

# Evaluation of ultrasonography for measurement of skin thickness in Shar-Peis

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**Objective**—To determine whether high-frequency diagnostic ultrasonography is useful for assessment of skin thickness in Shar-Peis.

**Animals**—10 healthy Shar-Peis and 10 healthy Beagles used as controls.

**Procedures**—Ultrasonographic examination of the skin was performed on 4 cutaneous sites by use of a 13-MHz linear-array transducer, and the mean of 3 measurements was calculated. Ultrasonography results were compared with histologic findings of skin specimens stained with H&E, Alcian blue at a pH of 2.5, and Masson trichrome stains, with histometric measurements of skin thickness made by use of a microscope, and with measurements of skin thickness made by use of a plicometer. Ultrasonography results were also compared via age and sex of selected animals.

**Results**—A clear correlation was detected between ultrasonography results and results of histologic and histometric analysis in both groups. In Shar-Peis, no correlation was found between ultrasonography results and age and sex, whereas in Beagles, a weak positive correlation was found only between skin thickness in dorsal cervical and frontal (on the rostral margins of the supraorbital processes) regions and age. A positive overall correlation was found in Shar-Peis between measurements made via ultrasonography and plicometry.

**Conclusions and Clinical Relevance**—Ultrasonography was a useful tool to assess skin thickness, and in Shar-Peis, it might be considered a valid alternative to invasive methods such as histologic examination to objectively estimate the severity of hereditary cutaneous hyaluronosis. (*Am J Vet Res* 2012;73:220–226)

Shar-Peis are unique among dog breeds because of their skinfolds, a feature that is hereditary in origin.<sup>1</sup> Tight thick wrinkles normally extend over the body at the puppy stage, then typically decrease as the dog grows older. Indeed, as seen in a comparable human disorder associated with generalized folding and thickening of the skin, it may be reasonable to speculate that the normal age-related decrease in the ratio of body surface area to weight could be the underlying mechanism for the changes in wrinkling noted with age.<sup>2</sup>

This unusual wrinkling and thickening of the skin has been considered to be the consequence of an abnormal deposition of mucin; therefore, the disease entity is found in textbooks under the name of cutaneous mucinosis. Recently, by use of histochemistry and an in vitro model, the main component of mucin has been determined to be

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## ABBREVIATION

HCH Hereditary cutaneous hyaluronosis

hyaluronic acid,<sup>3,4</sup> a large unsulfated glycosaminoglycan that, once synthesized, is extruded out of fibroblast cells where it contributes to the assembly and organization of the extracellular matrix. Therefore, the term HCH seems to describe the phenomenon more accurately.

Nevertheless, to the authors' knowledge, no studies have been performed in Shar-Peis to assess the severity of hyaluronic acid deposition and the relative increase in skin thickness in an objective and noninvasive manner. In humans, ultrasonography is considered a versatile, painless, low-risk, and noninvasive procedure that provides real-time visual information about skin thickness and skin density. It has been extensively used in inflammatory disorders characterized by thickening and fibrosis of skin such as systemic sclerosis,<sup>5,6</sup> in preoperative tumor thickness measurements,<sup>7,8</sup> and in the evaluation of age-related changes in skin thickness.<sup>9</sup> Moreover, in cutaneous sclerosis, several other mechanical instruments (eg, elastometer, twistometer, cutometer, and plicometer) have been also used to exert a controlled physical force on skin and to better assess cutaneous involvement.<sup>10</sup>

In veterinary medicine, ultrasonography has also been used to characterize benign and malignant skin tumors<sup>11</sup> and to evaluate skin thickness<sup>12</sup> and changes

in skin thickness in relation to hydration status and fluid distribution in dogs.<sup>13</sup> Additionally, the plicometer has found application in the examination of the effects of diet on skin biophysical variables.<sup>14</sup>

The objective of the study reported here was to determine whether ultrasonographic imaging could be a useful instrument to determine skin thickness in Shar-Peis. Additionally, the investigation sought to correlate ultrasonographic results with age and sex of selected animals to understand whether these variables might affect skin thickness. Finally, the last objective was to correlate skinfold measurements obtained via a plicometer with ultrasonography results.

## Materials and Methods

**Study population**—The study was performed with 20 dogs, of which 10 were Shar-Peis from 2 Shar-Pei breeders in Catalunya, Spain, and 10 were Beagles (controls) from the blood bank of the Veterinary Faculty of Universitat Autònoma de Barcelona and from the Beagle kennel of UNIVET Veterinary Diagnostic Service. The Shar-Pei group included 6 sexually intact adult females and 4 sexually intact adult males, ranging in age from 1 to 3 years (mean  $\pm$  SD, 1.8  $\pm$  0.9 years) and in body weight from 16 to 22 kg (mean  $\pm$  SD, 18.4  $\pm$  2.4 kg). On clinical examination, all of the Shar-Peis were considered affected by HCH of variable severity. Nine Shar-Peis were phenotypically classified as in conformity with the American standard of the breed, characterized by pronounced loose skin covering the forehead and extending to the neck, withers, and hind limbs. Only 1 Shar-Pei was classified as in conformity with the more traditional Chinese standard, characterized by tight wrinkles covering only the withers and the forehead. The Beagle group included 7 sexually intact adult females and 3 males, ranging in age from 3 to 9 years (mean  $\pm$  SD, 5.8  $\pm$  2.4 years) and in body weight from 8 to 14 kg (mean  $\pm$  SD, 11.1  $\pm$  2.6 kg). All dogs were considered healthy on the basis of results of clinical examination, CBC, and total protein analysis. A routine urine analysis was also performed in each dog. All procedures outlined in this project were approved by the breeders of Shar-Peis used in this study and by the Ethical Committee of the Universitat Autònoma de Barcelona prior to initiation of the study.

**Ultrasonographic measurements of the skin**—A B-mode real-time ultrasonographic examination of skin thickness was performed with an ultrasonography machine<sup>a</sup> fitted with a 13-MHz linear array transducer (frequency range, 4 to 13 MHz). Four regions, (frontal [on the rostral margins of the supraorbital processes], dorsal cervical [neck], sacral, and left metatarsal) were selected. After hair clipping, skin was gently cleaned with ethanol to remove any cutaneous surface residues and an abundant amount of acoustic coupling gel<sup>b</sup> was applied between the skin surface and the transducer to position the skin on the focal zone of the probe. Ultrasonographic examinations were performed by placing the transducer perpendicular to the skin, and the following order of skin examination was adopted: frontal region halfway along the line connecting the rostral

margins of the supraorbital processes, dorsal region of the neck at the junction between the second and third cervical vertebrae, and the sacral region halfway along the line connecting the left and right tuber coxae. During examination, animals were manually restrained in sternal recumbency. The dorsal surface of the metatarsus of the left hind limb was examined after positioning the animal in right lateral recumbency. Once the surface epidermis, epidermis-dermis, and dermis-subcutis interfaces were identified, only the full skin thickness (a composite of epidermis and dermis) was measured by use of the electronic caliper of the ultrasonography machine. Three measurements were performed, each at a distance of approximately 5 mm. Magnified images (width, 30 mm; depth, 22 mm) were evaluated.

**Plicometer examination**—In the same areas selected for ultrasonography examination, skinfold (plica) measurements were performed in triplicate by use of a digital plicometer<sup>c</sup> with a digital display and capability to measure from 1 to 100 mm. An assistant pinched the skin between the thumb and forefinger, and one of the investigators gently positioned the left arm of the instrument first, followed by the right arm, equipped with the sensor, into contact with the skinfold. After the closure of the arms of the instrument, an audible signal indicated that the measurement has been obtained. Whereas the nearly compression-free ultrasonography measurements were considered the sum of epidermal plus dermal thicknesses, skinfold thickness measurements were considered the sum of a double layer of skin together with underlying fatty tissue.

**Histologic examination**—Skin samples from each of the 4 ultrasonographic examination points were collected during local anesthesia by use of a 6- to 8-mm punch biopsy or via excisional biopsy. Samples were immediately fixed in neutral-buffered 10% formalin solution for 24 hours, then embedded in paraffin. Sections were stained for histologic examination with H&E, Alcian blue (pH, 2.5), and Masson trichrome stains. Analysis of skin thickness on H&E-stained samples was performed by use of a microscope<sup>d</sup> equipped with a camera.<sup>e</sup> The images were collected by use of software,<sup>f</sup> and histometric measurements of skin thickness were performed.

**Statistical analysis**—Statistical analysis was performed with commercial statistical software.<sup>g</sup> A 1-way ANOVA test was used to evaluate hypotheses regarding differences in the mean values for skin thickness between Shar-Peis and Beagles obtained via ultrasonographic and plicometer measurements, respectively. Differences in each selected region were calculated by use of a Tukey-Kramer multiple comparisons test.

A Pearson correlation test and regression analysis were used to evaluate the relationship between ultrasonography measurements and age, sex, and plicometer results. A *P* value was computed with a Student *t* test. In all tests, values of *P* < 0.05 were considered significant.

## Results

**Ultrasonographic measurements**—In accordance with a previous study,<sup>12</sup> 3 layers were observed

ultrasonographically in both Shar-Peis and Beagles. First, there was a superficial well-defined hyperechoic layer at the interface between the coupling gel

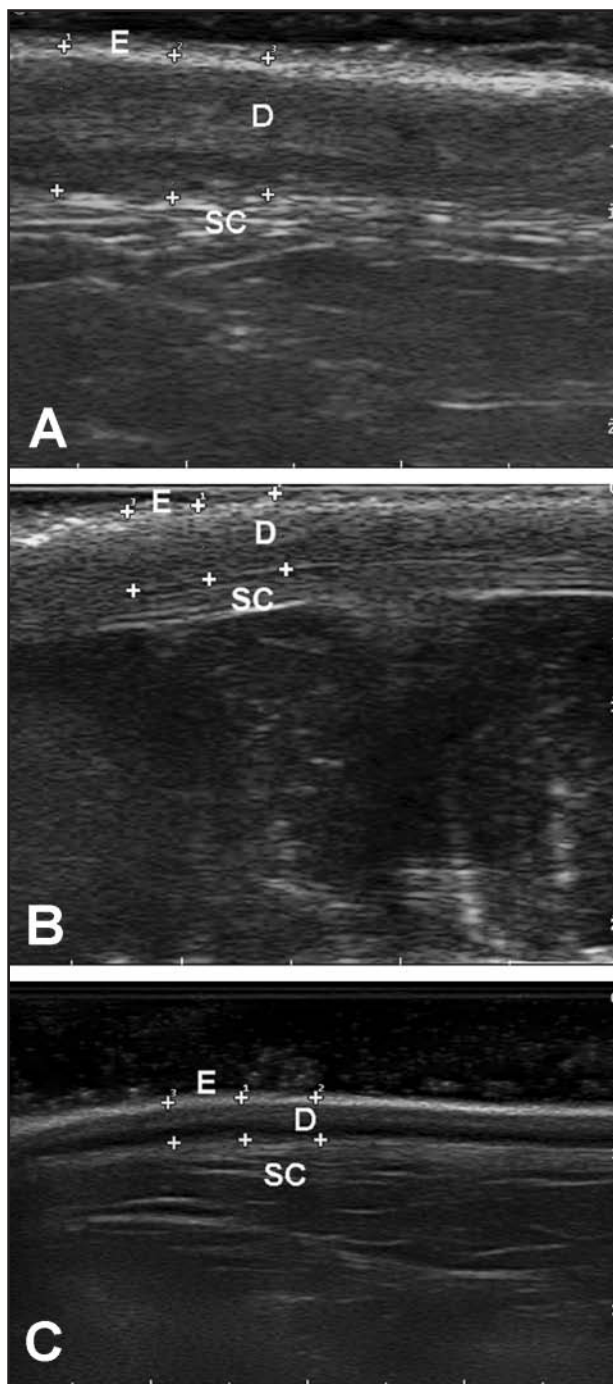


Figure 1—Ultrasonographic appearance of the skin in the sacral region in Shar-Peis (A and B) and a control dog (C). Three layers were distinguished in each image: a well-defined superficial hyperechoic layer corresponding to epidermal entry echo (E), a less echogenic layer corresponding to epidermis plus dermis (D), and a deep layer containing linear hyperechoic images corresponding to subcutaneous tissue (SC). A—Notice the fine granular hyperechoic texture in the second layer (epidermis plus dermis) of a Shar-Pei of American standard. B—Notice that the second layer of a Shar-Pei of Chinese standard is uniformly and more densely hyperechoic. C—Notice that 2 distinct bands of different echogenicity are observed in the dermis of a control dog (Beagle).

and the skin corresponding to epidermal entry echo. Beneath this was a less echogenic layer of variable intensity corresponding to the epidermis plus dermis and then a deep layer corresponding to subcutaneous tissue and containing thin linear hyperechoic areas superimposed on a nonhomogeneous hypoechoic pattern. In Shar-Peis, hyperechogenicity with a fine granular echotexture was observed in the second layer (epidermis plus dermis) of all dogs of American standard (Figure 1), whereas in the Shar-Pei of Chinese standard, the appearance of the second layer was uniformly and more densely hyperechoic. In Beagles, the echogenicity of the second layer was quite variable, ranging from a low to high echogenic pattern with a finely homogeneous echotexture. Particularly, in 6 of 10 Beagles, a double-layered appearance of the dermis, with a superficial band more echogenic than the deeper band, was detected.

In Shar-Peis, mean  $\pm$  SEM skin thickness was  $4.10 \pm 56$  mm, as opposed to  $2.23 \pm 41$  mm in Beagles, and was greatest in the sacral region, followed by the dorsal neck, frontal, and metatarsal regions. In Beagles, skin

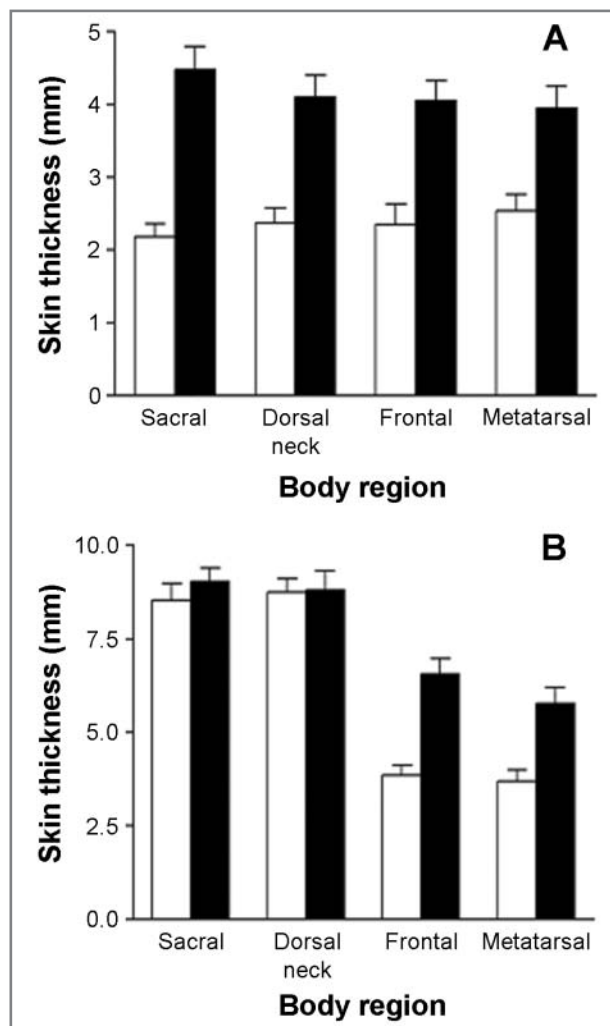


Figure 2—Comparisons (mean  $\pm$  SEM values) of ultrasonographic (A) and plicometer (B) skin thickness measurements between Shar-Peis (black columns) and Beagles (white columns) at 4 body regions (sacral, dorsal neck, frontal, and metatarsal).

thickness was greatest at the metatarsal region, followed by the dorsal neck, frontal, and sacral regions. A significant ( $P < 0.001$ ) difference between Shar-Peis and Beagles was detected in the sacral, dorsal neck, and frontal regions, whereas in the metatarsal region, this difference was also significant ( $P = 0.01$ ) but less pronounced (Figure 2). Nevertheless, with the multiple comparison test, no significant differences in skin thickness among the 4 regions were detected in either group. In Shar-Peis, no correlation was found between ultrasonography results in any of the 4 body regions and age or sex, whereas in Beagles, a positive correlation was found between age and ultrasonography results in the dorsal neck ( $r = 0.666$ ;  $P = 0.036$ ) and frontal ( $r = 0.750$ ;  $P = 0.012$ ) regions; however, no correlation was found when ultrasonography results were correlated with sex.

**Plicometer measurements**—Three plicometer measurements of cutaneous plica thickness were made in all dogs (Figure 2). Among the examined cutaneous regions, the largest plica thickness was evident in the sacral region followed by the dorsal neck, frontal, and metatarsal regions in Shar-Peis, whereas in Beagles, the dorsal neck region was thickest, followed by the sacral, frontal, and metatarsal regions. A significant difference between Shar-Peis and Beagles was detected only in the frontal region ( $P < 0.001$ ) and metatarsal region ( $P < 0.05$ ). By use of the multiple comparison test, significant ( $P < 0.001$ ) differences in plica thickness were detected in both groups, especially between the sacral and frontal regions and the sacral and metatarsal regions. In Shar-Peis, a weak positive correlation was found between ultrasonographic and plicometer measurements of each individual region, but this correlation was clearly positive overall ( $r = 0.538$ ;  $P = 0.004$ ; Figure 3). In contrast, in Beagles, the correlation between ultrasonography and plicometer results was significant in each individual region but not significant ( $P = 0.302$ ) overall ( $r = 0.167$ ).

**Histologic examination and histometric measurements**—In Shar-Peis of American standard, dermal collagen fibers were separated by a diffuse pale gray-pink substance corresponding to mucinous material (Figure 4). In the Shar-Pei of Chinese standard, scarce mucinous material was detected between collagen fibers, whereas in control dogs, this material was not found.

Histometric measurements of skin thickness were

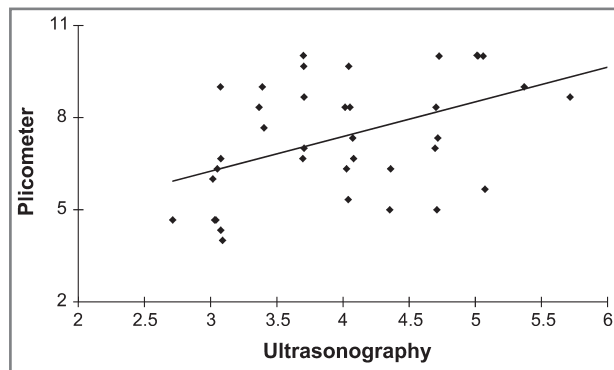


Figure 3—Graph of the correlation between results of ultrasonography (mm) and use of a plicometer (mm) for measurement of skin thickness in Shar-Peis.

strongly correlated with ultrasonographic results. Skin thickness was greatest in Shar-Peis of American stan-

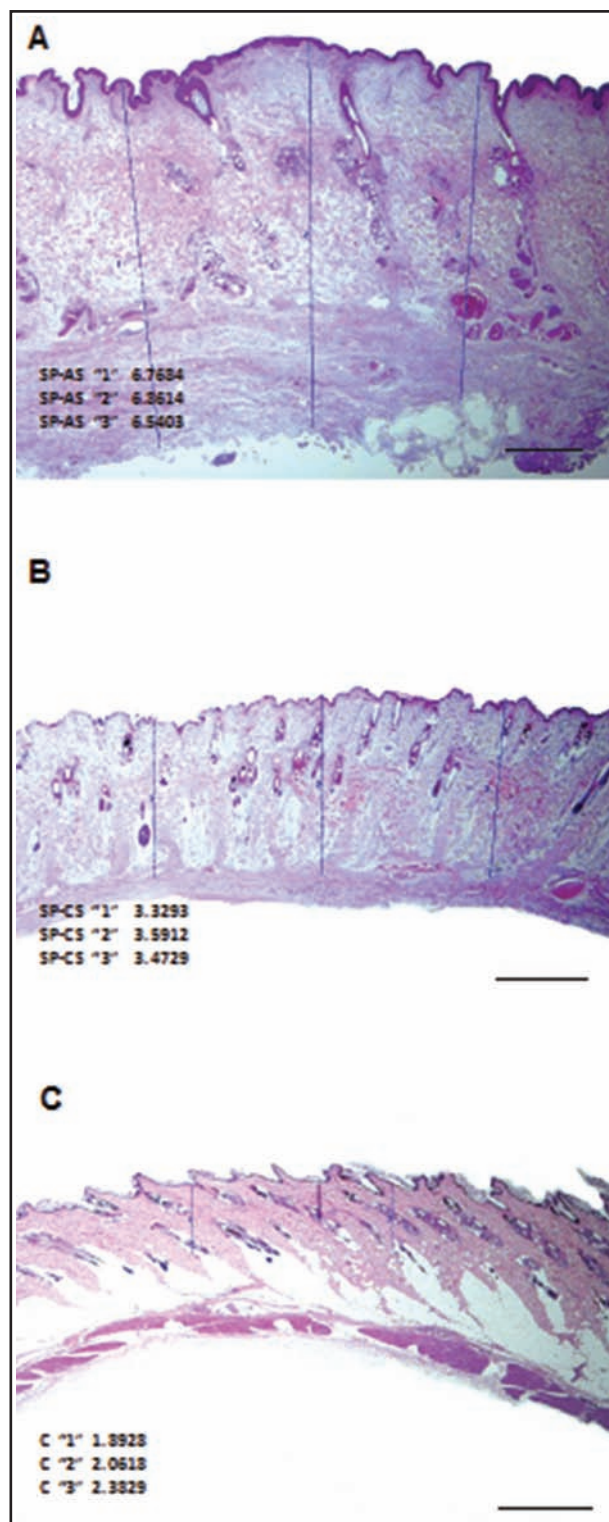


Figure 4—Photomicrographs of skin sections from the frontal region of 2 Shar-Peis and a Beagle. A diffuse pale, pink substance is present in the dermis of a Shar-Pei of American standard with the greatest skin thickness within the group of Shar-Peis (SP-AS; A). This finding is less evident in the Shar-Pei of Chinese standard (SP-CS; B) and not evident in the Beagle (C). On the left of each image are reported 3 measurements (mm) of the skin thickness performed via microscopy. H&E stain; bar = 500  $\mu$ m.

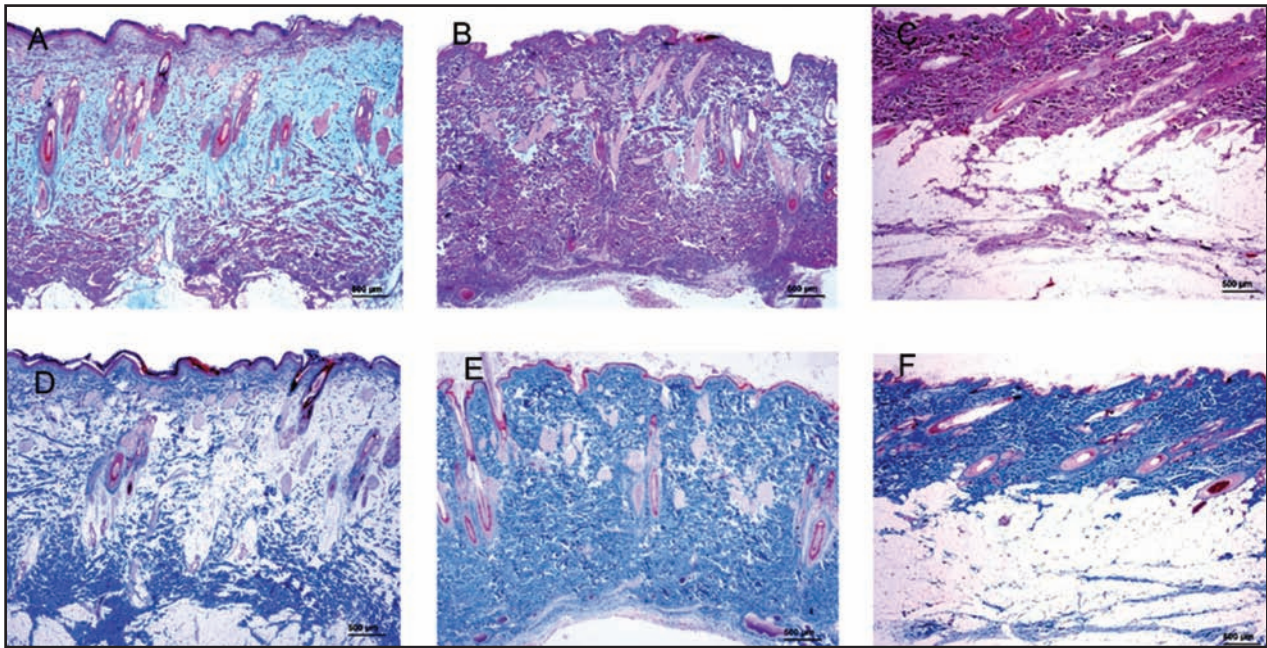


Figure 5—Photomicrographs of skin sections of 2 Shar-Peis and a Beagle obtained from the dorsal neck region. In the dermis of the Shar-Pei of American standard, collagen fibers are scattered within a basophilic material with characteristics of hyaluronic acid (A). This finding is less evident in the Shar-Pei of Chinese standard (B) and not evident in the Beagle (C). Alcian blue stain; bar = 500  $\mu$ m. Notice that in the Shar-Pei of American standard (D), collagen fibers stain an intense turquoise and are less densely distributed in comparison with the Shar-Pei of Chinese standard (E) and the Beagle (F). Masson stain; bar = 500  $\mu$ m.

dard, followed by the Shar-Pei of Chinese standard and Beagles.

By use of Alcian blue staining at a pH of 2.5, in the dermis of Shar-Peis, collagen fibers were scattered among a network of basophilic material, considered to be a characteristic of acid glycosaminoglycans such as hyaluronic acid.<sup>4</sup> This finding was clearly evident in Shar-Peis of American standard (Figure 5), whereas in the Shar-Pei of Chinese standard, it was less evident. In control dogs, this material was detected in some skin samples in small amounts. By use of Masson staining, collagen fibers (stained an intense turquoise) were less densely distributed in Shar-Peis of American standard in comparison with the Shar-Pei of Chinese standard and control dogs.

## Discussion

The purpose of this study was to determine the reliability of ultrasonography as a noninvasive method for measurement of skin thickness in Shar-Peis, as a possible indicator of HCH. In this breed, the diagnosis of HCH has long relied only on clinical observation and palpation, supported by histologic findings.<sup>3,15</sup> Recently, however, ultrasonography has been used to evaluate normal canine skin<sup>12</sup> and changes in canine skin thickness in relation to hydration status and fluid distribution.<sup>13</sup>

Ultrasonography involves the detection of reflected sound waves through tissues that possess inherently different acoustic properties. In particular, echoes in the dermis are the result of the reflection of the ultrasonography waves at the boundaries between dermal components, such as collagenous and reticular fibers, dermal ground substance, sebaceous and sweat glands, and the surrounding water-rich ground substance.<sup>16</sup> For example, a decrease in the echogenicity of the der-

mis may be caused by an excess of fluid in the interstitium separating the collagen fibers with consequent distension of the fiber network.<sup>17</sup> Therefore, the resulting ultrasonography image consists of regions of different echogenicity, which correlate to different histologic components of the skin.<sup>18</sup>

Furthermore, selection of the appropriate transducer is an important aspect of soft tissue ultrasonography. Indeed, in humans, 20-MHz scanners are used for measuring skin thickness and assessing inflammatory skin disorders, whereas 7.5- to 15-MHz scanners are generally used in dermatologic oncology.<sup>17</sup>

In accordance with Diana et al,<sup>12,13</sup> a 13-MHz linear array transducer was used in the present study. In all dogs, a superficial well-defined hyperechoic layer corresponding to the coupling gel skin surface interface was observed. Its echogenicity was considered to depend on the thickness of the stratum corneum and the amount of air trapped between the keratinized cells. A second less echogenic and thicker layer corresponding to the remaining epidermal layers plus the dermis was visible, followed by a third hypoechoic layer interspersed with hyperechoic linear areas running mostly parallel to the skin and representing subcutaneous tissue. In Shar-Peis of American standard, the second layer had a fine granular hyperechoic texture in all selected body regions in comparison with the Shar-Pei of Chinese standard in which the second layer appeared uniformly and densely hyperechoic. Dermal echogenicity is related to the various components of the dermis (dermal ground substance, collagen and elastic fibers, and glands), which may be responsible for echogenic images with differing intensities.<sup>12</sup> In this study, the differences in dermal echogenicity observed between Shar-Peis of American standard and the Shar-Pei of Chinese standard may have been related to dif-

ferences in hyaluronic acid deposition and the ability of this molecule to form a viscoelastic network for fibers in the dermis.

In contrast, in Beagles, the echogenicity of the second layer was characterized by echoes of various intensities and a fine and dense echotexture. Furthermore, in more than half of Beagles, a 2-layered appearance of the epidermis plus dermis (with the deeper layer less echogenic) was also observed. In accordance with previous reports,<sup>12,13</sup> this finding was probably related to differences in dermal fluid storage among dogs.

Ultrasonographic results were further corroborated by histologic results. In Shar-Peis of American standard, material believed to be hyaluronic acid was spread widely between collagen fibers that were normally arranged, whereas in the dermis of the Shar-Pei of Chinese standard, this material was sparsely distributed; this material was not detected in the dermis of controls. A clear correlation was found between results of histometric measurements of skin samples stained with H&E and results of ultrasonographic measurements of skin thickness.

As expected, Shar-Peis of American standard, which are known for their abundant wrinkles, had greater skin thickness, compared with the Shar-Pei of Chinese standard, known for less wrinkles, and with Beagles, in which wrinkles are not normally present. In dogs, skin thickness has been reported to decrease dorsally to ventrally in the trunk and proximally to distally in the limbs, with the thickest skin located on the head, dorsum of the neck, back, and sacrum.<sup>19</sup> Ultrasonographic results of this study indicated that skin thickness in Shar-Peis was greatest in the sacral area followed by dorsal neck, frontal, and metatarsal regions; in Beagles, the region of thickest skin was the metatarsal, followed by dorsal neck, frontal, and sacral regions. Therefore, it may be hypothesized that breed and other factors may have an influence on skin thickness in different body regions. For example, in humans, skin thickness is under the control of hormonal factors such as estrogens, which increase dermal hydroscopic properties, probably through enhanced synthesis of dermal hyaluronic acid,<sup>20–22</sup> and aging, which modifies the viscoelastic properties of skin by increasing the breakdown of extracellular matrix components.<sup>23,24</sup>

As a consequence, in the population of the present study, we investigated whether a correlation might exist between sex and dermal thickness and whether decreased glycosaminoglycan content with consequent skin atrophy would occur in older individuals. Contrary to expectations, no correlation was found in Shar-Peis and Beagles between ultrasonographically measured skin thickness and sex, whereas only in Beagles was a weak correlation detected between ultrasonographic results at dorsal neck and frontal regions and age. Although this study was conducted with only 2 breeds to minimize inter- and intraindividual variability, failure to find a correlation might have occurred because of the small number of individuals and variability in mean age between the 2 groups. In the future, better correlated results might be obtained by use of a larger study population and by standardization of hormonal status in homogeneously aged groups of animals.

Ultrasonography has also been used in dogs to measure subcutaneous fat and to predict total body fat.<sup>25,26</sup> However, in the present study and as reported,<sup>12</sup> a clear demarcation of the distal subcutaneous tissue boundary useful for making measurements was not obtained in ultrasonographic images. This may have occurred because subcutaneous tissue projects into the overlying dermis as papillae adiposae that surround hair follicles, sweat glands, and vasculature and form attachments to the underlying fibrous skeletal components.<sup>19</sup> The use of a plicometer, an instrument that in humans is typically used for the assessment of nutritional status in cross-sectional studies<sup>27,28</sup> as well as in growth hormone replacement therapy and follow-up of medical conditions such as obesity, is seen as an option to obviate this limitation. Most studies involving body composition assessments deal with the correlation of skinfold thickness to percentage body fat and are based on prediction equations geared to mathematical relationships.

In both groups of animals, the present study was limited to the simple measurement of skin thickness in triplicate. Despite high variability of the results from each region, in Shar-Peis, a positive correlation was found between ultrasonographic and plicometer measurements overall. In contrast, Beagles had low variability in each region but high variability overall that was reflected in the correlation coefficient. Analysis suggested that this variability of correlation between ultrasonographic and plicometer results could be the consequence of changes in the compressibility of subcutaneous tissues associated with breed, age, and nutritional status. Therefore, further studies are necessary that use a larger number of animals and probably also include more body locations.

Results of the present study indicated that a noninvasive diagnostic tool such as ultrasonography can be used to assess skin and that it is a simple, valid alternative to other more invasive methods of investigation, such as histologic examination, to objectively estimate the severity of HCH in Shar-Peis, as judged via skin thickness.

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- a. Esaote ultrasound machine (MyLab 70), Esaote España SA, Barcelona, Spain.
  - b. Polaris II ultrasound gel, GE Medical Systems, España SA, Barcelona, Spain.
  - c. Rimmeter digital skinfold meters, Robert Pringle Engineers Ltd, Harlow, Essex, England.
  - d. Leica MZ FLIII, Leica Microsystems, Wetzlar, Germany.
  - e. Leica DC500 CCD camera, Leica Microsystems, Wetzlar, Germany.
  - f. Universal Imaging MetaMorph, version 5.1, Universal Imaging Corp, Downington, Pa.
  - g. GraphPad InStat, version 3.05, GraphPad Software Inc, San Diego, Calif.
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## References

1. Akey JM, Ruhe AL, Akey DT, et al. Tracking footprints of artificial selection in the dog genome. *Proc Natl Acad Sci U S A* 2010;107:1160–1165.
2. Ramsden CA, Bankier A, Brown TJ, et al. A new disorder of hyaluronan metabolism associated with generalized folding and thickening of the skin. *J Pediatr* 2000;136:62–68.
3. Zanna G, Docampo MJ, Fondevila D, et al. Hereditary cutaneous systemic mucinosis in Shar-Pei dogs is associated with increased hyaluronan synthase-2 mRNA transcription by cultured dermal fibroblasts. *Vet Dermatol* 2009;20:377–382.

4. Zanna G, Fondevila D, Bardagi M, et al. Cutaneous mucinosis in Shar-Pei dogs is due to hyaluronic acid deposition and is associated with high levels of hyaluronic acid in serum. *Vet Dermatol* 2008;19:314–318.
5. Hesselstrand R, Scheja A, Wildt M, et al. High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease. *Rheumatology (Oxford)* 2008;47:84–87.
6. Akesson A, Hesselstrand R, Scheja A, et al. Longitudinal development of skin involvement and reliability of high frequency ultrasound in systemic sclerosis. *Ann Rheum Dis* 2004;63:791–796.
7. Machel L, Belot V, Naouri M, et al. Preoperative measurement of thickness of cutaneous melanoma using high-resolution 20 MHz ultrasound imaging: a monocenter prospective study and systematic review of the literature. *Ultrasound Med Biol* 2009;35:1411–1420.
8. Songra AK, Ng SY, Farthing P, et al. Observation of tumour thickness and resection margin at surgical excision of primary oral squamous cell carcinoma—assessment by ultrasound. *Int J Oral Maxillofac Surg* 2006;35:324–331.
9. Waller JM, Maibach HI. Age and skin structure and function, a quantitative approach (I): blood flow, pH, thickness, and ultrasound echogenicity. *Skin Res Technol* 2005;11:221–235.
10. Czirják L, Foeldvari I, Müller-Ladner U. Skin involvement in systemic sclerosis. *Rheumatology (Oxford)* 2008;47(suppl 5):v44–v45.
11. Nyman HT, Nielsen OL, McEvoy FJ, et al. Comparison of B-mode and Doppler ultrasonographic findings with histologic features of benign and malignant mammary tumors in dogs. *Am J Vet Res* 2006;67:985–991.
12. Diana A, Preziosi R, Guglielmini C, et al. High frequency ultrasonography of the skin of clinically normal dogs. *Am J Vet Res* 2004;65:1625–1630.
13. Diana A, Guglielmini C, Fracassi F, et al. Use of high-frequency ultrasonography for evaluation of skin thickness in relation to hydration status and fluid distribution at various cutaneous sites in dogs. *Am J Vet Res* 2008;69:1148–1152.
14. Young LA, Dodge JC, Guest KJ, et al. Age, breed, sex and period effects on skin biophysical parameters for dogs fed canned dog food. *J Nutr* 2002;132:1695S–1697S.
15. von Bomhard D, Kraft W. Idiopathic mucinosis cutis in Chinese Shar Pei dogs: epidemiology, clinical features, histopathologic findings and treatment [in German]. *Tierarztl Prax Ausg K Klientiere Heimtiere* 1998;26:189–196.
16. Gniadecka M, Quistorff B. Assessment of dermal water by high-frequency ultrasound: comparative studies with nuclear magnetic resonance. *Br J Dermatol* 1996;135:218–224.
17. Jemec GBE, Gniadecka M, Ulrich J. Ultrasound in dermatology. Part I. High frequency ultrasound. *Eur J Dermatol* 2000;10:492–497.
18. Aspres N, Egerton IB, Lim AC, et al. Imaging the skin. *Australas J Dermatol* 2003;44:19–27.
19. Scott DW, Miller WH, Griffin CE. Structure and function of the skin. In: Scott DW, Miller WH, Griffin CE, eds. *Muller and Kirk's small animal dermatology*. 6th ed. Philadelphia: WB Saunders Co, 2001;1–70.
20. Giacomoni PU, Mammone T, Teri M. Gender-linked differences in human skin. *J Dermatol Sci* 2009;55:144–149.
21. Stevenson S, Thornton J. Effect of estrogens on skin aging and the potential role of SERMs. *Clin Interv Aging* 2007;2:283–297.
22. Verdier-Sévrain S, Bonté F, Gilchrist B. Biology of estrogens in skin: implications for skin aging. *Exp Dermatol* 2006;15:83–94.
23. Farage MA, Miller KW, Berardesca E, et al. Clinical implications of aging skin: cutaneous disorders in the elderly. *Am J Clin Dermatol* 2009;10:73–86.
24. Callaghan TM, Wilhelm KP. A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part I: cellular and molecular perspectives of skin ageing. *Int J Cosmet Sci* 2008;30:313–322.
25. Wilkinson MJA, McEwan NA. Use of ultrasound in the measurement of subcutaneous fat and prediction of total body fat in dogs. *J Nutr* 1991;121:47–50.
26. Morooka T, Niiyama M, Uchida E, et al. Measurement of the back fat layer in Beagles for estimation of obesity using two-dimensional ultrasonography. *J Small Anim Pract* 2001;42:56–59.
27. Orphanidou C, McCargar L, Birmingham CL, et al. Accuracy of subcutaneous fat measurement: comparison of skinfold calipers, ultrasound, and computed tomography. *J Am Diet Assoc* 1994;94:855–858.
28. Stevens-Simon C, Thureen P, Barrett J, et al. Skinfold caliper and ultrasound assessments of change in the distribution of subcutaneous fat during adolescent pregnancy. *Int J Obes Relat Metab Disord* 2001;25:1340–1345.