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# Progressive heart failure after myocardial infarction in mice

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**Abstract** We tested the hypotheses that myocardial infarction in mice would lead to progressively worsening heart failure 12–18 weeks later and that exercise testing would provide a suitable means to evaluate left ventricular function sequentially. C57BL/6 mice (n = 69) underwent left coronary artery ligation (n = 50) or thoracotomy without ligation (n = 19). Sixteen animals (32%) died within 24 h of coronary ligation. Twenty additional animals (40%) died between days 3 and 14, and these mice showed infarct sizes of >50% of the left ventricle. Fourteen animals (28%) that survived two weeks underwent echocardiography and treadmill testing 12 and 18 weeks after infarction, with no further mortality. Mice were then killed, morphometric assessment made, infarct size evaluated, and myocardial norepinephrine content and expression of BNP and ANF measured. Mice with infarcts >30% of the left ventricle (n = 6; 12% of original cohort) had left ventricular dilation ($p < 0.0001$) and hypertrophy ($p < 0.001$), impaired left ventricular systolic function ($p < 0.0001$) and reduced exercise duration ($p = 0.03$) and total work ($p = 0.03$) 12–18 weeks after infarction. Mice with infarcts <30% of the left ventricle (n = 8; 16% of original cohort) had no significant functional changes or left ventricular remodeling. Hearts from mice with infarcts >30% had reduced myocardial norepinephrine levels (MI <30%: 177 ± 54 pg/mg, n = 6; MI >30%: 66 ± 14 pg/mg wet weight, n = 4; $p = 0.005$) and increased mRNA content of BNP ($p < 0.03$) and ANF ($p = 0.023$). Coronary artery occlusion in mice provides a relevant model of clinical heart failure that is progressive and can be assessed by sequential exercise testing, providing a means to study the development of heart failure and its treatment.

**Key words** Coronary artery occlusion – echocardiography – transgenic mice – animal models – exercise testing

## Introduction

Animal models of heart failure due to experimentally induced myocardial infarction (MI) have been described in rats, dogs, and pigs (1, 9, 16). In recent years mice have emerged as a desirable species to examine because transgenic lines provide opportunities to study heart failure in a unique manner. A number of murine models of heart failure have been developed either by cardiac-directed overexpression, or, alternatively, knocking out specific genes (10). These models resemble clinical heart failure, but the absence of etiological similarity to clinical heart failure and other concerns regarding transgene expression limits their fidelity. The most common cause of clinical heart failure is myocardial infarction (MI) – infarct-

BRC 344

associated heart failure in mice, therefore, may provide a more relevant model for study.

LV functional decrements and remodeling after MI have been studied by histological examination two weeks (12) by hemodynamics in anesthetized mice six weeks (18), by echocardiography nine weeks (8), by isolated perfused hearts six months (6), and by magnetic resonance imaging two weeks after myocardial infarction (21). A potential shortcoming of previous studies, however, is their failure to assess overall cardiovascular performance sequentially in unsedated animals – an important consideration in determining whether LV dysfunction is also associated with heart failure *per se* following MI. Furthermore, long-term evaluation of exercise performance, myocardial norepinephrine content (3, 4), and myocardial expression of brain natriureitic peptide (BNP; 20) – all useful signs of heart failure – have not been studied in mice after MI.

We tested the hypotheses that myocardial infarction in mice would lead to progressively worsening heart failure 12–18 weeks later and that exercise testing would provide a suitable means to evaluate dynamic left ventricular function sequentially. Mice were studied sequentially after MI (thoracotomy and left coronary ligation), using exercise treadmill testing, echocardiography, and morphometric and biochemical and molecular analyses.

## Methods

### Animals

Animal use was in accordance with institutional and NIH guidelines. Sexually mature male C57BL/6 mice (10–16 weeks old; 24.3 ± 0.2 g) were used. Fifty mice underwent thoracotomy and coronary artery ligation (MI group); 19 mice underwent thoracotomy but no coronary ligation (Control group). The duration of the study was 18 weeks. All animals surviving 18 weeks were killed for morphometric analysis.

### Myocardial infarction

Mice were anaesthetized with intraperitoneal injection of ketamine (100 μg/g) and xylazine (5 μg/g) and placed supine on a surgical board. A midline anterior cervical skin incision was made and the trachea exposed by blunt dissection. The tongue was retracted with forceps, and the trachea intubated with a 22-gauge angiocatheter. Position of the endotracheal tube was confirmed by direct visualization. Pressure controlled ventilation was initiated at 15 cm $H_2O$. A 1.5 cm vertical left parasternal skin incision was then made, underlying pectoralis muscles retracted, and the chest cavity entered through the fourth intercostal space. Using hooked retractors, adjacent ribs were retracted to expose the heart, and a 9-0 prolene suture (tapered needle) was placed around the proximal left coronary artery and then ligated. LV blanching indicated successful occlusion of the vessel. The chest was then closed by suturing together adjacent ribs (6-0 nylon), and the skin closed as a separate layer.

### Echocardiography

Echocardiography was performed in anesthetized mice as previously reported (19) at 12 and 18 weeks after operation. Animals were anesthetized with intraperitoneal injection of ketamine (100 μg/g) and midazolam (3 μg/g). Using a pediatric 12 MHz linear probe (Agilent Technologies) a parasternal short axis view was obtained as a guide for LV M-mode imaging at the papillary muscle level. The M-mode images were digitized on the optical disc (HP 5500). Using the HP 5500 standard software, LV dimensions were traced in both end-diastole and end-systole in short and long axis views and LV volumes determined using the modified Simpson method and fractional area change (FAC) and LV mass were calculated.

### Exercise treadmill testing

Mice were placed on a motor-driven treadmill (Columbus Instruments, Columbus Ohio). Mice were allowed to acclimate for 5 min, and then run at a starting speed of 10 m/min with increments of 5 m/min every 2 min. The starting grade was 5 degrees, which was increased manually by 5 degrees every 2 min. Exercise was stopped when animals reached maximal effort. Maximal effort was defined (5, 7) when 1) mice sat on the shocker for >15 seconds; or 2) mice sat on the shocker and were unsuccessful in attempts to get on the treadmill >15 seconds. This occurred only during the last 30–45 seconds of the run. The same investigator ran all mice on the same days 12 and 18 weeks after infarction. Reproducibility of assessment of maximal work was examined in 14 animals that underwent the same exercise protocol on two different occasions 6 weeks apart with very similar results ($r = 0.8$; $p < 0.006$)

### Morphometry

Wet weights of LV, RV, atria, lung and total body weights were obtained in animals found dead and in electively killed animals. Heart weights were normalized to body weight and tibial length for comparison between Control and MI groups.

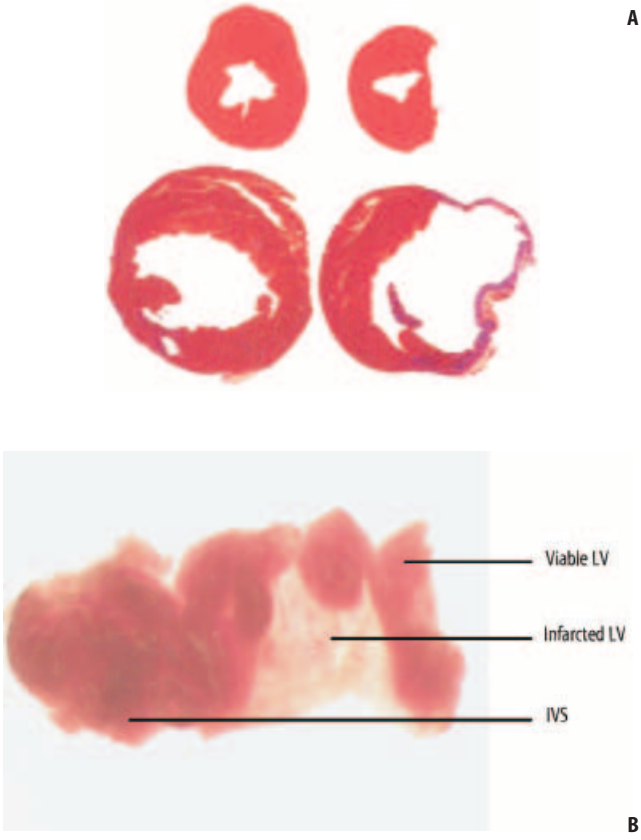


**Fig. 1** **A** Short axis transections of left ventricle four weeks after coronary artery occlusion (below). The infarct is evident in the rightward section of the lower portion of the figure, and there is obvious chamber enlargement and hypertrophy of the remaining wall. Sections of a normal mouse heart are shown for comparison (above). **B** Histological appearance of mouse heart following myocardial infarction, without staining. Clear demarcation of infarct (33%) and viable myocardium is displayed.

To assess infarct size, atria and RV were removed and the LV opened by a mid septal long axis incision. The LV was then laid flat between two glass slides. Pictures of the endocardial and epicardial surfaces were taken by a digital camera mounted on the dissecting microscope. A representative image is shown in Fig. 1. The infarcted area was measured by planimety using the SCION imaging software and expressed as percentage of total surface area of the LV.

### Myocardial norepinephrine content

Scar from the infarcted region of the LV was excised and discarded. LV septal tissue samples were homogenized in 0.1 M Tris pH 7 using a polytron. The samples were then centrifuged (6000 x g; 10 min; 4 °C). Fifty microliters of supernatant was assayed for catecholamines using a radio-enzymatic method (11).

### Myocardial BNP and atrial natriuretic factor (ANF) expression

Left ventricular samples distal to the infarcted region or in a similar region in control animals were used. Total RNA was extracted using RNAzol, suspended in formazol and resolved by standard gel electrophoresis as described previously (17). ANF and BNP cDNA probes were used to detect specific mRNA using Northern blotting. Membranes were exposed to X-ray film 24–72 hours and assessed by densitometry. GAPDH and β-actin were used to assess loading conditions.

### Statistical analysis

Data are reported as group mean ± 1 SEM. To examine the differences in progression of signs of heart failure, mice that survived for 18 weeks after myocardial infarction were divided into two similar-sized groups based on infarct size, using 30% infarction of the LV as the cut-point. The mean infarct size between these two groups was, as expected, different (MI > 30% group – mean infarct size 36 ± 3%; MI < 30% group – mean infarct size, 16 ± 3%; $p = 0.004$). Values obtained from these two groups were compared with values from the sham-operated control group ($n = 17$) using one-way analysis of variance. If the p value for the overall analysis of variance was < 0.05, we performed a single *post hoc* comparison between the two infarct groups. The null hypothesis was rejected when $p < 0.05$ (two tails). All data were acquired and analyzed without knowledge of whether animals were in the control or two MI groups.

## Results

### Infarct size and morphometry

Survival at 24 hours was 70% for the infarct group and 95% for controls. In the infarct group, of those alive at 24 hours, 57% died within the first 2 weeks (40% of the total initial cohort). Fifty percent of the deaths in the first post-operative week were due to LV rupture. Mean infarct size in this group was 65% (infarct area in fresh infarcts, less than 4 days, could not be well visualized), and these mice had congested lungs, pleural effusions and dilated hearts. Infarct size in mice that survived 18 weeks ranged from 10 – 45%. There were no deaths between week 2 and week 18. Mice with infarctions < 30% of the LV (range 10 – 29%; median 19%; mean 16 ± 3%) showed no signs of acute or chronic heart failure (normal exercise duration and total work, normal lung weights, no pleural effusions and no LV dilation). Ninety percent of the control group survived the 18-week study.

**Table 1** Morphometric assessment

| | Control n = 17 | MI < 30 % n = 8 | MI > 30 % n = 6 | p value ANOVA |
|---|---|---|---|---|
| Body weight (g) | 28.1 ± 0.7 | 27.5 ± 1.3 | 27.5 ± 1.0 | 0.87 |
| Heart weight (mg) | 130.7 ± 2.8 | 138 ± 9.0 | 168.0 ± 12.0* | 0.0001 |
| LV wet weight (mg) | 97.1± 2.1 | 101.2 ± 7.7 | 117.7 ± 5.6 | 0.016 |
| Lung (mg) | 133.7 ± 2.3 | 140.2 ± 13.0 | 158.2 ± 15.4 | 0.15 |
| Tibial length (mm) | 17.9 ± 0.1 | 18.0 ± 0.1 | 18.1 ± 0.2 | 0.46 |
| HW: tibial length (mg/mm) | 7.3 ± 0.2 | 7.7 ± 0.5 | 9.2 ± 0.5* | 0.0025 |
| LV: tibial length (mg/mm) | 5.4 ± 0.1 | 5.6 ± 0.4 | 6.5 ± 0.3* | 0.02 |
| Lung: tibial length (mg/mm) | 7.5 ± 0.1 | 7.8 ± 0.7 | 8.7 ± 0.9 | 0.231 |

*MI < 30 %* myocardial infarction group with less than 30% of the left ventricle infarcted; *MI > 30 %* myocardial infarction group with greater than 30% of the left ventricle infarcted; *LV* left ventricle; *Heart* represents weight of whole heart including both atria and ventricles; *HW* whole heart weight; Values represent mean ± 1 SEM. P values from analysis of variance.
* p < 0.05 *post hoc* t-test (two tails), > 30 % MI group vs < 30 % MI group

Confirming our pilot studies (Fig. 1), myocardial infarctions > 30% of the LV (range 30 – 48%; median 35%; mean 36 ± 3%) were associated with increased heart and LV weight to body weight (BW) ratios, as well as increased RV to BW and lung to BW ratios (Table 1 and Fig. 2) – no statistically significant changes were noted in animals with MI < 30% of the LV.

There were excellent linear correlations between percent infarct size measured by this method and LV end-diastolic diameter (12 weeks: $r^2 = 0.65$; $p < 0.0005$; 18 weeks: $r^2 = 0.66$; $p = 0.0007$) and fractional area change (12 weeks: $r^2 = 0.61$; $p < 0.003$; 18 weeks: $r^2 = 0.63$; $p = 0.002$).

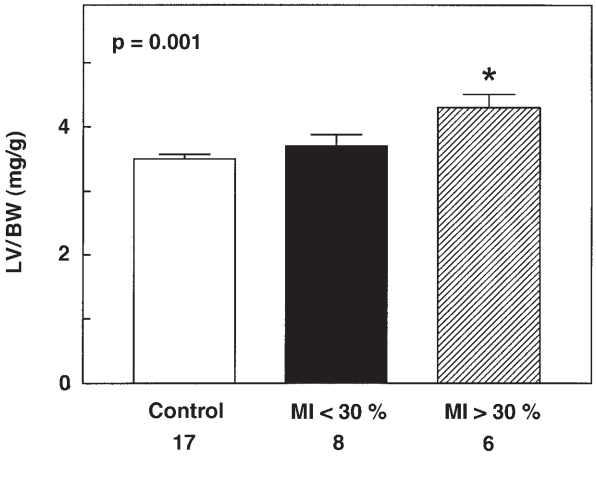


**Fig. 2** Animals with larger myocardial infarctions (MI > 30% of the left ventricle), had increased left ventricular to body weight ratios. Other indicators of morphometry changes are shown in Table 1. *LV* left ventricle, *BW* body weight. Bars represent mean values, error bar denotes 1 SEM. P value from analysis of variance; * p = 0.046, *post hoc* t-test (MI > 30 % vs MI < 30 %).

**Table 2** Echocardiography

| | Control n | MI < 30 % n | MI > 30 % n | p value ANOVA |
|---|---|---|---|---|
| EDD @ 6 Wk (mm) | 3.26 ± 0.11<br>4 | 4.13 ± 0.26<br>5 | 4.61 ± 0.84<br>2 | |
| EDD @ 12 Wk (mm) | 3.31 ± 0.09<br>10 | 3.46 ± 0.16<br>8 | 4.95 ± 0.38***<br>6 | 0.0001 |
| EDD @ 18 Wk (mm) | 3.39 ± 0.10<br>10 | 3.43 ± 0.21<br>7 | 5.05 ± 0.46***<br>6 | 0.001 |
| EDV @ 6 Wk (μl) | 16 ± 2<br>2 | 21 ± 2<br>4 | 52 ± 12<br>2 | |
| EDV @ 12 Wk (μl) | 19 ± 2<br>5 | 19 ± 3<br>7 | 70 ± 15***<br>6 | 0.0001 |
| EDV @ 18 Wk (μl) | 23 ± 2<br>5 | 32 ± 5<br>7 | 92 ± 25***<br>6 | 0.0001 |
| FAC @ 6 Wk (%) | 55 ± 1<br>2 | 54 ± 1<br>4 | 34 ± 12<br>2 | |
| FAC @ 12 Wk (%) | 58 ± 2<br>5 | 57 ± 3<br>7 | 25 ± 8***<br>6 | 0.0001 |
| FAC @ 18 Wk (%) | 55 ± 1<br>6 | 45 ± 1<br>7 | 17 ± 6***<br>6 | 0.0001 |
| LV Mass @ 6 Wk (mg) | 74 ± 4<br>4 | 96 ± 2<br>4 | 143 ± 47<br>2 | |
| LV Mass @ 12 Wk (mg) | 75 ± 2<br>3 | 75 ± 3<br>4 | 161 ± 22*<br>6 | 0.01 |
| LV Mass @ 18 Wk (mg) | 89 ± 3<br>10 | 90 ± 7<br>7 | 153 ± 24**<br>6 | 0.002 |

*MI < 30 %* myocardial infarction group with less than 30 % of the left ventricle infarcted; *MI > 30 %* myocardial infarction group with greater than 30 % of the left ventricle infarcted; *EDD* end-diastolic diameter; *EDV* end-diastolic volume; *FAC* fraction of area change; *Wk* weeks after myocardial infarction; *LV* left ventricle. Values represent mean ± 1 SEM. P values from analysis of variance. *p < 0.05, **p < 0.01 ***p < 0.001 *post hoc* t-test (MI > 30 % vs MI < 30 %); statistical analysis not performed when n < 3

## Echocardiography

Table 2 shows the results of serial echocardiography at 12 and 18 weeks following intervention. Mice with infarct size subsequently shown to be > 30% of the LV showed LV dilation and reduced fractional area changes 12 and 18 weeks after MI – changes that were substantially greater than those observed in animals with MI < 30% of the LV.

In a limited number of animals, studies were also conducted 6 weeks after MI (Control, 4; MI < 30% 5; MI > 30%, 2). Although limited sample size at the early time point did not permit statistical analysis, data suggested that LV mass, EDD and EDV were enlarged following large MI 6 weeks after infarction, but not to the degree that was obtained 12 and 18 weeks after MI. Mice with smaller infarctions did not show significant changes in these parameters 6 weeks after MI (Table 2).

**Table 3** Exercise capacity

| | Control n = 14 | MI < 30 % n = 8 | MI > 30 % n = 6 | p value ANOVA |
|---|---|---|---|---|
| ETT time, 12 Wk (minutes) | 8.2 ± 0.5 | 7.2 ± 0.6 | 6.6 ± 1.0 | 0.21 |
| ETT time, 18 Wk (minutes) | 7.2 ± 0.4 | 6.1 ± 1.1 | 4.5 ± 0.8 | 0.03 |
| ETT maximal work, 12 Wk (watts) | 14.2 ± 1.8 | 9.9 ± 1.4 | 8.8 ± 2.4 | 0.11 |
| ETT maximal work, 18 Wk (watts) | 10.8 ± 1.6 | 7.2 ± 1.5 | 4.3 ± 1.1 | 0.03 |

*MI < 30 %* myocardial infarction group with less than 30 % of the left ventricle infarcted; *MI > 30 %* myocardial infarction group with greater than 30 % of the left ventricle infarcted; *ETT* exercise treadmill testing; *Wk* weeks after myocardial infarction. Values represent mean ± 1 SEM. P values from analysis of variance

## Exercise treadmill testing

Large infarcts (MI > 30 %) were associated with a tendency for reduced exercise duration at 12 weeks, which further deteriorated by 18 weeks (p = 0.03) (Table 3 and Fig. 3). Maximal work achieved 18 weeks after MI was reduced by 33 % after MI < 30 % and by 60 % in animals with MI > 30 % of the LV (p = 0.03). Between 12 and 18 weeks, exercise tolerance deteriorated by more than 50 % in animals with MI > 30 % of the LV. In general, mice with MI < 30 % of LV showed smaller changes in exercise performance than did animals with MI > 30 % compared to the control group (Fig. 3).

## Myocardial norepinephrine content

Larger infarcts were associated with > 2-fold decreased myocardial norepinephrine content (p < 0.005; Fig. 4). Likewise, myocardial dopamine, a precursor of norepinephrine, showed a 4-fold decrease (p < 0.02). No significant change was noted in myocardial norepinephrine or dopamine content between the Control group and animals with smaller infarcts.

## Myocardial BNP and ANF expression

Larger infarcts were associated with increased expression of left ventricular BNP (p < 0.03; Fig. 5). Left ventricular expression of ANF was also increased (p = 0.023; Fig. 5). No significant change was noted in BNP or ANF expression in myocardial samples from animals with smaller infarctions (MI < 30 %).

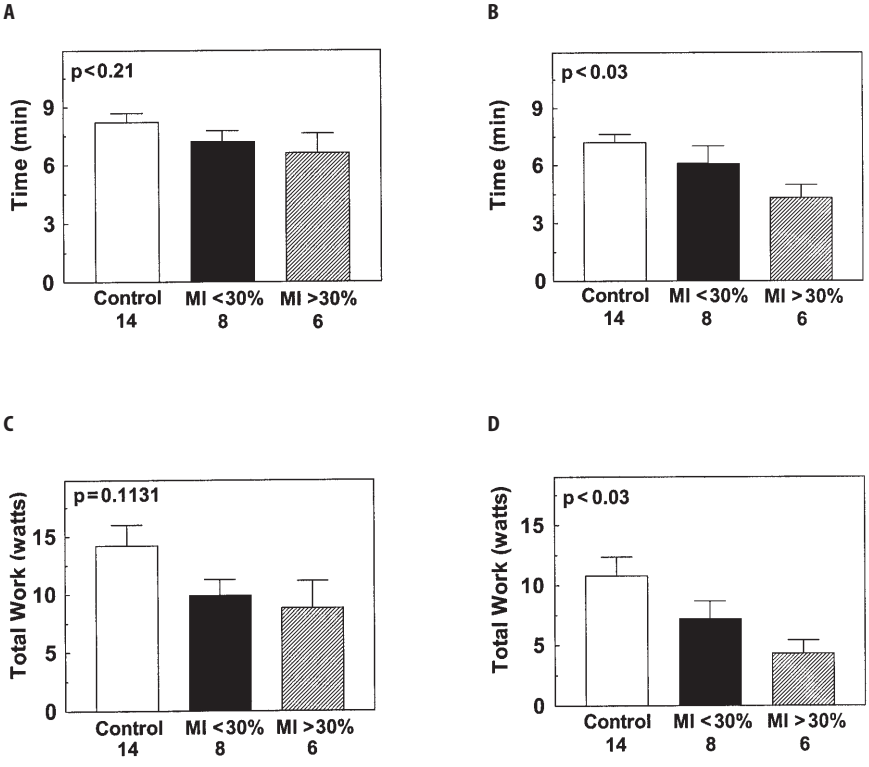


**Fig. 3** Exercise treadmill testing conducted 12 weeks (**A** and **C**) and 18 weeks (**B** and **D**) after myocardial infarction. Both ETT time and total work achieved decline between 12 and 18 weeks in mice with larger myocardial infarctions (MI > 30 %). Bars represent mean values, error bar denotes 1 SEM. P value from analysis of variance.

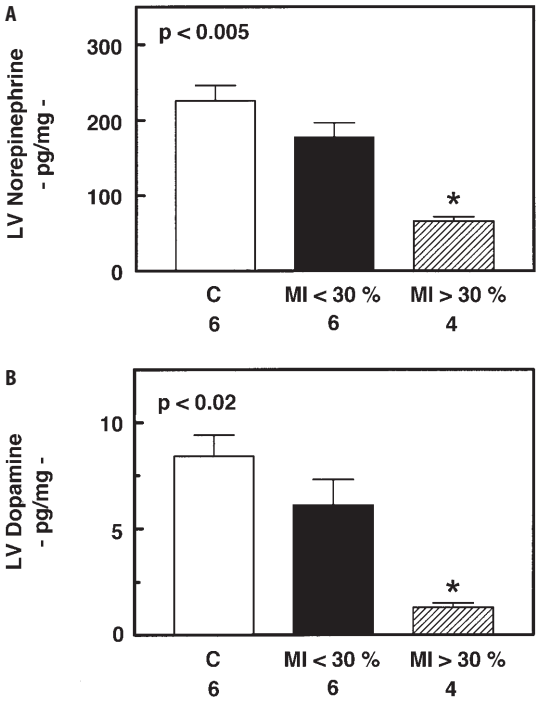


**Fig. 4** Animals with larger myocardial infarctions (MI > 30 % of the left ventricle) had reduced amounts of left ventricular (LV) norepinephrine (**A**) and dopamine (**B**). Epinephrine content (not shown) was unchanged. Bars represent mean values, error bar denotes 1 SEM. P value from analysis of variance; * p < 0.02, *post hoc* t-test (MI > 30 % vs MI < 30 %).

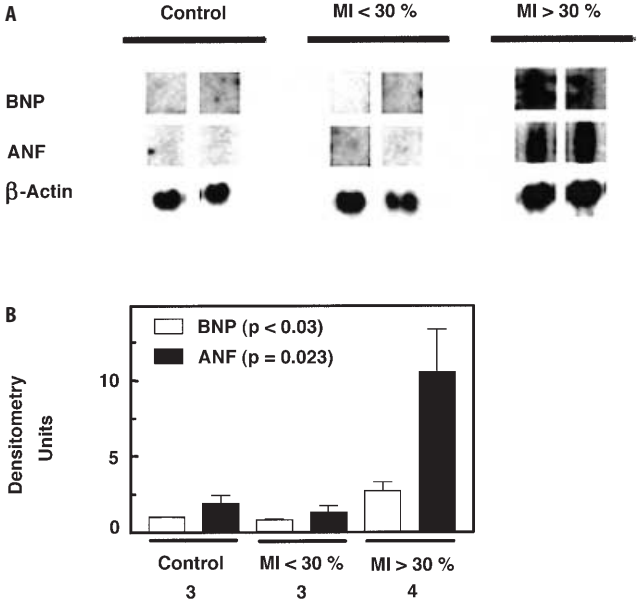


**Fig. 5** ANF and BNP mRNA expression was increased in left ventricular samples from animals with large infarcts (MI > 30 %). **A** Representative Northern blots of samples from two animals in each of the three study groups. **B** Data from all animals (normalized to β-actin expression) are summarized in the graph. Bars represent mean values, error bar denotes 1 SEM. P values are from analysis of variance.

## Discussion

This study provides sequential physiological and biochemical data regarding the progression of heart failure after myocardial infarction in mice. A goal of the study was to determine whether exercise treadmill testing would be a useful indicator of heart failure in this model. Exercise testing, a useful means to assess patients with heart failure, provides one of the sole methods of obtaining physiologically relevant data from conscious unsedated mice. In this regard, it may serve a suitable in-life indicator of heart failure, and provide a means to know when an animal should be treated (pharmacological agent, transgene activation or transgene delivery) in the setting of interventional studies that employ mice with heart failure from any cause. In addition to elucidating the functional impairment following large myocardial infarction, we also examined traditional biochemical and molecular indicators of heart failure. Large infarcts were associated with reduced myocardial content of norepinephrine, and increased expression of BNP and ANF in the heart. These three elements – impaired exercise performance, reduced myocardial norepinephrine content, and increased expression of myocardial BNP and ANF – mimic clinical heart failure due to myocardial infarction (2–4, 13, 17). An additional finding in the present study is that signs of heart failure, in animals with large myocardial infarctions that survive the first two weeks, appear to progress between week twelve and week eighteen.

The rationale for more complete evaluation of the long-term sequelae of MI in mice was two-fold. First, since myocardial infarction is the leading cause of clinical heart failure, we evaluated serially the development of heart failure after myocardial infarction in mice. Implicit in this decision is our view that such a model would have advantages over murine models that emanate from cardiac-directed overexpression or knockout of specific genes. A second advantage of the model is that it addresses a pitfall of cross breeding paradigms using transgenic mice, in which a so-called treatment effect may simply indicate that heart failure has been prevented from developing due to interactions between two transgenes during growth and development. By using infarct-associated heart failure in transgenic mice with cardiac-directed overexpression of a therapeutic transgene, or in mice with regulated expression of a therapeutic transgene, one can circumvent the shortcomings of cross breeding paradigms. The increasing number of transgenic mice strains provides ample opportunity to study heart failure, and an infarct-associated model of murine heart failure is desirable.

Important factors in evaluation of clinical heart failure include symptoms, physical examination, laboratory findings, and functional assessment, including measurement of exercise tolerance. The functional classification

in clinical heart failure has been the most important determinant of prognosis. Yet many of these elements (symptoms, physical examination, and laboratory findings) are impossible to obtain from mice or are severely limited by the inadequate amounts of blood that can reasonably be sampled over time. This underscores the importance of obtaining assessment of exercise capacity, thereby providing a useful parameter that should reflect overall cardiovascular function. The data provided in the current manuscript establish exercise treadmill testing as a useful in-life assessment for evaluating the extent of heart failure in this model.

LV structure and function after myocardial infarction in mice has been studied by histological examination two weeks (12), by hemodynamics in anesthetized mice six weeks (15, 18), by echocardiography nine weeks (8), by isolated perfused hearts six months (6), and by magnetic resonance imaging two weeks after myocardial infarction (21). These studies have provided useful information regarding the presence of LV dysfunction following MI, but have two potential shortcomings in documenting the presence of heart failure. First, heart failure is not defined solely by the presence of LV dysfunction. Second, the methods used require sedation and restraint and generally do not allow serial assessment of cardiovascular performance in individual animals. In the present study exercise testing – performed on unsedated unrestrained mice serially – was used to determine whether, in addition to LV dysfunction evident on echocardiograms, there was also evidence for heart failure *per se*.

Our finding that an infarct size greater than 30% of the LV is required to induce substantial adverse structural remodeling and functional decrements is in agreement with previously published data (6, 14, 18). In the current study we found, eighteen weeks after the intervention, that surviving mice with larger infarcts (but not smaller infracts) had myocardial hypertrophy, LV chamber dilation and tended to have heavier lungs, suggesting pulmonary congestion. A decrement in LV function (reduced fractional area change) was evident 6 weeks after large infarctions, but took 18 weeks to develop in animals with smaller infarctions. Exercise performance was significantly impaired at 12 weeks only in the large infarct group, and showed further steep decline from 12 to 18 weeks, suggesting that compensatory structural mechanisms are exhausted at 12 weeks, leading to more rapid decline in function thereafter.

The animals that died within the first two weeks after myocardial infarction showed evidence of severe congestive heart failure. While large infarcts (greater than 50% of LV) were lethal, small infarcts (less than 30% of LV) are not sufficient to induce adverse remodeling and functional impairment. Our data suggests that a narrow range of infarct size (30 – 50%) and 12 – 18 weeks are required to induce sufficient adverse LV remodeling leading to heart failure.

One limitation of the model is that of the animals that survive two weeks after myocardial infarction, just 40% develop signs of severe heart failure. However, the time and expense in generating these animals is entirely acceptable compared to models of heart failure in other species. For example, the current study was completed for a total animal cost of $600 US, and it took only 5 days to induce infarcts in the 50 animals used in this study. The advantage of having a suitable murine model of infarct-associated heart failure – *vis-à-vis* potential usefulness of transgenic lines – counterbalances other potential limitations.

In conclusion, this study describes a murine model of heart failure based on myocardial infarction – the most common cause of clinical heart failure. We have shown that exercise treadmill testing provides a useful means to evaluate sequentially the presence of heart failure in conscious unsedated animals. In addition reduced myocardial norepinephrine content and increased expression of myocardial BNP and ANF – elements also seen in clinical heart failure – are also present in this model. Finally, substantial additional LV remodeling and functional decrements occur in animals with larger infarctions, between week 12 and week 18.

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