Vasoconstrictor role of cyclooxygenase-1-mediated prostacyclin synthesis in non-insulin-dependent diabetic mice induced by high-fat diet and streptozotocin

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Zhu N, Liu B, Luo W, Zhang Y, Li H, Li S, Zhou Y. Vasoconstrictor role of cyclooxygenase-1-mediated prostacyclin synthesis in non-insulin-dependent diabetic mice induced by high-fat diet and streptozotocin. Am J Physiol Heart Circ Physiol 307: H319–H327, 2014. First published May 30, 2014; doi:10.1152/ajpheart.00022.2014.—This study tested the hypothesis that in diabetic arteries, cyclooxygenase (COX)-1 mediates endothelial prostacyclin (PGI2) synthesis, which evokes vasoconstrictor activity under the pathological condition. Non-insulin-dependent diabetes was induced to C57BL/6 mice and those with COX-1 deficiency (COX-1−/− mice) using a high-fat diet in combination with streptozotocin injection. In vitro analyses were performed 3 mo after. Results showed that in diabetic aortas, the endothelial muscarinic receptor agonist ACh evoked an endothelium-dependent production of the PGI2 metabolite 6-keto-PGF1α, which was abolished in COX-1−/− mice. Meanwhile, COX-1 deficiency or COX-1 inhibition prevented vasoconstrictor activity in diabetic abdominal aortas, resulting in enhanced relaxation evoked by ACh. In a similar manner, COX-1 deficiency increased the relaxation evoked by ACh in nitric oxide synthase-inhibited diabetic renal arteries. Also, in diabetic abdominal aortas and/or renal arteries, both PGI2 and the COX substrate arachidonic acid evoked contractions similar to those of nondiabetic mice. However, the contraction to arachidonic acid, but not to PGI2, was abolished in vessels from COX-1−/− mice. Moreover, we found that 3 mo after streptozotocin injection, systemic blood pressure increased in diabetic C57BL/6 mice but not in diabetic COX-1−/− mice. These results explicitly demonstrate that in the given arteries from non-insulin-dependent diabetic mice, COX-1 remains a major contributor to the endothelial PGI2 synthesis that evokes vasoconstrictor activity under the pathological condition. Also, our data suggest that COX-1 deficiency prevents or attenuates diabetic hypertension in mice, although this could be related to the loss of COX-1-mediated activities derived from both vascular and nonvascular tissues.

CYCLOOXYGENASE (COX), which exists mainly as COX-1 and COX-2 isoforms, metabolizes arachidonic acid (AA) in the vasculature to produce prostacyclin (PGI2), which acts on PGI2 (IP) receptors to mediate vasodilation and protect vessels from the development of disease (4, 6, 28). Decreased production of PGI2 has been suggested to cause an increased incidence of cardiovascular events (11, 49). However, PGI2 may also act on thromboxane prostaglandin (TP) receptors on smooth muscle to mediate vasoconstriction and function as an endothelium-derived contracting factor (EDCF) (9, 19, 23, 24, 34, 47, 48, 50). In fact, the vasomotor reaction to PGI2 may be modulated by the functionally opposing TP and dilator IP receptors (9, 21, 47, 50), and an EDCF-like action of PGI2 can result from little or low functional presence of vasodilator IP receptors that leads to a major activation of TP receptors (10, 23–25, 42). Also, COX-2 has been commonly considered a major source of endothelial PGI2 synthesis (11); however, in some vascular beds, COX-1 functions as the major COX form (16, 17), mediating endothelium-dependent contraction (23–25, 39, 41) or relaxation (21, 30, 38) under normal physiological conditions. On the other hand, the precise role of COX-1 in diseased vessels, such as in diabetes, still remains to be elucidated clearly.

In diabetic rats and/or mice, COX-2-derived prostanoids have been suggested to cause abnormal vasoconstrictor responses or to account for the development of endothelium-derived vasoconstrictor activity (2, 12, 32, 33, 43). Indeed, COX-2, which has been identified as an inducible form, increases in diseases or pathophysiological conditions, including diabetes (1, 5, 15, 20, 35, 38, 40). On the other hand, COX-1-derived PGI2 has been found to mediate endothelium-derived vasoconstrictor activity or to increase the stiffness of resistance arteries in a rat or mouse model of hypertension, respectively (10, 44). Also, there are studies indicating that COX-1 can also be upregulated, and, in some diabetic arteries, inhibition of COX-1 abolishes endothelium-dependent contraction evoked by a Ca2+ ionophore (8, 36, 37). We speculate that in the given diabetic arteries, COX-1 may remain as a major COX form, mediating endothelial PGI2 synthesis to evoke vasoconstrictor activity under the pathological condition. However, the above-mentioned results were obtained with COX-1 inhibitors, which might have effects independent of their intended target (3). In addition, the association of COX-1-mediated endothelium-dependent contraction with PGI2 synthesis in diabetic arteries remains to be clearly elucidated. In fact, the activity of PGI2 synthase (PGIS) has been previously suggested to decrease, and this, together with upregulated COX-2, can cause endothelial dysfunction and/or result in the development of endothelium-derived vasoconstrictor activity in diabetes (5, 54, 55). Also, although COX-2 inhibition could be beneficial for diabetic hypertension (2), how the loss of COX-1 influences diabetic complications, such as hypertension, remains largely to be understood.

Therefore, in this study, non-insulin-dependent diabetes was induced to C57BL/6 mice and those with COX-1 deficiency (COX-1−/− mice) to determine the contribution of COX-1 and PGI2 synthesis to endothelium-derived vasomotor reactions under the pathological condition in the abdominal aorta and

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renal arteries, where PGI₂ normally mediates vasoconstrictor activity (23, 25). Also, experiments were performed to determine how COX-1 deficiency influences the development of diabetic complications, such as hypertension.

**MATERIALS AND METHODS.**

*Chemicals and solutions.* Nω-nitro-L-arginine methyl ester (L-NAME), ACh, phenylephrine (PE), AA, and the nonselective COX inhibitor indomethacin were purchased from Sigma (St. Louis, MO). PGI₂, the TP receptor antagonist SQ-29548, and the COX-1-selective inhibitor FR-122047 were from Cayman Chemical (Ann Arbor, MI). L-NAME, PE, ACh, FR-122047, and AA were dissolved in distilled water (pured with N₂ for dissolving AA), and PGI₂ was dissolved in carbonate buffer (50 mM, pH 10.0). Indomethacin and SQ-29548 were dissolved in DMSO at 2,000-fold working concentrations [the final concentration of DMSO was 0.05% (vol/vol)].

The compositions of physiological salt solution (PSS) and 60 mM K⁺-PSS (K⁵⁺) were as previously described (51).

*Mice, induction of diabetes, and tissue preparation.* All procedures performed on mice conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and were approved by the Institutional Animal Research and Use Committee of Shantou University.

C57BL/6 and COX-1⁻/⁻ mice were as previously described (23). Non-insulin-dependent diabetes (a more common type of diabetes) was induced to C57BL/6 or COX-1⁻/⁻ mice in a similar manner by high-fat diet and streptozotocin (STZ) injection using a regimen modified from that previously described (27). Briefly, mice were fed a high-fat diet (21.1% crude protein, 19.1% crude fat, 43.4% carbohydrate, 40% calories in fat, SLAC, Shanghai, China) beginning at 5 wk of age. A single intraperitoneal injection of 100 mg/kg STZ (in 0.05 M citrate buffer, pH 4.5, Sigma) was given 4 wk after. Blood glucose of age. A single intraperitoneal injection of 100 mg/kg STZ (in 0.05 M citrate buffer, pH 4.5, Sigma) was given 4 wk after. Blood glucose was measured using an OneTouch Blood Glucose Monitoring System (Johnson & Johnson, Brunswick, NJ) after tail cuffing at 2 wk and 3 mo after STZ injection. In addition, age-matched C57BL/6 mice fed on normal chow were used as positive controls for COX-1-mediated activity and/or contrast for the abnormalities developed in diabetic mice.

For in vitro analyses, diabetic C57BL/6 or COX-1⁻/⁻ mice (3 mo after STZ injection) or age-matched nondiabetic C57BL/6 mice were killed by CO₂ inhalation. With the assistance of a binocular microscope, aortas and renal arteries were dissected free of adherent tissues. For functional experiments, the nonbranching area of the abdominal aorta (the section below the renal arteries) and main stems of the renal artery were cut into 1-mm rings (3–4 abdominal aortic or 2 renal arterial rings could be obtained from a single mouse) as previously described (24, 25). In addition, in some experiments, mesenteric arteries were also isolated for the control response of PGI₂-induced relaxation (21).

**Measurement of systemic blood pressure.** Blood pressure was monitored monthly after STZ injection with a noninvasive, computerized tail-cuff system (ALC-NIBP, ALCBIO, Shanghai, China). Mice were first accustomed to tail-cuff blood pressure measurements for 3 consecutive days, and blood pressure was then measured on the fourth day. Mean arterial blood pressure (MAP), which was taken from the averaged value of three measurements, was used for analysis. Values of age-matched, nondiabetic mice were obtained in a similar manner.

**Analyses of renal pathology.** After mice had been killed, kidneys from both sides were weighed and expressed as a ratio of body weight [kidney-to-body weight ratio (KBR)]. For microscopic pathological examination, kidneys were fixed with 10% formalin, embedded in paraffin, and then sliced into 4-µm-thick sections. After placement on slides, sections were stained with periodic acid-Schiff (PAS) and then analyzed with NIH Image software (NIH freeware, Bethesda, MD). The mesangial matrix area represents the PAS-positive and nuclei-free area in the mesangium, whereas the glomerular area was obtained by tracing the borders of the capillary loop. The relative mesangial area was obtained by dividing the mesangial area with the glomerular area, and the value for each mouse was averaged from at least 10 glomeruli.

**Analyses of the vascular response.** Analyses of vascular function were performed as previously described (25, 51). Briefly, the vascular ring was mounted between two tungsten wires in an organ bath filled with PSS aerated with 95%O₂-5% CO₂ and maintained at 37°C. One wire was stationary, whereas the other wire was connected to a force transducer (AE 801, Kronex). Thereafter, vessels were stimulated with 60 mM K⁺ every 15 min, and the resting tension was adjusted stepwise to an optimal level (~250 or 300 mg for the renal artery and abdominal aorta, respectively), at which point the response to 60 mM K⁺ was maximal and reproducible.

To remove the influence of nitric oxide (NO), some vessels were treated with the NO synthase (NOS) inhibitor L-NAME (1 mM), under which the response of arteries appears similar to that in endothelial NOS⁻/⁻ mice (53). Inhibitors [L-NAME, FR-122047, SQ-29548, and indomethacin] were added 30 min before the vessel was contracted with an agent and kept in the solution throughout the experiment. For the response elicited by an agent under baseline (unprecontracted) conditions was expressed relative to that of 60 mM K⁺, whereas during the contraction evoked by PE at the amount indicated was expressed relative to the value immediately before the application of the agent.

**Assay of 6-keto-PGF₁α.** The PGI₂ metabolite 6-keto-PGF₁α (with a molecular mass of 370) was determined by HPLC-mass spectroscopy (HPLC-MS), with which signals of other COX-related products [PGE₂ and PGD₂, molecular mass of 352; PGI₂, molecular mass of 354; and the thromboxane A₂ (TXA₂) metabolite thromboxane B₂, molecular mass of 370 with a longer retention time than 6-keto-PGF₁α] are separated from 6-keto-PGF₁α, and can be simultaneously monitored (23, 52) using a previously described protocol (22). Aortas were cut open and rinsed of blood components. In some experiments, the endothelium was removed by a moistened cotton swab with the help of a binocular microscope. Aortic strips (two preweighed whole sections of the aorta for each single measurement) were incubated with PSS at 37°C for 30 min followed by exposures to PSS (1,000 µU) or PSS containing ACh (10 µM) or AA (10 µM, 37°C) for 15 min each. The extraction of sample and standard solutions and HPLC-MS measurements were performed as previously described (22). The amount of 6-keto-PGF₁α was calculated from the area of the signal against that of a standard curve and expressed as nanograms per milligram of wet tissue.

**Protein detection.** Expressions of COX-1, PGIS, and β-actin (as internal controls) were detected by Western blot analysis in intact abdominal aortas and kidney tissues (renal cortex or medulla). Anti-PGIS (polyclonal, rabbit, 1:2,000 dilution) and anti-COX-1 (polyclonal, rabbit, 1:1,000) antibodies were purchased from Cayman Chemical (Ann Arbor, MI), and anti-β-actin (polyclonal, rabbit, 1:2,000 dilution) antibody was bought from Santa Cruz Biotechnology (Santa Cruz, CA). Immunocomplexes were visualized with reaction solution from an ECL plus kit (Amersham, Buckinghamshire, UK) and detected using Kodak X-ray film (XBT-1, Xiamen, China). In some experiments, the expression of PGIS, which appeared as a single band in Western blots, was detected by immunohistochemistry in sections obtained from diabetic COX-1⁻/⁻ kidneys using a previously described protocol (25).

**Measurement of blood insulin level.** In some experiments, the blood insulin level was measured 3 mo after STZ injection. Briefly, the mouse was anesthetized with pentobarbital sodium (50 mg/kg ip), and blood was collected, using a syringe, from the right ventricle after a thoracotomy. After coagulation, serum (10 µl) was used for insulin
measurements with a mouse/rat insulin EIA kit (Millipore, Billerica, MA) according to the manufacturer’s instructions.

Data analysis. Data are expressed as means ± SE from numbers or pools of vessels from different animals (n). A Student’s t-test (unpaired, two tails) was used to compare the difference between two means for statistical evaluation. When more than two means were compared, one-way ANOVA followed by Dunnett’s post hoc test or paired, two tails) was used to compare the difference between two pools of vessels from different animals (n = 5 for each, P < 0.05). Values were obtained in an observer blinded manner and are expressed as means ± SE; n = 10 for each group. Diabetic C57BL/6 [wild type (WT)/D] or diabetic cyclooxygenase (COX)-1-deficient [knockout (KO)/D] mice were euthanized 3 mo after streptozotocin injection. WT/N, age-matched, nondiabetic C57BL/6 mice.

RESULTS

Development of hyperglycemia, diabetic complications, and effects of COX-1 deficiency. In all diabetic mice, hyperglycemia (>18 mM) developed 2 wk after STZ injection. To determine whether complications developed and how they were influenced by COX-1 deficiency, renal pathology and systemic blood pressure were determined in diabetic C56BL/6 and COX-1−/− mice (induced to diabetes in pairs, 10 for each mouse strain). Whereas blood glucose was higher, the body weight in both diabetic C56BL/6 and COX-1−/− mice was lower than that of age-matched, nondiabetic C57BL/6 mice (Table 1). However, the blood glucose levels and body weights were not significantly different between the two diabetic strains (Table 1). In addition, compared with the control nondiabetic mice, both diabetic C57BL/6 and COX-1−/− mice showed enlargement of kidneys or increased KBRs with similar extents of mesangial expansion, as revealed by the relative mesangial area obtained by PAS staining (Table 1).

On the other hand, either kidney weight (0.45 ± 0.02 vs. 0.58 ± 0.04 g in diabetic C57BL/6 mice) or KBR (Table 1) was smaller in diabetic COX−1−/− mice than in diabetic C57BL/6 mice. It should be noted that the KBR of nondiabetic, 5-mo-old COX−1−/− mice (14.0 ± 0.17 mg/g, n = 5) was comparable with that of C57BL/6 mice (Table 1). In addition, MAP, which remained unchanged (97.1 ± 3.49, 96.5 ± 1.43, and 90.5 ± 2.7 mmHg in diabetic C57BL/6, diabetic COX−1−/−, and nondiabetic C57BL/6 mice, respectively, P > 0.05) for up to 2 mo after STZ injection increased in diabetic C57BL/6 mice but not in diabetic COX−1−/− mice before death (Table 1). Meanwhile, insulin levels of diabetic C57BL/6 and COX−1−/− mice did not change significantly compared with nondiabetic mice (1.68 ± 0.09 and 1.57 ± 0.12 vs. 1.37 ± 0.11 ng/ml, respectively, n = 5 for each, P > 0.05), consistent with the nonsulin-dependent property of the disease models (27).

Effects of COX-1 deficiency on PGII2 synthesis and/or relaxation to ACh in diabetic mouse aortas. Next, we examined the production of the PGII2 metabolite 6-keto-PGF1α and the response evoked by ACh (10 μM) in aortas from diabetic C57BL/6 or COX−1−/− mice. Under basal (unstimulated with ACh) conditions, no product was present in the reaction solution (data not shown). However, in aortas of diabetic C57BL/6 mice, stimulation with 10 μM ACh produced 6-keto-PGF1α in amounts comparable with that of nondiabetic mice (Fig. 1A). In addition, 6-keto-PGF1α was the only product detected using HPLC-MS (Fig. 1B, top); however, it was not detected in vessels from COX−1−/− mice (n = 3; Fig. 1B, bottom) that had been stimulated with ACh or in those from diabetic C57BL/6 mice denuded of endothelium (n = 3; data not shown).

Meanwhile, in diabetic C57BL/6 abdominal aortas contracted with 10 μM PE (to achieve a sustained contraction of 80–100% that evoked by 60 mM K+; the maximal contraction to 60 mM K+ or 2 μM PE was 496 and 426 mg, respectively), the relaxation evoked by ACh (10 μM) was blunted by a force development (Fig. 1, C and D, top), similar to that of nondiabetic mice (where the maximal contraction to 60 mM K+ or 2 μM PE was 432 and 385 mg, respectively). However, such a force was absent in vessels from COX−1−/− mice (the maximal contraction evoked by 60 mM K+ and 2 μM PE was 398 and 374 mg, respectively), resulting in an enhanced relaxation, which had a significant lower tension than that of C57BL/6 mice within 6 mins after the application of ACh (Fig. 1, C and D, bottom). A similar enhancing effect was obtained in diabetic C57BL/6 vessels with the addition of 1 μM of the selective COX-1 inhibitor FR-122047 (Fig. 1C). On the other hand, under baseline conditions, ACh did not induce any response in vessels from either diabetic C57BL/6 or COX−1−/− mice (data not shown).

Effect of COX-1 deficiency on responses evoked by ACh in diabetic arteries with NOS inhibition. The above functional analyses suggest that COX-1 mediates a vasoconstrictor activity that is overcome by the relaxing action mainly derived from the endothelial NO concomitantly activated (23). To substantiate this, experiments were further performed on NOS-inhibited diabetic C57BL/6 or COX−1−/− vessels. As shown in Fig. 2A, in l-NAME-treated abdominal aortas of diabetic C57BL/6 mice, ACh, at both low (0.5 μM or 5-fold the minimal concentration for ACh to evoke contraction) and maximal (10 μM) concentrations (53), evoked contractions with forces similar to those of nondiabetic mice (Fig. 2A). However, no contraction was obtained with 10 μM ACh in diabetic COX−1−/− vessels (Fig. 2B, bottom, n = 5; top shows a control response) or in diabetic C57BL/6 abdominal aortas that had been denuded of endothelium or treated with the TP receptor antagonist SQ-29548 (n = 5 for each; data not shown).

In l-NAME-treated diabetic renal arteries, ACh did not evoke contraction under baseline conditions (data not shown),
Fig. 1. PGI₂ synthesis and vasomotor reaction evoked by ACh in diabetic C57BL/6 and cyclooxygenase (COX)-1 deficient (COX-1−/−) aortas. A: bar graph showing the amount of the PGI₂ metabolite 6-keto-PGF₁α evoked by ACh in aortas of diabetic C57BL/6 mice [wild type (WT)/D] compared with nondiabetic mice (WT/N). Values are expressed as means ± SE; n = 3. NS: not significant. B: representative traces showing the signal of 6-keto-PGF₁α evoked by ACh in WT/D aortas and its absence in counterparts from diabetic COX-1−/− mice [knockout (KO)/D]. The blue trace represents signals of molecular mass 370, in which only that of 6-keto-PGF₁α appeared, whereas the green and red traces represent those of molecular mass 354 (PGF₂α) and 306 (PGE₂ and PGD₂), where none were detected. C: time courses of relaxations evoked by ACh in abdominal aortas (AAo) from WT/N, WT/D, or KO/D mice that had been treated with 1 μM of the COX-1 inhibitor FR-122057 (WT/D + FR). Values are expressed as means ± SE; n = 5 for each. **P < 0.01, KO/D vs. WT/D mice. PE, phenylephrine.

possibly due to the masking effect of a relaxing activity mediated by EDHF, as previously shown in nondiabetic mice (25). Therefore, the effect of COX-1 deficiency on the ACh-induced NO-independent response was examined in L-NAME-treated diabetic renal arteries precontracted with 2 μM PE (to achieve a sustained contraction 80–100% of that evoked by 60 mM K⁺). As shown in Fig. 2, C and D, the relaxation in diabetic COX-1−/− renal arteries (which have a maximum of 346 or 304 mg of force in response to 60 mM K⁺ and 10 μM PE, respectively) was significantly enhanced compared with

Fig. 2. Vasomotor reaction evoked by ACh in WT/D and KO/D vessels with nitric oxide (NO) synthase inhibited. A: bar graph showing the contraction evoked by 0.5 or 10 μM ACh + N nitro-L-arginine methyl ester (L-NAME)-treated AAo from WT/D mice compared with WT/N mice. Values are expressed as means ± SE; n = 5. B: representative trace showing the absence of contraction evoked by ACh (10 μM) in L-NAME-treated AAo from KO/D mice. Top, control response from WT/D mice. C: time courses of relaxation evoked by ACh (10 μM) in L-NAME-treated WT/D or KO/D renal arteries (RA) that had been contracted with 2 μM PE and that of KO/D mice in the presence of the nonselective COX inhibitor indomethacin (KO/D + IND; 10 μM). Values are expressed as means ± SE; n = 5 for each. **P < 0.01, KO/D vs. WT/D mice. D: representative traces from C showing relaxation of RA from WT/D and KO/D mice.
that of diabetic C57BL/6 mice (in which the maximal force generated by 60 mM K\(^+\) or 10 \(\mu\)M PE was 329 and 296 mg, respectively). In addition, such an effect of COX-1 deficiency was unaltered by 10 \(\mu\)M of the nonselective COX inhibitor indomethacin (Fig. 2C).

Responses evoked by PGI\(_2\) and the function of IP and/or TP receptors in diabetic arteries. Since PGI\(_2\) is commonly considered to act on IP receptors to mediate relaxation, its effect on intact, NOS-uninhibited diabetic C57BL/6 abdominal aortas that had been precontracted with 2 \(\mu\)M PE (to achieve 40–60\% that induced by 60 mM K\(^+\), at which the relaxation caused by an agent would be readily detected) was then examined. As shown in Fig. 3A, PGI\(_2\) (0.03–0.3 \(\mu\)M) did not induce any relaxation but rather an increase of PE-induced contraction, similar to that we have previously reported in nondiabetic mice (23). No relaxation was even obtained in such vessels treated with 10 \(\mu\)M of the TP receptor antagonist SQ-29548 (Fig. 3A, middle), although a graded relaxing response with a maximal complete relaxation of PE-induced contraction was seen, as expected (21), in a similarly treated mesenteric artery from the same mouse (Fig. 3A, bottom).

Moreover, we noted that in the L-NAME-treated diabetic abdominal aorta, PGI\(_2\) evoked contraction under baseline conditions, which was to a similar extent as that of nondiabetic mice, and this again was abolished by SQ-29548 (Fig. 3B). Also, contractions to the TP receptor agonist U-46619 were comparable between diabetic and nondiabetic vessels (Fig. 3C). In addition, in L-NAME-treated renal arteries, which normally show a more potent contraction in response to the compound than some other arteries in mice (25), 1 \(\mu\)M PGI\(_2\) evoked contractions that were again not significantly different \((P > 0.05)\) between diabetic and nondiabetic mice (Fig. 3D).

Expression and/or function of COX-1 or PGIS in diabetic mice. Expressions of COX-1 and/or PGIS in diabetic mice were also examined. As shown in Fig. 4A, in abdominal aortas, expression of COX-1 was clearly seen; however, its level was significantly lower than that of nondiabetic control mice (Fig. 4B). A similar decrease of COX-1 was noted in the renal cortex and medulla (Fig. 4C). On the other hand, PGIS in the diabetic abdominal aorta was not significantly different from the nondiabetic abdominal aorta (Fig. 4, A and B). In addition, immunohistochemical staining of the kidneys showed that PGIS was present in diabetic COX-1\(^{-/-}\) mice (Fig. 4D).

Due to the inconsistence in COX-1 levels and ACh-induced responses between diabetic and nondiabetic mice (which was to a similar extent as that of nondiabetic mice, and this again was abolished by SQ-29548 (Fig. 3B). Also, contractions to the TP receptor agonist U-46619 were comparable between diabetic and nondiabetic vessels (Fig. 3C). In addition, in L-NAME-treated renal arteries, which normally show a more potent contraction in response to the compound than some other arteries in mice (25), 1 \(\mu\)M PGI\(_2\) evoked contractions that were again not significantly different \((P > 0.05)\) between diabetic and nondiabetic mice (Fig. 3D).

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COX-1 to increase in diabetic C57BL/6 mice but not in diabetic manner to that of nondiabetic mice. Moreover, MAP was noted suggesting that PGI2 could also be a major COX-1-derived endothelium-dependent production of the PGI2 metabolite was clearly demonstrated by the abolition of ACh-evoked activity but also suggest that COX-1 may function as an oligizing enzyme that produces PGI2 and evokes vasoconstrictor activity evoked by the endothelial muscarinic receptor agonist ACh was abolished by COX-1 deficiency. We also found that in diabetic abdominal aortas and/or renal arteries, PGI2 or the COX substrate AA (which stimulates in vitro PGI2 synthesis as well) evokes contraction in a similar manner to that of nondiabetic mice. Moreover, MAP was noted to increase in diabetic C57BL/6 mice but not in diabetic COX-1−/− mice. These results not only demonstrate that in the given diabetic arteries COX-1 remains as a major AA metabolizing enzyme that produces PGI2 and evokes vasoconstrictor activity but also suggest that COX-1 may function as an adverse modulator of diabetic hypertension in mice.

The role of COX-1 as a major COX form in diabetic aortas was clearly demonstrated by the abolition of ACh-evoked endothelium-dependent production of the PGI2 metabolite 6-keto-PGF1α in diabetic COX-1−/− mice. It should be noted that we confirmed the development of diabetes in C57BL/6 or COX-1−/− mice by hyperglycemia, renal pathology, and/or hypertension. In addition, our immunohistochemistry results revealed that PGIS is expressed in diabetic COX-1−/− vessels. Also, as we have previously shown in nondiabetic vessels, 6-keto-PGF1α is the only COX-related metabolite detected, suggesting that PGI2 could also be a major COX-1-derived product in diabetic arteries (23). Moreover, we noted that the relaxation evoked by ACh in the diabetic abdominal aorta was enhanced by COX-1 deficiency or COX-1 inhibition, further suggesting that COX-1, which has been previously shown to exist mainly in the endothelium of mouse arteries (23, 52), mediates a vasoconstrictor activity that is overcome by the dilator action derived largely from endothelial NO (23). Indeed, in such vessels, when NOS is inhibited, ACh evokes contraction that is abolished by COX-1 deficiency or endothelial denudation under baseline conditions.

Furthermore, we noted that even in the intact diabetic abdominal aorta with NOS uninhibited, PGI2 evokes not relaxation but rather an increase of force on PE-induced contraction, similar to results we have previously reported in nondiabetic mice (23). In fact, in NOS-inhibited vessels, PGI2 or the TP receptor agonist U-46619 evokes contraction, similar to that of nondiabetic mice. Interestingly, the amount of PGI2 evoked by ACh is above the concentration required to initiate a vasoconstrictor activity (1 ng/mg 6-keto-PGF1α can be translated into 2.7 μmol PGI2 produced in 1 kg vessel tissue). In addition, PGH2, the intermediate of PGI2 synthesis that may diffuse outside the endothelium, can also cause contraction by acting on TP receptors before being converted to PGI2 in medial smooth muscle (10, 13, 52). Thus, the vasoconstrictor activity of COX-1 in diabetic aortas could be in an important part derived from PGI2 synthesis, owing to limited function of IP receptors, as we have previously suggested in normal mouse arteries (23). Moreover, we found that the relaxation evoked by ACh on PE-induced contraction in NOS-inhibited diabetic renal arteries was also enhanced by COX-1 deficiency. Meanwhile, nonselective COX inhibition did not add to the effect of COX-1 deficiency, implying little involvement of COX-2 in the vessels. As a result, the role of COX-1 as the major form is not limited to a specific vessel, and its PGI2 synthesis can also lead to vasoconstrictor activity in certain diabetic vessels of critical organs, including renal arteries. In further support of this idea, both PGI2 and AA evoked contraction in the diabetic abdominal aorta and/or renal arteries; however, only the response to AA was abolished by COX-1 deficiency.

Also of interest is that whereas MAP of either diabetic strain was similar to that of control mice up to 2 mo after STZ...
Fig. 5. PGI2 synthesis and/or contraction evoked by the COX substrate arachidonic acid (AA) in diabetic vessels. A: summary (n = 3 for each) of the PGI2 metabolite 6-keto-PGF1α, evoked by AA (10 µM) in aortas of WT/D mice compared with WT/N mice. B: summary (n = 5 for each) of contractions evoked by AA (10 µM) in L-NAME-treated AAo from WT/D, WT/N, and KO/D mice. In A and B, values are expressed as means ± SE. **P < 0.01 vs. control. C, bottom: representative trace showing the absence of contraction to AA (10 µM) in KO/D RA, where PGI2 still induces contraction. Top, control response from WT/D mice.

injection, it increased in diabetic C57BL/6 mice but not in diabetic COX-1−/− mice before death (3 mo after STZ injection). This further suggests that COX-1 deficiency, which did not alter normal blood pressure here and in a previous report (31), may favorably influence the development of diabetic hypertension, consistent with the vasoconstrictor role of COX-1 in vessels of diabetic mice, including renal arteries. Due to the size of the vessels, we were unable to perform experiments on smaller diabetic arteries; however, COX-1-mediated PGI2 synthesis has been previously shown to cause contraction in porcine small intrarenal arteries (interlobular arteries) or to increase the stiffness of resistance arteries (24, 44). In contrast, in some arteries, such as mouse mesenteric arteries where IP receptors have a substantial functional presence, PGI2 evokes relaxation (21, 26, 30, 38). Interestingly, in platelets, COX-1 also synthesizes TxA2, which can adversely influence renal and vascular function (6, 14, 31). As a result, the increased blood pressure in diabetic C57BL/6 mice but not in diabetic COX-1−/− mice may reflect that the beneficial effect of dilator activity of COX-1-derived PGI2 on diabetic hypertension is outweighed by the above-mentioned adverse activities derived from COX-1-mediated AA metabolism in vascular and nonvascular tissues, including synthesis of PGI2 or TxA2 in the vascular endothelium and platelets, respectively.

Therefore, our above results not only substantiate the results of a prior report on diabetic rat arteries with COX-1 inhibition (36) but also further suggest that PGI2 synthesis may lead to vasoconstrictor activity and contribute to the adverse effects of COX-1 on diabetic hypertension. Previously, COX-2 has been commonly suggested to be the major mediator of endothelium-derived vasoconstrictor activity, and its inhibition is considered to beneficially influence diabetic hypertension (2, 12, 32, 33, 43). However, COX-2 deficiency has also been suggested to cause hypertension and increase thrombotic events (49). In this regard, our results may suggest an alternative for pharmacological intervention of diabetic hypertension. It should be noted that the vasoconstrictor role of COX-2 in previous reports might be exaggerated. This is because that some of the COX-2 inhibitors used in previous studies can also inhibit COX-1-mediated endothelium-dependent contraction (22, 29). In addition, our results show that PGIS expression and 6-keto-PGF1α production evoked by AA or ACh are unaltered in diabetic aortas, which excludes that the decreased PGIS activity, which has been proposed to reverse the dilator activity of AA metabolism into contraction (33, 54, 55), is a major cause of endothelium-derived vasoconstrictor activity in diabetic mouse arteries.

However, some previous reports have indicated that hypertension can develop 1 or 2 wk after diabetic induction (18, 46). This could result from varied genetic background, altered methods of diabetic induction (46), or the presence of endothelial NOS deficiency (18). Also, we showed that in diabetic aortas, either the contraction or 6-keto-PGF1α evoked by AA was similar to that of control nondiabetic mice; however, the level of COX-1 was lower in diabetic aortas and kidneys. This might suggest an increased activity for the amount of COX-1 expressed. Thus, the lower level of COX-1 might be an adaptive mechanism to maintain an appropriate COX-1 activity under diabetic conditions, explaining why in this and previous reports the endothelium-mediated reaction (relaxation and/or contraction) in diabetic arteries was comparable with that of nondiabetic conditions (2, 7). In addition, our results revealed that the enlargement of kidneys in diabetic COX-1−/− mice was to a significantly lesser extent than in diabetic C57BL/6 mice. However, PAS staining showed that mesangial expansion was similar between the two diabetic mouse strains. Thus, further studies are required to determine whether the lesser extent of kidney enlargement reflects an improvement in renal pathology and contributes to the unaltered blood pressure in diabetic COX-1−/− mice (45).

In summary, our results explicitly demonstrate that in the given diabetic mouse arteries, COX-1 remains as a major contributor to the endothelium-dependent PGI2 synthesis that evokes vasoconstrictor activity under the pathological condition. Also, our data suggest that COX-1 deficiency prevents or attenuates diabetic hypertension, although this could be related
to the loss of COX-1–mediated activities derived from both vascular and nonvascular tissues.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.Z., B.L., Y. Zhang, H.L., and S.L. performed experiments; N.Z., B.L., W.L., Y. Zhang, and Y. Zhou analyzed data; N.Z., B.L., W.L., Y. Zhang, H.L., S.L., and Y. Zhou approved final version of manuscript; B.L. and Y. Zhou conception and design of research; Y. Zhou drafted manuscript; Y. Zhou edited and revised manuscript.

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