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

Antonio Cittadini,1 Raffaele Napoli,1 Maria Gaia Monti,1 Domenica Rea,1 Salvatore Longobardi,2 Paolo Antonio Netti,3,4 Marion Walser,5 Mariateresa Samà,6 Gianluca Aimaretti,6 Jörgen Isgaard,5 and Luigi Saccà1

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 performance of the failing heart and the progression of cardiac remodeling, increased perivascular factor-α, 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.

A bidirectional link exists between insulin resistance (IR) and chronic heart failure (CHF). IR predicts CHF independently of other risk factors (1). Conversely, IR often develops in CHF and associates with more severe symptoms and worse clinical outcome (2,3). IR also involves the myocardial tissue (4), where it downregulates glucose uptake. Because of the inhibited glucose uptake, free fatty acid (FFA) remains the preferred substrate oxidized by the cardiomyocytes, thus precluding the energetic advantage provided by glucose versus FFA oxidation. The reduced energy production and the metabolic inflexibility aggravate the performance of the failing heart and the progression of CHF (5,6). This is why IR is becoming a new target in the treatment of CHF (7,8).

Metformin and thiazolidinediones (TZDs) are effective antidiabetic options that have a proven efficacy to reduce IR. However, here lies the therapeutic conundrum: both drugs are either contraindicated or not recommended in the treatment of diabetes when CHF coexists. TZDs (both pioglitazone and rosiglitazone) are contraindicated because of the increased incidence of CHF as a result of their effect to increase renal sodium reabsorption and vascular permeability (9). Despite extensive epidemiological surveys and retrospective studies suggesting that the opposite might be true (10), no prospective study addresses the impact of TZDs on CHF progression.

With regard to metformin, recent data point to its potential benefit in CHF. Through the activation of AMP-activated protein kinase (AMPK), metformin enhances basal and insulin-stimulated glucose uptake in insulin-resistant cardiomyocytes (11). Diabetic patients with CHF treated with metformin show a more favorable clinical outcome, including reduced mortality, as compared with diabetic patients with CHF treated with other antidiabetic agents (10,12,13). Although retrospective in nature, this evidence comes from rather large patients groups. On the basis of these observations, the U.S. Food and Drug Administration has recently decided to change the status of contraindication to warning when metformin is used in patients with CHF (14). Simultaneously, there has been a renewed interest in clarifying the interaction of metformin with CHF pathophysiology in various experimental models (15–18). The results show beneficial effects of metformin in terms of left ventricular (LV) function. Whether a long-term treatment with metformin or rosiglitazone affects the development and the progression of CHF has not been explored. Accordingly, we tested this hypothesis in the spontaneously hypertensive, heart failure (HF) prone SHHF rat, which develops a progressive form of HF closely resembling the chronic setting of diabetic cardiomyopathy (19).

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-SHFF rats), and 10 received rosiglitazone (GlaxoSmithKline, Verona, Italy) at a dose of 2 mg/kg per day (R-SHFF 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 homeostasis model assessment: HOMA-IR = (fasting insulin [µU/mL] x fasting glucose [mmol/L]) / 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 (Vivid 7; GE Healthcare, Waukesha, WI) equipped with a 17.5 MHz transducer (20). All measurements were performed by an observer who was blinded to the protocol and were 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 (20,21). Perfusion was set at a constant flow of 12 mL/min/g heart weight with phosphate-free, Krebs–Henseleit buffer bubbled with 95% O2 and 5% CO2, temperature at 36°C, and pacing at 3.5 Hz. Coronary perfusion and LV pressures were measured with Capto SP 844 pressure transducers (MEMSCAP, Skopum, Norway). LV mechanical parameters were continuously measured with a water-filled latex balloon and acquired at 50% of Vmax as previously described (21). Incompliant (aortic) and outcompliant (2 pulmonary artery) O2 content was measured continuously by means of an O2 microelectrode (16-730A; Microelectrodes, Bedford, NH). MV02 was calculated as the product of arterial-venous O2 difference and coronary flow and expressed as mmol/L 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 lipid histochemistry. Cross sections 6-µm thick were deparaffinized and stained with hematoxylin-eosin (general morphology) or with picrosirius red (collagen content). All measurements were carried out as previously described (20). For cardiac neutral lipid content, frozen sections were stained with the Oil Red O method (22). Deparaffinized immunohistochemistry with the following antibodies: anti-tumor necrosis factor-α (TNF-α) (Endogen, Woburn, MA), anti–endothelial nitric oxide synthase (eNOS), and anti–endothelial nitric oxide synthase (eNOS) (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, Freemont CA), all diluted 1:100. The visualization was performed by avidin–biotin complex kit and diaminobenzidine (Pierce Biotechnology, Rockford, IL).

**Western blotting.** LV samples were prepared according to Axelsson et al. (24). Protein concentration were 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. Antigen–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 band 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; Cat. No. ab92724; 1:200; Santa Cruz, CA). 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.

**Statistical analysis.** The data are presented as mean ± 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, although it caused a 35% reduction of the HOMA-IR index, was not statistically significant. Serum FFA concentration as well as myocardial FFA content was significantly lower 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 intensively 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
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). MFVo2 was not different among the SHHF groups. However, we calculated the ratio of myocardial contractility to Vo2. 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

**TABLE 2**

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

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 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 (26,35) 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

**TABLE 3**

Echocardiographic parameters of Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats

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

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 studied extensively, and Table 4 summarizes some of these findings.

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial contractile performance and $MV_O2$ of isolated and perfused hearts of Sprague-Dawley, SHHF, M-SHHF, and R-SHHF rats</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>$n$</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Developed pressure (mmHg)</td>
</tr>
<tr>
<td>$dP/dt$ (mmHg/s)</td>
</tr>
<tr>
<td>$-dP/dt$ (mmHg/s)</td>
</tr>
<tr>
<td>Developed wall stress (kilodynes/cm$^2$)</td>
</tr>
<tr>
<td>$\tau$ (ms)</td>
</tr>
<tr>
<td>$MV_O2$ (µmol/min/g)</td>
</tr>
<tr>
<td>$dP/dt/MV_O2$</td>
</tr>
<tr>
<td>$\Delta$-developed pressure (mmHg)</td>
</tr>
</tbody>
</table>

$\tau$, time constant of exponential pressure decay; $dP/dt/MV_O2$, index of myocardial efficiency calculated as the ratio of $MV_O2$ to LV $dP/dt$; $\Delta$-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.
the object of renewed interest. In a mouse model of post-infarction 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.

FIG. 5. Myocardial VEGF protein content and VEGFR-2 phosphorylation using Western blotting and immunoprecipitation. A: Representative Western blot, with the corresponding histograms depicted in B. Metformin therapy significantly attenuated the marked reduction of VEGF and VEGFR-2 that occurred in the SHHF rats, while rosiglitazone had a neutral effect. C: Immunohistochemical images that confirm the biochemical data. VEGFR2 was well distributed in the capillary walls of M-SHHF rats compared with SHHF and R-SHHF groups. Cardiomyocytes of rats subjected to metformin therapy also revealed a diffuse immunoreaction for VEGF, whereas in the SHHF and R-SHHF rats, only a slight immunoreaction was found. Scale bar = 20 μm. C, Sprague-Dawley rats. (A high-quality digital representation of this figure is available in the online issue.)
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.

ACKNOWLEDGMENTS

GlaxoSmithKline (Verona, Italy) provided rosiglitazone and partial financial support to the study. No other potential conflicts of interest relevant to this article were reported.

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., E.J., and 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.L. interpreted molecular biology data and critically read the manuscript at all stages. L.S. designed and supervised the study, interpreted molecular biology data and critically read the manuscript, 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.

REFERENCES

7. Saccó L. Heart failure as a multiple hormonal deficiency syndrome. Circ Heart Fail 2009;2:151–156
23. Bogazzi F, Russo D, Raggi F, et al. Transgenic mice overexpressing growth hormone (GH) have reduced or increased cardiac apoptosis through activation of multiple GH-dependent or -independent cell death pathways. Endocrinology 2009;140:5758–5769
34. Rakshit RD, Marber MS. Nitric oxide: an emerging role in cardioprotection? Heart 2001;86:368–372


