High-frequency ultrasonography of the skin of clinically normal dogs

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Objectives—To assess the applicability of high-frequency diagnostic ultrasonography for evaluation and accurate measurement of the skin thickness of clinically normal dogs.

Animals—26 healthy dogs (12 sexually intact males, 13 sexually intact females, and 1 spayed female) of various breeds and ages.

Procedure—Ultrasonographic examination of the skin and histomorphometric analysis of skin biopsy specimens obtained from the same site were performed. A 13-MHz linear-array transducer was used to obtain a series of ultrasonographic images of the skin in the flank region; images were analyzed and measured by use of imaging software. Cutaneous biopsy specimens were placed in fixative and then stained with H&E and Masson trichrome stains. Histomorphometric analysis was performed by use of an image analyzer. Thickness of the epidermis and dermis of each specimen was evaluated by use of a semiautomatic procedure of quantification. Data obtained from ultrasonographic and histologic measurements were compared by use of the Pearson correlation test.

Results—The ultrasonographic pattern of canine skin was consistently characterized by 3 distinct, defined echogenic layers corresponding to the epidermal entry echo, epidermis and dermis, and subcutaneous tissues. A positive correlation was found between ultrasonographic and histologic measurements of skin thickness.

Conclusions and Clinical Relevance—Comparison between ultrasonographic and histologic appearance of the skin revealed that layering of canine skin (ie, epidermis and dermis) and the subcutaneous tissues may be recognized and measured by use of high-frequency ultrasonography. Thus, diagnostic ultrasonography may be a useful tool for the noninvasive evaluation of cutaneous disorders in dogs. (Am J Vet Res 2004;65:1625–1630)

Complete clinical evaluation of the canine integumentary system includes obtaining a patient’s medical history, a thorough physical examination (ie, direct inspection and palpation of the skin, hair, and appendages), and use of ancillary diagnostic aids. Diagnostic tests and laboratory procedures (eg, microscopic examination of hair or skin scrapings, cytologic evaluation of skin specimens, bacteriologic and fungal culture, examination of skin biopsy specimens, and allergy testing) are particularly useful whenever a definitive diagnosis cannot be achieved from analysis of the medical history and physical examination alone. Combined use of those diagnostic tests and laboratory procedures allows confirmation or elimination of various cutaneous disorders included in the differential diagnoses.

Various ultrasonographic techniques (eg, A-mode or pulsed ultrasonography) were first used for evaluation of the skin and subcutaneous tissue of humans in the late 1970s. Developments in ultrasonic instruments, particularly the advent of high-frequency transducers, have successively made B-mode ultrasonographic imaging of the skin of humans more feasible. The use of ultrahigh-frequency ultrasonographic transducers (ie, frequency of ≥20 MHz) allows the recognition of tiny normal structures (eg, cutaneous adnexa) or precise differentiation among the cutaneous layers. Their main field of application includes characterization of malignant cutaneous neoplasms, evaluation and monitoring of inflammatory disorders (eg, morphea), and quantification of reactions during allergy testing. Therefore, a thorough description of the ultrasonographic appearance of the human integumentary system has been reported and ultrasonography is a diagnostic tool widely used in human dermatology. On the contrary, few reports have described the application of diagnostic ultrasonography for examination of the skin in domestic animals. A description of the ultrasonographic image characteristics of the skin of cattle, examined at a frequency of 7.5 MHz, has been reported. Diagnostic ultrasonography has also been used for evaluation of the skin and subcutaneous tissues in a horse with cutaneous lymphangioma and in dogs with mammary gland tumors. Nevertheless, to the best of our knowledge, there are no reports that have described the ultrasonographic appearance of normal canine skin. The objectives of the study reported here were to describe the ultrasonographic image of normal canine skin and to correlate the ultrasonographic measurement of skin thickness with histomorphometric measurement of cutaneous samples obtained from the same body region.

Materials and Methods

Animals—Data were obtained from 26 clinically normal client-owned dogs (12 sexually intact males, 13 sexually intact females, 13 sexually intact females, and 1 spayed female) of various breeds and ages.
ally intact females, and 1 spayed female) examined at the Veterinary Hospital of the University of Bologna between January and December 2001. Dogs represented various breeds including 4 English Setters, 2 Yorkshire Terriers, and 1 each of Dalmatian, Boxer, Toy Poodle, Chow Chow, Siberian Husky, Italian Bloodhound, Zwergpinscher, Zwerschnauzer, English Pointer, St Bernard, Sharpei, Great Dane, Kerry Blue Terrier, Kuvasz, and Lagotto; there were 5 mixed-breed dogs. Dogs ranged from 2 to 13 years of age (mean ± SD, 7.3 ± 3.4 years) and from 4 to 75 kg (mean, 23.5 ± 16.6 kg). Dogs were considered healthy on the basis of normal results of physical examination, a CBC count, routine serum biochemical analysis, and urinalysis. Informed consent of owners was obtained. The protocol of the study was approved by the Ethical Committee of the University of Bologna.

Ultrasonography of the skin—One investigator (AD) performed B-mode real-time ultrasonographic examination of the skin of all dogs by use of a real-time ultrasound machine equipped with a high-frequency (13 MHz) linear-array transducer. For ultrasonographic scanning, the midportion of the region of the left flank of each dog was clipped and the skin surface gently cleaned with 70% isopropyl alcohol to remove cutaneous debris and sebum. The dogs were positioned in right lateral recumbency for the ultrasonographic examinations. Copious amounts of coupling gel were applied between the transducer and skin surface. The ultrasound beam was maintained strictly perpendicular to the skin surface while avoiding direct contact between the probe and the skin surface. Only gentle pressure was applied to minimize compression on the tissues beneath the probe. Three focuses, separated by a constant distance of 7 mm, were used. The gain control was set at 30%. All time-gain compensation controls were maintained at 80%. Enlarged images (width of 30 mm and height (ie, tissue depth) of 24 mm) were evaluated.

A series of images of the skin were obtained and stored on a magnetic optical disc for subsequent off-line evaluation. Descriptions and measurements of ultrasonographic images were successively performed by use of imaging software. In particular, appearance of the ultrasonographic patterns was compared to histologic images for the best interpretation of the various echogenic images. Two experienced investigators (AD and MC) performed all ultrasonographic measurements by use of the electronic calipers of the ultrasound machine. Measurements of the skin were made starting from the outer side of the skin surface to the clearly recognizable acoustic interface. The mean value of 3 measurements obtained from 3 points of the same ultrasonographic image was used for statistical analysis.

Histologic examination—Punch biopsy specimens (4 mm in diameter) were obtained from the same cutaneous sites examined ultrasonographically in each dog. Biopsy specimens were immediately placed in neutral-buffered 10% formalin. Tissue blocks were embedded in paraffin, and 2 serial sections were cut perpendicular to the skin surface; serial sections were cut at a thickness of 5 μm. The sections were stained with H&E to enable investigators to examine the normality of epidermal and dermal structures and Masson trichrome stain to enable examination of epidermal cells and delineation of the epidermis from the dermis.

Histomorphometric measurements of dermal and viable epidermal thickness, excluding the stratum corneum, were performed by use of a computer-assisted image-analysis system on sections stained with Masson trichrome. For each section, 5 fields were analyzed. In each field, 10 measurements of the epidermal thickness were obtained (distance between the innermost aspect of the stratum corneum and the dermal-epidermal junction). Only the cellular layer was considered because the acellular layer often was inadvertently removed during tissue processing. Isolation of exclusively the outermost portion of the cellular layer of the epidermis and the dermal-epidermal junction was possible because of the variation in Masson trichrome stain in the stratum corneum, viable epidermis, and dermis. Filtering techniques were also used and included a 490-nm band pass to better distinguish components and edges of the various cutaneous regions of interest. Use of the band pass allowed better equalization and dichotomy for the image analysis procedures, which consequently resulted in a more precise measurement. For each section, 5 measurements of epidermal thickness in each of 5 fields were made; the mean of these 5 measurements was divided by the length of the epidermis to yield the mean epidermal thickness. The same procedure was used to measure the dermal thickness. Finally, the sum of epidermal and dermal thickness determined by histologic examination was compared with ultrasonographic measurements.

Statistical analysis—Data were analyzed by use of a commercial statistical software package. A Pearson correlation test was performed to determine whether a relationship existed between ultrasonographic measurements of skin thickness obtained by the 2 investigators. Once we documented a strong correlation between measurements registered by the 2 investigators, the mean of measurements obtained by both investigators was used for subsequent analysis. Furthermore, a Pearson correlation test was performed to determine whether a relationship existed between the ultrasonographic and histologic measurements of the skin. Finally, a linear regression test was applied to evaluate whether the histologic measurement of skin thickness was related to body weight in each dog. Values of P < 0.05 were considered significant.

Results

Ultrasonographic features of canine skin had a consistent pattern characterized by 3 distinct and recognizable layers (Figure 1). First, a defined regular hyperechoic line was observed at the interface between the coupling gel and the skin. Under this, a less echogenic layer containing many various echogenic intensities was evident. More deeply, a thinner layer characterized by a nonhomogeneous hypoechoic pattern containing thin linear hyperechoic images was found. In 11 (42.3%) dogs, this deep layer was not uniform and appeared composed of 2 distinguishable bands with clearly differing echogenicity (Figure 2).

The distance between the first hyperechoic line and the acoustic interface between the 2 layers (ie, dermis and subcutis) beneath it ranged between 1,210 and 3,633 μm (mean ± SD, 1,933 ± 585 μm) for 1 investigator and between 1,267 and 3,570 μm (mean, 1,908 ± 570 μm) for the other investigator. Measurements obtained by both observers were highly correlated (r, 0.992; P < 0.001).

Slides stained with Masson trichrome were characterized by 4 distinct segments in the original color image (background, keratin, collagen, and cells; Figure 3). These were then filtered and automatically examined in black and white such that the cho-
sen component (epidermis or dermis) was separated from the other segments. Histologically, the thickness of the epidermis plus dermis ranged between 606 and 1,780 µm (mean, 1,481 ± 484 µm).

Results of skin measurement made by use of ultrasonography (measured by 2 investigators) and measurements of skin thickness derived from histologic sections were summarized (Table 1). A significant positive correlation \( r = 0.533; P = 0.01 \) was found between measurements of skin thickness obtained by use of ultrasonography and those derived by use of histologic examination.

Subcutaneous tissues were not included in ultrasonographic or histologic measurements. In fact, a clear demarcation of the distal subcutis boundary was lacking in the ultrasonographic images, whereas the boundary of the subcutis was not included in the biopsy specimens.

We did not detect a significant correlation \( r = 0.310; P = 0.122 \) between the histologic measurement of skin thickness and body weight.

Figure 1—Representative ultrasonogram of normal canine skin obtained by use of a 13-MHz linear-array transducer. Three distinct layers are clearly recognizable: epidermal entry echo (E), the epidermis plus dermis (D), and the subcutaneous tissues (S). A measurement of skin thickness was obtained (dotted line). Bar = 5 mm.

Figure 2—Ultrasonographic appearance of the skin in 11 of 26 dogs examined in the study. Notice that echogenicity of the second layer (epidermis plus dermis; D) is not uniform, and 2 distinct bands with differing echogenicity are recognizable (arrows). Bar = 5 mm. See Figure 1 for remainder of key.

Figure 3—Photomicrograph of a histologic section of normal canine skin (A) and digitized images of the epidermis obtained by use of a blue band pass filter (490 nm; B) and a red band pass filter (630 nm; C). Notice the cutaneous adnexa (asterisks). Panel A, Masson trichrome stain. Bar = 125 µm (A and C) and 15 µm (B). See Figure 1 for remainder of key.
Table 1—Measurements of skin thickness (epidermis plus dermis) determined from histologic examination of tissue sections and evaluation of ultrasonographic images obtained from 26 clinically normal dogs.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Weight (kg)</th>
<th>Histologic (µm)</th>
<th>Investigator 1 (µm)</th>
<th>Investigator 2 (µm)</th>
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<td>1,762</td>
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<td>1,947</td>
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</table>

Mean ± SD  
23.3 ± 16.6  1,481 ± 484  1,933 ± 585  1,908 ± 570

*Epidermis and dermis of these dogs were evident as 2 distinct ultrasonographic layers consisting of 2 bands with differing echogenicity.

Discussion

Analysis of results of the study reported here revealed that an ultrasonogram of normal canine skin comprises 3 consistent echogenic layers when scanned at a frequency of 13 MHz. The first hyperechoic line was created as a result of the differing acoustic impedance between the coupling gel and stratum corneum of the skin surface. This line corresponds to the epidermal entry echo described by human dermatologists, and its echogenicity depends on the thickness of the stratum corneum and the amount of air trapped between the keratotic scales.\(^17\) This first echogenic image is sufficiently strong to partially obscure details of the epidermis located beneath; therefore, this line does not correspond to the entire epidermal structure.\(^11,18-20\)

The second layer is less echogenic and considerably thicker than the epidermal entry echo. It corresponds to the epidermis and dermis, as evidenced by comparing the ultrasonographic images to the respective histologic sections. Nevertheless, definitive differentiation between epidermis and dermis was not possible with the ultrasonographic frequency used in this study. The echogenicity of this layer appeared quite variable, changing from a relatively low to a medium-high echogenic pattern, whereas the echotexture was finely homogeneous in all dogs. Dermal echogenicity is related to the various components of the dermis (ie, collagenous and reticular fibers, dermal ground substance, and sebaceous glands) that may cause echogenic images with differing intensities.\(^7,8,21\)

Recognition of skin adnexa (ie, hair follicles, shafts, and sebaceous glands) was not possible with the ultrasonographic frequency used in the study reported here. Because of their small dimensions,\(^8\) a frequency of at least 20 MHz is necessary for the clear recognition of these structures.

In humans, the dermis is composed of 2 layers: the papillary dermis, which is a more superficial and thinner layer that contains fine connective tissue, and the reticular dermis, which is deeper, thicker, and composed of large bundles of fibers in a water-rich interstitial medium. Thus, 2 distinct bands with differing echogenicity can be easily recognized in the human dermal layer.\(^11,22\) In contrast, a clearly defined demarcation between the papillary and reticular dermis is not evident in the skin of domestic carnivores.\(^23\) In the skin of humans, dermal echogenicity depends on the amount of collagen fibers (which increase dermis echogenicity) and water influx (which decreases dermal echogenicity, probably through distention of the fiber network).\(^21,24\) The 2 ultrasonographic patterns we observed in the dermal layer of dogs are probably related to the differing amounts of dermal fluid storage, which influences its echogenicity (Figures 1 and 2). In fact, an additional hypoechogenic band indicating a more...
abundant interstitial water content was observed in 11 dogs; in these dogs, there was a greater difference between ultrasonographic and histologic measurements of skin thickness. Therefore, a smaller (ie, thinner) histologic measurement was observed in these 11 dogs as a consequence of greater dehydration of the biopsy specimens.

The clearly recognizable and defined dermis-subcutis interface was probably related to the large difference in acoustic impedance between the dermis and subcutaneous layer.23 The third and thickest layer, characterized by an inhomogeneous hypoechoic pattern with thin linear hyperechoic bands, corresponded to the subcutaneous tissues. Its ultrasonographic appearance is related to the primarily adipose nature of the subcutis that contains connective septa.8,10,11

The reported mean thickness of the skin of dogs ranges from 0.5 to 5 mm. In general, it decreases in thickness from dorsal to ventral on the trunk and from proximal to distal on the limbs.23 The ultrasonographic thickness of the skin, which was consistently measured at the same body region, varies greatly in the dogs of the study reported here. In humans, thickness of the skin varies among parts of the body25 and may be influenced by several factors including age,26-28 sex,29 and distribution of body fluid.24,30,31 These factors are able to modify the degree of tension and elasticity of the skin. In particular, thickness of the skin depends on the amount of collagen fibers, cellular substances, and interstitial fluid. Intrinsic aging of the skin is accompanied by a decrease in skin thickness as a result of a reduction of water, proteins, and collagen content.27 Moreover, the skin of females is thinner than that of males26 because female sex hormones may influence water retention, thus resulting in changes in skin thickness.29 In addition to these factors, which may also be valid for dogs, it must be stressed that great polymorphism characterizes the various canine breeds, further influencing skin thickness.23-25 Accordingly, the greatest skin thickness in our study was detected for a Sharpei, which is not surprising given the abundant mucinous dermal intercellular material characteristic of this breed, whereas the lowest skin thickness measurements were found in a Zwergpinscher and a Toy Poodle. In contrast, no correlation was found between body weight and skin thickness of dogs in the study reported here.

A positive correlation was detected between cutaneous thickness measured by use of ultrasonography and measurements obtained by use of histologic examination. Histologic examination currently represents the criterion-referenced standard for the evaluation and measurement of skin layers.23 Histologic examination of skin samples allows accurate recognition of the various cutaneous and subcutaneous structures as well as precise measurement of skin thickness, but it is invasive and time is required for processing of samples. Furthermore, procedures for preparing tissues for histologic examination, including excision, fixation in formalin, dehydration in alcohol, and embedding in paraffin, may lead to alteration and distortion of a tissue’s natural state and may therefore affect results of thickness measurements.1 In contrast, ultrasonographic measurements are performed in vivo when the skin is under normal tension. Therefore, a 1:1 correlation between measurements performed in vivo and those obtained after tissue preparation cannot be expected. In 24 of 26 (92.3%) dogs in our study, the skin thickness determined histologically was lower, compared with that determined ultrasonographically. This fact was probably attributable to the influence of the aforementioned steps during preparation of histologic samples that resulted in shrinkage of skin tissues.

Ultrasonographic measurement of the skin performed independently by 2 investigators revealed no significant differences; instead, there was a high correlation between them. Therefore, the ultrasonographic measurement of skin thickness is highly reproducible without substantial variability among investigators who are skilled in the interpretation of ultrasonographic images.

We used a standardized technique for the ultrasonographic evaluation of canine skin. Analysis of our results revealed that high-frequency diagnostic ultrasonography is a useful tool for the noninvasive evaluation of skin tissues in dogs and the accurate measurement of skin thickness. Additional applications of ultrasonographic examinations may allow noninvasive characterization of skin tissues in dogs affected by various cutaneous disorders, similar to that already documented in human patients.

References