Biomarkers of Ageing in Dogs

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Short Report

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Abstract

Gerontology is a major research topic in veterinary medicine. Although several reports have shown that aging in companion dogs is similar to human aging, there are few reports on changes in biomarker levels in aged dogs. The purpose of this preliminary study was to evaluate the differences in biomarker levels between young and old companion dogs, focusing on cardiovascular and musculoskeletal biomarkers. The young dogs included those aged under 36 months (n = 16, 19.8 ± 9.3 months), while the old ones included those aged over 108 months (n = 16, 155.8 ± 22.8 months). All dogs were referred to our university due to patellar luxation. The mean weight of the young and old dogs was 2.9 ± 0.9 kg and 4.0 ± 1.1 kg, respectively. We measured the serum concentrations of brain-derived neurotrophic factor (BDNF), osteoprotegerin (OPG), angiotensin II (ANGII), and endothelin-1 (ET-1) in both groups. Although the concentrations of BDNF did not differ between the two groups, the OPG, ANGII, and ET- 1 levels were significantly higher in the old companion dogs than in the young dogs. The results showed that the concentration of some biomarkers may increase as dogs age, especially OPG, ANGII, and ET- 1. This may inspire further research on osteoporosis and cardiovascular diseases in elderly dogs.

Introduction

Gerontology is a major research topic in human and veterinary medicine. Epigenetics is the study of chemical features that attach to genes and affect their activity. In recent years, comparisons between dog and human methylomes have revealed a nonlinear relationship that translates dog years to human years. These studies also showed that the timing of major physiological milestones was similar between the two species (Hoffman et al. 2018; Sándor et al. 2019).

In a study of dogs, Alexander et al. (2018) reported that several inflammatory and immunological substances, such as immunoglobulin M and 8-hydroxy-2-deoxyguanosine, increased with age, but that immunoglobulin G and C-reactive protein levels did not change. Unlike mice and rats, companion dogs are affected by diverse spontaneously occurring diseases similar to those in humans, such as age-related neurological, cardiovascular, and musculoskeletal diseases (Inoue et al. 2015). In human geriatric medicine, there are common problems associated with the musculoskeletal system (such as sarcopenia and osteoporosis) (Lan et al. 2006; Marzetti et al. 2013), cardiovascular system (Dewhurst et al. 1991), and cognition (Miniksar et al. 2021). For example, osteoprotegerin (OPG) is steadily released from vascular endothelial cells in response to inflammatory stimuli, which can be an important factor in osteoporosis (Coulson et al., 2017). However, brain-derived neurotrophic factor (BDNF) has been reported to rescue synaptic and muscle function and select molecular mechanisms in muscles that might underlie improved cellular functions, especially those associated with cognition (Miniksar et al. 2021).

Major problems are also observed in the cardiovascular system in geriatric medicine. Angiotensin II (ANGII) is a well-known peptide hormone that causes vasoconstriction and elevates blood pressure, primarily through the ANGIII-converting enzymes in the lungs and endothelial cells, kidney epithelial cells, and the brain (Forrester et al. 2018). It is also associated with sarcopenia (Marzetti et al. 2013). Another

essential biomarker is endothelin-1 (ET-1), a potent endogenous vasoconstrictor produced mainly by the vascular endothelium (Yanagisawa et al. 1988). In a human study, the concentration of ET-1 in young individuals was significantly lower than that in older individuals (Maeda et al. 2003), suggesting the detrimental effects of various diseases associated with aging, including hypertension and metabolic syndrome (Yu et al. 2015). However, the levels of these biomarkers have not been measured in younger and older companion dogs. As such, the purpose of this preliminary study was to investigate the difference in each parameter between young and elderly companion dogs.

Materials And Methods

Animals

The dogs in this study were referred to the Rakuno Gakuen University between March 2020 and February 2021 due to medial patellar luxation. The definitions of young and old were as follows: young referred to dogs aged less than 36 months, while old referred to dogs aged over 108 months. All dogs weighed less than 5 kg, similar to those reported in a previous study (Sándor et al. 2019). This study was approved by the Rakuno Gakuen University, School of Veterinary Medicine Institutional Animal Care and Use Committee (approval No. VH19A10).

Measurements of biomarkers

Serum samples were collected from the saphenous vein and stored at − 80°C until the time of measurement. All measurements were performed commercially using canine enzyme-linked immunosorbent assay (ELISA) kits. Canine ET-1 levels were measured using a canine ET-1 ELISA kit (Cat No, MBS2606443), with a sensitivity of 5 pg/mL. BDNF levels were measured using a canine BDNF ELISA kit (Cat No, RK00516), with a sensitivity of 0.056 ng/mL. Canine ANGII levels were measured using an ANGII ELISA kit (Cat No, MBS705510), with a sensitivity of 4.68 pg/mL. Lastly, canine OPG levels were measured using a canine OPG ELISA kit (Cat No, MBS011402), with a sensitivity of 0.1.

Statistical analysis

Unpaired Student’s t-tests were used to compare the young and old dogs. Analysis of variance (ANOVA) with a post-hoc Tukey-Kramer test was used to determine sex differences in all young and old dogs. Differences were considered significant at $P < 0.05$.

Results

Animals

As shown in Table 1, the young group had a mean age of 19.8 ± 9.3 months, while the old group had a mean age of 155.8 ± 22.8 months. The body weight (kg) of the young group was 2.9 ± 0.9, lower than that

of the old group (4.0 ± 1.1). In the young group, there were eight males (six neutered, two intact) and eight females (four spayed, four intact). In the old group, there were eight males (four neutered, four intact) and eight females (four spayed, four intact). As for the breeds, the young group included mixed (n = 7), Toy poodle (n = 4), Chihuahua (n = 2), Pomeranian (n = 1), Maltese (n = 1), and a Pekingese (n = 1). Meanwhile, the old group included Pomeranian (n = 3), Miniature schnauzer (n = 3), Chihuahua (n = 2), Papillon (n = 2), Shiba inu (n = 2), Mixed (n = 2), Boston terrier (n = 1), and a Toy poodle (n = 1).

Serum concentrations of biomarkers

The serum concentration of the musculoskeletal biomarker OPG was significantly higher in the old group (1.7 ± 0.39 ng/mL) than in the young group (0.86 ± 0.47 ng/mL; $p < 0.05$). ANOVA also revealed significant differences between young males (1.19 ± 0.14 ng/mL) and young females (0.54 ± 0.17 ng/mL), young males and old males (1.92 ± 0.14 pg/mL), and young males and old females (1.43 ± 0.23 ng/mL), as seen in Fig. 1a. In contrast, the serum concentration of BDNF did not significantly differ among the four groups. The results were as follows: young, 3.39 ± 1.23 ng/mL; old, 3.35 ± 0.69 ng/mL; young males, 4.1 ± 1.36 ng/mL; young females, 2.6 ± 0.28 ng/mL; old males, 3.39 ± 0.67 pg/mL; and old females, 3.06 ± 0.11 ng/mL (Fig. 1b). As shown in Figs. 2a and 2b, the serum concentration of the cardiovascular biomarker ANG II was significantly higher in the old group (16.3 ± 3.0 pg/mL) than in the young group (10.5 ± 3.5 pg/mL; $p < 0.05$). Specifically, the ANGII levels were significantly elevated in the old males (16.0 ± 3.3 pg/mL) and females (16.7 ± 3.3 ng/ml) than in the young males (12.6 ± 1.8 pg/mL) and females (8.4 ± 3.6 pg/mL). Similarly, the serum concentration of ET-1 in the old group (34.0 ± 0.96 pg/mL) was significantly higher than that in the young group (30.68 ± 0.69 pg/mL; $p < 0.05$). Specifically, the old males (34.4 ± 1.1 pg/mL) and females (33.65 ± 0.69 ng/mL) had higher levels than the young males (30.7 ± 0.58 pg/mL) and females (30.63 ± 0.83 pg/mL).

Discussion

In this preliminary study, serum OPG, ANGII, and ET-1 concentrations were significantly higher in the older companion dogs than in the younger companion dogs. The BDNF levels did not differ between the two groups in our study. A decrease in serum BDNF levels is negatively correlated with cognitive decline and memory in dogs (Sechi et al. 2015). Most studies on aging involving laboratory beagles have compared those aged over and under seven years to investigate the cognitive decline associated with aging. However, beagles may present with short-term memory impairments as early as six years (Studzinski et al. 2006). There is also a neurobehavioral syndrome that may affect dogs aged over eight years referred to as canine cognitive dysfunction or cognitive dysfunction syndrome (Chapagain et al. 2018). In our preliminary study, none of the elderly dogs showed symptoms of cognitive dysfunction. Collectively, the onset time of cognitive dysfunction in dogs has remained controversial, necessitating an understanding of the relationship between BDNF levels and cognitive dysfunction syndrome in elderly dogs.

In our study, serum OPG concentrations were higher in the older dogs than in the younger dogs, a trend that has also been observed in humans (Coulson et al. 2017). Circulating levels of OPG are higher in older adult humans than in young humans and positively associated with whole-body bone mineral density, suggesting an association with osteoporosis (Coulson et al. 2017). There have been a few reports of osteoporosis in veterinary medicine (Weigel et al. 1981). Interestingly, our study showed sex-based differences in both the young and old dogs, which was also observed in a previous study (Hoffman et al. 2018), indicating that there might be sex-based differences in bone metabolism during aging. Thus, the spontaneous bone changes seen in canines during aging might be similar to those in humans. Our findings for the cardiovascular biomarkers ANGII and ET-1 might be similar to previous findings in humans (Yu et al. 2015; Forrester et al., 2018; Cabiati et al. 2020) and rats (Ishihata et al. 2006). The ANG-converting enzyme 2 activity in plasma and tissue is elevated in dogs with heart disease (Larouche-Lebel É et al. 2019). In addition, ET-1 is known to be a cardiovascular biomarker for aging in rats (Cabiati et al. 2020). Thus, although we did not evaluate the blood pressure or perform echocardiography or other general blood tests, we believe veterinary practitioners might need to test serum ANG II and ET-1 concentrations in elderly dogs in some clinical settings, such as for cardiac diseases (Prosek et al. 2004).

In the sex-based comparisons between young and old dogs, there were significant differences in all the parameters in our study, except for BDNF. In a different study, limited sex-based effects were noted in either longevity or causes of death in companion dogs, suggesting that the most significant sex-based differences in most canine populations may be due to the effects of neutering (Hoffman et al. 2018). In our study, we included both intact and neutered dogs. Thus, we should evaluate the effects of neutering in dogs in the future. The limitations of this study included the limited number of dogs in each group and the inclusion of only two broad categories (young and elderly companion dogs) for testing the concentration of each biomarker. We plan to conduct further studies on biomarkers associated with cardiovascular diseases and osteoporosis in the near future. In summary, this study shows that some musculoskeletal and cardiovascular biomarkers are present at higher levels in elderly dogs and should be considered by practitioners.

Declarations

Statement of animal ethics

The animal ethics for this study was approved by the Rakuno Gakuen University, School of Veterinary Medicine Institutional Animal Care and Use Committee (approval No. VH19A10).

Code or data availability

Our original research encouraged to include formal citations to datasets in article reference lists where deposited datasets are assigned Digital Object Identifiers (DOIs) by a data repository.

Consent to participate

We authorize the staff of to perform the procedure (blood collection). While we accept that all procedures will be performed to the best of the abilities of the staff at this hospital.

Consent to publication

The authors agree the consent to publication by Veterinary Research Communications. We understand our manuscript may change during the review and production processes. Also, once the article is published, I cannot remove my consent.

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Conflict of interest statement

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of this paper.

Author contributions

Mitsuhiro Isaka: principal investigator, study idea, study design, data analysis, and manuscript preparation; Hiroshi Ueno and Ryuji Araki: data collection.

Ethics approval

This study was approved by the Rakuno Gakuen University, School of Veterinary Medicine Institutional Animal Care and Use Committee (approval No. VH19A10).

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Table

Table Characteristics of dogs

	Young (n=16)	Older (n=16)
Age (months)	19.8 ± 9.3	155.8 ± 22.8
Body weight	2.9 ± 0.9	4.0 ± 1.1
Sex	male (n=8) casted (n=6) intact (n=2) female (n=8) neutered (n=4) intact (n=4)	male (n=8) casted (n=4) intact (n=4) female (n=8) neutered (n=4) intact (n=4)
Breed	MIX (n=7) T.Poodle (n=4) Chihuahua (n=2) Pomeranian (n=1) Maltese (n=1) Pekinese (n=1)	Pomeranian (n=3) M. schnauzer (n=3) Chihuahua (n=2) Papillon (n=2) M.Shiba (n=2) Mix (n=2) B. Terrier (n=1) T. Poodle (n=1)

Figures

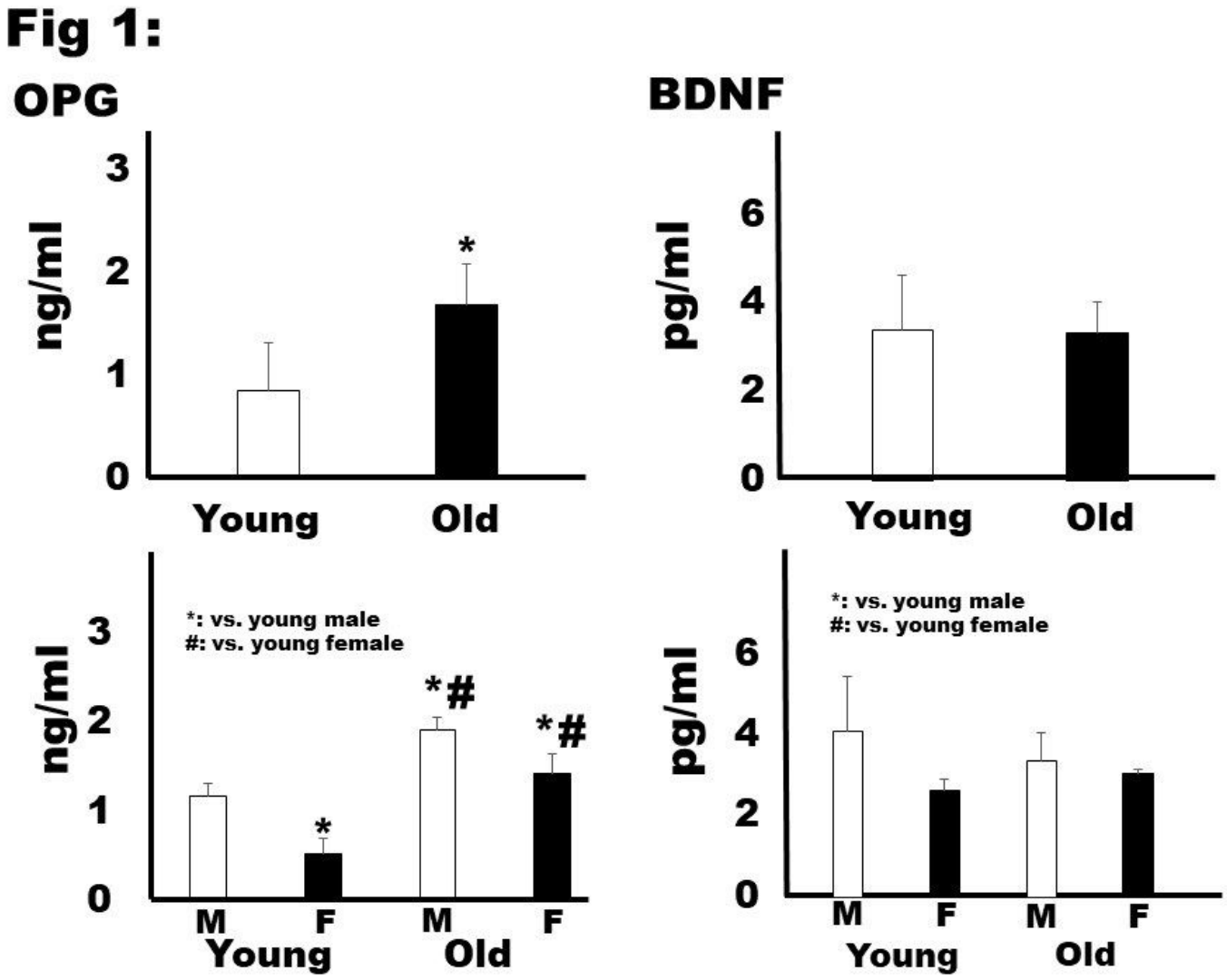

Figure 1

Comparison of musculoskeletal system biomarkers by sex and age a. OPG, osteoprotegerin; b. BDNF, brain-derived neurotrophic factor

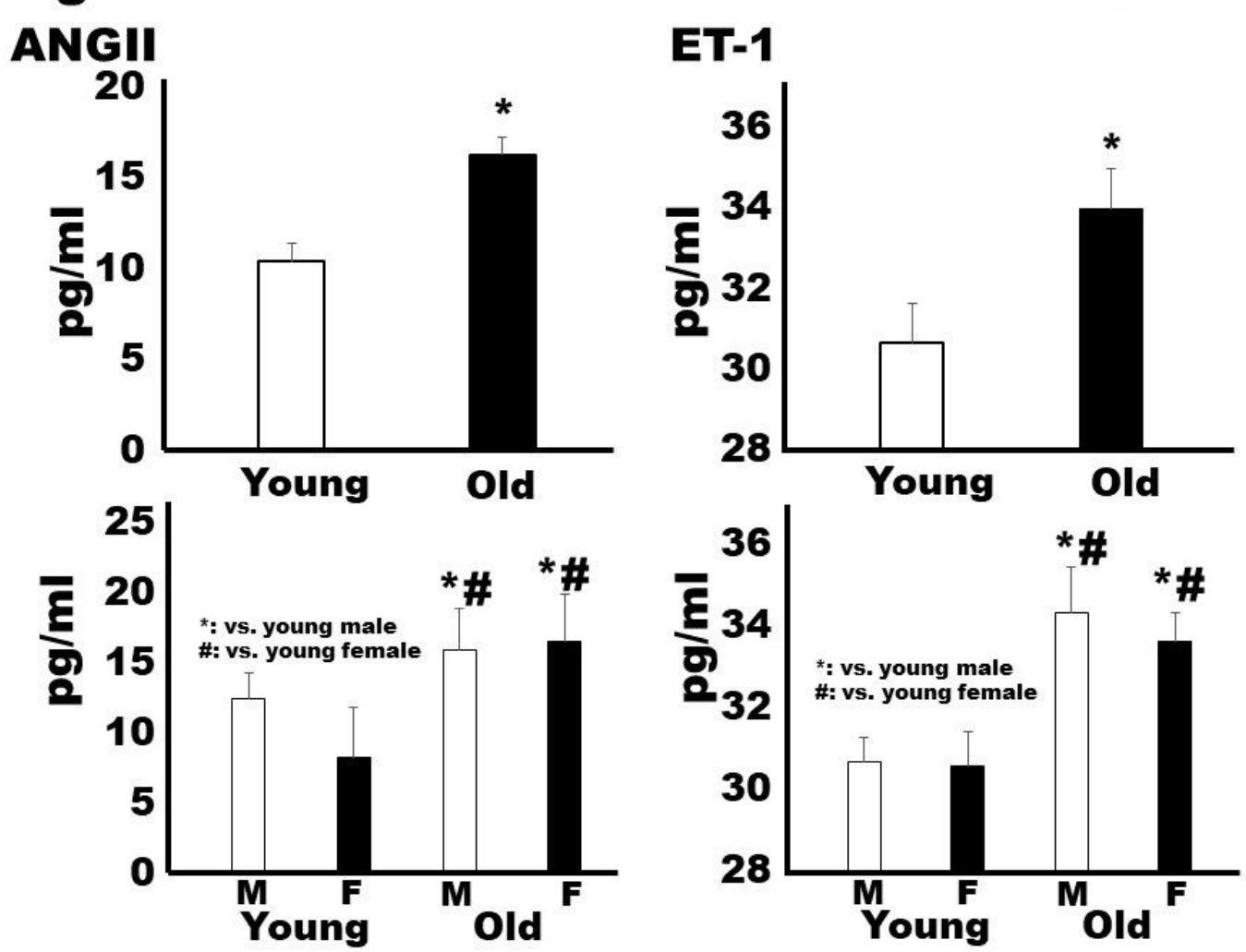

Figure 2

Comparison of cardiovascular system biomarkers by sex and age a. ANGII, angiotensin II; b. ET-1, endothelin-1