# Effects of Transmyocardial Laser Revascularization by Using a Prototype Pulsed $CO_2$ Laser on Contractility and Perfusion of Chronically Ischemic Myocardium in a Porcine Model

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**The purpose of this study was to test a new prototype pulsed $CO_2$ laser to be used for transmyocardial laser revascularization (TMR). We wanted to determine whether it can reduce thermal damage and mitigate induced ischemia with improvement in contractile reserve of the heart as evidenced by contrast echocardiography at rest and under dobutamine stress. TMR is an emerging surgical strategy for treatment of myocardial ischemia not amenable to conventional percutaneous or surgical revascularization. Eleven pigs underwent ameroid occluder placement at the origin of the circumflex coronary artery. Six weeks later, occlusion of the circumflex coronary artery was documented. TMR was then carried out on 10 pigs by using a prototype pulsed $CO_2$ laser that delivered 8–12 joules in 1.5 ms with a spot size of 1 mm. Six weeks after TMR, the pigs were restudied. The animals developed significant ischemia after 6 weeks of ameroid occlusion, at rest ($p = 0.01$) and at peak stress ($p = 0.004$). Wall motion for the ischemic segments improved significantly 6 weeks after TMR at peak stress ($p = 0.02$). TMR results in an improvement in wall motion in our model of chronic ischemia and improves wall motion score index more during induced stress than at rest. *ASAIO Journal* 2000; 46:786–791.**

Transmyocardial laser revascularization (TMR) is an emerging therapeutic strategy designed to enhance myocardial perfusion by using a high energy laser beam to create channels from the epicardium to endocardium in the hope that oxygenated left ventricular blood will directly perfuse the ischemic myocardium. This technique is based on the theory of the reptilian model of circulation where a significant amount of direct transmyocardial perfusion is present in alligator hearts.[1]

Blood in the reptilian heart is delivered to the myocardium through an extensive vascular network composed of intramyocardial sinusoids that directly connect to the ventricular muscle.[2] Although an analogous network of myocardial sinusoids exists in humans, it perfuses only the inner myocardium.

Myocardial needle puncture was performed in 1950 by Sen *et al.* in an attempt to create transmural channels to deliver oxygenated left ventricular blood directly, into the myocardial sinusoids.[3,4] However, there was premature channel closure due to fibrous tissue in-growth.[5] Mirhoseini *et al.* in 1982 proposed the use of a $CO_2$ laser to create transmural channels to minimize local tissue destruction and fibrosis, and thereby improve channel patency.[6–8]

Use of TMR has subsequently been demonstrated in some clinical studies[9,10] to improve the functional class of angina pectoris and relative endocardial perfusion in patients with ischemic heart disease. There is a disparity between clinical responses and objective end-points, especially the lack of consistent improvement in myocardial perfusion studies in patients with subjective clinical improvement.

The purpose of this ongoing study is to determine whether transmyocardial laser revascularization (TMR) can lessen induced ischemia and improve contractile reserve as evidenced by contrast echocardiography at rest and under dobutamine stress.

## Materials and Methods

### *Acute Studies*

Initially, five animals were used to determine the optimal pulse width and laser energy necessary to get full thickness penetration of the porcine myocardium. The optimal laser settings were two rapid fire impulses of 750 $\mu$s each, with a total pulse width of 1.5 ms, and the laser energy set between 8 and 12 joules to gain full penetration of the left ventricular myocardium determined by intraoperative transesophageal echocardiography. This translates to a peak power ranging from 5,000 to 8,000 watts. The acute experiments were also used to demonstrate the safety of the laser impulse being fired on during all phases of the ECG cycle.

### *Chronic Studies*

Eleven juvenile domestic pigs underwent ameroid occluder placement at the origin of the circumflex coronary artery after a baseline selective left coronary angiogram and an epicardial contrast echocardiogram. Six weeks later, we documented complete occlusion of the circumflex coronary artery with a left coronary angiogram (**Figure 1**), and quantified the wall motion score index (WMSI) and perfusion status of the affected segments of myocardium by using contrast enhanced epicardial echocardiography at rest and with dobutamine stress. TMR was done through a left anterior thoracotomy on 10 of the

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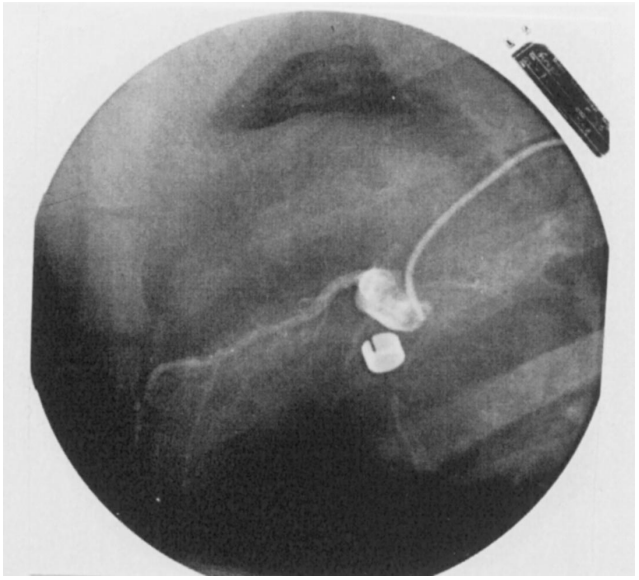

**Figure 1.** Selective left coronary angiogram done 6 weeks after occluder placement, showing complete occlusion of the origin of the circumflex by the ameroid occluder.

animals by using a prototype pulsed $CO_2$ laser by using 8–12 joules energy, with a pulse width of 1.5 ms and a spot size of 1 mm. Full thickness penetration of the myocardium was confirmed by using intraoperative transesophageal echocardiography that showed intracavity microbubble formation. The mean number of confirmed laser channels drilled per animal was 25 ± 5 holes made in the ischemic segments approximately 8–10 mm apart. Two of the animals were studied 2 weeks after TMR, after left coronary angiography and a resting/stress epicardial contrast echocardiogram. Eight pigs were studied 6 weeks after TMR. The pigs were restudied by using a selective left coronary angiogram (**Figure 2**) and resting/stress epicardial echocardiography was done with and without contrast to elucidate WMSI and perfusion status. One animal was treated as a control without laser treatment.

*Histopathology*

All the hearts after acute injury, 2 weeks after TMR, or 6 weeks after TMR were sent for histopathology. Measurements of channel size were performed on 31 acute channels with three sections through each channel, for a total of 93 measurements. They were assessed for the thermal zone of damage by using birefringence microscopy. In this technique, the normal myocardial tissue is birefringent and, therefore, looks bright through crossed polarizers, whereas the thermally denatured myocardial tissue loses its birefringence and, therefore, looks dark. This method allows ready identification of the thermally damaged zones. Measurements were made radially from the edge of the channel to the outer edge of the thermal zone.

Hearts were studied for progression of the healing process at 2 weeks and 6 weeks after TMR. Histopathology was done on areas containing the suspected channel regions in groups. Within each group the myocardium was sliced at 2 to 3 mm intervals in a plane parallel to the epicardial and endocardial surfaces. Level 1 was closest to the epicardium and level 4 was closest to the endocardium, with levels 2 and 3 in the intervening space.

*Left Coronary Angiography*

Selective left coronary angiography was performed at three instances for each animal by means of the femoral artery by using a Judkin's catheter. The initial baseline, before insertion of the ameroid occluder, demonstrated a normal left circumflex system. Six weeks after occluder placement, angiography was done, with TMR done after angiography in this session. Six weeks after TMR, angiography was redone.

*Epicardial Echocardiography*

Epicardial echocardiography was performed through a left anterior thoracotomy at baseline or preoccluder phase, and repeated at the induced ischemia stage or before TMR phase 6 weeks later and lastly, 6 weeks later during the post-TMR phase. An apical two chamber view documented regional wall motion in segments 13, 14, 15, 16, 17, and 18. Segments 17 and 18 were affected by the occlusion of the left main circumflex coronary artery. Regional wall motion was scored as follows: (1) normal wall motion and systolic thickening (endocardial excursion of >5 mm and systolic thickening of 25%), (2) indicates hypokinesia (systolic thickening <5 mm), (3) indicates akinesia (absence of systolic wall thickening), and (4) indicates dyskinesia (systolic wall thinning and outward motion). The wall motion score index (WMSI) was calculated at rest and at peak stress induced with dobutamine infusion.

*Myocardial Contrast Echocardiography*

The ischemic myocardium at risk was analyzed by using contrast enhanced two-dimensional echocardiography before

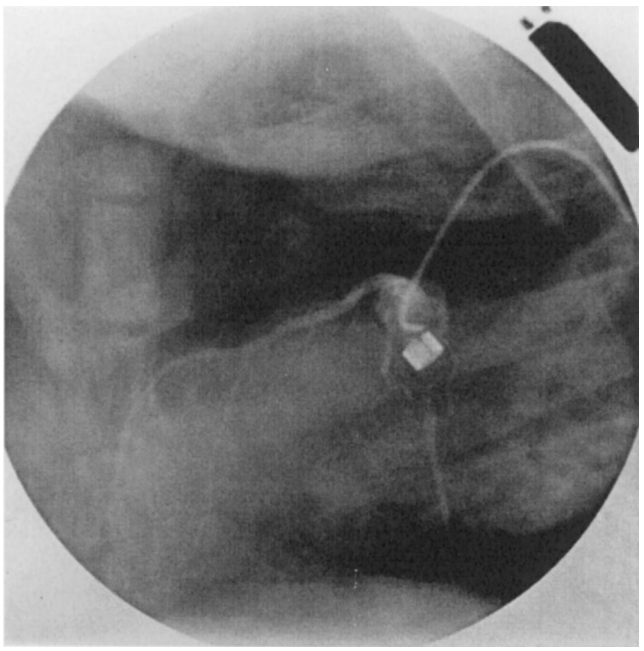

**Figure 2.** Selective left coronary angiogram done 6 weeks after transmyocardial laser revascularization (TMR), showing complete occlusion of the circumflex, with retrograde filling of the distal circumflex from collaterals.

occluder placement, before TMR, and at 6 weeks after TMR to assess myocardial perfusion abnormalities. Measurements were made at rest and at peak stress.

A lipid-fluorocarbon echo contrast agent (Aerosomes MRX 115 Ima Rx Pharmaceutical Corp., Tucson, AZ) was injected intravenously at a dose of 0.01 ml/kg. Aerosomes MRX 115 are perfluropropane gas microbubbles stabilized within a lipid bilayer. Their mean diameter is 2.5 $\mu$m, and more than 99% of the bubbles are $<10$ $\mu$m in diameter. Left ventricular long axis images were obtained with an ultrasound system and a 5 MHz transducer SSH 140 phased array (Toshiba, Tokyo, Japan). After the echocardiographic images were optimized, gain and gray scale settings were kept constant throughout the experiment. The echocardiographic images were recorded on super VHS videotape for later analysis. Off-line video densitometry with a 256 gray scale level was performed with a computerized system (Macintosh IICi computer, Apple, Cupertino, CA), by using NIH Image version 1.49.

### *Data Analysis*

Results are expressed as mean value ± SD, unless otherwise specified. Comparisons were made by using paired Student's t-tests. Differences were considered significant at $p < 0.05$.

## Results

### *Acute Studies*

The effects of the pulsed prototype $CO_2$ laser were studied in the various phases of the ECG cycle. When the laser impulse was synchronized with the P wave, it consistently induced an extra impulse without a sustained hemodynamic effect. The laser impulse synchronized on the R-wave; between the R and T waves had no effect. When the impulse was synchronized on the T wave, it sometimes induces an extra impulse without any sustained effect. At no time did this laser cause any life threatening ventricular arrhythmias when fired during any phase of the ECG cycle.

### *Histopathology*

The pattern and progression of TMR by using this laser can be characterized based on the acute, 2 week, and 6 week animals. Measurements of the diameter of each acute channel were made using standard microscopic techniques. Statistical analysis of the measurements of the channel diameters resulted in a mean value of 806 $\mu$m and a standard deviation of 256 $\mu$m. The acute channels were characterized by well defined spaces devoid of endothelial lining, but filled with blood, fibrin, and curled necrotic myocytes. Surrounding this channel were basophilic myocytes demonstrating abundant contracture band changes. These changes were caused by the thermal energy damage (**Figure 3**). Analysis and radial measurement of the thermally damaged zones resulted in a mean of 16 $\mu$m and a standard deviation of 10 $\mu$m.

After 2 weeks, many of the TMR regions continued to show a central patent zone. The spaces were filled to variable degrees by blood, fibrin, and granulation tissue. Multinucleated giant cells, histiocytes, and granulation tissue surrounded these blood filled spaces.

After 6 weeks the central zones were filled with granulation and fibrous tissue containing myofibroblasts and chronic inflammatory cells. Variable numbers of vascular elements including sinusoids and small arteries; ectatic capillaries were seen in some sites (**Figures 4 and 5**).

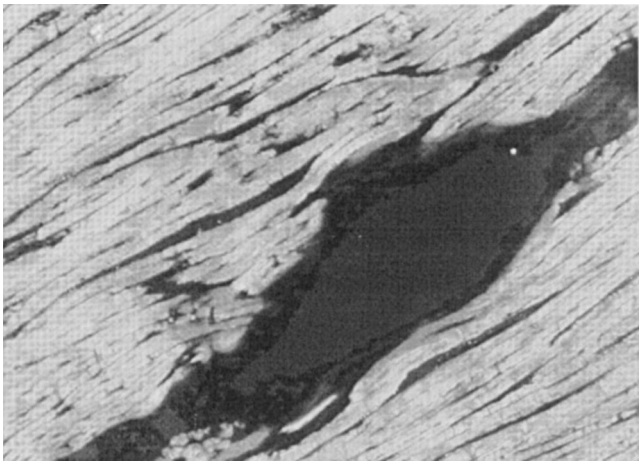

**Figure 3.** Polarized light micrograph showing thermal damage around an acute channel from a CircuLase laser impulse. The central channel is ~800 $\mu$m in diameter with thermal damage of ~10 $\mu$m (*in vitro*).

### *Left Coronary Angiography*

All 11 pigs used in the study with the ameroid occluder had complete occlusion of the main circumflex coronary artery at 6 weeks and developed ischemic changes (**Figure 1**). Six pigs developed collaterals at this phase, with evidence of retrograde opacification of the distal circumflex coronary artery. Two pigs were studied 2 weeks after TMR to study the progression of channel formation. One animal was studied as a control without TMR. Eight pigs completed the study, and all animals demonstrated collateral formation at the end of the study (**Figure 2**). This high degree of compensation can be

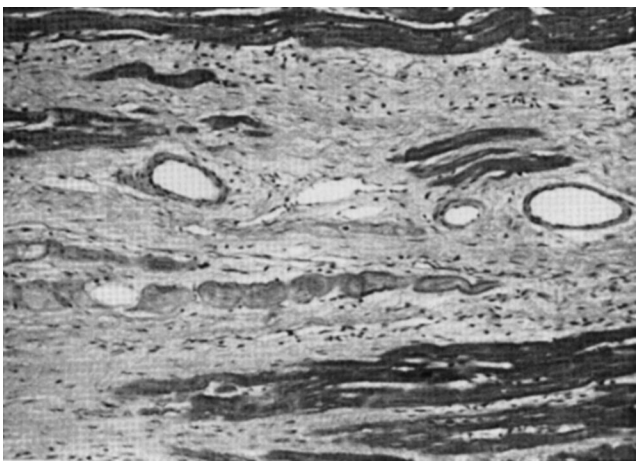

**Figure 4.** A 5× trichome stained section of lased myocardium 6 weeks after TMR at level 2 contains an elliptical zone of fibrosis containing entrapped myocytes and dilated vascular spaces. This focus measures up to 0.68 mm in thickness, and no patent channel is found.

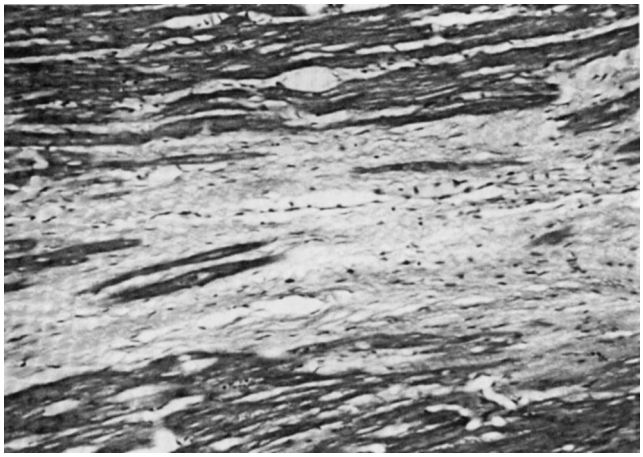

**Figure 5.** A 5× trichome stained section of lased myocardium 6 weeks after TMR at level 3 containing a single potential elliptical zone of fibrosis containing dilated vascular spaces. It measures up to 0.8 mm in thickness, and no central passage is found. This zone is composed of dilated sinusoids, telangiectatic capillaries, and small arteries embedded in loose mature fibrous collagenous matrix containing scattered myofibroblasts, fibroblasts, and occasional inflammatory cells.

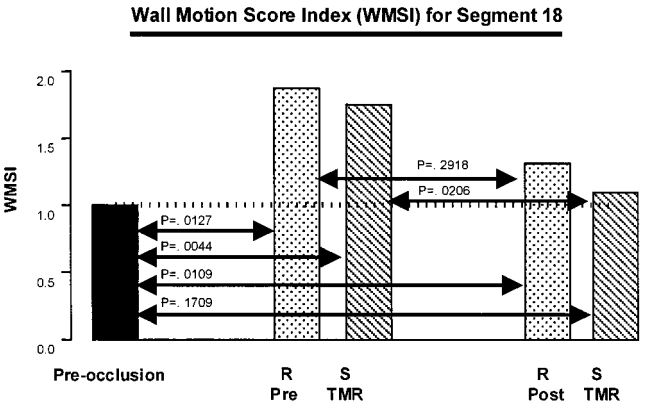


**Figure 6.** Wall motion score index (WMSI) at both rest and peak dobutamine stress for segment 18 before and after TMR, demonstrating postoperative improvement in WMSI of the segment at rest and at peak stress. The animals developed significant ischemia and, therefore, decreased wall motion after 6 weeks of ameroid occlusion at rest ($p = 0.01$) and at peak stress ($p = 0.004$). Significant improvement in WMSI was observed between pre-TMR WMSI and post-TMR stress tests ($p = 0.02$). Wall motion returns to near normal during post-TMR stress testing, from a WMSI of 1 at baseline to 1.09 during post-TMR stress. R, resting; S, stress.

explained, as our model was a juvenile, growing pig. The control animal developed collaterals at the end of 12 weeks, but its wall motion did not return to normal despite the collaterals, suggesting that collateral formation did not fully compensate and restore wall motion abnormalities.

*Epicardial Echocardiography*

The results of mean WMSI for segments 18 and 17 are shown in **Table 1** and in **Figures 6 and 7**. Segment 18 was ischemic to a greater degree than segment 17 and demonstrated significant improvement after TMR.

*Myocardial Contrast Echocardiography*

The ischemic myocardium was analyzed by using contrast enhanced two-dimensional echocardiography at both rest and peak stress to assess myocardial perfusion abnormalities. This technique correlates well with resting perfusion defects to sestamibi single photon emission computed tomography[11,12] and identification of hibernating myocardium.[13,14] For contrast during off-line video densitometry, because the actual density varied with the injection, animal, and instrument settings, we found it best to look at the difference between the average for ischemic/lased segments 17 and 18 and compare this with the average for the nonischemic unlased segments 13 and 14. Although there was a decrease in perfusion to segment 17 and, especially, 18 on the magnitude of 20–25 video density units (VDU) on a gray scale of 256 units, we could not find any statistical significance in our data to show improvement in myocardial perfusion status after TMR, and at times a change in texture characteristic of scaring was visualized.

**Table 1. Results of Mean Wall Motion Score Index for Segments 18 and 17***

| | Resting Echocardiography | | | Stress Echocardiography | |
|---|---|---|---|---|---|
| Segments | Baseline | Before TMR | After TMR | Before TMR | After TMR |
| 18 | 1 | 1.87 | 1.31 | 1.75 | 1.10 |
| 17 | 1 | 1.44 | 1.13 | 1.19 | 1.10 |

* TMR = transmyocardial laser revascularization.

## Discussion

There are currently two laser modalities approved by the FDA for the surgical approach to TMR. The 800 watt $CO_2$ laser

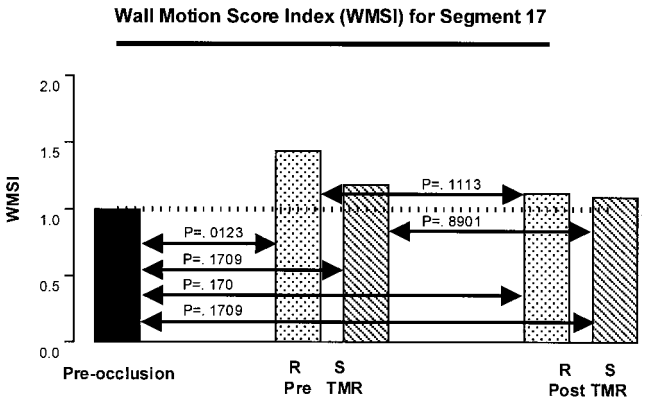


**Figure 7.** The WMSI at rest and at peak dobutamine stress for segment 17 before and after TMR, demonstrating postoperative improvement in WMSI of the segment at rest and at peak stress. The animals developed significant ischemia in segment 17 and, therefore, a decrease in wall motion after 6 weeks of ameroid occlusion at rest ($p = 0.01$) but not at peak stress ($p = 0.17$). There was no significant improvement of segment 17 by WMSI after TMR shown. Wall motion returned to near normal/baseline during post-TMR at rest to 1.13, and to 1.10 during post-TMR stress testing. R, resting; S, stress.

(PLC Medical systems, Inc.) and the Ho:YAG laser (Eclipse Surgical Technologies, Inc.). The eximer laser Xe:Cl (Medlas) is currently in clinical use in Europe. The 800 watt $CO_2$ laser has a pulse width of ~38 ms and an energy of 30 joules. This laser is synchronized to fire during the refractory period of the ECG cycle ~100 ms after the R wave. Unless the laser is synchronized to pulse within this safety window, the patient can experience premature ventricular beats that may lead to potential harmful arrhythmias, including ventricular tachycardia and ventricular fibrillation. Mirhoseini *et al*.[6] have showed that the pulse of a $CO_2$ laser will produce arrhythmias during the vulnerable period of the cardiac cycle unless the duration of the pulse is of the order of 1 ms. Kadipasaoglu[15] has shown that the number of arrhythmias occurring within the first 60 heart beats of the laser pulse were significantly higher for the holmium:YAG laser firing at 5 pulses/sec unsynchronized (as in the Eclipse commercial laser), and for the 50 ms PLC $CO_2$ laser pulse synchronized with the T-wave, compared with the PLC $CO_2$ laser synchronized with the R-wave.

The prototype laser used in this study is a 1.5 ms pulsed $CO_2$ laser with two rapid fire impulses of 750 $\mu$s each using 8–12 joules. This laser does not require ECG synchronization, and causes less thermal damage in the acute phase as it is faster and uses less energy, therefore, decreasing the area of fibrosis in the long-term. An estimation of the central channel size by using the prototype laser was ~800 $\mu$m, and the extent of thermal damage was ~10–20 $\mu$m (**Figure 7**). In comparison, a polarized light micrograph showing thermal damage from the PLC laser[16] (Jansen *et al*.), reveals a central channel of ~500 $\mu$m with thermal damage for ~ 150 $\mu$m in the surrounding tissue.

The current data demonstrates that TMR improves wall motion in a model of chronic ischemia and improves WMSI more during induced stress than at rest, possibly by recruiting hibernating myocardium.[17] This is an ongoing study and, therefore, our results are preliminary, although they agree with the current literature[17,18] on the beneficial effects of TMR.

The precise mechanisms for the beneficial effects remain elusive; the success of TMR is based on the supposition of improved regional blood flow to the ischemic myocardium. It has not been conclusively demonstrated by our study that myocardial perfusion improves after TMR. Histologic evidence of channel patency has not been shown, although increase in collateral formation and neovascularization has been demonstrated in other animal models.[19,20] In our model, we observed neovascularization within the scar of the TMR channels, but its contribution to myocardial perfusion is not proven.

One animal that did not develop ischemia showed stiffening of the myocardium after TMR, suggesting that TMR may cause scar formation and limit myocardial distensibility and dilatation, with possible contraction of the ischemic segments and tethering to improve wall motion.

### *Study Limitations*

The major limitation of this study is the relatively small animal cohort (eight pigs). We also have not completed a control group of animals and have one sham control. The model is a growing juvenile pig that triples in weight at the end of 12 weeks. Because growing myocardium is resilient to ischemia, one of every four pigs developed ischemia after 6 weeks of ameroid occlusion of the main circumflex coronary artery, and many animals were not suited for the study. Six of eight animals had pre-TMR collaterals, and all the animals demonstrated collateral formation at the end of the study 6 weeks after TMR. It is difficult to assess without a control group whether this is a natural response in this juvenile model or neovascularization in response to TMR.

## Conclusion

TMR results in an improvement in wall motion in our model of chronic ischemia and improves WMSI more significantly during induced stress than at rest, possibly by recruiting hibernating myocardium

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