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Use of a colorimeter is a viable method to measure melanin and erythema content in the context of laser beam attenuation by use of a class IV laser in different tissues in dogs

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OBJECTIVE
Patient factors may alter laser photon attenuation, but these factors have not been adequately evaluated in live dogs. Our objective was to evaluate class IV laser beam attenuation (LBA) by canine tissues using a colorimeter to evaluate melanin and erythema indices. We hypothesized that greater melanin and erythema indices and unclipped hair would increase LBA, and these properties would vary among tissues.

ANIMALS
20 client-owned dogs.

PROCEDURES
Between October 1 and December 1, 2017, colorimeter measurements and LBA in various tissues before and after clipping overlying hair were evaluated. Data were analyzed using generalized linear mixed models. Statistical significance was set at $P < .05$.

RESULTS
LBA was greater in unclipped (98.6 ± 0.4%) than clipped hair (94.6 ± 0.4%). The least LBA occurred in the pinna (93%) while the greatest occurred in the caudal vertebra (100%) and caudal semitendinosis muscles (100%). Each mm of tissue thickness resulted in LBA of 11.6%. Each unit increase in melanin index resulted in a 3.3% increase in LBA. There was no association of LBA with erythema index.

CLINICAL RELEVANCE
To our knowledge, this is the first study that evaluated LBA by different tissues in live dogs using a colorimeter to evaluate melanin and erythema indices. We recommend clipping hair prior to photobiomodulation to decrease laser beam attenuation and using increased laser doses in thicker tissues and dogs with high melanin content. The colorimeter may be helpful in customizing patient treatment dosimetry. Future studies are necessary to determine therapeutic laser doses for adequate photobiomodulation effects.

Therapeutic lasers are widely used for treatment of various conditions in veterinary patients despite limited supporting literature regarding efficacy.[1,2] Therapeutic lasers create physiologic changes at the cellular level driven by light, or photobiomodulation.[1] Lasers emit a monochromatic, coherent, and collimated light that allows photon absorption targeted to specific wavelength-dependent chromophores, or photon acceptors, within tissues.[1] ATP synthesis occurs following photon absorbance by mitochondria. Stimulation of mitochondrial chromophores causes nitric oxide dissociation from cytochrome-C oxidase at the terminal chain in mitochondria and cell membranes.[3] This results in increased production of oxygen and formation of proton gradients activating flavomononucleotides, producing ATP.[3] Production of ATP augments cell-to-cell signaling and increases intracellular calcium leading to enhanced mitochondrial function, cell proliferation, and bone production.[3–5] Photobiomodulation studies have shown positive effects on wound healing, muscle regeneration, and chronic and acute pain conditions, including osteoarthritis.[6–9]

Laser penetration may be inhibited by extrinsic and intrinsic factors. Extrinsic factors include laser power, wavelength, and mode of application (pulsed versus continuous). Intrinsic factors include patient characteristics such as skin melanin and erythema content, presence or absence of hair, blood flow, tissue type, and tissue thickness. The optimal wavelength of laser energy for greatest tissue penetration, and the least amount of scattering and surface absorbance is generally between 600 and 1,200 nm.[10] Despite optimal wavelength selection and mode of application, intrinsic factors exist, such as hair, dermis, epidermis, and subcutaneous tissues, that could affect laser absorbance, transmission, and scatter of photons. All of these factors result in varying amounts of laser beam attenuation (LBA), potentially leading to subtherapeutic laser doses at the intended target tissue site.[11]

Despite the widespread empirical clinical use of laser therapy in veterinary patients, there is a lack of information regarding the penetration of laser energy through areas covered by hair, skin (both pigmented and non-pigmented), muscle, cartilage, and bone in live dogs. Recent invasive live animal studies evaluating the penetration depth of a 830 nm low intensity laser, and a 50 Hz superpulsed and multiple wavelength laser on living dog tissues found that on-contact laser application showed improved light penetration to deeper tissues whereas the non-contact technique showed no difference between 1 and 5 cm tissue depth.[12,13] Hochman-Elam et al[14] recently reported on the impacts of laser power, wavelength, coat color and the presence of hair during laser application in live dogs. The study found higher laser transmission occurred with a class IV 810/980 nm wavelength laser at 0.5 W compared with a class IIIb 904 nm laser. There were also significant differences in laser transmission of photons through tissues among white, brown, and black coats, with less transmission occurring with increasing coat pigment.[14] Higher powers allowed greater transmission and it was noted that coat length did not affect transmission.[14] Another study evaluated class IV laser penetration through equine cadaveric skin and found significantly higher laser transmission for both light-colored skin and clipped hair.[15] Interestingly, higher transmission for lighter colored skin occurred using an 800 nm wavelength compared to greater transmission of medium- and dark-colored skin using the 970 nm wavelength laser.[15] This is not a surprising finding, especially when considering the absorption coefficients of hemoglobin, water, and melanin, wavelengths of common medical lasers, and the relationship between average melanin with the corresponding scattering coefficient.[16,17] In a recent study looking at human melanin content and scattering coefficients, a chromameter was used to measure melanin content in different human subjects and was found to be an appropriate instrument for predicting the spectroscopic behavior of different skin types based on melanin and hemoglobin content.[16] Another study using human cadaveric skin revealed that approximately 66% of LBA occurred after only traveling 0.78 mm and that most laser radiation was absorbed within the first 1 mm of skin.[18] Despite the frequent use of laser therapy for dermatological conditions and superficial wound healing, laser therapy is also used for conditions of deeper tissues, including musculoskeletal and neurologic conditions.[1]

Experimental studies evaluating melanin as a barrier to photon penetration have shown varying concentrations of melanin absorb light in different amounts.[19] In canine skin, melanin concentrations are greatest in the superficial epidermal layers which may result in increased absorbance of photons, thus reducing the number of photons that can pass into deeper dermal tissues. Melanin may therefore reduce the effectiveness of laser therapy. This was validated by Hochman-Elam et al[14] in live dogs who found that transmission (penetration) of laser light was decreased in dogs with darker pigmented skin. Objective methods that use colorimetry technology to quantify melanin and erythema levels in the skin and haired surface are available. These tools may be useful to guide laser treatment protocols by using erythema and melanin densities to alter laser doses, but this has not been evaluated in veterinary studies.

The primary goals of our study were to noninvasively measure laser penetration through various tissues in live dogs with different colored skin, and penetration through tissues with unclipped and clipped hair. A secondary goal was to use a colorimeter to evaluate melanin and erythema indices and their effects on laser penetration. We hypothesized that 1) laser penetration would vary with tissue type, 2) there would be a difference in laser attenuation in clipped and unclipped hair of the various tissues, and 3) a melanin/erythema index could be obtained and would have correlation with laser penetration.

Materials and Methods

Inclusion criteria

Twenty adult, client-owned dogs weighing between 15 and 32 kg were included in this prospective study. The study was approved by the Institutional Animal Care and Use Committee at The University of Tennessee College of Veterinary Medicine. Prior to enrollment in the study, signed owner consent was obtained and a physical examination was performed on each dog to rule out systemic disease. Seven sites were chosen to receive laser application in each dog: 1) the middle of the right pinna, 2) right triceps muscle group at its widest point, 3) right skin inguinal region, 4) tail immediately distal to tail base through a caudal vertebra, 5) tail (caudal vertebra intervertebral space), 6) right common calcaneal tendon, and 7) right caudal semitendinosis (thigh) muscles.

Assessment of tissue thicknesses

Vernier calipers (Vernier calipers; Bel-Art Products) were used to measure the tissue thickness (in mm) at each tissue site prior to laser therapy. The middle of the right pinna, right triceps muscle group at the level of maximum thickness, right inguinal skin fold between the body wall and thigh, tail (through a caudal vertebra), tail (through a caudal intervertebral space), common calcaneal tendon midway between the tuber calcaneus and musculotendinous junction, and caudal thigh muscles midway between the tuber ischium and stifle joint were measured. Each site was measured 3 times and the mean tissue thickness was recorded. Care was taken to avoid deformation and compression of tissues during measurements.

Laser calibration and wavelength detection

A commercially available Class IV solid state (diode) laser operating at wavelengths 810/980 nm (CTC-12; LiteCure) was used for all laser measurements. A continuous wave (CW) beam was delivered through an optical fiber to a handpiece. The laser handpiece, fitted with a 2.75 cm X 14.75 cm deep tissue attachment head, was fixed in a stationary position in a custom jig. The aperture diameter is 1 cm^2 and emits a focused beam resulting in a 1-cm^2 spot diameter when in contact with the tissue surface. The deep tissue attachment was used to record the strength of the emission passing through a calibration volume of saline in voltage. This value, recorded in millivolts (mV), was the value that was transmitted through the saline calibration volume, allowing for a calculation of the laser power that was absorbed by the saline. To convert the values from voltage to watts, a calibration curve was created to ensure that the photodiode (OPM; PDA10CF; ThorLabs) was accurately detecting optical power **(Table 1)**. The photodiode diameter is 0.2 mm^2 in diameter, with a wavelength detection between 800 and 1,700 nm, and energy detection of 150 mHz BW. Laser beam penetration measurements were recorded from 0.5 W to 5 W to determine a calibration curve using a constant volume and thickness of sterile 0.9% saline.

Table 1—Conversion table developed on the basis of a calibration curve to ensure accurate conversion from voltage detected (photodiode voltage detected) to watts (power) to calculate the detectable optical power (DOP) when evaluating class IV laser penetration through tissues of 20 nonsedated client-owned dogs between October 1, 2017, and December 1, 2017.

Class IV laser setting (W)	Photodiode voltage detected (V)	Power (W)
0.5	10.58562404	0.466
1	22.53713505	0.899
1.5	32.09834386	1.251
2	45.75721359	1.662
2.5	55.3184224	2.074
3	65.5625747	2.478
3.5	72.39200956	2.79
4	77.85555745	3.25
4.5	86.05087929	3.77
5	105.9573815	4.24

R^2 = 0.9976; y = 0.0413x - 0.0843.

Assessment of melanin and erythema index at designated tissue sites

A colorimeter (ColorMeter DSM II; Cortex Technology) was applied in a perpendicular fashion to the center of each tissue site prior to laser application before and after hair clipping to measure the melanin and erythema index. Triplicate measurements were obtained and recorded. The colorimeter was calibrated weekly according to the manufacturer's guidelines.

Measuring laser penetration through designated tissue sites

The laser was positioned in the jig in a manner that maintained skin contact with both the laser and photodiode (PD; **Figure 1**). Each patient was placed in a standing position during measurements (patients were not sedated). The laser was then turned on and the detected maximum optical power (MOP) was recorded to determine the baseline MOP without tissue measurement **(Figure 2)**.

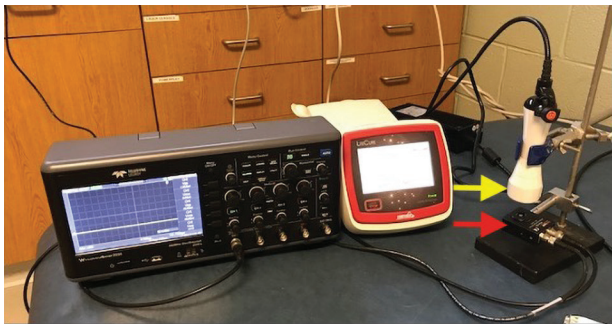

Figure 1—Image of the custom jig (right) constructed to hold a laser probe (yellow arrow) to the surface of each anatomic site of interest and to hold a photodiode (PD; red arrow) exactly 90° to the laser beam and in contact with far surface of the site when evaluating class IV laser penetration through tissues of 20 nonsedated client-owned dogs between October 1, 2017, and December 1, 2017. The PD was attached to an oscilloscope (left) for signal detection.

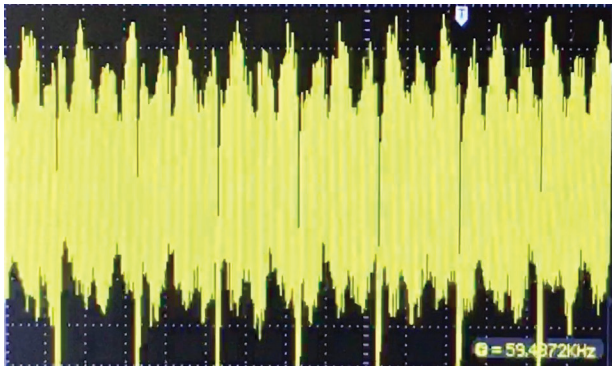

Figure 2—Representative oscilloscopic image of an active signal of the detected optical power (DOP) while the laser was applied to an anatomic site of one of the dogs described in Figure 1. The laser power, represented by the yellow undulating waveform, penetrated through the dog's tissue and to the PD. The x-axis represents seconds, and the y-axis represents millivolts. The root mean square values of millivolts were obtained from the oscilloscope screen.

After the baseline MOP was recorded, the laser was positioned across the first tissue site, the right pinna. The laser was turned on and 3 measurements, 1 second apart, were obtained and recorded from the oscilloscope. The attenuation of power was then obtained by subtracting the transmitted power to the photodiode (DOP) from the maximal optimal power (MOP) detected without tissues. The difference of these 2 numbers (MOP-DOP) was expressed as a percentage of tissue laser power attenuation. The root mean square values of millivolts were obtained from the oscilloscope screen. This is a standard measurement which approximates the average value of a waveform.

Percent LBA by tissues was determined for power settings of 0.5, 1, 3, and 5 W. The laser was similarly applied to the remaining sites determined in the following order for each dog: right triceps muscle group, right inguinal region, tail (through a caudal vertebra), tail (through a caudal intervertebral space), right common calcaneal tendon, and right caudal mid-thigh muscles.

After data were collected from all sites, an approximately 4 cm^2 region of hair was clipped over the previously tested sites on both sides of the tissues. The colorimeter was again used to determine the melanin and erythema indices as previously described. The laser was then positioned perpendicular to each of the sites and the transmission was determined in triplicate in a similar fashion to previous measurements.

Statistical analysis

All statistical analyses were performed using a statistical software (SAS/STAT software version 9.4; SAS Institute Inc). Generalized linear mixed models were fit to the data to investigate the association between the dependent variable, LBA, and the following independent variables: hair clipping (clipped vs not clipped), melanin index, erythema index, tissue type (pinna, triceps, inguinal region, caudal vertebra intervertebral space, caudal vertebra, distal common calcaneal tendon, proximal thigh), tissue thickness, and laser power (0.5, 1, 3 and 5 W). Patient identification was specified as the subject in the repeated statement of the model. The model fit was performed in 2 steps. In the first step, unadjusted associations between each of the independent variables listed above and LBA were investigated by fitting models with each independent variable at a time. The second step involved fitting a multivariable model that adjusted for the effect of each independent variable for the other variables in the model. Least square means and SEs for each categorical variable were estimated. Two-way comparisons between levels of categorical variables were also assessed and multiple comparisons were adjusted for using the Tukey-Kramer method. Statistical significance was set at $P < .05$.

Results

Patient characteristics

A total of 20 client-owned dogs were included in the study. No adverse events were observed. Mean weight was 25.6 kg (range, 16 to 32 kg). A variety of breeds were assessed, including mixed breed (10), Labrador Retriever (2), Australian Shepherd (2), Golden Retriever (1), German Shepherd Dog (1), Standard Poodle (1), Australian Kelpie (1), Shetland Sheepdog (1) and Greyhound (1). Body condition scores ranged between 4/9 and 7/9. All patients were deemed healthy for study purposes. A total of 840 LBA measurements were obtained prior to and after hair clipping at the 7 sites.

Effects of laser power

Mean LBA was significantly ($P < .001$) higher for the laser power output of 0.5 W (99.3%) and 1 W (98.5%) versus 3 W (95.8%) or 5 W (92.7%), and results were significantly ($P < .05$) higher for the laser power output of 3 W versus 5 W. Laser output power (watts) was indirectly associated with mean LBA, with higher power having less LBA (greater penetration) of laser ($P < .001$).

Effects of tissue thickness

Tissue thickness had a significant association with LBA ($P < .0001$). Each mm of tissue thickness increased LBA by 11.6%.

Effects of tissue site

Tissue site was significantly associated with LBA ($P < 0.001$). The average thickness of the pinna, triceps, inguinal region, tail through a caudal vertebra, tail through a caudal vertebra intervertebral space, common calcaneal tendon, and caudal thigh were: 3.9 (± 2.2) mm, 6.4 (± 4.1) mm, 4.5 (± 1.9) mm, 21.6 (± 5.3) mm, 10.0 (± 2.9) mm, 8.0 (± 2.0) mm, 42.5 (± 23.4) mm respectively. Laser beam attenuation was greatest in the proximal thigh (100%), caudal vertebra (100%), distal intervertebral space (97.44%), and triceps muscle (97.41%) and the differences between these values were not significant. The next greatest laser beam attenuation was in the calcaneal tendon (94.88%). The difference between the triceps muscle, distal intervertebral disc space and calcaneal tendon was not significant. The least laser beam attenuation occurred in the inguinal region (93.44%) and pinna (92.98%) and the difference between these 2 tissues and the calcaneal tendon was not significant.

Melanin index

Based on the final multivariable model, the melanin index was significantly ($P < .001$) associated with LBA. One unit increase of melanin index was associated with a 3.3% decrease in laser penetration. The mean melanin index across all sites and dogs prior to clipping was 107.4 ± 51 and post-clipping was 69.0 ± 28.

Erythema index

Erythema index had no association ($P < .274$) with LBA. The mean erythema index across all sites and dogs prior to clipping hair was 8.5 ± 8.3 and post-clipping was 6.3 ± 3.5.

Unclipped versus clipped hair

Sites with clipped hair had significantly ($P < .001$) greater laser penetration and lower mean tissue LBA (94.6 ± 0.4%, and 5.4% penetration) than LBA in non-clipped hair (98.6 ± 0.4%, and 1.4% penetration) when all sites were combined.

Discussion

We accept the hypothesis that laser penetration would vary with tissue type. We further accept the hypothesis that there would be a difference in laser beam penetration between clipped and unclipped hair of the various tissues. While our study also showed promising results using a colorimeter to identify a correlation between laser beam attenuation and melanin content, statistically significant results were not evident with erythema indices. To our knowledge, this is the first study that evaluates LBA by different tissues in live dogs using a colorimeter to evaluate melanin content and erythema indices. Similar penetration studies have been performed in live human skin. However, there are relatively few studies that have measured penetration in deeper tissues, especially in dogs.[20,21]

Photons are reflected, absorbed, scattered, or transmitted through skin and tissues.[22] One factor affecting the degree of laser photon penetration is the wavelength of the laser. Planck's Law describes the relationship between wavelength and tissue penetration by photons.[23] Photon delivery to deeper tissues is greater with longer wavelengths. In a study evaluating photon beam attenuation through rat skin, penetration of skin was only 20% of the applied laser energy when a 810 nm laser was used as compared to 38% when a 904 nm laser was used.[24] Another recent study looked at laser penetration in human Achilles tendons by comparing a 810 nm laser and a 904 nm laser.[25] The study revealed that the 904 nm wavelength laser had greater tissue penetration compared to a 810 nm laser.[25] Our study evaluated a laser with a 980 nm wavelength because we wished to study LBA of tissues with thickness that may be used for clinical treatment. Differences between devices in the distribution of photon energy in tissue (fluence distribution) can be partly attributed to wavelength, but the optical properties of the tissue, irradiance (power density of the beam) as determined by power output of the laser, the surface area irradiated by the beam, and fluence (the total amount of energy delivered to the area) as determined by the irradiance and duration of time of laser exposure also play important roles.

The distance between the probe and tissue of interest may affect laser penetration. A recent study evaluated Class IV laser penetration through thoracolumbar tissues in canine cadavers and found that placement of the laser probe directly on skin resulted in up to 67% greater light transmission compared to off contact with the skin; however, tissue thickness was not reported.[26] Decreasing the amount of distance between the laser and the target tissues may reduce photon scattering and reflection from the skin and could result in a higher number of photons delivered to tissues. In addition, there may be some beneficial effects of mild tissue compression regarding laser penetration.[27] On-contact application of the probe to the skin may be superior for laser transmission in canine cadavers and is one reason that we used on-contact application in our study.

Power, measured in watts, also affects tissue penetration by increasing the total number of photons administered to tissue in a given time. Previous studies show a direct relationship between power and degree of laser penetration, as was confirmed in our present study as well.[14] In our study laser power applied to tissues varied from 0.5 W to 5 W. When 5 W is applied, the dose of laser (total number of photons) during a 1 second application is 5 J. While a dose of 3 to 10 J/cm^2 has been previously suggested in companion animals for musculoskeletal conditions, there are few veterinary studies describing doses for various conditions, the maximum power (irradiance) of laser, or the maximum dose (fluence) of laser that can be safely applied to patients.[4,14] For this reason, it is difficult to make specific recommendations regarding doses that should be applied to different areas of the body without further penetration, efficacy, and safety studies.

The Modified Beer-Lambert Law explains transmission and propagation of light through biological media.[22,28,29] To quantify the effects of chromophores on tissue attenuation, a differential pathlength factor (DPF) is determined by taking the average distance that a picosecond light pulse travels across tissue.[22] In tissues, applying the Modified Beer-Lambert Law assumes that attenuation of laser energy is a function of a DPF and depends on absorption and scattering coefficients, scattering phase function, and interoptode distance between the source and detector. The accuracy of this equation depends on how variable the pathlength is.[22] To date, nearly all studies of the relationship of laser penetration through tissues have been of cadaveric tissue, which are deficient in blood and therefore hemoglobin.[30] Because there is no blood flow in cadaveric tissue, it is uncertain how blood flow through live tissues affects photon beam attenuation and penetration. Given that blood, water and melanin are the major absorbing components in live tissues, laser beam attenuation (LBA) detection in live tissues would likely be less when compared to cadaveric tissue.[12] A strength of our study is that we measured LBA in live dogs. The mechanism of photobiomodulation is thought to heavily rely on photon absorption by cytochrome C oxidase (CCO).[31] While there are recent studies looking at the effects of CCO levels on oxygenation, there are none using cadavers and comparing those values to live animals.[32] Future studies looking at laser energy and its penetration through live tissues as compared to cadaveric tissues may be useful in determining doses and clinical uses of laser therapy.

The relationship between LBA and tissue thickness is believed to be a logarithmic process; photon penetration decreases exponentially as tissue thickness increases.[33] The optical penetration depth of tissue (δ) is the distance into tissue where the incident irradiance is decreased by 63%. Previous studies looking at the optical penetration depth of tissue (δ) found that this value is 3 to 5 mm in non-melanin containing tissue and 1 to 2 mm in melanin containing tissue.[17] In our study, we objectively measured LBA through tissues of various thickness and com-

position with different melanin and erythema (related to hemoglobin) content. Overall, tissue thickness was significantly associated with LBA in the unadjusted statistical model. However, when other factors were accounted for in the adjusted mixed model (clipping, melanin content, and tissue type), tissue thickness was not significantly associated with LBA. This may have been skewed by the fact that different tissue types were similar in thickness to each other, and when the model corrected for tissue type, this may have affected LBA to a greater degree than continuous data of tissue thickness for all tissue types combined. For example, the thickness of the pinna ranged from 2 to 6.5 mm and had a LBA of 93% while thigh muscle thickness ranged from 11 to 87 mm and had 100% LBA of laser energy. Our results are consistent with results of cadaveric tissue that report an increasing attenuation of the beam as the photons move through an increasing thickness of tissue.[34,35] Unfortunately, our study used live dogs which precluded the detection of laser energy at specific increments of tissue thickness because of technical challenges in placing photosensors at various depths in live tissues without affecting variables such as blood flow. Further, it is unknown how many photons are needed to create a photobiomodulatory effect in tissues. No dose titration studies have been performed in veterinary medicine for various conditions that have evaluated photon penetration, tissue depth, tissue composition, and melanin or hemoglobin content. Recommendations from manufacturers are based on mathematical models using cadaveric tissues with clinical data largely lacking. Nevertheless, we believe that the effect of tissue thickness may be very important when treating areas with tissues greater than 1 cm thick. Further research is needed to characterize the effect of tissue thickness on laser penetration and how to appropriately adjust laser therapy to account for tissue thickness.

In addition to tissue thickness, tissue type also likely affects attenuation of laser energy. Tissue beam attenuation of light is affected by the light's propensity to be reflected, transmitted, scattered, or absorbed by that particular tissue type.[17] A recent study evaluated therapeutic laser penetration through the inguinal skin and common calcaneal tendon in live dogs and found differences in LBA between the tissues.[14] However, there were also differences in tissue thickness, similar to our findings. Each biological tissue inherently contains tissue geometry and indices that can uniquely scatter laser photons. Complete descriptions of tissue geometry of tendon, fat, skin, and bone are lacking in veterinary medicine, although canine muscle has been studied.[29] The sites evaluated for laser penetration in our study included tissues of varying thicknesses and composition, including skin, muscle, cartilage, tendon, and bone, in addition to fat and connective tissues associated with these sites. The lowest LBA (greatest penetration) of laser occurred in the pinna (93%), inguinal region (93.4%), and calcaneal tendon sites (95%). These regions have less subcutaneous tissue, muscle, and bone compared to the other sites evaluated. These tissues were also thinner than other tissues, yet the majority of laser light was still absorbed by these thinner tissues. This is consistent with a human cadaveric skin study where most of the laser was absorbed in the first 1 mm of tissue.[18] The proximal thigh and caudal vertebra had complete LBA of laser power (0% penetration). The thickness and composition of these tissues apparently created complete obstruction of photon travel.

Chromophore tissue composition, including water, hemoglobin, melanin, carotene, collagen, amino acids, and other proteins are present in variable amounts in canine tissues.[36] Melanin, hemoglobin, and deoxyhemoglobin are thought to be some of the most common chromophores in living tissues that absorb laser energy. Melanin content increases LBA, and therefore decreases laser penetration based on objective colorimeter melanin index values. Given that this was a noninvasive study, information on penetration depth and therefore light dosimetry is absent from this study. Although the colorimeter used in our study has only been evaluated in human skin to estimate melanin content, it likely has correlation with melanin content in canine skin, especially since dark skinned dogs have a higher melanin content compared with lighter skinned dogs.[37] Our results suggest that dogs with higher melanin content have greater LBA and decreased penetration. This has previously been validated with findings of melanin absorption coefficients at 810 nm (147 μ_a) and 980 nm (76 μ_a) lasers.[17] For this reason, a higher dose of laser energy may be necessary in highly pigmented skin so that adequate numbers of photons reach target tissues. Caution should be used when applying high laser power to darkly pigmented dogs because photon energy absorbed by melanin may be converted to thermal energy, and therefore result in excessive heating of superficial tissues. Although there were no changes in LBA regarding the erythema index in our study, all dogs had healthy skin and pathologic erythematous lesions were not observed, potentially skewing any results related to erythema index. Future studies should evaluate the relationship between erythema index and laser penetration with varying degrees of erythematous conditions, such as in dogs with skin incisions. The colorimeter has not been used to evaluate hair, and therefore caution should be used in interpreting colorimeter data obtained with dog hair. In the current study, darker pigmented skin and hair had a higher melanin index (107.4 ± 51) prior to clipping compared to after clipping (69.0 ± 28), suggesting that hair may overestimate melanin content measures. Colorimeter and laser technology both use light, and individual hairs of dogs have the ability to create scatter at the hair-skin surface which may affect the results seen in our study.

Our study revealed the highest overall differences in LBA (4%) between clipped and non-clipped tissues, with clipping resulting in less beam attenuation (greater penetration), similar to findings of other studies.[14,15] This is likely due to the removal of the physical barrier between the laser and the skin. Individual hairs contribute to photon absorption, reflection and scat-

tering at the site where the laser photons strike the treatment site. It is possible that dark-colored hair absorbs photons, while white hair may reflect or scatter light. Any material or external substances that interfere with laser energy delivery influence the amount of photons reaching the skin surface, decrease the overall penetration of the laser, and therefore, any biological effects of photobiomodulation. Because coat hair color, density, and inherent properties vary between different individuals and breeds, it would be very difficult to standardize irradiance regimes without removing the overlying hair. For this reason we recommend removing all hair prior to laser application to improve fluence distribution within the tissue. Our results are similar to other studies that support the importance of clipping hair to improve delivery of laser energy to tissues.[14,15] It may also be important to clip hair so that skin color and pigmentation may be evaluated when making treatment dose decisions.

There are several limitations of our study, including using a heterogeneous canine population with inherent differences in tissue thickness and hair characteristics, and the inability to determine the depths of tissues at which LBA occurred. This was a noninvasive study using live dogs that precluded photosensors being inserted at various tissue depths. Furthermore, it is highly likely that insertion of photosensors in live tissue would alter blood flow and affect results. Another limitation is that healthy dogs were recruited for the study and dogs with dermatological conditions, healing wounds, or incisions may have altered erythema indices more than were measured in this study. Greater erythema indices in these conditions may influence laser transmission, and therefore our results should not be extrapolated to these conditions. Finally, only a single wavelength combination was assessed; other wavelengths may have different penetration of photons in tissues. Nevertheless, this study should provide insight and information to design further prospective studies to measure laser penetration and the effects of photobiomodulation in clinical conditions.

Future studies are needed to determine the efficacy of photobiomodulation therapy for various clinical conditions, the minimum threshold of laser energy needed to create a photobiomodulatory effect, dose titration (including toxic effects), and the effects of laser power on specific clinical conditions. In addition, studies to assess the effect of wavelength on photobiomodulation, the relationship between wavelength and fluence levels for photobiomodulation, and whether or not a ceiling effect exists in which further increases in fluence do not increase the photobiomodulation effect or perhaps even cause toxic effects are needed. Based on our study, consideration should be given to clipping hair, tissue thickness, tissue type, and the amount of melanin in the skin when performing photobiomodulation therapy.

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