Metformin Prevents the Development of Chronic Heart Failure in the SHHF Rat Model

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Insulin resistance is a recently identified mechanism involved in the pathophysiology of chronic heart failure (CHF). We investigated the effects of two insulin-sensitizing drugs (metformin and rosiglitazone) in a genetic model of spontaneously hypertensive, insulin-resistant rats (SHHF). Thirty SHHF rats were randomized into three treatment groups as follows: 1) metformin (100 mg/kg per day), 2) rosiglitazone (2 mg/kg per day), and 3) no drug. Ten Sprague-Dawley rats served as normal controls. At the end of the treatment period (12 months), the cardiac phenotype was characterized by histology, echocardiography, and isolated perfused heart studies. Metformin attenuated left ventricular (LV) remodeling, as shown by reduced LV volumes, wall stress, perivascular fibrosis, and cardiac lipid accumulation. Metformin improved both systolic and diastolic indices as well as myocardial mechanical efficiency, as shown by improved ability to convert metabolic energy into mechanical work. Metformin induced a marked activation of AMP-activated protein kinase, endothelial nitric oxide synthase, and vascular endothelial growth factor and reduced tumor necrosis factor-α expression and myocyte apoptosis. Rosiglitazone did not affect LV remodeling, increased perivascular fibrosis, and promoted further cardiac lipid accumulation. In conclusion, long-term treatment with metformin, but not with rosiglitazone, prevents the development of severe CHF in the SHHF model by a wide-spectrum interaction that involves molecular, structural, functional, and metabolic-energetic mechanisms.

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RESEARCH DESIGN AND METHODS

Animal model. All experimental procedures were approved by the animal care committee of the University Federico II and conformed to the Guide for the Care and Use of Laboratory Animals. A total of 40 rats, aged 24 weeks, were used for this study. Of these, 30 were SHHF rats and 10 were control, Sprague-Dawley rats. Both were obtained from Charles River Laboratories (Milan, Italy). Ten SHHF rats received metformin at a dose of 100 mg/kg per day (M-SHHF rats), and 10 received rosiglitazone (GlaxoSmithKline, Verona, Italy) at a dose of 2 mg/kg per day (R-SHHF rats). Metformin and rosiglitazone were dissolved in the drinking water. The remaining 10 SHHF rats received drinking water alone and were used as metformin and rosiglitazone controls (SHHF rats). All animals were inspected daily and weighed weekly. The treatment period lasted 12 months.

Noninvasive arterial blood pressure measurement. Rats were placed in a plastic restrainer and blood pressure (BP) was measured with the tail cuff.
method (MK2000; Muromachi Kikai, Tokyo, Japan) without warming the animals. A 7-day training before measurement was performed.

**Measurement of blood glucose, insulin, and FFAs.** The plasma concentrations of glucose and insulin were determined under fasting conditions. IR was determined by hyperinsulinaemic euglycaemic clamp (HOMA-IR = fasting plasma glucose [mmol/L] × fasting serum insulin [µIU/mL]/22.5. Serum FFA concentration was measured by spectrophotometric enzymatic assay (Wako Chemicals, Richmond, VA).

**Echocardiography.** Transthoracic echocardiograms were performed according to previously described methods using a high resolution imaging system for small animals (Vevo 770, VisualSonics Inc., Toronto, ON, Canada) equipped with a 17.5 MHz transducer (20). All measurements were performed by an observer who was blinded to the protocol to and based on the average of three to six consecutive cardiac cycles.

**Isolated whole-heart experiments.** Animals were killed by deep anesthesia (zolazepam and tiletamine, 20 mg/kg), and the heart was rapidly excised, immersed in ice-cold Krebs–Henseleit buffer, weighed, and mounted in a Langendorff apparatus (ADInstruments, Bella Vista, NSW, Australia), as previously described (20). Perfusion was set at a constant flow of 12 mL/min with phosphate-free, Krebs–Henseleit buffer bubbled with 5% O₂ and 5% CO₂, temperature at 36°C, and pacing at 3.5 Hz. Coronal perfusion and LV pressures were measured with a water-filled latex balloon and acquired at 50% of Vmax as previously described (21). Incomparing (aortic) and outgoing (pulmonary artery) O₂ content was measured continuously by means of an O₂ microelectrode (16–730A; Microelectrodes, Bedford, NH). MVO₂ was calculated as the product of arterial-venous O₂ difference and coronary flow and expressed as mmolO₂ per minute per gram of LV. All LV parameters were digitized by a PowerLab/LabChart Pro system (ADInstruments).

**Histology.** Myocardial tissue was formalin fixed and paraffin embedded for morphometry and immunohistochemistry or frozen in Tissue Tek OCT compound (ProSciTech, Kirwan, QLD, Australia) for histochemistry. Cross sections 6-µm thick were deparaffin and stained with hematoxylin-cosin (general morphology) or with picrosirius red (collagen content). All measurements were carried out as previously described (20). For cardiac neutral lipids, frozen sections were stained with the Oil Red O method (22). Deparaffin and immunohistochemistry with the following antibodies: anti-tumor necrosis factor-α (TNF-α) (Endogen, Woburn, MA), anti-endothelial nitric oxide synthase (eNOS), or anti-CD68 (NeoMarkers, Freemont CA), all diluted 1:100. The visualization was performed by avidin-biotin complex kit and diaminobenzidine (Pierce Biotechnology, Rockford, IL). Morphometric analysis was performed using a Nikon Eclipse 1000 microscope with Nikon NIS-Elements Basic Research software. Two observers blinded to the experimental protocols carried out all measurements independently. The DNA fragmentation test was performed using the Annexin-V-Fluos Staining Kit and activated Caspase III antibody (Roche Diagnostics, Mannheim, Germany) with DAPI nuclear contrast, as validated by Cittadini et al. (20) and Bogazzi et al. (23).

**Western blotting.** LV samples were prepared according to Axel sen et al. (24). Protein concentration was determined by the BCA Protein Assay Kit (Pierce Biotechnology) using BSA as a standard.

For Western blot, 15 µg protein was separated on 4–20% Novex Tris-Glycine gels (Invitrogen) and electrophoretically transferred to polyvinylidene difluoride membranes in a tank buffer system with Novex Tris-Glycine Transfer Buffer (Invitrogen). Membranes were blocked in 5% BSA in 0.05% Tween 20 in 0.1 mol/L Tris-buffered saline, pH 7.5, and incubated for 60 and 30 min with primary and horseradish peroxidase–labeled secondary antibodies, respectively. Antibody complexes were visualized by a chemiluminescence kit (ECL Advance Western Blotting Detection Kit; Amersham Biosciences, Cologno Monzese, Italy), which subsequently were detected with Hyperfilm ECL (Amersham Biosciences). The detected immunoreactivities were scanned and analyzed as integrated optical density using Scion ImagePC (Scion Corporation, Frederick, MD). The density of a protein sample was always compared with that of samples within the same gel. Four different primary antibodies were used: a polyclonal rabbit antibody against the AMPK-α1 and -α2 isoforms (Cell Signaling, Danvers, MA), a rabbit antibody against phosphorylated T172 of the AMPK-α-subunit (Cell Signaling), an anti–vascular endothelial growth factor (VEGF) rabbit polyclonal antibody (1:500; Santa Cruz Biotechnology, San Francisco, CA), and a monoclonal mouse antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon Millipore, Billerica, MA). Each amount of each protein (densitometric values) was standardized against the corresponding GAPDH. Equal protein loading of VEGF was confirmed by reprobing the membranes with a mouse monoclonal antibody to α-tubulin (1:1,000; Calbiochem, Rome, Italy). The effects of VEGF overexpression on phosphorylation of VEGF receptor 2 (VEGFR-2) was explored by means of immunoprecipitation–Western blot analysis.

**TaqMan gene expression.** Total RNA was extracted from heart tissue using the Tri Reagent solution (Ambion, Austin, TX). cDNA was prepared from 250 ng total RNA, using conditions recommended by the supplier (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Austin, TX). Quantitative real-time PCR analysis was performed using an ABI 7300 Sequence Detection System (Applied Biosystems). Predesigned, gene-specific primers and probes were used to detect each gene (Applied Biosystems). The following rat TaqMan Gene Expression Assays were used: GPDH (assay Rn00856922_m1), sarco/endoplasmic reticulum Ca²⁺-ATPase 2a (SERCA2a) (Assay ID: Rn00665782_m1), Na-Ca exchanger 1 (Slc8a1, assay Rn00570627_m1), eNOS3 (Nos3, assay ID: Rn01233831_s1), eN-muscarinic acetylcholine receptor complex (Myb6, assay Rn00583031_m1), phospholamban (PLB) (Assay ID: Rn01434045), and atrial natriuretic peptide (Nppa, assay Rn00561661). For all primers, more details are available at http://www.appliedbiosystems.com. The amount of each transcript was normalized to the amount of GPDH expressed in the same sample. All samples were analyzed in duplicates. The relative comparative cycle threshold method was used to analyze the real-time quantitative PCR data (User Bulletin 2: Applied Biosystems).

**Statistics.** Data are reported as means ± SD. Comparison between M-SHHF or R-SHHF rats and control SHHF rats was performed by the unpaired t test with Bonferroni correction. P < 0.05 was considered significant.

**RESULTS**

At age 18 months, SHHF rats showed the structural and functional features of a severely decompensated HF, in agreement with previous reports that provide the original characterization of this model (19). All SHHF and M-SHHF rats survived until the end of the study, when they were aged 18 months. In contrast, two R-SHHF rats died at age 16 and 17 months because of pulmonary edema, as determined by autopsy. In all tables and figures, the statistical comparison is made among the three SHHF groups. Data from control Sprague-Dawley rats were included merely to provide the reference values of the parameters under study in aged 18 months healthy controls and were not used for statistical comparisons.

As shown in Table 1, SHHF rats were characterized by high values of arterial BP, which was significantly reduced by both metformin and rosiglitazone at 8 and 12 months. SHHF rats were severely insulin resistant, as documented by their HOMA-IR index that was four times higher than that of Sprague-Dawley controls. As expected, metformin attenuated IR considerably, while the effect of rosiglitazone was not significant. Both aspects were unmarkedly reduced in M-SHHF compared with SHHF and R-SHHF rats.

The myocardial histological features are presented in Table 2 and Figs. 1 and 2. Cardiac hypertrophy developed in the SHHF rats and was not affected by either treatment, as shown by the similar heart weights and cardiomyocyte diameters. The SHHF heart showed extensive interstitial remodeling, consisting of collagen accumulation, particularly in the perivascular area, and reduced capillary density. In both M-SHHF and R-SHHF rats, the collagen volume fraction and the type I and type III components were not different from SHHF rats. However, in M-SHHF rats, the fraction of collagen accumulated in the perivascular area was significantly smaller than in SHHF rats and capillary density was substantially preserved, while rosiglitazone led to more marked perivascular fibrosis. Additional features of the SHHF myocardium were the marked presence of inflammatory cells and TNF-α expression and the intensely active apoptotic process. Both aspects were unaffected by rosiglitazone but were attenuated by metformin. Moreover, metformin reduced drastically the accumulation of fat droplets (Fig. 3) and stimulated the expression of eNOS that reached levels >10 times higher than those of...
**TABLE 1**

| Metabolic and hemodynamic characteristics of Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats | Baseline | 4 months | 8 months | 12 months |
|---|---|---|---|---|
| **Body weight (g)** | 403 ± 16 | 363 ± 18 | 364 ± 17 | 354 ± 13 |
| **Systolic BP (mmHg)** | 19 ± 6 | 18 ± 6 | 18 ± 6 | 18 ± 6 |
| **Diastolic BP (mmHg)** | 13 ± 5 | 17 ± 7 | 15 ± 7 | 13 ± 5 |
| **Glucose (mg/dL)** | 90 ± 5 | 89 ± 8 | 89 ± 8 | 88 ± 3 |
| **Insulin (mU/mL)** | 80 ± 5 | 79 ± 8 | 79 ± 8 | 78 ± 3 |
| **HOMA-IR** | 1.5 ± 0.5 | 1.4 ± 0.3 | 1.3 ± 0.2 | 1.2 ± 0.1 |
| **Serum FFA (mmol/L)** | 15 ± 4 | 13 ± 3 | 13 ± 3 | 12 ± 2 |
| **Carnitine (µmol/mg)** | 1.4 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.1 ± 0.1 |

SHHF rats. In striking contrast to metformin’s effects, rosiglitazone accentuated lipid accumulation that was four times higher than in SHHF rats and decreased eNOS to levels that were even lower than those of the normal Sprague-Dawley rats.

The data of LV morphology and function are summarized in Table 3. Compared with control rats, SHHF rats exhibited LV dilation and reduced systolic function. Moreover, the higher systolic pressure associated with eccentric remodeling led to a pronounced increase of systolic wall stress. Metformin attenuated LV dilation, and since wall thickness was not affected by metformin, the relative wall thickness (RWT = 2 [posterior wall thickness/LV internal dimension]) was slightly increased. As a consequence of the increased RWT and the lowered arterial BP, LV meridional peak systolic wall stress, 0.334 × [LV pressure × (1 + posterior wall thickness/LV internal dimension)], was drastically reduced by metformin. This is a positive event because in vivo measured wall stress is inversely related to LV performance, at variance with LV stress measured in the isolated heart (see below) that is an entirely different parameter reflecting intrinsic contractility (21). Treatment with rosiglitazone did not attenuate either LV remodeling or wall stress.

The data on myocardial contractility and energetics are shown in Table 4. As compared with normal rats, SHHF rats showed an extensive functional impairment that involved both the systolic performance (developed pressure, stress, and maximum dP/dt [rate of rise of LV pressure]) and the indices of ventricular relaxation (−dP/dt and τ). Rosiglitazone did not improve systolic dysfunction and deteriorated even further LV relaxation (τ). In contrast, metformin exerted beneficial effects on all aspects of LV dynamics. Both contractility and relaxation were improved, and the relative indices were very close to those observed in control animals. As a prototype, developed wall stress, which is considered a faithful index of LV intrinsic contractility independent of load, increased to normal values after metformin treatment (21). MV0₂ was not different among the SHHF groups. However, we calculated the ratio of myocardial contractility to MV0₂. This is a reliable index of myocardial efficiency, which is the ability of the myocardium to convert metabolic energy into mechanical work. Myocardial efficiency was strongly depressed in the SHHF and R-SHHF groups, whereas metformin was able to restore it to levels comparable to those of control rats. We also examined the contractile reserve by measuring LV developed pressure in response to the elevation of extracellular calcium from 2.0 to 4.0 mmol/L. In the R-SHHF group, the contractile reserve was markedly impaired, whereas in the M-SHHF rats, it remained at levels comparable to those of the control rats (Table 4).

As shown in Fig. 4, Western blot analysis revealed a marked increase of the ratio of phosphorylated-to-total AMPK in the M-SHHF group compared with the other study groups. TaqMan quantification of gene expression demonstrated a significant upregulation of eNOS by metformin compared with the other SHHF groups. Moreover, the SERCA2 transcript levels tended to be lower in the R-SHHF group, leading to a significant increase of the PLB-to-SERCA2 ratio compared with the other SHHF groups. We next determined myocardial VEGF protein content and VEGFR-2 phosphorylation using Western blotting and immunoprecipitation. SHHF rats displayed a remarkable decrease of VEGF and VEGFR-2 phosphorylation compared with the control group. While rosiglitazone did not influence VEGF
downregulation, metformin significantly increased myocardial VEGF and VEGFR-2 phosphorylation, restoring them to almost normal levels (Fig. 5).

**DISCUSSION**

This is the first study that directly compares the long-term metformin and rosiglitazone effects in an animal model of hypertensive, insulin-resistant CHF that closely mirrors the clinical condition observed in many patients with long-term diabetes. We demonstrate that metformin treatment has a profound beneficial impact on LV remodeling and function, whereas rosiglitazone exerts largely deleterious effects. Metformin reduced IR, FFA levels, and myocardial lipid accumulation. Perivascular fibrosis was reduced by metformin, and capillary density was preserved in parallel with improved myocardial perfusion.

| TABLE 2 | Structural and molecular features of the heart in Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats |
|----------|-----------------------------------------------|
|          | C | SHHF | M-SHHF | R-SHHF |
| n        | 10 | 10   | 10     | 8      |
| Heart weight/body weight (mg/g) | 3.31 ± 0.78 | 4.82 ± 0.82 | 5.70 ± 0.44 | 5.32 ± 0.51 |
| Collagen volume fraction (%) | 4.1 ± 1.3 | 13.6 ± 5.8 | 15.9 ± 3.4 | 16.7 ± 3.1 |
| Type I collagen (%) | 3.8 ± 0.01 | 12.1 ± 5.4 | 13.6 ± 4.0 | 15.6 ± 3.1 |
| Type III collagen (%) | 0.2 ± 0.01 | 1.5 ± 0.3 | 2.2 ± 1.3 | 1.0 ± 0.6 |
| Perivascular collagen (%) | 15 ± 6 | 47 ± 10 | 34 ± 13* | 68 ± 12† |
| Cardiomyocyte diameter (µm) | 19.8 ± 3.0 | 33.1 ± 5.9 | 34.8 ± 3.3 | 30.8 ± 3.6 |
| Capillary density (n/mm²) | 1,683 ± 143 | 1,091 ± 341 | 1,245 ± 505 | 991 ± 313 |
| Apoptotic index (apoptotic nuclei/10⁶) | 2 ± 1 | 40 ± 11 | 20 ± 7† | 37 ± 6 |
| Lipid droplet index (% area of lipid droplets) | 0.11 ± 0.06 | 14.1 ± 1.7 | 4.9 ± 1.6† | 56.2 ± 3.6† |
| TNF-α (% area of positive cells) | 6.7 ± 0.9 | 84.1 ± 3.7 | 27.3 ± 2.2† | 89.4 ± 13.7 |
| eNOS (% area of positive cells) | 2.5 ± 1.0 | 6.4 ± 2.1 | 78.3 ± 5.8† | 1.1 ± 0.7† |

C, Sprague-Dawley rats. ‡P < 0.05 vs. SHHF. †P < 0.01 vs. SHHF.

**FIG. 1.** A: Perivascular collagen content was examined on histological sections stained with picrosirius red. In each study group, collagen content was also expressed as a percentage of perivascular area (bar graph). M-SHHF rats displayed a significant reduction of perivascular collagen content when compared with placebo or R-SHHF rats. B: Representative stainings of cardiomyocyte apoptosis detected by annexin (green) and activated Caspase III immunoreaction (red). Graph bars show the apoptotic index in study groups. Of note, rosiglitazone-treated animals display several myocardial areas characterized by massive apoptotic process not only in cardiomyocytes (R-SHHF, background picture) but also in lymphocytes and endothelial cells, as shown in the superimposed microphotograph (R-SHHF, white framed box). Scale bar = 20 µm. C, Sprague-Dawley rats. *P < 0.05 vs. SHHF. (A high-quality digital representation of this figure is available in the online issue.)
with attenuated reduction of myocardial VEGF content. In addition, metformin increased AMPK activation and eNOS expression and reduced markedly the apoptotic process. From the functional point of view, metformin attenuated LV remodeling by reducing LV dilation and wall stress. Systolic and diastolic function, whether examined in vivo or in vitro, were improved by metformin, and both the myocardial contractile reserve and myocardial efficiency were restored to normal levels. In contrast, rosiglitazone augmented perivascular fibrosis and reduced capillary density, markedly potentiated myocardial lipid accumulation, inhibited eNOS expression, and increased the PLB-to–SERCA2 ratio. Although the study was not planned to look at survival, it is noteworthy that two rats died in the rosiglitazone group because of pulmonary edema.

Metformin is endowed with pleiotropic effects, predominantly mediated through the activation of AMPK (25,26). In turn, AMPK acts as a metabolic master shift in response to energy depletion, orchestrating a metabolic response aimed at preserving ATP content (26). In addition, AMPK also acts as an antiproliferative, antifibrotic, and antiapoptotic agent. The data of the current study support the concept that emerged from clinical studies (UK Prospective Diabetes Study [UKPDS]) that the cardioprotective effects of metformin are partly independent of glycemic control (25–27).

One of the mechanisms of the beneficial effect of metformin is the marked reduction of myocardial lipid accumulation. It is well accepted that lipotoxicity is indeed a hallmark of diabetic heart in rodents and humans (28,29). Intracellular lipid accumulation induces initial cardiac hypertrophy followed by LV dysfunction and premature cell death, the so-called lipid-induced programmed cell death. Such sequence of events has been characterized by several human and animal studies and recapitulated by Chiu et al. (30) in an elegant transgenic model. Our SHHF rats exhibit the typical features of IR cardiomyopathy complicated by a marked increase of myocardial lipid droplets and apoptosis. It is plausible that metformin, by reducing circulating FFA and improving IR, attenuated the futile, ATP-wasting FFA cycle that occurs when the myocardium is exposed to high plasma FFA concentrations (31). Metformin is known to enhance basal and insulin-stimulated glucose uptake in IR cardiomyocytes (11), which promotes FFA oxidation, thus reducing lipotoxicity.

We also observed a significant reduction of perivascular fibrosis in the metformin group. Fibrosis develops in HF in response to increased wall tension and inflammatory cytokines. Therefore, the significant reduction of LV wall stress and TNF-α myocardial content observed in the metformin group provides two novel antifibrotic mechanisms of metformin. In this regard, it has been previously shown that metformin interferes with collagen deposition by reducing transforming growth factor-β (16) via a cross-talk with extracellular signal–related kinase (32).

The data on VEGF myocardial content point to another novel mechanism of metformin. SHHF rats had significantly decreased myocardial VEGF expression and VEGF phosphorylation, which were both markedly increased by...
Metformin. Progressive attenuation of VEGF myocardial expression is a seminal event in IR cardiomyopathy, and VEGF gene therapy is able to reverse the cardiac phenotype (33). Taken together, enhanced VEGF myocardial signaling may represent the pathophysiological underpinning for metformin induced angiogenesis, which translates into a significant increase of capillary density, with attendant salutary consequences on LV architecture and function.

Another likely mechanism by which metformin improved LV remodeling in our model is the stimulation of nitric oxide (NO) production, which plays a pivotal role in the regulation of vascular tone and cardiac function (34). We found a dramatic increase of myocardial eNOS content through stimulation of eNOS gene transcription in the metformin group. It is well known that AMPK increases eNOS activity and, in turn, NO bioavailability (28,35) and that NO is endowed with cardioprotective properties that include reduction of apoptosis, oxidation, and inflammation and improvement of mitochondrial function (34). Relevant to the current findings is the intriguing hypothesis that AMPK stimulation of eNOS may represent a link between metabolic adaptations and cardiovascular function under stress conditions, such as CHF, by increasing glucose uptake and promoting GLUT4 translocation (31).

Another interesting finding of the current study was the effect of metformin to improve myocardial efficiency, which reflects the ability of the cardiomyocytes to handle the metabolic energy. A major determinant of myocardial efficiency is LV wall stress, and metformin indeed markedly reduced wall stress. It is also possible that the improved myocardial IR, the reduced FFA flux, and the NO effect to reduce VO₂ and energy demand through cyclic GMP (31) may have contributed to the observed improvement of myocardial energetics.

Rosiglitazone worsened myocardial lipid accumulation and perivascular collagen deposition. The molecular phenotype of R-SHHF rats was characterized by the reduction of eNOS protein expression and increase of the PLB-to-SERCA2 ratio. This novel finding may account for the negative effects of rosiglitazone on LV contractile reserve and mechanical efficiency in our CHF model, in view of the pivotal role of SERCA2 and of its negative regulator PLB on calcium handling in CHF (36). Moreover, in the R-SHHF rats, the reduced eNOS protein expression in the context of impaired NO bioavailability (31).

**FIG. 3.** Myocardial lipid content was assessed on frozen sections with Oil Red O histochemistry. Lipid droplets were stained in bright red. M-SHHF rats showed significant reduction of myocardial lipid content when compared with SHHF and R-SHHF groups. Scale bar = 20 μm. C, Sprague-Dawley rats. (A high-quality digital representation of this figure is available in the online issue.)

**TABLE 3**

| Echocardiographic parameters of Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats |
|---------------------------------|-------------|-------------|-------------|-------------|
| n                              | C (10)      | SHHF (10)   | M-SHHF (10) | R-SHHF (8)  |
| LV end-diastolic diameter (mm)  | 6.6 ± 3     | 8.7 ± 2     | 7.5 ± 0.6   | 9 ± 1       |
| LV end-systolic diameter (mm)   | 3.1 ± 2     | 5.4 ± 1     | 4.2 ± 0.9†  | 5.8 ± 0.8   |
| Fractional shortening (%)       | 51 ± 7      | 38 ± 14     | 46 ± 9      | 35 ± 7      |
| Posterior wall thickness (mm)   | 1.5 ± 0.4   | 1.4 ± 0.3   | 1.5 ± 0.6   | 1.3 ± 0.8   |
| Anterior wall thickness (mm)    | 1.6 ± 0.4   | 1.4 ± 0.3   | 1.4 ± 0.4   | 1.4 ± 0.2   |
| RWT                             | 0.47 ± 0.2  | 0.32 ± 0.2  | 0.42 ± 0.2  | 0.28 ± 0.1  |
| Peak systolic wall stress (kilodynes/cm²) | 93 ± 24 | 308 ± 42 | 210 ± 36† | 299 ± 27 |

C, Sprague-Dawley rats. †P < 0.01 vs. SHHF.
of progressing CHF may have negatively affected pathologic LV remodeling. In this context, experimental evidence supports the concept that eNOS limits LV remodeling and dysfunction and modulates extracellular matrix proteins under chronic pressure overload (37) and that targeted eNOS overexpression attenuates cardiac dysfunction and improves survival in ischemic cardiomyopathy (38). There was no evidence of fluid retention in the R-SHHF rats, but two animals died over the treatment period. Autopsy documented pulmonary edema, which might have been secondary to either hypertensive bursts or acute LV decompensation due to ischemia or arrhythmias.

**Comparison with previous studies.** It was the UKPDS that first revealed a cardioprotective effect of metformin, consisting of 39% reduction in the incidence of myocardial infarction (27). The cardiac effects of metformin have been

### TABLE 4
Myocardial contractile performance and $MV_O^2$ of isolated and perfused hearts of Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats

|                     | C         | SHHF      | M-SHHF    | R-SHHF    |
|---------------------|-----------|-----------|-----------|-----------|
| $n$                 | 10        | 10        | 10        | 8         |
| Developed pressure (mmHg) | 97 ± 6    | 54 ± 26   | 88 ± 35*  | 50 ± 20   |
| $dP/dt$ (mmHg/s)     | 1,435 ± 80| 1,001 ± 86| 2,182 ± 96† | 1,000 ± 600|
| $-dP/dt$ (mmHg/s)    | −1,270 ± 198| −590 ± 109| −1,074 ± 438† | −600 ± 220|
| Developed wall stress (kilodynes/cm²) | 33 ± 2   | 18 ± 12   | 33 ± 10†  | 25 ± 10   |
| $\tau$ (ms)         | 60 ± 8    | 72 ± 3    | 66 ± 10   | 95 ± 6†   |
| $MV_O^2$ (μmol/min/g) | 20 ± 4    | 46 ± 19   | 35 ± 16   | 44 ± 23   |
| $dP/dt/MV_O^2$       | 71.05 ± 4.2 | 21.85 ± 1.8 | 61.43 ± 4.1† | 22.88 ± 0.9 |
| Δ-Developed pressure (mmHg) | 26.03 ± 2.4 | 20.01 ± 4.4 | 31.98 ± 1.9† | 10.5 ± 2.8† |

C, Sprague-Dawley rats; $\tau$, time constant of exponential pressure decay; $dP/dt/MV_O^2$, index of myocardial efficiency calculated as the ratio of $MV_O^2$ to LV $dP/dt$; Δ-developed pressure, index of myocardial contractile reserve, as assessed as the response of the developed pressure to graded increase of Krebs buffer calcium concentration from 2.0 to 4.0 mol/L. *$P < 0.05$ vs. SHHF. †$P < 0.01$ vs. SHHF.

![FIG. 4. A: TaqMan gene expression of protein involved in calcium handling and contractility. eNOS was significantly upregulated by metformin, while R-SHHF rats displayed a significant increase of the PLB-SERCA2 ratio. As expected, Western blot analysis of phosphorylated (p) and total AMPK ratio (B) showed a marked increase in the ratio of pAMPK to total AMPK in M-SHHF rats. A representative blot is depicted. MHC, major histocompatibility complex; ANP, atrial natriuretic peptide; NCX1, Na-Ca exchanger 1; C, Sprague-Dawley rats. *$P < 0.05$ vs. SHHF.](image-url)
the object of renewed interest. In a mouse model of postinfarction HF, metformin treatment for 4 weeks improved ventricular function, an effect that was mediated through activation of AMPK and eNOS (15). Similar beneficial effects on LV remodeling and function were recently reported by Wang et al. (18) in a rat model of postinfarction HF. In a canine model of HF induced by rapid ventricular pacing, metformin treatment for 4 weeks improved cardiac function (16). In these studies, the beneficial effect of metformin was observed in metabolically normal animals in which HF was experimentally induced in a short time frame and there was little time for metabolic determinants to exert deleterious cardiovascular effects. Thus, the extrapolation of the data to the cardiac complications in diabetic humans remains uncertain. Our model is characterized by marked IR and spontaneous HF. More important, the impact of metformin on these abnormalities was tested over a long treatment period, as may occur in most diabetic patients receiving metformin.

Shoghi et al. (39) reported that rosiglitazone enhances glucose and diminishes FFA use. In contrast, Baranowski et al. (40) found increased lipid accumulation in the rat heart despite concomitant reduction of plasma FFA availability after administration of pioglitazone, suggesting that a mismatch between the rate of FFA uptake and oxidation was responsible for lipid accumulation. We and others did not find evidence for increased cardiac volumes after rosiglitazone administration, whereas Blasi et al. (41) reported augmented LV diastolic dimension and increased urinary aldosterone excretion and ratio of heart to body weight. A recent study by Goltsman et al. (42) concludes that rosiglitazone treatment was not associated with worsening of fluid retention or cardiac status in rats with experimental volume-overload CHF but, rather, an improvement of renal handling of salt and water. In contrast to our data, a recent study by Kravchuk et al. (43) did not find evidence of a cardioprotective effect of metformin. However, differences in animal models (streptozotocin-induced diabetes vs. SHHF rat), treatment duration (only 3 days vs. 12 months), and methods to assess cardiac function may underlie this apparent discrepancy. Consistent with the current data is a study by Lygate et al. (44), who observed increased mortality and no effects on LV remodeling in the rat model of postinfarction HF after rosiglitazone administration, whereas in normal rats, rosiglitazone enhanced myocardial contractility (44). The authors could provide no explanation for the increased mortality and suggested as a potential mechanism either arrhythmias or FFA reduction with subsequent energy starvation. The findings of the current study are congruent with most of the available literature since they do not show any beneficial effect of rosiglitazone on LV pathologic remodeling. Our study further expands on previous findings by showing reduction of LV contractile reserve and efficiency and deleterious molecular changes consisting of reduced myocardial eNOS expression and raised PLB-to-SERCA2 ratio.
Clinical implications. Not only is IR related to the severity and etiology of CHF but, more important, it also has been shown to be an independent risk for mortality in patients with CHF. Therefore, strategies to correct such metabolic defect represent potential means to improve CHF prognosis. The current study provides further pathophysiologically underpinnings to the concept that metformin not only should be used without restrictions in patients with CHF but even recommended for its cardioprotective potential. Considering that metformin still carries a black box warning from the U.S. Food and Drug Administration against its use in treating diabetes in HF patients, principally based on the remote risk of lactic acidosis, we believe the time has come to implement clinical trials aimed at confirming the robust evidence from experimental studies.

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A.C. and R.N. designed the study, interpreted data, and wrote the manuscript. M.G.M. performed in vivo and ex vivo experiments and light microscopy and analyzed data. D.R. maintained the rat colony and performed in vivo experiments. S.L. performed serological experiments. P.A.N. provided reagents and gave conceptual advice. M.W., M.S., and G.A. performed molecular biology experiments. J.I. interpreted molecular biology data and critically read the manuscript at all stages. L.S. designed and supervised the study, interpreted data, and wrote the manuscript. L.S. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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