METFORMIN IMPROVES CARDIAC FUNCTION IN A NONDIABETIC RAT MODEL OF POST-MI HEART FAILURE

Meimei Yin, Iwan C. C. van der Horst, Joost P. van Melle, Cheng Qian, Wiek H. van Gilst, Herman H. W. Silljé, and Rudolf A. de Boer

University Medical Center Groningen, Thorax Center, Department of Cardiology, University of Groningen, The Netherlands

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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 injury (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 measurement. 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 N$_2$O-O$_2$. 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 thickness (PWT) were obtained.

<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>TCTAACCAGCATTTCTCTC</td>
</tr>
<tr>
<td>eNOS</td>
<td>GACCTTTAGGAAAGTACGGCAATGCA</td>
<td>GTOAAAGGGACTATCTGTGATGCTC</td>
</tr>
<tr>
<td>Collagen I</td>
<td>ACAAAGTACGCTTACCATGG</td>
<td>AAGTTTCAGGTGTGAATCCG</td>
</tr>
<tr>
<td>Procollagen</td>
<td>GAGGGCAGTGGCTCTCCTTT</td>
<td>GGGCTTGGACTTGATGG</td>
</tr>
<tr>
<td>36B4</td>
<td>GGTGCCTCAGTGGCTCCTC</td>
<td>GGACGGCGAAATGACATGG</td>
</tr>
</tbody>
</table>

ANP, atrial natriuretic peptide; eNOS, endothelial nitric oxide synthase; collagen I, collagen type I; 36B4, acidic ribosomal phosphoprotein P0.
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/dmax and dP/dmin, 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 snap frozen in liquid nitrogen. A midpapillary slice of the LV was fixed in 4% paraformaldehyde overnight and paraffin embedded. The deparaffinized 3-μm sections were stained with picrosirius red/Fast green, as described (35). The infarct size was calculated as percentage of the scar length to the total LV circumference. The images were obtained with a Leica microscope and analyzed by using appropriate software (Image-pro plus, version 4.5.0.29).

Furthermore, sections were stained with a Gomori’s silver staining to allow visualization of the cardiomyocytes membranes. We measured cell size from transversally cut cardiomyocytes in the border zone of the infarcted area using image analysis software (Zeiss KS400, Germany). Finally, we examined the collagen volume fraction in sections of the LV free wall, after excluding vessels. Tissue sections were stained with Masson’s trichrome stain to evaluate the extent of interstitial fibrosis, modified from previously described methods (7). The area of stained tissue was calculated as a percentage of the total area within a field by using imagescope software.

Biomolecular assays. Arterial blood was collected with EDTA-coated centrifuge tubes. After centrifugation at 3,000 rpm for 15 min at 4°C the plasma was divided into different tubes, snap frozen in liquid nitrogen, and stored for future additional analysis. Metformin was measured by HPLC in the Pharmacy laboratory of University Medical Centre Groningen. Insulin levels were measured at 12 wk after surgery by use an ELISA kit (rat/mouse insulin 96-well plate assay, Millipore). The plasma active GLP-1 and adiponectin levels were measured with the ELISA kits from Linco Research and Millipore. Myocardial glycogen content was measured by using the Enzym-Chrom Glycogen Assay Kit (BioAssay Systems, Hayward, CA) according to manufacturer’s protocol.

Real-time PCR. Total RNA from the border zone tissue of infarction was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) and RNA concentrations were measured with a nano-drop device. First-strand cDNA was prepared by using a random primmer mix and thereafter used as a template for quantitative real-time reverse-transcriptase-PCR (25 ng/reation). mRNA levels are expressed in relative units based on a standard curve obtained by using a calibrator cDNA mixture. All measured mRNA expression levels were corrected for 36B4 reference gene expression. Primer sequences can be found in Table 1.

Western blot analysis. Myocardial tissue samples (75 mg), taken from the area-at-risk portion of the LV, were homogenized in 1 ml RIPA buffer (50 mM Tris pH 8.0, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 150 mM NaCl) containing phosphatase inhibitor cocktail 1 (Sigma), protease inhibitor (Roche), and 1 mM PMSF. Protein concentrations were quantified with the DC protein assay (Bio-Rad Laboratories, Veenendaal, the Netherlands) with bovine albumin as a standard. Equal amounts of protein were loaded onto polyacrylamide-SDS gels and, after electrophoresis, proteins were transferred to a PVDF membrane. The membrane was blocked for 1 h with 5% nonfat milk in PBST and probed with primary antibodies overnight at 4°C. The following primary antibodies were used: phospho-AMPKα Thr172 (1:500, Cell Signaling Technology); AMPKα (1:1,000, Cell Signaling Technology); p-Akt Ser473 (1:1,000, Cell Signaling Technology); Akt (1:1,000, Cell Signaling Technology); phospho-eukaryotic elongation factor-2 (Thr56) (1:1,000, Cell Signaling Technology); Akt (1:1,000, Cell Signaling Technology); eEF2 (1:1,000, Cell Signaling Technology); caspase-3 (1:1,000, Cell Signaling Technology); GAPDH (1:20,000, Fitzgerald Industries International, Acton, MA). Immunoblots were next processed with secondary antibodies for 1 h at room temperature followed by enhanced chemiluminescence detection using an ECL-Plus chemiluminescence reagent kit (Amersham).

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 values <0.05 were considered statistically significant.

Table 2. Body weight, LV weight and body weight ratio, infarct size and hemodynamic parameters

<table>
<thead>
<tr>
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<th>Sham</th>
<th>Sham + Metformin</th>
<th>MI</th>
<th>MI + Metformin</th>
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<tr>
<td>Active GLP-1, pm</td>
<td>6.71 ± 0.43</td>
<td>5.65 ± 0.33</td>
<td>5.75 ± 0.21</td>
<td>5.58 ± 0.27</td>
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<td>Adiponectin, μg/ml</td>
<td>15.77 ± 0.49</td>
<td>16.18 ± 0.74</td>
<td>15.45 ± 0.55</td>
<td>14.93 ± 0.58</td>
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</table>

All values are means ± SE. MI, myocardial infarction; GLP-1, glucagon-like peptide-1.

Table 3. Body weight, LV weight and body weight ratio, infarct size and hemodynamic parameters

<table>
<thead>
<tr>
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<th>Sham</th>
<th>Sham + Metformin</th>
<th>MI</th>
<th>MI + Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW baseline, g</td>
<td>320 ± 9</td>
<td>314 ± 6</td>
<td>313 ± 4</td>
<td>313 ± 4</td>
</tr>
<tr>
<td>BW 12 wk, g</td>
<td>433 ± 15</td>
<td>399 ± 9</td>
<td>428 ± 11</td>
<td>394 ± 12</td>
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<tr>
<td>LVW/BW</td>
<td>2.21 ± 0.08</td>
<td>2.38 ± 0.02</td>
<td>2.23 ± 0.04</td>
<td>2.47 ± 0.04†</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>0</td>
<td>0</td>
<td>38.0 ± 2.2*</td>
<td>29.6 ± 3.2*‡</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>303 ± 8</td>
<td>273 ± 8*</td>
<td>311 ± 10</td>
<td>274 ± 8†</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>116 ± 3</td>
<td>105 ± 3*</td>
<td>105 ± 3*</td>
<td>102 ± 2</td>
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<tr>
<td>DBP, mmHg</td>
<td>79 ± 3</td>
<td>71 ± 3*</td>
<td>76 ± 1*</td>
<td>70 ± 2</td>
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<tr>
<td>LVESP, mmHg</td>
<td>117 ± 3</td>
<td>106 ± 3*</td>
<td>106 ± 3*</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>11 ± 2</td>
<td>14 ± 5</td>
<td>18 ± 4</td>
<td>15 ± 2</td>
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<tr>
<td>dP/dmax, mmHg/s</td>
<td>7,199 ± 299</td>
<td>5,444 ± 264*</td>
<td>5,836 ± 390*</td>
<td>5,166 ± 166</td>
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<tr>
<td>dP/dmin, mmHg/s</td>
<td>−8,399 ± 553</td>
<td>−6,496 ± 524*</td>
<td>−5,516 ± 669*</td>
<td>−5,488 ± 197</td>
</tr>
</tbody>
</table>

All values are means ± SE. BW, body weight; LVW, left ventricle weight; LVW/BW, the ratio of left ventricular weight and body weight; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dmax and dP/dmin, the maximal rate of increase and decrease of left ventricular pressure, respectively. †Statistically significant difference compared with sham-operated rats (P < 0.05). ‡Statistically significant difference compared with MI control rats (P < 0.05).
statistically significant. SPSS (PASW, Chicago, IL) version 18.0 was used to perform all statistical analyses.

**RESULTS**

*Effect of metformin on metabolic parameters.* To confirm that metformin was consumed by the rats and entered the bloodstream, 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).

![Graphs showing metabolic parameters](https://via.placeholder.com/150)

**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/dtmax and dP/dtmin, 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).

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

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).

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 AMPK 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.

Fig. 4. Effects of metformin on protein expression. Protein levels of phosphorylation of AMPKγThr172, phosphorylation of eEF2 Thr56, phosphorylation of p70S6K Thr389, and GLUT4 measured in the border zone of the MI tissue are shown for all groups. A: metformin significantly increased the phosphorylation of AMPKγThr172 after 12 wk both in sham and MI operated groups. B: metformin significantly increased the phosphorylation of eEF2 Thr56 12 wk after MI. C: metformin did not affect the phosphorylation of p70S6K Thr389 among all groups. D: 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.

Until recently, metformin was contraindicated in HF, mainly because of concerns about lactic acidosis. However, epidemiological data suggest that metformin is safe and may even be the first choice antidiabetic drug in HF patients with diabetes. In a case-control study in patients with diabetes and HF (n = 1.633), metformin was associated with a reduced all-cause-mortality (adjusted odds ratio 0.72; P = 0.003) (21). In diabetic patients with newly established HF (n = 1.305), metformin monotherapy was associated with a reduced 1-yr mortality compared with sulfonylurea treatment (25). In HF patients admitted to the hospital (n = 16.417), metformin use was associated with a lower 1-yr mortality compared with treatment with insulin or sulfonylurea (24.7 vs. 36.0%, P <
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 cardio-protective 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-
formin-treated animals. This suggests an insulin sensitizing effect of metformin, resulting in insulin level reductions, lower myocardial glycerol content, and enhanced GLUT4 expression, while maintaining normal plasma glucose levels. These observations suggest an improved myocardial glucose uptake, but we did not measure this. Except for insulin, plasma levels of other metabolic hormones did not change, indicating that metformin did not generate major systemic changes. Nevertheless, weight gain in metformin-treated animals was reduced, and this could not be simply related to changed food uptake. Although, we cannot exclude that nutrient uptake in the intestine has changed, our observation that metformin activates AMPK might point more into the direction of increased cellular metabolism. As described, AMPK is an enzyme that is activated in response to changes in cellular energy metabolism, for instance in muscle contraction, in ischemia, or by pharmacological agents like metformin (26). In our study, metformin treatment led to an increased phosphorylation of AMPK, resulting in an increased phosphorylation of one of its downstream targets, eEF2. Moreover, activated AMPK has a fundamental role in glycerogen synthesis and gluolysis (36), which is supported by our study showing that long-term metformin treatment causes a decreased glycerogen content in the myocardium, an observation that is common to most antidiabetic drugs (19). 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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