Enhanced NF-κB Activity Impairs Vascular Function Through PARP-1-, SP-1-, and COX-2–Dependent Mechanisms in Type 2 Diabetes

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Type 2 diabetes (T2D) is associated with vascular dysfunction. We hypothesized that increased nuclear factor-κB (NF-κB) signaling contributes to vascular dysfunction in T2D. We treated type 2 diabetic (db/db−/−) and control (db/db+/+) mice with two NF-κB inhibitors (6 mg/kg dehydroxymethylepoxyquinomacin twice a week and 500 μg/kg/day IKK-NBD peptide) for 4 weeks. Pressure-induced myogenic tone was significantly potentiated, while endothelium-dependent relaxation (EDR) was impaired in small coronary arterioles and mesenteric resistance artery from diabetic mice compared with controls. Interestingly, diabetic mice treated with NF-κB inhibitors had significantly reduced myogenic tone potentiation and improved EDR. Importantly, vascular function was also rescued in db/db−/− and db/db+/+ double knockout mice compared with db/db−/− mice. Additionally, the acute in vitro downregulation of NF-κB-p65 using p65NF-κB short hairpin RNA lentivirus in arteries from db/db−/− mice also improved vascular function. The NF-κB inhibition did not affect blood glucose level or body weight. The RNA levels for Sp-1 and eNOS phosphorylation were decreased, while p65NF-κB phosphorylation, cleaved poly(ADP-ribose) polymerase (PARP)-1, and cyclooxygenase (COX)-2 expression were increased in arteries from diabetic mice, which were restored after NF-κB inhibition and in db/db−/− and db/db+/+ mice. In the current study, we provided evidence that enhanced NF-κB activity impairs vascular function by PARP-1−, Sp-1−, and COX-2–dependent mechanisms in male type 2 diabetic mice. Therefore, NF-κB could be a potential target to overcome diabetes-induced vascular dysfunction. Diabetes 62:2078–2087, 2013

Diabetes-induced vascular dysfunction is a major clinical problem that is responsible for morbidity and predisposes patients to a variety of cardiovascular diseases (1,2). Vascular endothelial and smooth muscle cell dysfunction are early events in diabetes, characterized by impaired nitric oxide (NO) pathway signaling and potentiation of pressure-induced myogenic tone (3–6). The loss of vascular endothelial NO bioavailability in diabetes results in vasospasm, platelet aggregation, leukocyte adhesion, vascular smooth muscle proliferation, and induction and progression of atherosclerosis (7–9) associated with increases in activity of the proinflammatory transcription factor nuclear factor-κB (NF-κB) (7). The activation of the NF-κB pathway regulates gene expression of cytokines and chemotactic and matrix proteins and induces cell proliferation resulting in the induction and progression of vascular disease (10).

It has been shown that hyperglycemia induces cyclooxygenase (COX)-2 expression through NF-κB pathway (11). This concept is supported by previous studies showing that COX-2 induction is primarily mediated through the activation of the NF-κB pathway (12,13). It has been reported that NF-κB subunits interact with poly(ADP-ribose) polymerase (PARP)-1 in the nucleus and then both bind to DNA to modulate gene expression (14). Recently, we have demonstrated that PARP-1 activity is enhanced in the vasculature in type 2 diabetes and is involved in the impairment of vascular function (15). It also has been shown that NF-κB regulates inflammatory cytokines through the transcription factor Sp-1 (16,17). Thus, the role and mechanism of NF-κB in vascular dysfunction in type 2 diabetes are important questions that remain unanswered. Therefore, in this study we determined whether enhanced NF-κB activity impairs vascular function in type 2 diabetes by PARP-1−, Sp-1−, and COX-2–dependent mechanisms and confirmed that the effect of NF-κB is not specific to one vascular bed by including coronary and mesenteric resistance arteries (MRAs).

RESEARCH DESIGN AND METHODS
All experiments were performed according to the American Guidelines for the Ethical Care of Animals and were approved by Tulane University Health Sciences Center Animal Care and Use Committee. Type 2 diabetic male mice (db/db−/−) (8–to-10-week-old males) and their homologous controls were purchased from The Jackson Laboratory (Bar Harbor, ME), housed in groups of five mice, and maintained at a temperature of 23°C with 12h light/dark cycles. Mice were fed on a solid standard diet (Na+ content 0.4%) and water. Mice were divided into six groups: 1) control mice infused with saline (n = 10), 2) control mice that received dehydroxymethylepoxyquinomacin (DHMEQ) (an NF-κB inhibitor, 6 mg/kg p.i. injection twice a week) for 4 weeks (n = 10), 3) control mice that received IKK-NBD peptide (an NF-κB inhibitor, 500 μg/kg/day daily i.p. injection) for 4 weeks (n = 10), 4) db/db−/− mice infused with saline (n = 10), 5) db/db−/− mice treated with DHMEQ (6 mg/kg i.p. injection twice per week) for 4 weeks (n = 10), and 6) db/db−/− mice treated with IKK-NBD peptide (500 μg/kg/day i.p. daily) for 4 weeks (n = 10). The body weight and blood glucose levels were recorded weekly during the experimental period. Blood glucose measurements were obtained from tail blood samples using a blood glucose meter (Prestige Smart System HDI; Home Diagnostic, Fort Lauderdale, FL) in all groups of mice after a 6h fast as previously described (18). Systolic blood pressure was measured by the tail-cuff machine as previously described (19).

At the end of the treatment period, mice were anesthetized with isoflurane and blood samples were collected from carotid artery into containing heparin

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diabetes.diabetesjournals.org DIABETES, VOL. 62, JUNE 2013 2079

mol/L) and sodium nitroprusside (SNP) (10

solution bubbled with carbogen at 37°C and pH = 7.4, arteries were stretched

After precontraction with PE (10

p65NF-

Arteries from MRA were mounted in a small-vessel dual-chamber myograph

MRA and CAs from diabetic mice were carefully


determined using the 2-


test (two tailed) for comparing two groups,

RESULTS

General parameters. Blood glucose levels and body weight were elevated in diabetic mice (383.1 \pm 28.8 mg/dL and 43.3 \pm 0.6 g, respectively) compared with control mice (121.1 \pm 1.8 mg/dL and 25.7 \pm 0.5 g) but were not

In vitro experiments. MRAs and CAs from diabetic mice were carefully cleaned of fat and connective tissue and then cut into rings (2 mm in length). MRAs were mounted in a small-vessel dual- chamber myograph for measurement of isometric tension. After a 30-min equilibration period in PSS solution bubbled with carbogen at 37°C and pH = 7.4, arteries were stretched to their optimal lumen diameter for active tension development. After a second 30-min equilibration period, the vessels were exposed to phenylephrine (PE) (10^{-5} mol/L) and the presence of functional endothelium was assessed by the ability of ACh (10^{-6} mol/L) to induce relaxation.

For determination of the role of endothelial NO synthase (eNOS) and COX-2 in the impaired EDR in diabetic mice, MRAs were incubated with NS 398 (10^{-4} mol/L) and apocynin (100

Drugs. PI hydrochloride, ACh, L-NAME, apocynin, and NS398 were obtained from Sigma-Aldrich, St. Louis, MO) for cells. A total of 1 μg DNase I-treated RNA was reverse transcribed into cDNA using the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) with random hexamers in a 20-

The machinery used was to normalize the transfection efficiency. In another set of experiments, the EDRs were only transfected with 1 μg of the expression vector pCMV4p65 and incubated with normal glucose (5 mmol/L) or high glucose (25 mmol/L), cells were lysed in lysis buffer (Promega), and luciferase and renilla activities were measured. The renilla activity was used to normalize the transfection efficiency. In another set of experiments, the EDRs were only transfected with 1 μg of the expression vector pCMV4p65 and incubated with normal glucose (5 mmol/L) or high glucose (25 mmol/L), cells were lysed in lysis buffer (Promega), and luciferase and renilla activities were measured.

Statistical analysis. Results are expressed as means ± SEM. Concentration response curves were analyzed using the GraphPad Prism 4.0 software (GraphPad, La Jolla, CA). One-way or two-way ANOVA was used to compare each parameter when appropriate. Comparisons between groups were performed with t tests when the ANOVA test was statistically significant. Values of P < 0.05 were considered significant. Differences between specified groups were analyzed using the Student t test (two tailed) for comparing two groups, with P < 0.05 considered statistically significant.

RESULTS

General parameters. Blood glucose levels and body weight were elevated in diabetic mice (383.1 ± 28.8 mg/dL and 43.3 ± 0.6 g, respectively) compared with control mice (121.1 ± 1.8 mg/dL and 25.7 ± 0.5 g) but were not affected by the NF-κB inhibitors or in dominant-negative knockout mice (db/db p65NF-κB/−/− and db/db PARP-1/−/−), indicating that enhanced NF-κB and PARP-1 activity are involved in type 2 diabetes–induced vascular complication (Table 1). Systolic blood pressure was similar in all groups (Table 1).

M. KASSAN AND ASSOCIATES
TABLE 1
Blood glucose, body weight, and systolic blood pressure measurements

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dL)</th>
<th>SBP (mmHg)</th>
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<tr>
<td>Control</td>
<td>25.79 ± 0.58</td>
<td>131.1 ± 1.89**</td>
<td>101.1 ± 1.09</td>
</tr>
<tr>
<td>Control plus DHEEQ</td>
<td>27.98 ± 1.46</td>
<td>145.6 ± 5.18**</td>
<td>105.6 ± 1.11</td>
</tr>
<tr>
<td>Control plus IKK-NBD peptide</td>
<td>25.56 ± 0.71</td>
<td>137 ± 9.0**</td>
<td>107 ± 0.12</td>
</tr>
<tr>
<td>db−/db− plus DHEEQ</td>
<td>43.39 ± 0.67*</td>
<td>383.8 ± 28.87</td>
<td>103 ± 0.87</td>
</tr>
<tr>
<td>db−/db− plus IKK-NBD peptide</td>
<td>40.89 ± 0.73</td>
<td>409.8 ± 28.42</td>
<td>109 ± 0.42</td>
</tr>
<tr>
<td>db−/db− plus p50NF-kB−/−</td>
<td>37.86 ± 0.2</td>
<td>401 ± 24.8</td>
<td>101 ± 1.8</td>
</tr>
<tr>
<td>db−/db− p50NF-kB−/−</td>
<td>38.50 ± 2.90</td>
<td>310.33 ± 38.12</td>
<td>103 ± 0.12</td>
</tr>
<tr>
<td>db−/db− PARP−1−/−</td>
<td>39.51 ± 4.51</td>
<td>320.12 ± 16.24</td>
<td>102 ± 1.24</td>
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Data are means ± SE. SBP, systolic blood pressure. *P < 0.05 for db−/db− vs. control, treated db−/db−, and double knockout; **P < 0.05 for control vs. db−/db−, treated db−/db−, and double knockout.

or peptide had no effect on the control group. Additionally, NF-kB inhibitors reduced interleukin-6 levels and TNF-α in the diabetic group with no effect on insulin and cholesterol. Interestingly, all of these parameters were reduced in double knockout mice (db−/db− p50NF-kB−/− and db−/db− PARP−1−/−) (Supplementary Fig. 1).

NF-κB and coronary and MRA reactivity. Myogenic tone was significantly increased in the CA and MRA from diabetic mice compared with control mice and was normalized after NF-κB inhibition (Fig. 1A and B). The EDR was also impaired in CA and MRAs from diabetic mice and was rescued after NF-κB inhibition (Fig. 1C and D). To strengthen our data, we examined myogenic tone and the EDR in CAs and MRAs from diabetic mice and was normalized after NF-κB inhibition (Fig. 1E–H).

We did not observe any effect on myogenic tone or EDR in CAs or MRAs from control mice treated with DHEEQ and IKK-NBD peptide (Supplementary Fig. 2A–D). The inhibition of eNOS with L-NAME reduced the EDR in all groups of mice (Supplementary Fig. 2E and F).

Effect of acute EGFRtk inhibition and downregulation of p65NF-κB on CA reactivity. To determine whether NF-κB is downstream of EGFRtk and that the acute downregulation of NF-κB improves coronary vascular function in diabetes, we incubated CAs from db−/db− mice with the EGFRtk inhibitor (AG1478, 1 μmol/L) for 4 h. Our results show a significant improvement in EDR associated with a reduction in NF-κB activity (Figs. 1I and 2G).

Furthermore, in isolated coronary artery from db−/db− mice transfected with p65NF-kB shRNA lentiviral particles for 4 h, there was a significant reduction in p65NF-kB expression and improvement in coronary EDR (Figs. 1J and 2K). As a control experiment, CA from db−/db− mice transfected with scrambled shRNA did not show improved coronary EDR (Fig. 1J).

NF-κB and signaling. To confirm the effect of NF-κB inhibition, we performed Western blot analysis and found that NF-κB activity was elevated in db−/db− mice compared with db−/db− mice treated with DHEEQ and IKK-NBD peptide and compared with the double knockout db−/db− p50NF-kB−/− and db−/db− PARP−1−/− mice (Fig. 2A, D, and H). The EDR was significantly improved in MRA isolated from db−/db− mice treated with COX-2 inhibitor (Fig. 3E and F and Table 1). These data were also associated with reduction in cleaved PARP-1 and COX-2 expression in MRA from db−/db− p50NF-kB−/− mice (Fig. 4F and K). Furthermore, Western blot analysis revealed that in MRA from db−/db− PARP−1−/− mice, PARP-1 was absent and this was associated with reduced p65NF-kB phosphorylation or COX-2 expression or with an increase in eNOS phosphorylation (Fig. 4F, H, J, and L). Endothelium-independent relaxation.
in response to SNP in MRA was similar in control, db⁻/db⁻, and db⁻/db⁻ p65NFκB⁻/⁻ mice (Supplementary Fig. 4A).

**Effect of acute EGFRtk inhibition and downregulation of p65NF-κB on MRA reactivity.** Isolated MRAs from db⁻/db⁻ mice treated with EGFRtk inhibitor significantly improved the EDR (Fig. 3G and H and Supplementary Table 1). Western blot analysis confirmed the reduction in p65NF-κB phosphorylation in MRA (Fig. 4M) indicating that p65NF-κB is downstream of EGFRtk. The acute downregulation of p65NF-κB in isolated MRAs from db⁻/db⁻ mice transfected with p65NF-κB shRNA lentiviral particles significantly improved the EDR, which is inhibited by L-NAME (Fig. 3H and Supplementary Table 1). Western blot analysis confirmed the downregulation of p65NF-κB in MRA transfected with p65NF-κB shRNA lentiviral particles (Fig. 4N). Endothelium-independent relaxation in response to SNP in MRA showed no difference between controls, db⁻/db⁻, and db⁻/db⁻ transfected with lentivirus shRNA p65NF-κB (Supplementary Fig. 4B).

**Effect of high glucose and overexpression of p65NF-κB on eNOS promoter.** To elucidate the mechanism by which eNOS expression and activity were decreased in diabetic, we studied the Sp-1 expression known as downstream signaling of NF-κB. Thus, the mRNA levels of Sp-1 were significantly reduced in coronary artery isolated from db⁻/db⁻ mice treated with high glucose and/or transfected with p65NFκB (Supplementary Fig. 4B). Interestingly, db⁻/db⁻ mice treated with NF-κB inhibitor significantly increased Sp-1 expression in CAs (Fig. 5A). In primary ECs isolated from CAs, transfected with a reporter plasmid containing the eNOS promoter, the stimulation of these cells with high glucose and/or overexpression of p65NF-κB significantly reduced Sp-1 expression (Fig. 5B). EDR in response to cumulative doses of ACh (10⁻⁸ to 10⁻⁴ mol/L) in coronary arteries and MRAs from control and db⁻/db⁻ mice treated with or without DHMEQ and IKK-NBD (C and D) and db⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice (G and H). *P < 0.05 for db⁻/db⁻ vs. control, db⁻/db⁻ treated with DHMEQ or IKK-NBD; †P < 0.05 for db⁻/db⁻ vs. db⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice. EDR in response to SNP in MRA showed no difference between groups (E and F).

FIG. 1. Effect of the NF-κB inhibition on myogenic tone and EDR in coronary and MRAs. Pressure-induced myogenic responses in coronary arteries and MRAs from control and type 2 diabetic (db⁻/db⁻) mice treated with or without DHMEQ and IKK-NBD (IKK) peptide (A and B) and db⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice (E and F). *P < 0.05 for db⁻/db⁻ vs. control, db⁻/db⁻ treated with DHMEQ or IKK-NBD; †P < 0.05 for db⁻/db⁻ vs. dB⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice. EDR in response to cumulative doses of ACh (10⁻⁸ to 10⁻⁴ mol/L) in coronary arteries and MRAs from control and db⁻/db⁻ mice treated with or without DHMEQ and IKK-NBD (C and D) and db⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice (G and H). *P < 0.05 for db⁻/db⁻ vs. control, db⁻/db⁻ treated with DHMEQ or IKK-NBD; †P < 0.05 for db⁻/db⁻ vs. db⁻/db⁻ p65NFκB⁻/⁻ and db⁻/db⁻ p50NF-kB⁻/⁻ mice.
and myogenic tone were significantly improved in type 2 diabetic mice, we and other investigators have demonstrated an increase in myogenic tone (15,34,35). Interestingly, in the current study we observed that the augmented myogenic tone in diabetic mice is the result of enhanced NF-κB activity (39). In addition, it has been proposed that cultured porcine coronary EC function is impaired with senescence, associated with enhanced NF-κB activity (39). In this study, we demonstrated that type 2 diabetes is associated with increased vascular NF-κB activity and impaired vascular function. Thus, the inhibition of NF-κB activity with different approaches (pharmacologic, double-knockout mouse models and downregulation of protein expression by NF-κB shRNA lentiviral particles) significantly improved EDR in CA and MRA. Importantly, the effect of NF-κB activity inhibition is not specific to one vascular bed.
FIG. 3. Effect of the NF-κB inhibition on EDR in MRAs. Key representative traces showing EDR curves to ACh from control and type 2 diabetic mice (db/db) treated with or without DHMEQ or IKK-NBD peptide, db/dbp50NF-KB/−, mice, and db/dbpPARP-1/− mice (A). EDR in response to cumulative doses of ACh (10^{-8} to 10^{-5} mol/L) in MRAs precontracted with PE (10^{-5} mol/L) from control and db/db treated with or without DHMEQ or IKK-NBD peptide (B) and incubated with or without apocynin (Apo) (NADPH oxidase inhibitor) (C) or NS 398 (COX-2 inhibitor) (D). *P < 0.05 for db/db vs. control, db/db treated with DHMEQ or IKK-NBD. &P < 0.05 for db/db treated with DHMEQ or IKK-NBD vs. control. EDR in MRA from control, db/db, db/dbp50NF-KB/−, and db/dbpPARP-1/− (E) and incubated with COX-2 inhibitor, NS 398 (F), and db/db incubated with either AG1478 (EGFRtk inhibitor) (G) or P65NF-kB shRNA lentiviral particles (H). *P < 0.05 for db/db vs. control, db/dbp50NF-KB/−, or db/dbpPARP-1/−. #P < 0.05 for db/dbp50NF-KB/− vs. control, db/dbpPARP-1/−. $P < 0.05 for db/db vs. db/db plus AG1478. @P < 0.05 for db/db, db/db plus scrambled vs. db/db plus P65 shRNA.
FIG. 4. Western blot analysis and quantitative data in homogenized MRAs from control and type 2 diabetic mice (db/db) treated with or without DHMEQ (DHM) or IKK-NBD (IKK) showing P-eNOS and T-eNOS (A) P-p65, T-p65 (B), c-PARP-1 and T-PARP-1 (C), COX-2 (D), and β-actin. $P < 0.05$ for db/db vs. control, control treated with DHMEQ or IKK-NBD, db/db treated with DHMEQ or IKK-NBD. #P < 0.05 for db/db, db/db treated with DHMEQ or IKK-NBD vs. control, control treated with DHMEQ or IKK-NBD. Western blot analysis in MRA from control, db/db, and db/db p50NF-kB2/2 showing P-eNOS, T-eNOS (E), p-50 (G), c-PARP-1 and T-PARP-1 (I and J), COX-2 (K and L), and β-actin and db/db PARP-1-/- showing P-eNOS, T-eNOS (F), P-p65 and T-p65 (H), T-PARP-1 (J), COX-2 (L), and β-actin. Western blot analysis in MRA from db/db incubated with either AG1478 (EGFRtk inhibitor) or p65NF-kB shRNA lentiviral particles, showing P-p65, T-p65 (M and N), and β-actin. P, phosphorylated; T, total; C, active.
indicating the critical importance of the NF-κB pathway in the global regulation of vasculature function in type 2 diabetes. It is also interesting to note that NF-κB inhibition did not reduce blood glucose and insulin levels or body weight, suggesting that enhanced NF-κB is a consequence of type 2 diabetes.

It is well-known that active NF-κB interacts with PARP-1 and translocates to the nucleus to target promoters (41). In addition, we previously reported that inhibition of PARP-1 activity improved vascular function in type 2 diabetes (15). In the current study, we found an increase in PARP-1 activity in arteries from type 2 diabetic mice. The inhibition of PARP-1 activity in db/db mice, using genetic deletion, significantly improved vascular function. In addition, the inhibition of the NF-κB pathway reduced PARP-1 activity, indicating that NF-κB regulates vascular function in type 2 diabetes by a PARP-1–dependent mechanism. These results are supported by studies showing an improvement in vascular function in double knockout db/db−/−PARP-1−/− mice.

To further investigate the downstream mechanism of NF-κB and PARP-1 and because of the importance of the transcription factor Sp-1 in the regulation of eNOS promoter activity, we studied Sp-1 expression. Interestingly, we found that Sp-1 mRNA levels were significantly reduced and were rescued by the inhibition of NF-κB in CAs from type 2 diabetic mice. In addition, in vitro studies show that overexpression of p65NF-κB in transfected primary ECs or incubation with high glucose reduced eNOS promoter activity and eNOS and Sp-1 mRNA levels. Our results are in agreement with studies indicating that the transcription factor Sp-1 binds to the eNOS promoter (43). In addition, it has recently been demonstrated that p65NF-κB subunit interacts with Sp-1 and negatively regulates gene expression (44). Therefore, our results suggest that enhanced NF-κB signaling impairs vascular function by PARP-1– and Sp-1–dependent mechanisms.

To provide information about the mechanism of impaired vascular function caused by enhanced NF-κB activity in diabetes, we observed that vascular COX-2 expression was enhanced in db/db mice. These data are consistent with studies showing the induction of COX-2 in type 2 diabetes (45,46).

The NF-κB is a ubiquitous family of transcription factors that also control the expression of genes involved in the inflammatory response, such as COX-2. Our studies showed that inhibition of NF-κB activity in db/db mice reduced
COX-2 expression indicating that COX-2 is downstream to NF-κB. In addition, the in vitro inhibition of COX-2 improves vascular function in db/\textit{db} mice. These data indicate that inhibition of NF-κB improved vascular function by a COX-2–dependent mechanism.

Although a previous population-based study showed that COX-2 inhibitors might lead to increase the risk of myocardial infarction in the general population (47), it has been shown that a post hoc analysis for different coxibs revealed a significant association with incident atrial fibrillation for etoricoxib but not for celecoxib. These results indicate that the issue is mostly related to the nature of COX-2 inhibitors (48).

In summary, our study demonstrates the importance of NF-κB in the regulation of vascular function in type 2 diabetes by PARP-1–, Sp-1–, and COX-2–dependent mechanisms (Supplementary Fig. 5). Therefore, NF-κB could be a potential target for a novel therapeutic strategy to reverse diabetes-induced vascular dysfunction.

Novelty and significance. Although there are studies involving NF-κB in diabetes, the proposed study is innovative, in our opinion, because our approach provided direct evidence that enhanced NF-κB activity causes vascular dysfunction in terms of myogenic tone and EDR by PARP-1–, Sp-1– and COX-2–dependent mechanisms in two different vascular beds. There is a paucity of studies that explore the role of the NF-κB pathway and the mechanism (PARP-1–Sp-1–COX-2) by which type 2 diabetes impairs the regulation of arteries, which leads to heart disease.

Emerging evidence from experimental and clinical research indicates that an NF-κB pathway plays pivotal roles in cardiovascular diseases. Lack of such knowledge is a fundamental problem because without it, endothelial dysfunction that causes coronary artery disease will still present a high risk for myocardial infarction in diabetic patients. Thus, our ex vivo and in vitro data clearly indicate an enhanced NF-κB pathway associated with impaired EDR in three vascular beds. Importantly, the inhibition of NF-κB activity improves vascular EDR. Thus, in our study we demonstrated the role of enhanced NF-κB activity in the dysfunction of vascular EDR by PARP-1–Sp-1– and COX-2–dependent mechanism.

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M.K., S.-K.C., and M.G. conducted experiments and wrote the manuscript. A.B. generated PARP-1 knockout mice. K.U. generated and provided DHMEQ. M.T. and S.B. participated in discussion and wrote the manuscript. K.M. was the principal investigator, designed the research, and wrote the manuscript. K.M. is the guarantor of the work and, as such, had full access to all the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis.

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