; Dog; Fatty acids; Mammary plasia; Red meat; Retinol; Selenium.

Canine mammary tumors are encountered frequently in veterinary practice; their annual incidence rate in intact female dogs is approximately 260 per 100,000 dogs at risk.[1] Ovariectomy performed before 2.5 years of age decreases the risk for mammary tumors in female dogs, but other risk factors remain obscure, especially those related to nutritional habits.[2] Sonnenschein and others[3] reported that among ovariohysterectomized dogs, obesity early in life increased the risk of mammary tumors, and the percentage of energy derived from fat in the diet was lower in the cases than in the controls. This is in contrast with what has been observed in humans, in which, generally, the consumption of fat is associated with a higher risk for breast cancer.[4–8]

In addition to the information derived from the dietary questionnaires obtained in this study, plasma concentrations of retinol and selenium were measured. Likewise, in order to assess the intake of individual fatty acids, which cannot be determined by use of dietary interviews alone, the profile of fatty acids in subcutaneous adipose tissue was analyzed, because it usually reflects that of the diet.[9]

The association between fat intake and mammary cancer in humans has been confirmed in numerous studies in laboratory rodents.[10–12] From the latter it seems that the polyunsaturated fatty acids primarily promote carcinogenesis,[10–12] but data from studies in humans are contradictory.[13–15] Among micronutrients, intake of retinol[16] and selenium[17] are inversely correlated with several types of cancer in humans. The consumption of these nutrients can be estimated by determining their concentrations in plasma.[18]

In the case–control study reported here, the relationship between diet and canine mammary tumors and dysplasias was investigated by means of univariate and multivariate analysis. The population included lived in the Madrid area (Spain) and differed from that studied in Philadelphia (Pennsylvania, USA) by Sonnenschein and others[3] in that the proportion of sexually intact female dogs and the caloric intake derived from home-prepared foods in the diets were higher.

Materials and Methods

Dogs

Control Groups. Both a healthy control group and a hospital control group were studied. The healthy control group consisted of 44 female dogs, 5–13 years of age, presented for follow-up examination after minor disorders or for vaccination to the Small Animal Clinic of Complutense University and 4 private practices in Madrid. Physical examination revealed no signs of disease.

The hospital control group of 42 female dogs was of the same age distribution as the healthy controls; these dogs were presented to the Small Animal Clinic for disorders other than endocrine, metabolic, gynecologic, or neoplastic diseases. Ophthalmologic disorders, chronic cardiac diseases, acute intestinal disorders, and orthopedic disorders were the most common problems in these dogs.

Case Group. The cases consisted of 102 female dogs in which dysplasias or tumors of the mammary gland were diagnosed in the clinic during 1992 and 1993. The age range was between 5 and 13 years. Clinical staging was done according to the TNM system, which describes the tumor's size (I), the extent of the spread to the lymph nodes (N), and the extent of distant metastasis (M) to other organs.[19] Both physical examination and radiographic evaluation of the thorax were performed to detect distant metastases. Pathologic examination of the tumors was done on the basis of the World Health Organization's classification for dysplasias and tumors of the mammary gland in domestic animals.[20] The case group was divided into a "benign" group consisting of 48 female dogs with at least 1 dysplastic lesion or benign tumor of the mammary gland, and a "malignant" group con-

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sisting of 54 female dogs with at least 1 malignant mammary tumor. Forty-two dogs had local disease, 10 had lymph node metastases, and 2 had distant metastases at presentation.

Dietary History Interviews

We solicited the voluntary participation of the owners in a study of canine nutrition and health, without revealing the specific aims of the study to avoid possible bias in their answers. An interview about the dog's diet and reproductive history was performed at the beginning of the 1st examination and was reviewed later by the same person. When review of the questionnaire revealed that some data were missing, an additional interview was carried out by telephone (n = 20). The interviews for 10 healthy controls and 4 hospital controls were conducted by 2 practitioners who knew the case–control status of the dog and the aim of the study, but were not aware of the hypotheses being tested. These 14 questionnaires were also reviewed in the manner described above.

The questionnaire consisted of the following:

1. The dog's age, weight, and height. Weight and height were measured when the dog entered the study.
2. The breed, size, and body conformation. Size was categorized by weight in kg as toy (≤5.0), miniature (5.1–11.3), median (11.4–23.0), large (23.1–39.9), or giant (≥40). Body conformation was categorized as thin, normal, or obese at the time of the examination, 1 year before the examination, and at 1 year of age. Assessment of body conformation at the time of presentation consisted of a clinical determination and a readjustment using the standard weights of dog breeds. "Underweight" was defined when ribs were visible, waist easily noted, and abdominal tuck evident. "Normal weight" included dogs with palpable ribs without excess fat covering, and waist observed when viewed from above. "Obese" were dogs with excessive fat covering ribs, with fat deposits over the lumbar area, waist barely visible to absent, and abdominal tuck scarcely apparent to absent.
3. Reproductive history, including whether the dog was ovariohysterectomized or not, and the age of ovariohysterectomy; age at 1st estrus in months; number of full-term pregnancies and age at the 1st pregnancy; and treatments to prevent estrus. All of this information was used to characterize the dogs and to identify possible confounders.
4. Dietary history. Based on a quantitative food frequency questionnaire, the owners were asked about the habitual diet over the past year, focusing primarily on that of the last month. These dietary data allowed calculation of the total intake per day and the relative proportions of daily intake provided by commercial food and homemade meals. The intake of commercial food was recorded in terms of the frequency; quantity; and brand of canned, dry, and semimoist foods, including products used as treats. The intake of homemade meals was described in terms of the frequency per week, amount per day, and method of preparation (raw, boiled, or fried) of 64 possible foods. These foods were selected on the basis of interviews in a preliminary study to determine the foods consumed most frequently by dogs in Madrid. The owner was also asked whether foods not included in the questionnaire were consumed frequently by the dog. The intake of fats (butter, margarine, different types of oils), vitamin and mineral supplements, and table scraps were included. The dietary questionnaire concerned food consumed during the month preceding the examination, but the approximate date when this diet had been started was also recorded and the dogs were included for analysis only if they had been receiving the recorded diet for at least the last year. Dietary data for 13 dogs (5 cases, 5 healthy controls, and 3 hospital controls) were excluded for this reason.

Dietary Profiles

Dietary data obtained from the questionnaires were introduced into the Small Animal Nutritionist Program[21] to calculate a nutrient profile. Caloric and macronutrient contents of food were recorded for the year before patients entered the study. This information was obtained from commercial dog food manufacturers and from a standard reference on analysis of Spanish table foods.[22] The dietary profile consisted of total daily caloric intake; the percentage of calories provided by commercial and table foods; the percentage provided by fat, protein, and carbohydrates; and the percentage provided by the following 5 food items: red meat, poultry, liver, dairy products, and fruits and vegetables. These food items correspond to groups of foods with similar and characteristic nutrient properties. The outcome of the comparisons between cases and controls regarding these items could mirror an effect of the nutrient composition of the group of foods. One or more of 19 other foods were introduced into the analysis when owners reported that these foods had been eaten frequently by their dogs.

Samples for Nutritional Analysis

Serum samples were collected from both cases and controls at the time of the 1st clinical examination. A sample of fat was also obtained in the following manner. A small fold of skin on the dorsal lumbar area was lifted slightly and a 20-gauge Butterfly® needle was inserted at an angle of 60° into the subcutaneous fat. Vacuum was applied with a 20-ml syringe and the needle was gently moved back and forth until fat was obtained in the plastic tube attached to the needle. The tube was sealed and stored in a sealed plastic bag at −20°C until the analyses of fat were performed. The method is similar to that used by Beynen and Katan[23] in humans.

Analyses of Nutritional Indicators

Serum selenium and retinol concentrations and the fatty acid profile in subcutaneous adipose tissue were determined in the Department of Laboratory Animal Science of the School of Veterinary Medicine, Utrecht University (The Netherlands).

Serum selenium concentration. The serum selenium concentration was measured in 47 cases and in 38 healthy and 27 hospital controls, all selected at random, by the method of Koh and Benson.[24]

Serum Retinol Concentration. The serum retinol concentrations was determined in 24 cases and in 19 healthy and 7 hospital controls, selected at random, by the method of Roodenburg and others.[25]

Fatty Acid Profile of Adipose Tissue. The fatty acid profile of subcutaneous adipose tissue was analyzed by the method of Beynen and Katan[23] in 42 cases, 16 healthy controls, and in 18 hospital controls selected at random.

Statistical Analyses

Variables included in the study were evaluated and compared for cases and control groups using BMDP Statistical Software.[26] Continuous variables were analyzed with the BMDP7D program, using the analysis of variance (ANOVA) F test if the variances were equal, and the Welch or Brown–Forsythe tests if not. Levene's test was used to analyze the variances. Multiple comparisons between groups were performed with the Student–Newman–Keuls test. Likewise, a nonparametric analysis of variance was obtained with the Kruskal–Wallis test using the BMDP3S program. The χ^2 test was used to compare categorical variables between cases and control groups using the BMDP4F program. Correlation between continuous variables was performed with BMDP6D. The value $P \leq .05$ was considered significant.

Multivariate logistic regression analysis to evaluate the risk of mammary tumor development was performed with the BMDPLR program. The P values were .05 to enter and .15 to exit.

Table 1. Descriptive findings in 102 cases and 44 healthy and 42 hospital controls.

	Cases		Healthy		Hospital		
	n	%	n	%	n	%	*P* Value
Breed							
Purebred	59	57.8	26	59.1	27	64.3	
Mixed breed	43	42.2	18	40.9	15	35.7	.1
Breed size							
Toy and miniature	36	35.3	14	31.8	13	31.0	
Medium	30	29.4	11	25	5	11.9	.1
Large and giant	36	35.3	19	43.2	24	57.1	
Reproductive status							
Intact	91	89.2	36	81.8	36	85.7	
Ovariectomized	11	10.8	8	18.2	6	14.3	.5

Results

Data on age, breed, reproductive status, and body conformation and dietary variables were evaluated in 102 cases, 44 healthy controls, and 42 hospital controls. The results are given as mean ± standard error of the mean (SEM).

Descriptive and Reproductive Data

The descriptive data for the animals are summarized in Table 1. The age (years) at diagnosis of the cases was 9.8 ± 0.2 (n = 102). The age in healthy controls was 8.0 ± 0.4 (n = 44) and in hospital controls was 8.3 ± 0.3 (n = 42). The difference in mean age between cases and individual control groups was significant ($P < .001$), as was the difference between cases and both combined control groups ($P < .05$).

Dogs of mixed breed were the most numerous (40% in total) in cases as well as in control groups. The distribution of breeds was similar among the 3 groups of dogs ($P = .18$). Likewise, the distribution of size among the 3 groups of dogs was not statistically different ($P = .11$).

Ten percent of the cases were ovariohysterectomized, as were 18% of the healthy controls and 14% of the hospital controls. In view of the small numbers involved, the possible influence of ovariectomy on mammary tumor development could not be evaluated.

Differences between cases and controls regarding the age at the 1st full-term pregnancy, the number of pregnancies, and the hormonal treatments to prevent estrus were not significant. Among the reproductive variables, only the difference in age at the 1st estrus was nearly significant among the 3 groups ($P = .09$), being lower in cases (8.6 ± 0.6 months) than in healthy controls (10.3 ± 0.7 months) ($P < .05$). However, the difference between cases and hospital controls (8.6 ± 0.6 months) was not significant ($P = .1$).

Body Conformation

The distribution of body conformation (underweight, normal, or obese) of cases and controls at 1 year of age, at 1 year before entering the study, and at the time of presentation is shown in Table 2. Forty percent of the cases were reported to have been obese at 1 year of age, compared with 11% of the healthy controls and 14% of the hospital controls; this difference was significant ($P = .0001$). A sim-

Table 2. Body conformation at 1 year of age, at 1 year before diagnosis, and at the time of presentation of cases and controls. Dogs with missing information at 1 year of age are not included (1 case and 1 hospital control).

Body Conformation	Cases		Healthy Controls		Hospital Controls		
	n	(%)	n	(%)	n	(%)	*P* Value
At 1 year of age							
Underweight	20	19.8	6	13.6	5	12.2	
Normal	41	40.6	33	75.0	30	73.2	
Obese	40	39.6	5	11.3	6	14.6	.0001
At 1 year before presentation							
Underweight	9	8.8	2	4.5	2	4.8	
Normal	36	35.3	29	65.9	20	47.6	
Obese	57	55.8	13	29.5	20	47.6	.01
At presentation							
Underweight	3	2.9	1	2.3	0	0	
Normal	42	41.2	29	65.9	19	45.2	
Obese	57	55.9	14	31.8	23	54.8	.06
Total	102	100	44	100	42	100	

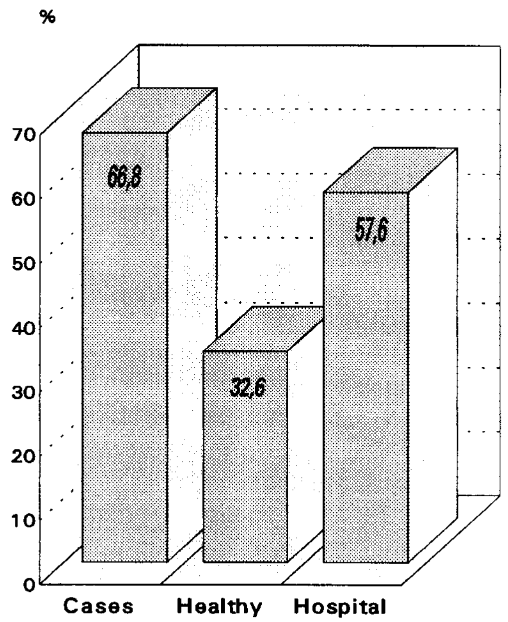

Fig. 1. The percentage of total caloric intake provided by home-prepared foods in cases and controls.

ilar comparison between cases and controls at 1 year before diagnosis also revealed a significant difference ($P = .01$). At the time of diagnosis, the difference in body conformation between cases and both control groups was not significant ($P = .06$).

Dietary Factors

Commercial Versus Homemade Food. The proportions of daily food intake provided by commercial foods and by homemade meals and table food were analyzed in terms of calories. Within each group, both cases and controls, the contribution of commercial and home-prepared food to total caloric intake ranged from 0 to 100%. The contribution of homemade food to total caloric intake in cases was 67 ± 4% (n = 97), in healthy controls it was 33 ± 7% (n = 39), and in hospital controls it was 58 ± 6% (n = 39). The difference between cases and healthy controls was significant ($P < .01$), but not that between cases and hospital controls. The percentage of total caloric intake provided by home-prepared food in cases and controls is showed in Figure 1.

Carbohydrate, Fat, and Protein. The percentages of dietary calories provided by carbohydrates, fat, and protein in the diets of 171 dogs are summarized in Table 3. The dietary data of 4 dogs were excluded in the subsequent analyses because the amounts of food reported in the dietary questionnaire were unprecise. The differences in the percentage of calories provided by carbohydrates and protein between cases and healthy controls or hospital controls were not significant. The percentage of dietary calories provided by fat was higher in cases than in healthy and hospital controls, but the difference was not significant ($P = .07$, $P = .1$, respectively).

In the 171 diets analyzed, a positive linear correlation existed between the percentage of calories provided by fat and the percentage provided by home-prepared food (linear regression, $r = 0.17$, $P = .031$). The percentages of calories provided by protein and carbohydrates were not linearly correlated with those provided by either commercial or homemade food.

Specific Foods. Red meat was defined as any type of beef, pork, lamb, or horse meat except liver. The contribution of calories by red meat was significantly different in the 3 groups ($P < .007$, ANOVA F test). Calories from red meat in cases (13.2 ± 1.9) were significantly different than in healthy controls (5.7 ± 2.2), but compared to the hospital controls (6.9 ± 2.3) the difference was not significant. The difference between cases and both control groups combined was significant ($P < .05$).

Poultry meat was defined as any edible part of the chicken except liver. The percentage of dietary calories provided by poultry meat was 9.0 ± 1.5 in cases, 6.6 ± 2.6 in healthy controls, and 10.5 ± 2.4 in hospital controls. Differences were not significant using the parametric F test. The difference among the 3 groups and that between cases and healthy controls was significant using the nonparametric Kruskal–Wallis test ($P = .05$).

The difference in the contribution of dietary calories by liver (beef, pork, lamb, chicken) between cases (2.3 ± 0.9) and hospital controls (2.4 ± 1.4) was not significant, nor was that between cases and healthy controls (1.0 ± 0.7).

Dairy products were defined as milk and milk products such as yogurt and cheese. The difference in their contribution to caloric intake was not significant when cases (3.4 ± 0.7) were compared with healthy controls (2.6 ± 0.8) and hospital controls (4.7 ± 1.1).

Vegetables and fruits were considered together. The difference in their contribution to caloric intake between cases (2.8 ± 0.6) and healthy controls (2.3 ± 0.7) was not significant, nor was that between hospital controls (2.9 ± 0.7) and cases.

Nutritional Indicators and Mammary Tumor Risk

Serum Selenium Concentration. The mean serum selenium concentration in the cases (n = 47) was 0.300 ± 0.007 μg/mL (range 0.213–0.434 μg/mL). The mean serum

Table 3. Percentages (mean ± SEM) of dietary calories provided by carbohydrates, fat, and protein in 171 dogs. The *P* values of F and Kruskal–Wallis tests are identical.

	Cases (n = 93)	Healthy Controls (n = 39)	Hospital Controls (n = 39)	*P* Value
% Energy from carbohydrates	43.0 ± 1.4	45.1 ± 2.3	44.4 ± 1.9	.6
% Energy from fat	31.0 ± 1.3	26.6 ± 1.6	29.5 ± 1.5	.07
% Energy from protein	26.1 ± 0.8	28.3 ± 1.3	26.1 ± 1.1	.2

Table 4. Development of mammary tumors: multivariate analyses including significant variables. Final steps.

Term Entering	Coefficient	SE	Exponential (coefficient)	Confidence Interval	*P* Value	Goodness of Fit χ^2
			Cases and combined control groups (n = 167)			
Age	0.273	0.076	1.31	1.13–1.53	.0002	
Body condition at 1 year	1.512	0.43	4.54	1.91–10.8	.0002	0.11
Red meat	0.029	0.011	1.03	1.01–1.05	.0076	
			Cases and hospital control group (n = 130)			
Age	0.265	0.093	1.30	1.08–1.57	.003	
Body condition at 1 year	1.425	0.547	4.16	1.41–12.3	.004	0.6
Red meat	0.027	0.014	1.03	0.99–1.06	.04	
			Cases and healthy controls (n = 129)			
Homemade food	0.018	0.005	1.02	1.01–1.03	.0004	
Body condition at 1 year	1.458	0.604	4.30	1.30–14.2	.008	0.35
Age	0.268	0.097	1.31	1.08–1.59	.001	

selenium concentration in the healthy control group (n = 38) was 0.292 ± 0.007 μg/mL (range 0.218–0.405 μg/mL) and in the hospital group (n = 27) was 0.308 ± 0.010 μg/mL (range 0.139–0.398 μg/mL). The differences were not significant.

Serum Retinol Concentration. The mean serum retinol concentration in the cases (n = 24) was 60 ± 4 μg/dL (range 24–102 μg/dL). The mean serum retinol concentration in healthy controls (n = 19) was 79 ± 10 μg/dL (range 26–209 μg/dL) and in hospital controls (n = 7) was 102 ± 25 μg/dL (range 41–222 μg/dL). The difference between cases and the control groups combined was significant. The difference between the mean values of cases and hospital controls was significant ($P < .05$), but not between cases and healthy controls. Using the nonparametric tests, differences did not reach the level of significance.

Fatty Acid Profile in Adipose Tissue

The differences in intake of essential n-3 and n-6 fatty acids between cases and controls were not significant, nor were the differences between C16:0 and C18:1, which reflect de novo fatty acid synthesis. Nevertheless, using the nonparametric Kruskal–Wallis test the difference in C18:1 fatty acid content between cases and healthy controls reached the statistical level of significance ($P < .05$), but differences between cases and hospital controls or both groups combined were not significant ($P = .1$).

Diet-Related Variables and Pathological Findings

The differences in body conformation and the dietary variables between cases with dysplasia and benign tumors and cases with malignant tumors were not significant. However, the difference in the contribution by vegetables and fruit to caloric intake between cases with malignant tumors (2.5 ± 0.9, n = 48) and cases with dysplasia and benign tumors (3.0 ± 0.6, n = 44) was significant ($P < .05$). The differences in body conformation and dietary variables between cases with malignant tumors of different clinical stage were not significant.

Multivariate Analyses

These analyses require the use of dogs with complete ascertainment for all the variables, and missing data decreased the number of dogs included. Thus, several analyses were done, but those including the maximum number of dogs are presented. Body conformation at 1 year of age, at 1 year before diagnosis, and at diagnosis was grouped in 2 categories (underweight and normal versus obese).

In a 1st set of multivariate analysis including the majority of variables, when we compared cases between the 2 control groups (n = 110), significant variables were age ($P < .0001$), percentage of home-prepared food ($P = .003$), body conformation at 1 year ($P = .007$), and percentage of poultry meat ($P = .03$). Results of analyses between cases and individual control groups were similar to the following analyses.

A 2nd set of analyses were done including the following significant variables in the univariate analysis: age; body conformation at 1 year of age, at 1 year before examination, and at the moment of presentation; percentage of energy from fat, from red meat, and from poultry meat; and percentage of energy from homemade meals (n = 167). Results of this 2nd set of multivariate analyses are shown in Table 4, in which the exponential coefficient corresponds to the factor that multiplies the odds ratio of probability (case) when the selected variable increases 1 unit for continuous variables, or changes from absence to presence for categorical variables.

When cases were compared with the 2 control groups combined, age emerged as an independent and significant variable in step 1. In step 2, body conformation at 1 year of age, and percentage of energy from read meat in step 3, were the other selected variables. Thus, older age, obesity at 1 year of age, and high percentage of energy from red meat were independent variables related to the development of mammary tumors.

Using the hospital control group for comparison with cases, older age (step 1), obesity at 1 year of age (step 2), and high percentage of energy from red meat (step 3) were the independent significant variables associated with high risk for mammary neoplasms. When the comparison was performed between cases and healthy controls, high energy from home-prepared meals (step 1), older age (step 2), and obesity at 1 year of age (step 3) were the independent selected variables that increase the risk of mammary tumors.

Discussion

As has been stated previously, only 1 study is published on dietary factors and development of canine mammary tumors.[3] The dogs in the present study were living in the Madrid area (Spain) and differed from those studied in Philadelphia by Sonnenschein and others[3] in that the proportion of dogs that were not ovariohysterectomized was higher (86% versus of 44%) and the proportion of the caloric intake derived from home-prepared foods was higher (53% versus 35%). Furthermore, we not only used a dietary questionnaire to assess macronutrient intake but also measured the fatty acid composition of subcutaneous adipose tissue and the plasma concentrations of retinol and selenium. Determining the intake of individual fatty acids by use of dietary questionnaires alone is not possible, but the profile of fatty acids in subcutaneous adipose tissue can reflect that of the diet.[9]

The influence of ovariectomy on the development of mammary tumors could not be evaluated in this study. Differences in other reproductive variables between cases and controls were not significant, with the exception of the age at 1st estrus. Estrus at an early age was significantly related to risk, as has been found by others.[27] Similarly, late menarche and early menopause have been found to protect against breast cancer in humans.[28]

In this study body conformation before presentation was assessed retrospectively by the owners, which limilts the interpretation of this results. Obesity at 1 year of age and obesity 1 year before diagnosis were both significantly related to risk of developing mammary tumors. Obesity at diagnosis in cases and obesity at examination in controls were not significantly related. Similar findings were reported in another study of canine mammary tumors.[3]

It has been proposed that overnutrition during the adolescent years in humans is an important determinant of subsequent breast cancer incidence.[29,30] The lower frequency of obesity observed in vegetarian populations, which suggests a lower caloric intake, may lead to a delay in the onset of menarche[31] and may also influence hormonal status at other times of life.[29]

Obesity at 1 year of age was reported significantly more often among cases than controls. This led us to the hypothesis that obesity at a young age might modify the onset of sexual maturity in female dogs and their hormonal status later in life, which might enhance mammary carcinogenesis. In addition, if mechanisms that have been proposed in humans[32] are also operative in mammary tumors in dogs, the somewhat higher occurrence of obesity in the cases than in the controls 1 year before diagnosis could also have enhanced carcinogenesis by modifying the concentrations and availability of female sex hormones. However, the effect of sex steroid hormone exposure, specially early in life, could not be evaluated in the present study.

The lack of a relationship between mammary tumor risk and obesity at the time of diagnosis might have been influenced by the population of controls included. A high proportion of healthy controls were clinically considered as normal weight, and hospital controls as obese.

The cases were found to have consumed a significantly higher proportion of homemade food than had the healthy controls or the healthy and hospital controls combined. This finding should be interpreted with caution, with regard to the question of whether commercial food per se is protective against mammary tumors, because of the potential influence of other factors. For example, in most of the dogs eating homemade food, a high frequency of consumption of table scraps and treats occurred.

Commercial foods were generally lower in fat content than homemade foods, in which fat usually provided more than 40% of the calories. Furthermore, we have found a correlation between the proportion of fat and of home-prepared food in the diets.

The differences in the percentage of calories provided by carbohydrates and protein between cases and either healthy or hospital controls or the 2 control groups combined were not significant. This is in agreement with the findings of a previous study in dogs.[3]

Dietary fat is the macronutrient about which there is the greatest confusion with regard to its effect on development and progression of breast cancer in humans. Epidemiologic evidence supporting an association between high fat intake and the risk of breast cancer was reviewed,[33] and a recent study confirms this association.[34] In an ecological study a positive relationship was found, especially at the age of 50 years and above,[8] but in 2 large cohort studies, no positive association was found between fat intake and cancer incidence.[35,36] In this study, the percentage of calories provided by fat in the cases was higher than in the healthy control group, but the difference was not significant. These findings reflect the already described proportionately higher consumption of homemade foods by the cases than the controls and the higher content of fat in homemade foods than in commercial foods.

We found mammary tumor risk to be positively associated with the intake of red meat and negatively associated with the intake of poultry meat. Particulary, the intake of red meat emerged as an independent factor associated with the risk in the multivariate analysis. Epidemiologic studies have revealed an association between human breast cancer and the consumption of beef and pork;[37,38] meat;[39,40] and sausage, eggs, and meat.[34,41]

However, we have not found an association between consumption of vegetables and fruits and mammary tumor development. Higher consumption of vegetables and fruits is associated, although not universally, with a decreased risk of cancer at most sites in humans, especially epithelial cancers of the alimentary and respiratory tracts.[42] The intake of crude fiber, carotene and vitamin C, and fruits and vegetables[43–46] has been inversely associated with breast cancer risk. A recent epidemiologic study revealed that a low-fat diet is associated with a dietary pattern characterized by high intakes of vitamin C, carbohydrates, fiber, poultry, fruits, and vegetables, and this could obviously have an influence in studies of the relation of dietary fat to cancer risk.[47]

We found vitamin A intake, as reflected by serum retinol concentrations, to be negatively associated with mammary tumor risk. This is an indication that vitamin A could protect against the development of mammary tumors in dogs. This finding is similar to that in some epidemiologic studies of breast cancer in humans.[48–51] However, we found no significant difference in serum selenium concentration between cases and controls. Similar findings have been reported in some studies in human breast cancer[52,53] but not in others.[15]

A significant difference was not found between cases and controls in intake of fatty acids, which is in agreement with findings in an epidemiologic study of breast cancer in humans.[54] The finding of a higher content of C18:1 fatty acid in cases than in healthy controls, which may reflect de novo fatty acid synthesis, should be interpreted with caution, because the content in cases was similar to that in hospital controls.

In the multivariate analyses using cases and control groups combined and cases and the hospital control group, older age, obesity at 1 year of age, and a high consumption of red meat were variables independently related to the risk for mammary neoplasms. In the multivariate analysis using cases and the healthy control group, a higher percentage of home-prepared food, older age, and obesity at 1 year of age were the variables that emerged independently related to mammary tumor development, probably because the percentage of home-prepared food was significantly higher in the case group that in the healthy control group.

In conclusion, obesity at an early age and 1 year before diagnosis are positively associated with the risk of canine mammary tumors. The intake of homemade foods is related to a higher prevalence of mammary tumors than is the intake of commercial foods. A higher risk is associated with consumption of beef and pork than with poultry. Serum retinol concentrations were significantly higher in controls than in cases but serum selenium concentrations and the concentrations of fatty acids in subcutaneous adipose tissue did not differ significantly between cases and controls. Multivariate analysis revealed that older age, obesity at 1 year of age, higher percentage of red meat intake, and, less significantly, a higher proportion of home-prepared food were independently associated to a higher risk for canine mammary tumors. On the basis of this study, diet at an early age cannot be excluded to influence mammary tumorigenesis. More similar studies on diet and canine mammary tumors are necessary to provide evidence that nutritionally related factors influence mammary tumorigenesis in the dog.

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