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Renal cyclooxygenase-2 in obese Zucker (fatty) rats

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Background. Cyclooxygenase (COX) isoforms, COX-1 and COX-2, are involved in production of prostanoids in the kidney. Increases in renal COX-2 expression have been implicated in the pathophysiology of progressive renal injury, including type 1 diabetes. Thromboxane A_2 (TxA_2) has been suggested as the key mediator of these effects resulting in up-regulation of prosclerotic cytokines and extracellular matrix proteins. Unlike type 1 diabetes, renal COX has not been studied in models of type 2 diabetes.

Methods. Renal cortical COX protein expression, and urinary excretion of stable metabolites of prostaglandin E_2 (PGE_2) and TxA_2, in association with metabolic parameters, were determined in 4-and 12-week-old Zucker fatty rats (fa/fa rat) (ZDF4 and ZDF12), a model of type 2 diabetes, and in age-matched littermates with no metabolic defect (Zucker lean) (ZL4 and ZL12).

Results. Western blotting revealed increased COX-2 expression in ZDF4 as compared to ZL4 ($245 \pm 130\%$) ($P < 0.05$). This increase in COX-2 was even more apparent in 12-week-old ZDF rats ($650 \pm 120\%$) ($P < 0.01$). All groups of rats demonstrated COX-2–positive cells in typical cortical localizations [macula densa, thick ascending loop of Henle (TALH)]. In contrast to COX-2, COX-1 expression was 30% lower in ZDF12. These changes in COX expression were associated with enhanced urinary excretion of prostanoids, in parallel with the development of metabolic abnormalities. Moreover, increases in prostanoid excretion in ZDF12 were in part reduced by wortmannin (100 μg/kg), used as inhibitor of insulin signaling.

Conclusion. Renal cortical COX-2 protein expression and function were increased in ZDF rats, as compared to controls, whereas COX-1 exhibited opposite regulation. The changes in COX-2 paralleled metabolic abnormalities, and were at least in part a four consequence of hyperinsulinemia. These abnormalities may play a role in renal pathophysiology in this model of type 2 diabetes.

The role of cyclooxygenase (COX) metabolites of arachidonic acid in the development of renal alterations, and in the pathogenesis of diabetic nephropathy in type 1 diabetes, has been suggested in a number of clinical studies [1–3], as well as in experimental models of diabetes [4–9].

Two isoforms of COX have been identified, COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and performs housekeeping functions in the vascular system. In the normal adult kidney, COX-1 has been localized to arteries and arterioles, glomeruli, and collecting ducts [10, 11]. In contrast, COX-2 operates as an inducible enzyme with low or undetectable levels in most tissues, and its expression can be markedly increased by a number of inflammatory, mitogenic, and physical stimuli [12, 13]. Although considered to be an inducible enzyme, COX-2 is constitutively expressed in occasional renal cells of the thick ascending loop of Henle (TALH) and in the region of the macula densa of the rat kidney, and in podocytes in the human kidney [11, 14, 15].

Increased COX-2 expression and activity has been described in several models of progressive renal disease, including models of type 1 diabetes [8, 9]. Long-term studies have demonstrated beneficial effects of selective COX-2 inhibitors in these experimental conditions on the development of proteinuria and renal structural damage [8, 16, 17] [abstract; Komers R, *J Am Soc Nephrol* 13:166A, 2002]. These renoprotective effects could be attributable to inhibition of thromboxane A_2 (TxA_2) production and consequent beneficial effects on mRNA expression of transforming growth factor-β (TGF-β), and molecular markers of glomerular injury, such as type III type IV collagen [8, 17]. Consequently, COX-2–derived metabolites are likely to play a role in the development of diabetic nephropathy.

Nephropathy is also a major problem in type 2 diabetes [18]. Various animal models have been reported that could help to elucidate the pathophysiology of nephropathy in type 2 diabetes [19]. One such model is the Zucker diabetic fatty rat (fa/fa) (ZDF) [20]. These rats are metabolically well characterized: an autosomal-recessive mutation of the fa gene, encoding the leptin receptor, results in hyperphagia, obesity, and hyperlipidemia [21].

Key words: Zucker rat, diabetic nephropathy, cyclooxygenase-2, hyperinsulinemia, thromboxane A2.

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Similar to patients with type 2 diabetes, ZDF rats display insulin resistance, hyperinsulinemia, and impaired glucose tolerance, and slowly progressive increases in blood glucose levels [22].

ZDF rats develop progressive albuminuria and glomerulosclerosis later in the course of nephropathy [23]. However, unlike the streptozotocin (STZ) diabetic rats, renal COX-2 expression and function have not been studied in models of type 2 diabetes.

To address this issue, we determined renal protein expression, localization, and regulation of COX-2, together with expression of COX-1, in 4- and 12-week-old ZDF fa/fa rats and in age-matched control Zucker lean rats (ZL) that do not develop the diabetic phenotype.

METHODS

ZL and ZDF rat were obtained from Charles River (Sulzfeld, Germany). Renal cortical expression of COX isoforms, immunohistochemical localization of COX-2, and urinary excretion of stable metabolites of prostaglandin E_2 (PGE_2) and TxA_2, together with metabolic and renal parameters were studied in six male ZDF rats at the ages of 4 and 12 weeks, and in six age-matched ZL rats as controls.

To further explore possible role of hyperinsulinemia in regulation of renal cortical COX-2 in ZDF rats, additional groups of 12-week-old ZDF and ZL were administered with intraperitoneal injection of the phosphatidyl inositol-3-kinase (PI3K) inhibitor wortmannin [24][100 μg/kg body weight in 15% dimethyl sulfoxide (DMSO)] (Cell Signaling Technology, Inc., Beverly, MA, USA) or with vehicle (15% DMSO). Immediately after wortmannin or vehicle administration, the rats ($N = 5$ in each group) were placed into metabolic cages to obtain timed urine samples for analysis of urinary excretion of PGE_2 and TxB_2. General physical and metabolic parameters were measured in separate groups of wortmannin-or vehicle-treated ZL and ZDF ($N = 5$ in each group), sacrificed 90 minutes after injection. In previous in vivo studies conducted by Gao et al [25], substantially lower dose of wortmannin (15 μg/kg body weight) injected intravenously blocked insulin-induced increases in Akt activity, and inhibited beneficial effects of insulin on apoptosis in a rat model of myocardial infarction.

All experiments were carried out with the approval of, and in accordance with the regulations of, the Institutional Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine. The animals were housed with a light-dark cycle of 12 hours each, and with free access to food (standard chow) and water. The blood and tissues were harvested without previous food restriction.

Immunoblotting and immunohistochemistry

The rats were sacrificed with cervical dislocation and the blood was collected into chilled tubes for determinations of glucose levels and plasma insulin concentrations. After collection of blood samples, the kidneys were exposed via midabdominal incision, removed, decapsulated, divided into cortical and medullary portions, and snap frozen in liquid nitrogen for Western blot analysis. The half of the left kidney was immersed in 10% formalin for immunohistochemistry.

To obtain whole cell homogenates, kidney cortices were homogenized in RIPA buffer containing 50 mmol/L Tris, 150 mmol/L NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 1.0% Triton X-100, and protease inhibitors (leupeptin 20 μg/mL and benzamidine 20 μg/mL), and centrifuged at 12000 $\times$ g for 30 minutes at 4°C. The resulting supernatant was aliquoted and saved at −70°C until analysis. Total protein content in fractions was determined by BCA analysis (Pierce Chemical Co., Rockford, IL, USA).

Immunoblotting was performed as previously described [9]. In brief, denatured proteins were separated through an SDS-polyacrylamide gel and transferred to polyvinylidine difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were washed and then blocked overnight with Tris-buffered saline, plus 0.05% Tween-20 (TBS-T) containing 5% nonfat dry milk. Following blocking, membranes were again washed, and incubated overnight with rabbit polyclonal antimurine COX-2 or COX-1 antisera (Cayman Chemical, Ann Arbor, MI, USA) diluted 1:800 in TBS-T. Immunodetection was accomplished by incubating membranes with a goat antirabbit-IgG secondary antibody conjugated with horseradish peroxidase (HRP) for 45 minutes (1:100,000) (Pierce Chemical Co.) in TBS-T containing 5% nonfat dry milk. Visualization was performed with enhanced chemiluminiscence (ECL) Western-blotting kit (Supersignal West Dura) (Pierce Chemical Co.) according to the manufacturer's instructions. Resultant films (Eastman Kodak Co., Rochester, NY, USA) (Scientific Imaging Systems, New Haven, CT, USA) were scanned using a flatbed scanner and images analyzed with NIH Image software. The membranes were then stripped, reblocked, and reincubated for 1 hour at room temperature with goat antiactin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by 45-minute incubation with antigoat-IgG secondary antibody conjugated with HRP (1:4000) (Santa Cruz Biotechnology), and reaction with ECL as described above.

For immunohistochemical analysis, the fixed kidneys were processed as previously described [9]. The fixed kidney halves were dehydrated through a graded series of ethanols, embedded in paraffin, sectioned at 4 μm thickness, and placed onto glass slides. The same

Table 1. Physical and renal characteristics in 4-week-old and 12-week-old lean Zucker (ZL) and fatty diabetic Zucker (ZDF) rats

	Number	Body weight *g*	Right kidney weight *g*	Right kidney weight/100 g body weight	Creatinine clearance *mL/min*	Urinary protein excretion μg/*min*
ZL 4 weeks	5	103 ± 2	0.43 ± 0.01	0.42 ± 0.01	ND	ND
ZDF 4 weeks	6	131 ± 6[a]	0.51 ± 0.01[b]	0.40 ± 0.02	ND	ND
ZL 12 weeks	5	358 ± 9	1.13 ± 0.05	0.31 ± 0.01	0.93 ± 0.13	5.6 ± 0.9
ZDF 12 weeks	6	538 ± 25[b]	1.30 ± 0.04[b]	0.24 ± 0.01[‡]	0.92 ± 0.10	13.6 ± 3.0[c]

[a] $P < 0.01$; [b] $P < 0.001$ vs. ZL of the same age; [c] $P < 0.05$.

antibody as described above was used for immunohistochemical detection of COX-2 in ZDF and ZL rats. Sections were deparaffinized, and pretreated by steaming in 10% Citra buffer (BioGenex, San Ramon, CA, USA). After blocking, the slides were incubated overnight at 4°C with primary antibody (diluted 1:100) or with the same concentration of nonimmune mouse IgG as a control. Endogenous peroxidase activity was blocked with 3% H_2O_2 solution in methanol. The primary antibody was localized using the Vectastain ABC-Elite peroxidase detection system (Vector Laboratories, Burlingame, CA, USA). This was followed by reaction with diaminobenzidine (DAB) as chromogen and counterstaining with hematoxylin (Sigma Chemical Co., St. Louis, MO, USA). Sections of each diabetic kidney were processed in parallel with appropriate control tissue.

Urinary excretion of COX metabolites

Within 5 days prior to sacrifice, the 12-week-old ZL and ZDF rats, and 12-week-old wortmannin- or vehicle-treated ZL and ZDF rats underwent timed urine collections in metabolic cages with free access to food and water. The urine was collected in volumetric tubes immersed in a mixture of ice and dry ice. After completion of collections, the urine was immediately stored at −70°C and kept frozen until further analysis. Urinary PGE_2 and its metabolites, and urinary concentrations of TxB_2, a stable metabolite of TxA_2, were analyzed using enzyme immunoassay (EIA) (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Analytical methods

Blood glucose levels were measured by the glucose oxidase assay (Pliva-Lachema, Czech Republic). Serum insulin concentrations were measured using a rat insulin RIA kit (Amersham Biosciences, Piscataway, NJ, USA). Serum triglyceride concentrations were determined by standard enzymatic methods (Pliva-Lachema), and serum nonesterified fatty acids (NEFA) were measured using an acyl-coenzymeA oxidase–based colorimetric kit (Roche Diagnostics, Basel, Switzerland). Plasma and urinary creatinine were measured using commercially available kit (Pliva-Lachema). Urinary protein concentrations were measured spectrophotometrically after reaction with perchloric acid (Pliva-Lachema).

Statistical analysis

Data are expressed as mean ± SEM. All analyses were performed by analysis of variance (ANOVA) followed by the Scheffé test. Differences in responses to treatments between control and diabetic rats were tested by two-way repeated measures ANOVA, using Statview SE and Graphics software (Brainpower, Calabasas, CA, USA). A P value of less than 0.05 was viewed as statistically significant.

RESULTS

General physical, renal, and metabolic parameters in ZL and ZDF rats

General physical and renal parameters in ZL and ZDF rats are summarized in Table 1. ZDF rats demonstrated increased body and renal weight gain, although the kidney/body weight ratio was not different in 4-week-old ZL and ZDF, and even decreased in 12-week-old ZDF as compared to ZL of the same age. There were no differences in creatinine clearance between the 12-week-old ZL and ZDF groups.

However, urinary protein excretion was slightly, but significantly increased in 12-week-old ZDF rats compared with ZL.

Metabolic parameters in 4- and 12-week-old rats are shown in Table 2. ZDF rats demonstrated progressive increases in plasma insulin concentrations in parallel with increased serum triglycercide and NEFA concentrations. Blood glucose levels were slightly but significantly increased in both age groups of ZDF as compared to ZL.

Protein expression of COX isoforms and prostanoid excretion

As shown in Figure 1A, ZDF rats demonstrated increases in renal cortical COX-2 protein expression as compared to ZL rats. The difference in COX-2 expression between the ZL and ZDF groups was more prominent with increasing age. In all groups of rats, immunoreactive COX-2 was localized in typical localizations in macula densa cells, and in the TALH. In ZL, COX-2

Table 2. Metabolic characteristics in lean Zucker (ZL) and fatty diabetic Zucker (ZDF) rats

	Plasma insulin nmol/L	Blood glucose mmol/L	Triglyceride mmol/L	Nonesterified fatty acids mmol/L
	[nmol/L]	[mmol/L]	[mmol/L]	[mmol/L]
ZL 4 weeks	0.40 ± 0.04	4.0 ± 0.1	0.6 ± 0.1	ND
ZDF 4 weeks	0.74 ± 0.07[a]	5.5 ± 0.6b	1.4 ± 0.1[a]	ND
ZL 12 weeks	0.48 ± 0.09	5.3 ± 0.1	0.9 ± 0.1	0.43 ± 0.02
ZDF 12 weeks	1.81 ± 0.19[c]	6.7 ± 0.3‡	4.2 ± 0.4[c]	0.54 ± 0.02[c]

[a] $P < 0.01$; [b] $P < 0.05$; [c] $P < 0.001$ vs. ZL of the same age.

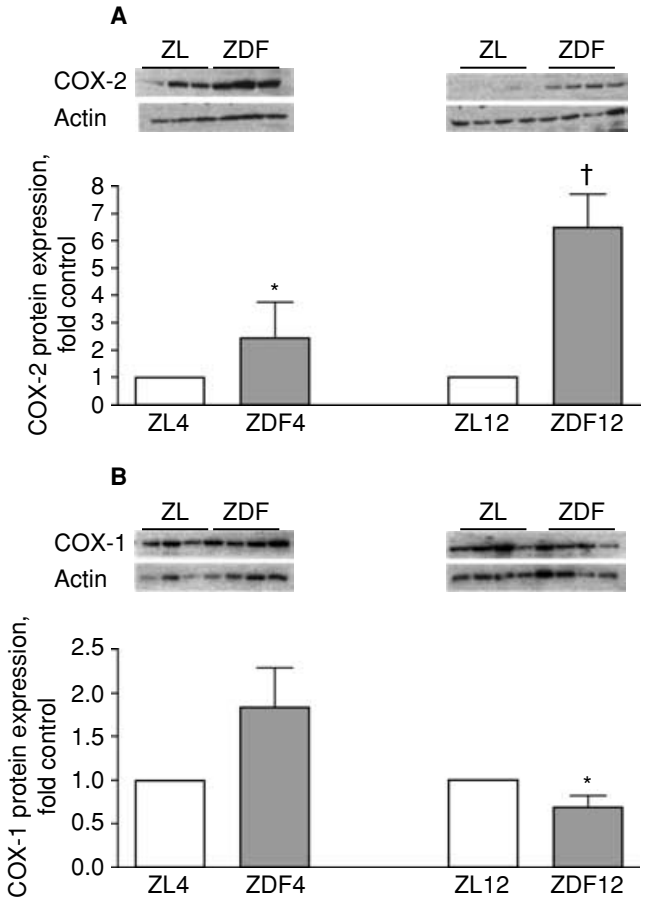

Fig. 1. Renal cortical cyclooxygenase (COX)-2 and COX-1 expression in Zucker lean (ZL) and fatty diabetic rats (ZDF). (*A*) Increased COX-2 expression was apparent already in 4-week-old ZDF rats (ZDF4) and further increased in 12-week-old ZDF rats (ZDF12) as compared to age-matched lean counterparts (ZL4 and ZL12). The upper panels show representative blots. (*B*) There were no significant differences in COX-1 expression between the 4-week-old ZDF and ZL rats. In contrast, COX-1 expression was lower in 12-week-old ZDF rats as compared to age-matched lean counterparts. The insets show representative blots. *$P < 0.05$ vs. ZL; †$P < 0.01$ vs. ZL.

immunoreactivity in both locations was found in occasional cells, whereas ZDF rats demonstrated clusters of COX-2–positive cells (Fig. 2). In contrast to COX-2, there were no significant differences in COX-1 expression between ZL and ZDF rats at age 4 weeks (Fig. 1B). Moreover, in 12-week-old ZDF rats, COX-1 expression was lower than in their age-matched ZL counterparts ($P < 0.05$).

To assess the impact of observed changes in protein expression of renal COX isoforms on production of COX-derived metabolites, further studies determined urinary excretion of PGE_2 and TxB_2, a stable metabolite of TxA_2, in 12-week-old rats. Urinary excretion of both metabolites was increased in ZDF as compared to ZL rats (Fig. 3).

Acute effects of wortmannin on PGE_2 and TxB_2 excretion

Further experiments focused on the possible role of hyperinsulinemia in modulation of COX activity in ZDF rats. Additional groups of 12-week-old ZL and ZDF rats were administered with wortmannin or with vehicle. Wortmannin acts as an inhibitor of PI3K, an important intermediate in insulin signaling [26]. The differences in physical and metabolic parameters between the vehicle-treated ZL and ZDF rats (Table 3) as well as the differences in PGE_2 and TxB_2 excretion (Fig. 4) between the vehicle-treated ZL and ZDF rats were similar as in the previous protocol. Wortmannin had no effect on these parameters in ZL. In contrast, wortmannin-treated ZDF rats demonstrated significantly lower TxB_2 excretion and a similar trend in PGE_2 excretion (Fig. 4), as compared to their vehicle-treated counterparts. These changes were associated with an increase in plasma triglyceride and a decrease in NEFA concentrations (Table 3).

DISCUSSION

In the present studies, ZDF rats, studied as a model of type 2 diabetes, demonstrated a progressive increase in COX-2 expression in the renal cortex as compared to lean controls. In contrast to COX-2, COX-1 expression was lower in 12-week-old ZDF. These changes in expression of COX isoforms in ZDF rats were associated with increased urinary excretion of both PGE and TxB_2, determined as a stable metabolite of TxA_2. The changes in expression and activity of COX isoforms in ZDF rats were associated with metabolic abnormalities characteristic of the insulin-resistant state, increased kidney weight, and mild proteinuria; however, the kidney/body weight ratio

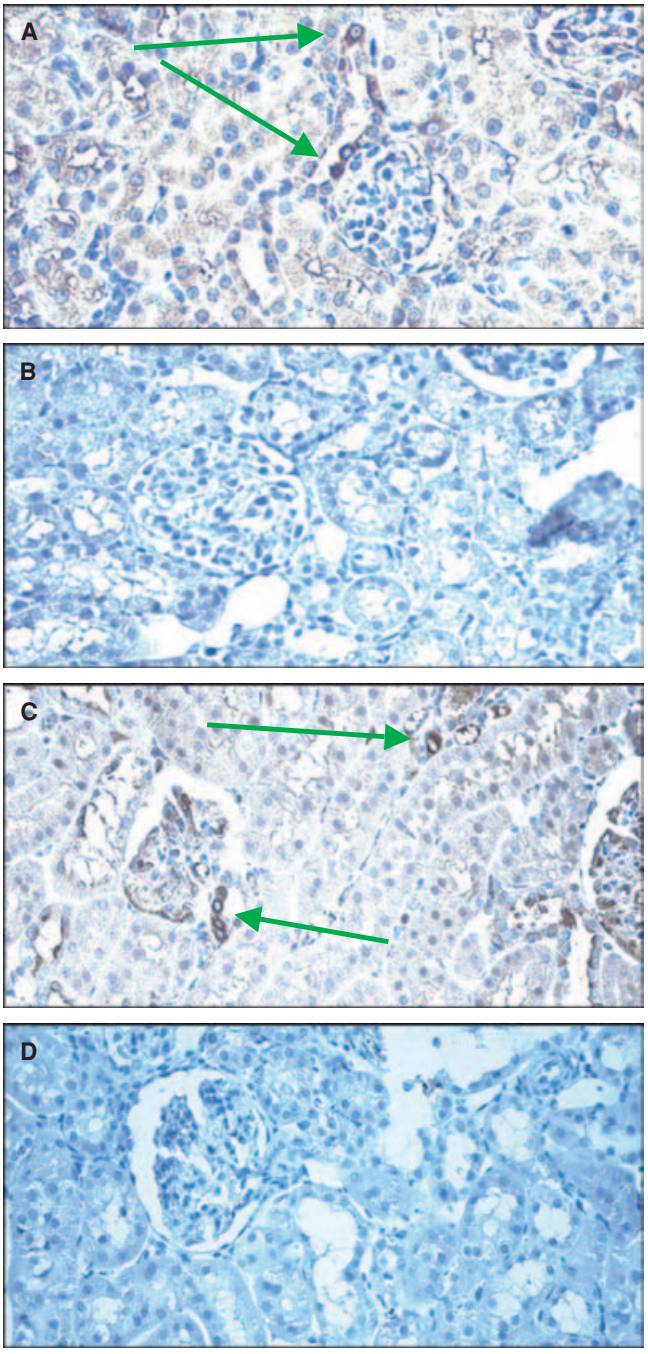

Fig. 2. Renal cortical immunohistochemical localization of cyclooxygenase (COX)-2 in Zucker lean (ZL) (*A*) and fatty diabetic rats (ZDF) (*C*). Representative images in 12-week-old rats are shown. Both in ZL and ZDF rats, COX-2 was localized in the cells of macula densa and in thin ascending limb of Henle (TALH) (arrows, 200×). (*B* and *D*) Control adjacent sections incubated with nonimmune IgG.

in 12-week-old animals was lower than in lean controls due to marked obesity.

Our present findings demonstrate another model of progressive renal injury that is associated with increased cortical COX-2 expression and activity. Considering the documented role of COX-2–derived metabolites in the pathophysiology of progressive renal disease, these observations suggest that COX-2 might be involved in the pathophysiology of renal disease in the ZDF rat. Evolution of nephropathy in ZDF has been well described. Extensive studies by Kasiske et al [23] and Coimbra et al [27] have shown that the first detectable changes in glomerular morphology suggestive of glomerulosclerosis and significant proteinuria occur at about 4 to 5 months of age, whereas more subtle changes characterized by glomerular mesangial matrix expansion and albuminuria occur by 14 weeks of age. Thus, similar to findings in some other models of progressive glomerulosclerosis, such as the rat remnant kidney model [28], fawn-hooded hypertensive rats [29], or type 1 diabetic nephropathy [8, 9], increased renal expression of COX-2 and enhanced generation of prostanoids in ZDF rats precedes the development of glomerulosclerosis. Furthermore, in the present studies it coincided with early development of proteinuria.

An increase in renal cortical COX-2 expression in ZDF rats corresponds to previous reports by our group as well as by other investigators, suggesting marked increases in renal COX-2 expression and function in models of type 1 diabetes [8, 9]. However, there are striking differences between the metabolic environments associated with COX-2 up-regulation in the present and in previous studies. Unlike the studies in type 1 diabetes, changes in COX-2 expression in ZDF were not closely associated with hyperglycemia, since at 12 weeks of age, ZDF rats demonstrated only mild hyperglycemia, and their younger counterparts had blood glucose levels still within the normoglycemic range. Therefore, some other factors must contribute to COX dysregulation in the ZDF. The increase in COX-2 expression, as observed in ZDF rats, paralleled the development of insulin resistance and metabolic abnormalities characterized by progressive obesity, hyperinsulinemia, serum triglyceride, and NEFA concentrations. Therefore, a progressive increase in COX-2 expression is likely linked to the development of the metabolic syndrome.

Hyperinsulinemia is one of the most striking metabolic/hormonal abnormalities in the ZDF. Therefore, we embarked on further studies to explore the role of this factor in the enhanced prostanoid excretion in this model of type 2 diabetes. To inhibit the major insulin signaling pathway, the rats were administered the inhibitor of PI3K, wortmannin. This intervention significantly reduced TxB_2 excretion in ZDF rats, albeit the change in PGE did not reach statistical significance. These findings, together with the lack of effect of wortmannin in ZL rats, suggest that enhanced COX-2 activity in ZDF is in part insulin-dependent. Although PI3K is not activated only by insulin, but by a number of other factors, hyperinsulinemia is such a prominent feature in ZDF rats that effects of wortmannin could be attributable to inhibition of insulin signaling.

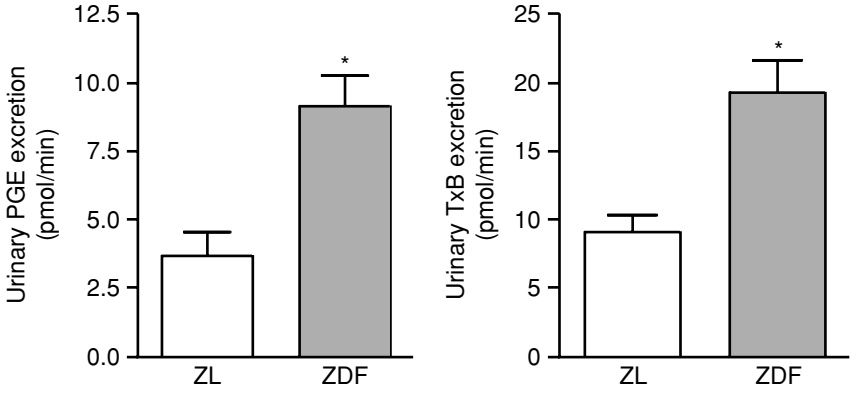

Fig. 3. Urinary excretion of stable metabolites of prostaglandin E (PGE) and thromboxane A_2 (TxA_2) in 12-week-old fatty diabetic Zucker (ZDF) and lean Zucker (ZL) rats as controls. ZDF demonstrated increased excretion of both COX metabolites as compared to ZL. $^*P < 0.05$.

Table 3. Physical and metabolic characteristics in vehicle- and wortmannin-treated lean Zucker (ZL) and fatty diabetic Zucker (ZDF) rats

	Number	Body weight *g*	Right kidney weight *g*	Right kidney weight/100 g body weight	Blood glucose *mmol/L*	Triglyceride *mmol/L*	Nonesterified fatty acids *mmol/L*
ZL vehicle	5	345 ± 11	1.06 ± 0.06	0.31 ± 0.01	4.4 ± 0.1	0.67 ± 0.10	0.30 ± 0.04
ZDF vehicle	5	508 ± 22[a]	1.23 ± 0.04[b]	0.24 ± 0.01[a]	5.8 ± 0.2[a]	2.82 ± 0.12[c]	0.50 ± 0.04[c]
ZL wortmannin	5	355 ± 10	1.05 ± 0.03	0.30 ± 0.01	4.6 ± 0.1	0.59 ± 0.07	0.24 ± 0.04
ZDF wortmannin	5	516 ± 17[a]	1.17 ± 0.05[b]	0.23 ± 0.01[a]	6.1 ± 0.2[a]	4.52 ± 0.47[c]	0.29 ± 0.03[d]

[a] $P < 0.001$ vs. ZL; [b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.01$ vs. ZDF-vehicle.

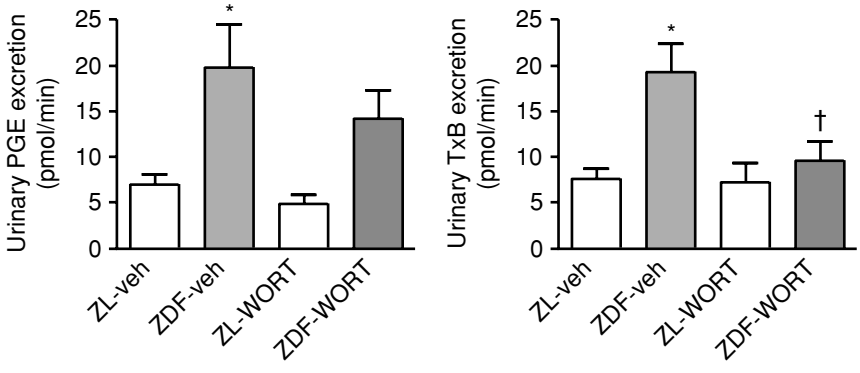

Fig. 4. Urinary excretion of stable metabolites of prostaglandin E (PGE) and thromboxane A_2 (TxA_2) in 12-week-old fatty diabetic Zucker (ZDF) and lean Zucker (ZL) treated with wortmannin or with a vehicle. Vehicle-treated ZDF (ZDF-veh) demonstrated increased excretion of both cyclooxygenase (COX) metabolites as compared to ZL (ZL-veh). Wortmannintreated ZDF (ZDF-WORT) rats demonstrated significantly lower TxB_2 excretion as compared to vehicle-treated counterparts. In contrast to ZDF, wortmannin induced no changes in TxB_2 excretion in ZL (ZL-WORT). $^*P < 0.05$ vs. ZL.

Analysis of metabolic parameters in wortmannin-treated ZDF also showed marked reduction in plasma NEFA concentrations, together with higher triglyceride concentrations. Detailed discussion of this phenomenon is beyond the scope of this paper, but these changes could be related to decreased activity of hormone-sensitive lipoprotein lipase in adipose tissue [30]. Furthermore, these observations could be considered as additional proof of effective PI3K inhibition by wortmannin administered at a given dose. Since NEFA act as precursors for synthesis of arachidonic acid [31], and therefore, as precursors of a spectrum of COX metabolites, the reduction in NEFA levels observed in response to wortmannin administration in ZDF rats could also contribute to attenuation in TxB_2 excretion. Thus, in addition to insulin, enhanced COX-2 activity in ZDF rats could be also attributable to increased NEFA.

Although the experiments assessing the acute effects wortmannin on COX enzymatic activity suggest the link between the hormonal and metabolic characteristics of metabolic syndrome and renal COX dysregulation, it should be noted that this interpretation is based on just a few measurements of urinary prostaglandins and metabolic parameters. Therefore, these data should be viewed as exploratory and supportive. To provide conclusive evidence, future studies should focus on measurements of COX-2 activity and expression, together with indicators of renal injury in models of type 2 diabetes, after long-term modulation of these metabolic/hormonal risk factors.

COX-1 was not the major focus of the present studies. However, our finding of decreased expression of this isoform in 12-week-old ZDF rats may have pathophysiologic significance. Considering the documented

localization of this isoform in collecting ducts [11], it has been hypothesized that COX-1–derived metabolites are involved in natriuresis. For example, pressure natriuretic responses were inhibited by indomethacin, but not by a selective COX-2 inhibitor [32]. Reduction in COX-1 expression in ZDF rats could contribute to later development of hypertension in this model by decreased natriuretic responses.

Lessons derived from studies focusing on renal COX-2 regulation may provide additional clues for determination of factors characteristic both for the metabolic syndrome and for modulation of COX-2 expression in the renal cortex. COX-2 expression and function in macula densa cells is stimulated by decreased sodium chloride concentration in tubular fluid. In particular, chloride ion tubular concentrations seem to be important for COX-2 regulation. Insulin is a potent stimulator of chloride reabsorption up-stream from macula densa [33, 34]. Therefore, progressive hyperinsulinemia in ZDF rats may be associated with changes in ionic content of tubular fluid sensed by macula densa cells leading to up-regulation of COX-2. In this context, Schnyder et al [35] have recently provided persuasive evidence that insulin signaling pathways involved in insulin-induced sodium reabsorption in proximal tubular cells are not affected by insulin resistance.

Previous studies by Vora et al [36] have suggested that a significant proportion of ZDF rats develop hydronephrosis later in the course of nephropathy. This phenomenon may interfere with identification of renal pathophysiologic mechanisms specific for metabolic syndrome. Theoretically, development of hydronephrosis could trigger processes that would lead to COX-2 up-regulation. Our present studies were deliberately performed at early stages of the disease, when both age groups of ZDF do not display hydronephrosis.

CONCLUSION

We report complex changes in renal cortical expression of COX isoforms in ZDF rats as compared to lean controls. These changes were characterized by a progressive increase in COX-2; COX-1 expression was lower in 12-week-old ZDF rats. These changes in expression of COX isoforms in the ZDF rats were associated with increased urinary excretion of both PGE and TxB_2, determined as a stable metabolite of TxA_2. The changes in COX-2 expression and activity paralleled the development of metabolic abnormalities characteristic of insulin resistant states. Further experiments with wortmannin suggested the link between hormonal/metabolic factors characteristic for type 2 diabetes (hyperinsulinemia and increased plasma NEFA concentrations) and COX-2 up-regulation and enhanced prostanoid excretion. With respect to the documented roles of COX in the regulation of kidney function and in renal pathophysiology, the observed alterations in COX expression and function could contribute to the later development of nephropathy in this model of type 2 diabetes. Furthermore, the data provide a rationale for studies of additional metabolic/hormonal mechanisms operating in the pathophysiology of diabetic nephropathy.

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