In vitro evaluation of the distribution of blood flow within a canine bipedicled latissimus dorsi muscle flap

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**Objective**—To identify the predominant perforating artery in the canine latissimus dorsi muscle and demonstrate that perfusion of the predominant perforating artery improves blood flow in segments of the latissimus dorsi muscle that are located distally from the thoracodorsal artery.

**Sample Population**—Latissimus dorsi muscles dissected from 7 dogs.

**Procedures**—Colored microspheres were used to determine the degree of perfusion of the latissimus dorsi muscle via the thoracodorsal artery, predominant perforating artery, or the thoracodorsal artery and predominant perforating artery together. The latissimus dorsi muscle was divided into 4 proximal to distal segments relative to the thoracodorsal artery (segments A, B, C, and D, respectively).

**Results**—The perforating artery, located at the level of the fifth intercostal space, predominantly supplied perfusion to segments B, C, and D. The number of microspheres received by segment C was significantly higher when the thoracodorsal artery and perforating artery were used for muscle perfusion (181.40 ± 44.90 microspheres/300 g of tissue for every 3,000 spheres injected), compared with use of the thoracodorsal artery alone (60.00 ± 13.70 microspheres/300 g of tissue for every 3,000 spheres injected).

**Conclusions and Clinical Relevance**—Blood flow via the predominant perforating artery improves perfusion to the middle part of the latissimus dorsi muscle in dogs. A bipedicled latissimus dorsi muscle flap would provide a healthier muscle for cardiac assist in the treatment of dilated cardiomyopathy in dogs. (Am J Vet Res 2003;64:1255–1259)

Cardiomyoplasty and aortomyoplasty are the 2 forms of long-term cardiac assist for patients with heart failure.1,2 Long-term results from cardiomyoplasty have been dismal, because the latissimus dorsi muscle that is wrapped around the heart degenerates.3-7 During long-term cardiomyoplasty, the skeletal muscle flap undergoes fiber atrophy, fat infiltration, and fibrosis.8-10 Ischemia is the most important factor that contributes to degeneration of the latissimus dorsi muscle during long-term cardiac assist.11,12 The latissimus dorsi muscle receives its blood supply from the thoracodorsal artery and other branches from the intercostal arteries (perforating arteries), which perforate the chest wall and enter the caudal portions of the latissimus dorsi muscle. Because this cardiac assist procedure requires dissection of the latissimus dorsi muscle on its neurovascular pedicle (thoracodorsal artery, vein, and nerve), perforating arteries are ligated during elevation of the skeletal muscle for cardiomyoplasty or aortomyoplasty. The procedure results in a reduction of blood flow by 70 to 90% within the muscle.13 Reduction of blood flow within the muscle follows a proximal to distal gradient, with the distal portion being the most severely affected.4

Blood flow to the latissimus dorsi muscle has been improved with in situ prestimulation, vascular delay, or both.5,14 However, both procedures require 2 surgical interventions, which delays the benefit of cardiac assist in patients with advanced heart failure. Improvement of the blood supply of the muscle flap could be accomplished in 1 procedure by creating a bipedicled muscle flap. It would require the creation of a vascular anastomosis between 1 of the arteries of the muscle flap and a peripheral artery. The purposes of the study reported here were to identify the predominant perforating artery in the canine latissimus dorsi muscle and demonstrate that perfusion of the predominant perforating artery improves blood flow in segments of the latissimus dorsi muscle that are located distally from the thoracodorsal artery.

**Materials and Methods**

**Animals**—Latissimus dorsi muscles were harvested from 7 research dogs that were euthanatized for reasons unrelated to this study. Euthanasia was performed with IV injection of pentobarbitral (88 mg/kg). Five minutes before euthanasia, each dog received heparin sodium (100 U/kg, IV). The latissimus dorsi muscles were harvested immediately after euthanasia.

**Instrumentation**—The thoracodorsal artery and predominant perforating artery of each muscle were dissected free from surrounding tissue. The other intercostal perforating arteries and veins were ligated. The thoracodorsal and perforating arteries were cannulated with a 20-gauge catheter. A suture of No. 1 silk was placed around the artery and the catheter to maintain the catheter in place.

Sterile saline (0.9% NaCl) solution was placed in a sterile 5 L glass aspirator bottle. Ten milliliters of Tween 80 was added to the saline solution. The glass aspirator bottle was closed with a stopper. The stopper was equipped with a connection to a pressure gauge and to an air cylinder. Pressure over the sterile saline solution in the glass aspirator bottle was maintained during the entire experiment to 100 mm Hg by adjusting the airflow from the air cylinder. The bottom

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glass hose connection was connected to the catheters in the thoracodorsal and perforating arteries. Both arteries were flushed with sterile saline solution for 10 minutes. Sutures were placed around any major arteries that were leaking serosanguineous fluid. After 10 minutes of perfusion, an efflux of clear fluid was observed from the thoracodorsal vein.

**Microvascular perfusion**—After 10 minutes of continuous flushing through both arteries, perfusion of the latissimus dorsi muscle was maintained only via the thoracodorsal artery (Fig 1). One million red microspheres (15-µm diameter) were injected into the thoracodorsal artery. The same operation was repeated on the perforating artery with 1 million blue microspheres (15-µm diameter). Lastly, 2 million yellow microspheres (15-µm diameter) were injected into the thoracodorsal and perforating arteries. The latissimus dorsi muscle was separated into 4 proximal to distal segments (A, B, C, and D, respectively; Fig 1). Three tissue specimens were harvested from each segment of the muscle. Muscle specimens were processed for microspheres retrieval.

The procedure used for processing tissue and recovery of microspheres was adapted from Kowallik et al. Tissue specimens were carefully dissected free from fat and weighed. Specimens were cut into portions of 0.3 to 1 g and placed into 15 mL screw-cap glass tubes. Seven milliliters of a 4M KOH solution containing 0.05% Tween 80 was added to each sample for digestion of the tissue. After digestion overnight, microspheres were retrieved by filtration through a filter (10-µm diameter pores) placed in an analytical vacuum filter holder on a filtering flask. The filter that contained the colored microspheres was placed in a 1.5 mL Eppendorf tube. Dye from the microspheres was extracted with 500 µL of dimethylformamide added to the Eppendorf tube. The Eppendorf tube was centrifuged at 2,000 X g for 5 minutes. The supernatant was transferred into a spectrophotometer glass cell for photometric absorption analysis. Samples were placed in a spectrophotometer, and absorbance was measured at 672, 594, 535, 448, and 370 nm. Recovery standards for each color were made with the dye extracted from 3,000 microspheres. Absorbance was measured at similar wave-lengths. The number of microspheres retrieved from each muscle specimen was calculated with a matrix-inversion software program. The number of spheres retrieved was normalized to an equivalent of 1,000 microspheres injected. For each muscle specimen, the number of spheres retrieved was divided by the weight of the specimen. The number of microspheres from the 3 specimens in each segment was added. Therefore, number of microspheres retrieved for each segment is reported as per 300 g of tissue for every 3,000 spheres injected.

**Statistical analysis**—Normal distribution of the number of spheres per 100 g of tissue was evaluated with a Kolmogorov Smirnov test. Data were not normally distributed. A Wilcoxon signed rank test was used to evaluate the effect of using the thoracodorsal artery or the thoracodorsal and perforating arteries on the normalized number of microspheres in the tissue specimen. The level of significance was set at value of P < 0.05.

**Results**

The perforating artery located at the level of the fifth intercostal space was consistently the predominant perforating artery identified. A long segment of the artery could be dissected in the intercostal space. The thoracodorsal artery supplied predominantly segments A and B of the latissimus dorsi muscle (Fig 2). The perforating artery supplied predominantly segments B, C, and D. The perforating and thoracodorsal arteries together supplied predominantly segments A

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**Figure 1**—Diagram of the experimental apparatus used in vitro to perfuse segments A, B, C, and D of the latissimus dorsi muscle via the thoracodorsal artery, fifth intercostal perforating artery, or both.
and B. Perfusion of segment C was increased 3 fold when the thoracodorsal and perforating arteries were used for muscle perfusion.

The mean number of microspheres retrieved in the muscle was not significantly ($P = 0.960$) different when the thoracodorsal artery was used for muscle perfusion (464.00 ± 130.00 microspheres/300 g of tissue for every 3,000 spheres injected) versus the thoracodorsal and perforating arteries (418.00 ± 94.00 microspheres/300 g of tissue for every 3,000 spheres injected). The number of microspheres received by segment A when the thoracodorsal artery was used for muscle perfusion (1,185.00 ± 342.00 microspheres/300 g of tissue for every 3,000 spheres injected) was significantly ($P = 0.043$) higher than when the thoracodorsal and perforating arteries were used (875.50 ± 236.60 microspheres/300 g of tissue for every 3,000 spheres injected). The number of microspheres received by segment B when the thoracodorsal artery was used for muscle perfusion (547.70 ± 207.00 microspheres/300 g of tissue for every 3,000 spheres injected) was not significantly ($P = 0.735$) higher than when the thoracodorsal and perforating arteries were used (555.50 ± 181.50 microspheres/300 g of tissue for every 3,000 spheres injected). The number of microspheres received by segment C when the thoracodorsal and perforating arteries were used for muscle perfusion (181.40 ± 44.90 microspheres/300 g of tissue for every 3,000 spheres injected) was significantly ($P = 0.028$) higher than when the thoracodorsal artery was used (60.00 ± 13.70 microspheres/300 g of tissue for every 3,000 spheres injected). The number of microspheres received by segment D when the thoracodorsal and perforating arteries were used for muscle perfusion (58.50 ± 14.80 for every 300 g of tissue for every 3,000 spheres injected) was not significantly ($P = 0.128$) different than when the thoracodorsal artery was used (58.50 ± 14.80 microspheres/300 g of tissue for every 3,000 spheres injected).

**Discussion**

The perforating artery from the fifth intercostal space is the most prominent artery entering the latissimus dorsi muscle in dogs. Windisch et al also identified a prominent perforating artery in the cranial dorsal part of the latissimus dorsi muscle, which seems similar to the perforating artery identified in our study.

In our study, the number of microspheres retrieved in the latissimus dorsi muscle flap after perfusion via the thoracodorsal artery followed a similar cranial to caudal gradient as has been found in several other in vivo experiments. This would indicate that the distribution of microspheres is similar in vitro and in vivo.

In our study, in vitro continuous flow perfusion of the latissimus dorsi muscle via the thoracodorsal and fifth intercostal perforating arteries resulted in a 3-fold increase in the delivery of microspheres to the middle to distal muscle segments. This finding compares favorably with results using other techniques to improve perfusion of the latissimus dorsi muscle. Vascular delay allows an improvement of 20 to 90% of the blood flow in the middle segment of the latissimus dorsi muscle. Maintaining perfusion of the fifth intercostal perforating artery has the potential to improve blood flow to the middle part of the latissimus dorsi muscle more efficiently than vascular delay.

Perfusion of the distal muscle segment did not improve in our study. It has been shown in in vitro studies that perforating arteries only perfuse a portion of the latissimus dorsi muscle. However, intramuscular anastomoses between the perforating arteries from intercostal arteries and the thoracodorsal artery develop in the latissimus dorsi muscle of sheep after 3 weeks of chronic electrical stimulation. Development of intramuscular anastomoses improves perfusion of the distal segment of the latissimus dorsi muscle. Similarly, intramuscular anastomoses are likely present in the canine latissimus dorsi muscle, but we could not demonstrate their existence in our acute in vitro study.

Perfusion of the proximal segment of the latissimus dorsi muscle was not improved when both the thoracodorsal and fifth intercostal perforating arteries were used in our study. Perfusion of the most proximal...
segment of the latissimus dorsi muscle was limited with the use of the perforating artery alone. It has been shown\textsuperscript{11,21} that the collateral circulation from the perforating artery does not contribute to the blood supply of the proximal part of the latissimus dorsi muscle. Blood flows coming from each artery to the proximal segment are in opposite directions, which might result in a negative effect on the total blood flow in the proximal segment. In our study, we attempted to reproduce the normal anatomy; however, we did not mimic the normal hemodynamic physiologic changes, because we did not have a pulsatile flow. We perfused the muscle with a pressure of 100 mm Hg, which is close to the mean arterial pressure in an awake dog. The mean arterial pressure is the driving pressure in tissue perfusion. It would have been interesting to repeat the experiment perfusing the perforating artery first and then the thoracodorsal artery to see whether the order of perfusion of the different arteries had an effect. Most likely it does not, because the perforating artery does not contribute substantially to the blood flow in segment A. The use of microspheres to evaluate blood flow to an organ is considered the gold standard technique.\textsuperscript{11,21} Repeated injections of microspheres of different colors have been used to evaluate blood flow in various organs with different ischemic conditions.\textsuperscript{20,25-29} We used a high number of microspheres per injection in our study to improve the accuracy of the technique in evaluating perfusion of the muscle flap.\textsuperscript{21} Microspheres might aggregate in capillaries with low flow and give a high value for blood flow.\textsuperscript{20,25-29} We pretreated the muscle with Tween 80 to prevent aggregation of microspheres.\textsuperscript{20,25-29} The reduction of blood flow in segment A might represent a negative effect from the creation of a bipedicled muscle flap. However, the proximal part of the latissimus dorsi muscle is not wrapped around the heart in vivo during cardiac assist; therefore, it does not contribute to the treatment.

Use of muscles from cadavers has several limitations. First, we could not evaluate the effect of muscle contraction on blood flow. Because it has been shown that muscle contraction has an effect on blood flow to the distal part of the muscle flap, it would be interesting to evaluate this effect with a bipedicled muscle flap.\textsuperscript{11,12} Another limitation is the lack of vascular tone and response to neuromediators that most likely play an important role in the distribution of blood flow within the latissimus dorsi muscle during electrical stimulation and cardiac assist.

On the basis of the findings in our acute in vitro study, perfusion of a perforating artery seems to improve the blood supply of the middle and distal segments of the latissimus dorsi muscle. Creation of a vascular anastomosis between the thoracodorsal and perforating arteries from the fifth intercostal space could be performed at the time of initial surgery.

\textsuperscript{2}Insite, Infusion Technology Systems Inc, Sandy, Utah.
\textsuperscript{3}Glass aspirator, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{4}Tweeze 80, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{5}Pressure gauge, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{6}Dye track Micropsheres, Triton Technology, San Diego, Calif.
\textsuperscript{7}Glass tubes, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{8}Potassium hydroxide, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{9}Filter membranes, Triton Technology, San Diego, Calif.
\textsuperscript{10}Millipore vacuum filter holder, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{11}Filtering flask, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{12}Eppendorf tube, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{13}Dimethylformamide, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{14}Ultimicro spectrophotometer glass cell, Fischer Scientific, Pittsburgh, Pa.
\textsuperscript{15}Spectrophotometer, Beckman 640, Beckman Coulter, Fullerton, Calif.
\textsuperscript{16}Matrix Inversion software, Triton Technology, San Diego, Calif.

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