Probiotic Administration Attenuates Myocardial Hypertrophy and Heart Failure Following Myocardial Infarction in the Rat

Gan et al: Probiotic Treatment Attenuates Heart Failure

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Abstract

**Background**—Probiotics are extensively used to promote gastrointestinal health and emerging evidence suggests that their beneficial properties can extend beyond the local environment of the gut. Here, we determined whether oral probiotic administration can alter the progression of post-infarction heart failure.

**Methods and Results**—Rats were subjected to six weeks of sustained coronary artery occlusion and administered the probiotic *Lactobacillus rhamnosus* GR-1 or placebo in the drinking water *ad libitum*. Culture and 16s rRNA sequencing showed no evidence of GR-1 colonization or a significant shift in the composition of the cecal microbiome. However, animals administered GR-1 exhibited a significant attenuation of left ventricular hypertrophy based on tissue weight assessment as well as gene expression of atrial natriuretic peptide. Moreover, these animals demonstrated improved hemodynamic parameters reflecting both improved systolic and diastolic left ventricular function. Serial echocardiography revealed significantly improved left ventricular parameters throughout the six week follow-up period including a marked preservation of left ventricular ejection fraction as well as fractional shortening. Beneficial effects of GR-1 were still evident in those animals in which GR-1 was withdrawn at four weeks suggesting persistence of the GR-1 effects following cessation of therapy. Investigation of mechanisms showed a significant increase in the leptin to adiponectin plasma concentration ratio in rats subjected to coronary ligation which was abrogated by GR-1. Metabonomic analysis showed differences between sham control and coronary artery ligated hearts particularly with respect to preservation of myocardial taurine levels.

**Conclusions**—The study suggests that probiotics offer promise as a potential therapy for the attenuation of heart failure.

**Key Words**: heart failure, cardiac hypertrophy, probiotics, microbiota, gut-heart axis
There is strong evidence that the nature of the microbiome plays an important role in the regulation of health and disease.¹ For example, there is evidence that cardiovascular disease can be modulated by changes in the gut microbiota, possibly due to alterations in the production of gut-derived hormones which exert cardiovascular effects and based on this, producing changes in the gut microbiota has been suggested as a possible approach for the treatment of heart failure.² Probiotics, defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host, exert numerous health benefits likely due to changes in the gut microbiota.²⁻⁵ However, there is a paucity of data with respect to the potential benefit of probiotics on cardiovascular health. Recently Lam and coworkers provided the first evidence that probiotics may be cardioprotective by showing that administration of a commercially-available beverage containing the probiotic Lactobacillus (L.) plantarum 299v twenty four hours before subjecting rats to thirty minutes ischemia and two hours reperfusion, produced a 27% reduction in infarct size and improved postischemic left ventricular function after reperfusion by 35%.⁶ A major consequence of myocardial infarction, particularly in the absence of timely tissue salvage by reperfusion is the development of cardiac hypertrophy and heart failure which occurs through chronic complex persistent remodelling subsequent to the initial insult.⁷ Although advances in therapy have improved survival rates from the initial myocardial infarction, mortality rates from heart failure remain high and are expected to rise.⁸ We hypothesized that probiotic treatment can alter the course of heart failure development following infarction, independently of myocardial salvage by reperfusion.
Methods

Animals

Male Sprague-Dawley rats weighing approximately 250 grams were randomly assigned to one of six treatment groups as outlined in the following section. The protocol was approved by the Animal Use Subcommittee of the University of Western Ontario and procedures adhered to the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada).

Induction of Heart Failure

Heart failure was induced as described previously. Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg bw, i.p.), intubated, and artificially ventilated using a rodent respirator (model 683, Harvard Apparatus). A left thoracotomy was performed and the heart was gently exposed. To induce myocardial infarction, the left main coronary artery was ligated approximately 3mm from its origin using a firmly tied silk suture. For sham operation, the ligature was placed in an identical fashion but not tied. Buprenorphine (0.03 mg/kg bw, i.p.) was immediately administered to all animals after surgery for pain management. All animals were housed singly per cage following surgery. The studies were completed after a total of six weeks of sustained CAL or sham surgery at which time animals were subjected to final echocardiography and catheter-based hemodynamic assessment before euthanasia.

Echocardiography

Echocardiography and body weight determinations were performed on each animal immediately prior to surgery and every two weeks thereafter until sacrifice. Rats were anesthetized with 2% isoflurane and echocardiography was performed as previously described using a Vevo 770 high-

4
resolution in vivo microimaging system equipped with a real-time microvisualization scan head of 17.5 MHz (VisualSonics, Toronto, Ontario, Canada). M-mode 2-dimensional echocardiography images were obtained from the parasternal short axis. Images were analyzed using the Vevo 770 Protocol-Based Measurements software and calculations for the dimensions of the left ventricle (LV) diameter. Doppler measurements were taken to determine peak early diastolic filling velocity (E wave), peak late diastolic filling velocity (A wave), and E/A ratios.

**Hemodynamic Measurements**

Hemodynamic measurements were taken six weeks after surgery. Animals were anesthetized with pentobarbital sodium (50 mg/kg bw). An anterior thoracotomy was performed and the LV was catheterized retrogradely via the right carotid artery using a 2.0F P-V Mikro-Tip catheter (Millar Instruments, Houston, TX) as previously described. Left ventricular pressure-volume relationships (P-V loops) were obtained by increasing afterload by a gradual occlusion of the ascending aorta.

**Probiotic Culture and Dosing Regimen**

Resuscitation and subsequent propagation of *L. rhamnosus* GR-1 was conducted in MRS broth (18 hours, 37°C under anaerobic conditions, GasPak™ BD). The organisms were then streaked onto MRS agar (Difco™ BD, Franklin Lakes, NJ). Single colonies were selected and used to inoculate 3 ml of MRS broth (incubated as above) and in turn used to inoculate 500 ml MRS broth. The cells were centrifuged (1600 x g for 20 minutes) and the pellet was resuspended in 25 ml sterile skim milk (10% NFDM, Nestlé, Vevey, Switzerland) to a concentration of $3.0 \times 10^{10}$ CFU/ml. Aliquots of 100 μL of GR-1 in skim milk, or skim milk only for control groups were
stored at -20°C until use. GR-1 was administered in the drinking water ad libitum immediately after completion of surgery in order to provide an approximate daily dose of $1 \times 10^9$ CFUs based on water consumption of approximately 50 ml/day. For most studies, treatments were carried out for the entire six week follow-up period, although an additional study entailed the probiotic being withdrawn after four weeks and replaced with control drinking water for the remaining two weeks. Each of the six treatment groups had a sample size of 10.

**Plasma analyses of Leptin and Adiponectin**

Immediately following hemodynamic measurements and prior to sacrifice, blood was collected directly from the heart and kept on ice. Plasma samples were brought to room temperature and assayed for leptin and adiponectin using commercially available ELISA kits (Enzo Life Sciences, Plymouth Meeting, PA).

**Heart Weight Measurement and Tissue Processing**

Hearts were removed after hemodynamic measurements and weighed. Fifty to one hundred mg of tissue from the left ventricle (non-ischemic region) was collected and stored at -80°C for later determination of ANP expression as an index of hypertrophy and metabonomic analysis.

**Cecum Digesta Sample Collection and Analyses**

After sacrifice, the cecum was removed immediately. Cecum digesta (0.3 g each) was resuspended in 1 mL sterile 1X phosphate buffered saline. Serial dilutions ($10^3$-$10^6$) were made and 10 µL of each dilution were drop-plated on MRS agar (Difco™, BD) containing 32 µg/mL fusidic acid (Sigma-Aldrich, Oakville, Ontario, Canada) sterilized using a 0.2 µm filter. Plates
were incubated at 37°C anaerobically (GasPak™, BD) for 48 hours. GR-1 colonies were identified and enumerated.

The hypervariable V6 region of the 16S rRNA gene from each DNA sample was amplified using left forward 5’ primers each tagged with a unique barcode sequence as previously described. PCR was performed as described and amplification products were quantified using Qubit® to determine DNA concentration, and equal molar quantities were mixed and sequenced using the Ion Torrent platform (Life Technologies, Carlsbad, CA). Raw sequence data were filtered, processed, and analysed as previously described. Taxonomic assignments were made using Seqmatch from the Ribosomal Database Project which were verified using the Greengenes database. Taxonomic assignments were arranged and presented using QIIME for 16S rRNA analysis. Communities from each sample were compared using weighted UniFrac beta-diversity analysis.

1H NMR spectroscopic analysis of heart tissue

Tissue extractions were performed as previously described. Briefly, 30 mg of heart tissue was dissected from the left ventricle and homogenized in 300 μL of chloroform:methanol (2:1). The homogenate was combined with 300 μL of water, vortexed and centrifuged (13,000 g for 10 minutes) to separate the aqueous and organic phases. Water was removed by vacuum and reconstituted in 550 μL of phosphate buffer (pH 7.4) in 100% D2O containing 1mM of the internal standard, 3-(trimethylsilyl)-[2,2,3,3,2H4]-propionic acid. For each sample, a standard one-dimensional NMR spectrum was acquired with water peak suppression using a standard pulse sequence (recycle delay-90°-t1-90°-tsm-90°-acquire free induction decay). RD was set as 2 s,
the 90° pulse length was 7.7 μs, and the mixing time (t_m) was 10 ms. For each spectrum, eight dummy scans were followed by 128 scans with an acquisition time per scan of 2.91 s and collected in 64K data points with a spectral width of 20 ppm.

1H NMR spectra were manually corrected for phase and baseline distortions and then digitized using an in-house MATLAB (version R2009b, The Mathworks Inc, Natick, MA) script. Spectra were aligned as described previously17 to adjust for subtle shifts in peak position and each spectrum was normalized using a probabilistic quotient approach. Principal components analysis (PCA) was performed with Pareto scaling in MATLAB using scripts (Korrigan Sciences Ltd Maidenhead, UK). The contribution of each metabolite to sample classification was visualized by back-scaling transformation, generating a correlation coefficient plot. These coefficient plots are colored according to the significance of correlation to treatment with red indicating high significance and blue indicating low significance. For all models, one orthogonal component was used to remove systematic variation unrelated to class. Predictive performance was assessed using a 7-fold cross validation approach and the Q^2Y (goodness of prediction) values are provided.

Statistical analysis

Data reported are means ± S.E. Data were analyzed using a one-way ANOVA followed by a post hoc Tukey test to determine the effect of CAL and potential influence of GR-1. Echocardiographic data were analyzed using 2-way ANOVA with repeated measures and a post hoc Tukey test. All variables analyzed were assumed to be approximately normally distributed. Differences were considered significant when P < 0.05.
Results

Effect of treatments on body weight gain

None of the treatments exerted any effect on body weight growth throughout the six week post-surgery period with identical body weights observed throughout the six week post-surgery period, irrespective of treatment (not shown).

Effect of Probiotic Administration on Intestinal Microbial Composition

A total of 242 distinct organisational taxonomic units (OTU) groupings were identified. *L. rhamnosus* was not detected in any of the 60 individual samples (Figure 1A). Overall, there was no distinct grouping of community compositions in any of the 60 samples with respect to probiotic administration nor was there distinct grouping of communities in cecum digesta samples of rats that received CAL surgery versus sham surgery (Figure 1B and 1C) suggesting no changes in the microbial composition of the gut. *L. rhamnosus* GR-1 was readily cultivated from fresh cecum digesta samples on semi selective MRS agar containing fusidic acid. Presumptive colonies were identified based on size and morphology and enumerated (Table 1). Colonies with GR-1-like morphology were not detected in samples of rats on the control vehicle treatment (containing only water or skim milk). Thus, when taken together the data in Table 1 and Figure 2 demonstrate that GR-1 was present and alive in the cecum (Table 1), yet did not colonize or change the existing composition of the cecum microbiota (Figure 1).

Probiotic Supplementation Attenuates Cardiac Hypertrophy

CAL significantly increased left ventricular (LV) weight and produced a marked increase in gene expression of atrial natriuretic peptide (ANP) thus indicating a robust hypertrophic response at
the end of the six week follow-up period (Figure 2). However, animals treated with GR-1 showed near-normalized LV to body weight ratio and significantly reduced ANP expression.

**Probiotic Supplementation Attenuates LV Dysfunction Following Coronary Artery Ligation**

E/A ratios, indicative of trans-mitral valve blood flow properties, were increased in rats subjected to CAL although this was significantly attenuated by GR-1 (Figure 3 A and B). In addition, LV internal diameters during systole (LVIDs) and diastole (LVIDd) were significantly increased during the six week CAL period although these were significantly blunted by GR-1 treatment (Figure 3 C to E). CAL induced a significant reduction in both ejection fraction and fractional shortening of approximately 25% and 30%, respectively at the end of the six week post infarction period although these effects were significantly attenuated by GR-1 treatment (Figure 3 F and G).

Hemodynamic analyses indicate significant systolic and diastolic dysfunctions in animals subjected to six weeks of CAL (Figure 4). These effects were significantly but not completely reduced by probiotic treatment with respect to all parameters measured.

Pressure-volume loops for these experiments are shown in Figure 5, panels A to D. The end systolic pressure volume relationship (ESPVR) was substantially less steep in animals subjected to six weeks CAL whereas the end diastolic pressure volume relationship (EDPVR) was increased indicating a reduction in contractile function and increased diastolic stiffness, respectively. However, these changes were significantly attenuated by GR-1 treatment. As
shown in Figure 5, panels E and F, preload recruitable stroke work (PRSW) an index of LV contractility, was significantly depressed in control infarcted animals whereas the isovolumetric relaxation time (T\textsubscript{au}) was increased, although these responses were significantly attenuated by probiotic administration.

**Probiotic Supplementation Prevents the Increased Leptin to Adiponectin Plasma Concentration Ratio**

Figure 6 shows that CAL significantly increased plasma leptin concentrations in the control group but not the GR-1 group whereas adiponectin levels were unaffected. Surprisingly, a trend towards elevated leptin levels was observed in sham-operated animals provided GR-1, although the differences were not statistically significant. Assessment of the leptin to adiponectin plasma concentration ratio revealed a significant two-fold increase in this relationship. The increase in plasma leptin concentrations and the corresponding increase in the leptin to adiponectin ratio seen six weeks after CAL were completely prevented by GR-1 treatment.

**Probiotic Supplementation Improves Cardiac Metabolic Profile**

Based on PCA, differences were observed between the cardiac metabolic profiles of sham-operated and CAL animals treated with water (Figure 7). This metabolic distinction was weakened when CAL animals received milk (Figure 8B) and the probiotic GR-1 (Figure 7 C). Pair-wise orthogonal projection to latent structure-discriminant analysis (OPLS-DA) models reinforce this observation. A model with good predictive strength was returned comparing the water sham and water CAL animals (Figure 7D, Q\textsuperscript{2}Y = 0.498, P < 0.001). Here, sustained CAL increased the amount of creatinine in the heart tissue and decreased the amount of glutamine, alanine, taurine, scyllo-inositol, inosine and total creatine. Models with weaker predictive ability
were obtained when comparing water sham with milk CAL animals ($Q^2Y = 0.2112, P = 0.07$) and water sham with probiotic CAL animals ($Q^2Y = 0.2298, P = 0.09$) indicating both milk and GR-1 dampened the metabolic consequences of CAL.

**Maintenance of Anti-remodelling Effect Following Probiotic Withdrawal**

We also conducted experiments in which the probiotic GR-1 was administered for only a four week period and then withdrawn for the remaining two weeks. These results are summarized in Table 2 and show that the reduction in hypertrophy and the associated improvement in left ventricular function are evident two weeks after GR-1 withdrawal. Indeed, the improvement in many parameters was very similar to that seen in animals treated with GR-1 for the entire six week post-CAL period.
Discussion

In this report we show that administration of a probiotic attenuates post-infarction remodelling and heart failure in rats subjected to sustained CAL. *L. rhamnosus* GR-1 was selected because of its immune-modulatory activity via the gut as well as our extensive experience with this probiotic strain. A preliminary echocardiography-based study carried out in our laboratory but not reported here, demonstrated identical benefit of GR-1 and the probiotic *L. plantarum* 299v in rats subjected to four weeks of CAL. Thus, the beneficial effect of GR-1 is likely shared by other probiotics.

The anti-remodelling effect of GR-1 was evidenced by improved hemodynamic systolic and diastolic properties, improved cardiac function assessed by serial echocardiography and reduced hypertrophy whereas GR-1 alone was completely without effect in sham-operated rats nor did it reduce blood pressure in either sham or coronary artery ligated animals, thus precluding a potential benefit of afterload reduction. The benefit of GR-1 likely involves a direct influence on the myocardium possibly secondary to reductions in gastrointestinal-derived pro-hypertrophic factors. This distant site concept has received particular attention in terms of the “gut-brain axis” in which probiotic administration alters mood behaviour through effects on the central nervous system. The possibility exists that similar gut-derived messages may affect the cardiovascular system.

Emerging evidence suggests that metabolic therapy could confer benefit for treating heart failure in view of numerous alterations in intermediary metabolism seen in the failing myocardium. Myocardial metabonomic assessment showed that CAL produced a number of distinct
myocardial changes including elevations in creatinine and reductions in tissue taurine, glutamine, alanine, scyllo-inositol, inosine and total creatines which were attenuated by probiotic treatment, and to a lesser degree by skim milk. The reduction in creatine levels in the failing myocardium has been demonstrated in both experimental and clinical heart failure, likely secondary to changes in the creatine transporter. However, creatine deficiency produced by deletion of the biosynthetic enzyme guanidinoacetate N-methyltransferase failed to exert any effect on postinfarction survival or LV remodelling and dysfunction after CAL. Thus, at present the functional significance of creatine in the failing myocardium and its preservation by probiotic administration is difficult to appreciate particularly as this relates to postinfarction remodelling and the evolution to heart failure.

The other potential benefit of probiotic administration in the postinfarcted myocardium revealed from metabonomic analyses is the preservation of myocardial taurine content. While taurine is the most abundant amino acid in the heart its physiological role is far from clear. However, taurine likely plays an important role in heart failure especially as taurine deficiency results in LV dysfunction, an effect reversed by dietary taurine supplementation. Taurine can directly inhibit hypertrophy produced by angiotensin II in ventricular myocytes and taurine administration improves LV function in heart failure patients. Overall however, the precise role of taurine in postinfarction remodelling requires further investigation particularly as it pertains to taurine preservation with probiotic administration in the postinfarcted myocardium.

Emerging evidence suggests that adipokines, including leptin and adiponectin, play important roles in cardiovascular regulation and modulate the progression of cardiovascular disease.
Of particular relevance to our study, a significant reduction in plasma concentrations of the pro-
satiety adipokine leptin in rats provided with a probiotic beverage was recently reported
suggesting that this mediated the cardioprotective effect of probiotic administration on infarct
size reduction, a finding reinforced by reversal of cardioprotection with exogenously-
administered leptin.\footnote{6} With respect to heart failure, various studies have shown that leptin exerts
hypertrophic effects under different experimental conditions.\footnote{28} Furthermore, clinical studies
have shown that heart failure is associated with hyperleptinemia,\footnote{30, 31} and elevated leptin has
been proposed as a risk factor for heart failure.\footnote{31} A recent report showed implicated leptin to the
development of heart failure in obese men with no history of pre-existing coronary heart
disease,\footnote{32} suggesting that leptin directly contributes to the development of heart failure in obese
individuals.\footnote{33} Our study showed a significant increase in plasma leptin concentrations in rats
subjected to CAL which was prevented by GR-1 with no effect on adiponectin concentrations.
At the same time, it was surprising to observe that plasma leptin levels tended to increase in
sham-operated animals provided GR-1 when compared to their respective control group. As
such, it is difficult at the present time to assign a specific role for leptin, or indeed other
adipokines, to the salutary effect of probiotic administration on heart failure. However we
believe that absolute plasma concentration values of individual adipokines may be of lesser
importance than their concentrations relative to each other. Indeed, in this regard there is now
extensive evidence in both animal and clinical studies that the leptin to adiponectin ratio
represents a stronger index for a number of cardiovascular- and metabolic-related morbidities
than each component alone.\footnote{34-36} Our results show for the first time, a substantial increase in the
leptin to adiponectin ratio in animals subjected to CAL which was normalized by probiotic
administration. Whether this reflects a cause and effect relationship with respect to the ability of
GR-1 to ameliorate heart failure cannot be definitively ascertained. Extending our finding to the clinical scenario must be done with caution, but our data suggest a potential mechanism where probiotics may slow the progression of heart failure.

A potential limitation of our study is that we did not employ irradiated GR-1 as a control group in order to demonstrate the necessity for live bacteria or that the effects reported here were not due to an immunological effect that could take place with dead bacteria. We also did not detect major changes in the gut microbial composition between treatment groups, which suggests that the GR-1 strain did not colonize and that this is not a prerequisite for its beneficial effect.

Preliminary unpublished studies by our group suggest that probiotics could directly attenuate the hypertrophic response, possibly via the release of anti-hypertrophic factors. In this regard, ventricular myocytes co-cultured with GR-1 demonstrated improved viability over time, although of more relevance to the present study, these myocytes were completely unresponsive to the hypertrophic effect either of the $\alpha_1$ adrenoceptor agonist phenylephrine or hydrogen peroxide. These studies are currently attempting to identify a GR-1 derived factor(s) as a potential anti-hypertrophic agent(s).

In summary, the present study is the first to report salutary effects of probiotic administration to rats subjected to prolonged coronary artery occlusion culminating in cardiac hypertrophy as well as systolic and diastolic left ventricular dysfunction. The underlying mechanisms for these effects are likely very complex and multifaceted but initial evidence suggests improved myocardial metabolic status including tissue taurine preservation as well as a favourable reduction in the leptin to adiponectin plasma concentration ratio. Although hearts were not
reperfused in the current study, the possibility cannot be ruled out that infarct size reduction contributed to the salutary effect of GR-1 on left ventricular function. Whether our findings apply to other animal species or human heart failure remains to be determined. However, the widespread availability of probiotic preparations may facilitate their testing as a treatment for heart failure particularly in combination with existing therapies. The potential benefit of this conjoint approach includes improvement of therapeutic efficacy as well as the possibility of reduced dosing of existing medications thus minimizing their potential for adverse effects. These concepts warrant further study.

Acknowledgments

We acknowledge the assistance of Julia Di Bella and Jordan Bisanz for providing their technical expertise.

Sources of Funding

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Disclosures

None.
References

Table 1. Cultivation of *L. rhamnosus* GR-1 from cecum digesta with different treatment groups six weeks after surgery

<table>
<thead>
<tr>
<th>Treatment Group (n=10)</th>
<th>CFU/ml GR-1</th>
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<tr>
<td>Sham surgery/water</td>
<td>Not detected</td>
</tr>
<tr>
<td>Sham surgery/GR-1</td>
<td>$8.71 \times 10^7$</td>
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<tr>
<td>Coronary artery ligation/water</td>
<td>Not detected</td>
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<tr>
<td>Coronary artery ligation/GR-1</td>
<td>$6.73 \times 10^7$</td>
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<tr>
<td>Coronary artery ligation/skim milk</td>
<td>Not detected</td>
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<tr>
<td>Coronary artery ligation/4 weeks GR-1 + 2 weeks skim milk</td>
<td>$7.56 \times 10^7$</td>
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Table 2. Indices of hypertrophy, hemodynamics and echocardiographic parameters in animals subjected to six weeks CAL and treated for the first four weeks with *L. rhamnosus* GR-1.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CAL</th>
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<tr>
<td><strong>Hypertrophy:</strong></td>
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<td></td>
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<tr>
<td>LVW/BW (g/g)</td>
<td>0.943 ± 0.02</td>
<td>1.36 ± 0.04</td>
<td>1.09 ± 0.03</td>
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<td>ANP/18s (pg/g)</td>
<td>0.94 ± 0.39</td>
<td>8.87 ± 0.73</td>
<td>4.17 ± 0.73</td>
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<tr>
<td><strong>Hemodynamics:</strong></td>
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<tr>
<td>LVEESP (mm Hg)</td>
<td>118 ± 2.9</td>
<td>74.2 ± 2.6*</td>
<td>91.4 ± 2.4#</td>
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<td>LVEDP (mm Hg)</td>
<td>2.85 ± 0.36</td>
<td>10.6 ± 0.75*</td>
<td>7.1 ± 0.9#</td>
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<td>+dP/dt (mm Hg/sec)</td>
<td>8874 ± 451</td>
<td>4982 ± 247*</td>
<td>6184 ± 409#</td>
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<td>-dP/dt (mm Hg/sec)</td>
<td>7732 ± 140</td>
<td>4948 ± 409*</td>
<td>6636 ± 380#</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>386 ± 8.9</td>
<td>384 ± 10.9</td>
<td>386 ± 11.5</td>
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<tr>
<td>Cardiac output (ml/min)</td>
<td>51.3 ± 1.4</td>
<td>28.4 ± 1.7*</td>
<td>39.1 ± 2.5#</td>
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<tr>
<td>Stroke volume (μl)</td>
<td>132 ± 4.8</td>
<td>73 ± 6.1*</td>
<td>102 ± 7.39#</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>105 ± 0.84</td>
<td>95.2 ± 2.11*</td>
<td>98.5 ± 1.56</td>
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<td>SBP (mm Hg)</td>
<td>123 ± 1.55</td>
<td>109 ± 3.26*</td>
<td>111 ± 2.58</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>98 ± 1.47</td>
<td>89 ± 2.29</td>
<td>93 ± 1.93</td>
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<tr>
<td>ESPVR (mm Hg/μl)</td>
<td>0.678 ± 0.67</td>
<td>0.35 ± 0.07*</td>
<td>0.594 ± 0.04#</td>
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<td>EDPVR (mm Hg/μl)</td>
<td>0.042 ± 0.009</td>
<td>0.078 ± 0.008*</td>
<td>0.056 ± 0.009#</td>
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<td>PRSW (mm Hg)</td>
<td>81.6 ± 5.0</td>
<td>56.0 ± 2.67*</td>
<td>69.5 ± 3.81#</td>
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<td>Tau (msec)</td>
<td>8.5 ± 0.61</td>
<td>12.5 ± 1.07*</td>
<td>9.90 ± 0.04</td>
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<td><strong>Echocardiography:</strong></td>
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<td></td>
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<tr>
<td>E/A ratio</td>
<td>1.38 ± 0.04</td>
<td>1.91 ± 0.20*</td>
<td>1.58 ± 0.06#</td>
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*P < 0.05 compared to Sham
#P < 0.05 compared to CAL
<table>
<thead>
<tr>
<th>Measure</th>
<th>Sham Group</th>
<th>CAL Treatment Group</th>
<th>CAL No Treatment Group</th>
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<tr>
<td>LVIDd</td>
<td>7.73 ± 0.22</td>
<td>8.58 ± 0.22*</td>
<td>7.92 ± 0.17#</td>
</tr>
<tr>
<td>LVIDs</td>
<td>4.27 ± 0.24</td>
<td>5.48 ± 0.25*</td>
<td>4.45 ± 0.19#</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>76.3 ± 2.10</td>
<td>62.3 ± 2.65*</td>
<td>71.0 ± 2.19#</td>
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<tr>
<td>Fractional shortening (%)</td>
<td>47.23 ± 0.24</td>
<td>35.39 ± 2.47*</td>
<td>46.23 ± 2.13#</td>
</tr>
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</table>

Data are presented as mean ± SEM (N=10). *P<0.05 versus sham group. #P<0.05 versus CAL no treatment group.
Figure Legends

Figure 1. Microbiome analysis of cecum digesta. (A) Heat map displaying the 50 most abundant OTUs detected in cecum digesta samples. (B) Weighted β-diversity UniFrac analysis-generated PCoA plots display dissimilarities in community compositions of each sample. (C) PCoA plot comparing the communities from animals on GR-1 treatment versus control treatment (skim milk or water).

Figure 2. Reduction of hypertrophy by L. rhamnosus GR-1. Top panel shows the left ventricle to body weight ratios (LV/BW) in animals subjected to six weeks of coronary artery ligation or to sham procedure. Corresponding values for LV gene expression of the hypertrophic marker atrial natriuretic peptide (ANP) as a ratio to 18S expression are provided in the bottom panel. Data are presented as mean ± SEM (N=10). *P<0.05 versus sham group. #P<0.05 versus CAL no treatment group.

Figure 3. Serial echocardiography analyses following CAL. (A, B), representative Doppler images for transmitral velocity with corresponding quantified results, respectively. (C-E), M-mode images and corresponding diastolic and systolic left ventricular internal diameters (LVIDd, LVIDs) quantified data, respectively. (F, G) Ejection fractions and fractional shortening data obtained biweekly throughout the six week post-ligation period. *P<0.05 versus CAL + GR-1 and sham group; +P<0.05 from week zero. For E/A ratios values at two and six weeks were significantly different from week 0 whereas all values were significantly higher from week 0 for both LVIDd and LVIDs.
**Figure 4.** Hemodynamic assessment six weeks post CAL. Data are presented as mean ± SEM (N=10). *P<0.05 versus respective sham group. #P<0.05 versus control CAL group. LVSP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt\(_{\text{max}}\) maximum of the first derivative of LV pressure development over time; -dP/dt\(_{\text{min}}\), minimum of the first derivative of LV pressure over time; MAP, mean arterial pressure; SBP, systolic blood pressure; diastolic blood pressure; DBP, diastolic blood pressure.

**Figure 5.** Pressure-volume (P-V) relationships, preload-recruitable stroke work (PRSW) and isovolumetric relaxation time (Tau) six weeks post CAL. Panels A to D show P-V relationships in sham animals (A), animals subjected to CAL (B) or both conditions in animals fed GR-1 (C,D). For each treatment group the end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) values are shown. PRSW and Tau are shown in panels E and F, respectively. Data are presented as mean ± SEM (N=6-8). *P<0.05 versus respective sham group. #P<0.05 versus control CAL group.

**Figure 6.** Plasma leptin and adiponectin concentrations the leptin-adiponectin ratio six weeks post CAL. Data are presented as mean ± SEM (N=8). *P<0.05 versus control sham group. #P<0.05 versus control CAL group.

**Figure 7.** Pair-wise comparisons of the metabolic profiles from water-treated sham-operated hearts with the different treated CAL hearts. PCA scores plots comparing water-treated sham-operated hearts with (A) water-treated CAL hearts, (B) milk-treated CAL hearts and (C) GR-1 treated CAL hearts. (D) OPLS-DA coefficients plot highlighting metabolic variation between
water-treated sham operated hearts versus water-treated CAL hearts. OPLS-DA model $Q^2_Y = 0.498$ and $p < 0.001$ (10,000 permutations).
Figure 1
**Figure 3**

(A) Examples of echocardiographic images showing diastolic filling patterns, with E/A ratios indicated.

(B) Graph showing the E/A ratio over weeks with significant differences indicated.

(C) Examples of LV images demonstrating LV end-diastolic and end-systolic dimensions (LVIDs and LVIDd) over weeks.

(D) Graph showing changes in LVIDs with significant differences indicated.

(E) Graph showing changes in LVIDd with significant differences indicated.

(F) Graph showing changes in ejection fraction with significant differences indicated.

(G) Graph showing changes in fractional shortening with significant differences indicated.
**Figure 4**

Graphs showing changes in various hemodynamic parameters:
- **LVSP**: Left Ventricular Systolic Pressure
- **LVEDP**: Left Ventricular End Diastolic Pressure
- **MAP**: Mean Arterial Pressure
- **SBP**: Systolic Blood Pressure
- **+dP/dt_max**
- **-dP/dt_min**
- **Cardiac Output**
- **Stroke Volume**
- **DBP**: Diastolic Blood Pressure

Bars represent control and GR-1 groups, with significant differences indicated by asterisks (*) and hash symbols (#).

Legend:
- Sham
- CAL
Figure 5

Control GR-1

Sham CAL

Sham+GR-1

CAL+GR-1
Plasma Leptin (ng/ml)

Control | GR-1
---|---
Sham | CAL

Plasma Adiponectin (ng/ml)

Control | GR-1
---|---

Plasma Leptin/Adiponectin

Control | GR-1
---|---

Figure 6
Figure 7
Probiotic Administration Attenuates Myocardial Hypertrophy and Heart Failure Following Myocardial Infarction in the Rat
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