Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure

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Yin M, van der Horst IC, van Melle JP, Qian C, van Gilst WH, Silljé HH, de Boer RA. Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure. Am J Physiol Heart Circ Physiol 301: H459–H468, 2011. First published May 13, 2011; doi:10.1152/ajpheart.00054.2011.—Metformin is the first choice drug for the treatment of patients with diabetes, but its use is debated in patients with advanced cardiorenal disease. Epidemiological data suggest that metformin may reduce cardiac events, in patients both with and without heart failure. Experimental evidence suggests that metformin reduces cardiac ischemia-reperfusion injury. It is unknown whether metformin improves cardiac function (remodeling) in a long-term post-MI remodeling model. We therefore studied male, nondiabetic, Sprague-Dawley rats that were subjected to either myocardial infarction (MI) or sham operation. Animals were randomly allocated to treatment with normal water or metformin-containing water (250 mg·kg⁻¹·day⁻¹). At baseline, 6 wk, and 12 wk, metabolic parameters were analyzed and oral glucose tolerance tests (OGTT) were performed. Echocardiography and hemodynamic parameters were assessed 12 wk after MI. In the MI model, infarct size was significantly smaller after 12-wk metformin treatment (29.6 ± 3.2% vs. 38.0 ± 2.2%, P < 0.05). Moreover, metformin resulted in less left ventricular dilatation (6.0 ± 0.4 vs. 7.6 ± 0.6 mm, P < 0.05) and preservation of left ventricular ejection fraction (65.8 ± 3.7% vs. 48.6 ± 5.6%, P < 0.05) compared with MI control. The improved cardiac function was associated with decreased atrial natriuretic peptide mRNA levels in the metformin-treated group (50% reduction compared with MI, P < 0.05). Insulin resistance did not occur during cardiac remodeling (as indicated by normal OGTT) and fasting glucose levels and the pattern of the OGTT were not affected by metformin. Molecular analyses suggested that altered AMP kinase phosphorylation status and low insulin levels mediate the salutary effects of metformin. Altogether our results indicate that metformin may have potential to attenuate heart failure development after myocardial infarction, in the absence of diabetes and independent of systemic glucose levels.

Metformin decreases blood glucose by enhancing insulin sensitivity, inducing greater peripheral uptake of glucose, and decreasing hepatic glucose output while lowering plasma insulin concentrations (17, 33). The cardioprotective effects of metformin can, however, not be attributed to the glucose-lowering effects alone (12). Recent experimental studies suggested ancillary potential mechanisms. More specifically, the protective effects may be conferred via the AMP-activated protein kinase (AMPK) pathway (3, 12, 27, 29, 31, 38), which is activated by metformin. Especially, in ischemia-reperfusion (I/R) models, metformin was shown to decrease the cardiomyocyte apoptosis (16, 29), to improve endothelial function by increasing NO production (12, 29, 39), to maintain myocardial energy production during ischemia (13), and to affect lipid metabolism (15, 32, 37). Clinical studies show that metformin may reduce plasma dipeptidyl peptidase-4 activity and increase circulating levels of glucagon-like peptide 1 (GLP-1), which is an incretin hormone that has protective effects on the heart and the vasculature (4, 23, 28). Experimental studies employing a pressure-overload model in mice and a pacing model in dogs showed that metformin attenuates cardiac fibrosis by inhibiting collagen synthesis (29, 38).

These experimental studies strongly suggest that metformin reduces I/R injury. However, little evidence is available to support the use of metformin in chronic cardiac remodeling, e.g., after myocardial infarction (MI) and in HF.

To further investigate the role of metformin in HF we conducted a long-term study to determine the effects of metformin on cardiac function and metabolic parameters in a post-MI rat model.

METHODS

Design of the study. Sprague-Dawley rats (weight 250–260 g, Harlan) were randomly allocated to left coronary artery ligation (MI) or sham surgery. The sham procedure was identical to the MI group except that the ligation was not tied (6). Two days before surgery, rats were fed metformin-containing water (250 mg·kg⁻¹·day⁻¹) or normal water, and this was continued for a period of 12 wk, rendering four experimental groups: sham (n = 7), sham+metformin (n = 7), MI (n = 8), and MI+metformin (n = 9). The animals were further fed ad libitum with a standard rat diet and housed in groups of four to five rats under a 12:12-h light-dark cycle. All animals received standard care, and the experimental protocol was reviewed and approved by the local Animal Ethical Committee of the University Medical Centre Groningen.

At baseline, week 6, and week 12, an oral glucose tolerance test (OGTT) was performed. At week 12, cardiac function was determined by echocardiography. After 12 wk the rats were euthanized after measurement of invasive hemodynamic parameters, and the hearts were rapidly excised, weighed, and prepared for histochemistry and molecular analysis.

Oral glucose tolerance test. An OGTT was performed in nonanesthetized rats. After 16-h fasting, glucose (2 g/kg as a 50% solution) was given orally to the animal. Blood was obtained from the tail to
measure glucose levels at several time points: 0, 5, 15, 30, 60, and 120 min after the glucose administration. Glucose levels were measured with a blood glucose monitor (Accu-Check, Roche).

Echocardiographic measurements. Echocardiographic measurements were performed by an individual blinded researcher to the treatment groups. Rats were anesthetized with 2.5% isoflurane in a gas mixture of N2O-O2. The M-mode and 2D echocardiography images were obtained with a high-resolution system (Vivid 7, GE, Healthcare, and Chalfont St Giles, UK), by use of a 10 MHz transducer. Diastolic and systolic measurements of left ventricle (LV) internal dimensions (LVIDd and LVIDs, respectively), as well as measurements of the thickness of the interventricular septum (IVSs, IVSd) and posterior wall.

Table 1. Primer sequences used in real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>5’-3’ Forward</th>
<th>5’-3’ Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>ATGGGCTCCTTCTCCATCAC</td>
<td>TCTACCGGAGTCTCTTCTC</td>
</tr>
<tr>
<td>eNOS</td>
<td>GACCTTTAAAGAGGAAAGGGGAAATGCA</td>
<td>GTGGAAGGAACTATCCCTTGTATGGCTC</td>
</tr>
<tr>
<td>Collagen I</td>
<td>ACAGGGTAGCCTACATGG</td>
<td>AATTTCCGGGTGGACCTGC</td>
</tr>
<tr>
<td>Procollagen</td>
<td>GAGGGCGAGTGGCTGGCTT</td>
<td>GGGCTGTGGACCTTGACTTC</td>
</tr>
<tr>
<td>36B4</td>
<td>GGGCTCAGTGGCTGCTC</td>
<td>GGAAGGGGCAAATGGGATGG</td>
</tr>
</tbody>
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ANP, atrial natriuretic peptide; eNOS, endothelial nitric oxide synthase; collagen I, collagen type I; 36B4, acidic ribosomal phosphoprotein P0.

Fig. 1. Metformin levels, glucose levels, insulin levels, myocardial glycogen content, and body weight gain at 12 wk after myocardial infarction (MI) surgery. A: serum metformin (Met) levels (top); fasting glucose levels (middle), and plasma insulin level (bottom). B: oral glucose tolerance tests were performed at baseline, 6 wk, and 12 wk after MI surgery. No significant differences were found among all groups. C: myocardial glycogen content. D: use of metformin is associated with attenuated body weight gain. *P < 0.05 vs. sham group; #P < 0.05 vs. MI group.
(LVW, LVPWd) were recorded. LV fractional shortening (FS \%) was calculated as $FS = \frac{LVIdd - LVIds}{LVIdd} \times 100\%$. LV ejection fraction (EF \%) was calculated by using the Teichholz method of estimated LV volumes (20).

**Hemodynamic measurement.** Before euthanasia, rats were anesthetized and invasive hemodynamics were measured as described (7). Briefly, a microtip catheter with pressure transducer (2-Fr, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the LV cavity. After 5 min stabilization, the heart rate, LV end-systolic (LVESP) and end-diastolic pressures (LVEDP), and developed LV pressure ($dLVp = LVESP - LVEDP$) were recorded. The parameters of the maximal rates of increase and decrease in developed LV pressures ($dP/dt_{max}$ and $dP/dt_{min}$, respectively) were determined.

**Procurement of heart tissue for infarct size, cardiomyocyte size, and interstitial fibrosis measurement.** After euthanasia, hearts were rapidly excised and arrested in diastole in 2 M ice-cold KCl. The right ventricle and atria were removed. The basal and apical parts of the LV were rapidly excised and arrested in diastole in 2 M ice-cold KCl. The right ventricle and atria were removed. The basal and apical parts of the LV were rapidly excised and arrested in diastole in 2 M ice-cold KCl. The right ventricle and atria were removed.

**Real-time PCR.** Total RNA from the border zone tissue of infarcted rats was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) and RNA concentrations were measured with a NanoDrop device.

**Statistical analysis.** All data are expressed as means ± SE. Differences among groups were tested by one-way analysis of variance with a two-sided Dunnett t-test as post hoc test, taking the MI group as a comparator. Comparison between groups sham and sham + metformin was tested by independent t-tests. $P < 0.05$ were considered statistically significant.
RESULTS

Effect of metformin on metabolic parameters. To confirm that metformin was consumed by the rats and entered the blood stream, serum metformin levels were measured. In both treatment groups (sham + metformin and MI + metformin), the serum levels of the drug were ~3 mg/l, whereas no metformin could be detected in nontreated groups (Fig. 1A, top). Because metformin is an oral antihyperglycemic agent that may lower the blood glucose levels, fasting glucose levels were determined and OGTTs were performed. No differences were found in the fasting glucose levels between the groups at baseline, weeks 6 and 12 after surgery (12-wk results shown in Fig. 1A, middle). Moreover, no differences could be identified in the OGTTs at these intervals (Fig. 1B), suggesting that glucose uptake from the plasma was not altered and that long term remodeling after MI did not generate insulin resistance in these rats. Blood plasma insulin levels were also determined and were significantly decreased in both metformin-treated groups compared with nontreated groups (Fig. 1A, bottom). Since

![Graph showing serum metformin levels](image1)

![Graph showing OGTT results](image2)

![Graph showing blood plasma insulin levels](image3)

Fig. 2. Effects of metformin on echocardiographic parameters. A and B: interventricular septum diameters (IVS) in diastole (d) and systole (s), respectively. C and D: left ventricular internal dimensions (LVID) in both diastole and systole, respectively. E: left ventricular ejection fraction. F: fractional shortening of the left ventricle. *P < 0.05 vs. sham group; #P < 0.05 vs. MI group.
glucose levels were unaltered, this suggests that metformin sensitized insulin responsiveness in metformin-treated animals. Myocardial glycogen content in both metformin-treated groups was significantly decreased compared with nontreated groups (Fig. 1C). We also observed a lower body weight gain in metformin-treated animals (both sham and MI) compared with the untreated animals (Fig. 1D). Food intake was analyzed, but no consistent differences were observed in time, and therefore this lower gain in weight did not appear to be linked to food intake (data not shown). Therefore, we decided to analyze additional plasma parameters controlling metabolism. In particular active GLP-1 and adiponectin plasma levels were determined by ELISA. No differences were, however, observed in these parameters among the groups (Table 2), suggesting that the differences in weight gain are generated at a different level.

**Cardiac and hemodynamic parameters.** Infarct size was significantly smaller in the MI + metformin group compared with the MI control group (MI + metformin 29.6 ± 3.2% vs. MI control 38.0 ± 2.2%, P < 0.05) (Table 3 and Fig. 6B). As expected, MI caused significant reduction of blood pressure, LVESP, dP/dt max and dP/dt min, but no improvement was observed after a 12-wk treatment with metformin. However, it is of note that sham-operated metformin-treated animals also had significantly lower blood pressure and LV pressure, possibly related to decreased oral (water, food) intake. The LVEDP was increased in the MI group compared with the sham group and was slightly decreased in the MI + metformin group compared with the MI group (Table 3).

The presence of MI was accompanied by wall thinning of the anterior wall (Fig. 2, A and B) as determined by echocardiography. Treatment with metformin significantly attenuated this wall thinning. Furthermore, 12 wk after MI, severe LV dilatation was observed as shown by the increased LVIDd and LVIDs, and this resulted in lower FS and LVEF. Again, metformin significantly attenuated LV dilatation and partially prevented LV dysfunction (Fig. 1, C–F).

**Cardiac gene expression.** To assess the molecular changes induced by long-term metformin treatment, we measured cardiac gene expression of atrial natriuretic protein (ANP), endothelial nitric oxide synthase (eNOS), procollagen, and collagen I by RT-PCR (Fig. 3). The ANP mRNA level was significantly increased after MI compared with sham (P < 0.05), but in metformin-treated MI animals this increase in ANP expression was significantly attenuated (Fig. 3A). The eNOS mRNA level was markedly decreased in the MI group compared with sham, and metformin partially prevented this sharp decrease in eNOS mRNA levels in MI animals (Fig. 3B). Similar positive metformin effects were found for procollagen and collagen I expression, which were significantly increased in the MI group compared with sham, and with a trend to a limited increase in the metformin-treated group (Fig. 3, C and D).

**Cardiac protein expression.** To study the molecular effects of metformin treatment, the phosphorylation of AMPK, eEF2, and p70S6K were measured in the heart, as well as GLUT-4 protein levels (Fig. 4). Furthermore, the cleaved (active) form of the apoptotic protease caspase-3, AMPK isoforms α1 and

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**Fig. 3.** Quantitative real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was conducted and expression was measured of myocardial atrial natriuretic peptide (ANP; A), endothelial nitric oxide synthase (eNOS; B), procollagen (C), and collagen I (D), respectively (mRNA corrected for 36B4 mRNA level). The relative corrected values are shown for each group. *P < 0.05 vs. sham group; #P < 0.05 vs. MI group.
α2, and phosphorylated Akt were measured (Fig. 5). Metformin treatment led to a significant increased phosphorylation of AMPKαThr172 in both treatment groups (Fig. 4A), as well as an increased phosphorylation of eEF2 (Fig. 4B), whereas there was a nonsignificant difference found in the AMPK down-stream protein p70S6K phosphorylation (Fig. 4C). GLUT4 expression is decreased in the MI group after 12 wk MI; metformin restored this. *P < 0.05 vs. sham group; #P < 0.05 vs. MI group; n = 7–9 for all groups.

**DISCUSSION**

Our study demonstrates that long-term metformin administration is associated with preserved cardiac function in a rat model of post-MI cardiac remodeling. The key findings are that long-term (12 wk) metformin treatment after MI significantly attenuated cardiac remodeling post-MI, evidenced by 1) reduced MI size; 2) improved LV geometry (measured by echocardiography); 3) less increase in molecular correlates of LV remodeling (among others, less ANP and collagen production); and 4) measures of improved glucose and cellular energy metabolism.

LV hypertrophy and interstitial fibrosis. The cross-sectional area of cardiomyocytes was increased in both MI groups compared with sham groups, and metformin treatment had no effect on the cardiomyocyte hypertrophy in the post-MI heart failure model (Fig. 6A). This contrasts with the ratios of LV weights corrected for body weights reported in Table 3, which probably is explained by differences in body weights rather than LV weight.

Post-MI heart failure is accompanied by increased fibrosis, which was nonsignificantly reduced by metformin (Fig. 6C).
All-cause readmission and HF hospitalization were also less common in diabetic patients treated with metformin than in those not treated with an insulin-sensitizing drug. Although results from prospective randomized placebo-controlled clinical trials are lacking, these data strongly suggest that metformin use is associated with a better outcome in clinical HF in diabetic patients.

It has been shown that metformin exerts cardioprotective effects in various experimental setups, although mostly acute cardiac damage models. First, Solskov et al. (31) reported that metformin exerted a protective effect on the rat heart subjected to cardiac I/R injury in an in vitro Langendorff setup. This study indicated that, at least in vitro, metformin could exert protective effects when administered before a coronary occlusion was inflicted. Another acute study confirmed the cardioprotective effect of metformin in mice subjected to cardiac I/R model in vivo, also when administered during the reperfusion stage (2). This indicated that also in vivo, metformin exerts protective effects after MI, which was confirmed in another study with a longer follow-up, showing improved LV function and survival in mice subjected to I/R injury (12). Moreover, metformin was shown to attenuate cardiac fibrosis induced by pressure overload in a mouse model and inhibited collagen synthesis (38). Interestingly, in dogs in a model of rapid pacing metformin could also prevent progression of HF (29). The precise actions of metformin on the heart are still not fully elucidated, but a central role of activation of AMPK, which is a regulator of cellular energy formation, has been proposed (2, 12, 29, 38).

Our study is unique in that it used a model of chronic (instead of temporary) ligation of a coronary artery, which leads to large myocardial infarction and cardiac remodeling that resembles the human sequel of events. We herein show that chronic metformin treatment is associated with a decreased extent of myocardial damage, possibly due to both necrosis and apoptosis resulting in reduced MI size, which is associated with less remodeling over time. Metformin has been shown to inhibit apoptosis, as reported in a dog model of heart failure (29). We found that cleaved caspase-3 levels were increased post-MI, as reported before (8); however, the use of metformin only nonsignificantly reduced caspase-3 levels. Increased levels of the anti-apoptotic protein Akt are associated with suppression of AMPK phosphorylation, which can be restored by metformin (18). On the other hand, Sasaki et al. (29) showed that metformin treatment causes decreased Akt phosphorylation in failing hearts. In our study, the use of metformin was not associated with meaningful differences in phosphorylated levels of Akt. Therefore we conclude that the beneficial effects of metformin cannot, or can only marginally, be explained by differences in apoptosis. We furthermore confirm the inhibitory effect of metformin on fibrosis, evidenced in multiple experimental models, indicating that this is a general met-
formin-associated cardiac phenomenon and not only linked to pressure overload model and pacing mode (29, 38). In our study, we observed a decreased expression of collagens and reduced fibrosis.

In support of another study (12), the cardioprotective effects appeared to be independent of antihyperglycemic effects of metformin. In fact, fasting glucose levels and OGTTs were similar in all groups, despite lower insulin levels in the met-
function (data not shown). Since metformin affects various did not cause side-effects like hypoglycemia and renal dys-
safety and efficacy in HF, e.g., in a post-MI trial. We recently evidence that metformin should be prospectively tested for its nondiabetic rats with post-MI heart failure. We postulate that metformin exerts a protective effect on cardiac remodeling in this study. The dose of metformin used in this study was limited. We started administration of metformin 2 days before MI, so we cannot separate early and late protective effects in AMPK downstream effectors in the heart, rather than changes altogether, these effects might suggest that the activation of certain cellular metabolic pathways to maintain energy supply. Interestingly, caloric restriction confers cardioprotective effects, while maintaining normal plasma glucose levels. These all these effects suggest that metformin may restore the cellular energy flux by inhibition of processes that switch off the ATP-consuming pathways to protect the stressed heart (14).

We observed a strong insulin-lowering effect of chronic metformin treatment. It has become apparent that a strong association exists between hyperinsulinemia and incident heart failure. A recent paper provided proof that cardiac insulin signaling itself also exacerbates cardiac remodeling (30). Therefore, part of the beneficial effects that we report herein may be due to low insulin levels and signaling.

AMPK is activated during caloric restriction and stimulates cellular metabolic pathways to maintain energy supply. Interestingly, caloric restriction confers cardioprotective effects, and metformin-treated animals and long-term dietary restriction animals have similar gene expression patterns (9). Together, these effects might suggest that the activation of certain AMPK downstream effectors in the heart, rather than changes in cellular metabolism itself, confers cardioprotective effects.

Limitations of the study. Sample size and thus power is limited. We started administration of metformin 2 days before MI, so we cannot separate early and late protective effects in this study. The dose of metformin used in this study was comparable to that in other studies in rodents and humans (200–350 mg·kg⁻¹·day⁻¹) (1, 39). We established this dose did not cause side-effects like hypoglycemia and renal dysfunction (data not shown). Since metformin affects various targets, including AMPK and many downstream targets, the study design does not allow to prove causality between the molecular targets and functional outcome.

Conclusions. In summary, our study demonstrated that metformin exerts a protective effect on cardiac remodeling in nondiabetic rats with post-MI heart failure. We postulate that AMPK and low insulin levels are important mediators in the protective effects. These findings underscore the potential beneficial effects of metformin in HF and provide further evidence that metformin should be prospectively tested for its safety and efficacy in HF, e.g., in a post-MI trial. We recently launched such a trial (Metformin to Reduce Heart Failure After Myocardial Infarction; NCT01217307).

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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