FULL PAPER *Surgery*

Contrast-enhanced ultrasonography to assess blood perfusion of skeletal muscles in normal dogs

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ABSTRACT. This study evaluated perfusion of skeletal muscle using contrast enhanced ultrasonography in humerus, radius, femur and tibia in normal dogs. Contrast enhanced ultrasonography for each region was performed after injecting 0.5 mL and 1 mL of contrast medium (SonoVue) in every dog. Blood perfusion was assessed quantitatively by measuring the peak intensity, time to the peak intensity and area under the curve from the time–intensity curve. Vascularization in skeletal muscle was qualitatively graded with a score of 0–3 according to the number of vascular signals. A parabolic shape of time–intensity curve was observed from muscles in normal dogs, and time to the peak intensity, the peak intensity and area under the curve of each muscle were not significantly different according to the appendicular regions examined and the dosage of contrast agent administered. This study reports that feasibility of contrast enhanced ultrasonography for assessment of the muscular perfusion in canine appendicular regions.

KEY WORDS: contrast enhanced ultrasonography, contrast medium, muscle

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Bone fracture can be healed through direct or indirect process according to the stability and the gap between the fragments [7, 10]. Although some characteristics including the bone fracture, decrease of the gap between fragments, callus formation and fixation devices can be evaluated on radiography, neovascularization and blood perfusion in the fracture site, which are one of the most important factors for fracture healing, can't be estimated [19]. Because the bone changes follow after blood supply in fracture healing, we hypothesized that perfusion of surrounding soft tissues can be used as an earlier indicator of hemodynamic changes during bone healing. Several previous studies discussing power Doppler, dynamic contrast-enhanced magnetic resonance imaging and scintigraphy have reported that evaluation of the neovascularization can be helpful for predicting whether the condition is toward normal fracture healing, delayed union or nonunion [4, 6, 33, 36, 37]. Contrast enhanced ultrasonography (CEUS) can detect blood flow with low volume and slow velocity in smaller vessels through harmonic signals from a microbubble contrast agent in real time after injecting the contrast medium [15]. Dynamic blood perfusion can be visually evaluated based on the bright signal formed by contrast agent at a low mechanical index. In addition, blood velocity and volume can be assessed quantitatively based on the time − intensity curve (TIC) generated from CEUS.

In veterinary medicine, the quantitative assessments using the TIC on CEUS have been applied to various organs, including the canine liver [3, 23–25], spleen [26], lymph nodes [40], adrenal gland [28, 29], kidney [8, 13, 39], pancreas [14, 32] and superficial tumors [27]. Neovascularization is one of the most important factors for fracture healing. Therefore, microcirculation in skeletal muscles can be used as an early indicator of hemodynamic changes during fracture healing. However, no study has applied CEUS to skeletal muscles in veterinary medicine. Although applying CEUS to musculoskeletal regions is just beginning even in humans, CEUS has been used to assess perfusion of skeletal muscle in various conditions including acute muscle injury, peripheral arterial disease, diabetes mellitus with peripheral arterial disease and ischemia and also in osseous and osseocutaneous free flaps [1, 2, 5, 9, 11, 16–18, 21, 22, 30, 31].

This study was performed to establish the protocol of contrast enhanced ultrasonography for the surrounding soft tissue of thoracic and pelvic limbs as a preliminary study for estimating perfusion in skeletal muscles of fracture cases. In the quantitative parameters of CEUS, the peak intensity (PI) and area under the curve (AUC) represented for blood volume, and the time to peak intensity (TP) represented for blood velocity.

MATERIALS AND METHODS

Eight 2–3-year-old male beagles (mean body weight; 10.51 ± 1.48 kg, range; 9.7–11.5 kg) were used. All dogs were clinically healthy based on a physical examination, complete blood counts, biochemistry, urinalysis, thoracic and abdominal radiography, and abdominal ultrasonography. There were unremarkable findings on lateral and craniocaudal radiographs of thoracic and pelvic limbs. The dogs were housed individually and were provided commercial food and water *ad libitum*.

After a 24 hr fast, the dogs were given intramuscular in-

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jections of a combination of 1.5 mg/kg zolazepam/tiletamine (Zoletil, Virbac, Carros, France) and 0.03 mg/kg medetomidine (Domitor, Orion Corp., Espoo, Finland). Blood pressure was recorded before and after anesthesia.

Conventional B-mode ultrasonography and CEUS were conducted in left humerus, radius, femur and tibia using the same 10 MHz linear probe and ultrasound equipment (ProSound Alpha 7, Aloka, Tokyo, Japan). The dog was positioned in a right lateral recumbency, and each examining limb was extended toward the operator and fastened. A conventional B-mode examination was performed to localize the scanning region for CEUS. The transducer was placed over the lateral plane from the shoulder joint to the humeral diaphysis proximally covered by the triceps muscle; the lateral plane from the proximal radial metaphysis to the diaphysis proximally covered by the extensor digitorum communis muscle and distally by the abductor pollicis longus muscle; the lateral plane from the stifle joint to the femoral diaphysis proximally covered by the tensor fasciae latae muscle and distally by the vastus lateralis muscle; or the lateral plane from the stifle joint to tibial diaphysis proximally covered by the tibialis cranialis, respectively. The longitudinal image of each bone surface including muscle was displayed on a monitor, with positioning so that the proximal metaphysis was on the right and the diaphysis was on the left. Color Doppler ultrasonography was performed to evaluate the presence of vessels. Second higher harmonics were performed in the extended pure harmonic detection mode for CEUS. The transmitted energy was reduced to a magnitude of 7% with a 0.07 mechanical index. Intrusion depth was set to 2.0–3.5 cm to visualize the muscle according to the appendicular regions. The pulse repetition frequency was set to 15 Hz with a 61% gain value. Contrast medium (SonoVue, Bracco, Milan, Italy) was administered by bolus injection into the cephalic vein via a three way stopcock and a 20 gauge intravenous catheter. A 0.5 m*l* (2.5 mg) or 1 m*l* (5 mg) of contrast medium was injected through a 1 m*l* syringe into each dog, immediately followed by a bolus injection of 5 m*l* saline. At the same time, dynamic sequences were stored for up to 110 sec by keeping the transducer in the same location. CEUS was completed in all dogs within 2 hr after opening the contrast medium bottle, and the contrast medium was agitated before injection into other dogs. A single appendicular region was examined in each dog on the same day, and the other regions were examined at 2-day intervals. All CEUS was performed by one examiner (OJY), and quantitative and qualitative evaluations of all CEUS were performed three times by two reviewers (OJY, JSH) blinded to the scanned region. All dynamic cine loops were evaluated using the installed software (SOP-ALPHA7-14, Aloka), which displayed the acoustic intensity according to time. Region of interest (ROI) in an arc shape of 1.1 × 2.9 mm was manually defined over the muscle to acquire the TIC. Motion correction was manually performed frame by frame. Quantitative corresponding parameters of the TIC, including PI, AUC and TP, were obtained from ROI. PI was measured with the greatest acoustic intensity. TP was defined as the duration from the start of contrast agent injection to PI. AUC was calculated as the area of the TIC using integration.

The protocol used in this study was approved by the Animal Care and Use Committee of Chonnam National University, and the animals were cared for in accordance with the Guidelines for Animal Experiments of Chonnam National University.

A statistical analysis was performed using the SPSS statistical program (SPSS Statistics Version 21, IBM Corp., Armonk, NY, U.S.A.). Analysis of reproducibility between reviewers was performed using the intra-class correlation coefficient test (ICC). ICC values were evaluated by the criteria: poor reliability, <0.4; fair to good, 0.4–0.75; and excellent, >0.75 [35]. Repeated-measures analysis of variance was used to investigate the intra- and inter- reproducibility coefficients of the TIC parameters including PI, TP and AUC and inter- reproducibility coefficients of vascularity score among four appendicular regions in normal dogs. A $P<0.05$ was considered significant for all analyses. Data are reported as mean ± standard deviation.

RESULTS

The mean blood pressure in eight dogs was 145.5 ± 7.52 mmHg before anesthesia and 126.38 ± 5.90 mmHg after anesthesia. Transient increase of vascular intensity and then disappearance was easily observed from all skeletal muscles after injecting the contrast medium (Fig. 1). The blood perfusion of skeletal muscle was reliably assessed using CEUS.

A total of 1,711 frames of CEUS were obtained from ROI over 110 sec, and a typical parabolic shape to the TIC was generated from muscle area (Fig. 2). The mean values of PI, AUC and TP measured from muscle were not significantly different among the four appendicular regions (Table 1). No significant difference in mean peak intensities, areas under the curve or TP was observed within each skeletal muscle of the four regions, according to the dosage of contrast medium. In quantitative analysis, the mean intra- and inter-reproducibility coefficients of PI, AUC and TP are represented in Table 2.

DISCUSSION

Assessing blood perfusion is meaningful to predict prognosis or monitor progression of fracture healing. The imaging modality has to be sensitive enough to detect low perfusion for an accurate assessment of blood flow in muscle, because normal skeletal muscle perfusion is very low at rest [41]. The ultrasound contrast medium, in principle, stays strictly intravascularly, and each single microbubble of contrast medium can be detected using CEUS, independent of velocity and volume of blood flow [5]. The blood volume measured with CEUS has a positive correlation with histologic capillarization [41]. Thus, CEUS has the capacity to assess skeletal muscle perfusion accurately not only at rest under normal conditions but also in fracture healing. A second-generation contrast agent was selected in this study, because this agent coated with inert gas is more stable allowing

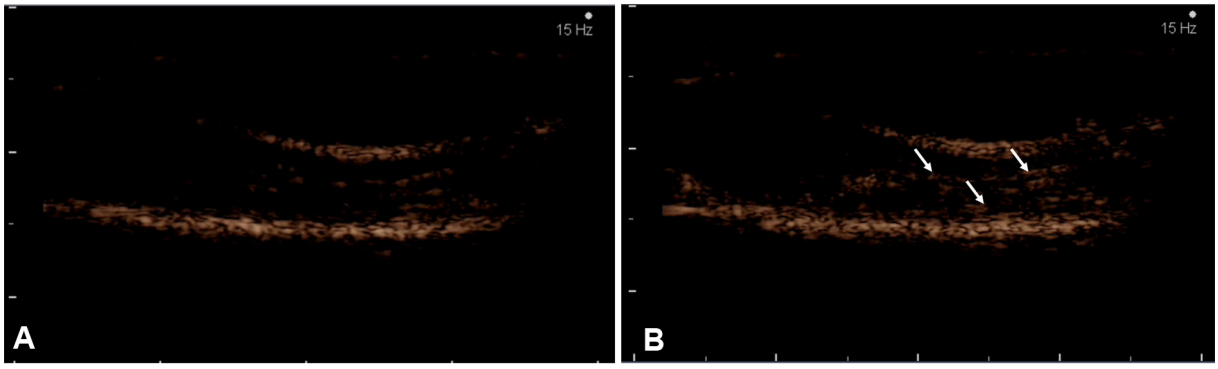

Fig. 1. Contrast enhanced ultrasonography on harmonic detection mode before (A) and after (B) contrast agent injection at the skeletal muscle of the humerus. Transient increase of vascular intensity (arrows) and then disappearance was easily observed from all skeletal muscles after injecting the contrast medium.

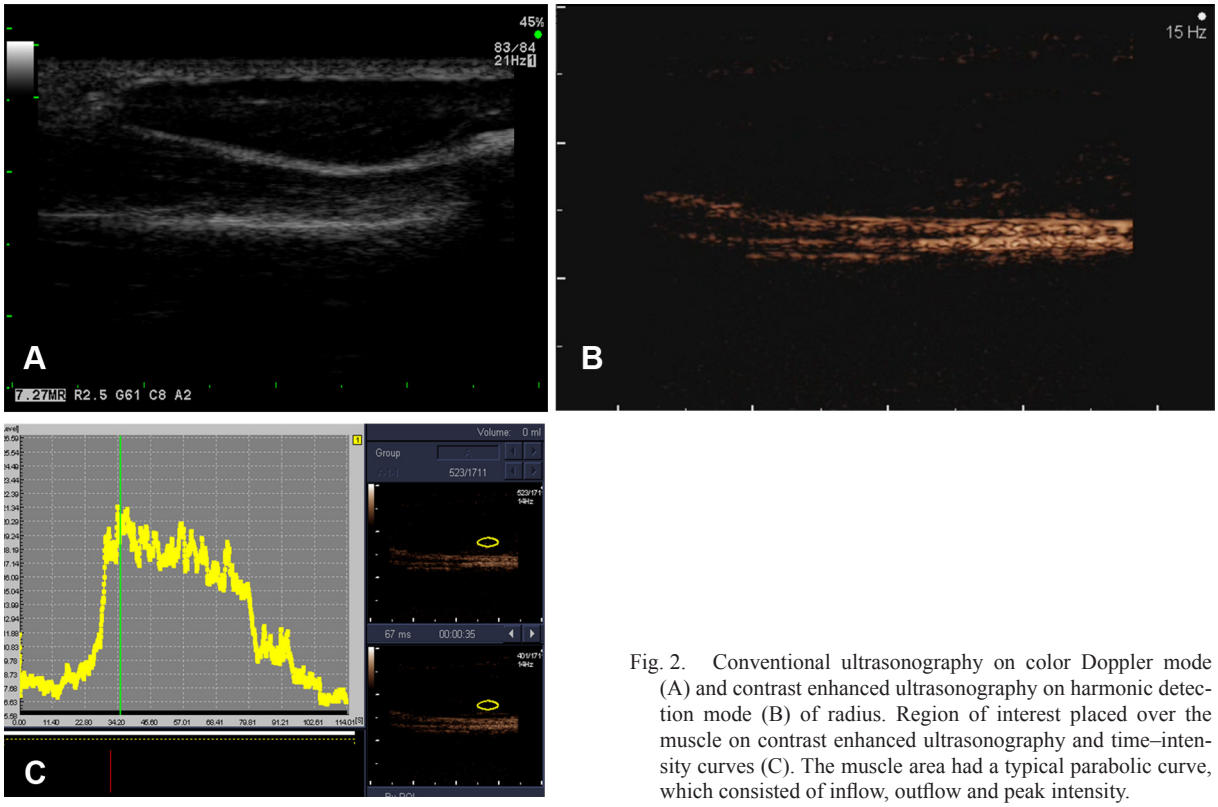

Fig. 2. Conventional ultrasonography on color Doppler mode (A) and contrast enhanced ultrasonography on harmonic detection mode (B) of radius. Region of interest placed over the muscle on contrast enhanced ultrasonography and time–intensity curves (C). The muscle area had a typical parabolic curve, which consisted of inflow, outflow and peak intensity.

longer persistence in the blood stream than first-generation agents coated with air. The size of sulfur hexafluoride-filled microbubbles is uniform in this contrast medium, and this agent has prolonged and excellent stability with a vial time of 6 hr and a half time of 6 min in peripheral blood [27]. In contrast to the first-generation contrast agents which become disrupted and release a large amount of acoustic emission at a high mechanical index, low mechanical index imaging using second-generation agents minimizes the microbubble burst to enhance the contrast effect and produces a nonlinear oscillation which provides for harmonic frequencies for a longer duration [27]. These non-destructive contrast agents allow for continuous real-time imaging and quantitative evaluation of blood perfusion by TIC analysis.

The mean values of PI and AUC represent the blood volume, and TP determines blood velocity [12]. PI and AUC in skeletal muscle tended to be higher in the humerus and femur than those in the radius and tibia, but no significant difference was observed. TP in the thoracic limbs tended to be more rapid (about 4 min) than that in the pelvic limbs; however, no significant difference among the 4 regions was observed. Thus, blood volume and velocity in skeletal

Table 1. Mean values of time-intensity curve parameters in appendicular muscles after injection of 0.5 m*l* or 1 m*l* contrast agent

	PI (level)		AUC		TP (msec)	
	0.5 m*l*	1 m*l*	0.5 m*l*	1 m*l*	0.5 m*l*	1 m*l*
Humerus	28.84 ± 9.69	22.62 ± 4.20	1,642.39 ± 643.23	1,474.06 ± 372.80	40,763 ± 10,274	38,576 ± 10,983
Radius	19.86 ± 8.92	16.15 ± 4.44	1,402.64 ± 851.85	838.91 ± 366.67	37,649 ± 5,976	43,101 ± 11,463
Femur	23.25 ± 14.36	28.31 ± 10.37	1,786.42 ± 1,279.55	2,041.50 ± 769.36	43,969 ± 5,976	38,576 ± 8,281
Tibia	16.40 ± 6.18	18.50 ± 1.60	1,143.29 ± 475.52	1,309.10 ± 280.76	42,890 ± 10,373	45,770 ± 6,707

Data are mean and standard deviation.

Table 2. Mean intra- and inter-reproducibility coefficients of peak intensity, area under the curve and time to peak intensity of the soft tissue in each appendicular bone region using 0.5 m*l* or 1 m*l* contrast agent in appendicular muscles

	0.5 m*l*						1 m*l*					
	Intra-reproducibility coefficients			Inter-reproducibility coefficients			Intra-reproducibility coefficients			Inter-reproducibility coefficients		
	PI	TP	AUC	PI	TP	AUC	PI	TP	AUC	PI	TP	AUC
Humerus	0.759	0.827	0.835	0.871	0.864	0.806	0.859	0.972	0.935	0.871	0.984	0.906
Radius	0.906	0.808	0.832	0.755	0.952	0.860	0.906	0.998	0.942	0.765	0.962	0.860
Femur	0.924	0.970	0.931	0.933	0.678	0.927	0.924	1.000	0.971	0.86	0.678	0.927
Tibia	0.742	0.744	0.801	0.899	0.751	0.856	0.772	0.774	0.841	0.95	0.891	0.885

muscle were not significantly different in the 4 appendicular regions. The vascularity score in the skeletal muscle was qualitatively assessed. Although a statistical analysis was not performed in this study, CEUS seemed to be more sensitive than color Doppler for detecting small vessels and low blood velocities, because CEUS showed distinct vessel signals, not presented by color Doppler image in skeletal muscle. In addition, there are no Doppler-specific artifacts, such as aliasing and blooming in CEUS. Severe soft tissue trauma and circulatory disturbances are one of the common causes of impediment to successful bone healing, because the fracture site is initially supplied blood by extraosseous angiogenesis. In addition, excessive damage to nutrient and periosteal vessels passing through cortical bone lead to delayed union [34]. Therefore, quantitative TIC analysis of skeletal muscular vascularization could provide early prognostic information about fracture healing.

The effect of dosage on perfusion of skeletal muscles was investigated. A standard protocol for using contrast agent in skeletal muscle is not established, even in humans, and many different doses, such as 2.4 m*l*, 4.8 m*l* and 5 m*l*, with diverse injection methods including bolus injection and continuous rate infusion have been applied [1, 2, 9, 11, 16–18, 20]. In the present study, there was no significant difference in PI, AUC or TP between the two doses. Consequently, 0.5 m*l* of contrast medium may be more practical for assessing skeletal muscle perfusion.

Generally, ultrasonographic contrast media are safe and well tolerated [27]. In this study, there was no side effect with CEUS using SonoVue in dogs. All TIC values including PI, AUC and TP had similarly high reproducibility. CEUS was safe, non-invasive imaging method with short examination times. Furthermore, quantitative assessment of skeletal muscle perfusion using TIC values had accuracy and objectivity with high reproducibility. There are some limitations in this study. First, this study was performed under general anesthesia, and blood volume and velocity may be different from those of patients without anesthesia [38]. The pharmacological effects of anesthetics itself can influence the TIC values [38]. In a previous study, the vasodilation effect of anesthetics altered local blood pressure and heart rate, and TP in liver was about 11.6 sec faster in dogs without anesthesia than for those under anesthesia [23]. Second, it was difficult to assess blood perfusion accurately in some muscular areas containing fascia, ligaments and tendons, because they show hyperintensity.

Although there were some limitations, CEUS is expected to be an excellent modality for evaluating changes of blood perfusion in skeletal muscle. This study provided quantitative reference value of muscular perfusion including blood volume and velocity in normal dogs. A 0.5 m*l* aliquot contrast medium is suggested as a practical dose for assessing the skeletal muscle. The CEUS protocol for evaluating the blood perfusion of skeletal muscles can be used as basic information for further studies evaluating the blood perfusion of skeletal muscles in various pathologic states including bone fracture using CEUS.

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REFERENCES

1. Amarteifio, E., Krix, M., Wormsbecher, S., Demirel, S., Braun, S., Delorme, S., Kauczor, H. U., Böckler, D. and Weber, M. A. 2013. Dynamic contrast-enhanced ultrasound for assessment of

therapy effects on skeletal muscle microcirculation in peripheral arterial disease: pilot study. *Eur. J. Radiol.* **82**: 640–646. [Medline] [CrossRef]
2. Amarteifio, E., Wormsbecher, S., Demirel, S., Krix, M., Braun, S., Rehnitz, C., Delorme, S., Kauczor, H. U. and Weber, M. A. 2013. Assessment of skeletal muscle microcirculation in type 2 diabetes mellitus using dynamic contrast-enhanced ultrasound: A pilot study. *Diab. Vasc. Dis. Res.* **10**: 468–470. [Medline] [CrossRef]
3. Baron Toaldo, M., Diana, A., Bettini, G., Donato, P. D., Linta, N. and Cipone, M. 2013. Imaging diagnosis-cholangiocellular adenoma: contrast-enhanced ultrasonographic findings of a benign tumor mimicking malignant neoplasia in a dog. *Vet. Radiol. Ultrasound* **54**: 71–74. [Medline] [CrossRef]
4. Barros, J. W., Barbieri, C. H. and Fernandes, C. D. 2000. Scintigraphic evaluation of tibial shaft fracture healing. *Injury* **31**: 51–54. [Medline] [CrossRef]
5. Calliada, F., Campani, R., Bottinelli, O., Bozzini, A. and Sommaruga, M. G. 1998. Ultrasound contrast agents: basic principles. *Eur. J. Radiol.* **27**: S157–S160. [Medline] [CrossRef]
6. Caruso, G., Lagalla, R., Derchi, L., Iovane, A. and Sanfilippo, A. 2000. Monitoring of fracture calluses with color Doppler sonography. *J. Clin. Ultrasound* **28**: 20–27. [Medline] [CrossRef]
7. Cruess, R. L. and Dumont, J. 1975. Fracture healing. *Can. J. Surg.* **18**: 403–413. [Medline]
8. Dong, Y., Wang, W., Cao, J., Fan, P. and Lin, X. 2013. Quantitative evaluation of contrast-enhanced ultrasonography in the diagnosis of chronic ischemic renal disease in a dog model. *PLoS ONE* **8**: e70337. [Medline] [CrossRef]
9. Duerschmied, D., Olson, L., Olschewski, M., Rossknecht, A., Freund, G., Bode, C. and Hehrlein, C. 2006. Contrast ultrasound perfusion imaging of lower extremities in peripheral arterial disease: a novel diagnostic method. *Eur. Heart J.* **27**: 310–315. [Medline] [CrossRef]
10. Frost, H. M. 1989. The Biology of Fracture Healing: An Overview for Clinicians. Part I. *Clin. Orthop. Relat. Res.* 283–293. [Medline]
11. Genovese, E. A., Callegari, L., Combi, F., Leonardi, A., Angeretti, M. G., Benazzo, F., D'Angelo, F. and Fugazzola, C. 2007. Contrast enhanced ultrasound with second generation contrast agent for the follow-up of lower-extremity muscle-strain-repairing processes in professional athletes. *Radiol. Med. (Torino)* **112**: 740–750. [Medline] [CrossRef]
12. Greis, C. 2011. Quantitative evaluation of microvascular blood flow by contrast-enhanced ultrasound (CEUS). *Clin. Hemorheol. Microcirc.* **49**: 137–149. [Medline]
13. Haers, H., Daminet, S., Smets, P. M. Y., Duchateau, L., Aresu, L. and Saunders, J. H. 2013. Use of quantitative contrast-enhanced ultrasonography to detect diffuse renal changes in Beagles with iatrogenic hypercortisolism. *Am. J. Vet. Res.* **74**: 70–77. [Medline] [CrossRef]
14. Johnson-Neitman, J. L., O'Brien, R. T. and Wallace, J. D. 2012. Quantitative perfusion analysis of the pancreas and duodenum in healthy dogs by use of contrast-enhanced ultrasonography. *Am. J. Vet. Res.* **73**: 385–392. [Medline] [CrossRef]
15. Klauser, A. S. and Peetrons, P. 2009. Developments in musculoskeletal ultrasound and clinical applications. *Skeletal Radiol.* **39**: 1061–1071. [Medline] [CrossRef]
16. Krix, M., Krakowski-Roosen, H., Amarteifio, E., Fürstenberger, S., Delorme, S., Kauczor, H. U. and Weber, M. A. 2011. Comparison of transient arterial occlusion and muscle exercise provocation for assessment of perfusion reserve in skeletal muscle with real-time contrast-enhanced ultrasound. *Eur. J. Radiol.* **78**: 419–424. [Medline] [CrossRef]
17. Krix, M., Weber, M. A., Kauczor, H. U., Delorme, S. and Krakowski-Roosen, H. 2010. Changes in the micro-circulation of skeletal muscle due to varied isometric exercise assessed by contrast-enhanced ultrasound. *Eur. J. Radiol.* **76**: 110–116. [Medline] [CrossRef]
18. Krix, M., Weber, M. A., Krakowski-Roosen, H., Huttner, H. B., Delorme, S., Kauczor, H. U. and Hildebrandt, W. 2005. Assessment of skeletal muscle perfusion using contrast-enhanced ultrasonography. *J. Ultrasound Med.* **24**: 431–441. [Medline]
19. Latta, L. L., Sarmiento, A. and Tarr, R. R. 1980. The rationale of functional bracing of fractures. *Clin. Orthop. Relat. Res.* 28–36. [Medline]
20. Mancini, M., Di Donato, O., Saldalamacchia, G., Liuzzi, R., Rivellese, A. and Salvatore, M. 2013. Contrast-enhanced ultrasound evaluation of peripheral microcirculation in diabetic patients: effects of cigarette smoking. *Radiol. Med. (Torino)* **118**: 206–214. [Medline] [CrossRef]
21. Mueller, S., Gosau, M., Wendl, C., Prantl, L., Wiggermann, P., Reichert, T. E. and Jung, E. M. 2012. Postoperative evaluation of microvascularization in mandibular reconstructions with microvascular flaps–First results with a new perfusion software for contrast-enhanced sonography (CEUS). *Clin. Hemorheol. Microcirc.* **52**: 187–196. [Medline]
22. Mueller, S., Meier, J. K., Wendl, C. M., Jung, E. M., Prantl, L. and Gosau, M. 2012. Mandibular reconstruction with microvascular re-anastomosed fibular free flaps–Two complementary methods of postoperative transplant monitoring. *Clin. Hemorheol. Microcirc.* **52**: 141–151. [Medline]
23. Nyman, H. T., Kristensen, A. T., Kjelgaard-Hansen, M. and McEvoy, F. J. 2005. Contrast-enhanced ultrasonography in normal canine liver. Evaluation of imaging and safety parameters. *Vet. Radiol. Ultrasound* **46**: 243–250. [Medline] [CrossRef]
24. O'Brien, R. T. 2007. Improved detection of metastatic hepatic hemangiosarcoma nodules with contrast ultrasound in three dogs. *Vet. Radiol. Ultrasound* **48**: 146–148. [Medline] [CrossRef]
25. O'Brien, R. T., Iani, M., Matheson, J., Delaney, F. and Young, K. 2004. Contrast harmonic ultrasound of spontaneous liver nodules in 32 dogs. *Vet. Radiol. Ultrasound* **45**: 547–553. [Medline] [CrossRef]
26. Ohlerth, S., Dennler, M., Ruefli, E., Hauser, B., Poirier, V., Siebeck, N., Roos, M. and Kaser-Hotz, B. 2008. Contrast harmonic imaging characterization of canine splenic lesions. *J. Vet. Intern. Med.* **22**: 1095–1102. [Medline] [CrossRef]
27. Ohlerth, S. and O'Brien, R. T. 2007. Contrast ultrasound: general principles and veterinary clinical applications. *Vet. J.* **174**: 501–512. [Medline] [CrossRef]
28. Pey, P., Daminet, S., Smets, P. M., Duchateau, L., de Fornel-Thibaud, P., Rosenberg, D. and Saunders, J. C. H. 2013. Contrast-enhanced ultrasonographic evaluation of adrenal glands in dogs with pituitary-dependent hyperadrenocorticism. *Am. J. Vet. Res.* **74**: 417–425. [Medline] [CrossRef]
29. Pey, P., Vignoli, M., Haers, H., Duchateau, L., Rossi, F. and Saunders, J. H. 2011. Contrast-enhanced ultrasonography of the normal canine adrenal gland. *Vet. Radiol. Ultrasound* **52**: 560–567. [Medline] [CrossRef]
30. Prantl, L., Pfeifer, C., Geis, S., Gosau, M. and Jung, E. M. 2011. Osteocutaneous free flaps: a critical analysis of quantitative evaluation of bone microcirculation with contrast-enhanced high resolution ultrasound (hrCEUS) and TIC analysis. *Clin. Hemorheol. Microcirc.* **49**: 251–259. [Medline]
31. Prantl, L., Pfister, K., Kubale, R., Schmitt, S., Stockhammer, V.,

Jung, W., Zorger, N., Herold, T., Nerlich, M., Stehr, A. and Jung, E. M. 2007. Value of high resolution ultrasound and contrast enhanced US pulse inversion imaging for the evaluation of the vascular integrity of free-flap grafts. *Clin. Hemorheol. Microcirc.* **36**: 203–216. [Medline]
32. Rademacher, N., Ohlerth, S., Scharf, G., Laluhova, D., Sieber-Ruckstuhl, N., Alt, M., Roos, M., Grest, P. and Kaser-Hotz, B. 2008. Contrast-enhanced power and color Doppler ultrasonography of the pancreas in healthy and diseased cats. *J. Vet. Intern. Med.* **22**: 1310–1316. [Medline] [CrossRef]
33. Rawool, N. M., Goldberg, B. B., Forsberg, F., Winder, A. A. and Hume, E. 2003. Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound. *J. Ultrasound Med.* **22**: 145–153. [Medline]
34. Remedios, A. 1999. Bone and bone healing. *Vet. Clin. North Am. Small Anim. Pract.* **29**: 1029–1044 v. [Medline] [CrossRef]
35. Roberts, C. T. and Richmond, S. 1997. The design and analysis of reliability studies for the use of epidemiological and audit indices in orthodontics. *Br. J. Orthod.* **24**: 139–147. [Medline] [CrossRef]
36. Schoierer, O., Bloess, K., Bender, D., Burkholder, I., Kauczor, H. U., Schmidmaier, G. and Weber, M. A. 2014. Dynamic contrast-enhanced magnetic resonance imaging can assess vascularity within fracture non-unions and predicts good outcome. *Eur. Radiol.* **24**: 449–459. [Medline] [CrossRef]
37. Tang, M. X., Mulvana, H., Gauthier, T., Lim, A. K., Cosgrove, D. O., Eckersley, R. J. and Stride, E. 2011. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. *Interface Focus* **1**: 520–539. [Medline]
38. Waller, K. R., O'Brien, R. T. and Zagzebski, J. A. 2007. Quantitative contrast ultrasound analysis of renal perfusion in normal dogs. *Vet. Radiol. Ultrasound* **48**: 373–377. [Medline] [CrossRef]
39. Wang, Y., Cheng, Z., Li, J. and Tang, J. 2010. Gray-scale contrast-enhanced ultrasonography in detecting sentinel lymph nodes: An animal study. *Eur. J. Radiol.* **74**: e55–e59. [Medline] [CrossRef]
40. Weber, M. A., Krakowski-Roosen, H., Hildebrandt, W., Schröder, L., Ionescu, I., Krix, M., Kinscherf, R., Bachert, P., Kauczor, H. U. and Essig, M. 2007. Assessment of metabolism and microcirculation of healthy skeletal muscles by magnetic resonance and ultrasound techniques. *J. Neuroimaging* **17**: 323–331. [Medline] [CrossRef]
41. Ziegler, L. E., O'Brien, R. T., Waller, K. R. and Zagzebski, J. A. 2003. Quantitative contrast harmonic ultrasound imaging of normal canine liver. *Vet. Radiol. Ultrasound* **44**: 451–454. [Medline] [CrossRef]