

The Number of Channels during Laser Transmyocardial Revascularization Can Alter Myocardial Function in the Isolated Rat Heart

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Abstract: Background and Objectives: Heart failure is a major potential complication in transmyocardial revascularization (TMR). The objective of this study was to assess the effect of TMR on myocardial function. **Study Design/Materials and Methods:** Two experiments were performed in normal rat myocardium, one using myocardial muscle strips, the other using a whole heart preparation. TMR was performed using Ho:Yag laser (2100 nm, 3 Hz, 280 mJ/pulse) via 0.6 mm core optical fiber. Myocardial muscle strips (n=14) were paced with 1 Hz/80 mV pulse current at a rate of 60 beats/min. A whole heart Langendorff set up (n=37) was used to perfuse the myocardium via a cannula in the aorta. Also, heat shock proteins (hsp) were measured by Western Blotting and ATP measured by a standard enzymatic method (n=10). **Results:** Using the myocardial strip preparation, Frank-Starling curves demonstrated a decrease in contractility compared to baseline by 42% after 20 TMR channels (0.55 ± 0.26 vs. 0.32 ± 0.12 g/cm²; p<0.05). In the whole heart preparation, myocardial contractility decreased by 21% after 20 TMR channels (5.8 ± 0.9 vs. 4.6 ± 1.5 g/heart; p<0.05). After 50 TMR channels/heart, hsp70 decreased by 78% and ATP increased by 32%. **Conclusions:** Lasing 20 channels/heart caused a significant decrease in contractility in normal rat myocardium while ATP levels increased. [The Journal Of American Science. 2007;3(4):72-80]. (ISSN: 1545-1003).

Keywords: channel numbers; heart failure; transmyocardial revascularization

1. Introduction

Coronary artery disease continues to be the leading medical cause of death worldwide. However, the development of newer technologies to treat myocardial ischemia has resulted in increased survival of patients with persistent and chronic ischemic heart disease. Many of these patients have diffuse coronary artery disease that is not amenable to further bypass surgery or angioplasty.

Transmyocardial revascularization (TMR) using laser irradiation is a technique reserved for refractory angina that is not responsive to the traditional therapy (1). The rationale for the procedure was based on the reptilian heart that receives its main blood supply from direct myocardial-ventricular channels. However, more recent studies suggest that most TMR channels had become chronically occluded. An alternative hypothesis to explain improvement of patient symptoms has been attributed to neorevascularization that occurs locally around the scarred TMR channels that may be perfusing ischemic muscle. Despite the controversies about mechanism of action, a major concern with the use of TMR is causing or worsening of heart failure by further loss of myocardial tissue. Thus, the number of channels that need to be placed should be defined in order to optimize the potential clinical benefits.

The aim of this study is to evaluate the relationship between the number of lased TMR channels and myocardial contractility as well as biochemical response of the myocardium to TMR.

2. Materials and Methods

TMR was performed on the rat heart tissues using a Ho:Yag (2100 nm, 3 Hz, 280 mJ/pulse) (Optipulse, Trinedyne, Irvine, CA) laser to create a full thickness channel in the myocardium. Laser irradiation for TMR was performed using a Ho:Yag laser delivered via a 0.6 mm core optical fiber.

2.1 Transmyocardial Revascularization

Sixty-one female Sprague Dawley rats (300-400 grams) were used in this study. Heparin (1000 unit/kg, I.M., Sigma, St. Louis, MO, USA) was administered 1 hr prior to anesthesia to prevent intracardiac thrombus. General anesthesia was induced using pentobarbital (80 mg/kg I.M., Abbott Laboratories, North Chicago, IL) and then the heart was rapidly excised via a mid-line chest incision. The hearts were kept in oxygenated PBS at 0°C until use (2). A total of 14 hearts were used to make myocardial muscle strips and 37 hearts were set up as a Langendorff preparation. An additional 10 hearts were used for biochemical

analysis (3,4).

Myocardial Strip Preparation: The myocardial strip model was made by excising both atria and right ventricles. Then, an incision from the apex of the left ventricle to the base on both sides was made while leaving the apex intact. Each side of the left ventricular muscle strip was then fixed to a post and an electrode was used to stimulate the myocardium. Electrical stimulation was delivered from a square wave pulse generator (1 Hz/80 mV/pulse). The preparation was immersed in PBS and allowed to stabilize for over 1 hr. prior to TMR (Figure 1). Twenty TMR channels were placed in the muscle followed by 10 min rest intervals until myocardial contractility deteriorated, and the channels were evenly distributed along the length of the muscle segment.

Whole Heart Langendorff Preparation: In this set up, 37 hearts were perfused using oxygenated PBS via the coronary circuit after cannulating the ascending aorta as demonstrated in Figure 2. The perfusion pressure was maintained at a mean of 60 mmHg. After 1 hr of perfusion, laser irradiation was performed in 23 hearts with laser salvos (4 channels \times 5; and 10 channels \times 3) and 10 min rest interval after each salvo. The hearts were perfused for up to 6 hr. Fourteen hearts served as control and were treated in the same fashion without lasing.

Myocardial Contractility Measurement: The Frank-Starling curves were measured to evaluate myocardial contractility. A basic physical property of cardiac muscle stipulates that if cardiac muscle is stretched, it will result in greater force of contractility. This continues to increase with stretching until the muscle fails. This process allows the detection of very early myocardial failure prior to changes that can be noted at resting conditions. Contractility was measured using a transducer attached to a strain gauge sutured to the cardiac apex. The electronic signal was then converted to mechanical displacement on a strip chart recorder. Various preloads (0, 1, 2, 3 g) were used to elicit a Frank-Starling response obtained after each salvo of TMR channels.

2.2 Biochemical Analysis

Heat shock proteins (hsp25, 60, 70, 90) and ATP were measured in ten hearts. Three hearts were used as control-control measurements without perfusion. An additional three hearts were first perfused with the PBS in the Langendorff set up for 6 hr and then homogenized for hsp and ATP measurements. Another three hearts were perfused for 1h and then 50 TMR channels were made followed by 5 hr perfusion prior to homogenization for hsp and ATP measurement. One heart was heated in a waterbath at 42°C for 15 min then kept at room temperature (23°C) for 6 hr prior to hsp and ATP measurement (5, 6).

Heat Shock Protein Measurement: Myocardial tissue was homogenized in 3 \times volume (W/V) of buffer extract under ice. The homogenized sample was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was collected. Heat shock proteins (hsp25, 60, 70 and 90) from the supernatant were detected by Western Blotting using monoclonal heat shock protein antibodies (hsp25, 60 and 90 antibodies, Sigma, St. Louis, MO; hsp70 antibody, StressGen Biotechnologies Corp, Victoria, BC, Canada). Secondary antibody was measured by the alkaline phosphatase method.

Measurement of ATP: The myocardial ATP content was measured according to Beutler (7). Briefly, homogenized heart tissue was treated as above. A 0.2 ml of the supernatant was mixed with 0.8 ml of sample modified Tris buffer (Tris-HCl 100 mM, EDTA 0.5 mM, MgCl₂ 2 mM, NADP 0.4 mM, glucose 1 mM, G-6-PD 0.3 U/ml, pH 8.0). Baseline readings were taken at OD_{340 nm} and 37°C. A 0.01 ml of hexokinase (400 U/ml) was added and readings were repeated at OD_{340 nm} at 37°C until a constant value was reached. ATP content in the sample was derived by comparison to a standard ATP curve.

2.3 Statistical Analysis

Data were reported as means \pm S.D. and compared by a paired Student's t-test. A p<0.05 was considered significant. The SigmaStat statistical software was used to calculate statistical comparison.

3. Results

3.1 Myocardial Strip Studies

Contractility: The myocardial strip studies (n=14) had normal Frank-Starling curves at baseline.

Following lasing of 20 channels/strip there was a significant decline in the Frank-Starling response. At a preload of 1.2 g/cm² lasing compared to non-lasing, myocardial contractility decreased by an average of 42% after 20 channels (0.55 ± 0.26 vs. 0.32 ± 0.12 g/cm²; p<0.05). Following 60 channels there was 67% decrease (0.55 ± 0.26 vs. 0.18 ± 0.11g/cm²; p<0.03) and after 180 channels there was 84% decrease (0.55 ± 0.26 vs. 0.09 ± 0.02 g/cm²; p<0.01) (Figure 3). The ratios of myocardial contractility at a preload of 1.2 g/cm² to non-preload levels at baseline and after 20, 60, 120 and 180 channels are shown in Figure 4. Following the placement of 120 channels, there was almost complete obliteration of the Frank-Starling response. After each lasing, the myocardial contractility decreased immediately but then gradually recovered following 10 min of rest. Although initially these achieved baseline contractility they became attenuated with progressive laser salvos.

3.2 Whole Heart Langendorff Preparation

In the whole heart Langendorff preparation, the Frank-Starling curves were normal at baseline (Figure 5). After each set of TMR channels the force of myocardial contractility decreased following lasing at each of the preload settings (0-3 g) (Figure 6). Maximum contractility of the hearts was at 3 g preload. Compared to baseline, the maximum myocardial contractility after lasing decreased significantly by 21% after 20 channels (5.8 ± 0.9 vs. 4.6 ± 1.5 g/heart; p<0.05), by 22% after 30 channels (5.4 ± 1.2 vs. 4.2 ± 1.3 g/heart; p<0.05), by 27% after 40 channels (5.1 ± 0.6 vs. 3.7 ± 1.1 g/heart; p<0.002), and by 32% after 50 channels (5.0 ± 1.5 vs. 3.4 ± 1.0 g/heart; p<0.02) (Figure 7). However, after 30 min of rest following lasing, myocardial function returned to baseline (4.1 ± 1.4 vs. 3.4 ± 0.9; p=ns). Unlike the muscle strips, the whole heart preparation seemed to hold up for longer periods without deterioration.

3.3 Biochemical Analysis

Heat Shock Proteins: hsp25, 60 and 70 were expressed in the all groups but there were no significant differences in expression of hsp25 and 60 amongst the groups. However, after lasing in group 3 hsp70 expression decreased by 78% (0.87 ± 0.10 vs. 0.19 ± 0.03; p<0.01) and hsp70 expression was highest in Group 4 (Figure 8) (Table 1). There was no expression of hsp90 detected in any of the four groups.

ATP Content: ATP content was 165.6±29.6 nmol/g rat heart (n=5) without lasing and 218.9±35.5 nmol/g rat heart after 50 channels/heart. The laser increased ATP content in rat heart tissue by 32% (p<0.02).

Table 1. Relative Heat Shock Proteins Expression

	Group 1	Group 2	Group 3	Group 4
hsp25	+	+	+	+
hsp60	+	+	+	+
hsp70	+	++	+	++++
hsp90	-	-	-	-

Group 1 = Three normal hearts excised; Group 2 = Three hearts perfused with PBS in the Langendorff set up for 6 hr before hsps measurement. Group 3 = Three hearts lased with 50 channels, perfused for 6 hr and then hsps measured. Group 4 = One heart heated in a waterbath at 42°C for 15 min then kept at room temperature at 23°C for 6 hr prior to hsps measurement.

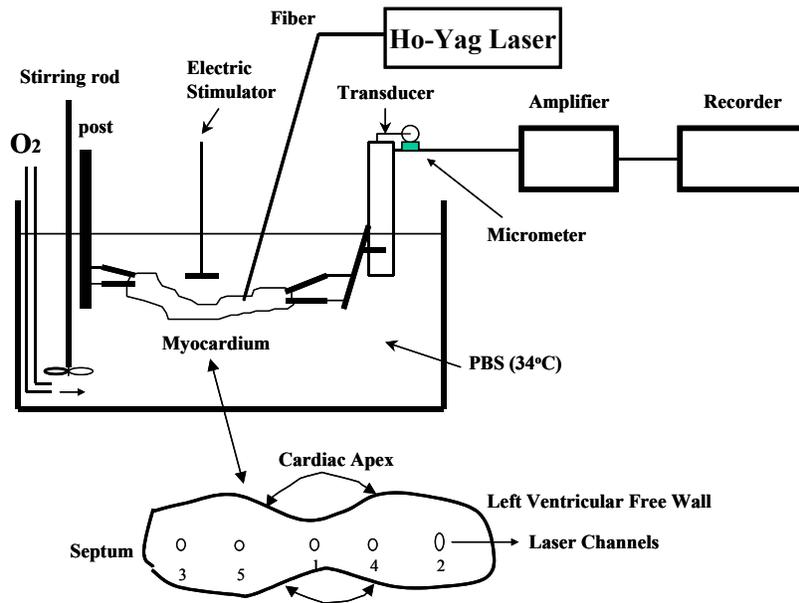


Figure 1. Set up of the myocardial strip model demonstrating the left ventricle dissected but still attached at the apex in the center of the strip preparation with the septum and ventricular free wall opened. Lasing was delivered via an optical fiber and placed in the numbered sequence shown on the muscle strip 1-5 with 1 being the first lased area.

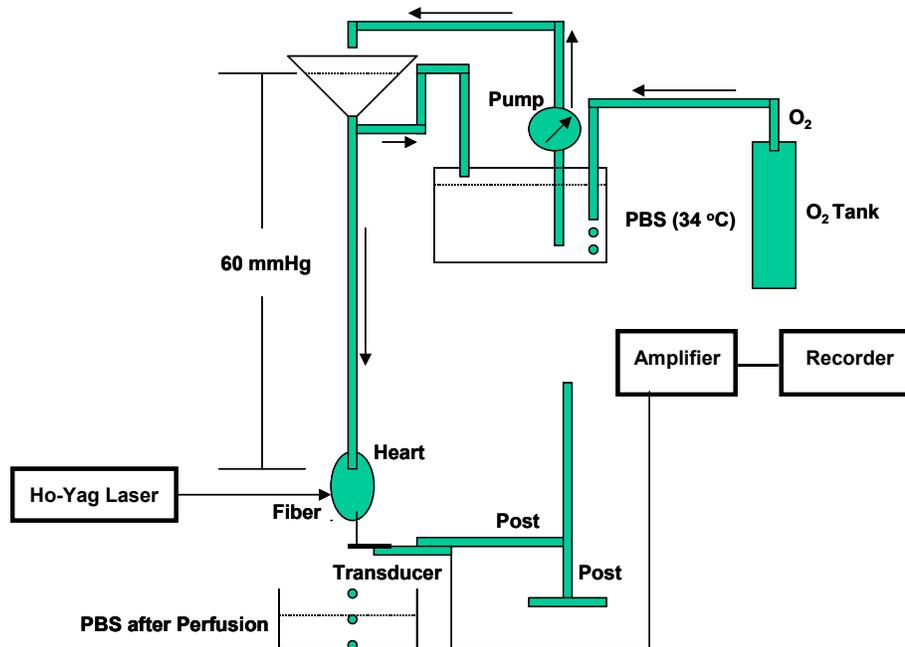


Figure 2. Set up of Langendorff whole heart preparation demonstrating the left ventricle being perfused by oxygenated PBS and the apex attached to a strain gauge to measure contractility of the myocardium with varying tension from 0–3 g.

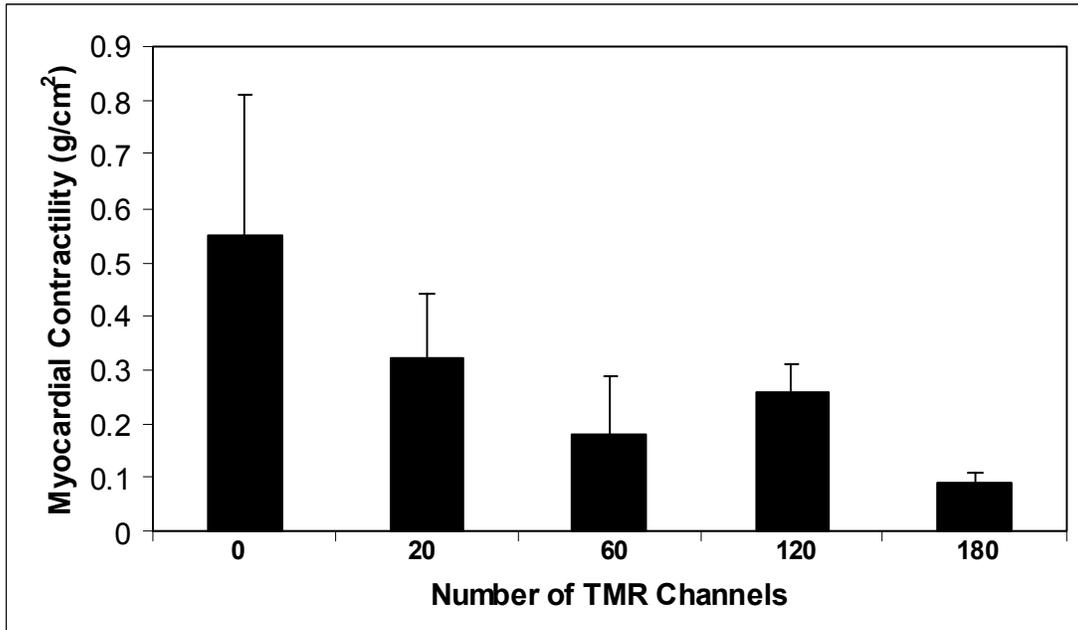


Figure 3. Bar graph demonstrating effect of laser channels on myocardial contractility in the myocardial strip model at a preload of 1.2 g/cm². This demonstrates a significant reduction of contractility after 20 channels.

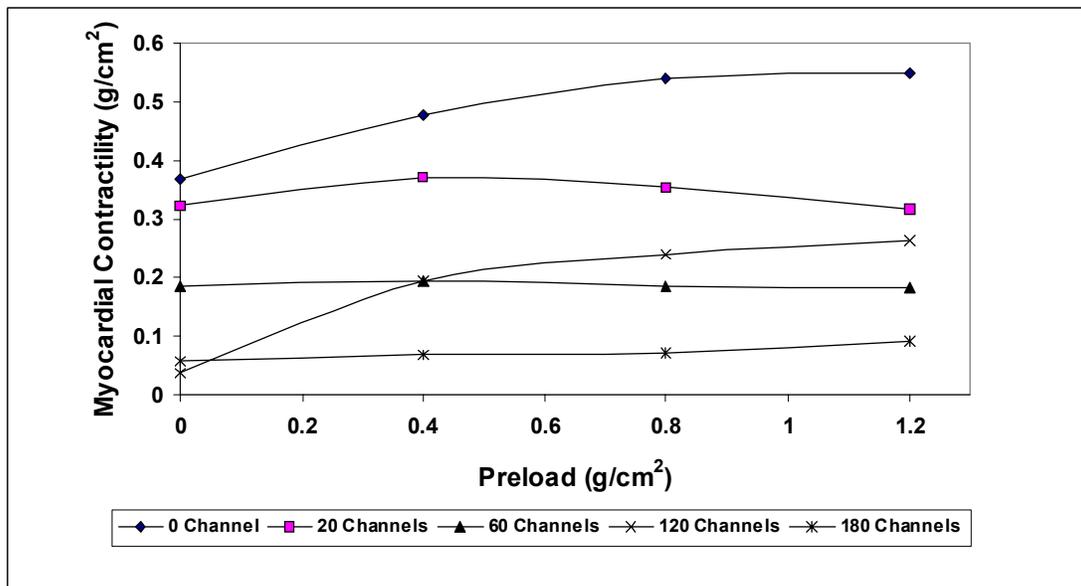


Figure 4. Frank-Starling curves of myocardial contractility at various preloads in the myocardial strip model. The curves represent an average of several Frank-Starling measurements with various TMR channels (0–180). The points on the curves represent an average of 14 experiments at each of the preloads. There is significant drop in contractility with increasing number of channels.

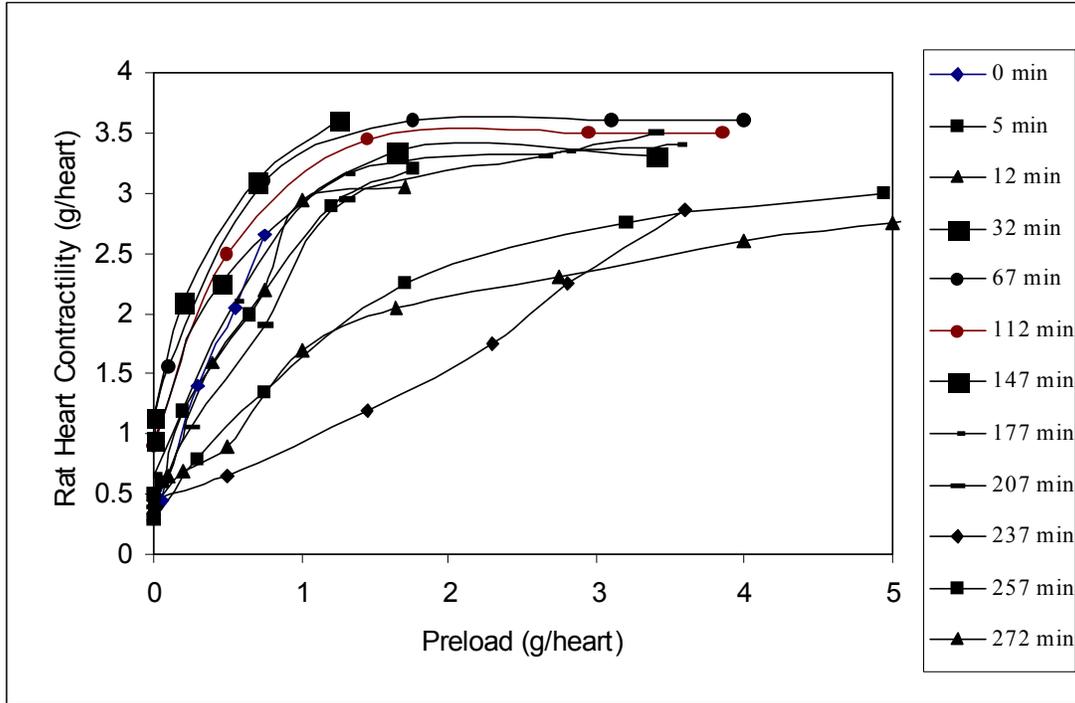


Figure 5. Example of control Frank-Starling curves in a normal non-lased rat heart with varying preloads (0–5 g). The Frank-Starling curves are stable for over 2.5 hr.

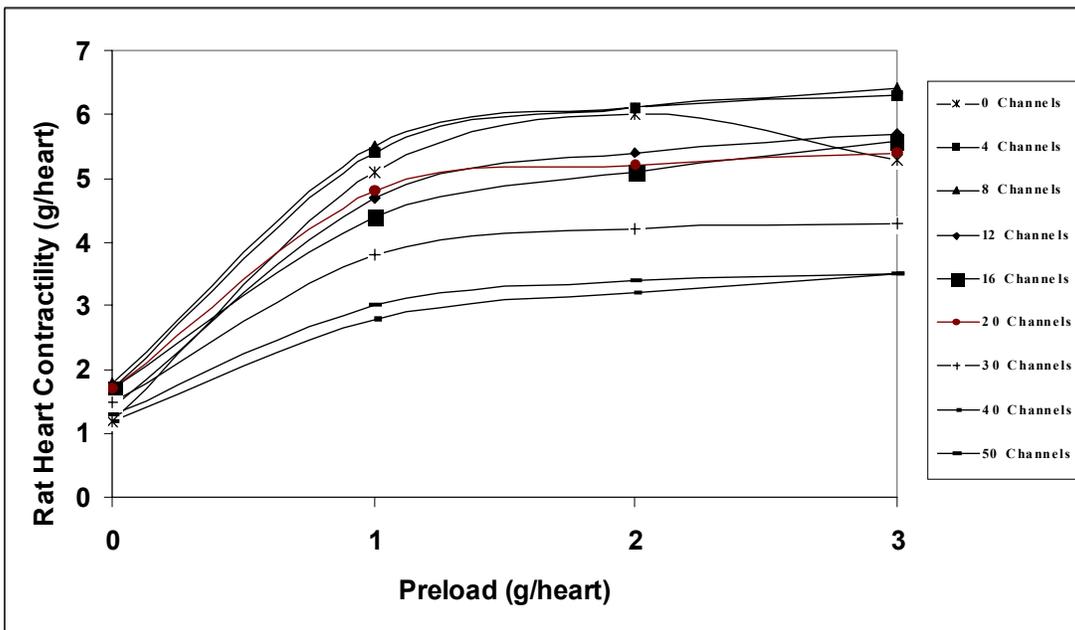


Figure 6. Example of Frank-Starling curves in a normal rat heart after lasing (0–50 TMR channels) at varying preloads (0–3 g). Each curve is representative of different TMR channels made.

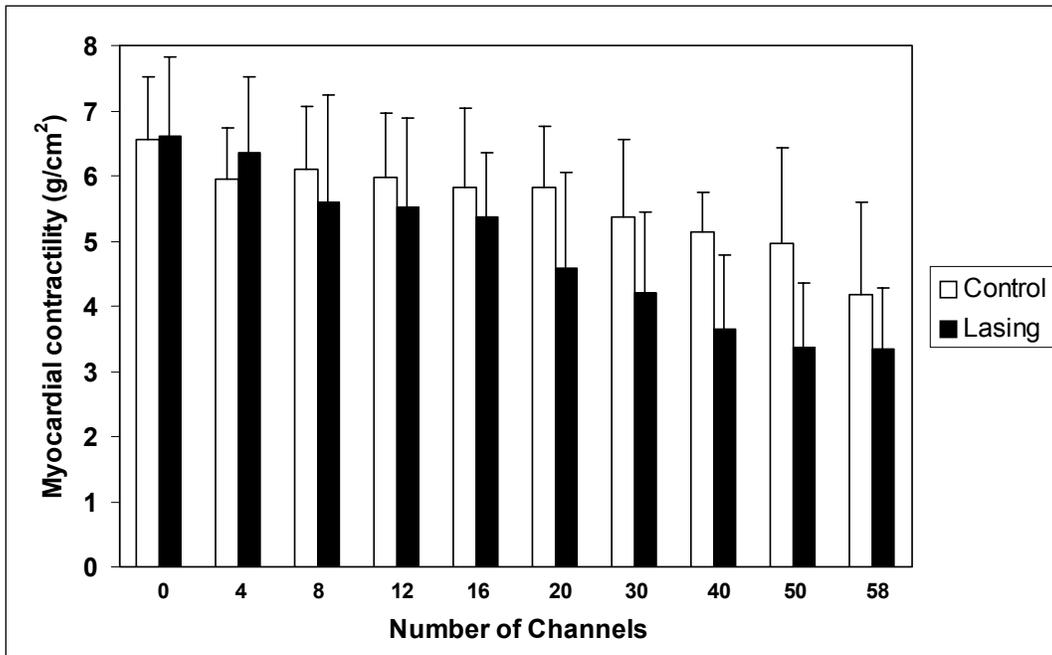


Figure 7. Bar graph of average of myocardial contractility in the Langendorff set up (TMR: n=23; Control: n=14). These demonstrate that there is significant reduction in contractility after 20 channels.

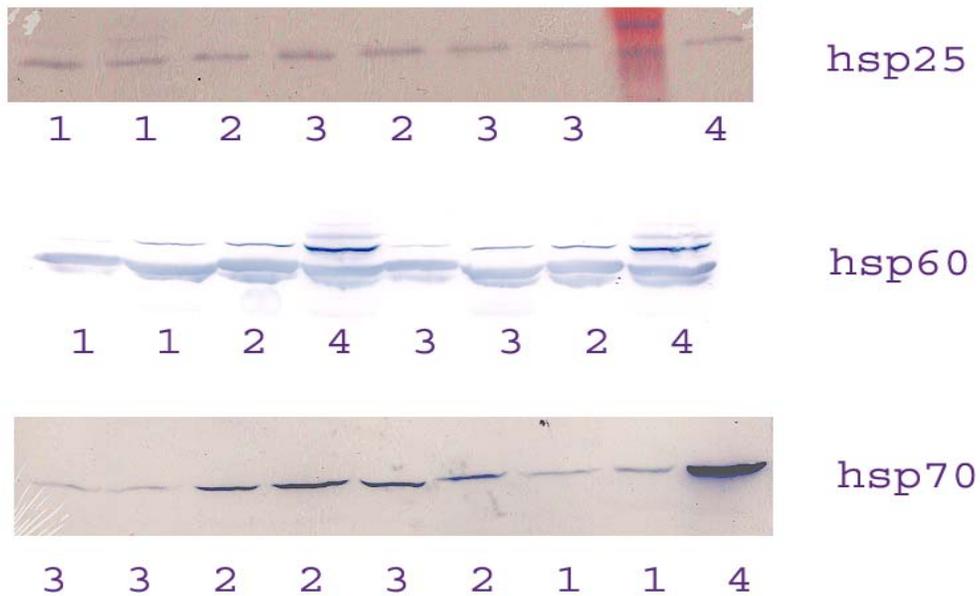


Figure 8. Heat shock proteins expression measured using Western Blotting. 1 = control-control; 2 = control; 3 = TMR (50 channels); 4 = heated heart (42°C). These data demonstrate that hsp 70 is maximally expressed in Group 4 and low in Group 3.

4. Discussion

The basic mechanics that regulate myocardial contractility include four factors: (1) The preload or the Frank-Starling mechanism that sets a passive load which establishes the initial muscle length of the cardiac fibers prior to contraction; (2) The after-load, which is the sum of all the loads against which the myocardial fibers must shorten during systole; (3) The inotropic state of the heart, which is reflected in the velocity of shortening capacity of the myocardium at a given instantaneous load and; (4) The heart rate or frequency of contraction. The above four factors are interrelated in the intact organism. For example, fiber length appears to influence quantitatively the number of active force-generating sites in the myocardium, whereas a change in the contractility is related to a qualitative change in the force generated by the sites i.e., their activations, with or without a change in their number (8).

In ischemic hearts with prior scarring from myocardial infarctions, the basic myocardial properties may be altered or suppressed. Thus, the additional loss of myocytes by TMR could worsen the contractility. So far, TMR has been performed clinically without knowing the exact number of channels needed for the full benefits of the procedure or the number of channels that can lead to heart failure. The results of this research demonstrate that a significant decrease in myocardial contractility occurs with lasing of more than 20 channels in the normal rat heart (13 channels/g wet heart tissue). This suggests that there exists a limit beyond which excessive number of channels will lead to loss of vital cardiac function. Because the average human heart is about 300 g an extrapolated number of channels from this rat heart study would be in excess of 3900 channels/human heart. Although this is entirely a theoretical number most TMR applications have used 4 channels per square cm. Thus, it is suggested that perhaps many more channels are needed for adequate perfusion results. As this was an *in vitro* study, it could be only a reference to the *in vivo* application, rather than a real prescription in the clinical application. The studies were done on normal isolated rat hearts, therefore, the results can not really be extrapolated into ischemic model or *in vivo* hearts, especially for human. The suggestion for clinical study to increase the channel density in ischemic myocardium should be done with caution. It should also state that this was done in normal heart model and that the same result may not be seen in ischemic myocardium. Specifically we were not using a laser with specifications that is seen in the clinical arena.

Cardiac biochemistry greatly influences intrinsic myocardial contractility. Heat and other injuries induce expression of heat shock proteins that are a category of stress proteins. Isolating the rat hearts by extraction from the chest causes severe stressful conditions in the *ex vivo* set up. Because hsp70 in the lased hearts was lower than in the non-lased control hearts then lasing may have possibly compensated for the stress of the isolated condition. It is possible that the TMR channels provided a route for the oxygenated buffered solution to support the stressed myocytes. However, it is also possible that the laser inhibited hsp70 expression or enhanced its degradation.

The result that lased hearts have higher ATP content supports the above hypothesis that some perfusion could be occurring that enhanced energy metabolism. This data is yet the most important with regards to TMR. This is further supported by the myocardial contractility recovery over time. However, this is in contradiction with the findings of Reuthebuch et al, who demonstrated that there was no beneficial effect on the high phosphate and lactate content after TMR in acute myocardial ischemia in pigs (9). This may be due to the fact that acutely ischemic tissue is inhibited from making adequate ATP.

Overall, this study provides direct evidence that up to 13 laser channels/g of myocardium could be tolerated without significant decrease in myocardial contractility in the normal rat heart. Also, the biochemical data suggest that the number of channels created enhanced ATP production. As currently applied in the clinical setting, perhaps not enough TMR channels are being created to achieve consistent benefit to patients. This may in part explain some of the conflicting observations noted in the clinical literature regarding the efficacy of TMR (10-14). Therefore it would be important to maximize the number of laser channels to make TMR a more effective procedure. Further clinical studies on ischemic myocardium with a much higher channel density may be desirable to evaluate TMR benefits.

The total quantity of ATP in the human body is about 0.1 mole. The majority of ATP is not usually synthesized *de novo*, but is generated from ADP by the aforementioned processes. Thus, at any given time, the total amount of ATP + ADP remains fairly constant. The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily which is around 50 to 75 kg. Typically, a human will use up their body weight of ATP over the course of the day (15). This means that each ATP molecule is recycled 1000 to 1500 times during a single day ($100 / 0.1 = 1000$). ATP cannot be stored, hence its consumption being followed closely by its synthesis (16). From the dynamic consideration of ATP synthesis there could be physiological effect for the increasing of ATP after TMR (32% increasing).

However, some reports showed that several days are required for de novo ATP synthesis. For example, several papers by Reimer & Jennings describe the time-course of ATP-changes and even 4 days after a 15 minute occlusion in dogs ATP content was still decreased at 88% of control (17). The confliction should be researched further.

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