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ORIGINAL RESEARCH

Long-Term Dipeptidyl Peptidase 4 Inhibition Worsens Hypertension and Renal and Cardiac Abnormalities in Obese Spontaneously Hypertensive Heart Failure Rats

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BACKGROUND: The long-term effects of dipeptidyl peptidase 4 (DPP4) inhibitors on blood pressure and cardiovascular and renal health remain controversial. Herein, we investigated the extended (>182 days) effects of DPP4 inhibition in a model of spontaneous hypertension, heart failure, diabetes mellitus, obesity and hyperlipidemia.

METHODS AND RESULTS: Adult obese spontaneously hypertensive heart failure rats (SHHF) were implanted with radio transmitters for measurement of arterial blood pressures. Two weeks later, SHHF were randomized to receive either a DPP4 inhibitor (sitagliptin, 80 mg/kg per day in drinking water) or placebo. At the end of the radiotelemetry measurements, renal and cardiac function and histology, as well as other relevant biochemical parameters, were assessed. For the first 25 days, mean arterial blood pressures were similar in sitagliptin-treated versus control SHHF; afterwards, mean arterial blood pressures increased more in sitagliptin-treated SHHF ($P<0.000001$). The time-averaged mean arterial blood pressures from day 26 through 182 were 7.2 mm Hg higher in sitagliptin-treated SHHF. Similar changes were observed for systolic (8.6 mm Hg) and diastolic (6.1 mm Hg) blood pressures, and sitagliptin augmented hypertension throughout the light-dark cycle. Long-term sitagliptin treatment also increased kidney weights, renal vascular resistances, the excretion of kidney injury molecule-1 (indicates injury to proximal tubules), renal interstitial fibrosis, glomerulosclerosis, renal vascular hypertrophy, left ventricular dysfunction, right ventricular degeneration, and the ratios of collagen IV/collagen III and collagen IV/laminin in the right ventricle.

CONCLUSIONS: These findings indicate that, in some genetic backgrounds, long-term DPP4 inhibitor treatment is harmful and identify an animal model to study mechanisms of, and test ways to prevent, DPP4 inhibitor–induced pathological conditions.

Key Words: dipeptidyl peptidase 4 inhibitors ■ heart damage ■ hypertension ■ kidney damage ■ spontaneously hypertensive heart failure rats

Dipeptidyl peptidase 4 (DPP4) inhibitors (DPP4Is; eg, sitagliptin, saxagliptin, and alogliptin) are a widely used class of antidiabetic drugs that increase incretin levels by inhibiting their metabolism by DPP4. Because activation of incretin receptors augments insulin release and suppresses glucagon secretion, DPP4I therapy improves glucose homeostasis.[1,2] There is considerable evidence, however, that DPP4Is yield suboptimal cardiovascular and renal outcomes in patients with type 2 diabetes mellitus. For example, the SAVOR–TIMI 53 (Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus [SAVOR]–Thrombolysis in Myocardial Infarction [TIMI] 53) trial reported that saxagliptin significantly increased

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CLINICAL PERSPECTIVE

What Is New?

- Herein, we show that extended (>182 days) treatment of spontaneously hypertensive heart failure rats (a model of the metabolic syndrome with a spontaneously hypertensive rat genetic background) with a dipeptidyl peptidase 4 inhibitor (DPP4I) increases blood pressure and damages the heart and kidneys.

What Are the Clinical Implications?

- In patients with type 2 diabetes mellitus, direct glucagon-like peptide-1 receptor agonists are antihypertensive and improve cardiovascular outcomes; in contrast, in patients with type 2 diabetes mellitus, DPP4Is, which indirectly activate glucagon-like peptide-1 receptors, underperform relative to direct glucagon-like peptide-1 receptor agonists with respect to target-organ protection; likely, this is because DPP4Is exert a complex mix of beneficial and harmful effects.
- As shown in the present study, in some contexts, long-term treatment with DPP4Is actually causes harm.
- The present study identifies an animal model that could reveal the mechanisms that compromise the beneficial effects of DPP4Is in patients with type 2 diabetes mellitus; a better understanding of such mechanisms would inform how to optimize therapy with DPP4Is and would suggest other approaches for treating and preventing cardiovascular and renal diseases in patients with type 2 diabetes mellitus.

Nonstandard Abbreviations and Acronyms

DBP	diastolic blood pressure
DPP4	dipeptidyl peptidase 4
DPP4I	dipeptidyl peptidase 4 inhibitor
GLP-1RA	glucagon-like peptide-1 receptor agonist
HR	heart rate
MABP	mean arterial blood pressure
PH	pulmonary hypertension
SBP	systolic blood pressure
SHHF	spontaneously hypertensive heart failure rats
SHR	spontaneously hypertensive rats
WKY	Wistar-Kyoto rats

hospitalization for heart failure, induced renal abnormalities, and tended to increase all-cause mortality.[3] Although an analysis of the EXAMINE (Examination of Cardiovascular Outcomes With Alogliptin vs Standard of Care in Patients with Type 2 Diabetes Mellitus and Acute Coronary Syndrome) study concluded that alogliptin does not increase overall hospitalization for heart failure,[4] this analysis did reveal a significantly increased rate of hospitalization for heart failure in a subgroup of patients without a history of heart failure who were treated with alogliptin.[4] Indeed, a US Food and Drug Administration advisory committee concluded that alogliptin increases hospitalization for heart failure. In line with this conclusion, several observational studies concluded that DPP4Is increase hospitalization for heart failure.[5,6] A comprehensive meta-analysis involving 54 trials enrolling 74 737 patients noted a near-significant 10.6% increased risk of hospitalization for heart failure in patients taking DPP4Is.[7] Moreover, Chen et al[8] concluded that sitagliptin had no cardiovascular benefits, but did increase the risk of recurrent myocardial infarction and coronary revascularization, and the PROLOGUE (Program of Vascular Evaluation under Glucose Control by DPP-4 Inhibitor) study found no evidence that sitagliptin decreases the progression of carotid intima-medial thickness.[9] At best, cardiovascular outcome trials suggest that DPP4Is do not improve cardiovascular mortality, nonfatal myocardial infarction, or nonfatal stroke.[2] This is in stark contrast to glucagon-like peptide-1 receptor agonists (GLP-1RAs), which clearly afford cardiovascular and renal benefits in patients with type 2 diabetes mellitus by directly activating incretin receptors.[2]

A better understanding of why DPP4Is underperform, relative to GLP-1RAs, with regard to improving cardiovascular and renal outcomes in patients with type 2 diabetes mellitus may reveal important clues as to causes of, and treatments for, cardiovascular and kidney diseases and may suggest ways to improve outcomes with DPP4I therapy. What is needed to achieve these goals is a reliable animal model in which DPP4Is enhance glycemic control yet worsen cardiac and renal outcomes. By using such an animal model, it may be possible to clarify the following: (1) which biochemical systems activated by DPP4Is neutralize the beneficial cardiovascular and renal effects of DPP4Is in patients; (2) which patients would benefit most from DPP4I therapy; and (3) what cotherapies could be combined with DPP4Is to improve patient outcomes.

Unfortunately, in stark contrast to diabetic patients, animal models of diabetes mellitus overwhelming and consistently show markedly improved renal and cardiovascular outcomes with DPP4I therapy.[10–22] However, our recent work demonstrates context-dependent effects of long-term treatment with sitagliptin on blood pressure in animal models. In this regard, we observed that sitagliptin increases arterial blood pressure in spontaneously hypertensive rats (SHR), has little effect on blood pressure in normotensive Wistar-Kyoto rats (WKY), and is

antihypertensive in rats with a polygenetically driven version of the metabolic syndrome (Zucker diabetic Sprague-Dawley rats).[23] Because these studies used the same drug, same dose, and same experimental design, yet yielded qualitatively different effects on blood pressure, we concluded that the effects of DPP4Is are context dependent.[24]

The fact that sitagliptin worsens hypertension in SHR, but does not adversely affect blood pressure in WKY or Zucker diabetic Sprague-Dawley rats, motivated the present study to investigate the "extended" long-term effects of sitagliptin in obese spontaneously hypertensive heart failure rats (SHHF). Because obese SHHF have an SHR genetic background, they should be sensitive to the adverse effects of DPP4Is. Moreover, because SHHF have a phenotype that includes the metabolic syndrome (obesity, diabetes mellitus, dyslipidemia, and hypertension) and cardiac and renal pathological features and dysfunction,[25] they should be more sensitive to the negative cardiovascular and renal effects of DPP4Is and should better model the demographics of patients often prescribed DPP4Is. Therefore, we hypothesized that this animal model may be ideal for revealing the negative effects of DPP4Is. The current study shows unequivocally that, in the appropriate genetic background (context), the extended administration of DPP4Is increases blood pressure and worsens cardiac and renal structure and function. These findings have implications for the current use of DPP4Is and suggest that obese SHHF may be a useful preclinical model for achieving a better understanding of the pharmacological features of DPP4Is. This better understanding may, in turn, improve treatment paradigms with DPP4Is and identify novel targets for drugs aimed at preventing and treating cardiovascular and renal diseases.

METHODS

For data and for additional information on analytic methods or study materials, contact Dr Jackson at edj@pitt.edu.

This study used 20 male, obese SHHF obtained from Charles River (Wilmington, MA). We selected to use male SHHF for this study because our goal was to examine the chronic effects of DPP4 inhibition in the background of the metabolic syndrome, type 2 diabetes mellitus, and increased risk of cardiac and renal disease. This phenotype, we reasoned, represents the main clinical demographic receiving DPP4Is and would be the most likely to reveal the harmful consequences of long-term DPP4 inhibition. More important, male obese SHHF express the metabolic syndrome with overt type 2 diabetes mellitus early in life and by 10 to 12 months of age express overt heart failure. In contrast, obese female SHHF do not develop overt diabetes mellitus and have preserved organ function, even at 12 months of age. For a comparison of obese male SHHF to other models of the metabolic syndrome and to other "control" rat strains, see review by Tofovic and Jackson.[25] The Institutional Animal Care and Use Committee approved all procedures. The investigation conforms to National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

In addition to carefully selecting the appropriate phenotype for this study, we also carefully considered the optimal experimental design with respect to both age of the animals on initiation of treatments and duration of treatments. Our goal herein was to treat adult animals with DPP4Is for as long as possible (to mimic life-long antidiabetic therapy in adult humans), while avoiding loss of animals attributable to death (which would threaten validity of results). This was an important consideration because by 12 months of age, male obese SHHF are near the end of their life span, making terminal experiments difficult. To optimize our experimental design, on arrival at 8 weeks of age, SHHF were acclimated for 4 weeks (now 12 weeks of age) and then implanted with radio transmitters (TA11PA-C40; Data Sciences International, St Paul, MN) for long-term monitoring of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP), and heart rate (HR) with a Data Sciences International radiotelemetry system, as previously described.[26] Blood pressures and HRs were captured throughout the 24-hour light-dark cycle. After a 2-week run-in period to allow animals to fully recover from surgery, 10 SHHF (now 14 weeks of age) were randomized to sitagliptin (80 mg/kg per day; Merck, Kenilworth, NJ) in drinking water and 10 were provided drinking water without sitagliptin. The concentration of sitagliptin in the drinking water was adjusted weekly, according to average body weight and water intake.

In humans, at steady state, 100 mg of sitagliptin per day (the standard clinical dose) suppresses plasma DPP4 activity by >90% throughout most of the dosing interval.[27] Therefore, in the present study, we sought to use a dose of sitagliptin that would approach this level of inhibition of plasma DPP4 activity. Our rationale for using 80 mg/kg per day in the current study was 3-fold. First, Reichetzeder and colleagues showed that in rats a sitagliptin dose of 30 mg/kg per day causes <50% inhibition of plasma DPP4 activity,[28] a finding indicating that 30 mg/kg per day of sitagliptin is insufficient to mimic the level of DPP4 inhibition achieved with the standard sitagliptin dose in humans. Second, Giannocco and coworkers showed that in adult SHR, 80 mg/kg per day

of sitagliptin blocks 88% of plasma DPP4 activity.[29] This indicates that 80 mg/kg per day, while not totally suppressing DPP4 activity, approaches the efficacy of the standard sitagliptin dose in humans. Third, we previously showed that sitagliptin at 80 mg/kg per day blocks plasma DPP4 activity in SHR and Zucker diabetic Sprague-Dawley rats by 70% and increases glucagon-like peptide-1 levels by 60%.[23] This finding is consistent with the results reported by Giannocco and colleagues. Therefore, in the current study, we chose to use 80 mg/kg per day of sitagliptin because this dose provides a percentage inhibition of DPP4 activity comparable to that observed in humans with the standard dose of 100 mg per day.

The radiotelemetry phase of the study lasted 182 days (26 weeks). During this phase of the study, one control rat died of unknown causes on day 47, and the blood pressure signal in another control rat began to decay on day 51 and soon thereafter was lost; however, this rat remained healthy and was continued in the study for downstream measurements. Therefore, complete 182-day radiotelemetry results were obtained and are reported for 8 control SHHF and 10 sitagliptin-treated SHHF. In the 10 sitagliptin-treated rats, oral sitagliptin treatment was maintained until the final disposition of the animal.

After the radiotelemetry phase of the study, terminal experiments were conducted in the 19 animals. This process required approximately 4 weeks. Thus, the SHHF at the end of the study were 10 to 11 months of age (ie, near the end of their life span but still healthy). After an overnight fast, tail vein blood samples were obtained for measurement of blood glucose (10009582, Glucose Colorimetric Assay Kit; Cayman Chemical, Ann Arbor, MI) and glycated hemoglobin (Bayer A1C Now+Multi-Test Blood Glucose Monitor, Whippany, NJ). Next, rats were placed in metabolic cages for 3 days. After 2 days of acclimation, body weights and 24-hour food intakes, water intakes, and urine volumes were determined. During the next few weeks, each of the 19 rats (1 rat per workday) was prepared for short-term hemodynamic studies. In this regard, rats were weighed and then anesthetized with thiobutabarbital (90 mg/kg IP). Body temperature was monitored with a rectal temperature probe and maintained with a heating plate and heat lamp. Polyethylene cannulas were inserted into the trachea (polyethylene-240) to facilitate respiration and into the femoral artery (polyethylene-50) for measurement of MABP using a digital blood pressure analyzer (BPA 200; Micro-Med, Inc, Louisville, KY). A polyethylene-50 cannula was inserted into the femoral vein, and an intravenous infusion of 0.9% saline (50 μL/min) was initiated to maintain volume status.

Before any further manipulations that might have destabilized the rat, measurements of right ventricular (RV) pressure-time variables were taken. Briefly, a pressure transducer catheter (SPR-513; Millar Instruments, Houston, TX) connected to a Millar MPCU-200 pressure conductance system was advanced into the RV via the right jugular vein and right atrium. After a 15-minute stabilization period, RV pressure-time variables were recorded for 10 minutes and the Millar probe was removed from the RV. Then, an admittance pressure catheter (Transonic Systems, Ithaca, NY) was inserted into the left ventricle (LV) via the right carotid artery (closed chest) for 5 minutes for measurement of LV pressure-time variables using an ADVantage Pressure System (ADV500 PV System; Transonic Systems). Next, a polyethylene-10 catheter was placed in the left ureter for urine collection, and a transit-time flow probe (1RB; Transonic Systems) was placed on the left renal artery for monitoring renal blood flow using a flowmeter (T206; Transonic Systems). After a 1-hour stabilization period, urine was collected for 90 minutes and divided into 4 aliquots: 1 for creatinine (500701, Creatinine Colorimetric Assay Kit; Cayman Chemical), 1 for sodium and potassium (IL-943 flame photometer; Instrumentation Laboratory, Lexington, MA), 1 for urinary albumin (Rat Albumin ELISA Kit; Genway, San Diego, CA), and 1 for the renal injury biomarkers kidney injury molecule-1 (ab119597, Rat KIM-1 ELISA Kit; Abcam, Cambridge, MA) and neutrophil gelatinase-associated lipocalin (ab207925, Rat Lipocalin-2 ELISA Kit; Abcam). Also, renal blood flow and MABP were monitored during the 90-minute urine collection and averaged for calculation of renal vascular resistance (renal vascular resistance=MABP/renal blood flow). At the end of the short-term measurements, a 1-mL blood sample was obtained for measurement of hematocrit and plasma creatinine, sodium, potassium, cholesterol (10007640, Cholesterol Fluorometric Assay Kit; Cayman Chemical), triglycerides (1000303, Triglyderide Colorimetric Assay Kit; Cayman Chemical), plasma interleukin-6 (R6000B, Rat IL-6 Quantikine ELISA kit; R&D Systems, Minneapolis, MN), and plasma interleukine-1β (IL-1β; RLB00, Rat IL-1beta Quantikine ELISA Kit; R&D Systems).

At the end of the short-term study, a catheter was placed in the LV and the rat's cardiovascular system was flushed with 60 mL of 0.9% NaCl to remove blood. Organs were removed and weighed, and a part of the RV and LV and one kidney were stored at −80°C until assayed for extracellular matrix proteins (including collagens I, III, IV, and VI, fibronectin, and laminin) using LifeSpan Biosciences (Seattle, WA) ELISA kits (LS-F5638, Rat Collagen I ELISA Kit; LS-F5562, Rat Collagen III ELISA Kit; LS-F4368, Rat Collagen IV ELISA Kit; LS-F8977, Rat Collagen VI ELISA Kit; LS-F24937, Rat Fibronectin ELISA Kit; and LS-F6465, Rat Laminin ELISA Kit). Also, part of the RV and LV and one kidney were placed in 4% paraformaldehyde for histological analysis, and the tibia was harvested and its

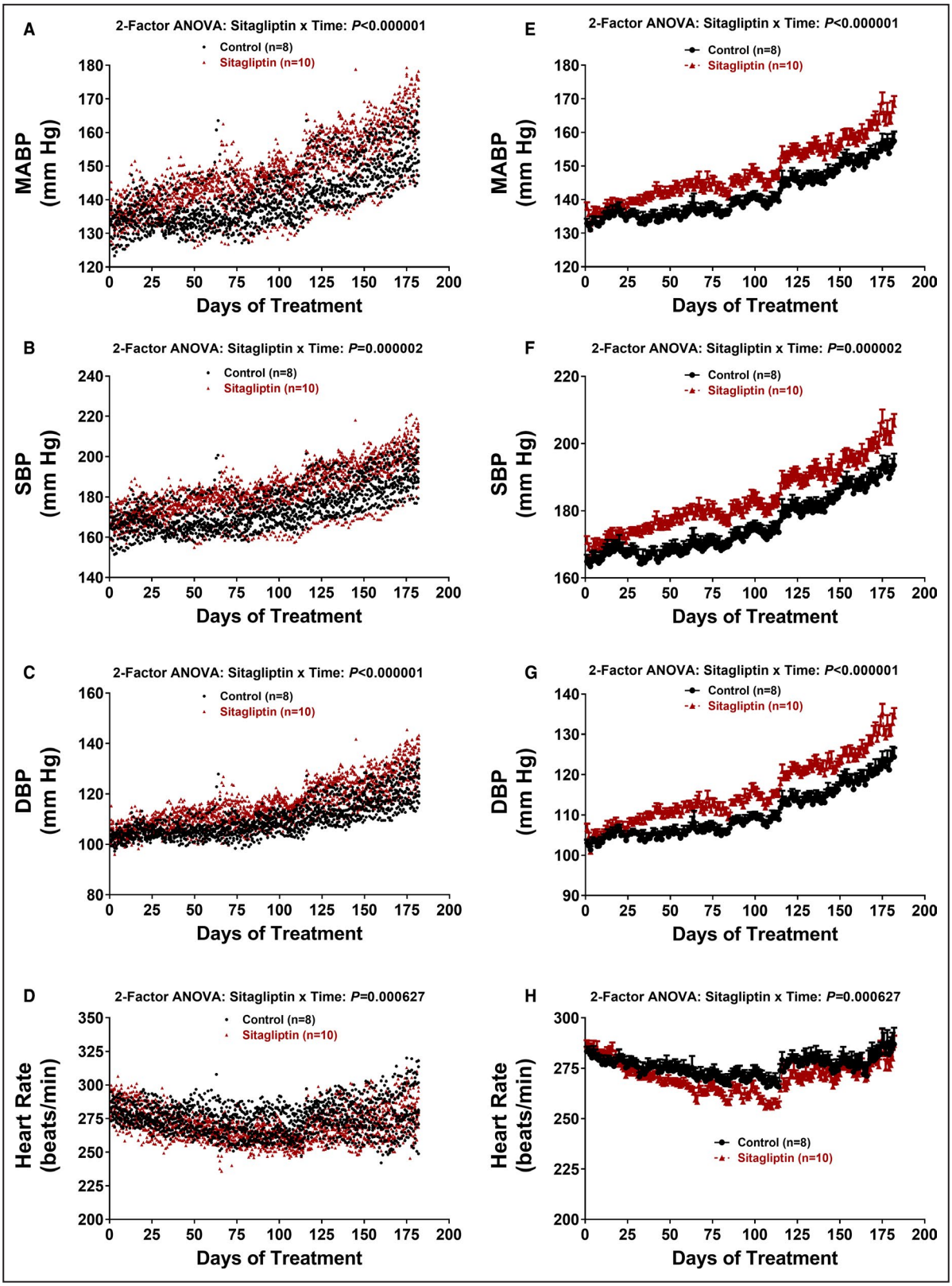

Figure 1. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on mean arterial blood pressure (MABP) (A, E), systolic blood pressure (SBP) (B, F), diastolic blood pressure (DBP) (C, G), and heart rate (D, H) in obese spontaneously hypertensive heart failure rats.

Results are expressed as both scatterplots (3276 data points for each variable, with each data point representing a 24-hour average of 144 readings) and, for ease of visualization, line graphs (means and SEM for n number of rats).

length measured for normalization purposes. Tissues in paraformaldehyde were submitted to the University of Pittsburgh's pathology laboratory for preparation of paraffin blocks and production of 9 slides per kidney (3 hematoxylin and eosin stains, 3 periodic acid–Schiff stains, and 3 trichrome stains) and 9 slides each for the RV and LV (3 hematoxylin and eosin stains, 3 picrosirius red stains, and 3 trichrome stains). Stained slides were sent to Nationwide Histology (Veradale, WA) for detailed histological analysis and scoring of the heart and kidney histopathological features (best to worst: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4) by a blinded veterinarian pathologist, as previously described.[30]

Statistical Analysis

Statistical analyses were conducted using NCSS Statistical Software version 19.0.2 (Kaysville, UT). Radiotelemetry data were analyzed using a nested 2-factor ANOVA in which one factor was treatment (plain drinking water versus sitagliptin in drinking water, fixed factor) and the other factor was time (days of treatment, repeated-measures fixed factor). Rat number was included in the analysis as a factor nested under treatment. Predetermined contrasts involving only 2 groups were conducted using a 2-tailed Student *t*-test, except for ratios that were tested for significance using a nonparametric Mann-Whitney *U* test. A few data points were missing because of death of animal, insufficient urine volume, or lost samples; however, all available data were included in the analyses. $P<0.05$ was considered statistically significant. For parametric statistics, values are presented as means and SEM; for nonparametric statistics, values are presented as medians and interquartile range (IQR).

RESULTS

Figure 1A–1D are scatterplots showing each of the 3276 data points for 24-hour MABPs, SBPs, DBPs, and HRs, respectively; for clarity, corresponding line graphs are provided in Figure 1E–1H. At baseline and for the first 25 days of radiotelemetry, MABPs, SBPs, DBPs, and HRs were similar in control versus sitagliptin-treated SHHF. Thereafter, however, MABPs, SBPs, and DBPs were elevated in the sitagliptin group compared with the control group. The significant 2-factor ANOVA treatment×time interaction ($P<0.000001$) indicates that the effects of sitagliptin on blood pressures depended on the duration of treatment. Time-averaged (day 26 through day 182) MABPs, SBPs, and DBPs were 7.2, 8.6, and 6.1 mm Hg higher in sitagliptin-treated SHHF compared with control SHHF. HRs gradually declined in both the sitagliptin-treated and control rats and more so in the sitagliptin rats (2-factor ANOVA, treatment×time interaction, $P=0.000627$). By the end of the 182-day radiotelemetry study, however, HRs had returned to baseline in both groups. Sitagliptin similarly affected daytime (Figure 2A–2C and 2E–2G) and nighttime (Figure 3A–3C and Figure 3E–3G) blood pressures. Sitagliptin did not affect daytime HRs (Figure 2D and 2H), yet did lower temporarily nighttime HRs (Figure 3D and 3H).

At the end of the radiotelemetry study, both groups of SHHF appeared healthy and had similar body weights, water intakes, food intakes, and urine outputs (Figure 4A–4D, respectively). Although fasting (Figure 5A) and postprandial (Figure 5B) blood glucose levels were not significantly different in sitagliptin-treated versus control SHHF, fasting glycated hemoglobin levels were significantly ($P=0.0455$) lower in SHHF treated long-term with sitagliptin (Figure 5C). Interestingly, total plasma cholesterol levels were ($P=0.0365$; Figure 5D), and plasma triglycerides levels tended to be ($P=0.0576$; Figure 5E), increased by extended long-term sitagliptin treatment; whereas plasma sodium (Figure 6A) and potassium (Figure 6B) were unaffected. With regard to inflammatory cytokines, long-term sitagliptin did not affect plasma interleukin-6 (Figure 6C) but did markedly reduce plasma IL-1β levels ($P=0.0470$; Figure 6D).

Despite the fact that body weights were similar in the 2 groups (Figure 7A), extended long-term treatment with sitagliptin caused renal hypertrophy, as evidenced by significantly greater unnormalized kidney weights ($P=0.0102$; Figure 7B) and kidney weights normalized to tibia length ($P=0.0118$; Figure 7C) in sitagliptin-treated SHHF. Unnormalized (Figure 7D) and normalized (Figure 7E) heart weights tended to be greater in sitagliptin-treated SHHF; however, these differences were not statistically significant. Similarly, unnormalized (Figure 7F) and normalized (Figure 7G) LV weights tended to be greater in sitagliptin-treated SHHF; yet again, these differences were not statistically significant. Unnormalized RV weights (Figure 7H) and normalized RV weights (Figure 7I) were not affected by sitagliptin treatment.

With regard to kidney function, renal blood flows (Figure 8A) tended ($P=0.0626$) to be lower in sitagliptin-treated SHHF, whereas MABPs tended ($P=0.0870$) to be higher during the terminal studies (Figure 8B), a finding consistent with the radiotelemetry results. Notable, renal vascular resistances were markedly increased ($P=0.0250$) by sitagliptin (Figure 8C). Although sitagliptin caused renal vasoconstriction, glomerular filtration rate and renal excretory function were preserved (see Figure 8D–8H for plasma creatinine, creatinine clearance, urine output, sodium excretion, and potassium excretion, respectively). Nonetheless, there was evidence, both biochemical and histological, of kidney damage by

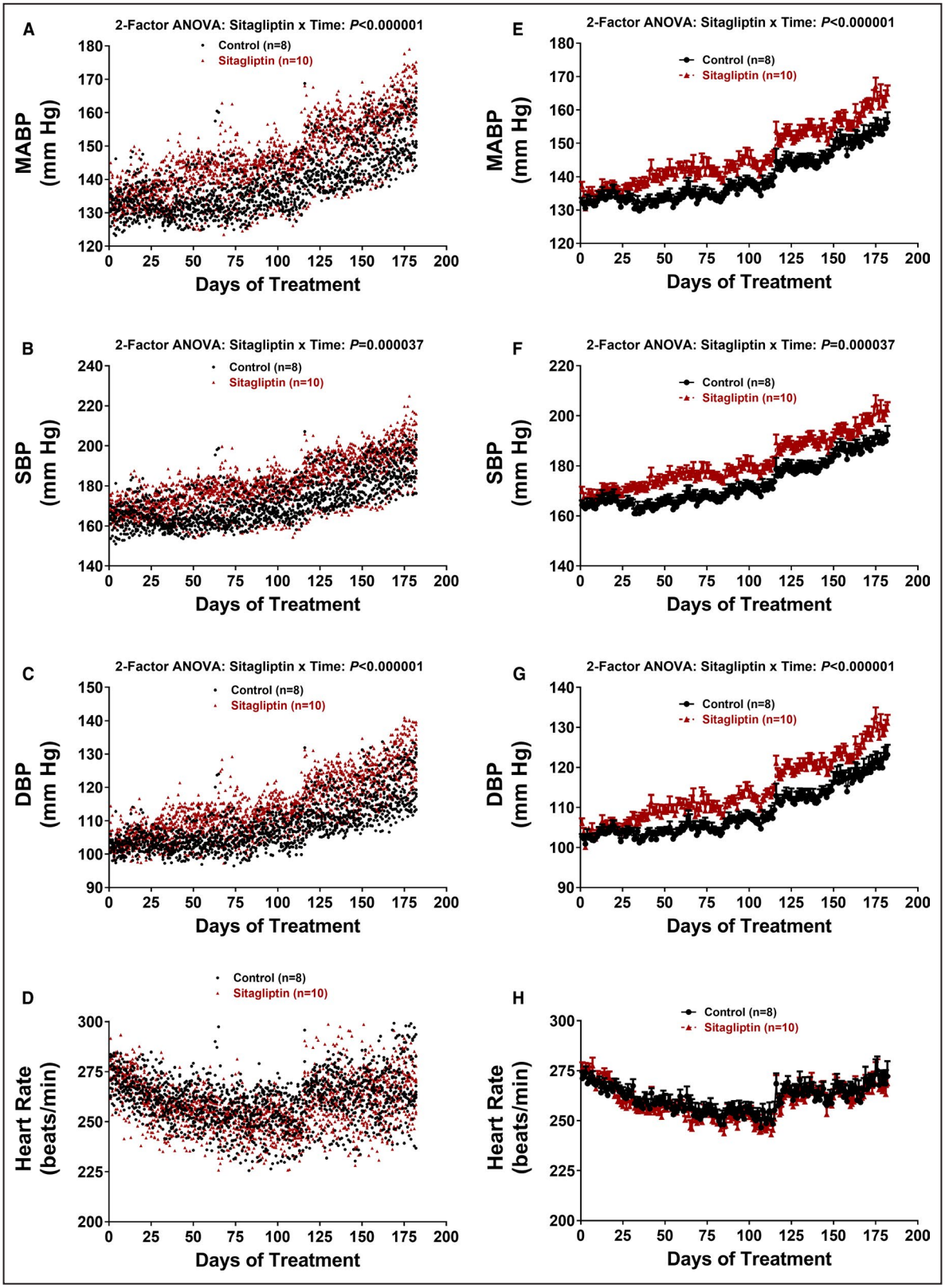

Figure 2. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on daytime mean arterial blood pressure (MABP) (A, E), systolic blood pressure (SBP) (B, F), diastolic blood pressure (DBP) (C, G), and heart rate (D, H) in obese spontaneously hypertensive heart failure rats.

Results are expressed as both scatterplots (3276 data points for each variable, with each data point representing a 12-hour average of 72 readings) and, for ease of visualization, line graphs (means and SEM for n number of rats).

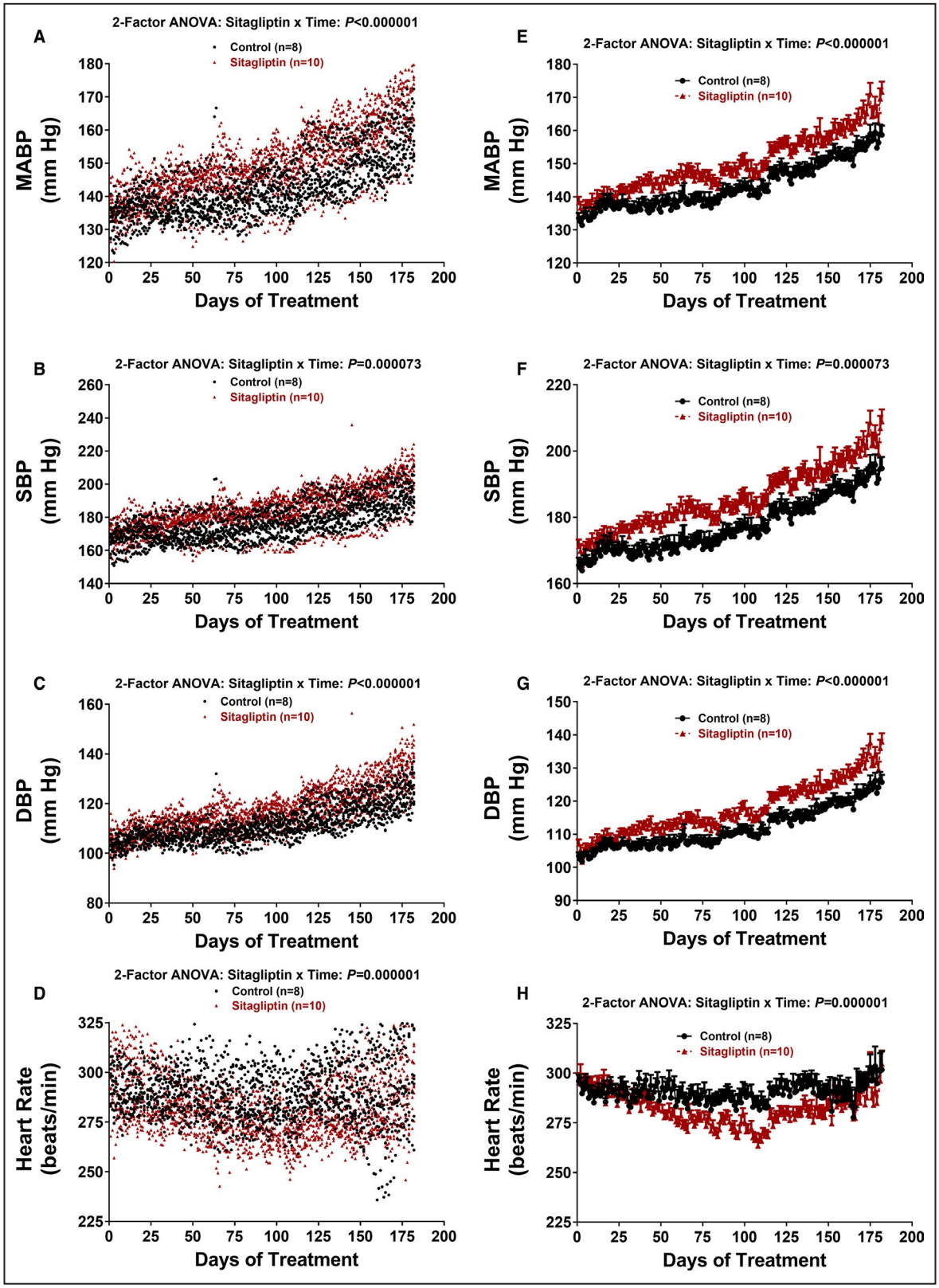

Figure 3. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on nighttime mean arterial blood pressure (MABP) (A, E), systolic blood pressure (SBP) (B, F), diastolic blood pressure (DBP) (C, G), and heart rate (D, H) in obese spontaneously hypertensive heart failure rats.
Results are expressed as both scatterplots (3276 data points for each variable, with each data point representing a 12-hour average of 72 readings) and, for ease of visualization, line graphs (means and SEM for n number of rats).

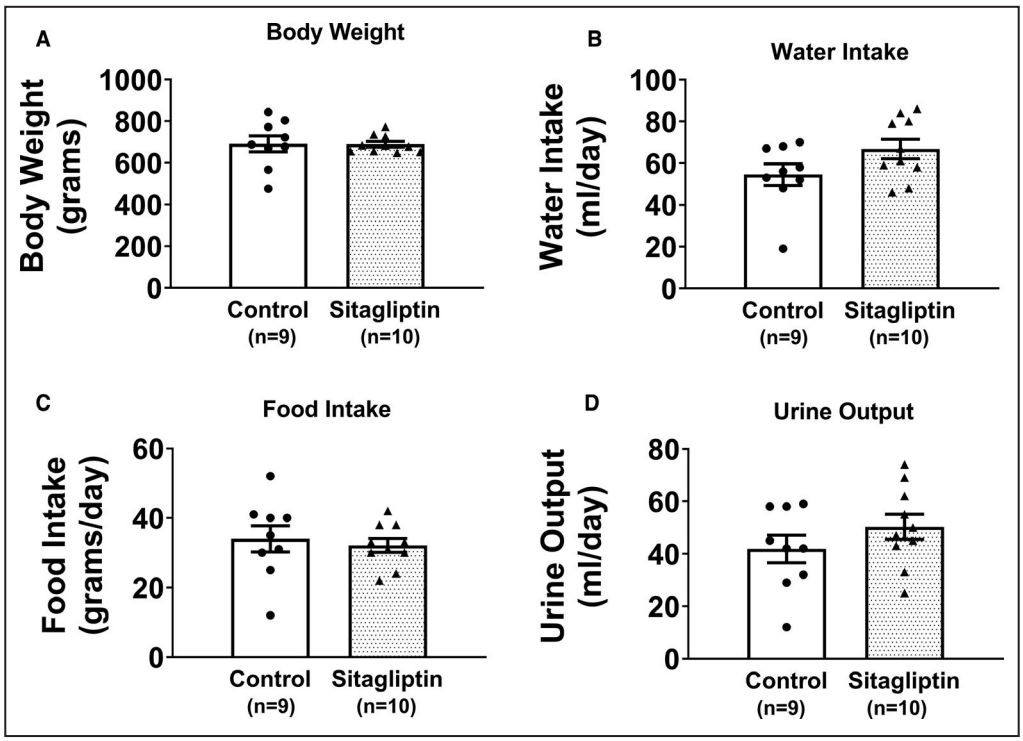

Figure 4. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on body weight (A), water intake (B), food intake (C), and urine output (D) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

long-term sitagliptin. For example, long-term sitagliptin treatment caused a >2-fold increase (P=0.0009) in the urinary excretion of kidney injury molecule-1 (Figure 9A), a biomarker for damage of proximal tubules, and worsened interstitial fibrosis, as evaluated by both hematoxylin and eosin (P=0.0191; Figure 9B) and trichrome (Figure 9C; P=0.0301) staining. Long-term sitagliptin treatment also worsened glomerulosclerosis (Figure 9D; P=0.0156) and caused renal vascular hypertrophy (P=0.0318; Figure 9E), but did not increase neutrophil gelatinase-associated lipocalin (Figure 9F) or albumin (Figure 9G) excretion. Figures 10 and 11 show representative micrographs illustrating renal histopathological features induced by long-term sitagliptin treatment in SHHF.

With respect to cardiac histopathological features, long-term sitagliptin did not affect LV degeneration (Figure 12A), vasculopathy (Figure 12B), inflammation (Figure 12C), or fibrosis (Figures 12D–12F). Nonetheless, long-term sitagliptin impaired LV systolic function, as evidenced by a sitagliptin-induced elevation of LV end-diastolic pressure (P=0.0015; Figure 13A) and reduction of the maximum rate of increase in LV pressure normalized to LV end-diastolic pressure (P=0.0080; Figure 13B). Sitagliptin also impaired LV diastolic function, as evidenced by a reduction in the maximum rate of decrease in LV pressure (P=0.0253; Figure 13C), maximum rate of decrease in LV pressure normalized to maximum LV pressure (P=0.0059; Figure 13D), and LV Tau (time constant for LV relaxation; P=0.0166; Figure 13E). Although indexes of RV systolic (Figures 13F and 13G) and diastolic (Figures 13H and 13I) function were not adversely affected by long-term sitagliptin, sitagliptin did worsen RV degeneration (P=0.0287; Figure 14A) and RV vasculopathy (P=0.0219; Figure 14B) and tended to increase RV inflammation (P=0.0535; Figure 14C); however, there was no evidence of increased RV fibrosis in sitagliptin-treated SHHF (Figures 14D–14F). Figure 15 shows representative micrographs illustrating RV degeneration induced by long-term sitagliptin treatment in SHHF.

For specific extracellular matrix proteins, sitagliptin did not significantly alter tissue levels of collagen I, III, IV, or VI, fibronectin, or laminin in the RV (Figure 16A), LV (Figure 16B), or kidney (Figure 16C). However, sitagliptin did affect the balance of extracellular matrix proteins in the RV. In the RV, the ratio of collagen IV/collagen III was increased from a median of 0.89 (IQR, 0.24) in control SHHF to 1.06 (IQR, 0.73) in sitagliptin-treated SHHF (P=0.0172). Likewise, the ratio of collagen IV/laminin was increased from a median of 0.61 (IQR, 0.22) in control SHHF to 0.87 (IQR, 1.22) in sitagliptin-treated SHHF (P=0.0172).

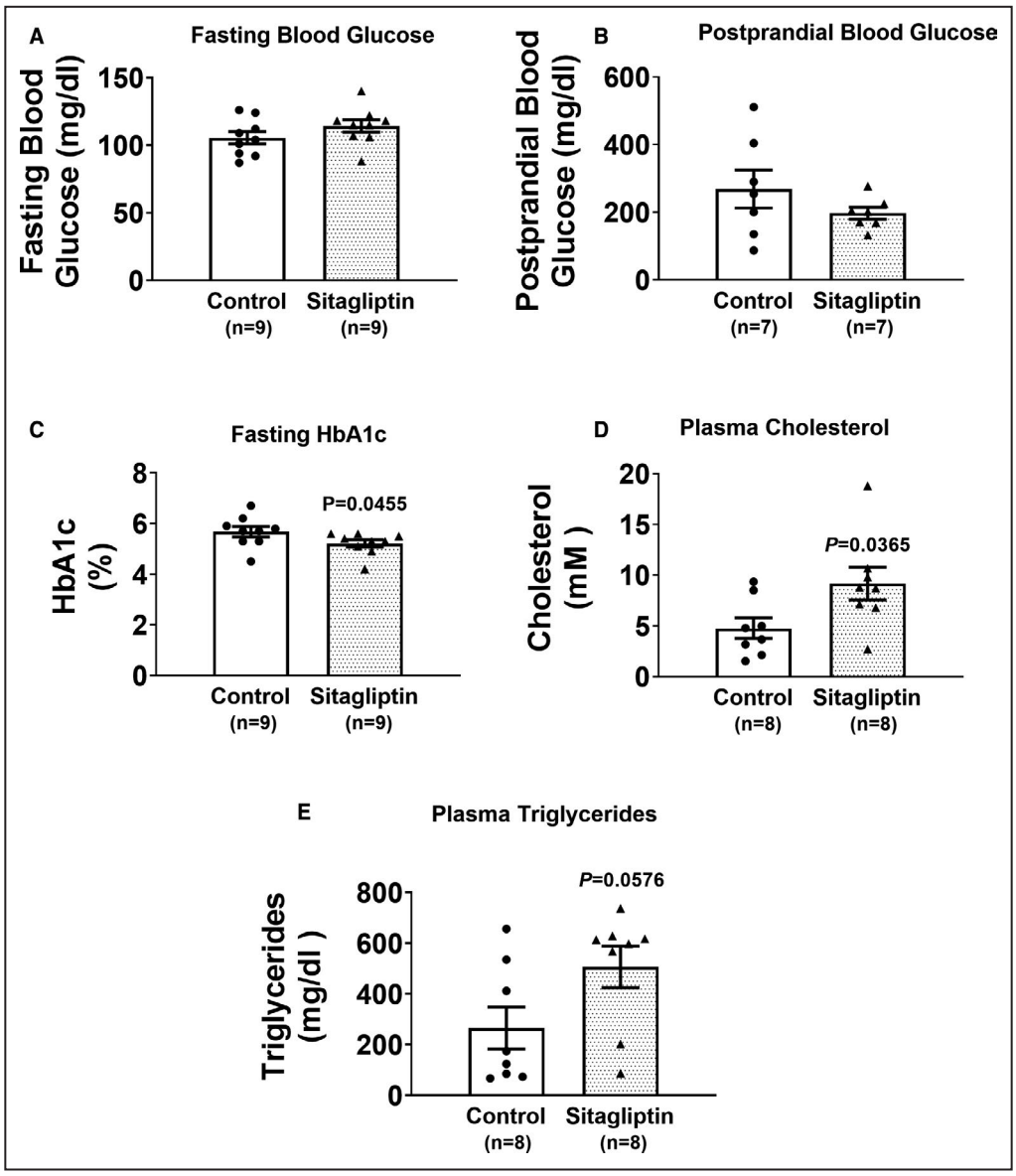

Figure 5. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on fasting blood glucose (A), postprandial blood glucose (B), fasting glycated hemoglobin (HbA1c) (C), plasma cholesterol (D), and plasma triglycerides (E) in obese spontaneously hypertensive heart failure rats.

Scatterplots include means and SEM for n number of rats.

DISCUSSION

DPP4Is are widely used antidiabetic drugs that indirectly activate incretin receptors, are well tolerated, and provide moderate glycemic control with minimal risk of hypoglycemia. DPP4Is are doubtless an important addition to the therapeutic armamentarium for the treatment of type 2 diabetes mellitus. Preclinical findings support the concept that DPP4Is should prevent target organ damage in patients with type 2 diabetes mellitus.[10–22] In contradiction to animal studies, however, cardiovascular outcome trials in patients indicate that at best DPP4Is do not reduce risk of cardiovascular or renal diseases,[31] and at worst may promote risk.[32] In contrast to DPP4Is, GLP-1RAs, which like DPP4Is also activate incretin receptors, reduce cardiovascular deaths, nonfatal myocardial infarctions, and nonfatal strokes in patients with an elevated cardiovascular risk profile.[31] Why the discrepancy between DPP4Is and GLP-1RAs? The answer to this question may increase our understanding of the role of other biochemical mechanisms that contribute to cardiovascular/renal diseases, may suggest novel approaches for the treatment and prevention of cardiovascular/renal diseases,

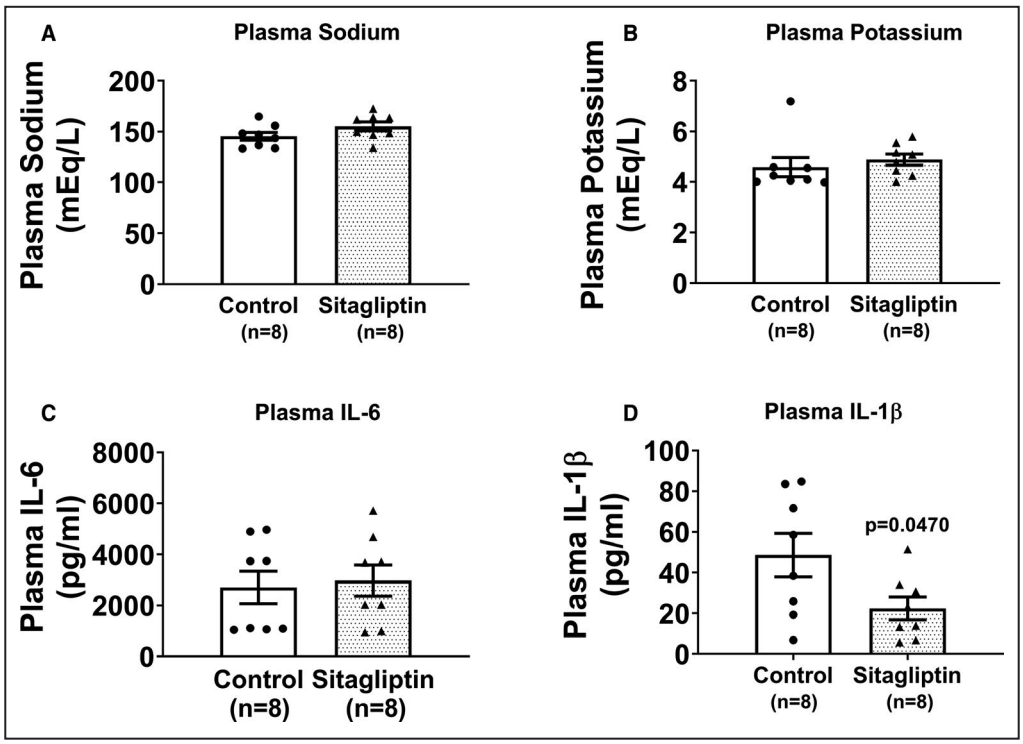

Figure 6. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on plasma sodium (A), plasma potassium (B), plasma interleukin-6 (IL-6) (C), and plasma interleukin-1β (IL-1β) (D) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

and may shed light on how to achieve in patients what can be achieved in animals with DPP4Is.

A better understanding of why DPP4Is underperform, relative to GLP-1RAs, in patients is hampered by the fact that currently no animal models have been identified that could be exploited to investigate the mechanisms of the negative effects of DPP4Is on the cardiovascular and renal systems. However, our recent studies suggest that rat models with an SHR genetic background may be sensitive to the adverse effects of DPP4Is. Indeed, in a previous study, we found that within a week of initiating treatment, sitagliptin increased arterial blood pressure in SHR, but not in WKY or Zucker diabetic Sprague-Dawley rats.[23]

Our in vitro work with SHR cells also underscores the concept that the adverse effects of DPP4Is may be greater in SHR.[33] In addition to augmenting incretin levels, inhibition of DPP4 increases levels of many other DPP4 substrates.[34–36] Prime examples are neuropeptide $Y_{1\text{-}36}$, peptide $YY_{1\text{-}36}$, and stromal cell-derived factor-1α, all of which are potent endogenous agonists of Gi-coupled receptors (Y_1 receptors[37,38] for neuropeptide $Y_{1\text{-}36}$ and peptide $YY_{1\text{-}36}$ and C-X-C chemokine receptor type 4 for stromal cell-derived factor-1α[39]). Our studies show that inhibition of the metabolism of these agonists by DPP4Is increases the activation of Gi-coupled receptors, which results in increased proliferation of, and collagen production by, cardiac fibroblasts, preglomerular vascular smooth muscle cells, and glomerular mesangial cells.[40,41] Germane to the current study is the fact that the effects of these peptide agonists on proliferation of, and collagen production by, cardiac fibroblasts, preglomerular vascular smooth muscle cells, and glomerular mesangial cells are greater in cells from SHR compared with cells from WKY.[33]

Together, our previous studies suggest that an animal model with SHR genetics may prove useful for investigating the harmful effects of DPP4Is. DPP4Is are used to treat type 2 diabetes mellitus, and patients with type 2 diabetes mellitus usually have the metabolic syndrome. For this reason, we chose to study the effects of a DPP4I in obese SHHF, an animal model that expresses the metabolic syndrome and has an enriched SHR genetic background.[25] We also reasoned that DPP4I-induced worsening of target organ damage in such a model would require long-term administration. Accordingly, we attempted an extended (>182 days) trial of sitagliptin in obese SHHF.

To our knowledge, this is the first study to examine, using radiotelemetry, the extended long-term effects

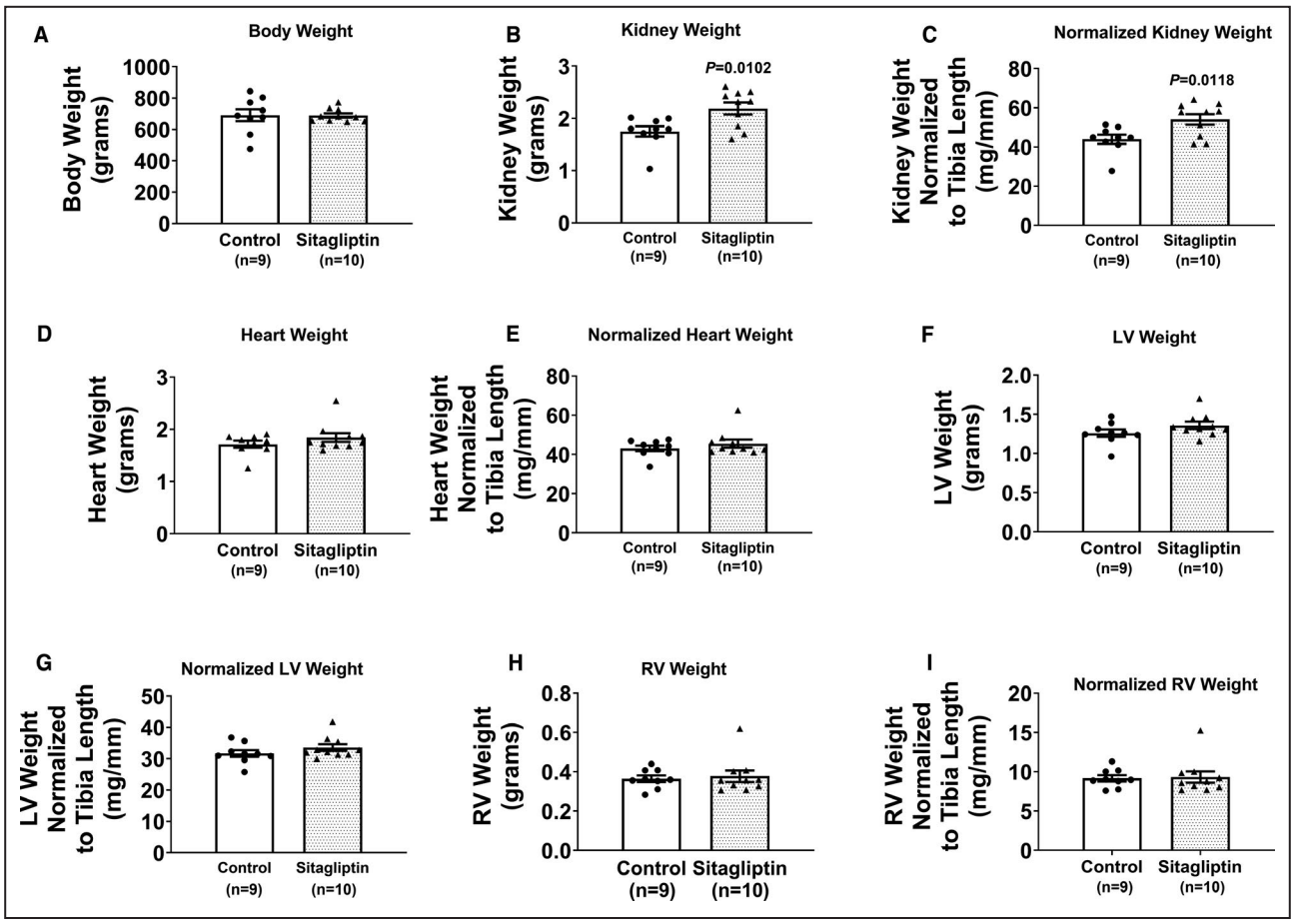

Figure 7. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on body weight (A), kidney weight (B), kidney weight normalized to tibia length (C), heart weight (D), heart weight normalized to tibia length (E), left ventricular (LV) weight (F), LV weight normalized to tibia length (G), right ventricular (RV) weight (H), and RV weight normalized to tibia length (I) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

of a DPP4I on blood pressure in animals. An important finding of the present study is that long-term inhibition of DPP4 clearly, significantly, and in a sustained manner increases blood pressure in obese SHHF. Moreover, our study also shows that if the duration of treatment is too short (<25 days for obese SHHF), this prohypertensive effect would be overlooked. Notably, the sitagliptin-induced increase in MABP in the present study was 7.2 mm Hg, which is similar to the increase of 9.2 mm Hg induced by 3 weeks of sitagliptin treatment in SHR.[23] Thus, our results herein resolve in the affirmative the controversy as to whether DPP4 inhibition under some circumstances can cause a sustained (indeed in obese SHHF, near life-long) increase in blood pressure. Interestingly, in patients, GLP-1RAs reliably lower blood pressure[31]; in contrast, overall, DPP4Is have a null effect on blood pressure. The present study sheds light on the disparate effects of GLP-1RAs versus DPP4Is on blood pressure by showing that under some circumstances DPP4Is increase blood pressure. Likely because of the heterogeneity of the patient population treated with DPP4Is, the expected antihypertensive effects attributable to activation of incretin receptors are cancelled by the prohypertensive effects established by the present study. In support of this conclusion, in short-term studies, Marney and coworkers report variable effects of DPP4 inhibition on blood pressure in humans, depending on the context.[42]

The present study also answers in the affirmative the question of whether under some circumstances long-term administration of DPP4Is can augment target organ damage, as reflected in worsening of histopathological features, function, or both. For example, herein, we observed increased RV degeneration and vasculopathy, with a strong trend (P=0.0535) for increased RV inflammation, in sitagliptin-treated SHHF. Also, long-term sitagliptin significantly increased RV expression of collagen IV relative to collagen III and laminin. As

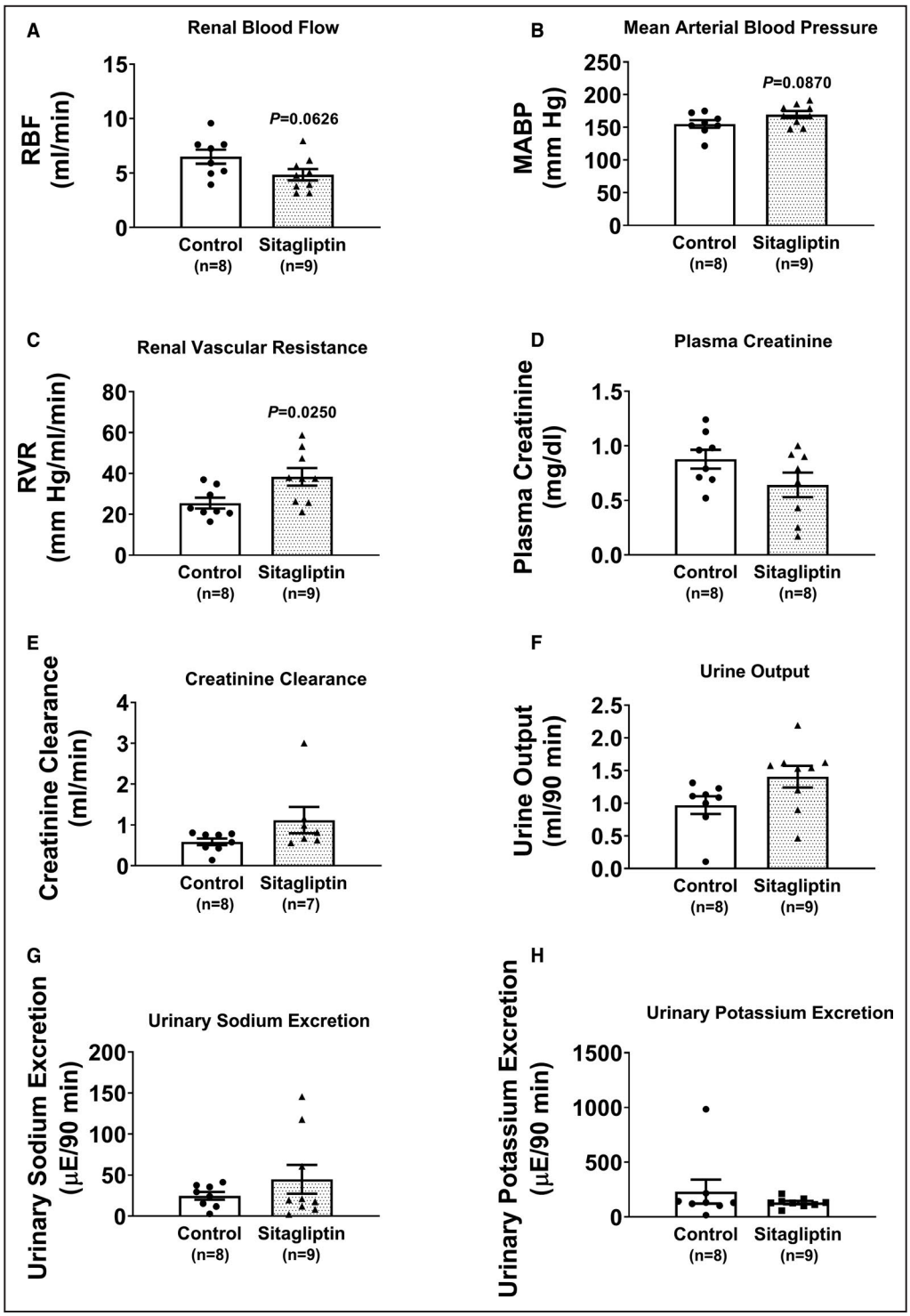

Figure 8. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on renal blood flow (RBF) (A), mean arterial blood pressure (MABP) (B), renal vascular resistance (RVR) (C), plasma creatinine (D), creatinine clearance (E), urine output (F), urinary sodium excretion (G), and urinary potassium excretion (H) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

discussed by Karsdal and coworkers,[43] changes in the balance, as opposed to absolute amounts, of various collagen types can contribute to disorders of the extracellular matrix. We did not detect, however, a worsening of RV diastolic or systolic function with long-term sitagliptin treatment. This may be

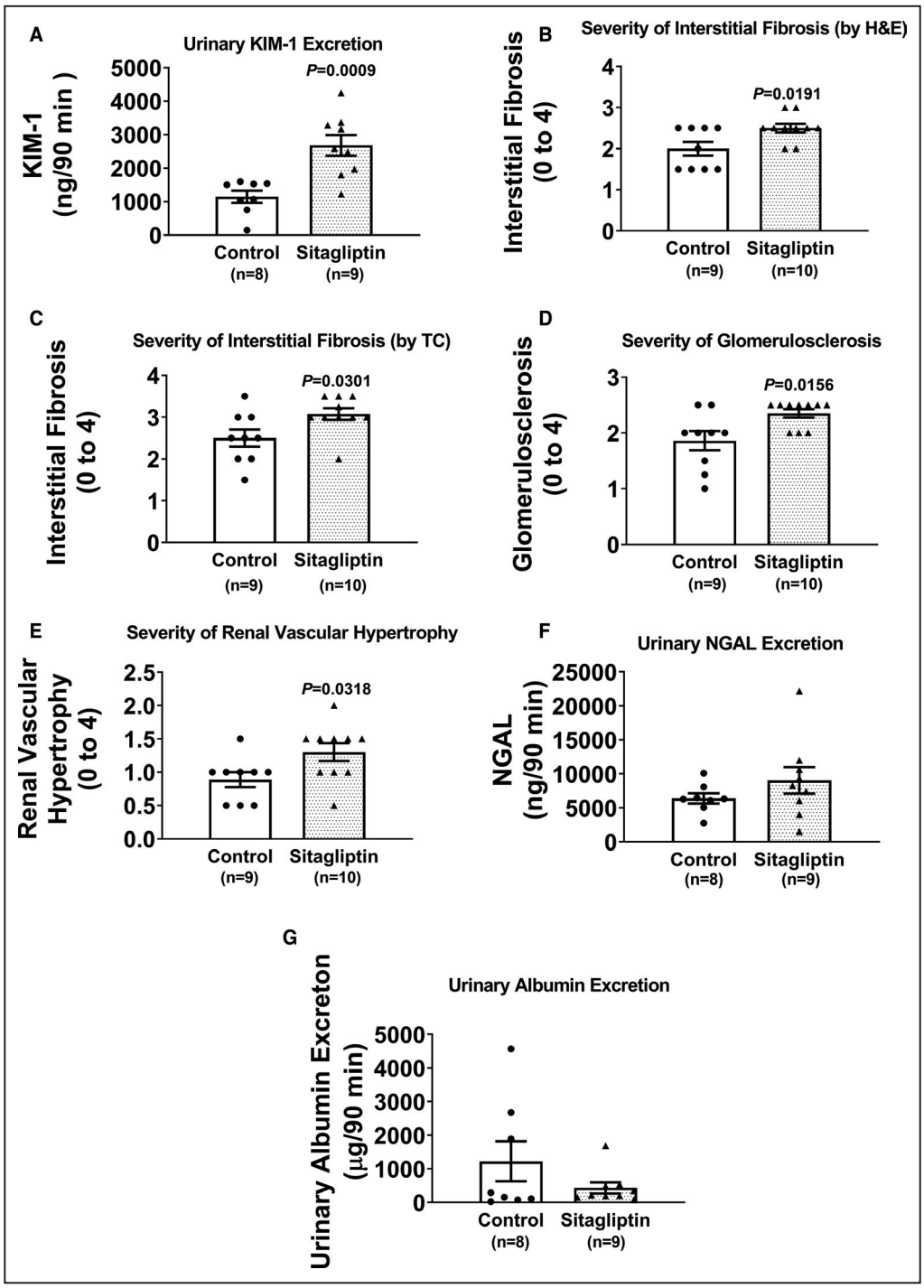

Figure 9. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on urinary kidney injury molecule-1 (KIM-1) excretion (A), severity of interstitial fibrosis, as assessed by hematoxylin and eosin (H&E) staining (B), severity of interstitial fibrosis, as assessed by trichrome (TC) staining (C), severity of glomerulosclerosis (D), severity of renal vascular hypertrophy (E), urinary neutrophil gelatinase-associated lipocalin (NGAL) excretion (F), and urinary albumin excretion (G) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

attributable to the fact that the histological changes in the RV induced by sitagliptin were too mild to significantly alter function. It is also conceivable that the changes in RV histological features relate to pulmonary hypertension (PH). Obesity, the metabolic syndrome, and heart failure increase the risk of PH.

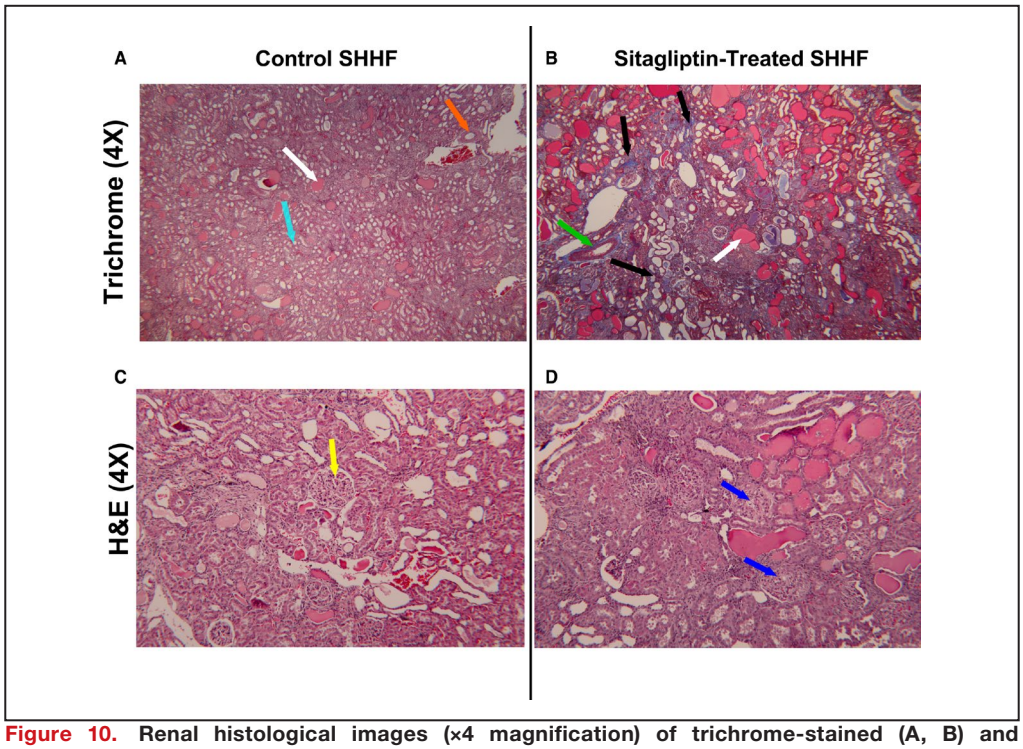

Figure 10. Renal histological images (×4 magnification) of trichrome-stained (A, B) and hematoxylin and eosin (H&E)–stained (C, D) kidney sections obtained from control (A, C) and sitagliptin-treated (80 mg/kg per day) (B, D) obese spontaneously hypertensive heart failure rats (SHHF).

Trichrome staining revealed diffuse renal interstitial fibrosis (note diffuse interstitial blue staining, as indicated by black arrows), moderate tubular dilation with presence of proteinaceous material (note red staining in tubules, as indicated by white arrow), and perivascular fibrosis (note perivascular blue staining, as indicated by green arrow) in sitagliptin-treated SHHF (**B**). Control SHHF (**A**) showed some proteinaceous material in the tubules (white arrow), but little interstitial fibrosis (turquoise arrow) or perivascular fibrosis (orange arrow). H&E staining showed glomeruli with abnormal morphological features in sitagliptin-treated SHHF (indicated by blue arrows) (**D**), but normal morphological features of glomeruli in control SHHF (indicated by yellow arrow) (**C**).

For example, some rats (model of the metabolic syndrome and heart failure with preserved ejection fraction that is generated by crossing a female Zucker diabetic fatty rat with a male SHHF) spontaneously develop moderate PH.[44] In the present study, control SHHF had elevated RV peak systolic

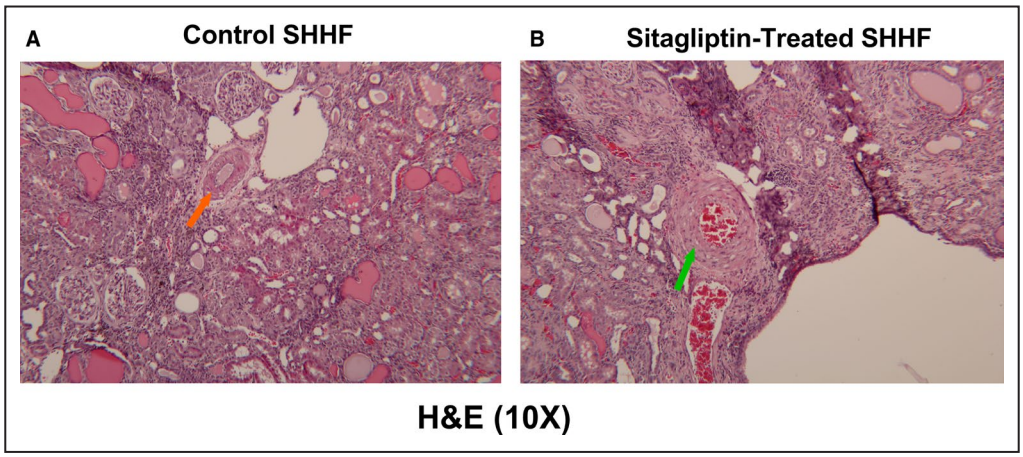

Figure 11. Renal histological images (×10 magnification) of hematoxylin and eosin (H&E)–stained kidney sections obtained from control (A) and sitagliptin-treated (80 mg/kg per day) (B) obese spontaneously hypertensive heart failure rats (SHHF).

H&E staining showed more severe renal vascular hypertrophy in sitagliptin-treated SHHF (green arrow) (**B**) vs control SHHF (orange arrow) (**A**).

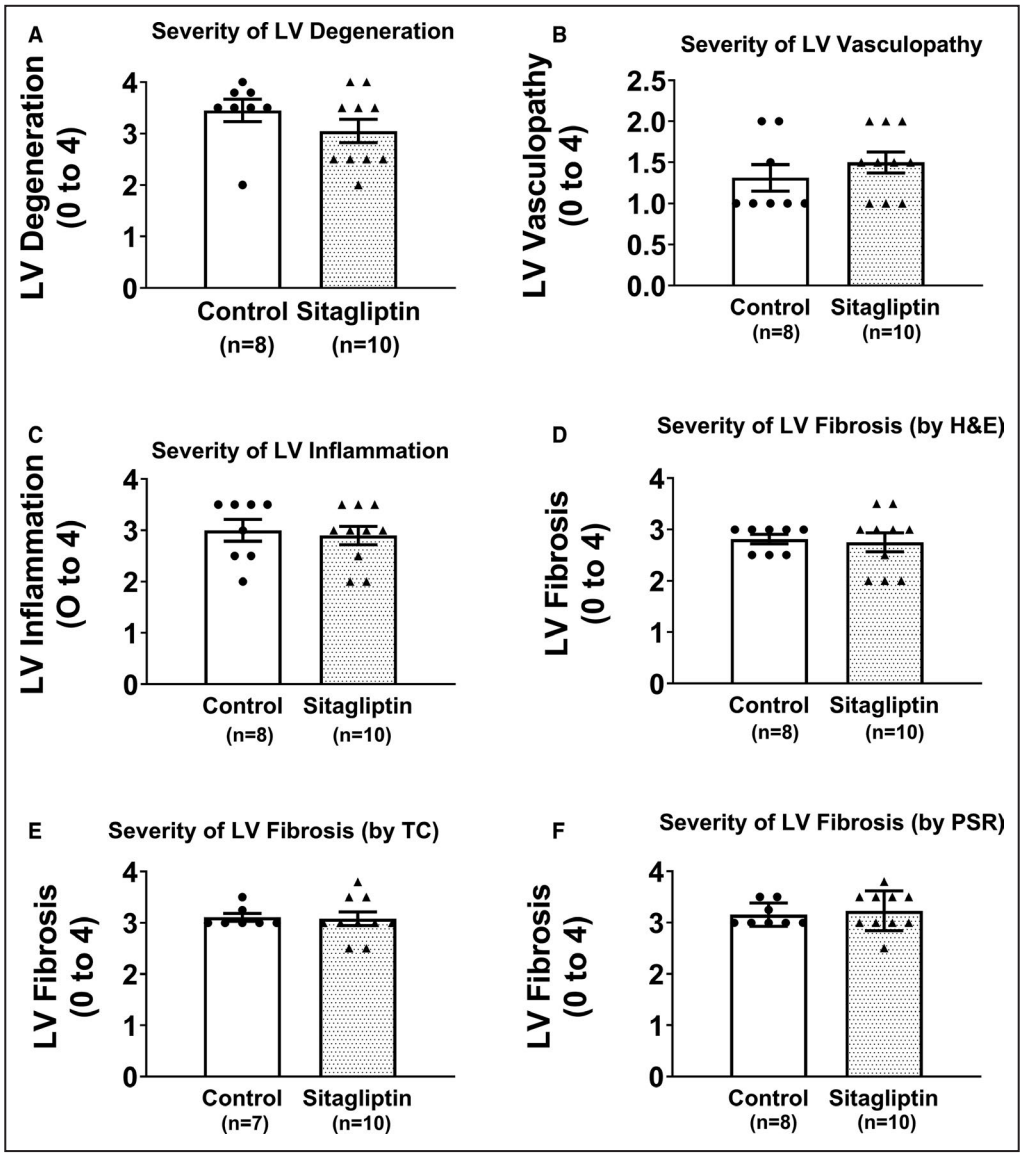

Figure 12. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on severity of left ventricular (LV) degeneration (A), LV vasculopathy (B), LV inflammation (C), and fibrosis (D–F), as assessed by hematoxylin and eosin (H&E) staining (D), trichrome (TC) staining (E), and picrosirius red (PSR) staining (F) in obese spontaneously hypertensive heart failure rats (SHHF).
Histopathological features were severe even in control obese SHHF and were no further worsened by sitagliptin treatment. Scatterplots include means and SEM for n number of rats.

pressures (33±1 mm Hg), and sitagliptin-treated SHHF tended to have even higher average levels (38±4 mm Hg). In fact, in 2 sitagliptin-treated SHHF, RV peak systolic pressures were extremely high (69 and 45 mm Hg) and exceeded all values in control SHHF. Currently, the effects of sitagliptin in patients with PH are unknown; but our findings herein warrant further investigation of DPPIs in models of angioproliferative PH.

Sitagliptin treatment also worsened both LV systolic and diastolic function. Therefore, we anticipated that long-term sitagliptin would also significantly worsen LV histopathological features. The explanation for why long-term sitagliptin worsened LV function yet did not worsen LV histopathological features likely relates to the fact that LV histopathological features were by the end of the study so severe in control SHHF that detecting an even more severe deterioration of LV histological features was not feasible. Indeed, by the end of the study, most obese SHHF already had severe LV degeneration and fibrosis.

With regard to the kidney, long-term DPP4 inhibition increased kidney weights, both absolute and normalized, a result consistent with renal injury in

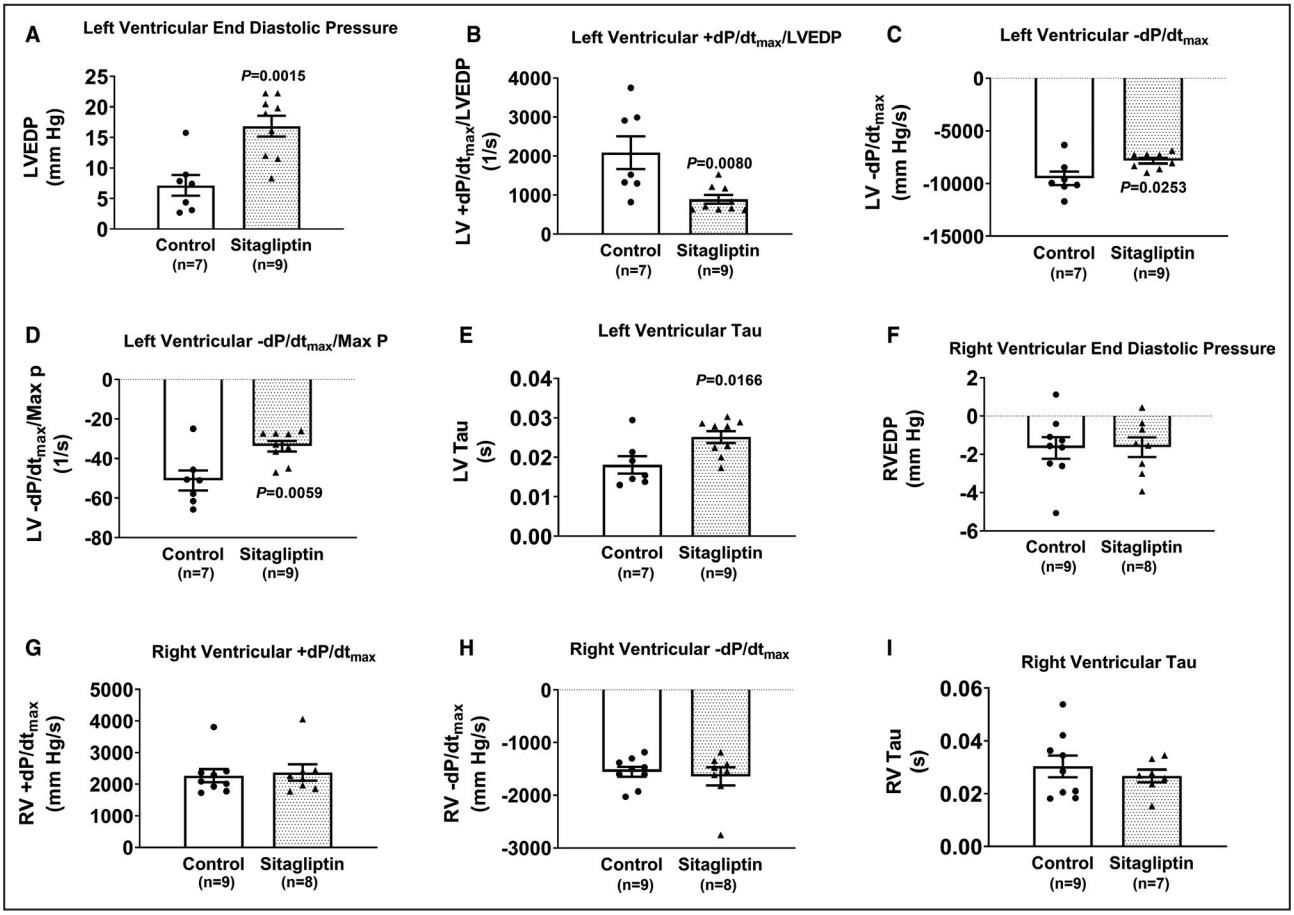

Figure 13. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on left ventricular (LV) end-diastolic pressure (LVEDP) (**A**), maximum rate of increase in LV pressure normalized to LVEDP (LV+dP/dt_{max}/LVEDP) (**B**), maximum rate of decrease in LV pressure (LV–dP/dt_{max}) (**C**), maximum rate of decrease in LV pressure normalized to maximum LV pressure (LV–dP/dt_{max}/Max P) (**D**), LV Tau (**E**), right ventricular (RV) end-diastolic pressure (RVEDP) (F), maximum rate of increase in RV pressure (RV+dP/dt_{max}) (**G**), maximum rate of decrease in RV pressure (RV–dP/dt_{max}) (**H**), and RV Tau (**I**) in obese spontaneously hypertensive heart failure rats. Scatterplots include means and SEM for n number of rats.

rats.[45–48] Kidney injury was confirmed by the significant increase in urinary kidney injury molecule-1 excretion, a well-accepted biomarker for damage to the proximal tubule, and by an increase in interstitial fibrosis, as evidenced by both hematoxylin and eosin and trichrome staining. In addition, long-term DPP4 inhibition worsened glomerulosclerosis and increased renal vascular hypertrophy, which likely explains the increased renal vascular resistance in sitagliptin-treated animals.

Interestingly, in the present study, long-term sitagliptin increased plasma total cholesterol levels in male, obese SHHF. This effect cannot be attributed to increases in food intake or body weight because these parameters were unchanged by long-term sitagliptin. In patients, however, the effects of sitagliptin on plasma cholesterol are highly variable,[49] perhaps because in clinical trials most patients are treated with multiple other "background" drugs, such as statins, metformin, and renin-angiotensin system inhibitors. The present study helps define the effects of sitagliptin on cholesterol levels in the absence of changes in food intake and body weight and without confounding interactions with other medications.

The negative effects of long-term sitagliptin were accompanied by 2 important positive outcomes, including the expected reduction in glycated hemoglobin and, unexpectedly, a marked decrease in the plasma levels of the inflammatory cytokine IL-1β. IL-1β plays a critically important role in promoting cardiovascular[50] and diabetic kidney disease[50]; indeed, in patients, administration of a monoclonal antibody targeting anti–IL-1β decreases cardiovascular events.[51] The present study suggests that if the negative effects of DPP4Is can be canceled by administration of other agents, the anti–IL-1β effects of DPP4Is could be harnessed to reduce the burden of cardiovascular and renal diseases. For example, a recent study by

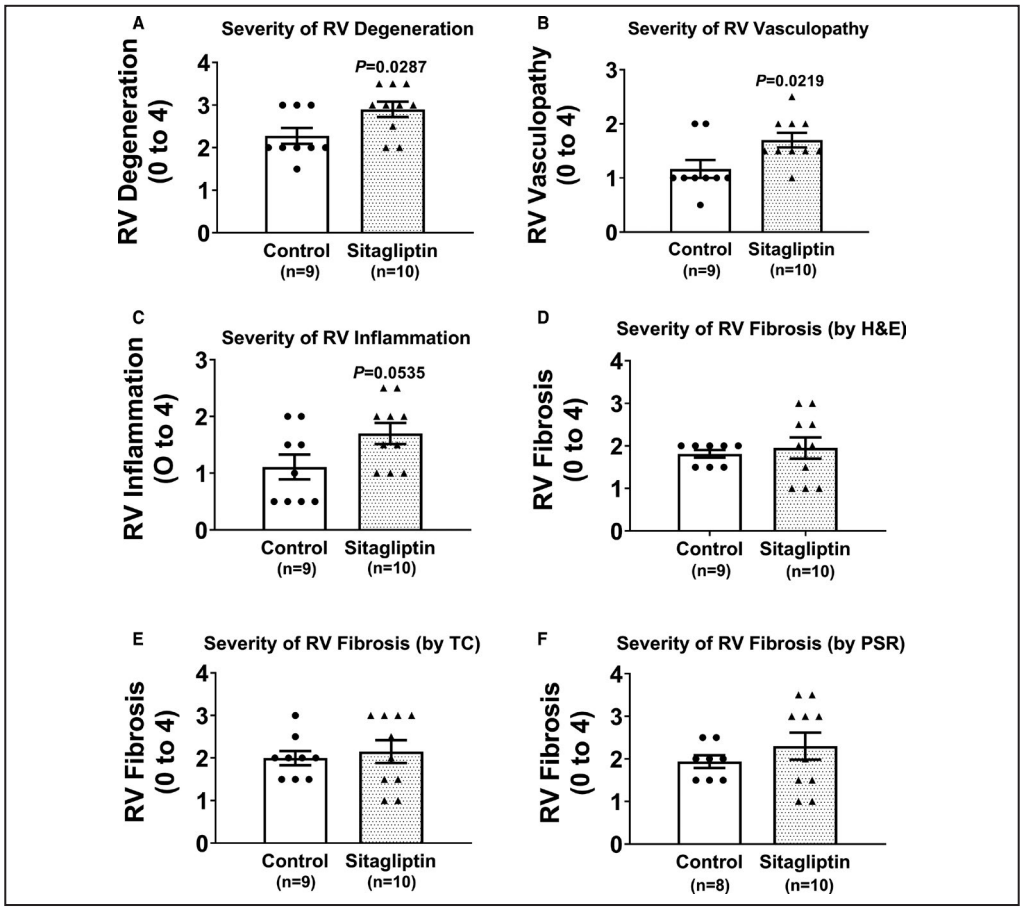

Figure 14. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on severity of right ventricular (RV) degeneration (A), RV vasculopathy (B), RV inflammation (C), and fibrosis (D–F), as assessed by hematoxylin and eosin (H&E) staining (D), trichrome (TC) staining (E), and picrosirius red (PSR) staining (F) in obese spontaneously hypertensive heart failure rats.
RV degeneration and vasculopathy were significantly worsened by long-term treatment with sitagliptin. Scatterplots include means and SEM for n number of rats.

Younis and coworkers demonstrated that coadministration of vildagliptin and metformin prevents the elevation of IL-1β in patients with type 2 diabetes mellitus with coronary artery disease.[52] Thus, the obese SHHF may be a useful model for studying ways to optimize the anti–IL-1β effects of DPP4Is in patients.

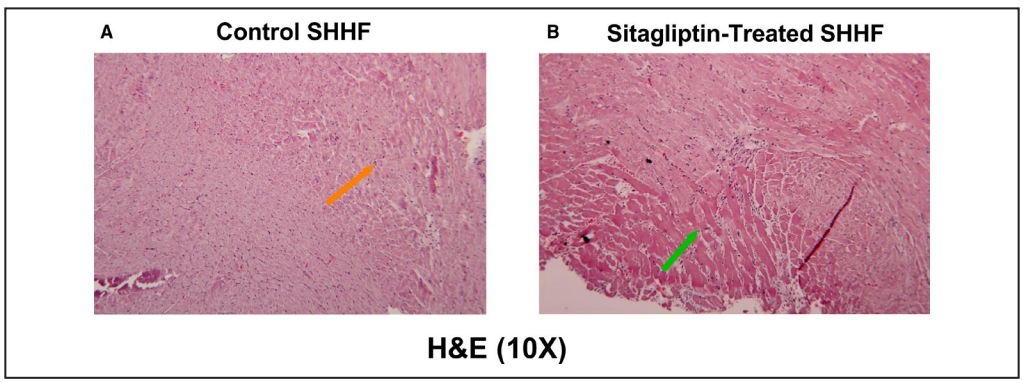

Figure 15. Right ventricular (RV) histological images (×10 magnification) of hematoxylin and eosin (H&E)–stained sections obtained from control (A) and sitagliptin-treated (80 mg/kg per day) (B) obese spontaneously hypertensive heart failure rats (SHHF).
H&E staining revealed areas of severe and diffuse degeneration of the RV in sitagliptin-treated SHHF (green arrow) (**B**), but mild and localized RV degeneration in control SHHF (orange arrow) (**A**).

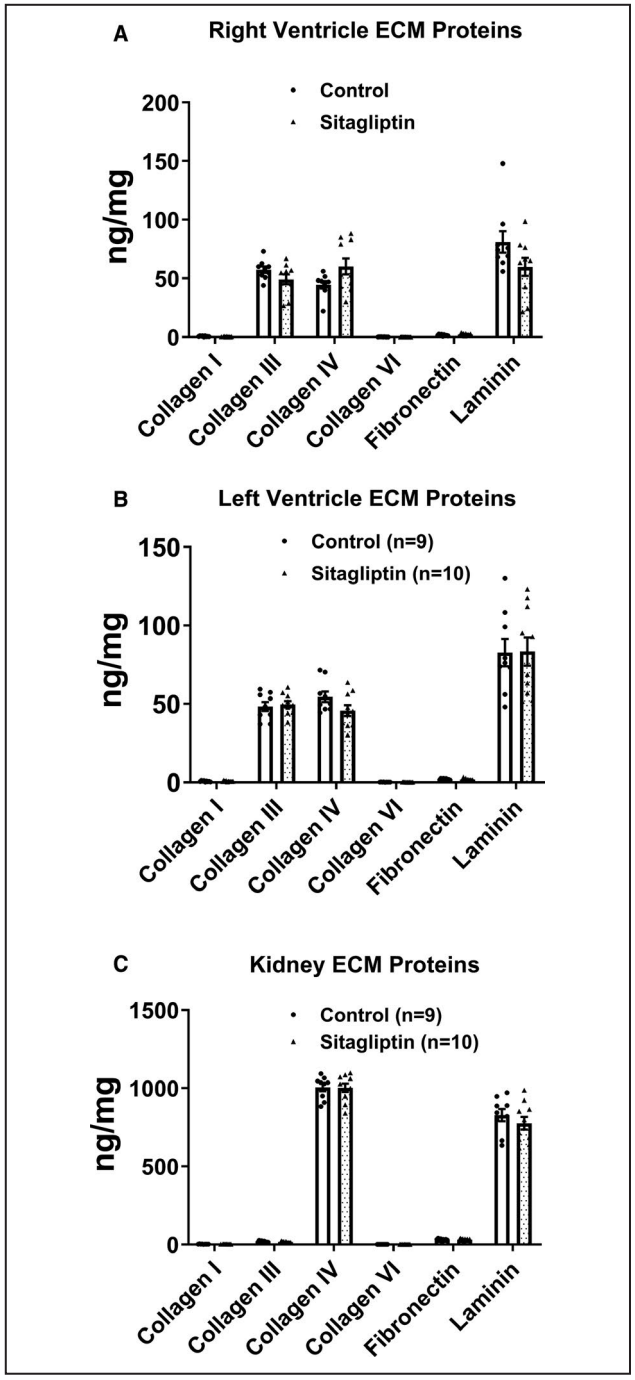

Figure 16. Effects of long-term treatment with sitagliptin (80 mg/kg per day) on levels of extracellular matrix (ECM) proteins (collagen I, III, IV, and VI, fibronectin, and laminin) in the right cardiac ventricle (A), left cardiac ventricle (B), and kidney (C) in obese spontaneously hypertensive heart failure rats.
Scatterplots include means and SEM for n number of rats.

In conclusion, the present study answers in the affirmative the question as to whether DPP4Is in some animal models can cause a significant and sustained (perhaps life-long) increase in blood pressure and can promote target organ damage/dysfunction. In patients, GLP-1RAs are antihypertensive and improve cardiovascular outcomes; yet, DPP4Is, which conceptually are indirect GLP-1RAs, are neither antihypertensive nor cardiovascular protective in patients. Likely, this reflects the reality that in patients DPP4Is exert a complex mix of beneficial and harmful effects, with the net effect being neutral. The present study identifies an animal model that could reveal the mechanisms that compromise the beneficial effects of DPP4Is. A better understanding of such mechanisms would inform how to optimize therapy with DPP4Is and would suggest other approaches for treating and preventing cardiovascular and renal diseases. Finally, the current study establishes that extended long-term treatment with DPP4Is markedly and significantly reduces plasma levels of IL-1β, an effect that has significant clinical potential.

ARTICLE INFORMATION

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Disclosures

None.

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