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Age, weight and circulating concentrations of total testosterone are associated with the relative prostatic size in adult intact male dogs

Bodil Ström Holst *, Sanna Nilsson [1]

Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07, Uppsala, Sweden

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ABSTRACT

Prostatic hyperplasia (PH) is an androgen-dependent condition associated with increased prostatic size that is common in intact dogs, and similar to the condition in men. In dogs, the increase in prostatic size is most prominent the first years, and after approximately four years (in beagles), a plateau is reached, and further growth is slower. Why the prostate continues to grow more in some individuals is not clear. Most testosterone in the circulation is bound to albumin or sex hormone binding globulin (SHBG) and only a minor part is unbound and biologically active. The binding to SHBG has higher affinity than that to albumin. In addition, SHBG has own biological functions, modifying testosterone action. The aim of the present study was to investigate if there is an association between relative prostatic size and the variables total testosterone concentration, SHBG concentration, an estimation of bioavailable testosterone: the ratio between testosterone and SHBG (free androgen index, FAI), estradiol concentration, the estradiol/testosterone ratio, dog age and dog weight. Hormone concentrations were measured in serum from 79 intact male dogs aged ≥ four years, weighing ≥ five kg. The size of the prostate was estimated using ultrasonography, and relative prostate size, S_{rel}, was calculated as the estimated size related to the normal size for a 4-year-old dog of the same weight. There as a negative correlation between testosterone concentration and age ($\rho = -0.27$, P = 0.018) and a positive correlation between age and S_{rel} ($\rho = 0.27$, P = 0.016) and between SHBG and weight ($\rho = 0.38$, P = 0.001). The FAI was negatively correlated with dog weight ($\rho = -0.32$, P = 0.004). There were no significant correlations between S_{rel} and SHBG or FAI or between estradiol or estradiol/testosterone and S_{rel}, age or weight. A multiple regression analysis showed significant associations between log S_{rel} and log testosterone concentration, log age and log weight of the dog, with an adjusted R^2 of 9.5%. Although the variables total testosterone concentration, age and weight of the dog were all significantly associated with S_{rel}, the coefficient of determination was low, indicating that they only explained a minor part of the prostatic size. The results support the analysis of total testosterone in studies of prostatic growth in the dog.

1. Introduction

Prostatic hyperplasia (PH), traditionally called benign prostatic hyperplasia [1,2], is the most common prostatic disease in the dog, making the dog a valuable model for comparative studies of the condition in humans [3]. One difference is that the canine prostate has no clear zones, and PH in the dog is characterized by hypertrophy and hyperplasia of the alveolar cells in all portions of the gland [4] whereas PH in men is most prevalent in the transitional zone [5]. In addition, carcinomas are very uncommon causes of prostatic enlargement in dogs compared to in men [6,7]. There is a breed predilection, and large breeds are more commonly predisposed to PH than small or miniature breeds [6,8–10]. Prostates with hyperplasia are generally larger than normal prostates [4], and prostate weight closely parallels the presence of histologically defined PH [11]. With increasing age, almost every intact male dog will develop PH [4], and because of this PH is the most common cause of enlarged prostates in the dog. Estimation of prostatic size is common when diagnosing PH. With increased size of the prostate, clinical signs, such as problems with defecation and urination, are more commonly reported [12], but the severity of clinical signs does not correlate with prostatic size [13]. Androgens are needed for the normal development of the prostate and for proliferation of

* Corresponding author.
E-mail address: Bodil.Strom-Holst@slu.se (B.S. Holst).
[1] Present address: Ekeby Djurpraktik, Ekeby Bruk Ing 2 M, SE-75263 Uppsala, Sweden, e-mail: sannaclaudianilsson@gmail.com

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prostatic cells, and PH is dependent on androgens [14]. Prostatic hyperplasia is thus a scourge of the intact male dog.

Although androgen concentrations tend to decrease with age [15], the prevalence of PH increases with age [4]. There is no apparent change in estrogen concentrations with age [15], and in dogs with PH, an increased estradiol/testosterone ratio has been described [9], possibly inducing the condition. It is also possible that a prolonged influence of androgens above a certain threshold induces PH. Generally, the total testosterone concentration is measured. However, only the free proportion may exert a biological effect [16]. The proportion of free testosterone is considered to be low, in humans 1–3% [17]. Most testosterone is transported in the bloodstream bound to sex hormone binding globulin (SHBG) with high affinity or to albumin with low affinity [18]. The ratio between total testosterone and SHBG is called the free androgen index (FAI), a measure of bioavailable testosterone [19].

SHBG is a glycoprotein, a homodimer that is produced by the hepatocytes in the liver [18]. SHBG binds to both estradiol and testosterone, the affinity to testosterone being several times stronger than that for estradiol [20]. By binding testosterone, SHBG regulates testosterone access to target cells, decreasing the free testosterone concentration and increasing the total testosterone concentration via the hypothalamic-pituitary feedback mechanism and via increased circulating ligand half-life [16]. In addition to being a carrier for testosterone, SHBG has own specific functions. There are high concentrations of SHBG in the glandular cells of the prostate [20]. There are also receptors for SHBG, R_{SHBG}, in the plasma membranes of the prostate glands [21]. Both estradiol and a metabolite to DHT (3-α-diol) may bind to and activate the SHBG-R_{SHBG} complex, leading to an increase in cellular cAMP [22]. Estradiol and 3-α-diol may also substitute for androgens, activating the androgen receptor via a signal transduction pathway involving cAMP activated by the SHBG- R_{SHBG} complex [23]. In addition, SHBG may modify the action of testosterone within the prostate [24] and kidney [25].

The aim of the present study was to investigate if there is an association between relative prostatic size and the variables total testosterone concentration, SHBG concentration, an estimation of bioavailable testosterone: the ratio between testosterone and SHBG (free androgen index, FAI), estradiol concentration, the estradiol/testosterone ratio, dog age and dog weight.

2. Material and methods

2.1. Dogs

Serum samples from a previous study, stored in −80 °C, were used [12]. The samples were from 79 dogs; 70 purebred dogs of 40 different breeds and 9 mixed breed dogs. All dogs were intact male dogs aged ≥ four years, weighing ≥ five kg and no dog had been treated with anti-androgens within the preceding six months Their mean age was 7.5 years (SD 2.6), range 4–13 years, and mean weight 24.5 kg (SD 12.2), range 5.6–48.6 kg. The prostates of the dogs had been examined by ultrasonography. The prostatic volume (V) for the individual dogs had been calculated according to Kamolpatana and co-workers as V = (1/2.6) * (Length * Width * Height) + 1.8 cm [26].

2.2. Analysis of testosterone (T), sex hormone binding globulin (SHBG), and estradiol

The total testosterone concentration in serum was analyzed using Immulite® 2000 (Siemens Healthcare Diagnostics, Erlangen, Germany), SHBG was analyzed using a commercial canine ELISA (Amsbio, Milton Park Abingdon, UK) and estradiol was also analyzed using a commercial canine ELISA (AssayGenie, Dublin, Ireland). There was not enough serum, leading to missing values, for one dog for the SHBG analysis and for five dogs for the estradiol analysis. All samples were analyzed in duplicate.

2.3. Statistical analysis

Statistical analyses were performed using a commercially available software program (Minitab 18, Minitab Inc., State College, PA, USA). A value of $P < 0.05$ was considered significant.

The relative prostate size, S_{rel} (size of the prostate related to the normal size for a 4 year old dog of the same weight), was calculated as previously described [12], and the FAI was calculated as S-total testosterone/S-SHBG.

Spearman's ρ was used to evaluate potential associations between the variables (dog age, dog weight, S_{rel}, testosterone, SHBG, FAI, estradiol and estradiol/testosterone). A two sample *t*-test was applied to compare the $LogS_{rel}$ between dogs with clearly increased SHBG concentrations and the other dogs.

After logarithmic transformation of all variables, a multiple regression analysis was applied to evaluate the effect of dog age, weight, testosterone, SHBG, FAI, estradiol and estradiol/testosterone on S_{rel}. There was no significant association between S_{rel} and estradiol or estradiol/testosterone ($P = 0.46$ for both). Because of this, and to keep as many dogs as possible in the model, estradiol and estradiol/testosterone were omitted from the final model. The residuals were normally distributed. Plots of residuals versus variables were checked and the variance inflation factors, VIF, were low (1.08–1.16), and the model was thus deemed appropriate.

2.4. Ethical considerations

The study was approved by the local animal ethical committee, permit number C89/14. Participating dog owners had given their informed written consent.

3. Results

The median concentration and inter-quartile range (IQR) was for testosterone 7.2 nmol/l (3.4–14.5), for SHBG 4.9 nmol/l (3.4–7.0) and for estradiol 4.5 nmol/l (3.9–5.2). There was a negative correlation between testosterone concentration and age ($\rho = -0.27$, $P = 0.018$) and a positive correlation between S_{rel} and age ($\rho = 0.27$, $P = 0.016$).

There was a positive correlation between SHBG and weight ($\rho = 0.38$, $P = 0.001$), with 11 dogs having clearly elevated SHBG concentrations (Fig. 1). The breeds of the dogs with high SHBG concentrations were (number of dogs with high SHBG/total number of dogs of this breed included in the study): Beauceron (1/1), Breton (1/1), Rough collie (1/1), Golden retriever (1/6), Irish setter (2/2), Rottweiler (3/3) Springer Spaniel (1/1) and German wirehaired pointer dog (1/1). The log S_{rel} did not differ significantly between the dogs with the highest SHBG concentrations and the rest of the dogs ($P = 0.32$). When they were removed, the association between SHBG and weight was not significant ($P = 0.17$).

The FAI was negatively correlated with dog weight ($\rho = -0.32$, $P = 0.004$). There were no significant correlations between S_{rel} and SHBG or FAI.

There were no significant correlations between estradiol or estradiol/testosterone and the age or weight of the dog, or with S_{rel}.

The final multiple regression model showed significant associations between log Srel and log age and weight of the dog, and log testosterone concentration (see Table 1). The model had an adjusted R^2 of 9.5%.

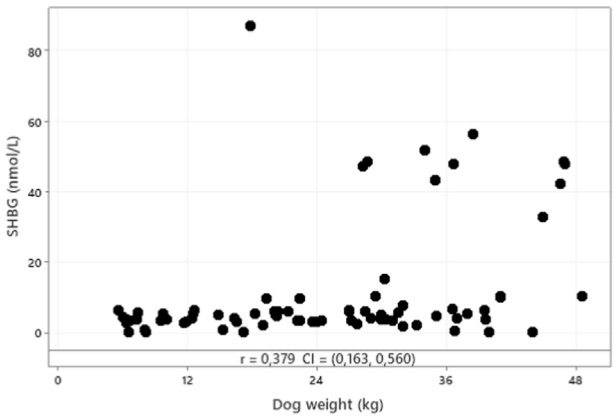

Fig. 1. Spearman correlation between dog weight and concentration of SHBG
SHBG = sex hormone binding globulin, r = correlation coefficient, CI = confidence interval.

4. Discussion

In the present study, prostatic size was positively associated with age, weight and testosterone concentration. Furthermore, the testosterone concentration was negatively correlated with age, the SHBG concentration was positively correlated with dog weight and the FAI was negatively correlated with weight. There were no significant associations between prostatic size and SHBG or FAI. Neither the serum estradiol concentration, nor the estradiol/testosterone ratio, was associated with age, weight or S_{rel}. It should be noted that in the present study, no diagnoses of PH were made, and the studied associations were with the relative size of the prostate. However, prostatic size is strongly associated to PH [11].

The increased prostatic size with age is in accordance with what has been previously described. All included dogs were more than 4 years old. This is the age up to which the major part of prostatic growth occurs, although prostatic growth continues at a reduced rate also after 4 years [11]. In the present study, prostatic size was calculated as a relative measure, related to what would have been normal for a dog of this weight at 4 years of age [12]. The positive association between prostatic size and dog weight is therefore not just an effect of larger prostates in larger dogs, but shows that the larger dogs have relatively larger prostates than smaller dogs. The present study included a variety of breeds, with only few dogs per breed. It has previously been described that most breeds predisposed to PH are medium to large size breeds [6,8–10], and the results of the present study extends this finding to a general association between relative prostatic size and dog weight. Why larger dogs, and especially certain breeds, have relatively larger prostates is not known. In the present study, there was a significant positive association between SHBG concentrations and dog weight. This could possibly indicate an effect of SHBG on prostatic growth and in the pathogenesis of PH. There are receptors for SHBG in the prostate and a suggested stimulatory effect on the prostate of the SHBG-R_{SHBG} complex [22]. The expression of SHBG in the prostate, and concentration in the serum, increases with age in rats [27] whereas a general decrease in serum SHBG concentration with age has been described in men [28]. However, there was no association between serum SHBG concentration and the size of the prostate in the present study.

In men, obesity and the metabolic syndrome have been associated with prostate enlargement [29,30]. Men with PH more often than controls had the metabolic syndrome, and also lower concentrations of estradiol and SHBG [2]. The inflammation that is associated with obesity has been described to induce an androgenic to estrogenic switch in the prostate gland, which may contribute to the association with prostatic enlargement [31]. The peripheral estradiol concentration was not associated to prostatic size in the present study. The body condition score of the dogs had not been recorded, and because many different breeds were represented, it was impossible to use weight as a proxy for body condition.

Another possibility for the relation between prostatic size and dog weight could be related to insulin-like growth factor (IGF-I). Circulating IGF-I concentrations differ between dog breeds of different sizes and are higher in larger dogs [32,33]. In a study including Labrador retriever and Rhodesian ridgeback dogs, Labrador retrievers with clinical signs of PH had significantly lower concentrations of IGF-I than dogs without such signs [34]. There was also a negative association between IGF-I and a marker of prostate volume, canine prostate specific esterase (CPSE) [34]. The possible role of IGF-I in prostatic growth and the development of PH in dogs remains to be evaluated.

The finding in the present study that prostatic size is related to the total testosterone concentration in mature and old dogs of many different breeds is interesting. Only one sample per dog was collected, and without preceding stimulation of the gonads. Testosterone concentrations fluctuate during the day [35,36], and these fluctuations may mask minor associations. Still, the testosterone concentration decreased with age in the present population of dogs 4 years and older. A positive association between testosterone concentrations and size of the prostate has previously been described in populations including younger dogs: Beagles 0.6–9 years old [11], and in Rhodesian ridgebacks, but not in Labrador retrievers, in a study on dogs 1.5–6 years old [10]. In a smaller study including 22 dogs, no association between the relative prostatic size and steroid concentrations (androgens, estrogens, progestins or corticosteroids) was found [37]. In men, there are conflicting results regarding testosterone concentration and prostatic size or PH. One study described high testosterone concentrations to be associated with PH [38] whereas another study reported a decreased risk of PH with increased testosterone concentrations. In the latter study, the finding was discussed as possibly related to a reduced activity of 5-α-reductase, leading to a decreased conversion of testosterone to the more active compound dihydrotestosterone (DHT) [39]. In a third study, prostatic size in men was correlated with age but not with testosterone concentrations [40]. In prostatic tissue from dogs with PH, the net formation of DHT is increased [41], and PH has also been associated with an increase in the estrogen/testosterone ratio [9]. In the present study, neither estradiol nor the estradiol/testosterone ratio was associated with age or the prostatic size. The role of estrogens in prostatic growth may vary depending on other hormones. In humans, the association between estrogens and prostatic size differs between men with low and men with high concentrations of free testosterone [42]. The results of the present study emphasize the role of testosterone in prostatic growth in mature and old dogs. In human medicine, it has been debated whether total testosterone or free

Table 1
The association between Log relative prostatic size (S_{rel}) and log age and weight of the dog, log concentration of testosterone (T) and sex hormone binding globulin (SHBG) and their ratio. Adjusted R^2: 9.5%.

Term	Coefficient	SE	T-Value	P-Value	VIF
Constant	−0,412	0,200	−2,06	0,043	
Log dog age	0,432	0,150	2,87	0,005	1,10
Log dog weight	0,1879	0,0918	2,05	0,044	1,16
Log T concentration	0,1227	0,0586	2,09	0,040	1,12
Log SHBG concentration	−0,0111	0,0415	−0,27	0,789	1,13
log T/Log SHBG	0,000007	0,000610	0,01	0,991	1,08

testosterone should be analyzed in clinical studies [17,19,20]. The present study supports the analysis of total testosterone, and not the bioavailable fraction (FAI), for studies of prostatic growth in the dog.

The samples had been stored at −80 °C for several years before analysis, and had been thawed previously for another study [12]. The use of stored samples enables new research questions to be investigated according to the principle of 3R [43]. All samples were treated the same way, steroids are stable during storage and after freeze-thawing [44], and SHBG has also been found to be stable during storage [45] and with minor changes related to freeze-thawing [46], supporting the validity of the results.

In the present study, the size of the prostates was estimated using ultrasound. PH is the most common cause of prostatic growth in the dog and thus the most likely cause for increased prostatic sizes. However, no diagnostic measures to diagnose PH were undertaken. The three factors age, weight of the dog and testosterone concentrations were all significantly associated with S_{rel}, but the R^2 of the model was low, and they thus only explain a minor part of the increase in prostatic size. There may be several explanations to this. It has been suggested that an increased sensitivity of the prostate to androgens and estrogens may be initiated early in life, long before PH actually develops [15], and the endocrine milieu when the dog has developed hyperplasia would then not be the same as that linked to the initiation of hyperplastic changes. Other factors contributing to PH have also been suggested. The environmental pollutant bisphenol A (BPA) has been associated to PH in the dog, probably via endocrine disruption [47]. Chronic inflammation has been suggested to be involved in the progression to PH in men [48] and is common in dogs with PH [4,49,50]. In men it has been described that those that have been diagnosed with prostatitis are at increased risk of later developing PH [51]. The mechanism by which inflammation induces hyperplasia is not clear, but growth factors such as fibroblast growth factor and neurotrophic factor are more commonly expressed in hyperplastic than in normal dog prostates [50]. Although testosterone is fundamental, other factors thus seem important for the prostatic growth.

5. Conclusion

A multiple regression analysis showed significant associations between the relative size of the prostate (the size related to the normal prostatic size of a 4-year-old dog of the same weight), and total testosterone concentrations, age and weight of the dog. The coefficient of determination was only 9.5%, and other factors thus contribute largely to the prostatic size. The SHBG concentration was correlated to the weight of the dogs, which is interesting because PH is more common in dogs of larger breeds. However, no association was detected between prostatic size and SHBG concentration, or between prostatic size and estradiol concentration or the estradiol/testosterone ratio. The results support the analysis of total testosterone in studies of prostatic growth in the dog.

CRediT authorship contribution statement

Bodil Ström Holst: Conceptualization, Supervision, Funding acquisition, Writing – original draft, Writing - Original draft preparation, Writing – review & editing, reviewing and editing. **Sanna Nilsson:** Investigation, Writing – original draft, Writing - Original draft preparation.

Declaration of competing interest

The authors declare no conflict of interest.

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