

Importance of Cyclooxygenase 2-Mediated Low-Grade Inflammation in the Development of Fructose-Induced Insulin Resistance in Rats

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Abstract

This study was designed to examine the role of cyclooxygenase (COX) 2-mediated low-grade inflammation in the development of fructose-induced whole body and muscular insulin resistance in rats. The rats were on regular or fructose-enriched diets for 8 weeks. Fructose-fed rats were further divided into 3 groups (n = 8 per group). There were fructose-fed rats, fructose-fed rats with nimesulide (a selective COX2 inhibitor, 30 mg/kg/day, gavage) and fructose-fed rats with celecoxib (a selective COX2 inhibitor, 30 mg/kg/day, gavage). The present result showed that fructose-induced time-dependent increases in systolic blood pressure and fasting plasma insulin and triglyceride levels were significantly suppressed in rats treated with nimesulide or celecoxib. The ratio of area under glucose curve divided by area under insulin curve obtained during the oral glucose tolerance test was significantly decreased in fructose-fed rats, which were markedly reversed in those co-treated with nimesulide or celecoxib. Accordingly, fructose-induced decrease in insulin-stimulated glucose uptake in soleus muscle was significantly reversed in those combined with nimesulide or celecoxib. Fructose-induced time-dependent increases in plasma 8-isoprostane and PGE metabolites were concomitantly suppressed by nimesulide or celecoxib co-treatment. The present study demonstrates that the COX2-mediated low-grade inflammation, especially mediated by increase in oxidative stress was important in the development of insulin resistance in fructose-fed rats.

Key Words: cyclooxygenase 2-mediated inflammation, oxidative stress, fructose, skeletal muscle, insulin resistance, rats

Introduction

Accumulating evidence has pointed to a correlative and causative relationship between inflammation and insulin resistance/type 2 diabetes (T2DM) (6, 28, 30). Inflammation induces the expression of a variety of

proteins, including prostaglandin-endoperoxide synthase-2 (PTGS2), also known as cyclooxygenase (COX) 2, the key enzyme in eicosanoid metabolism. Previous report has demonstrated the association of a promoter variant in the inducible COX2 gene (PTGS2) with type 2 diabetes mellitus in Pima Indian, indicating that

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COX-mediated inflammation may contribute to the development of T2DM in some individuals (22). Moreover, T2DM in elderly men has been related to COX-mediated inflammation and oxidative stress, reflected by enhanced prostaglandin formation and elevated 8-iso-prostaglandin $F_{2\alpha}$ (13).

High-dose aspirin has been documented to improve glucose metabolism in T2DM (20) and COX2 selective inhibitor such as celecoxib 200 mg daily increased insulin sensitivity in healthy subjects (11). In addition, El Midaoui *et al.* showed that dietary aspirin reduced insulin resistance, vascular superoxide formation and elevated blood pressure in rats on high dietary glucose for 3 weeks (7). Nevertheless, some case reports showed that COX2 inhibitors could induce hypoglycemic episode when overconsumed or taken in combination with oral hypoglycemic drugs (26, 32). These observations show that the changes in COX activity are involved in the regulation of glucose homeostasis under the states of normal and insulin resistance.

High dietary fructose consumption is increasingly being recognized as an important causative factor in the development of metabolic syndrome (29). Recent advances have uncovered some of the physiological and molecular mechanisms such as renin-angiotensin activation, NADPH oxidase and inflammation (29). A better understanding of the dietary and environmental factors that underly the current epidemic of the metabolic syndrome is critical to curbing the rise in the populations of metabolic syndrome and T2DM worldwide. Recent studies continue to highlight the key role played by fructose as an important diabetogenic nutrient, contributing to this growing epidemic (25). Thus, the fructose-induced insulin resistant rat is an appropriate animal model for evaluating the effect of COX2 inhibition on the pathogenesis of metabolic syndrome.

However, the involvement of COX2-mediated low-grade inflammation in the development of insulin resistance in metabolic syndrome and T2DM remains ambiguous (13, 22). Therefore, the present study was undertaken to examine the inhibitory effects of COX2 activation by selective COX2 inhibitors on the development of whole body and muscular insulin resistance in fructose-fed rats, an animal model of metabolic syndrome.

Materials and Methods

Animals

Male Sprague-Dawley rats (5-6 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan. The rats were housed in an animal center in National

Defense Medical Center, Taipei, Taiwan. All animals were handled and housed according to the guidelines and manual set by the Committee of the Care and Use of Laboratory Animals at this institute.

The rats were randomly assigned into 2 groups and fed separately either regular or 60% fructose-enriched diet (TD89247, Teklad Primer Labs, Madison, WI, USA) for 8 weeks. The rats on fructose-diet were further divided into 3 subgroups ($n = 8$ per group) combined gavage with vehicle, nimesulide (30 mg/kg/day, mesulid, a selective COX2 inhibitor, Helsinn Birex Pharmaceuticals Limited, Dublin, Ireland) or celecoxib (30 mg/kg/day, celebrex, a selective COX2 inhibitor, Pfizer Inc, New York, NY, USA) from week 0 to the end of the study. The compressed tablet or capsule content from commercial preparations of mesulid and celebrex were weighed and crushed into a fine suspension with physiological saline (NaCl, 0.9% w/v) based on the weight of active substance quoted per tablet or capsule. These suspensions were then diluted further with saline (10 mg/ml) to give appropriate amounts of the active substance. All suspensions were made immediately before use and were not stored. Control rats were given the same solution without COX2 inhibitor. The selected doses of celecoxib and nimesulid were based on the previous study about anti-inflammatory effect of celecoxib (31) and the selectivity of COX2 of celecoxib and nimesulid (9). In addition, the final effective doses were determined by our preliminary dose-dependent study of celecoxib and nimesulid on fructose-fed induced insulin resistance in rats.

Oral Glucose Tolerance Test (OGTT)

At the end of week 0, 4, and 8, experimental rats were subjected to an OGTT performed without anesthesia after a 24-h fasting period, as described by Whittington *et al.* (35). In brief, the appropriate amount of glucose solution (2 g/ml/kg body weight) was administered by gavage right after baseline blood sample was taken from a tail-vein. Following glucose administration, four more blood samples were collected at 30, 60, 90, and 120 min. The ratio of the area under glucose curve divided by the area of insulin curve attained during the OGTT is used to reflect whole body insulin sensitivity in this study.

Muscle Preparation and Measurement of 2-Deoxy Glucose (2-DG) Uptake into Soleus Muscle

Muscle preparation. The rats were anesthetized by an injection of pentobarbital sodium (100 mg/kg body weight, i.p.), and the soleus muscles were dissected out. Soleus muscles, which are composed primarily of slow twitch (type I red fibers), were split before

incubation. The soleus muscle (red muscle) has more abundant glucose transporter (GLUT) 4 protein expression than white muscle (3). The down-regulated in red skeletal muscle but not white skeletal muscle was also noted in the state of insulin resistance (3). The resulting muscle strips, with tendons still attached at both ends, weighed 15–22 mg. Soleus strips were allowed to recover for 1 h before subsequent incubation.

Measurement of 2-DG uptake. Split soleus muscles were incubated for 30 min at 35°C in a Dubnoff shaking incubator in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% radioimmunoassay-grade bovine serum albumin (BSA) in the presence or absence of purified porcine insulin (2 mU/ml, porcine insulin, Actrapid, Novo Nordisk A/S, Denmark). This concentration of insulin activates glucose transport maximally in our isolated muscle preparations (12).

2-DG uptake was measured by a modification of the method described previously for 3-MG (36). After the rinse, muscles were incubated for various lengths of time in 1.5 ml of KHB containing 1 or 8 mM 2-deoxy-D-[1,2-³H]glucose (2.25 µCi/ml, PerkinElmer Life and Analytical Sciences, Boston, MA, USA), 0.3 µCi/ml [U-¹⁴C] mannitol (PerkinElmer Life and Analytical Sciences, Boston, MA, USA), 2 mM sodium pyruvate, 0.1% BSA, and 2 mU/ml insulin. Osmolarity was kept constant in all experiments by varying the concentration of mannitol such that the sum of the 2-DG, mannitol, and pyruvate equaled 40 mosM. Muscles incubated for > 30 min were first incubated in media identical to that described above but without the radiolabeled mannitol and then transferred to media with [¹⁴C]mannitol for the final 20 min of the incubation. To terminate the assay, muscles were blotted at 4°C, clamp frozen, and stored at -80°C until processed.

Muscles were homogenized in 1 ml 10% trichloroacetic acid solution. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 g. Duplicate 100-µl aliquots of the muscle extract supernatant and of the incubation medium were counted for radioactivity, and the extracellular space and intercellular 2-DG concentration (µmol/ml intracellular water) were determined as previously described (36).

Separation of free and phosphorylated 2-DG. Because 2-DG is a glucose analog that is phosphorylated but not further metabolized in the intracellular tissue space, glucose uptake in individual tissues can be estimated by determining the individual tissue content of 2-[1,2-³H] DG-6-P and plasma 2-[1,2-³H] DG profile, which was fitted with a double exponential or linear curve. The concentration of phosphorylated 2-DG in tissues was calculated as described pre-

viously (24).

Body Weight and Blood Pressure Measurements

At the end of week 0, 2, 4, 6, and 8 in rats with different diet intervention, body weight was measured and systolic blood pressure (SBP) measurement was performed in conscious rats by indirect tail-cuff method (volume-oscillometric method) using a fully automatic blood pressure monitoring system (UR-5000, UEDA, Japan) as described before (17).

Chemical Analysis

Whole blood glucose levels were assayed by the glucose oxidase method with a YSI glucose analyzer (YSI 2300 Plus, Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma triglyceride levels were determined by using appropriate enzymatic colorimetric method (Roche Mira plus, Roche Diagnostic systems, Inc, Basel, Switzerland). Plasma insulin levels were measured by using a commercial rat enzyme immunoassay (EIA) kit provided by Mercodia (Mercodia AB, Uppsala, Sweden). The blood samples for measuring prostaglandin E metabolites (PGEM) and 8-isoprostanes were collected into polypropylene tube containing 0.95 ml EDTA (0.05 M) and 0.05 ml indomethacin (0.04 M) to inhibit platelet generation of prostaglandins *ex vivo* (10) and were analyzed with commercial rat EIA kits (Cayman Chemical, Ann Arbor, MI, USA).

Statistical Analysis

Statistical analysis was performed according to the repeated measurements of one-way analysis of variance (ANOVA) followed by Bonferroni test. A probability of $P < 0.05$ was taken to indicate a statistical difference between means. Values are expressed as means ± SEM.

Results

Changes of Body Weight, SBP and Blood Concentrations during the Time Course Study

As shown in Table 1, body weights were not different among rats fed regular or fructose-enriched diet. However, the body weights in fructose-fed rats treated with nimesulide (F_{M30}) or celecoxib (F_{C30}) for 8 weeks were significantly lower than those without treatment. Fructose feeding significantly increased SBP levels which were significantly suppressed in rats co-treated with nimesulide (F_{M30}) or celecoxib (F_{C30}) throughout the study. Fructose-induced elevation in fasting plasma insulin and triglyceride levels were

Table 1. Changes of body weight (BW), systolic blood pressure (SBP), plasma glucose (PG), insulin and triglyceride during the time course study

Group	Treatment	BW g	SBP mmHg	PG mmol/L	Insulin pg/ml	Triglyceride mg/dl
C	Weeks 0	188 ± 2	132 ± 5	6.0 ± 0.5	0.3 ± 0.1	83 ± 9
	Weeks 2	288 ± 5	132 ± 3	6.5 ± 0.2	0.4 ± 0.1	73 ± 13
	Weeks 4	357 ± 7	137 ± 3	6.1 ± 0.2	0.4 ± 0.0	79 ± 21
	Weeks 6	393 ± 7	137 ± 2	6.8 ± 0.2	0.4 ± 0.1	98 ± 18
	Weeks 8	424 ± 5	140 ± 2	5.8 ± 0.1	0.5 ± 0.1	104 ± 20
F	Weeks 0	203 ± 10	129 ± 1	6.0 ± 0.6	0.2 ± 0.0	96 ± 16
	Weeks 2	300 ± 11	147 ± 3*	7.1 ± 0.3	0.7 ± 0.2*	281 ± 36*
	Weeks 4	354 ± 22	151 ± 3*	6.4 ± 0.1	0.9 ± 0.2*	182 ± 61*
	Weeks 6	411 ± 17	153 ± 6*	6.9 ± 0.3	1.0 ± 0.2*	171 ± 37*
	Weeks 8	469 ± 20	156 ± 5*	6.7 ± 0.2	1.2 ± 0.3*	175 ± 30*
F _{M30}	Weeks 0	183 ± 3	129 ± 2	5.5 ± 0.3	0.2 ± 0.0	68 ± 8
	Weeks 2	283 ± 10	133 ± 2 [#]	5.7 ± 0.3	0.4 ± 0.0	113 ± 14 [#]
	Weeks 4	336 ± 6	139 ± 2 [#]	5.8 ± 0.3	0.3 ± 0.0 [#]	129 ± 25 [#]
	Weeks 6	366 ± 7	142 ± 2	5.5 ± 0.2	0.4 ± 0.2 [#]	128 ± 11 [#]
	Weeks 8	378 ± 7 [#]	143 ± 2 [#]	5.8 ± 0.3	0.3 ± 0.0 [#]	107 ± 10 [#]
F _{C30}	Weeks 0	188 ± 6	132 ± 3	4.5 ± 0.3	0.2 ± 0.0	114 ± 12
	Weeks 2	276 ± 8	132 ± 2 [#]	5.6 ± 0.3	0.4 ± 0.0	82 ± 8 [#]
	Weeks 4	336 ± 9	139 ± 2 [#]	6.0 ± 0.2	0.2 ± 0.0 [#]	99 ± 8 [#]
	Weeks 6	371 ± 10	143 ± 3	5.7 ± 0.2	0.3 ± 0.0 [#]	125 ± 11 [#]
	Weeks 8	386 ± 11 [#]	145 ± 3 [#]	5.7 ± 0.2	0.3 ± 0.0 [#]	108 ± 14 [#]

BW, body weight; SBP, systolic blood pressure; PG, plasma glucose level; C, rats on regular diet for 8 weeks; F, rats on fructose diet for 8 weeks; F_{M30}, fructose-fed rats co-treated with nimesulide, 30 mg/kg/day for 8 weeks; F_{C30}, fructose-fed rats co-treated with celecoxib, 30 mg/kg/day for 8 weeks; n = 8 in each group. **P* < 0.05 vs. C in the corresponding time point. [#]*P* < 0.05 vs. F in the corresponding time point. Data are means ± SEM.

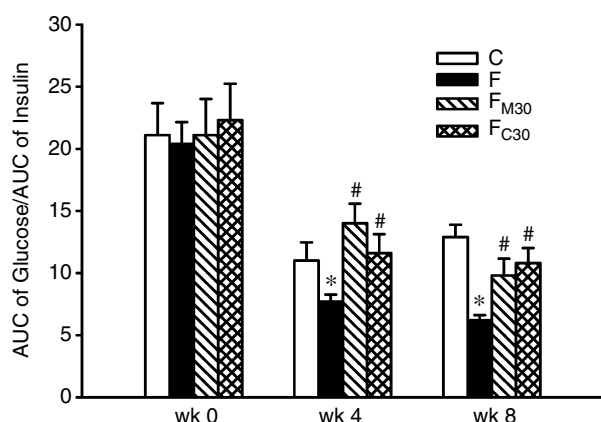


Fig. 1. The ratio of AUC of plasma glucose levels divided by AUC of plasma insulin levels under OGTT. C, control rats; F, fructose-fed rats; F_{M30}, fructose-fed rats co-treated with nimesulide, 30 mg/kg/day from week 0; F_{C30}, fructose-fed rats co-treated with celecoxib, 30 mg/kg/day from week 0; n = 8 in each group; **P* < 0.05 vs. C; [#]*P* < 0.05 vs. F.

significantly suppressed in those treated with nimesulide (F_{M30}) or celecoxib (F_{C30}). Plasma glucose levels were not different among groups.

OGTT

As shown in Fig. 1, after fructose feeding for 4 and 8 weeks, fructose-induced decrease in the ratio of area under glucose curve divided by area under insulin curve obtained during OGTT, an index of insulin sensitivity was significantly reversed in those treated with nimesulide (F_{M30}) or celecoxib (F_{C30}).

Insulin-Stimulated Glucose Uptake in Soleus Muscle

As shown in Fig. 2, the insulin-stimulated glucose uptake in isolated soleus muscle was significantly decreased in fructose-fed rats. Both nimesulide and celecoxib treatments significantly reversed fructose-induced reduction in insulin-stimulated muscular

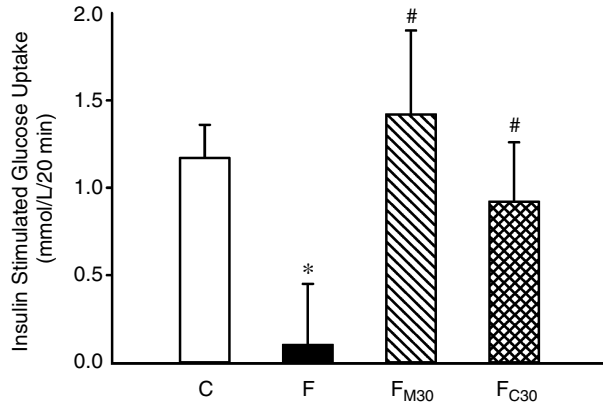


Fig. 2. Insulin-stimulated glucose uptake in isolated soleus muscle in rats at the end of the study. Other abbreviations and statistical notations see Fig. 1. * $P < 0.05$ vs. C; # $P < 0.05$ vs. F.

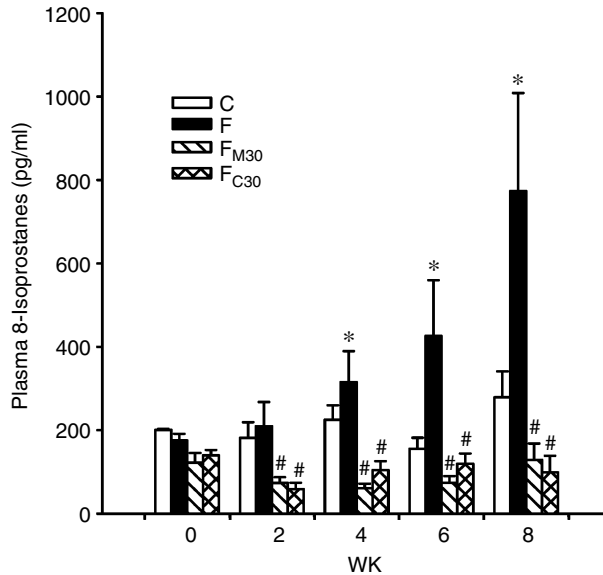


Fig. 3. Plasma 8-isoprostanes during the experimental period in rats. Abbreviations see Fig. 1. $N = 8$ in each group. * $P < 0.05$ vs. F; # $P < 0.05$ vs. F in the corresponding time point.

glucose uptake.

Plasma 8-Isoprostanes and PGE Metabolites

As shown in Fig. 3, plasma 8-isoprostanes were time-dependently increased in rats on fructose enriched diet. Both nimesulide and celecoxib treatments markedly suppressed fructose-induced increase in plasma 8 isoprostanes, the index of oxidative stress.

As shown in Fig. 4, plasma PGE metabolites were mild and gradually increased in fructose-fed rats in time-dependent manner, which were significantly suppressed in fructose-fed rats co-treated with nime-

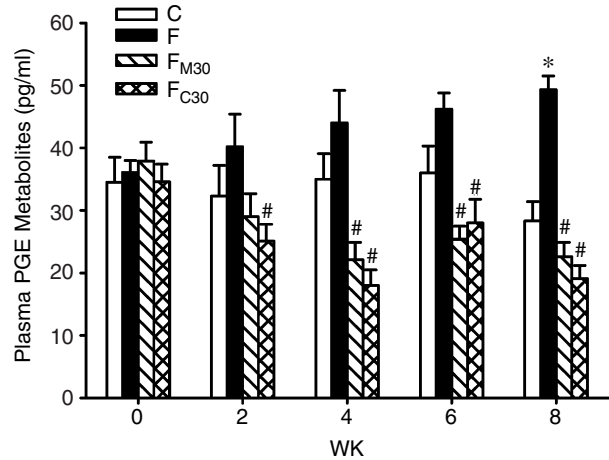


Fig. 4. Plasma PGE metabolites during the experimental period in rats. Abbreviations see Fig. 1. $N = 8$ in each group. * $P < 0.05$ vs. C; # $P < 0.05$ vs. C in the corresponding time point.

sulide (F_{M30}) or celecoxib (F_{C30}).

Discussion

The present results demonstrated that the COX2-mediated inflammation, especially *via* increase in oxidative stress in the development of fructose-induced whole body and muscular insulin resistance were markedly reversed in those co-treated with COX2 inhibitor, either nimesulide or celecoxib. In the meantime, fructose-induced time-dependent increase in plasma 8-isoprostane levels and PGE metabolites were also significantly suppressed in rats co-treated with selective COX2 inhibitor. The present result indicates that COX2-mediated low-grade inflammation might significantly contribute to the development of fructose-induced insulin resistance in rats, an animal model of metabolic syndrome.

COX2-mediated inflammatory responses, particularly production of reactive oxygen species (ROS), have been documented to significantly contribute to the development of hypertension (15, 27) and other cardiovascular diseases in clinical and animal studies (33). However, the role of COX2-mediated oxidative stress in the development of insulin resistance remained unclear. The present result demonstrated that COX2 inhibition significantly suppressed the development of fructose-induced whole body and muscular insulin resistance, implying the importance of COX2-mediated low-grade inflammation in the pathogenesis of insulin resistance in metabolic syndrome and T2DM. In support to our contention, a recent clinical study (13) shows that T2DM in elderly men is related to COX-mediated low-grade inflammation, reflected by enhanced prostaglandin formation. In this study, the oxidative

injury appeared in a later process in the pathogenesis of diabetes, possibly related to inflammation. Furthermore, it has been shown that a metabolite of thromboxane A₂, a COX-mediated product primarily formed in the thrombocytes, is associated with T2DM (4, 5). These observations indicate that COX2-mediated inflammatory response is important in the etiology of the development of insulin resistance in early stage of T2DM.

In addition, fructose-fed rat is an animal model of metabolic syndrome characterized by hyperinsulinemia/insulin resistance, hypertriglyceridemia and hypertension (16, 18). Previous study has demonstrated that COX-downstream prostaglandins, such as prostacyclin and thromboxane metabolites, were significantly increased in rats with fructose feeding for 7 weeks. Chronic thromboxane synthase inhibition prevents fructose-induced hypertension but not insulin resistance, suggested that COX2-mediated inflammatory responses may at least partially contribute to the pathogenesis of fructose-induced insulin resistant syndrome (10). The present study provides evidence to support the COX2-mediated low-grade inflammation and concomitant increase in oxidative stress are crucial for the development of fructose-induced insulin resistant syndrome.

Accumulating evidence suggests that COX2 may be differentially responsible for systemic PGE₂ production and the generation of reactive oxygen species (ROS) (19, 23, 34). The present result showed that co-treatment with nimesulide or celecoxib significantly suppressed fructose-induced increase in oxidative stress indicating by decreasing time-dependent increase in oxidative stress in fructose-fed rats. Oxidative stress has been proposed to causally link to the development of insulin resistance (8), and conversely, increases in prostaglandins of the E series are known to increase the sensitivity of glucose uptake to insulin in isolated, incubated skeletal muscle of the rats (20). Therefore, the anti-oxidative effect of selective COX2-inhibitor seems to play a predominant role in the suppression of fructose-induced whole body and muscular insulin resistance.

Our observation showed that nimesulide and celecoxib both could significantly reverse fructose-induced decrease in insulin-stimulated glucose uptake in soleus muscle. Soleus muscles are slow-twitch muscle fibers which usually account for ~50% or more of total muscle mass in humans and are more insulin responsive than fast-twitch fibers (2, 14, 21). Our result indicates the importance of COX2-mediated low-grade inflammation on muscular insulin resistance in humans.

The present result showed that both nimesulide and celecoxib significantly attenuated fructose-induced increase in BP, implying that the involvement of COX2-mediated inflammatory response in the development

of fructose-induced hypertension. This result is consistent with the above-mentioned study of Galipeau, *et al.* which showed the long-term inhibition of COX-2 downstream thromboxane synthase activation could prevent fructose-induced hypertension (10). Moreover, the short- and long-term COX2 inhibition has been reported to reverse endothelial dysfunction in patients with hypertension. However, it has also been clarified that the deleterious cardiovascular profile of COX2 inhibitors can be accounted for by inhibition of COX-dependent prostaglandin synthesis, especially the prostacyclin production (23). The clinical consequence of COX2 inhibition on the pathogenesis of cardiovascular diseases remains controversial and needs to be further investigated.

The limitation of this study is that the causal relationship between COX2-mediated downstream products such as ROS and PGE₂ and fructose-induced insulin resistance remains elusive in the present study. It needs to be further investigated. The discrepancy of glucose metabolism between human and rats would, at least partially, influence the data.

In conclusion, the present study demonstrates that the COX2-mediated low-grade inflammation, especially through increase in oxidative stress significantly contribute to the development of insulin resistance in fructose-fed rats, suggesting that it may play a pivotal role in the pathogenesis of metabolic syndrome and T2DM in humans.

Acknowledgments

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References

1. Alpert, E., Gruzman, A., Tennenbaum, T. and Sasson, S. Selective cyclooxygenase-2 inhibitors stimulate glucose transport in L6 myotubes in a protein kinase C δ -dependent manner. *Biochem. Pharmacol.* 73: 368-377, 2007.
2. Ariano, M.A., Armstrong, R.B. and Edgerton, V.R. Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* 21: 51-55, 1973.
3. Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M. and Zorzano, A. Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. *Biochem. J.* 282: 765-772, 1992.
4. Davi, G., Catalano, I., Averna, M., Notarbartolo, A., Strano, A., Ciabattini, G. and Patrono, C. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N. Engl. J. Med.* 322: 1769-1774, 1990.
5. Davi, G., Ciabattini, G., Consoli, A., Mezzetti, A., Falco, A., Santarone, S., Pennese, E., Vitacolonna, E., Bucciarelli, T., Costantini, F., Capani, F. and Patrono, C. *In vivo* formation of 8-

- iso-prostaglandin F_{2α} and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 99: 224-229, 1999.
6. Duncan, B.B., Schmidt, M.I., Pankow, J.S., Ballantyne, C.M., Couper, D., Vigo, A., Hoogeveen, R., Folsom, A.R. and Heiss, G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 52: 1799-1805, 2003.
 7. El Midaoui, A., Wu, R. and de Champlain, J. Prevention of hypertension, hyperglycemia and vascular oxidative stress by aspirin treatment in chronically glucose-fed rats. *J. Hypertens.* 20: 1407-1412, 2002.
 8. Eriksson, J.W. Metabolic stress in insulin's target cells leads to ROS accumulation – a hypothetical common pathway causing insulin resistance. *FEBS Lett.* 581: 3734-3742, 2007.
 9. FitzGerald, G.A. and Patrono, C. The coxibs, selective inhibitors of cyclooxygenase-2. *New Eng. J. Med.* 345: 433-442, 2001.
 10. Galipeau, D., Arikawa, E., Sekirov, I. and McNeill, J.H. Chronic thromboxane synthase inhibition prevents fructose-induced hypertension. *Hypertension* 38: 872-876, 2001.
 11. Gonzalez-Ortiz, M., Martinez-Abundis, E., Balcazar-Munoz, B.R. and Robles-Cervantes, J.A. Inhibition of cyclooxygenase-1 or -2 on insulin sensitivity in healthy subjects. *Horm. Metab. Res.* 33: 250-253, 2001.
 12. Hansen, P.A., Gulve, E.A. and Holloszy, J.O. Suitability of 2-deoxyglucose for *in vitro* measurement of glucose transport activity in skeletal muscle. *J. Appl. Physiol.* 76: 979-985, 1994.
 13. Helmersson, J., Vessby, B., Larsson, A. and Basu, S. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. *Circulation* 109: 1729-1734, 2004.
 14. Henriksen, E.J., Bourey, R.E., Rodnick, K.J., Koranyi, L., Permutt, M.A. and Holloszy, J.O. Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am. J. Physiol.* 259: E593-E598, 1990.
 15. Hermann, M., Camici, G., Fratton, A., Hurlimann, D., Tanner, F.C., Hellermann, J.P., Fiedler, M., Thiery, J., Neidhart, M., Gay, R.E., Gay, S., Luscher, T.F. and Ruschitzka, F. Differential effects of selective cyclooxygenase-2 inhibitors on endothelial function in salt-induced hypertension. *Circulation* 108: 2308-2311, 2003.
 16. Hsieh, P.S., Cheng, W.T., Liu, T.T., Loh, C.H. and Chang, B.C. Diminution of hypertriglyceridemia-induced pressor effect under hyperinsulinemic condition in normal and fructose-induced insulin resistant rats. *Chinese J. Physiol.* 50: 99-104, 2007.
 17. Hsieh, P.S. Attenuation of insulin-mediated pressor effect and nitric oxide release in rats with fructose-induced insulin resistance. *Am. J. Hypertens.* 17: 707-711, 2004.
 18. Hsieh, P.S. Reversal of fructose-induced hypertension and insulin resistance by chronic losartan treatment is independent of AT2 receptor activation in rats. *J. Hypertens.* 23: 2209-2217, 2005.
 19. Huang, Y., Liu, J., Wang, L.-Z., Zhang, W.-Y. and Zhu, X.-Z. Neuroprotective effects of cyclooxygenase-2 inhibitor celecoxib against toxicity of LPS-stimulated macrophages toward motor neurons. *Acta Pharmacol. Sin.* 26: 952-958, 2005.
 20. Hundal, R.S., Petersen, K.F., Mayerson, A.B., Randhawa, P.S., Inzucchi, S., Shoelson, S.E. and Shulman, G.I. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J. Clin. Invest.* 109: 1321-1326, 2002.
 21. Kern, M., Wells, J.A., Stephens, J.M., Elton, C.W., Friedman, J.E., Tapscott, E.B., Pekala, P.H. and Dohm, G.L. Insulin responsiveness in skeletal muscle is determined by glucose transporter (Glut4) protein level. *Biochem. J.* 270: 397-400, 1990.
 22. Konheim, Y.L. and Wolford, J.K. Association of a promoter variant in the inducible cyclooxygenase-2 gene (PTGS2) with type 2 diabetes mellitus in Pima Indians. *Hum. Genet.* 113: 377-381, 2003.
 23. McAdam, B.F., Catella-Lawson, F., Mardini, I.A., Kapoor, S., Lawson, J.A. and FitzGerald, G.A. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. *Proc. Natl. Acad. Sci. U.S.A.* 96: 272-277, 1999.
 24. Meszaros, K., Lang, C.H., Bagby, G.J. and Spitzer, J.J. Contribution of different organs to increased glucose consumption after endotoxin administration. *J. Biol. Chem.* 262: 10965-10970, 1987.
 25. Miller, A., Adeli, K., Miller, A. and Adeli, K. Dietary fructose and the metabolic syndrome. *Curr. Opin. Gastroenterol.* 24: 204-209, 2008.
 26. Perucca, E. Drug interactions with nimesulide. *Drugs* 46 Suppl 1: 79-82, 1993.
 27. Pitt, B., Pepine, C. and Willerson, J.T. Cyclooxygenase-2 inhibition and cardiovascular events. *Circulation* 106: 167-169, 2002.
 28. Pradhan, A.D., Manson, J.E., Rifai, N., Buring, J.E. and Ridker, P.M. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. [see comment]. *J. Am. Med. Assoc.* 286: 327-334, 2001.
 29. Rutledge, A.C., Adeli, K., Rutledge, A.C. and Adeli, K. Fructose and the metabolic syndrome: pathophysiology and molecular mechanisms. *Nutr. Rev.* 65: S13-S23, 2007.
 30. Schmidt, M.I. and Duncan, B.B. Diabetes: an inflammatory metabolic condition. *Clin. Chem. Lab. Med.* 41: 1120-1130, 2003.
 31. Smith, C.J., Zhang, Y., Koboldt, C.M., Muhammad, J., Zweifel, B.S., Shaffer, A., Talley, J.J., Masferrer, J.L., Seibert, K. and Isakson, P.C. Pharmacological analysis of cyclooxygenase-1 in inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 95: 13313-13318, 1998.
 32. Sone, H., Takahashi, A. and Yamada, N. Ibuprofen-related hypoglycemia in a patient receiving sulfonylurea. *Ann. Intern. Med.* 134: 344, 2001.
 33. Taddei, S., Virdis, A., Ghiadoni, L., Magagna, A. and Salvetti, A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 97: 2222-2229, 1998.
 34. Whelton, A., Schulman, G., Wallemark, C., Drower, E.J., Isakson, P.C., Verburg, K.M. and Geis, G.S. Effects of celecoxib and naproxen on renal function in the elderly. *Arch. Intern. Med.* 160: 1465-1470, 2000.
 35. Whittington, K.B., Solomon, S.S., Lu, Z.N. and Selawry, H.P. Islet allografts in the cryptorchid testes of spontaneously diabetic BB/Wor dp rats: response to glucose, glipizide, and arginine. *Endocrinology* 128: 2671-2677, 1991.
 36. Young, D.A., Uhl, J.J., Cartee, G.D. and Holloszy, J.O. Activation of glucose transport in muscle by prolonged exposure to insulin. Effects of glucose and insulin concentrations. *J. Biol. Chem.* 261: 16049-16053, 1986.