Hypertension Plus Diabetes Mimics the Cardiomyopathy Induced by Nitric Oxide Inhibition in Rats*

Rita C. Sampaio, BS; Jose E. Tanus-Santos, MD, PhD; Silvia E. S. F. C. Melo, PharmD; Stephen Hyslop, PhD; Kleber G. Franchini, MD, PhD; Iara M. Luca, PhD; and Heitor Moreno, Jr, MD, PhD

Study objectives: We compared the myocardial lesions caused by the long-term inhibition of nitric oxide (NO) biosynthesis with those associated with renovascular hypertension (two-kidney, one-clip model [2K-1C]) and superimposed streptozotocin-induced diabetes mellitus (DM).

Design: Prospective trial.

Setting: University laboratory.

Interventions: Male Wistar rats were classified into the following groups: (1) a control group; (2) the L-NAME group (treatment with the NO synthase inhibitor N\-nitro-L-arginine methyl ester [L-NAME], 75 μmol per rat per day, orally); (3) the 2K-1C group (renovascular hypertension); (4) the DM group (treatment with streptozotocin, 60 mg/kg via intraperitoneal route); and (5) the 2K-1C plus DM group (renovascular hypertension and streptozotocin-induced DM). Arterial BP was measured by a tail-cuff method for 3 weeks, after which histologic and stereological analysis of the heart was done and cardiac NO synthase type 3 (NOS3) levels were assessed by Western blotting. The circulating levels of nitrates/nitrites and thromboxane B₂ (TXB₂, the stable metabolite of thromboxane A₂) were also measured.

Results: In DM and 2K-1C rats, the myocardial lesions consisted mainly of recent myocardial infarcts, which were more severe in the 2K-1C plus DM group. In L-NAME–treated rats, multiple foci of reparative fibrosis and fresh myocardial necrosis resembled the severe lesions found in the 2K-1C plus DM group. Although NOS3 protein expression increased (19 to 44%; p < 0.05) in all treated groups, serum nitrate/nitrite levels decreased only in the L-NAME group and the 2K-1C plus DM group. These two groups also showed a more pronounced increase in TXB₂ concentrations.

Conclusions: These results indicate that the association of hypertension and DM mimics the alterations induced by L-NAME in rats, which suggests a role for NO in the pathophysiology of hypertensive-diabetic cardiomyopathy.

(CHEST 2002; 122:1412–1420)

Key words: diabetes mellitus; heart; hypertension; myocardial diseases; N\-nitro-L-arginine methyl ester; nitric oxide

*From the Departments of Pharmacology (Drs. Sampaio, Tanus-Santos, Melo, Hyslop, and Moreno) and Medicine (Dr. Franchini), Faculty of Medical Sciences, and Department of Histology (Dr. Luca), Institute of Biology, State University of Campinas, Campinas, Brazil.

Manuscript received October 16, 2001; revision accepted February 28, 2002.
Hypertension is a comitant synergistic factor. Moreover, there is an increased prevalence of hypertension in diabetic patients and vice versa, suggesting that both conditions commonly coexist.4

The relationship among hypertension, diabetes, and endothelial function is complex. Since hypertensive-diabetic rats showed more severe coronary microvascular abnormalities,5 hypertension was suggested to exacerbate diabetic cardiomyopathy by further impairing the blood supply to the myocardium.6 In both disorders, the decreased production or bioavailability of nitric oxide (NO) causes endothelial dysfunction7 that may involve coronary vessels. A dysregulation of cardiac myocyte NO synthase type 3 (NOS3) was suggested to be involved in hypertension-induced cardiac alterations,8 and an increased expression of a dysfunctional NOS3 has been reported in an experimental model of diabetes,9 thus indicating that the NO system has a crucial role in both conditions. We have previously described hypertensive cardiomyopathy secondary to endothelial dysfunction following administration of the NO synthase inhibitor N\textsuperscript{\textcircled{o}}-nitro-L-arginine-methyl ester (L-NAME).10

In the present study, we compared the myocardial alterations following the chronic inhibition of NO biosynthesis with those observed in hypertensive-diabetic rats. We also evaluated the responses to DM and renovascular hypertension alone (two-kidney, one-clip model [2K-1C]). We hypothesized that similar mechanisms could be activated in hypertensive-diabetic and L-NAME–induced cardiomyopathy. To examine the role of NO in the development of hypertensive-diabetic cardiomyopathy, the cardiac levels of NOS3 protein were evaluated by Western blotting and serum nitrate and nitrite concentrations were measured. Since thromboxane A\textsubscript{2} (TXA\textsubscript{2}), an eicosanoid produced by activated platelets and endothelium, is frequently involved in the pathophysiology of cardiovascular diseases,11 the plasma levels of thromboxane B\textsubscript{2} (TXB\textsubscript{2}), the stable metabolite of TXA\textsubscript{2}, were also quantified.

MATERIALS AND METHODS

Experimental Groups

Male Wistar rats (150 to 200 g at the start of the study) were provided by Central Animal Services of the university, and were classified as follows: (1) a control group (n = 14), sham-operated rats that received tap water alone; (2) the L-NAME group (n = 31), rats that received L-NAME in drinking water; (3) the 2K-1C group (n = 26), rats with a silver clip placed around one renal artery; (4) the DM group (n = 27), rats that received a single intraperitoneal (IP) dose of streptozotocin, 60 mg/kg, and (5) the 2K-1C plus DM group (n = 26), rats that received a single dose of streptozotocin and had one renal artery clipped. The animals were killed after 3 weeks. This investigation conformed to the National Research Council guidelines.

Induction of Goldblatt II Hypertension

Rats were anesthetized with sodium pentobarbital, 40 mg/kg IP, a left flank incision was done to allow a silver clip (0.2-mm inner diameter) to be placed around the renal artery.12 In sham-operated control rats, the clip was removed immediately after being fixed around the vessel and the kidney was repositioned in the abdomen.

Long-term Treatment With L-NAME

L-NAME was dissolved in the drinking water at a concentration of 1.2 mM to give a daily intake of approximately 75 μmol per rat per day.10 The average daily intake of water and food did not differ significantly among the five groups.

Streptozotocin Administration

Diabetes was induced with an IP injection of streptozotocin, 60 mg/kg, dissolved in citrate buffer.

BP Measurements

Arterial BP was measured twice a week by a tail-cuff method,13 and the mean of these two determinations was considered to be the mean for that week. The same procedure was applied to weight gain.

Heart Weight Indexes

After 3 weeks of treatment, the rats were killed with a lethal dose of sodium pentobarbital, and the heart was removed, washed with saline solution, and then fixed in 10% formalin for 24 h. Subsequently, the atria were removed and the ventricles weighed to obtain the heart weight (HW). The left ventricular weight (LVW) and right ventricular weight (RVW) were determined after separating the ventricles. The HW index (HWI) was calculated by dividing the HW by the body weight (BW). Correspondingly, the LVW index (LVWI) and the RVW index (RVWI) were calculated by dividing the LVW and RVW by the BW.

Histologic Analysis

The left and right ventricles were cut into five equidistant rings perpendicular to the long axis of the heart. All rings were embedded in paraffin, and 5-μm sections were stained with hematoxylin-eosin. For each rat, one section of each of the five ventricular rings was studied by light microscopy. Cardiomyocyte size was determined by measuring the cell diameters by using an optical microscope system supplied with a graduated eyepiece micrometer and a 100 × objective (1,000 × magnification). Fifteen cells randomly selected from the subepicardial, midmyocardial, and subendocardial regions were measured in each animal of the different experimental groups. The investigator responsible was unaware of the corresponding groups for slides examined.

Stereological Procedures

Myocardial lesions were evaluated quantitatively using a stereological method that allowed the blind analysis of lesions...
resulting from recent infarcts, ie, focal lesions showing the onset or progress of death in cardiac myocytes, as well as the presence of fibrosis in scar tissue. In this method, the counting grid consisted of a combination of points and parallel lines (in this case, a 10 × objective with a graticule containing 100 points and 10 parallel lines). Each point corresponded to the center of a polygon of known area. The distance between the lines served as the point of reference for calibrating the system and also for determining the area of the lesion. For sample areas with a square format, the area (distance squared) was given by the formula:

\[ A = \Gamma \left( \frac{A}{P} \right) \times PT \]

where A was the area of the test point, \( \Gamma \) (Ap) was the sum of areas of the test points used, and PT was the total number of test points.

Western Blotting of Myocardial NOS3

For Western blotting, the thoracic cavity was opened and the heart rapidly removed. The left ventricle was minced coarsely and immediately homogenized in approximately 10 volumes of solubilization buffer (1% Triton-X 100, 100 mM Tris-HCl, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM ethylenediamine tetra-acetic acid, 2 mM phenylmethylsulfonyl fluoride, and 0.1 mg of aprotinin per milliliter) at 4°C using a polytron PT40S generator (model PT 10/35; Brinkman Instruments; Westbury, NY) operated at maximum speed for 30 s. The extracts were centrifuged at 10,000g at 4°C for 30 min to remove insoluble material. The resulting supernatant was used for the assay. Protein concentrations were determined with a dye-binding assay using Bio-Rad reagent (Bio-Rad Laboratories; Richmond, CA) and bovine serum albumin (BSA) as the standard. Equal amounts of total protein were for all samples diluted with Laemmli sample buffer containing 100 mM dithiothreitol and heated in a boiling water bath for 4 min, after which they were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (8% polyacrylamide gels) in a mini-square format, the area (distance squared) was given by the formula:

\[ A = \Gamma \left( \frac{A}{P} \right) \times PT \]

where A was the area of the test point, \( \Gamma \) (Ap) was the sum of areas of the test points used, and PT was the total number of test points.

Plasma Measurements

**Thromboxane B2:** Venous blood samples were collected from the tail vein into tubes containing ethylenediamine tetra-acetic acid at baseline and 3 weeks after treatment. The plasma was separated by centrifugation and stored at 20°C until assayed. Plasma samples were extracted using C18s reverse-phase cartridges (Waters Corporation; Milford, MA), and the TXB2 levels were determined with a commercial enzyme immunoassay (Cayman Chemical; Ann Arbor, MI). The detection limit of this assay was 13.3 pg/mL.

**Nitrites and Nitrites:** The circulating levels of nitrates/nitrites were used to estimate NO production. Blood samples from a caudal vein were collected into heparinized tubes at baseline and 3 weeks after treatment. The plasma was separated by centrifugation and stored at 20°C until assayed. Plasma nitrates/nitrites levels were determined spectrophotometrically using a commercial assay kit (Cayman Chemical). Briefly, plasma samples were filtered at 4°C by centrifugation through filters with a molecular weight cutoff of 10,000 (Centricron; Millipore; Milford, MA). After the addition of nitrate reductase to convert nitrates to nitrites, Griess reagent was added to the mixture and the nitrite concentrations were determined by measuring the resulting absorbance at 550 nm in a SpectraMax 340 multilwell plate reader (Molecular Devices; San Diego, CA). The nitrite concentrations of the samples were calculated by reference to a standard curve of nitrite run in parallel. The detection limit of this assay was 2.5 μM.

**Insulin:** Insulin levels were determined at baseline and 3 weeks after treatment using a commercial enzyme immunoassay ( rat insulin enzyme immunossay kit; SBI-BIO, Massy, France).

**Creatinine:** Renal function was assessed by measuring creatinine levels by the Jaffe method at baseline and 3 weeks after treatment.

Statistical Analysis

Results are expressed as mean ± SE. Analysis of variance (ANOVA) for repeated measurements was used to assess the differences in BW and tail-cuff pressure (TCP). One-way ANOVA was used to compare HW, LVW, RVW, HW1, LVW1, and RVW1. When ANOVA results were significant, the Duncan test was used to determine the differences among groups. In all cases, p < 0.05 was considered to be significant. All calculations were done using SigmaStat software (SPSS Science; Chicago, IL).

**RESULTS**

**BW and TCP**

Table 1 and Figure 1 show the BW and TCP, respectively. The two groups of rats treated with streptozotocin had a lower BW than the control group (p < 0.05) after 3 weeks. TCP increased equally in the L-NAME, 2K-1C, and 2K-1C plus DM groups (p < 0.05). TCP increased in the DM group after 3 weeks (p < 0.05).

**Cardiac Weight and Cardiac Weight Indexes**

Table 1 shows the HW, RVW, and LVW, and their respective weight indexes. There were no significant differences in these parameters in the control, L-NAME, and DM groups. In contrast, HW, HW1, LVW, and LVW1 increased after 3 weeks of treatment (p < 0.05) in the 2K-1C and the 2K-1C plus DM groups when compared to the control group.

**Histologic and Stereological Analysis**

There were no evident histologic lesions in the hearts of control rats. The heart of L-NAME–treated
rats showed unambiguous foci of reparative fibrosis consistent with organized myocytolytic necrosis. In addition, recent myocardial necrosis with granulation tissue showing blood vessels and many fibroblasts was also seen in these rats (Fig 2). Furthermore, some of these rats showed interstitial and perivascular cardiac fibrosis. The rats in the other three experimental groups had myocardial lesions consisting mainly of recent myocardial infarcts with dark coagulated myocytes, contraction bands, and dead myocytes lacking nuclei. Although similar lesions were observed in these three groups, the myocardial lesions in the DM group were greater than in the 2K-1C group. Even more widespread lesions were present in the 2K-1C plus DM group compared to the 2K-1C group and the DM group (Fig 3).

Figure 4 provides a quantitative comparison of the myocardial lesions in the different groups studied. In agreement with the qualitative histologic analysis, stereological analysis showed larger myocardial lesions in the DM group and the 2K-1C plus DM group compared to the 2K-1C group (p < 0.05).

The diameters of myocytes in the DM group were similar to those in control rats in the three myocardial regions (Table 2). In contrast, the diameters of midmyocardial and subepicardial myocytes were greater in the 2K-1C group and the 2K-1C plus DM group compared to the control group (p < 0.05 for both). A small increase in the diameter of midmyocardial myocytes was observed in the L-NAME group (p < 0.05).

**Western Blotting of Myocardial NOS3**

Figure 5 shows a representative Western blot of NOS3 in the hearts of rats from the different groups, as well as the average ± SEM values (n = 4) of densitometric readings obtained with anti-NOS3 antibody. Increased levels of NOS3 protein were seen in the 2K-1C, DM, and 2K-1C plus DM groups (19 to 23% higher than in the control group; p < 0.05). Treatment with L-NAME produced a more pronounced increase in the NOS3 protein levels (44% higher than in the control group; p < 0.05).

![Figure 1. TCP during the 3 weeks of study. ○ = control group (n = 16); ● = L-NAME group (n = 31); ■ = 2K-1C group (n = 21); □ = DM group (n = 27); ▼ = 2K-1C plus DM group (n = 20). The points represent the mean ± SEM. *p < 0.05 vs basal for L-NAME, 2K-1C, and 2K-1C plus DM groups. †p < 0.05 for all groups at week 3 vs control group.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21983/)

![Figure 2. L-NAME: subendocardial infarct showing a well-established granulation tissue with blood vessels, many fibroblasts, and little collagenization (Massons trichrome, original × 128).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21983/)
Plasma Measurements:

Nitrates and Nitrites: The circulating levels of nitrates/nitrites were unaltered in the control, 2K-1C, and DM groups, but decreased in the L-NAME and 2K-1C plus DM groups (p < 0.05 for both; Fig 6, top, A).

TXB₂: TXB₂ concentrations increased after 3 weeks in the four experimental groups (p < 0.05), but not in the control group (Fig 6, bottom, B). This increase was more marked in the L-NAME and 2K-1C plus DM groups.

Insulin, Glucose, and Creatinine: Table 3 shows the plasma insulin, glucose, and creatinine levels at baseline and after 3 weeks. There were no significant changes in the creatinine levels in all of the experimental groups (Table 3). Marked increases in glucose and decreases in insulin levels occurred in the DM group and the 2K-1C plus DM group (p < 0.05 for both).

Discussion

In the present study, the association of hypertension and DM resulted in more severe cardiomyopathy than would be anticipated with each condition. The severity of the hypertensive-diabetic cardiomyopathy resembled that seen after the inhibition of NO synthesis, thus suggesting a role for NO in both cardiomyopathies.

Although the histologic changes in the heart were qualitatively alike in the 2K-1C, DM, and 2K-1C plus DM groups, quantitative analysis indicated larger lesions in the last group. These findings are comparable to those found at autopsy in a large group of patients with either hypertension, DM, or both conditions. In this case, the hearts of patients with concomitant hypertension and DM showed more severe and generalized fibrosis than those of patients with hypertension or DM alone. The histologic analysis in the present study suggested that the severity of L-NAME–induced myocardial lesions was analogous to that in rats with DM and superimposed hypertension (the 2K-1C plus DM group). This suggestion is supported by the observation that, although the total area of myocardial lesions produced by L-NAME differed from that in the 2K-1C plus DM group, the multiple foci of reparative fibrosis and fresh myocardial necrosis seen after treatment with L-NAME resembled the more severe and extensive lesions present in the 2K-1C plus DM group. The multiple foci of reparative fibrosis seen after treatment with L-NAME were similar to those described in previous studies, and represented remotely infarcted myocardium. Thus, long-term inhibition of NO synthesis may cause an early activation of the pathophysiologic mechanisms underlying the more recent cardiac lesions found in the 2K-1C plus DM group.

An increase in BP enhances the mechanical stress on cardiomyocytes and is an important stimulus for cardiac hypertrophy. The similar rises in BP levels in the 2K-1C, 2K-1C plus DM, and L-NAME groups contrasted with the different grades of hypertrophic responses in these experimental groups, and suggested that mechanisms other than a pressure overload are also activated. Indeed, hypertrophy has been suggested to be related to humoral factors such as plasma renin activity or angiotensin II levels, but not to the mechanical overload.

The larger HW, HWI, LVW, and LVWI were associated with a significantly greater myocyte diameter in the midmyocardium and subepicardium in
the 2K-1C group and the 2K-1C plus DM group. However, no remarkable increases were observed in the cardiac indexes and myocyte diameter of L-NAME–treated rats. These findings confirmed previous studies demonstrating that ventricular hypertrophy and increased cardiomyocyte diameter occur in 2K-1C–treated rats or after aortic stenosis, but not after treatment with L-NAME.10,20–22 This lack of a hypertrophic response after treatment with L-NAME may be explained by a limitation imposed on ventricular growth attributed to a more pronounced loss of cardiomyocytes that undergo apoptosis after inhibition of NO biosynthesis.23 Interestingly, one study24 has demonstrated that different doses of L-NAME, which produced the same increase in systolic BP, resulted in fibrosis that was proportional to the dose of L-NAME administered. Thus, a more powerful inhibition of NO biosynthesis may cause more severe myocardial lesions in rats treated with increasing doses of L-NAME, or when DM is superimposed on hypertension.

Rats treated with streptozotocin, 60 mg/kg, gained weight and showed hypoinsulinemia and hyperglycemia as reported previously with a slightly lower dose of streptozotocin (55 mg/kg).6 The increase in the BP of diabetic rats agrees with previous findings showing a trend for arterial pressure to increase when glucose levels

<table>
<thead>
<tr>
<th>Regions</th>
<th>Control (n = 6)</th>
<th>L-NAME (n = 6)</th>
<th>2K-1C (n = 6)</th>
<th>DM (n = 6)</th>
<th>2K-1C Plus DM (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium, μm</td>
<td>12.8 ± 0.8</td>
<td>13.4 ± 0.2</td>
<td>13.7 ± 0.4</td>
<td>12.0 ± 0.8</td>
<td>13.7 ± 0.5</td>
</tr>
<tr>
<td>Midmyocardium, μm</td>
<td>13.0 ± 0.2</td>
<td>14.8 ± 0.8†</td>
<td>18.3 ± 1.6†</td>
<td>13.5 ± 0.5</td>
<td>18.2 ± 1.3†</td>
</tr>
<tr>
<td>Subepicardium, μm</td>
<td>10.3 ± 0.8†</td>
<td>12.2 ± 0.5</td>
<td>13.2 ± 0.4†</td>
<td>10.8 ± 0.7†</td>
<td>13.4 ± 0.7†</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.
†p < 0.05 vs control.
‡p < 0.05 vs the other two regions.
levels are not controlled.25 Importantly, levels of NOS3 protein were increased in diabetic rat hearts, maybe as a result of enhanced oxidative stress caused by hyperglycemia.26 Although similar increases (19 to 25%) in the levels of NOS3 protein were also observed in the 2K-1C group and the 2K-1C plus DM group, treatment with L-NAME produced the greatest increase in NOS3 protein (44% higher than the control group). Curiously, the increased expression of NOS3 did not produce concomitant changes in the plasma nitrate/nitrite levels in any of the all groups. The plasma nitrate/nitrite concentrations decreased only in the L-NAME and 2K-1C plus DM groups, which showed the most severe myocardial lesions. Although the precise relationship between serum nitrate/nitrite levels and NO production is still unclear,27 these results suggest an important decrease in NO bioavailability16 in both groups. Indeed, diabetic rats show impaired acetylcholine-induced aortic relaxation and nitrate/nitrite production attributed to an abnormal metabolism of NO.28 A reduced bioavailability of NO caused by a lack of substrates or cofactors may lead to the uncoupling of NOS3 or increased NO degradation by reactive oxygen species.9,29

Complex mechanisms regulate NOS3 at the transcriptional and posttranscriptional levels.30 Although NOS3 is expressed predominantly in coronary and endocardial endothelial cells, and to a lesser extent in cardiac myocytes,31 the regulation of the expression of this enzyme in the heart is not well studied, especially in pathophysiologic conditions. Increased expression of NOS3 was reported in the heart after banding of the aorta,32 or after the development of hypertension in spontaneously hypertensive rats.33 The increased NOS3 levels seen in the 2K-1C group and the L-NAME group did not agree with previous studies showing decreased levels of NOS3 messenger RNA in 2K-1C–treated rats,34 or after 6 weeks of treatment with L-NAME, 60 mg/kg/d.35 Differences in rat strain, and in the dose and duration of treatment with L-NAME, could explain the discrepancies between these studies. These same reasons could account for the extent and severity of the myocardial lesions seen here compared to previous studies.

A hypercoagulable or prothrombotic state, partially due to platelet abnormalities, has been described in hypertension36 and DM,37 and may involve changes in the bioavailability of NO. This is mainly because endogenous NO, besides controlling vascular tone, also regulates leukocyte adhesion and platelet aggregation. Thus, the more severe myocardial lesions and greater increases in TXB2 levels seen in L-NAME–treated and 2K-1C plus DM-treated rats may be partially explained by a more extensive reduction in the availability of NO in both groups. This greater reduction in NO availability may have increased platelet activation and released more TXA2 than in the other experimental groups. This suggestion is in line with studies demonstrating that hyperglycemia per se, beside inducing platelet hyperactivity,37 can also chemically inactivate NO,39 thereby potentiating the decreases of NO bioavailability seen in hypertension.7 In further support of this hypothesis, inhibitors of NO biosynthesis have been shown to enhance platelet aggregation.39 In addition, increased TXA2 production, indicative of platelet activation, has been found in acute coronary disease.40 Together, these findings point to a lack of NO as a key factor in the diabetic-hypertensive patients.

There is one limitation in the present study. The reduced NO availability we have shown in diabetic-hypertensive cardiomyopathy could be further confirmed by examining the effects of a treatment that increases the availability of NO. Such a treatment would probably result in a normalization or attenuation of alterations in diabetic-hypertensive cardio-

### Table 3—Plasma Creatinine, Glucose, and Insulin Concentrations After 3 Weeks in the Groups Studied*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 16)</th>
<th>L-NAME (n = 31)</th>
<th>2K-1C (n = 21)</th>
<th>DM (n = 27)</th>
<th>2K-1C Plus DM (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Week 3</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>114 ± 15</td>
<td>112 ± 11</td>
<td>111 ± 14</td>
<td>105 ± 20</td>
<td>150 ± 24</td>
</tr>
<tr>
<td>Basal</td>
<td>105 ± 10</td>
<td>84 ± 11</td>
<td>104 ± 13</td>
<td>361 ± 115†</td>
<td>427 ± 107†</td>
</tr>
<tr>
<td>Week 3</td>
<td>0.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.11†</td>
<td>0.0 ± 0.00†</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.
†p < 0.05 vs basal.
myopathy. Since this issue has not been previously addressed, further studies exploring this possibility are warranted.

The experimental data presented here are supported by a recently published clinical study comparing hypertensive diabetic, hypertensive, and healthy subjects. Interestingly, hypertensive diabetic patients had the highest plasma levels of von Willebrand factor and soluble P-selectin, which reflect a prothrombotic state associated with low NO availability. Therefore, a soluble P-selectin, which reflects a prothrombotic state, the highest plasma levels of von Willebrand factor and soluble P-selectin, which reflect a prothrombotic state, has been suggested to accelerate the renal disease.

In conclusion, our data support the hypothesis that the cardiac abnormalities found in hypertension are similar to found in other pathophysiologic conditions such as DM, probably because of the activation of related underlying mechanisms. Moreover, the combination of hypertension and DM enhanced the severity of these abnormalities to such an extent that the effect resembled dose-dependent L-NAME–induced cardiomyopathy. These findings suggest a role for the NO system in the pathophysiology of diabetic-hypertensive cardiomyopathy.

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