THE EFFECT OF BASIC FIBROBLAST GROWTH FACTOR ON THE BLOOD FLOW AND MORPHOLOGIC FEATURES OF A LATISSIMUS DORSI CARDIOMYOPLASTY

Previous studies designed to determine whether latissimus cardiomyoplasty could be used to revascularize ischemic myocardium showed that after operation the latissimus was ischemic and had severely deteriorated. This study was undertaken to determine whether basic fibroblast growth factor, a potent angiogenic peptide, would improve the vascularity of the latissimus and enhance collateral formation between the muscle of the cardiomyoplasty and ischemic myocardium. In goats, myocardial ischemia was induced with an ameroid constrictor and cardiomyoplasty performed. The latissimus was continuously stimulated electrically at 2 Hz for 6 weeks and given four weekly bolus injections of human recombinant basic fibroblast growth factor (80 μg infused into the left subclavian artery). In eight animals, rates of regional blood flow were measured and both the heart and latissimus were evaluated histochemically. The latissimus blood flow rate was 0.114 ± 0.029 ml/gm per minute, which was three times greater than that of historical controls (chronically stimulated latissimus cardiomyoplasty without basic fibroblast growth factor treatment; 0.042 ± 0.007 ml/gm per minute, $p < 0.05$). Associated with the improved blood flow, there was significantly less evidence of skeletal muscle fiber dropout and muscle fibrosis in the animals treated with basic fibroblast growth factor. Latissimus-derived collateral flow to ischemic myocardium developed in five of the eight goats and averaged 0.288 ± 0.075 ml/gm per minute. This flow was 42.8% ± 15.7% ($n = 5$) of the flow required by normal myocardium (which was 0.728 ± 0.095 ml/gm per minute). This value for latissimus-derived collateral blood flow was almost twice that of the historical controls (24.0% ± 3.9%), but the increase did not achieve statistical significance ($p = 0.08$). These results hold the promise that basic fibroblast growth factor treatment might enhance the formation of extramyocardial collaterals to the heart and improve skeletal muscle function. (J THORAC CARDIOVASC SURG 1996;111:19-28)

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After latissimus dorsi cardiomyoplasty, new blood vessels were formed between skeletal muscle and the heart in an experimental model of myocardial ischemia.[1] Blood flow through these collateral vessels averaged 25% of the flow required by normal myocardium when the latissimus was stimulated either acutely or chronically.[2, 3] However, chronic stimulation also decreased the blood flow of the latissimus dorsi muscle itself.[4] We postulated that infusion of an angiogenic factor would improve the vascularity and perfusion of the latissimus and

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would further enhance collateral blood flow to the heart. If this hypothesis were supported, then cardiomyoplasty might be used to revascularize the myocardium of patients whose conditions are presently inoperable because of extensive distal coronary disease.

Basic fibroblast growth factor (bFGF) was selected for infusion in this study because of its potent angiogenic activity.[5, 6] bFGF is present in practically all tissues,[7, 8] and it may play a physiologic role in regulating vascularity. Endogenous bFGF expression appears to be increased in electrically stimulated skeletal muscle[9] and in ischemic myocardium.[10, 11] We have also identified bFGF in chronically stimulated muscles used in cardiomyoplasties.[12] Thus it appears plausible that exogenous infusion of bFGF might improve the vascularity of skeletal muscle and might enhance the formation of extracardiac collaterals to ischemic myocardium.

Methods

Experimental design. We modified our goat model of cardiomyoplasty and chronic myocardial ischemia[3] to administer bFGF directly into the arterial supply of the latissimus dorsi. Histochemical and morphometric methods were used to determine whether bFGF increased the vascularity of the latissimus. Microsphere injections were used to measure the effect of bFGF on latissimus blood flow and latissimus-derived collateral flow. The animals used in this study were cared for following the "Principles of Laboratory Animal Care" prepared by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science (NIH Publication No. 85-23, revised 1985).

Latissimus dorsi cardiomyoplasty. Under sterile conditions, a latissimus dorsi muscle was prepared for cardiomyoplasty in 28 goats, as previously described.[3] An intramuscular electrode was woven around the ramifications of the thoracodorsal nerve (Medtronic Inc., Minneapolis, Minn.) and connected to a pulse generator (Itrel model 7421, Medtronic Inc.).

Chronic myocardial ischemia. Chronic myocardial ischemia was induced by placing an ameroid constrictor around a branch of the circumflex coronary artery. Over the course of 2 to 3 weeks the ameroid constrictor absorbs water and slowly occludes the coronary artery.

The area at risk for ischemia was defined by injecting microspheres into the left atrium during a temporary occlusion of the branch of the circumflex artery. This injection also served to measure preexisting intracoronary collateral flow to the risk area.

bFGF administration. A 4F silicone rubber catheter was inserted into the subclavian artery, upstream of the origin of the thoracodorsal artery, through a left supraclavicular incision. The catheter was secured with a purse-string suture and connected to a vascular access port hub implanted subcutaneously in the neck.

Bolus injections of 80 μg of human recombinant bFGF (Synergen, Boulder, Colo.) were administered into the subclavian artery four times between postoperative days 10 and 35. On the basis of the results of in vitro studies,[13] of the in vivo kinetic study reported by Edelman, Nugent, and Karnovsky,[14] and of other animal studies,[15-18] 80 μg of bFGF by this route was likely to be an effective dose to promote angiogenesis in the latissimus. To maintain patency, the catheter was flushed with 10 ml heparinized saline solution (4 units heparin per milliliter).

Latissimus dorsi stimulation. Chronic, continuous electrical stimulation was begun on postoperative day 7. The pulse generator was initially set at 0.5 Hz and 3 volts. The frequency was gradually increased to 2 Hz over 10 days. The voltage was adjusted to maintain a palpable contraction.

Hemodynamic and blood flow measurements. After the 6-week period of electrical stimulation, the animals were anesthetized with sodium pentobarbital and the lungs ventilated artificially with 100% oxygen. Arterial blood pressure and the electrocardiogram were monitored and the time-tension index (time-tension index is equal to systolic pressure times heart rate) was calculated as an index of myocardial oxygen consumption. Amounts of regional blood flow were measured by injecting 15 million microspheres of one color into the left atrium.

Latissimus-derived collateral blood flow to the heart was measured by simultaneously injecting 7.5 million microspheres of another color into the subclavian artery. The microspheres were 15 μm in diameter and were too large to recirculate. Therefore they could only enter the myocardium through collaterals with the latissimus. Latissimus-derived collateral flow was calculated according to the formula previously developed.[3]

Histologic analysis and morphometry. The general histologic appearance of the latissimus was assessed from sections stained with hematoxylin and eosin (H & E). Vascularity of the latissimus was determined from capillary/fiber density ratios calculated from morphometric data. Masson's trichrome stain was used to identify areas of myocardial infarction.

General latissimus morphologic features. The right and left latissimus muscles were evaluated subjectively from H & E–stained sections. Biopsy specimens were taken from the proximal, mid, and distal (attached to heart) latissimus dorsi and placed in formalin. The samples were embedded in Paraplast medium and 7 μm sections were cut. Each slide was reviewed at ×100 and ×200 magnification. The following qualitative observations were made: (1) general morphologic features, rated as excellent, good, or poor, (2) presence or absence of cellular infiltrate, and (3) fiber dropout or fatty changes. The percentage of muscle fibers replaced by fat was estimated and categorized as none, 1% to 25%, 26% to 50%, or greater than 50%. Fatty areas were unstained and had a size similar to that of the muscle fiber they replaced.

Quantitative assessment of the latissimus. In four animals, a quantitative evaluation of the latissimus was done by measuring capillary density, amount of extracellular collagen, fiber size, and the capillary/fiber ratio. Muscle biopsy specimens were taken from the mid latissimus of all

four animals. In two animals, biopsy specimens were also taken from the proximal and distal latissimus.

The biopsy specimens were placed in Carnoy's solution overnight, dehydrated through graded ethanols, cleared in xylenes, and embedded in Paraplast medium. Ten serial sections 5 μm in thickness were cut from each block, mounted on glass slides, deparaffinized, and rehydrated.

Sections were stained with (1) Griffonia simplicifolia agglutinin–B_4 (GSA-B_4) lectin[19, 20] (capillary density), (2) silver stain (extracellular collagen),[21] and (3) H & E (fiber size and density). Capillary/fiber ratio was calculated from the capillary and fiber densities. For each measurement, 10 random fields for each muscle slide were analyzed with computer-assisted techniques.

GSA-B_4 (Sigma Chemical Co., St. Louis, Mo.) conjugated to horseradish peroxidase was diluted 1:50 in phosphate-buffered saline (PBS) solution and incubated on the tissue sections for 2 hours in a humidified chamber at 37° C. The slides were thoroughly rinsed in PBS solution. Sites of bound lectin were visualized by incubation in a 3′,3′-diaminobenzidine–hydrogen peroxide substrate medium followed by two additional rinses in PBS.[22] For a negative control, sections were incubated with nonconjugated lectin.

The stained sections were mounted on an inverted microscope (IM-35, Ziess, Munich, Germany) and imaged at a final magnification of ×400. The image was input through a high-resolution video camera (model 68, Dage, Michigan City, Ind.) connected to a computer image analysis system (IBAS 2000, Ziess/Kontron, Munich, Germany). For each section, 10 random fields with an area 25,390 μm^2 per field were analyzed.

By computer-aided stereology, the number of capillaries per unit area (numeric density) was computed.[19, 22, 23] A computer-generated test frame automatically discriminated the capillary profiles and determined the number of profiles within the test area with the use of exclusion-edge principles.[24] Capillary diameter was calculated by measuring the area of a stained vessel, transforming this area into a circle, and computing the diameter. This method for determining capillary diameter was used to avoid computation errors for vessels in a transverse orientation.

H & E–stained sections were viewed under epifluorescent illumination to determine fiber size. The silver-stained sections were used to determine the amount of extracellular connective tissue. Computer-assisted morphometry was used for both determinations.

Myocardial infarction. After the animals were killed, the heart was removed. The ventricles and attached latissimus muscle were cut into four concentric rings. Full-thickness sections were embedded in paraffin and stained with Masson's trichrome stain. Sections were mounted on a Reichert Diastar microscope (Leica, Inc., Deerfield, Ill.) and imaged at ×25 magnification. The image was input through an RGB/YC/NTSC video camera (Worldwide Video, Inc., Boyertown, Pa.) connected to Bioquant System IV True Color Image Analysis system (R & M Biometrics, Nashville, Tenn.). Color thresholds were set manually to identify the portions of the section that were stained blue (for collagen) or red (for viable tissue). The system measured each area independently and calculated the percent of infarct in each section.

Statistical analysis. Comparisons were made with paired or unpaired *t* tests as appropriate. A Bonferroni correction was made for multiple comparisons.

Results

Technically satisfactory results were obtained in eight animals and are presented here. There were five perioperative deaths caused by technical difficulties at the first operation. Ten animals died of sudden death between day 4 and day 37, presumably because of ischemic myocardium unprotected by collateral formation. In two animals, the subclavian artery injection catheter failed and the animals did not receive bFGF. Ventricular fibrillation occurred in three animals after the induction of anesthesia for the final study, but before any hemodynamic or blood flow data could be obtained. The high mortality in the goat relative to that in the dog may be because of the almost complete lack of preexisting collaterals in the goat. All data are presented as mean plus or minus the standard error.

Physiologic data

Hemodynamics. Arterial blood pressure and heart rate were measured in all eight animals at the time that blood flow was measured. During the temporary occlusion of the coronary artery at the time of the first operation (referred to as acute occlusion), blood pressure was 79.8 ± 4.7 mm Hg and was significantly lower than the 102.6 ± 5.0 mm Hg ($p < 0.05$) observed during the measurements made after the coronary artery was occluded (referred to as chronic occlusion). The lower pressure during the acute occlusion is attributable to the vasodilator effects of isoflurane anesthesia. During the final study, the animals were anesthetized with sodium pentobarbital.

Heart rates during the acute and chronic occlusions were 88.8 ± 5.2 and 88.3 ± 6.5 beats/min, respectively. The time-tension index did not differ significantly during the acute and chronic occlusions: 9221 ± 847 and 10,528 ± 841 mm Hg · beats · min^{-1}, respectively.

Latissimus dorsi blood flows. Rates of blood flow to the latissimus dorsi muscles are presented in Table I. The left latissimus muscle blood flow in this study with bFGF treatment was approximately three times higher than that reported by Bailey and associates[3] for chronically stimulated latissimus not given bFGF ($p < 0.05$, unpaired *t* test).

The rates of blood flow to the unstimulated right latissimus dorsi muscles were not significantly different during the acute and chronic coronary artery

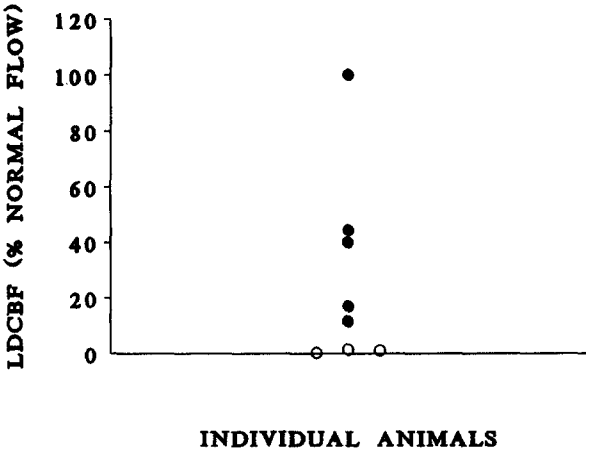

Fig. 1. Latissimus-derived collateral blood flow *(LDCBF)* to chronically ischemic myocardium expressed as percentage of flow delivered to normal myocardium. *Closed circles* represent animals that had development of significant collateral flow between latissimus and heart. *Open circles* represent animals that did not have such development.

Table I. *Latissimus dorsi muscle blood flow*

	Acute occlusion	*Chronic occlusion*
Left latissimus flow (ml/gm/min)		
Mean	0.0845*	0.1140†
SE	0.0292	0.0290
n	6	7
Right latissimus flow (ml/gm/min)		
Mean	0.0350	0.0290
SE	0.0174	0.0110
n	6	7

SE, Standard error.

* Significantly different from right latissimus during acute occlusion ($p < 0.05$). This elevation is an artifact secondary to mobilization of the muscle.

† During the chronic occlusion measurement, after 6 weeks of chronic 2 Hz stimulation, the rate of left latissimus flow was significantly different from that of right latissimus (unstimulated).

occlusions. During the chronic occlusion, the left latissimus was stimulated at 2 Hz and its flow was significantly higher than that of the unstimulated right latissimus.

Regional myocardial blood flow. Regional myocardial blood flow rates for the normal, chronic ischemic, and infarcted myocardium are presented in Table II. Blood flow rates measured in the chronic ischemic zone during acute occlusion of the coronary artery were low and indicated that there were few native collaterals.[25] After chronic occlusion, ischemic zone blood flow was about 75% of that in the normal myocardium, which indicated that collaterals, both intramyocardial and latissimus derived, had formed.

Table II. *Regional myocardial blood flow*

	Acute occlusion	*Chronic occlusion*
Normal myocardial blood flow (ml/gm/min)		
Mean	0.6676	1.0446
SE	0.1623	0.1648
n	6	8
Chronic ischemic myocardial blood flow (ml/gm/min)		
Mean	0.1281	0.7660
SE	0.0620	0.1704
n	6	8
Infarct blood flow (ml/gm/min)		
Mean	0.1228	0.1876
SE	0.0632	0.2235
n	3	5

SE, Standard error.

Blood flow to the infarcted area was as low in the final study as it was during the acute occlusion. Only five goats had infarcts (defined as regional myocardial flow less than 30% of normal zone flow at the seventh postoperative week).

Latissimus-derived collateral blood flow. The latissimus-derived collateral blood flow to ischemic myocardium was expressed as a percentage of blood flow received by normal myocardium because of variability of the reference withdrawal method. Individual data points for latissimus-derived collateral blood flow are presented in Fig. 1. Five of the eight animals *(closed circles)* showed development of collaterals between the latissimus and heart that contributed to coronary perfusion (flow greater than 10% of normal myocardial flow). Only the data from the animals with collaterals will be considered, to facilitate comparison with previously published data.[3]

The latissimus-derived collateral blood flow averaged 42.8% ± 15.7% of the flow delivered to the normal myocardium. In one animal, the latissimus delivered blood flow to the chronically ischemic myocardium that was equal to 100% of the normal myocardial flow. It is not known what factors caused some animals to have the development of such extensive collateral flow from the skeletal muscle graft, whereas in others extracardiac collaterals failed to develop. The total amount of flow delivered to the heart by the latissimus graft averaged 5.2 ±

Table III. *Comparison of the contribution of latissimus-derived collaterals with that of intracoronary collaterals*

	Stimulation alone	*Stimulation with bFGF*	
Blood flow (ml/gm/min)			
Normal myocardial flow	0.78	0.73	$p = 0.80$*
SE	0.13	0.10	
Ischemic myocardial flow	0.61	0.50	$p = 0.28$*
SE	0.09	0.03	
	$p = 0.07$†	$p = 0.09$†	
Percent of flow to ischemic myocardium by source of collateral flow			
Preexisting collaterals	33.2	12.3	$p = 0.03$*
SE	5.4	5.3	
New intracoronary collaterals	34.8	28.7	$p = 0.74$*
SE	10.4	14.5	
Latissimus-derived collaterals	32.0	59.0	$p = 0.08$*
SE	6.4	16.1	
n	10	5	

SE, Standard error.

* Independent *t* test comparing stimulation with and without bFGF.

† Paired *t* test comparing ischemic and normal myocardial flow.

1.5 ml/min ($n = 5$). Virtually all of this flow was delivered to the viable portion of the risk area.

Comparison of latissimus-derived and intracoronary collateral flow. The blood flow to ischemic myocardium was partitioned into the contributions from preexisting collaterals, newly developed intracoronary collaterals, and latissimus-derived collaterals, as described in the appendix. Table III compares the rates of flow during chronic occlusion from each source with those from our previous study in which bFGF was not infused.[3]

The means of the flows to the ischemic area tended to be lower than those of the normal zones, but were not significantly lower. The flow from preexisting collaterals was significantly lower in the bFGF-treated animals. We deduced from the lower "preexisting" collateral flow that the Ameroid constrictor must have been somewhat more proximal and hence must have more completely arrested flow to the risk area. The flow from intracoronary collaterals was about the same in both groups, which suggests that bFGF administered to the latissimus did not increase the development of intracoronary collaterals. The flow from the latissimus-derived collaterals in the bFGF-treated group was almost twice that in the group without bFGF and approached statistical significance. These data suggest that bFGF administration into the latissimus dorsi can stimulate the formation of extracardiac collaterals.

Morphologic data

Morphologic features of the right and left latissimus dorsi. The right latissimus dorsi was not mobilized or stimulated. It served as a control for the chronically stimulated left latissimus. The right latissimus dorsi was found to be of excellent quality, with no cellular infiltrate. There was no fiber dropout. Each specimen demonstrated a minimal amount of perimysial connective tissue.

Overall, similar muscle quality was observed in five of the eight left latissimus muscles and was rated as "excellent." Muscle quality was rated as "good" in the three other animals. Fiber dropout was less than 1% in one animal and less than 25% in the remaining seven animals. Cellular infiltrate was minimal in seven of eight animals and moderate in the remaining animal. There was no evidence of subintimal hyperplasia.

Quantitative assessment of the left and right latissimus muscles. Fig. 2 shows representative photomicrographs of serial sections of chronically stimulated left latissimus muscle treated with bFGF and unstimulated right latissimus. On the basis of the silver impregnation stain findings (*top panels*), there was no change in extracellular staining of the left latissimus (Fig. 2, *A*) when compared with unstimulated control right latissimus (Fig. 2, *B*). However, the muscle fiber size appeared to be reduced in the left latissimus. Capillary density appeared to be increased in the chronically stimulated left latissimus

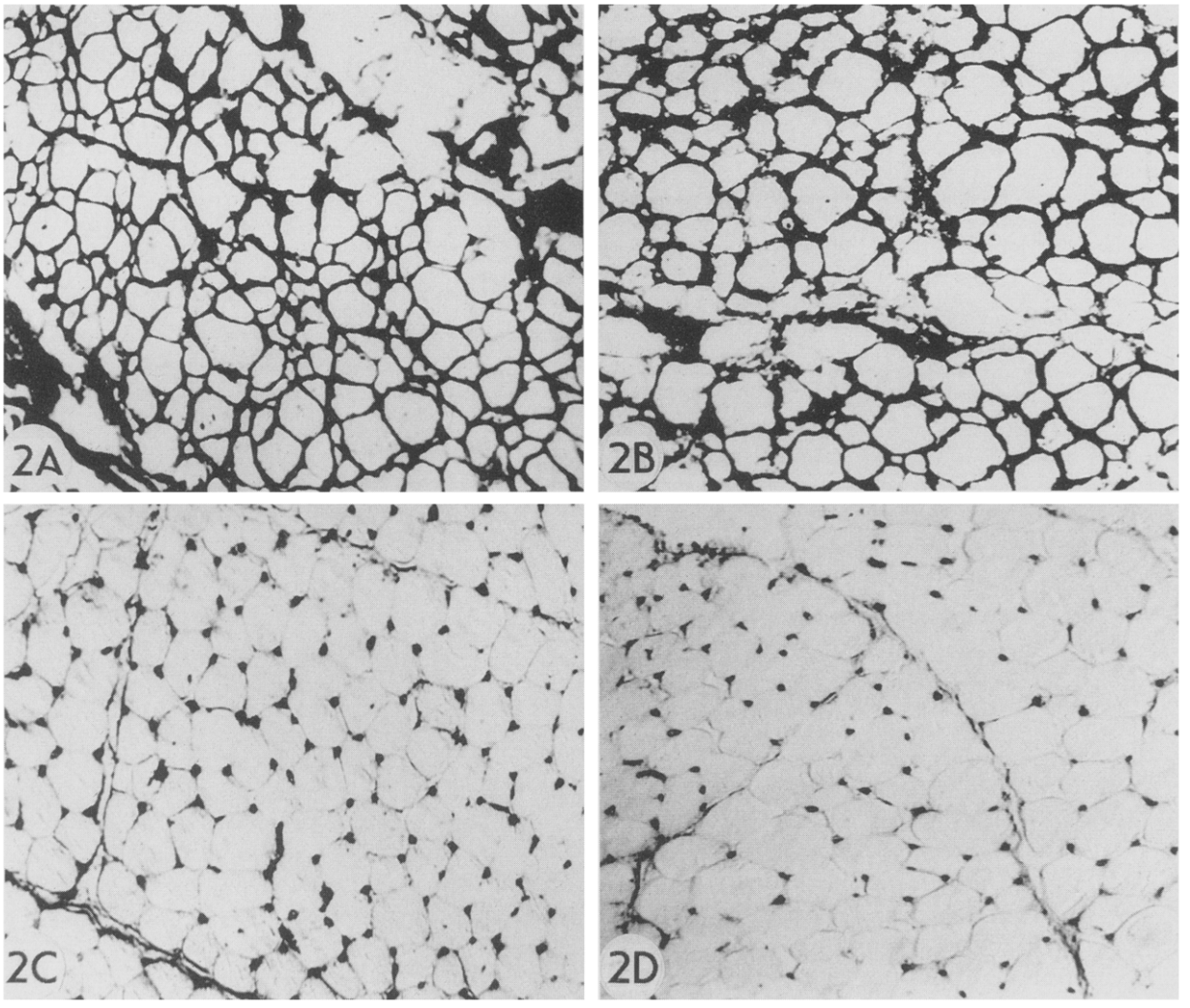

Fig. 2. Representative photomicrographs of sections taken from chronically stimulated left and unstimulated control right latissimus dorsi muscles (*left* and *right panels*, respectively). *Top panels* are muscle sections stained with use of silver-impregnation technique to quantitate extracellular matrix. *Lower panels* demonstrate muscle sections after lectin staining for capillaries.

(Fig. 2, *C*), when compared with that in the control muscle (Fig. 2, *D*).

The data for fiber size, amount of extracellular collagen, capillary density, and capillary/fiber ratio are presented in Table IV. There were no statistically significant differences in any of these values between the right and left latissimus. The lack of any significant differences is in agreement with our subjective conclusion that bFGF treatment protects against the deterioration usually seen in chronically stimulated muscles used in cardiomyoplasty.

For two animals, regional differences in the capillary/fiber ratio were examined. For the right (unstimulated) latissimus, the ratio was the same for the proximal, mid, and distal regions. However, the ratio for the proximal left (stimulated) latissimus was three times greater than that for the right latissimus and greater than that for the mid and distal regions of the left latissimus. The increase in the ratio was primarily as a result of higher capillary densities. Similar results were found in both animals.

Myocardial infarct. The total left ventricular weight was 73.27 ± 5.71 gm (n = 8). The risk area (chronically ischemic myocardium plus infarcted myocardium) was 25.8% ± 3.6% of the left ventri-

cle. Approximately one fourth of the myocardial risk area (28.4% ± 3.8%) was infarcted. All eight animals showed histologic evidence of infarct. Most of the infarct was in the subendocardial region.

Discussion

There are three principal findings of this study. First, bFGF treatment significantly improved the perfusion of the chronically stimulated latissimus muscle after cardiomyoplasty. Second, associated with the improved perfusion was an improved morphologic appearance of the latissimus when compared with that in previous reports.[3, 26-28] Third, there was a trend to an increased latissimus-derived collateral blood flow to chronically ischemic myocardium.

Vascularity of the latissimus. We postulated that bFGF would increase the vascularity, and hence the perfusion, of the latissimus. In this study, we compared the capillary density and capillary/fiber ratio of stimulated latissimus with those values in the right-sided control muscles in animals treated with bFGF. The capillary density and capillary/fiber ratio were elevated only in the proximal left (stimulated) latissimus in comparison with values in the unstimulated right latissimus. This effect has been reported by many others (see Hudlicka, Brown, and Egginton[29] for a review). Associated with the increased vascularity of the latissimus was an increase in its blood flow.

The failure of the mid and distal latissimus to show increased capillary/fiber ratios may be related to the fact that the circulation was compromised by severance of the perforating intercostal arteries during mobilization. Hudlicka and Price[30] have reported that the capillary/fiber ratio was not increased by chronically stimulating in situ skeletal muscles with compromised blood supplies. Nonetheless, the latissimus blood flow values in the mid and distal regions were elevated compared with those in the right latissimus and in historical controls. Therefore it appears that bFGF treatment maintained a level of vascularity that was sufficient to support a threefold increase in blood flow during chronic stimulation compared with that seen in response to chronic stimulation in muscles used in cardiomyoplasties without bFGF treatment.

Latissimus dorsi blood flows after mobilization. Immediately after mobilization of the left latissimus (acute occlusion measurement), the rate of left latissimus blood flow was significantly higher than that of the right ($p < 0.05$). This finding would appear to be inconsistent with the fact that the perforating intercostal arteries and other distal collaterals were severed. However, we have repeatedly observed elevated blood flow values in the muscle flaps immediately after mobilization, as have others.[1-4, 31, 32] This increase may be the result of an inflammatory response caused by the mobilization procedure, interruption of sympathetic nerves, or both of these events. Although we have no data that bear on the mechanism, it is clear that it was sufficient to overcome any deficit caused by compromising the vascular supply.

Table IV. *Summary of morphometric analysis*

	LLD	*RLD*
Fiber size (cross-sectional area,* μm^2)		
Mean	1365	1647
SE	121	115
Collagen content (percent of field)		
Mean	18.4	16.4
SE	1.7	1.0
n	4	4
Capillary density (no. of capillaries per mm^2)		
Mean	1222	1042
SE	114	247
n	4	4
Capillary/fiber ratio†		
Mean	2.59	2.26
SE	0.45	0.39
n	4	4

LLD, Chronically stimulated left latissimus dorsi (midregion); *RLD*, unstimulated right latissimus dorsi (midregion); *SE*, standard error.

*Calculated using an elliptical model from the major and minor axes of the individual fibers.

†Calculated as capillary density/fiber density. Fiber density calculated as the quotient of area not occupied by collagen or capillaries divided by fiber size.

Muscle morphologic features and correlation with muscle function. In the present study there was minimal fiber dropout, no significant increase in extracellular connective tissue, and no subintimal hyperplasia compared with the unstimulated right latissimus. These findings are in contrast to the evidence of latissimus degeneration seen in our historical controls[3, 4] and reported by Lucas and colleagues[33] and Kratz and colleagues.[27] These morphologic changes were correlated with impaired muscle function.[27] Because bFGF administration appears to have preserved the architecture of the muscle, it can be postulated that bFGF-treated muscles would have better function than muscles not treated with bFGF used in cardiomyoplasties.

Our finding of minimal subintimal hyperplasia is also in contrast to the reports of others. Severe intimal hyperplasia and proliferation of smooth

muscle cells in small arteries have been associated with both chronic stimulation[33] and infarcted myocardium treated with acidic fibroblast growth factor.[26] Reduced hyperplasia would contribute to better perfusion and muscle function.

Latissimus-derived collateral blood flow. bFGF augmented the amount of latissimus blood flow, but did not augment the amount of extramyocardial collateral blood flow. We postulated that bFGF infusion would augment collateral flow by increasing the vascularity and blood flow of the latissimus muscle and reducing infarct size.[4] However, the almost doubling of the contribution of the latissimus dorsi to perfusion of ischemic myocardium compared with results in historical controls[3] was not statistically significant.

Potential reasons bFGF would increase angiogenesis within the muscle, but not in the heart, may be related to the route, dosage, and schedule of bFGF administration. The dosage of bFGF to the latissimus appeared to be adequate. With an 80 μg bolus of bFGF, assuming a subclavian artery blood flow rate of 200 ml/min and a thoracodorsal artery blood flow rate of 8.5 ml/min, approximately 3.6 μg of bFGF was infused initially into the latissimus dorsi muscle. Baffour and associates[15] injected 1 to 3 μg bFGF per day directly into an ischemic hindlimb skeletal muscle and reported increased angiogenesis.

However, the dose received by the heart might have been too low. In our study, less than 3.6 μg of bFGF would have been received by the myocardium after recirculation and dilution by the cardiac output. This dose was smaller than that used by other investigators who demonstrated some beneficial effects of bFGF administration to the myocardium.[16-18] It is possible that an intracoronary route would have more vigorously stimulated the development of collaterals from the latissimus to the heart.

Because bFGF was given in periodic bolus injections, it is possible that its delivery might not have coincided with the most optimal time in the course of the development of myocardial ischemia. After administration, bFGF is bound to storage sites on the extracellular matrix, and it appears to be metabolized within 24 hours.[13] In contrast, the successful stimulation of angiogenesis within the latissimus could be attributed to the fact that it was continuously stimulated. Hence, bFGF would have been delivered to the latissimus during periods of increased oxygen demand that could not be met by its compromised vascular supply.

Possible mechanisms for the effect of bFGF on the latissimus. bFGF is a multifunctional peptide and it could improve muscle morphologic features through several different mechanisms. First, the neurotrophic effects[34] of bFGF might mitigate neurogenic damage induced by continuous electrical stimulation. Second, bFGF stimulates proliferation of myoblasts and may have stimulated regeneration of the injured skeletal muscle.[35-37] Thus bFGF might not necessarily prevent ischemic muscle damage, but rather might promote recovery from it. Third, bFGF is a potent angiogenic peptide,[5, 6] and it might accelerate capillary growth and improve muscle blood flow, before ischemic damage could occur.

bFGF is a member of a family of peptide growth factors that have been demonstrated to modulate angiogenesis during growth and development,[38, 39] but its only normal, physiologic role in adults appears to be during pregnancy, follicle development, and ovulation.[5] However, growth factors have been identified as having a prominent role in response to injury, ischemia, diabetes, and tumor growth.[5, 39] Recently, infusion of bFGF and of other angiogenic agents has been found to increase revascularization of ischemic skeletal muscle[15, 40, 41] and myocardium.[16-18, 42]

At present, the most likely mechanism for the effect of bFGF on a latissimus muscle used for cardiomyoplasty appears to be the acceleration of angiogenesis. There does not appear to be uniform histologic evidence of extensive fiber destruction and regeneration. However, conclusive evidence for prevention of muscle damage by enhancement of skeletal muscle blood flow requires further study.

Clinical implications. bFGF administration resulted in an improved perfusion of the latissimus. This finding is significant because chronic ischemic injury of the latissimus has been recognized as contributing to a lack of efficacy in both clinical[43-46] and experimental[27, 33, 47-50] cardiomyoplasties.

Some work has been done to overcome the problem of ischemia in the latissimus. A delay period between mobilization and the onset of electrical stimulation was shown to minimize ischemic damage,[49] but the complete prevention of damage was not demonstrated. The optimal length of the delay period and the optimal initial stimulation patterns have not been precisely defined. Stimulation of a mobilized muscle after clinical and experimental cardiomyoplasties still leads to ischemic muscle damage.

Study limitations. The main limitation of this study is that bFGF-treated muscles used in cardiomyoplasties were compared with historical control muscles not treated with bFGF. Further investigation will be required to confirm that bFGF is helpful in preventing skeletal muscle ischemia after a cardiomyoplasty.

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Appendix

In animals with a completely obstructed coronary artery, the total flow to the ischemic myocardium is delivered by preexisting intracoronary collaterals, new intracoronary collaterals, and the newly formed latissimus-derived collaterals. Preexisting collateral flow as a percentage of normal myocardial flow was determined by microsphere injection during the acute occlusion. At the final study, the same percentage of total flow is delivered to the ischemic myocardium. Therefore subtraction of the preexisting collateral flow and the latissimus-derived collateral flow from the total flow yields the flow via newly formed intracoronary collaterals.

Intracoronary collateral flow

= Total ischemic flow − Latissimus-derived collateral flow − Preexisting collateral flow

where preexisting collateral flow equals total ischemic flow × (acute occlusion ischemic zone flow)/(acute occlusion normal myocardial flow).