Acknowledgments

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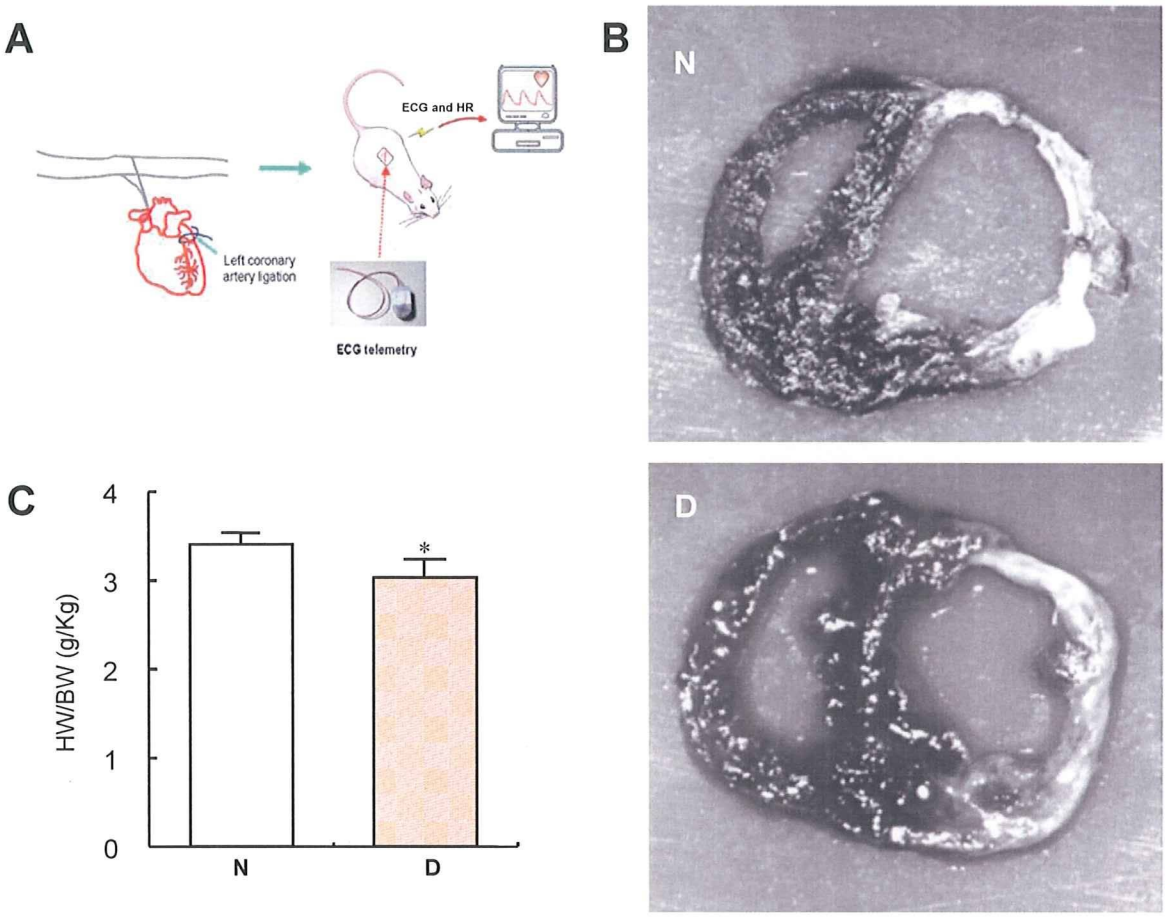
life-threatening arrhythmias: implications for clinical trials. *Circulation* 103: 2072-2077

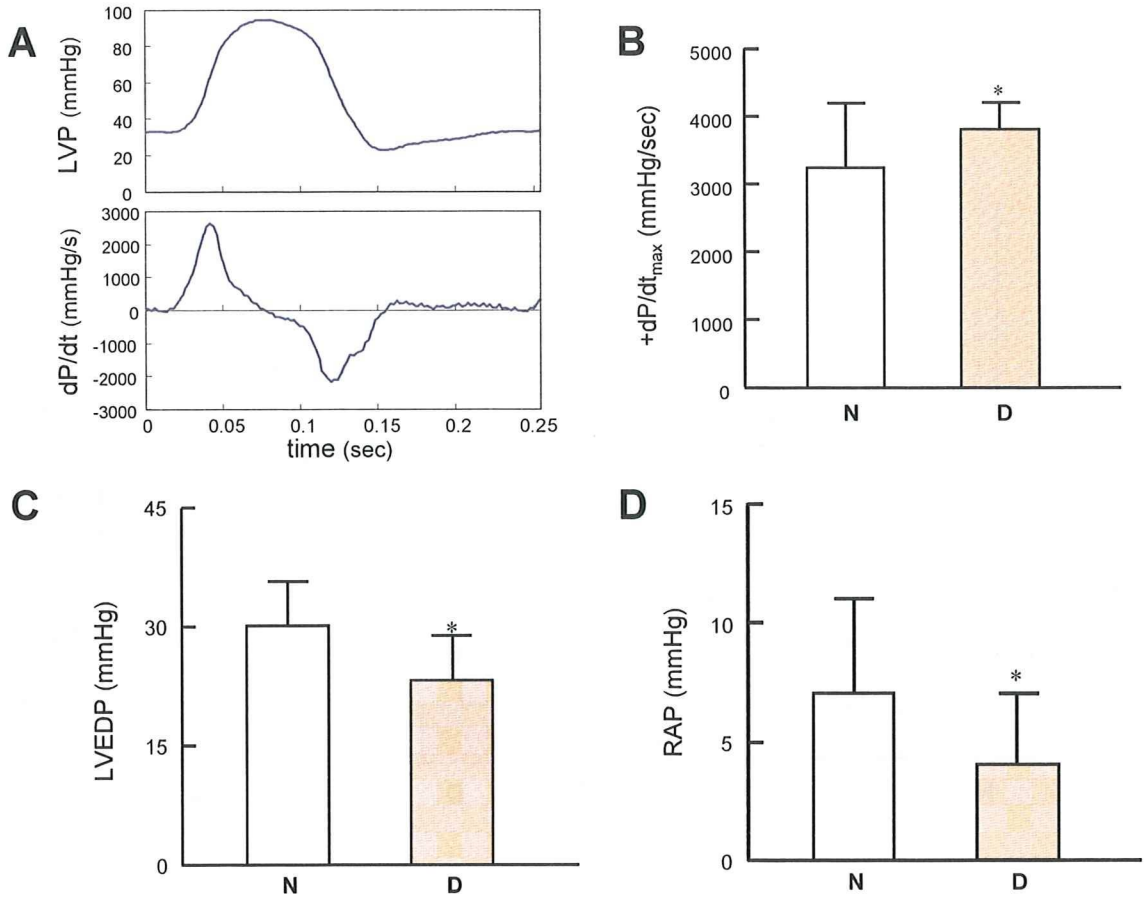
Figure legends

Figure 1.



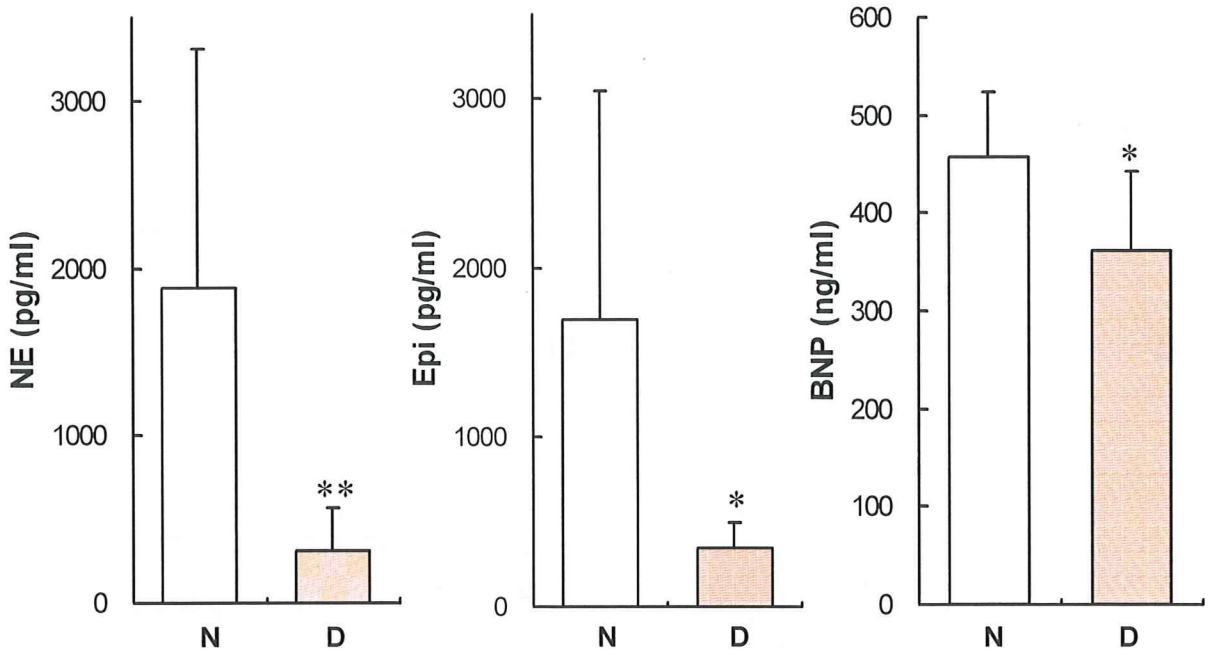
A: Schematic representation of the experimental design. Electrocardiogram was recorded continuously using a telemetric system. **B:** Ventricular sections of representative animals at week 6 of treatment. No significant difference in the size of myocardial infarction is observed between the donepezil group and the nontreated group. Compared with the nontreated heart (N), the donepezil-treated heart (D) showed thicker scar in the infarct area with more spared myocardium in the border area. **C:** Combined weight of left and right ventricles per body weight (HW/BW) at week 6 of treatment. Ventricular weight was significantly lower in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$

Figure 2.



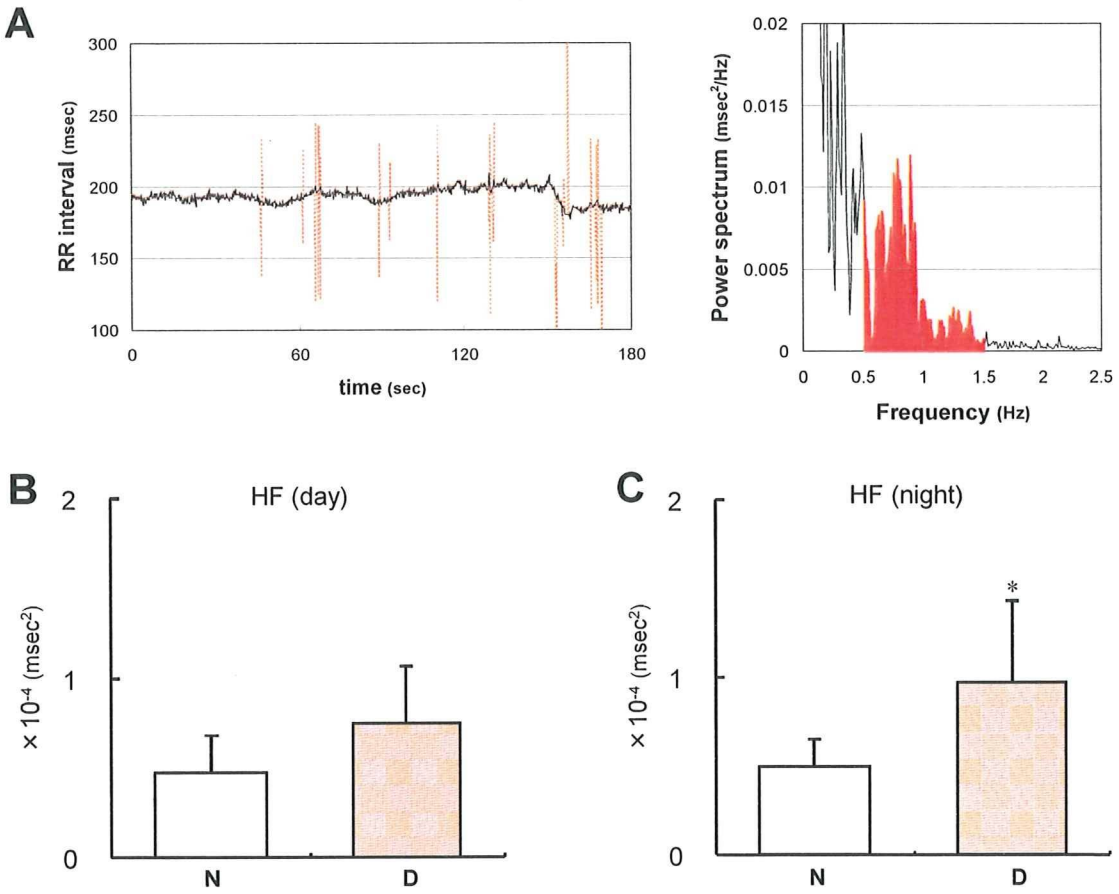
A: A representative example of left ventricular pressure waveform and its derivative in a nontreated rat. **B:** Maximal first derivative of left ventricular pressure (dP/dt_{max}) at week 6 of treatment. A significant increase in dP/dt_{max} was observed in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$ **C:** Left ventricular enddiastolic pressure (LVEDP) at week 6 of treatment. A significant decrease in LVEDP was observed in the donepezil group (shaded bar, D) compared to the nontreated control group (open bar, N). *: $p<0.05$ **D:** Right atrial pressure (RAP) at week 6 of treatment. A significant decrease in RAP was observed in the donepezil group (shaded bar, D) compared to the nontreated control group (open bar, N). *: $p<0.05$

Figure 3.



Blood concentrations of norepinephrine (NE), epinephrine (Epi) and brain natriuretic protein (BNP) at week 6 of treatment. Significant decreases in blood NE, Epi and BNP concentrations were observed in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$, **: $p<0.01$

Figure 4.



A: A representative example of time series of RR interval (left) and its power spectrum (right) in a donepezil-treated rat. RR intervals shown with dotted lines were judged as extrasystoles or post-extrasystoles and were removed before calculating power spectrum. Solid area indicates high frequency component (HF). **B:** HF of heart rate variability during the day. No significant difference in daytime HF value was observed between the donepezil group (shaded bar, D) and the nontreated group (open bar, N). **C:** High frequency component (HF) of heart rate variability during the night. A significant increase in nocturnal HF value was observed in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$ by t-test using log(HF) values

Metformin Prevents Progression of Heart Failure in Dogs
Role of AMP-Activated Protein Kinase

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Background—Some studies have shown that metformin activates AMP-activated protein kinase (AMPK) and has a potent cardioprotective effect against ischemia/reperfusion injury. Because AMPK also is activated in animal models of heart failure, we investigated whether metformin decreases cardiomyocyte apoptosis and attenuates the progression of heart failure in dogs.

Methods and Results—Treatment with metformin (10 μmol/L) protected cultured cardiomyocytes from cell death during exposure to H_2O_2 (50 μmol/L) via AMPK activation, as shown by the MTT assay, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling staining, and flow cytometry. Continuous rapid ventricular pacing (230 bpm for 4 weeks) caused typical heart failure in dogs. Both left ventricular fractional shortening and left ventricular end-diastolic pressure were significantly improved in dogs treated with oral metformin at 100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} (n=8) (18.6$\pm$1.8% and 11.8$\pm$1.1 mm Hg, respectively) compared with dogs receiving vehicle (n=8) (9.6$\pm$0.7% and 22$\pm$0.9 mm Hg, respectively). Metformin also promoted phosphorylation of both AMPK and endothelial nitric oxide synthase, increased plasma nitric oxide levels, and improved insulin resistance. As a result of these effects, metformin decreased apoptosis and improved cardiac function in failing canine hearts. Interestingly, another AMPK activator (AICAR) had effects equivalent to those of metformin, suggesting the primary role of AMPK activation in reducing apoptosis and preventing heart failure.

Conclusions—Metformin attenuated oxidative stress–induced cardiomyocyte apoptosis and prevented the progression of heart failure in dogs, along with activation of AMPK. Therefore, metformin may be a potential new therapy for heart failure. (***Circulation.*** **2009;119:2568-2577.)**

Key Words: AMP-activated protein kinase ■ heart failure ■ metformin ■ nitric oxide

Metformin is widely used as an antidiabetic drug with an insulin-sensitizing effect. A large-scale clinical trial (the UK Prospective Diabetes Study [UKPDS] 34) has shown that metformin therapy decreased the risk of cardiovascular death and the incidence of myocardial infarction associated with diabetes mellitus,[1] suggesting that this drug may be useful for patients who have both cardiovascular disease and diabetes mellitus. Eurich and colleagues[2] recently reported the results of a meta-analysis showing that metformin was the only antidiabetic agent to reduce all-cause mortality without causing any harm in patients who had heart failure and diabetes mellitus. These results suggest that a tight link exists between cardiovascular disease and diabetes mellitus and that metformin has a cardioprotective effect. Metformin is known to activate AMP-activated protein kinase (AMPK),[3–5] which is expressed in various tissues, including the myocardium, and plays a central role in the regulation of energy metabolism under stress conditions.[6] AMPK is activated by ischemia/reperfusion,[7–9] as well as in hearts with pressure overload hypertrophy[10] and subsequent heart failure.[11,12] In addition, Russell et al[9] have demonstrated that isolated hearts of AMPK-deleted mice show increased apoptosis and dysfunction after ischemia/reperfusion. Activation of AMPK by adiponectin also has been reported to protect cardiomyocytes against apoptosis and to attenuate myocardial ischemia/reperfusion injury in mice.[8] Furthermore, metformin has been reported to increase the production of nitric oxide (NO),[13–15] which is known to have various beneficial cardiovascular

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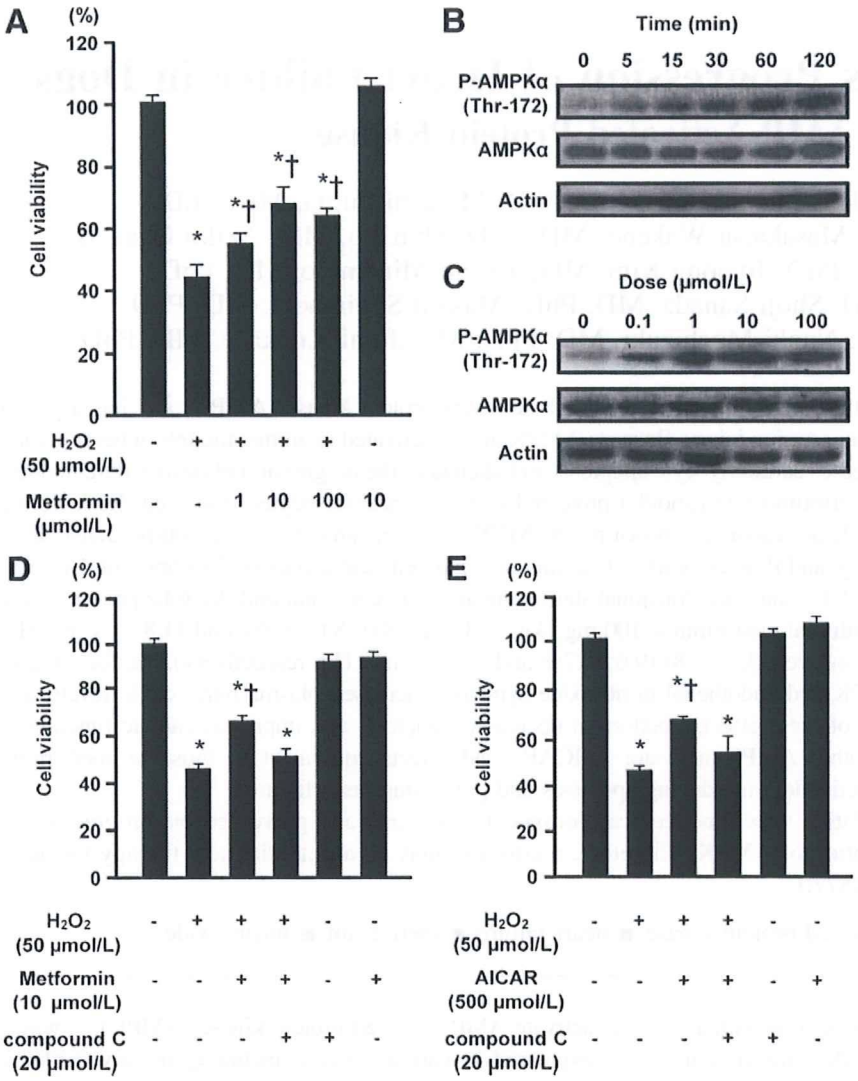

Figure 1. Effect of metformin on oxidative stress–induced cell death via AMPK activation in cultured rat cardiomyocytes. A, Cardiomyocyte viability after treatment with metformin (1, 10, or 100 μmol/L) and exposure to H_2O_2 (50 μmol/L). B, Time (0, 5, 15, 30, 60, 120 minutes) -dependent changes in AMPK phosphorylation in cardiomyocytes after treatment with metformin (10 μmol/L). C, Dose-dependent changes in AMPK phosphorylation in cardiomyocytes after treatment with metformin (0.1, 1, 10, or 100 μmol/L). D, Effect of an AMPK inhibitor (compound C; 20 μmol/L) on cardiomyocyte viability after treatment with metformin (10 μmol/L). E, Effect of an AMPK activator (AICAR; 500 μmol/L) on cardiomyocyte viability after treatment with metformin (10 μmol/L). Values are mean±SEM. P-AMPKα indicates phosphorylation of AMPKα. *$P<0.05$ vs no treatment; †$P<0.05$ vs H_2O_2 (50 μmol/L) treatment.

effects[16] and may alleviate mechanical or neurohormonal stress on the heart.

Clinical Perspective on p 2577

These findings led us to hypothesize that activation of AMPK by metformin may exert a cardioprotective effect under stress conditions. Accordingly, metformin might be a potential new treatment for cardiac failure because it activates AMPK and increases NO production. Therefore, we investigated the influence of metformin on apoptosis, an important feature of heart failure, using cultured neonatal cardiomyocytes exposed to H_2O_2 and the effect of metformin on the progression of pacing-induced heart failure in dogs, along with activation of AMPK.

Methods

Experimental procedures are described in the online-only Data Supplement.

Statistical Analysis

Results are expressed as mean±SEM. Comparison of changes between groups over time was performed by 2-way repeated-measures ANOVA. Other data were compared between groups by 1-way fractional ANOVA. The Tukey-Kramer test was used to correct for multiple comparisons. In all analyses, values of $P<0.05$ were considered to indicate statistical significance.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Metformin Attenuates Oxidative Stress–Induced Cell Death and Apoptosis in Cultured Cardiomyocytes via AMPK Activation

Cell viability was decreased in the presence of H_2O_2, as shown by the MTT assay, but this change was blunted by treatment with metformin in a dose-dependent manner (Figure 1A). Treatment with metformin (10 μmol/L) stimulated phosphorylation of AMPK in cultured cardiomyocytes in a time- and dose-dependent manner (Figure 1B and 1C). The effect of metformin on cell viability was blunted by cotreatment with compound C, an AMPK inhibitor (20 μmol/L) (Figure 1D). 5-Amino-4-imidazole-1-β-D-carboxamide ribofuranoside (AICAR; another AMPK activator) had an effect similar to metformin on cardiomyocyte viability after exposure to H_2O_2 (Figure 1E). These results suggested that

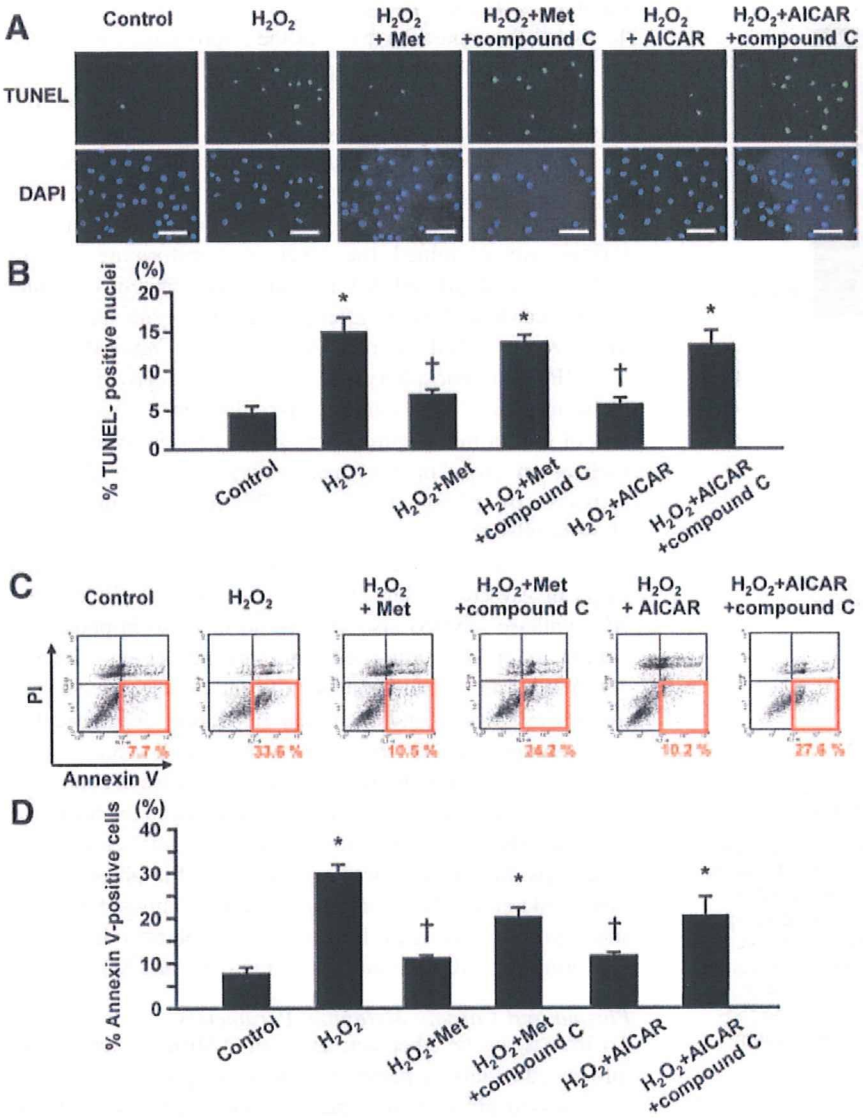

Figure 2. Effect of metformin on oxidative stress–induced apoptosis via AMPK activation in cultured rat cardiomyocytes. Representative (A) and quantitative (B) data on cardiomyocyte apoptosis obtained by TUNEL staining (n=3 in each experiment). Representative (C) and quantitative (D) data on cardiomyocyte apoptosis obtained by flow cytometry (n=3 in each experiment). Values are mean±SEM. PI indicates propidine iodide. *$P<0.05$ vs control; †$P<0.05$ vs H_2O_2 (50 μmol/L) treatment.

activation of AMPK protected cardiomyocytes against damage caused by H_2O_2.

H_2O_2 also increased cardiomyocyte apoptosis, as shown by the terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining and flow cytometry (annexin V–positive and propidine iodide–negative cells) (Figure 2A through 2D). Metformin pretreatment significantly reduced the extent of cardiomyocyte apoptosis compared with that in untreated control cells (Figure 2A through 2D). Treatment with compound C inhibited the effects of metformin and AICAR (which was similar to that of metformin) on apoptosis in cardiomyocytes exposed to H_2O_2 (Figure 2A through 2D). These results suggested that the activation of AMPK by metformin could prevent apoptosis of cardiomyocytes induced by H_2O_2.

Effect of Metformin on Cardiac Function in Dogs With Pacing-Induced Heart Failure

Cardiac Physiological and Pathophysiological Parameters

Four weeks after the rapid right ventricular (RV) pacing, left ventricular (LV) end-diastolic dimension, LV end-systolic dimension, LV fractional shortening, and LV ejection fraction of the pacing group showed significant deterioration compared with the sham group (Figure 3A and 3B). Treatment with metformin significantly reduced both LV dimensions and increased both LV fractional shortening and LV ejection fraction compared with the pacing group (Figure 3A and 3B). Before RV pacing, both mean aortic pressure and heart rate were similar in all groups, and these parameters did not change throughout the study (Table). Four weeks after the RV pacing, pulmonary capillary wedge pressure, mean pulmonary artery pressure, and LV end-diastolic pressure were all significantly higher in the pacing group compared with the sham group (Figure 4A and 4B). Metformin treatment significantly reduced pulmonary capillary wedge pressure, mean pulmonary artery pressure, and LV end-diastolic pressure compared with the pacing group (Figure 4A and 4B). Furthermore, cardiac output was decreased and systemic vascular resistance was increased in the pacing group compared with the sham group, whereas metformin increased cardiac output and decreased systemic vascular resistance compared with the levels in the pacing group (the Table).

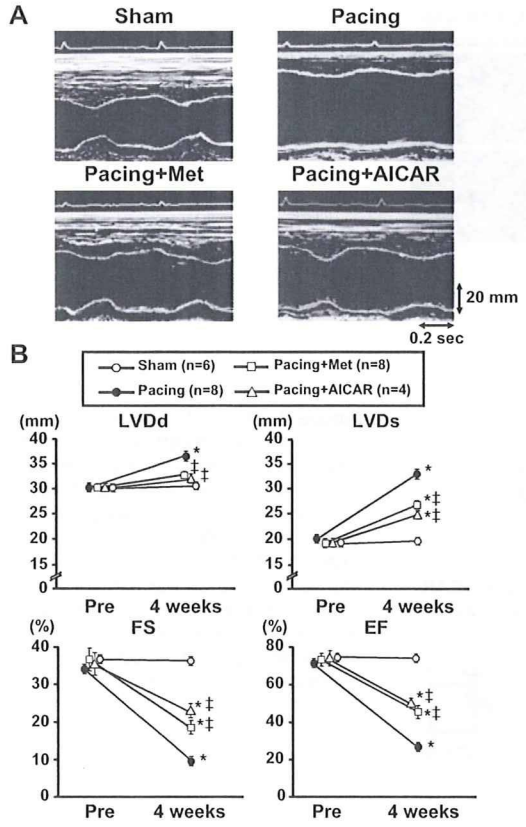

Figure 3. Effect of metformin on echocardiographic parameters. A, Representative M-mode echocardiograms obtained 4 weeks after sham surgery or after RV pacing. B, Echocardiographic parameters before and after sham surgery or after RV pacing in the sham group (n=6), pacing group (n=8), pacing plus metformin group (n=8), and pacing plus AICAR group (n=4). Values are mean±SEM. LVDd indicates LV end-diastolic dimension; LVDs, LV end-systolic dimension; LVFS, LV fractional shortening; and LVEF, LV ejection fraction. *$P<0.01$ vs sham group; ‡$P<0.01$ vs pacing group.

Importantly, the percentage of TUNEL-positive cells to total cells in LV myocardium in the pacing group increased compared with that in the sham group, which was blunted by treatment with either metformin or AICAR (Figure 5A through 5E).

Consistent with previous data,[17] no significant differences were found in body weight, the ratio of LV plus septal weight to body weight, and the ratio of RV weight to body weight among all groups (the Table).

To explore established markers of cardiac failure, we analyzed LV myocardial expression of the atrial natriuretic peptide and brain natriuretic peptide genes, which showed an increase in the pacing group, whereas metformin significantly suppressed this increase (Figure 6A and 6B). Metformin also significantly reduced the levels of angiotensin II and norepinephrine compared with the pacing group (the Table).

Pedometer counts were significantly reduced in the pacing group compared with the sham group, suggesting that heart failure led to reduced physical activities (the Table). Metformin increased the pedometer count compared with that in the pacing group. No differences in body fat were found among all groups (the Table).

Cardiac Molecular Parameters

To assess the molecular basis of the improvement in cardiac performance achieved by metformin administration for 4 weeks, we examined the collagen volume fraction in LV myocardium after staining with Masson's trichrome stain. Metformin reduced the collagen volume fraction compared with the pacing group (Figure 6C and 6D). To further investigate the mechanism of this antifibrotic effect of metformin, we examined the level of transforming growth factor-β1 (TGF-β1) mRNA associated with fibrosis in canine LV myocardium 4 weeks after pacing. Metformin suppressed the increase in TGF-β1 mRNA expression (Figure 6E).

AMPK was phosphorylated in the pacing group, and its phosphorylation was significantly enhanced by administration of metformin (Figure 7A and 7B). Phosphorylation was used as an index of enzymatic activity because AMPK is activated by phosphorylation.[18] This increase in AMPK phosphorylation was accompanied by augmented phosphorylation of acetyl-CoA carboxylase (ACC; a downstream target of AMPK) at Ser-79 (Figure 7A and 7C). Endothelial NO synthase (eNOS) also showed an increase in phosphorylation at Ser-1177 with metformin treatment (Figure 7A and 7D). Furthermore, metformin significantly upregulated eNOS mRNA expression and increased ΔNO (the difference between the plasma NO level before and after 4 weeks of RV pacing) compared with the pacing group (Figure 8A and 8B).

To investigate the level of insulin signaling in the heart, we examined the phosphorylation of Akt in the left ventricles in all groups. Significant increases were found in phosphorylation of Akt at Ser-473 in the pacing group compared with the sham group, and such increases were blunted by either metformin or AICAR treatment (Figure 8C and 8D).

Plasma and Cardiac Metabolic Parameters

To investigate whether activation of AMPK by metformin influenced metabolic parameters in the periphery or the heart, we assessed glucose and lipid metabolism after 4 weeks of pacing. Plasma free fatty acids tended to increase in the pacing group compared with the sham group, although no statistically significant difference was found. Fasting plasma levels of both glucose and lactate were similar among all groups (the Table). Both the fasting plasma insulin level and the homeostasis model assessment–insulin resistance value were significantly increased in the pacing group, whereas metformin reduced both parameters until they were similar to those of the sham group (the Table).

In the heart, both glucose extraction and the arterial–coronary sinus difference were increased in the pacing group compared with the sham group (the Table). In the pacing group, the free fatty acids extraction was not increased, but the arterial–coronary sinus difference tended to increase compared with the sham group (the Table). Lactate extraction and the arterial–coronary sinus difference were similar among all groups (the Table).

AICAR Mimics the Effect of Metformin in This Canine Pacing Model

To further confirm that activation of AMPK contributed to inhibition of the progression of heart failure, we administered

Table. Characteristics of the Dogs at 4 Weeks

	Sham Group (n=6)	Pacing Group (n=8)	Pacing+Metformin Group (n=8)	Pacing+AICAR Group (n=4)
Organ weight				
Body weight, kg	9.5±0.2	9.4±0.2	9.7±0.1	9.6±0.3
LV+septal weight, g	42±0.6	47.3±1.2	43.6±0.9	44.8±1.3
LV+septal weight/body weight ratio, g/kg	4.4±0.1	5.0±0.1	4.5±0.1	4.7±0.2
RV weight, g	14.7±0.5	15.6±0.6	15.0±1.2	14.7±1.0
RV weight/body weight ratio, g/kg	1.5±0.1	1.7±0.1	1.5±0.1	1.5±0.1
Hemodynamic parameters				
Mean aortic pressure, mm Hg	105±5	109±2	100±2	97±3.3
Heart rate, bpm	118±5	136±4	128±5	126±3.6
Cardiac output, L/min	2.6±0.1	1.6±0.1*	2.2±0.3†	2.2±0.3†
Systemic vascular resistance, dynes · s · cm^{-5}	3317±189	4769±235*	3775±334†	3763±237†
Plasma metabolic parameters				
Fasting glucose, mmol/L	5.3±0.3	5.3±0.1	5.3±0.1	5.3±0.2
Fasting insulin, μU/mL	14.2±3.3	67.6±13.7*	18.9±7.3†	24.4±10.5†
HOMA-IR	3.4±0.1	15.8±0.1*	4.4±0.1†	5.8±0.1†
Free fatty acids, μmol/L	305±67	716±68	554±101	595±69
Lactate, mmol/L	1.4±0.2	1.5±0.2	1.5±0.1	1.4±0.1
Cardiac metabolic substrates				
Glucose				
Arterial, mmol/L	5.8±0.1	6.4±0.2	6.6±0.1	6.6±0.4
Arterial–coronary sinus difference, mmol/L	0.6±0.1	1.6±0.3*	0.9±0.1	1.1±0.3
Extraction rate, %	10.5±1.2	28.6±4.7*	13.3±1.8	17.7±4.7
Free fatty acids				
Arterial, mmol/L	213.5±44.9	532.3±98.5*	312.8±56.6	294.5±22.8
Arterial–coronary sinus difference, mmol/L	90.4±13.2	153.7±20.6	99.0±9.1	103.2±20.6
Extraction rate, %	47.5±9.2	29.9±2.8	33.9±5.1	36.9±8.6
Lactate				
Arterial, mmol/L	1.8±0.1	1.9±0.3	2.3±0.7	1.8±0.8
Arterial–coronary sinus difference, mmol/L	1.2±0.3	1.0±0.2	1.3±0.5	1.1±0.4
Extraction rate, %	62.6±16.0	48.2±3.8	55.0±12.2	61.8±6.9
Plasma neurohormone levels				
Norepinephrine, pg/mL	34.9±13.0	195.9±21.3*	59.2±11.2†	79.3±8.9†
Angiotensin II, pg/mL	34.7±15.0	153.6±24.3*	78.1±14.8†	73.4±11.8†
Body fat and activity				
Body fat, %	13.7±1.2	18.7±2.9	16±1.2	14.3±0.8
Pedometer count	88 783±2899	64 541±2530*	78 423±3292†	77 716±1472†

HOMA-IR indicates homeostasis model assessment–insulin resistance. Values are mean±SEM.
*$P<0.05$ vs the sham group; †$P<0.05$ vs the pacing group.

another AMPK activator (AICAR at a dose of 5 mg/kg SC every other day) to dogs. As expected, AICAR reproduced the effects of metformin in this canine pacing model (Figures 3 through 8).

Discussion

To the best of our knowledge, this is the first study to demonstrate clearly that long-term (not short-term) oral administration of metformin, which is used as an antidiabetic agent worldwide, inhibits cardiac remodeling and prevents the progression of heart failure in dogs, along with increases in AMPK activation and NO production. Of course, we and others have previously shown that in rodent either AMPK activation or NO production attenuates myocardial ischemia/reperfusion injury in the ischemic model[7–9] and prevents cardiac remodeling in the pressure overload model.[11,12,19,20] However, it has been unclear whether AMPK or NO can modulate cardiac remodeling and inhibit the progression of heart failure in a canine model with another pathogenic mechanism that is not an ischemic or a pressure overload heart failure model. Therefore, we used a rapid pacing-induced heart failure dog model, which is considered to be similar to human dilated cardiomyopathy[21,22] and can be superimposed on translational study for human heart failure.

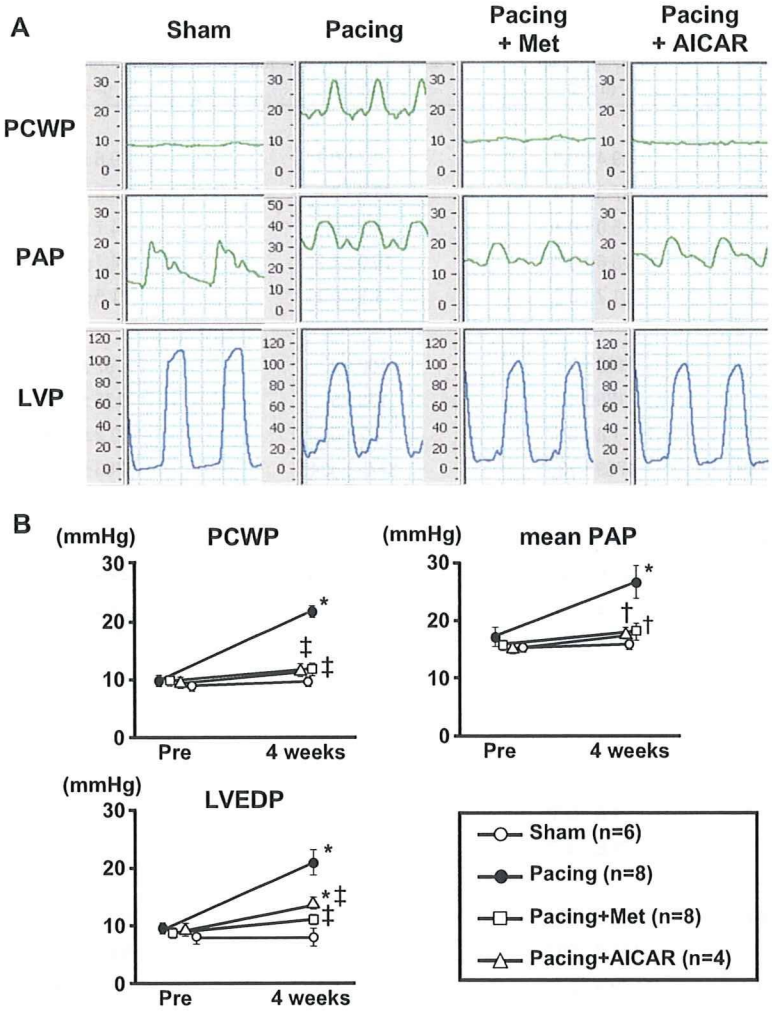

Figure 4. Effect of metformin on hemodynamic parameters. A, Representative graphs of hemodynamic parameters obtained at 4 weeks. B, Hemodynamic parameters before and after the 4-week study period in the sham (n=6), pacing (n=8), pacing plus metformin (n=8), and pacing plus AICAR (n=4) groups. Values are mean±SEM. PAP indicates pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; and LVEDP, LV end-diastolic pressure. *$P<0.05$ vs sham group; †$P<0.05$ vs pacing group; ‡$P<0.01$ vs pacing group.

Furthermore, we provide sufficient insight because dogs can be monitored more precisely for hemodynamic data than rodents.

Possible Cardioprotective Mechanism of Metformin Mediated via AMPK

Metformin has previously been shown to reduce high fat–induced apoptosis,[23] and AMPK has been reported to protect against hypoxic apoptosis in cardiomyocytes through attenuation of endoplasmic reticulum stress.[24] Consistent with these previous reports, we confirmed that metformin could ameliorate oxidative stress–induced apoptosis in cardiomyocytes. This effect was blunted by compound C, an AMPK inhibitor, suggesting that activation of AMPK was responsible for the inhibition of cardiomyocyte apoptosis. Furthermore, using a dog model, we demonstrated that metformin ameliorated the progression of heart failure induced by rapid RV pacing and decreased apoptosis in the LV myocardium, as indicated by TUNEL staining. Interestingly, AICAR, another AMPK activator, had effects almost identical to those of metformin, supporting that the activation of AMPK contributed to the observed cardioprotective effect. Indeed, AICAR also has been reported to reduce myocardial ischemia/reperfusion injury in humans and animals.[25,26] What processes following AMPK activation are involved in cardioprotection?

The first possibility is enhancement of NO production. Recchia et al[27] reported that basal cardiac NO release is decreased in dogs with heart failure induced by rapid pacing. We found that the difference in plasma NO levels between baseline and 4 weeks of RV pacing was significantly increased by metformin treatment compared with the pacing group. Metformin has been shown to phosphorylate AMPK at Thr-172 in cardiomyocytes and murine hearts,[4,5] whereas AMPK is known to phosphorylate eNOS at Ser-1177 in rat hearts,[28] resulting in an increase in NO production. Indeed, a recent report has indicated that short-term metformin treatment protects against myocardial infarction via AMPK-eNOS–mediated signaling in mice.[7] Other studies have suggested involvement of the AMPK-eNOS pathway in the response of endothelial cells to shear stress,[29] metformin,[30] and statins.[31] Consistent with these reports, we found that either metformin or AICAR promoted the phosphorylation of eNOS at Ser-1177 and increased both mRNA and protein levels of eNOS, possibly leading to increased plasma NO levels and reduced systemic vascular resistance. Although the precise mechanism of the effects of phosphorylation of AMPK by either metformin or AICAR on eNOS protein expression is not clear, these findings suggest that metformin or AICAR increased NO production, which improves endothelial

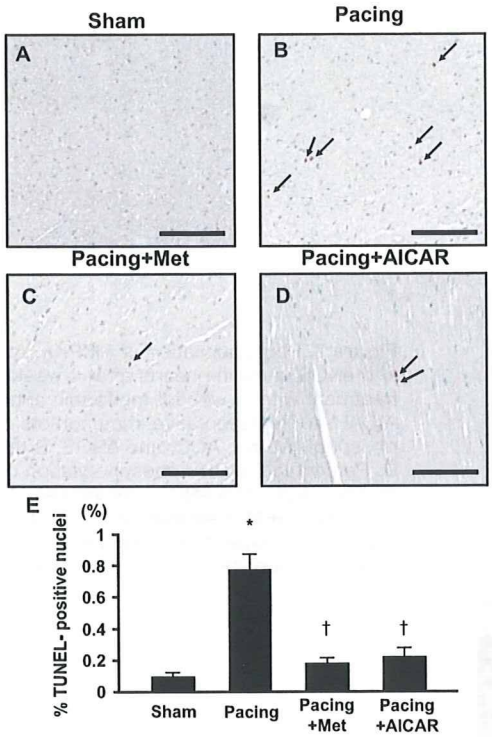

Figure 5. TUNEL staining of canine hearts at 4 weeks. Representative examples of TUNEL-stained hearts from sham (A), pacing (B), pacing plus metformin (C), and pacing plus AICAR (D) groups. Arrows indicate TUNEL-positive nuclei (brown). Scale bar=100 μm. E, Quantitative data on the percentage of TUNEL-positive nuclei to total cell nuclei. *$P<0.05$ vs sham group; †$P<0.05$ vs pacing group.

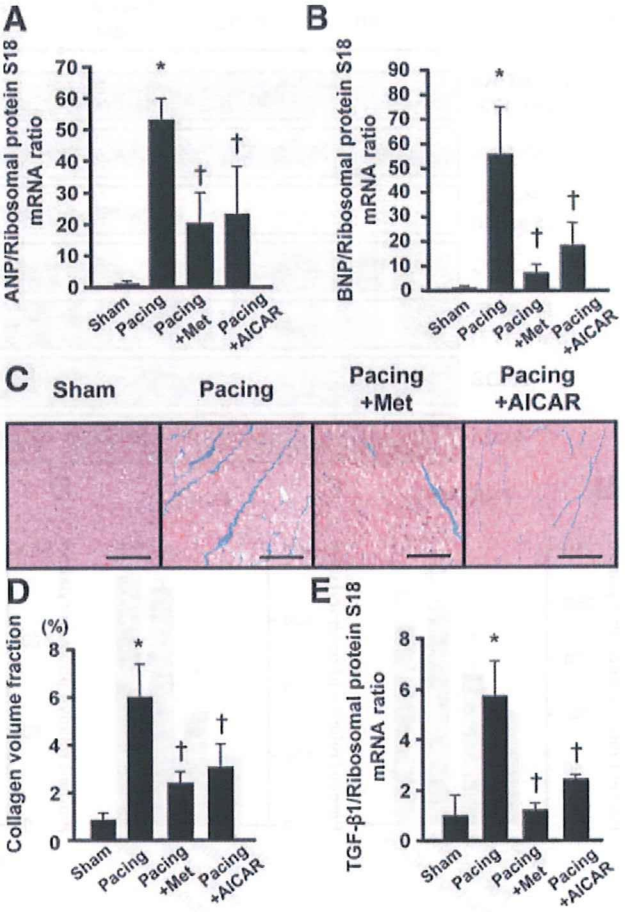

Figure 6. Natriuretic peptide expression, cardiac collagen volume fraction, and TGF-β1 expression. A, B, and E, Quantitative real-time reverse-transcriptase polymerase chain reaction analysis of myocardial atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and TGF-β1 expression, respectively. The mRNA values were corrected for the ribosomal protein S18 mRNA level. The sham group was arbitrarily assigned a value of 1.0. Results are mean±SEM. Representative results from 3 independent experiments are shown. *$P<0.05$ vs sham group; #$P<0.05$ vs pacing group. C, Representative histological appearance of LV myocardium stained with Masson's trichrome stain (light blue). Scale bar=100 μm. D, Collagen volume fraction in the LV myocardium. Values are mean±SEM. *$P<0.05$ vs sham group; †$P<0.05$ vs pacing group.

function. NO is believed to have various cardioprotective effects.[16] Therefore, enhancement of NO production by metformin via activation of AMPK may have contributed to alleviating the progression of heart failure induced by rapid RV pacing.

The second possibility is related to the improvement in insulin resistance. It is known that insulin resistance is associated with the progression of chronic heart failure, whereas chronic heart failure may provoke insulin resistance by increasing sympathetic activity, activating the renin-angiotensin system, or both.[32,33] We found that rapid RV pacing for 4 weeks induced heart failure and that metformin treatment improved insulin resistance (estimated by homeostasis model assessment–insulin resistance) compared with the pacing group, suggesting that the beneficial effect of metformin on heart failure mediated via AMPK may have been due in part to an improvement in insulin resistance.

The third possibility is the metabolic effects of AMPK activation. Both metformin and AICAR are reported to increase glucose extraction in heart,[34,35] which may decrease the severity of the failing hearts. However, we found a 2- to 3-fold increase in myocardial glucose extraction of pacing dogs, and metformin returned glucose extraction to the value of the sham group. Numerous studies have shown a switch from free fatty acids to glucose as the primary energy substrate in humans and animals with advanced heart failure,[27,36–38] suggesting that the reduction in glucose extraction by the improvement in heart failure by AMPK activation is likely to be greater than the induction of glucose extraction by direct activation of AMPK. The possibility exists that AMPK-induced glucose extraction triggers the improvement in heart failure, followed by the restoration of metabolic switch. On the other hand, we found that the net free fatty acids extraction of the pacing group tended to increase despite no statistical significance, which is consistent with the report by Paolisso et al[39] that myocardial free fatty acids extraction increased in patients with congestive heart failure[39] but is contrary to the reports of the metabolic switch.[27,36–38] The metabolic switch may differ in relatively acute or chronic heart failure and by the severity of heart failure.

The increased phosphorylation of Akt in the pacing group was attenuated in either the pacing plus metformin or the pacing plus AICAR group, suggesting that the levels of activation of insulin signaling decreased in either the

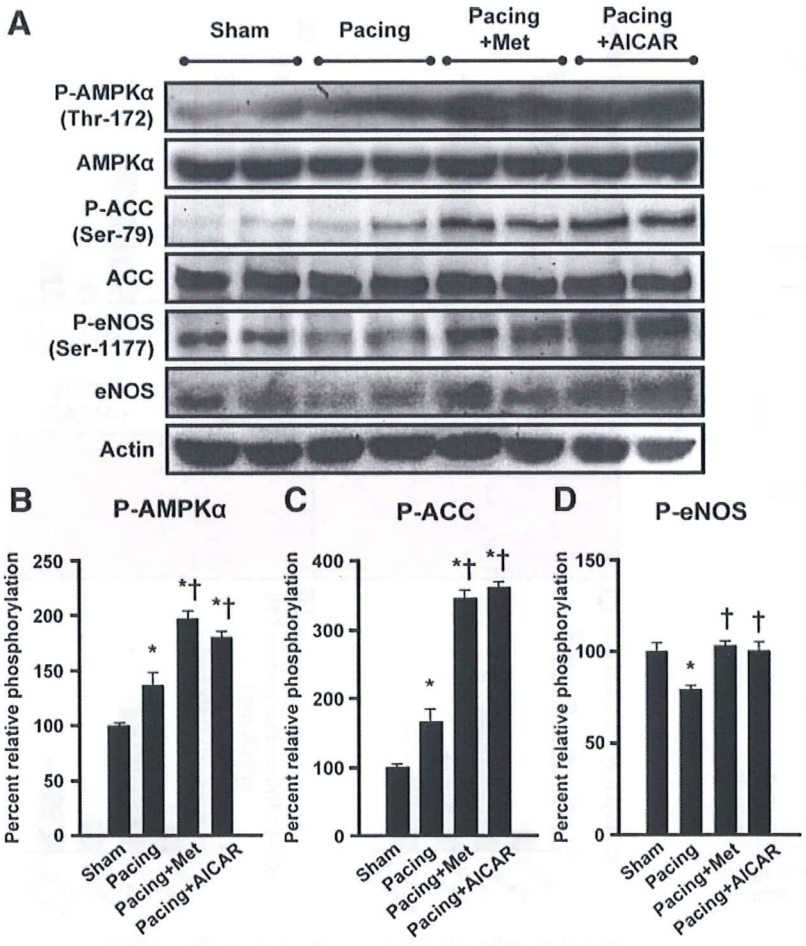

Figure 7. Phosphorylation of AMPKα, ACC, and eNOS in canine hearts after 4 weeks of treatment with or without metformin and AICAR. A, Representative immunoblots of phospho-AMPKα, ACC, and eNOS. B through D, Percentage relative phosphorylation of AMPKα, ACC, and eNOS, respectively. Values are mean±SEM. Representative results from 3 independent experiments are shown. *$P<0.05$ vs sham group; †$P<0.05$ vs pacing group.

metformin- or AICAR-treated group. Considering that glucose extraction was decreased in the pacing plus metformin and pacing plus AICAR groups and that AMPK was phosphorylated by either metformin or AICAR, which may increase in glucose extraction in the heart, the present data may be contradictory, but they are not contradictory when we consider the changes in phosphorylated Akt. The reason is that in this pacing-induced canine heart failure model, glucose extraction in the heart was influenced predominantly by insulin resistance, accompanied by the severity of heart failure, rather than AMPK phosphorylation, although further investigation on this issue is needed.

The fourth possibility is the antifibrotic effect of metformin. Several studies have indicated that AMPK activation inhibits protein synthesis through effects on both the eEF-2 and mTOR pathways.[40,41] We demonstrated that no significant difference in ventricular mass existed at autopsy among the groups. This dog pacing model has been reported to preserve wall thickness without hypertrophy or a consistent increase in heart weight, unlike the pressure overload model.[42] We found that metformin attenuated fibrosis and reduced the TGF-β1 mRNA level after 4 weeks of RV pacing compared with the pacing group. Metformin also improved representative markers of heart failure, including LV end-diastolic pressure, brain natriuretic peptide, angiotensin II, and norepinephrine. Although a number of factors may have contributed to the antifibrotic effect of metformin, our data suggest that inhibition of TGF-β1 by metformin has at least some role, resulting in the prevention of heart failure.

Taken together, these data suggest that metformin has a direct cardioprotective effect, has effects on the improvements of peripheral vascular system and insulin resistance, and inhibits fibrosis. All these actions might contribute to the improvement in the pathophysiology of heart failure, although we could not identify the exact role of each factor. It remains to be determined whether these results were a cause or consequence of improved cardiac function, especially in systemic effects of both insulin resistance and systemic vascular resistance.

Study Limitations

We found that the extent of phosphorylation of eNOS decreased despite the increase in the phosphorylated Akt in the pacing-induced failing canine hearts, which may be contradictory to previous reports that the phosphorylation of Akt leads to eNOS phosphorylation.[43,44] Because the signal transduction to modulate eNOS is unclear in the failing myocardium and the pathophysiological role and importance of Akt also are unclear, this discrepancy should be clarified in future studies.[45]

We need to consider the dose of metformin used in the present study, which was at least 3-fold higher than that used clinically. Nevertheless, adverse effects such as hypoglycemia and lactic acidosis were not detected during the experiment.

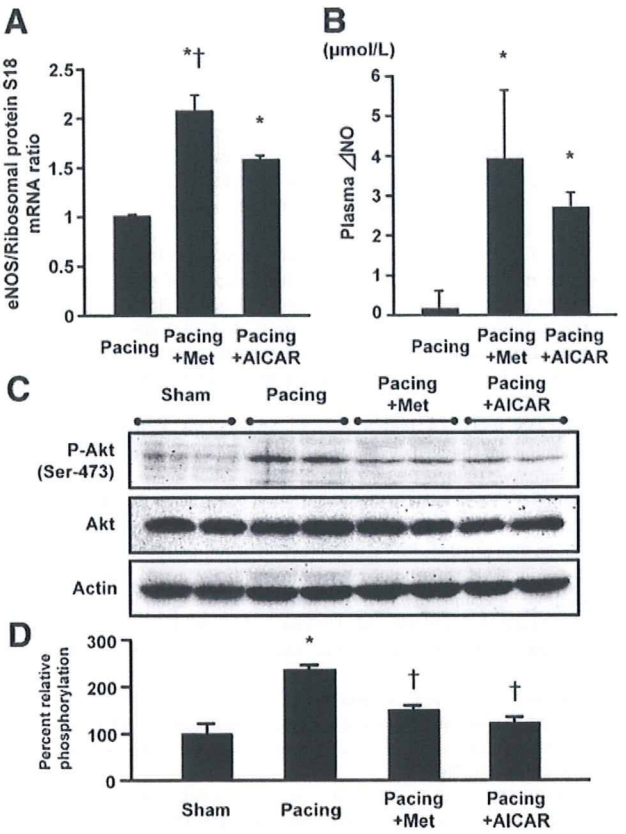

Figure 8. Effect of metformin on eNOS mRNA expression and plasma ΔNO levels, and phosphorylation of Akt in canine hearts. A, Quantitative real-time reverse-transcriptase polymerase chain reaction for eNOS mRNA. The mRNA levels were normalized to ribosomal protein S18 mRNA, and the pacing group was arbitrarily assigned a value of 1.0. B, Plasma ΔNO level after 4 weeks of RV pacing with or without metformin and AICAR administration. Values are mean±SEM. Representative results from 3 independent experiments are shown. *$P<0.05$ vs pacing group; †$P<0.05$ vs pacing plus AICAR group. C, Representative immunoblots of phospho-Akt. D, Percent relative phosphorylation of Akt. Values are mean±SEM. Representative results from 3 independent experiments are shown. *$P<0.05$ vs sham group; †$P<0.05$ vs pacing group.

Conclusions

We demonstrated that metformin prevents the progression of pacing-induced heart failure in dogs, along with the activation of AMPK. Metformin may offer a novel treatment strategy for heart failure.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Metformin is widely used as an antidiabetic drug with an insulin-sensitizing effect. A large-scale clinical trial (the UK Prospective Diabetes Study [UKPDS] 34) has shown that metformin therapy decreased the risk of cardiovascular death and the incidence of myocardial infarction associated with diabetes mellitus; metformin reduced the hemoglobin A_{1c} levels in treated patients to the same extent as in the other patients treated with conventional therapies. These results suggest that metformin might exert cardioprotective effects beyond its glucose-lowering action such as either activation of AMP-activated protein kinase (AMPK) or elevation of nitric oxide. Metformin is known to activate AMPK, which mediates potent cardioprotection against ischemia/reperfusion injury. AMPK also is activated in experimental failing myocardium, suggesting that activation of AMPK is beneficial for the pathophysiology of heart failure. The present study demonstrated that long-term oral administration of metformin prevents the progression of heart failure as indicated by hemodynamic and echocardiographic parameters. Metformin also promoted phosphorylation of both AMPK and endothelial nitric oxide synthase, increased plasma nitric oxide levels, and improved insulin resistance. As a result of these effects, metformin decreased apoptosis and improved cardiac function in failing canine hearts. Interestingly, another AMPK activator (AICAR) had effects equivalent to those of metformin, suggesting the primary role of AMPK activation in reducing apoptosis and preventing heart failure. Drugs that activate AMPK, especially metformin, may provide a novel strategy for the treatment of heart failure in clinical settings.