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Cancer Epidemiol Biomarkers Prev 2002;11:1434-1440.

Endogenous Gonadal Hormone Exposure and Bone Sarcoma Risk[1]

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Abstract

Although experimental and clinical evidence suggest that endogenous sex hormones influence bone sarcoma genesis, the hypothesis has not been adequately tested in an appropriate animal model. We conducted a historical cohort study of Rottweiler dogs because they frequently undergo elective gonadectomy and spontaneously develop appendicular bone sarcomas, which mimic the biological behavior of the osteosarcomas that affect children and adolescents. Data were collected by questionnaire from owners of 683 Rottweiler dogs living in North America. To determine whether there was an association between endogenous sex hormones and risk of bone sarcoma, relative risk (RR) of incidence rates and hazard ratios for bone sarcoma were calculated for dogs subdivided on the basis of lifetime gonadal hormone exposure. Bone sarcoma was diagnosed in 12.6% of dogs in this cohort during 71,004 dog-months follow-up. Risk for bone sarcoma was significantly influenced by age at gonadectomy. Male and female dogs that underwent gonadectomy before 1 year of age had an approximate one in four lifetime risk for bone sarcoma and were significantly more likely to develop bone sarcoma than dogs that were sexually intact [RR ±95% CI = 3.8 (1.5–9.2) for males; RR ±95% CI = 3.1 (1.1–8.3) for females]. χ^2 test for trend showed a highly significant inverse dose-response relationship between duration of lifetime gonadal exposure and incidence rate of bone sarcoma ($P = 0.008$ for males, $P = 0.006$ for females). This association was independent of adult height or body weight. We conclude that the subset of Rottweiler dogs that undergo early gonadectomy represent a unique, highly accessible target population to further study the gene:environment interactions that determine bone sarcoma risk and to test whether interventions can inhibit the spontaneous development of bone sarcoma.

Introduction

Osteosarcoma is the most frequently diagnosed bone tumor of adolescents and young adults (1–3). To date, little is known concerning etiology and risk factors for osteosarcoma. Limited geographic variation in the incidence of osteosarcoma suggests the importance of host factors such as gender or skeletal growth (4). Age-specific incidence data indicate an association between the pubertal growth spurt and development of bone sarcoma in adolescents (5–8). The hypothesis that rapid skeletal growth or large body size confers increased risk of bone sarcoma development has been proposed (6, 9, 10) but has not been rigorously tested in an appropriate animal model. Annual age-adjusted incidence rates for bone sarcoma are 1.0/100,000 males and 0.6/100,000 females (11). In one study, males were four times more likely than females to die within 2 years of bone sarcoma diagnosis (12). These data suggest that endogenous sex hormones may influence the development and biological behavior of these tumors.

Spontaneous osteosarcoma in pet dogs closely mimics its human counterpart in terms of skeletal location, metaphyseal involvement, aggressive biological behavior, high propensity for pulmonary metastases, and response to cytotoxic chemotherapy (13–15). An estimated 10,000 cases of bone sarcoma in pet dogs are diagnosed annually in the United States (13). An association between body size and bone sarcoma risk in dogs is well documented. Across different dog breeds, body size is the strongest predictor of risk for osteosarcoma (16, 17). However, no studies have used measures of body size such as adult height or weight obtained from individual dogs of the same breed to determine whether these factors significantly influence risk of bone sarcoma.

Humans do not frequently undergo gonadectomy. In contrast, pet dogs frequently undergo elective gonadectomy, providing a unique population to study the influence of endogenous sex hormones on spontaneous bone sarcoma development. Data collected from veterinary teaching hospitals suggested that both male and female neutered dogs were at increased risk for bone sarcoma (17). However, in that study, age at neutering was not available, and thus, duration of exposure to gonadal hormones for each dog could not be determined. To characterize the dose-response relationship between endogenous sex hormones and bone sarcoma risk, a study providing information on lifetime gonadal hormone exposure would be required.

To test the hypothesis that endogenous sex hormones significantly influence bone sarcomagenesis, we conducted a historical cohort study of Rottweiler dogs, a breed known to be at high risk for bone sarcoma. In addition, we determined whether adult height or body weight were significant risk factors for bone sarcoma between individuals of the same breed. Our results indicate that dogs undergoing early gonadectomy have a significantly higher risk of appendicular bone sarcoma, suggesting that sex hormones may be important modifiers of bone sarcoma development.

Received 12/26/01; revised 5/28/02; accepted 7/4/02.

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[1] This work was supported by The IAMS Company and the Animal Cancer Foundation. D. J. W. was supported, in part, by a Brookdale National Fellowship to Support Leadership in Gerontology.

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Materials and Methods

Study Population. We conducted an historical cohort study of Rottweiler dogs in the pet population that were followed over a significant proportion of their lifetime. Rottweiler dogs were targeted for this study because they represent a breed at high risk for appendicular bone sarcoma (17), a tumor bearing striking similarity to the osteosarcoma that affects children and adolescents. In November 1999, a questionnaire was mailed to 1,500 owners of Rottweiler dogs identified through 8 national Rottweiler breed specialty clubs.[3] In addition, the questionnaire was published in the national breed magazine, *The Rottweiler Quarterly* (18). Purebred Rottweiler dogs of any age that were alive on January 1, 1995, were eligible for study. Owners were asked to complete one questionnaire per eligible dog, and a maximum of five dogs could be entered per household. Data from 730 questionnaires returned by March 1, 2000, were used in this study.

Data Collection. With the assistance of a veterinarian, pet owners completed a 12-page questionnaire consisting of 62 questions encompassing six categories: general owner information; general dog information; development of bone sarcoma; familial history of cancer; exposure and trauma history; and health conditions confirmed by a veterinarian. General owner information included questions regarding number of Rottweilers owned, purpose of dog ownership (*e.g.,* pet or working dog), and place of residence. General dog information included questions pertaining to date of birth, gender, date of neuter, country of birth of this dog and two prior generations, housing conditions, body condition (immature and adult), adult body weight, adult height, growth rate, bone structure, diet, dietary supplements, vital status, and date and cause of death, if applicable. Questions pertaining to bone sarcoma included age at diagnosis, location of primary tumor, treatment, and survival. Familial cancer history consisted of questions pertaining to the owner's knowledge of the development of a bone tumor or other malignancy in siblings or first and second generation relatives. Vaccination history, chemical exposures, and trauma history were also obtained. The questionnaire also included a checklist of 40 health conditions (13 cancer-related and 27 noncancer-related) confirmed by a veterinarian. Pet owners were given the option to return the completed questionnaire anonymously or to include their name, address, and their veterinarian's contact information. Over 98% of returned questionnaires contained the identity of the owner and dog, including contact information, so that the accuracy of data could be verified.

Ascertainment of Bone Sarcoma. A diagnosis of bone tumor was reported for 133 dogs. Telephone follow-up with veterinarians and owners was conducted by two interviewers (D. M. C., D. L. S.) to obtain more detailed diagnostic information for any dog with a reported bone sarcoma. Medical records and available radiographs were reviewed by the authors (D. J. W., D. M. C.) for each dog with a reported bone tumor. Cases were included only if a diagnosis of appendicular bone sarcoma was supported by radiographic or histological evidence of bone sarcoma. We limited our study to the bone sarcomas of the appendicular skeleton because these tumors most closely resemble osteosarcoma of children and adolescents (13). Forty-seven dogs with a reported bone tumor were excluded from the analyses because medical records were incomplete, radiographs were not consistent with long bone sarcoma, or histopathology was inconsistent with osteosarcoma. The 47 dogs that were excluded did not differ significantly in terms of key characteristics from the subcohort of 86 dogs with appendicular bone sarcoma included in this analysis that satisfied the inclusion criteria. The subcohort without bone cancer included all 597 dogs in this cohort that were free of bone cancer.

Assessment of Gonadal Hormone Exposure and Other Risk Factors. Dogs were categorized on the basis of neuter status into four groups: castrated male; sexually intact male; spayed female; and sexually intact female. Lifetime gonadal hormone exposure of each dog was expressed in terms of total months of gonadal hormone exposure (*i.e.,* number of months sexually intact). We analyzed risk for bone sarcoma using months of gonadal hormone exposure as a continuous variable. In addition, we stratified dogs of each gender into four subgroups on the basis of their duration of gonadal exposure. This stratification enabled us to evaluate the dose-response relationship between duration of gonadal exposure and bone sarcoma risk within the study cohort. These subgroups included two biologically distinct groups representing the extremes of gonadal hormone exposure (*i.e.,* dogs neutered before skeletal maturation at <1 year of age and dogs that remained sexually intact for their entire lifetime). The remaining dogs that underwent gonadectomy after 1 year of age were dichotomized into two equal groups. For females, the gonadal hormone exposure subgroups were: spayed before 1 year of age; spayed between 1 and 5 years of age; spayed after 5 years of age; and sexually intact. For males, the gonadal exposure subgroups were: castrated before 1 year of age; castrated between 1 and 3.5 years of age; castrated after 3.5 years of age; and sexually intact.

Because body size is a potentially important risk factor for appendicular bone sarcoma development, we evaluated adult height and body weight for their possible association with bone sarcoma in Rottweiler dogs. Adult height (cm) and body weight (kg) were obtained from the questionnaire. To collect information on reproductive history, follow-up telephone interviews were conducted with owners of 332 female Rottweiler dogs that were sexually intact or spayed after 1 year of age. Successful interviews were obtained from 275 (83%) of attempted contacts. Data obtained included number of litters, date of whelping, number of puppies per litter, and whether the dog had ever received exogenous hormone treatment.

Statistical Analysis. Incidence of appendicular bone sarcoma was calculated for the entire population, for each gender-neuter category (castrated male, sexually intact male, spayed female, and sexually intact female), and for each gonadal hormone exposure subgroup. For each group, incidence rate of bone sarcoma was calculated by determining the number of bone sarcomas/10,000 dog-months at risk. To measure the strength of association between gonadal hormone exposure and bone sarcoma risk, RRs[4] and 95% CIs for bone sarcoma incidence rates were calculated. Sexually intact dogs were used as the reference group (RR = 1.0) for both males and females. χ^2 test for trend was used to analyze dose-response relationships across different lifetime duration of gonadal hormone exposure. Hazard ratios and 95% CIs were determined using Cox proportional hazard models to analyze lifetime duration of gonadal hormone exposure as a continuous variable. Risk factors asso-

[3] Medallion Rottweiler Club, Colonial Rottweiler Club, Delta Rottweiler Owners Club, Emerald Valley Rottweiler Club, Great Lakes Rottweiler Club, Gulfstream Rottweiler Club, Northstar Rottweiler Club, and Mile High Rottweiler Club.

[4] The abbreviations used are: RR, relative risk; CI, confidence interval.

Table 1 Description of population of Rottweilers included in a historical cohort study of bone sarcoma risk

No. of dogs	683
No. of households	402
Residence	
United States (45 states)	648 (94.9%)
Canada	35 (5.1%)
Status at time of questionnaire	
Alive	55%
Deceased	45%
Follow-up duration (mean; range)	
Dogs with bone sarcoma	8.8 (1.3–13.2) yr
Dogs without bone sarcoma	8.6 (1.7–15.6) yr
Age at Death (mean ± SD)	
Intact male	9.3 ± 2.5 yr
Castrated male	9.2 ± 2.5 yr
Intact female	7.5 ± 2.4 yr
Spayed female	9.8 ± 2.4 yr
Dogs with bone sarcoma	8.8 ± 2.0 yr
Dogs without bone sarcoma	9.5 ± 2.6 yr
Appendicular bone sarcoma	
Age at diagnosis (median; range)	8.0 (1.3–13.0) yr
Intact male	8.0 (5.0–12.0) yr
Castrated male	8.0 (5.0–13.0) yr
Intact female	7.5 (4.0–9.0) yr
Spayed female	9.0 (1.3–11.0) yr
Skeletal location	
Proximal humerus	32 (37.2%)
Distal radius	16 (18.6%)
Distal femur	10 (11.6%)
Distal tibia	9 (10.5%)
Other	19 (22.1%)
Cause of death (% of 305 deaths)	
Cancer	64.3%
Gastrointestinal disease	7.2%
Neurological disease	4.3%
Cardiovascular disease	3.9%
Old age	3.6%
Osteoarthritis	3.3%
Renal disease	2.6%
Endocrine disease	2.6%
Other	3.0%
Unknown	5.2%

ciated with bone sarcoma in univariate analysis at $P < 0.20$ were tested in multivariate Cox proportional hazards models. All data analyses were performed using standard computerized statistical software (SPSS Version 10.0 and Epi Info ver 6.04), and differences were considered to be statistically significant at $P < 0.05$.

Results

Baseline characteristics of the 683 dogs in this cohort are shown in Table 1. Eligible questionnaires were completed by owners of purebred Rottweilers from 402 households in 45 states across the United States and Canada. Approximately 45% of dogs were dead at the time the questionnaire was completed with a mean ± SD age at death of 9.3 ± 2.5 years. Mean ± SD age of dogs that were alive at the time of questionnaire was 8.1 ± 2.2 years. Cancer-related mortality was reported in 64.3% of Rottweiler dogs in this cohort. The most common noncancer causes of death were gastrointestinal diseases (7.2%), neurological diseases (4.3%), and cardiac diseases (3.9%).

Overall, the incidence of appendicular bone sarcoma in this cohort was 12.6%. Eighty-six cases were diagnosed during 71,004 dog-months follow-up. Overall, mean ± SD age at appendicular bone sarcoma diagnosis was 8.3 ± 1.9 years, which did not differ significantly between different gonadal hormone exposure categories. Tumors most often affected the forelimb. Proximal humerus and distal radius, the most frequent sites of appendicular bone sarcoma reported in large and giant breed dogs, were the most commonly affected skeletal sites in this population.

Females were more often diagnosed with bone sarcoma than males, however, the difference was not statistically significant [hazard ratio (95% CI) = 1.01 (0.66–1.55); $P = 0.97$] (Table 2). Age at gonadectomy significantly influenced risk for bone sarcoma. Both males and females that developed bone sarcoma were sexually intact for significantly fewer months than dogs that did not develop bone sarcoma (Table 2) [hazard ratios (95% CI) = 0.98 (0.98–0.99) for males and 0.98 (0.97–0.99) for females; $P < 0.0001$ for both]. In multivariate analysis, months intact remained significantly inversely associated with bone sarcoma risk after controlling for gender, adult height, and adult body weight ($P < 0.0001$; Table 3). For each additional month of being sexually intact, there was a 1.4% reduction in bone sarcoma risk.

To further evaluate the potential dose-response relationship between risk of bone sarcoma and gonadal hormone exposure, dogs were categorized into four subgroups for each gender based upon lifetime duration of exposure to gonadal hormones. Table 4 shows the incidence rate of bone sarcoma (per 10,000 dog-months) in each of the gonadal hormone exposure subgroups. There was a significant negative association between gonadal hormone exposure and risk of bone sarcoma (P for trend = 0.008 for males; 0.006 for females). In males, bone sarcoma incidence rate for dogs castrated before 1 year of age (lowest gonadal exposure) was 28.4 bone tumors/10,000 dog-months at risk, which was almost four times greater than the rate of bone sarcoma in sexually intact males [RR ± 95% CI = 3.8 (1.5–9.2); $P = 0.002$]. In females, bone sarcoma incidence rate in dogs spayed before 1 year of age (lowest gonadal exposure) was 25.1 bone tumors/10,000 dog-months at risk, which was more than three times greater than the rate in sexually intact females [RR ± 95% CI = 3.1 (1.1–8.3); $P = 0.02$]. The dose-response relationship between lifetime gonadal exposure and bone sarcoma risk in males is illustrated in Fig. 1, which shows the multivariate hazard function curves for each of the gonadal exposure subgroups.

Body size was evaluated as a possible risk factor for bone sarcoma in Rottweiler dogs. Adult height ranged from 58 to 76 cm (median = 66 cm) in 250 males and 48 to 79 cm (median = 61 cm) in 329 females. Despite the wide variation in adult height within the study population, this surrogate of skeletal growth was not significantly associated with bone sarcoma in males ($P = 0.15$) or females ($P = 0.97$). Adult body weight ranged from 36 to 68 kg (median = 50 kg) in 293 males and 27 to 73 kg (median = 40 kg) in 384 females. Similar to adult height, this measure of body size was not significantly associated with bone sarcoma in males ($P = 0.15$) or females ($P = 0.74$). In addition, when dogs were stratified into four gonadal exposure subgroups, adult height and body weight did not significantly contribute to bone sarcoma risk in males or females (data not shown). In multivariate analysis, adult height and body weight were not found to be significantly associated with bone sarcoma development (Table 3).

Because duration of gonadal exposure significantly influenced risk of bone sarcoma, we investigated further the reproductive history of female dogs in this study. The reproductive history of 275 female dogs that were sexually intact after 1 year of age was obtained by telephone interview of owners (Table 5). Fifty percent of female dogs had at least one litter during their lifetime and 50% were nulliparous. There was no signif-

Table 2 Univariate analysis of risk factors of appendicular bone sarcoma in a cohort of 683 Rottweiler dogs

Risk Factor	Dogs with bone sarcoma	Dogs without bone sarcoma	Hazard Ratio (95% CI)	*P*
Total no.	86 dogs	597 dogs		
Gender				
Male	35 dogs	259 dogs	1.00	
Female	51 dogs	338 dogs	1.01 (0.66–1.55)	0.97
Neuter status				
Intact male	10 dogs	120 dogs	1.00	
Castrated male	25 dogs	139 dogs	1.86 (0.89–3.87)	0.10
Intact female	5 dogs	64 dogs	1.00	
Spayed female	46 dogs	274 dogs	0.95 (0.38–2.40)	0.91
Lifetime gonadal exposure				
Months intact (mean ± SD)				
Male	53.1 ± 44.3 mo	71.1 ± 42.7 mo	0.98 (0.98–0.99)	<0.0001
Female	40.5 ± 34.0 mo	55.6 ± 35.0 mo	0.98 (0.97–0.99)	<0.0001
Body size				
Adult height (mean ± SD)				
Male	61.0 ± 2.8 cm	65.8 ± 2.8 cm	1.10 (0.97–1.26)	0.15
Female	24.0 ± 1.1 cm	61.0 ± 3.3 cm	1.00 (0.91–1.10)	0.97
Adult body weight (mean ± SD)				
Male	50.5 ± 7.6 kg	49.7 ± 5.7 kg	1.04 (0.99–1.10)	0.15
Female	40.9 ± 4.7 kg	40.6 ± 5.0 kg	1.01 (0.96–1.01)	0.74

Table 3 Multivariate cox proportional hazard models of bone sarcoma in a cohort of Rottweiler dogs

	Hazard ratio (95% CI)	*P*
Model 1 (n = 578 dogs)		
Gender	0.90 (0.48–1.69)	0.73
Months sexually intact	0.99 (0.98–0.99)	<0.0001
Adult height (cm)	1.02 (0.93–1.10)	0.73
Adult body weight (kg)	0.99 (0.94–1.05)	0.83
Model 2		
Male (n = 202 dogs)		
Months sexually intact	0.99 (0.98–1.00)	0.003
Adult height (cm)	1.11 (0.95–1.29)	0.21
Adult body weight (kg)	0.98 (0.90–1.07)	0.72
Female (n = 329 dogs)		
Mo sexually intact	0.98 (0.98–0.99)	0.001
Adult height (cm)	0.98 (0.89–1.08)	0.70
Adult body weight (kg)	0.99 (0.93–1.06)	0.75

icant difference in bone sarcoma risk between females with litters *versus* nulliparous females (P = 0.22). There were no significant differences between dogs with bone sarcoma and dogs without bone sarcoma with respect to number of years of reproductive activity, number of litters, or number of puppies. Age at first pregnancy was also similar between affected and nonaffected dogs (P = 0.47). Supplementation of nine females with exogenous hormones was not significantly associated with risk of bone sarcoma (P = 0.56).

Discussion

Comparative oncologists seek to test important hypotheses by studying the similarities and differences between the cancers that affect humans and animals. We focused on the appendicular osteosarcomas that naturally occur in Rottweiler dogs because of the striking biological similarities of this disease to its human counterpart. To our knowledge, this represents the first application of a spontaneous model of bone sarcoma to investigate the role of endogenous sex hormones in sarcomagenesis. In this study, we found a strong inverse association between lifetime exposure to gonadal hormones and risk of spontaneous bone sarcoma. Gonadal hormone exposure was a significant risk factor of bone sarcoma independent of adult body size, a previously recognized risk factor for bone sarcoma. Importantly, this study identifies a high-risk subpopulation of Rottweiler dogs that could be targeted to study whether modifications in lifestyle or environmental factors reduce the incidence of bone sarcoma.

Previous studies using dogs with spontaneous bone sarcoma have focused on the preclinical evaluation of novel therapeutics (19–23). We have turned our attention to studying possible host factors that contribute to the risk of bone sarcoma development. Using a historical cohort study design, we evaluated bone sarcoma risk in ~700 Rottweiler dogs living in North America. Rottweiler dogs were selected for this study because (*a*) their risk of bone sarcoma is very high compared with other breeds (17) and (*b*) >85% of their appendicular bone sarcomas are osteosarcoma.[5] Our previous work has demonstrated the feasibility of using questionnaires to generate reliable data on exposures (*e.g.*, diet, sex hormones, and environmental agents) and disease outcome in pet dogs (24–26).

Similar to the situation in humans, relatively little is known about factors that regulate bone sarcoma development in pet dogs. Because many pet dogs undergo elective castration or ovariohysterectomy as young animals, this population offers a unique opportunity to compare individuals that differ dramatically with respect to lifetime testicular or ovarian hormone exposure. Previous studies using pet dogs have clearly established a relationship between ovarian hormones and breast cancer risk (27, 28). We conducted this study to critically evaluate the dose-response relationship between gonadal hormone exposure and bone sarcoma risk because this could not be studied within the human population at risk for osteosarcoma. Our study, using a naturally occurring model of bone sarcoma, shows that risk of bone sarcoma is significantly increased by elective gonadectomy early in life. Exposure to endogenous sex hormones appears to be protective, as suggested by the high risk for bone sarcoma in male and female dogs that undergo

[5] D. J. Waters, unpublished data.

Table 4 Lifetime gonadal hormone exposure and bone sarcoma risk in a cohort of 683 Rottweiler dogs

	Dogs with bone sarcoma (no.)	Dogs without bone sarcoma (no.)	Total dog-months	Bone sarcoma incidence rate (95% CI)[a]	RR (95% CI)	*P*
Total population	86	597	71,004	12.1 (9.6–14.7)		
Gender						
Male	35	259	30,228	11.6 (7.8–15.4)	1.0	
Female	51	338	40,776	12.5 (9.1–15.9)	1.1 (0.7–1.7)	0.74
Male gonadal exposure subgroup						
Castrated before 1 yr of age	9	25	3,168	28.4 (9.8–47.0)	3.8 (1.5–9.2)	0.002
Castrated 1–3.5 yr of age	8	57	6,228	12.8 (3.9–21.8)	1.7 (0.7–4.3)	0.31
Castrated after 3.5 yr of age	8	57	7,632	10.5 (3.3–17.8)	1.4 (0.6–3.5)	0.48
Sexually intact	10	120	13,212	7.6 (2.9–12.3)	1.0	
				P trend = 0.008		
Female gonadal exposure subgroup						
Spayed before 1 yr of age	18	57	7,176	25.1 (13.5–36.7)	3.1 (1.1–8.3)	0.02
Spayed 1–5 yr of age	14	108	12,612	11.1 (5.3–16.9)	1.4 (0.5–3.8)	0.63
Spayed after 5 yr of age	14	108	14,856	9.4 (4.5–14.3)	1.2 (0.4–3.2)	1.00
Sexually intact	5	64	6,144	8.1 (1.0–15.3)	1.0	
				P trend = 0.006		

[a] Incidence rate expressed as number of bone sarcomas per 10,000 dog-months.

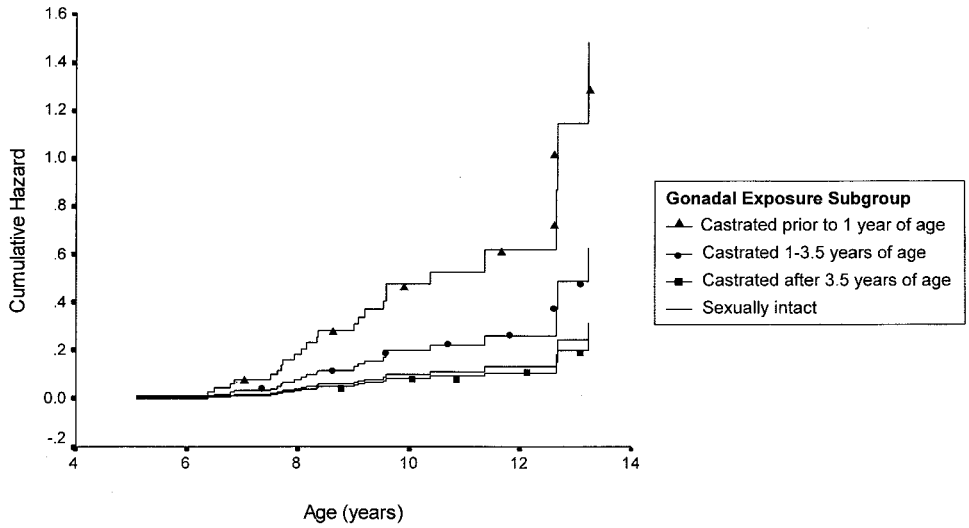

Fig. 1. Multivariate hazard functions for bone sarcoma risk in male Rottweiler dogs according to lifetime gonadal exposure.

gonadectomy within the first year of life. For each dog, we could accurately obtain age at gonadectomy, which provided a highly reliable measure of the duration of gonadal hormone exposure. The possibility that dogs with the same number of years of exposure to ovarian or testicular hormones had significant differences in circulating or target organ concentrations of sex hormones cannot be excluded because serum and tissue hormone concentrations were not measured.

There are very limited published data on Rottweiler dogs with which to compare the population that we studied. One study of dogs in Great Britain reported the median age at death in 101 Rottweiler dogs was 9.8 years (compared with 9.5 years in our study) and that Rottweilers had a >2-fold increased risk for cancer compared with other breeds (29). Our data were collected from the owners and veterinarians of purebred Rottweiler dogs in North America. The high participation rate in this study reflects the high degree of awareness and interest in the bone cancer predisposition of this breed. The dogs in this cohort underwent regular health examinations and received high-quality medical care for health problems. Although this study may overestimate the true incidence of bone sarcoma in the overall Rottweiler population, it is not clear how the strong inverse relationship that we found between gonadal exposure and bone sarcoma risk reflects bias attributable to questionnaire nonresponders. Pet owners who received questionnaires were never informed of the hypothesis that sex hormones might influence bone sarcoma risk. It is reasonable to conclude that nonresponders had a minimal effect on the most important implication of this work: the identification of a high risk target population for bone sarcoma prevention. We believe that the highly motivated pet owners who participated in this study were likely to be quite representative of those who would enroll their Rottweiler dogs in a bone sarcoma prevention trial.

Our finding that neutered Rottweiler dogs are at increased risk for bone sarcoma is consistent with the findings of Ru *et al.* (17). Using a computerized database from North American Veterinary Teaching Hospitals from 1980 to 1994, a case-control study of 3062 osteosarcoma cases and 3959 control dogs was conducted to evaluate risk factors of osteosarcoma in purebred dogs of various breeds. Neutered dogs were at 2.2 times (95% CI = 2.0–2.4) greater risk of osteosarcoma than sexually intact dogs (17). Because the database used in that

Table 5 Univariate analysis of reproductive risk factors of appendicular bone sarcoma in a cohort of 275 female Rottweiler dogs

Risk factor	Dogs with bone sarcoma	Dogs without bone sarcoma	*P*
Total no.	28 dogs	247 dogs	
Pregnancy			0.22
Yes	11 dogs	127 dogs	
No	17 dogs	120 dogs	
Duration of reproductive activity[a]	2.6 ± 1.5 yr	2.5 ± 1.5 yr	0.97
No. of litters	2.0 ± 0.9 litters	2.1 ± 1.1 litters	0.79
No. of live births	10.2 ± 6.5 puppies	11.9 ± 9.1 puppies	0.54
Age at first pregnancy	3.4 ± 1.2 yr	3.6 ± 1.1 yr	0.47

[a] Duration of reproductive activity = total number of years during which she was bred.

study provided no information on age at gonadectomy, the study could not evaluate bone sarcoma risk in terms of duration of gonadal hormone exposure. Our results indicate that dogs undergoing early gonadectomy have the highest risk for bone sarcoma development.

Little is known about how gonadal hormones or other host factors regulate sarcomagenesis. To date, there is no definitive evidence that a sequential multistep process, considered the hallmark of epithelial carcinogenesis (30), is operational in the transformation of mesenchymal cells. The possible mechanisms by which gonadal hormone exposure might protect against the development of bone sarcoma in both males and females are not immediately evident. Endogenous sex steroids such as estrogen and testosterone may serve as prodifferentiation agents that inhibit the malignant transformation of osteoblasts (31). Alternatively, the inverse association may be attributable to indirect effects of sex steroids on body conformation or physical activity. Although female dogs in the early gonadectomy subgroup reached the greatest height as adults, neither adult height nor body weight were significant risk factors for bone sarcoma. Finally, yet to be identified confounding factors unique to dogs that undergo elective gonadectomy before 1 year of age may account for this association. Gonadectomized female and male dogs lived longer than sexually intact dogs in this cohort (Table 1) and in a previous study (32), which might be expected to contribute to a higher overall cancer incidence associated with gonadectomy. However, in this cohort, there was no statistically significant difference in the overall cancer incidence rate in male or female dogs that underwent early gonadectomy before 1 year of age compared with sexually intact dogs (data not shown). Among all cancer diagnoses, bone sarcoma was overrepresented in the early gonadectomy subgroup, representing 27 of 45 (60%) cancer diagnoses compared with the sexually intact group in which only 15 of 66 (23%) cancer diagnoses were bone sarcoma. We found no evidence indicating that dogs in this cohort that underwent early gonadectomy received increased medical surveillance that might translate into increased likelihood of bone sarcoma diagnosis. In this population, there were no apparent differences in the frequency or intensity of veterinary services provided to dogs that underwent early gonadectomy and to those left sexually intact.

Several lines of evidence strengthen our confidence that the inverse association between gonadal hormone exposure and bone sarcoma risk may be causal. Because endogenous sex steroids are essential for skeletal homeostasis (33–38), the hypothesis that alterations in gonadal hormones might influence skeletal oncogenesis has biological plausibility. Secondly, our data in both males and females indicate a consistent inverse dose-response relationship between duration of gonadal exposure and incidence rate of bone sarcoma. Thirdly, there is temporal compatibility between exposure and outcome. Dogs that undergo gonadectomy within the first year of life have a greater risk of bone sarcoma than dogs that undergo gonadectomy later in life. Most Rottweiler dogs are diagnosed with bone sarcoma at age 8–10 years, and no dogs in our study developed bone sarcoma before the age of 1.3 years. Finally, our finding of the possible protective effect of gonadal hormones on bone sarcoma in this study is supported by a previous investigation using a different population of pet dogs (17).

Experimentally, exogenous sex hormones have been shown to suppress (6, 39) or promote (40–43) bone sarcoma development. The effect of exogenous estrogens on the development of radiation-induced bone sarcomas has been studied in mice after i.p. ^{90}Sr administration (43). Mice receiving s.c. estrogens had significantly increased incidence of bone tumors. In another study, mice fed estrogens (diethylstilbesterol and estradiol) had an increased number of spontaneous bone sarcoma (6 tumors in 1242 mice) compared with mice fed control diet (0 tumors in 356 mice; Ref. 40). No clear relationship between bone sarcoma development and dose or duration of dietary estradiol was found. The authors of that study acknowledged that the low incidence of spontaneous bone sarcoma (0.48%) in estrogen-fed mice resulted in inadequate power to reach statistical conclusions. Instead, we studied a population of Rottweiler dogs with a high incidence (12.6%) of spontaneous bone sarcoma. We focused on the role of endogenous sex steroids, rather than exogenous hormones. In contrast to these rodent studies, our results suggest that endogenous sex hormones have a protective effect on the spontaneous development of bone sarcoma within a dog breed that is programmed for high incidence of bone sarcoma. Although the gene-environment interactions that determine an individual's risk to develop bone cancer are poorly understood, our findings suggest that gonadal hormones are part of the internal environment that may significantly modify the risk for sarcomagenesis. For this reason, this work may have important implications for elucidating the complex interactions between genetic and environmental factors that regulate bone sarcomagenesis.

Thirty-five years ago, Tjalma (16) reported that the risk of bone sarcoma in large and giant breed dogs exceeded that of small breed dogs by as much as 185-fold. Although adult height and body weight are strong predictors of bone sarcoma between different breeds of dogs, the association between skeletal growth or body size and bone sarcoma risk had never been analyzed within a breed. In this study, using univariate and multivariate analyses, adult height and body weight were not significant risk factors of bone sarcoma development. In fact, neither the tallest nor heaviest adult Rottweiler dogs were at highest risk for bone sarcoma. Because adult height and body weight were owner reported, interobserver variation may have obscured significant between group differences in these parameters. Additional work is needed

to determine whether quantitative measures of the rate or duration of skeletal growth (*e.g.*, length of the radius or other long bones; age at physeal closure) are strongly associated with bone sarcoma risk in Rottweiler dogs.

In summary, this study found that male and female Rottweilers with the shortest lifetime gonadal exposure had the highest risk for bone sarcoma. Dogs that underwent early elective gonadectomy had a one in four lifetime risk of bone sarcoma development compared with a significantly reduced risk among dogs that were sexually intact throughout their lifetime. Although it remains unclear how endogenous gonadal hormones influence bone sarcoma development, our work provides the framework for selecting a target population for bone sarcoma prevention studies. We have identified a subgroup of Rottweiler dogs, recognizable as young adults, that are at high risk to subsequently develop spontaneous bone sarcoma. With the identification of this target population, practical clinical trials using pet dogs can be designed to test whether chemoprevention strategies can significantly delay or prevent the development of bone sarcoma. The conduct of such trials using pet dogs will further validate the use of the comparative approach to develop and test novel strategies that will decrease cancer-related mortality in humans.

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