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ABSTRACT

BACKGROUND: Prostanoids are a family of lipid mediators formed from arachidonic acid by cyclooxygenase enzymes and serve as biomarkers of vascular function. Prostanoid production may be different in males and females indicating that different therapeutic approaches may be required during disease.

OBJECTIVES: We examined sex-dependent differences in COX-related metabolites in genetically modified mice that produce a cyclooxygenase-2 (COX2) enzyme containing a tyrosine 385 to phenylalanine (Y385F) mutation. This mutation renders the COX2 enzyme unable to form a key intermediate radical required for complete arachidonic acid metabolism and provides a model of selective COX2 inhibition.

DESIGN AND METHODS: Mice heterozygous for the Y385F mutation in COX2 were mated to produce cohorts of wild-type, heterozygous, and COX2 mutant mice. We investigated whether the genotype distribution followed Mendelian genetics and studied whether sex-specific differences could be found in certain prostanoid levels measured in peritoneal macrophages and in urinary samples.

RESULTS: The inheritance of the COX2 mutation displayed a significant deviation with respect to Mendel's laws of genetics, with a lower-than-expected progeny of weaned COX2 mutant pups. In macrophages, prostaglandin E₂ (PGE₂) production following lipopolysaccharide (LPS) and interferon gamma (IFN γ) stimulation was COX2-dependent in both males and females, and data indicated that crosstalk between the nitric oxide (NO) and COX2 pathways may be sex specific. We observed significant differences in urinary PGE₂ production by male and female COX2 mutant mice, with the loss of COX2 activity in male mice decreasing their ability to produce urinary PGE₂. Finally, female mice across all 3 genotypes produced similar levels of urinary thromboxane (measured as 11-dehydro TxB₂) at significantly higher levels than males, indicating a sex-related difference that is likely COX1-derived.

CONCLUSIONS: Our findings clearly demonstrate that sex-related differences in COX-derived metabolites can be observed, and that other pathways (such as the NO pathway) are affected.

KEYWORDS: COX2, prostanoids, arachidonic acid metabolism, nitric oxide, sex specific

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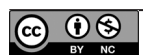
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Introduction

Prostanoids are a subclass of eicosanoids and include the prostaglandins, thromboxanes, and prostacyclins that are formed when the metabolism of arachidonic acid (AA) is initiated by 1 of 2 isozymes, commonly called cyclooxygenase (COX) enzymes, but officially known as prostaglandin-endoperoxide synthase (PTGS) or prostaglandin H₂ synthase (PGHS) enzymes. COX1 is the constitutive form of the enzyme occurring in most cells under normal basal conditions, whereas COX2 is readily induced upon exposure to inflammatory stimuli such as cytokines, bacterial endotoxins, or hormones. The crystal structures of both COX1 and COX2 reveal that both COX enzymes contain a prosthetic Fe^{III}-porphyrin in the peroxidase (POX) active site and a long hydrophobic channel, known as the cyclooxygenase (COX) binding channel, in which AA substrate binds.^{1,2} Both COX1 and COX2 are believed to occur as homodimeric proteins that are localized in the nuclear

membrane and the luminal surface of the endoplasmic reticulum.³ While structures and catalytic functions of COX1 and COX2 share similarities, they assume different physiological roles⁴ with COX1 regulating vascular tone⁵ and COX2 mediating inflammation, tumorigenesis, and atherosclerosis.^{6,7} The AA cascade is initiated by the actions of phospholipases leading to the release of AA from membrane phospholipids. AA, bound in the COX channel of either COX1 or COX2, is converted to a cyclic hydroperoxy endoperoxide, prostaglandin G₂ (PGG₂) and is reduced by the peroxidase (POX) active site to PGH₂.^{3,8,9} As represented in Scheme 1, catalysis is likely initiated by oxidation of the Fe^{III}-porphyrin by a peroxide and requires the involvement of a key tyrosine residue (Tyr 385) that is essential for COX activity.^{10,11} PGH₂ is the precursor to a number of other biologically active molecules. Scheme 1 depicts the downstream metabolites that were the focus of investigation in this study that include PGE₂ and thromboxane



stable metabolite and can serve as an indirect measurement of the systemic biosynthesis of TxA_2 . Full reviews of the diverse families of oxygenated metabolites produced from arachidonic acid, including those that have not been explored here, such as other prostaglandins (PGs), prostacyclin (PGI_2), leukotrienes (LTs), and lipoxins (LXs), can be found elsewhere.^{12,17}

Inflammation is often treated using non-steroidal anti-inflammatory drugs (NSAIDs), most of which are non-selective COX inhibitors, such as aspirin, ibuprofen, indomethacin, and naproxen. However, long-term use of non-selective COX inhibitors in disease states such as rheumatoid arthritis and gout has been associated with gastric damage and peptic ulcer formation.¹⁸ The development of COX2-specific inhibitors, such as Celecoxib (Celebrex) and Rofecoxib (Vioxx), were expected to relieve inflammation while sparing the ability of COX1 to produce prostaglandins that protect the gastric mucosa. Results from clinical trials evaluating COX2-specific inhibitors, however, attracted widespread attention due to an unanticipated incidence of myocardial infarction.¹⁹⁻²¹ As a result, Vioxx was withdrawn voluntarily from the world-wide market by Merck in 2004, and Celebrex now carries a “black-box” warning.²² Studies have revealed that while COX2 is induced by pro-inflammatory stimuli, it is also constitutively expressed in tissues such as the brain, kidney, pancreas, intestine, and blood vessels where it may function to maintain homeostasis.²³

To investigate further the physiologic roles of the COX1 and COX2 isoforms, both COX1- and COX2-null mice were previously developed.^{24,25} Surprisingly, the absence of COX1 was not sufficient to cause spontaneous stomach ulceration indicating that the relationship between COX inhibition and ulceration is complex.²⁴ However, COX1 null mice exhibited a decrease in AA-induced platelet aggregation fitting well with the theory that since platelets express COX1 and not COX2, elimination of COX1 would result in reduced thromboxane A_2 levels and thus, reduce the ability to induce blood clotting. On the other hand, the COX2-null mice were found to (i) possess a normal inflammatory response to treatments with tetradecanoyl phorbol or AA, (ii) show no innate gastrointestinal pathology, (iii) develop severe kidney disease, (iv) and be susceptible to peritonitis.²⁵ It was later shown that approximately 35% of the pups lacking COX2 died due a partially penetrant patent ductus arteriosus phenotype, although all the pups died if they were lacking both COX1 and COX2.²⁶

Thereafter, a mouse model that expresses a targeted knock-in mutation of COX2, in which tyrosine 385 was replaced by phenylalanine (Y385F), was developed.^{13,27} The Y385F mutation results in a COX2 enzyme that is still expressed but has no COX activity, although peroxidase (POX) activity is maintained.¹¹ The COX2 Y385F mutant mouse provides a model of selective COX2 inhibition, in that it mimics the effect of a COX2-selective drug that affects COX activity, without affecting the associated POX function. In this mouse model,

it was demonstrated that lipopolysaccharide (LPS)-stimulation of peritoneal macrophages induced COX2 mutant protein to the same degree as native COX2 protein in wild-type (WT) macrophages, with no compensatory mechanism of COX1 expression.¹³ Although COX2 mutant protein is expressed, COX activity is absent, but POX activity, as measured using *N,N,N,N*-tetramethyl-*p*-phenylenediamine (TMPD), remained intact and was no different to that observed in WT macrophages. A plausible explanation for the near normal neonatal survival of the COX2 Y385F mice compared to the COX2-null mice may be due to the formation of COX1-COX2 heterodimers in the ductus arteriosus, that is, an active catalytic subunit of COX1 combining with a mutant COX2 monomer that allows for remodeling at birth.¹³ An alternative hypothesis to COX1-COX2 dimer formation is that the peroxidase component of COX2, which remains intact in mutant mice, also has an, as yet, unexplored role in ductal homeostasis.¹³

Previously, we have shown that the FeCl_3 -induced injury model can be used to explore the hypothesis that male and female wild-type (WT) mice are not equally susceptible to thrombus formation.²⁸ Our results indicated that thrombosis formation in female WT mice was reduced compared to male WT mice and that this reduction could be correlated with increased induced NO synthase (iNOS) activity in female mice. During these studies, we also observed differential production of urinary prostanoid formation by male and female mice.²⁸

In this present study, we collected urine and elicited peritoneal macrophages that were stimulated with both LPS and interferon gamma ($\text{IFN}\gamma$) and studied whether sex-specific differences in the levels of prostanoids produced from wild-type and genetically modified COX2 Y385F mutant mice could be observed.

Materials and Methods

Mice

The breeding pairs of mice were originally obtained from Queen's University, Canada.¹³ The mice (from a mixed C57BL/6x129/Sv genetic background) contain a Y385F substitution in the enzyme cyclooxygenase 2 (COX2) that is expressed by the *Ptgs2* gene. Mice heterozygous for the Y385F mutation in COX2 ($\text{COX2}^{+/Y385F}$) were bred to obtain a cohort of WT ($\text{COX2}^{+/+}$), COX2 heterozygous ($\text{COX2}^{+/Y385F}$) and COX2 mutant ($\text{COX2}^{Y385F/Y385F}$) mice. The mice were genotyped for the *Ptgs2* allele by PCR analysis using kits (DNeasy® Blood & Tissue and Taq PCR Master Mix kits from Qiagen) and identified by tail tattoo. Primers that are complimentary to portions of exon-9 (EX9 Sense II TTG ACC AGA GCA GAG AGA TG, 20bp), exon-10 (EX10 Antisense II CCA GAT TTG AGG AGA ACA GAT G, 22bp), and neomycin (Neo Sense II GTT CCA CAT ACA CTT CAT TCT C, 22bp) were used. The primers were mixed in equal parts in the

Master Mix cocktail prior to PCR analysis of DNA extracted from murine tail tips. Wild-type (COX2^{+/+}) and COX2 mutant (COX2^{Y385F/Y385F}) mice gave rise to one band each, at ~566 and ~655 bp, respectively, whereas the COX2^{+/Y385F} mice gave rise to both bands by gel electrophoresis. The mice were fed a regular chow diet on an *ad libitum* basis.

Inheritance of the COX2 mutation

The census data that recorded the gender and genotype of the pups from breeding male and female COX2^{+/Y385F} mice over several years were analyzed to determine if the mutation introduced any deviation from Mendelian inheritance. The total numbers of male (193) and female (203) pups produced of each COX2^{+/+}, COX2^{+/Y385F} and COX2^{Y385F/Y385F} genotype were determined and a chi-square (χ^2) test was performed to assess if the observed data were compatible with the Mendelian model of inheritance.²⁹

Macrophage isolation, culture, and treatment

Macrophages were elicited from the intraperitoneal cavity following injection of sterile 4% thioglycollate medium (3 mL; Brewer Modified; Fluka) prepared in deionized water. After 4 to 5 days, the mice were euthanized under CO₂ gas, and the abdominal skin was washed with 70% ethanol. Peeling back the skin exposed the outside of the peritoneum, after which ice-cold phosphate-buffered saline (PBS; 10 mL) was injected into the cavity. The peritoneal cavity fluid was retrieved using a fresh syringe barrel, and the solution was centrifuged (1500 rpm for 5 minutes at 4°C). The supernatant liquid was removed, and the cells were washed in PBS (5 mL) and centrifuged (1500 rpm for 5 minutes at 4°C). The washing procedure was repeated a second time, and following centrifugation, the cells were re-suspended in Dulbecco's Modified Eagle Medium (DMEM; 2-6 mL) supplemented with 10% fetal calf serum (FCS), 1% glutamate, and 1% penicillin/streptomycin. The cells were counted using trypan blue and a hemacytometer, and then diluted appropriately with DMEM (containing supplements) to yield a cell population of approximately 5×10^6 cell/mL. The cells were dispensed into 6-well plates and allowed to adhere for 3 hours at 37°C with 5% CO₂, after which the cell culture medium was replaced with fresh DMEM (containing supplements) and the cells were incubated at 37°C with 5% CO₂ for 24 hours. In some cases, the cells were stimulated with lipopolysaccharide (LPS; from *Escherichia coli* 026:B6, 10 µg/mL; Sigma) and recombinant mouse interferon gamma (IFN γ ; 100 U/mL; Calbiochem) to induce COX2 gene transcription. In other cases, cells were also treated with the non-specific NOS inhibitor L-NMMA (L-N-monomethyl-arginine; 250 µM). After 24 hours, the supernatants were collected and stored at -80°C for later analysis. The cells were washed with PBS (1 mL), lysed with 0.1 N NaOH (500 µL), and the protein concentration determined at a later date by performing a bicinchoninic acid (BCA1; Sigma-Aldrich) assay, according to

manufacturer's instructions. A total of 30 mice were used in this portion of the study (14 female and 16 male mice). The average age of the female and male mice employed was 27.4 ± 1.0 weeks. The average weights of the female and male mice were 21.2 ± 0.5 g and 27.1 ± 0.8 g, respectively.

Urine collection

Mice were placed in metabolic cages and urine was collected over a 24 hours period. The urine was sterile filtered (using syringes capped with a 0.22 µm Millex GP filter unit), aliquoted, stored at -80°C and later analyzed. In this study, urine samples from a total of 70 mice were collected, with the following numbers of mice in the different groups: 13 female and 12 male COX2^{+/+} mice, 12 female and 13 male COX2^{+/Y385F} mice, and 12 female and 8 male COX2^{Y385F/Y385F} mice). The average age of the mice was 34.5 ± 2.5 weeks.

Prostanoid and nitrite/nitrate measurement

Enzyme immunoassay kits (Cayman Chemical) were used to measure PGE₂ and 11-dehydro TxB₂. The 11-dehydro TxB₂ kit measures both 11-dehydro TxB₂ and 11-dehydro-2,3-dinor TxB₂. The 11-dehydro TxB₂ monoclonal assay buffer supplied with the kit is designed to convert 11-dehydro TxB₂ into one conformation for more consistent results. Colorimetric assay kits (Cayman Chemical) were used to measure nitrate/nitrite and urinary creatinine. Urinary eicosanoid levels were normalized to creatinine.

Statistical analysis

Results are calculated as the mean \pm the standard error of the mean (SEM). Significant differences are determined by *t*-test, with $P < 0.05$ defined as statistically significant. In addition, where applicable, the data were subjected to a one-way ANOVA, or a two-way ANOVA (with replication) analysis followed by a Tukey's post-hoc assessment to determine whether differences between groups were statistically significant. The chi-square (χ^2) test was performed using Excel. The analyses were performed using Prism GraphPad (version 4) and Excel (versions 14.7.7 and 16.16.27) software.

Results

Inheritance of the COX2 mutation

An analysis of the census data of weaned pups resulting from breeding male and female COX2^{+/Y385F} mice over several years are provided in Table 1, and demonstrate a significant deviation from expected Mendelian genetics, indicating that the deviation must be due to some cause, and cannot be associated with chance alone.

It is evident that numbers of male COX2^{Y385F/Y385F} pups are especially low compared to female pups, indicating some sex-specificity. In contrast, previous studies noted that COX2^{Y385F/}

Table 1. Sex-specific inheritance of COX2 mutation.

GENOTYPE	TOTAL (MALE & FEMALE MICE) OBSERVED NUMBERS	TOTAL (MALE & FEMALE MICE) EXPECTED NUMBERS*	MALE MICE OBSERVED NUMBERS	MALE MICE EXPECTED NUMBERS*	FEMALE MICE OBSERVED NUMBERS	FEMALE MICE EXPECTED NUMBERS*
COX2 ^{+/+}	111	99	53	48.25	58	50.75
COX2 ^{+/Y385F}	242	198	127	96.5	115	101.5
COX2 ^{Y385F/Y385F}	43	99	13	48.25	30	50.75
<i>P</i> -value	4.81 × 10 ⁻¹⁰	-	1.63 × 10 ⁻⁸	-	3.49 × 10 ⁻³	-

A chi-square analysis of weaned female (203) and male (193) mice produced from breeding male and female COX2^{+/Y385F} mice to determine if the mutation introduced any deviation from Mendelian inheritance.

The expected numbers of mice (*) are based on the Mendelian pattern of inheritance: 25% COX2^{+/+}, 50% COX2^{+/Y385F}, 25% COX2^{Y385F/Y385F}.

Y385F mutant pups were obtained in the normal Mendelian ratio from heterozygous matings.¹³ With complete COX2 gene disruption, COX2 KO mice were also born at the expected Mendelian ratio, although 57% of the neonatal pups died within 48 hours due to failure of closure of the ductus arteriosus after birth.²⁷ Notably, COX2^{Y385F/Y385F} pups did not exhibit this problem, and it was suggested that the heterodimerization of COX1 and COX2 provided a compensatory mechanism to allow for normal closure that is not available to COX2-null mice. However, it was previously noted that the COX2^{Y385F/Y385F} pups frequently died within weeks of birth due to kidney malfunction and peritonitis.^{25,27} In our study, the pups were not disturbed during the first week of birth, and while occasional deaths were noted (and it is possible that some pups were cannibalized), these fatalities did not occur to the extent that would account for the low numbers of COX2^{Y385F/Y385F} pups. It is possible that the normal Mendelian ratio does result, but our observations may be skewed by some post-natal deaths and/or from prenatal lethality causing a significant reduction of COX2^{Y385F/Y385F} pups, which has been observed in other mouse models.³⁰ Since the numbers of COX2^{Y385F/Y385F} pups were low, it was necessary to conduct our research studies over several years in order to be able to study significant numbers of male and female mutant mice of the appropriate age. Further in-depth studies, however, are required to examine the extent of COX2 mutation involvement in prenatal fatality.

PGE₂ produced by macrophages from female and male mice

Macrophages were elicited from male and female COX2^{+/+}, COX2^{+/Y385F}, and COX2^{Y385F/Y385F} mice, and PGE₂ production was measured in the supernatant media after 24 hours under 4 different conditions: (i) control, and in the presence of (ii) L-NMMA, (iii) LPS/IFN γ , and (iv) LPS/IFN γ + L-NMMA (Figure 1). Previous studies have shown that crosstalk between the prostaglandin and NO pathways occurs, and for this reason, we investigated the effect of NO on prostaglandin levels by using the non-specific NOS inhibitor, L-NMMA.³¹⁻³³

As expected, the biggest response in PGE₂ levels was elicited upon LPS/IFN γ stimulation and was dependent on the presence of COX2. The highest PGE₂ levels, in response to LPS/IFN γ stimulation (in the presence and absence of L-NMMA), were produced by macrophages from mice in the following order: COX2^{+/+} > COX2^{+/Y385F} > COX2^{Y385F/Y385F}. In macrophages from COX2^{Y385F/Y385F} mice that are completely deficient in COX2 activity, LPS/IFN γ treatment (in the presence and absence of L-NMMA) failed to induce PGE₂ production above control levels. Thus, the response to LPS/IFN γ stimulation is COX2-specific, as expected. For cells treated with LPS/IFN γ (in the presence and absence of L-NMMA), there were significant differences that showed a significant correlation with both sex and genotype, as measured by 2-way ANOVA. Tukey's post hoc analysis indicated that a comparison of the values of any 2 different genotypes was statistically significant.

It has previously been found that NO production negatively regulates COX2 expression in response to inflammatory stimuli in smooth muscle cells and that iNOS gene deletion exaggerates COX2 induction.³¹ Thus, inhibition of NO synthase was found to upregulate LPS/IFN γ -mediated COX2 protein expression, although specific sex-dependent effects were not investigated.³¹ Our results indicated that the presence of L-NMMA significantly magnified LPS/IFN γ -mediated COX2 activity, but only in macrophages from female COX2^{+/+} and COX2^{+/Y385F} mice. Conversely, in macrophages from male COX2^{+/+} mice, we noted a significant decrease in PGE₂ production. These results indicate that the NO pathway does play a role in influencing PGE₂ levels, and that the effect appears to be sex dependent. Within a particular genotype, there were small differences between male and female responses within the same treatment group that were statistically significant in some cases, as determined by *t*-test and indicated in Figure 1.

Basal levels of PGE₂ production in control female mice across all 3 genotypes were similar and not significantly different. In contrast, basal PGE₂ production in control male mice across all 3 genotypes showed small differences that were statistically significant. PGE₂ production by macrophages from male

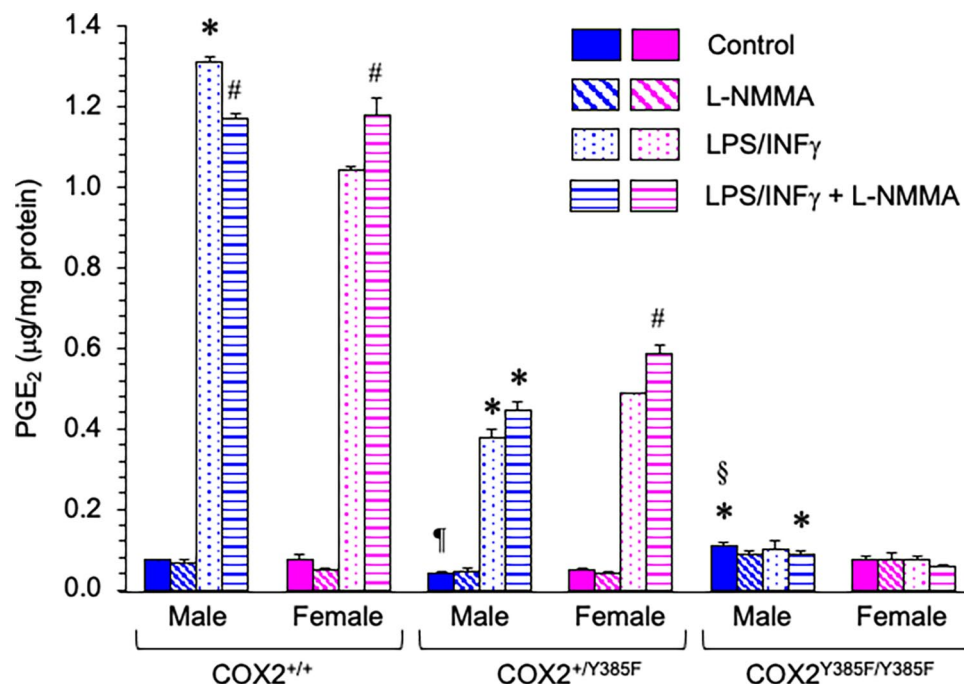


Figure 1. PGE₂ production by macrophages, elicited from male and female COX2^{+/+}, COX2^{+/Y385F}, and COX2^{Y385F/Y385F} mice under control conditions (solid) and in the presence of L-NMMA (diagonal lines), LPS/IFN γ (dots), and LPS/IFN γ + L-NMMA (horizontal lines). For each group, n=4-6 mice; *P < 0.05 compared to the female cohort of the same genotype in the same treatment group. #P < 0.05 following LPS/IFN γ + L-NMMA treatment compared to LPS/IFN γ treatment for the same genotype and sex. §P < 0.05 compared to male control COX2^{+/+} and COX2^{+/Y385F} mice. ¶P < 0.05 compared to male COX2^{+/+} mice.

COX2^{Y385F/Y385F} control mice was significantly higher than from male COX2^{+/+} and male COX2^{+/Y385F} mice. It is not clear whether the loss of complete function of COX2 in macrophages from male mice induces a compensatory mechanism whereby COX1 contributes to increased PGE₂ activity. Such a determination was beyond the scope of the current investigation.

The results indicate that PGE₂ produced above basal levels following LPS/IFN γ stimulation is COX2-derived, and that sex may also play a role in the levels observed. Since microsomal prostaglandin E synthase-1 (mPGES-1) plays a role in converting PGH₂ to PGE₂ in macrophages (Scheme 1), future studies to investigate the sex-dependence of this enzyme in macrophages would be useful.^{34,35} The results also demonstrate that inhibition of NOS activity by L-NMMA, may induce a sex-dependent response with regard to PGE₂ production, although further investigation is warranted.

Nitrite/nitrate produced by macrophages from female and male mice

Nitrite and nitrate levels, produced by macrophages elicited from male and female COX2^{+/+}, COX2^{+/Y385F}, and COX2^{Y385F/Y385F} mice, were measured in the supernatant media after 24 hours, under 4 different conditions: (i) control, and in the presence of (ii) L-NMMA, (iii) LPS/IFN γ , and (iv) LPS/IFN γ + L-NMMA.

Treating macrophage cells with LPS/IFN γ increased nitrite/nitrate levels in a sex- and genotype-dependent way, as measured by 2-way ANOVA (Figure 2). Tukey's post hoc

analysis indicated that in response to LPS/IFN γ stimulation, there were significant differences between genotypes. Interestingly, macrophages derived from both male and female COX2^{Y385F/Y385F} mice displayed a decreased ability to produce nitrite/nitrate following LPS/IFN γ stimulation, compared to COX2^{+/+} and COX2^{+/Y385F} mice. By *t*-test, the biggest statistical difference in sex-related nitrite/nitrate production by macrophages in response to LPS/IFN γ stimulation was observed between male and female COX2^{+/+} mice, with males producing a greater amount. The effect of LPS/IFN γ stimulation was abrogated by the co-addition of the non-specific NOS inhibitor, L-NMMA.

As shown in Figure 2, there were no statistically significant differences between nitrite/nitrate amounts measured in control macrophages and macrophages treated with L-NMMA or LPS/IFN γ + L-NMMA from female and male mice across all 3 genotypes.

Overall, the results indicate that LPS/IFN γ -induced NO production is COX2-dependent, with a loss of COX2 leading to impaired LPS/IFN γ -induced NO production. Furthermore, LPS/IFN γ -induced nitrite/nitrate production was sex-dependent, with macrophages from male COX2^{+/+} mice displaying significantly higher levels than their female cohorts.

Urinary PGE₂ produced by female and male mice

The COX2 Y385F mutant mouse provides a model of selective COX2 inhibition, and thus provides a way for us to examine the contribution of constitutive COX2 to urinary levels of

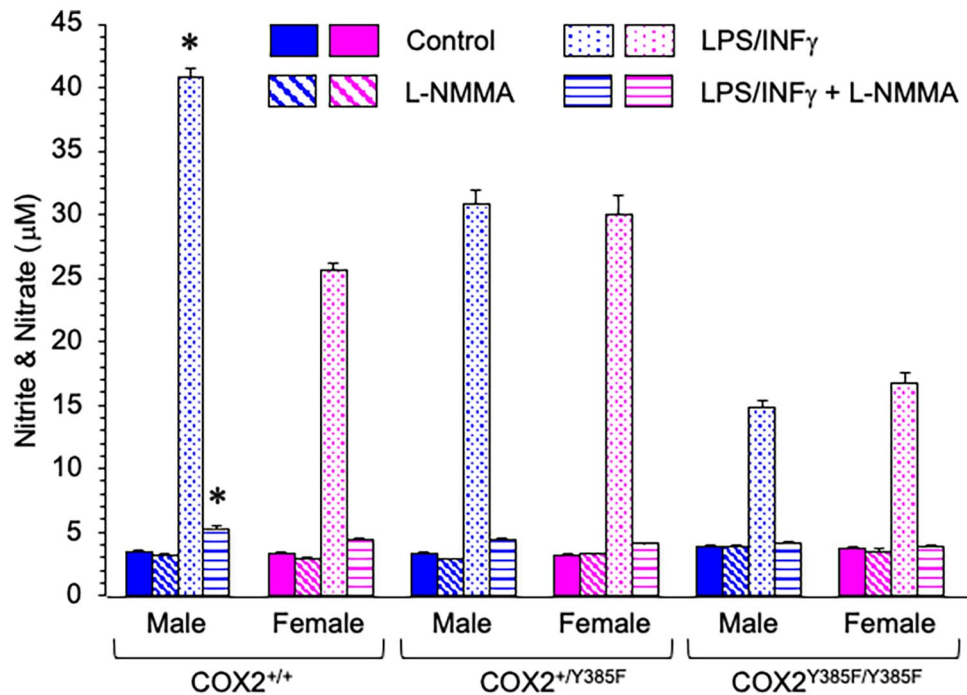


Figure 2. NO production by macrophages, measured as total nitrite (NO₂⁻) and nitrate (NO₃⁻), elicited from male and female COX2^{+/+}, COX2^{+/^{Y385F}} and COX2^{Y385F/Y385F} mice under control conditions (solid) and in the presence of L-NMMA (diagonal lines), LPS/IFN γ (dots), and LPS/IFN γ + L-NMMA (horizontal lines).

For each group, n=4-6 mice; *P < 0.05 compared to the female cohort of the same genotype in the same treatment group.

prostanoids under normal physiological conditions. Urinary PGE₂ levels were measured for male and female COX2^{+/+}, COX2^{+/^{Y385F}}, and COX2^{Y385F/Y385F} mice, and normalized to creatinine. As shown in Figure 3, slightly higher levels of urinary PGE₂ were measured for female mice in all genotypes compared to male mice, but the sex-dependent differences only reached significance for urinary PGE₂ obtained from male and female COX2^{Y385F/Y385F} mice.

A one-way ANOVA analysis for urinary PGE₂ obtained from male mice across the 3 genotypes revealed that PGE₂ levels are COX2-dependent, with a loss of COX2 activity resulting in a decreased ability to produce PGE₂. Female urinary PGE₂ levels remained indistinguishable across all 3 genotypes, and it thus, appears that this pathway under normal physiological conditions is COX2-independent for female mice. Further studies examining urinary PGE₂ levels under conditions of inflammation, when COX2 expression is induced, are warranted.

Urinary 11-dehydro TxB₂ produced by female and male mice

11-Dehydro-TxB₂ is a proven reliable biomarker of in vivo synthesis of TxA₂.^{36,37} Urinary 11-dehydro TxB₂ levels were measured for male and female COX2^{+/+}, COX2^{+/^{Y385F}} and COX2^{Y385F/Y385F} mice, and normalized to creatinine. As shown in Figure 4, female mice across all the 3 genotypes measured consistently and significantly higher levels of urinary 11-dehydro TxB₂ than the male cohorts. The COX2^{+/+}, COX2^{+/^{Y385F}}

and COX2^{Y385F/Y385F} genotype did not significantly affect the levels of urinary 11-dehydro TxB₂ measured for either male or female mice, indicating that COX2 does not affect the sex-dependent difference observed. Thus, the results suggest that the sex-related difference in urinary 11-dehydro TxB₂ production is COX1-derived rather than being COX2-dependent. It would be interesting to explore whether urinary 11-dehydro TxB₂ levels are altered under inflammatory conditions, when COX2 expression is induced.

Discussion

Prostanoids are oxygenated metabolites of arachidonic acid that play important roles in a large variety of physiological functions that include being mediators of suppressors and activators of inflammation. Since inflammation is implicated in many disease states, the identification of altered levels of prostanoids can serve as biomarkers in the progression of certain pathogenic processes.³⁷ Examples of diseases that involve inflammation and have been correlated with changes in eicosanoid or prostanoid levels include atherosclerosis,³⁸ cancer,³⁹ psoriatic arthritis,⁴⁰ asthma,⁴¹ chronic obstructive pulmonary disease (COPD),⁴² and neurodegenerative disorders such as amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases.^{43,44}

Tracking eicosanoid and prostanoid levels and correlating their levels to specific disease states can provide targets for the development of improved therapeutic protocols, but efficient treatment can be made complicated by the fact that there might be sex-related differences associated with eicosanoid

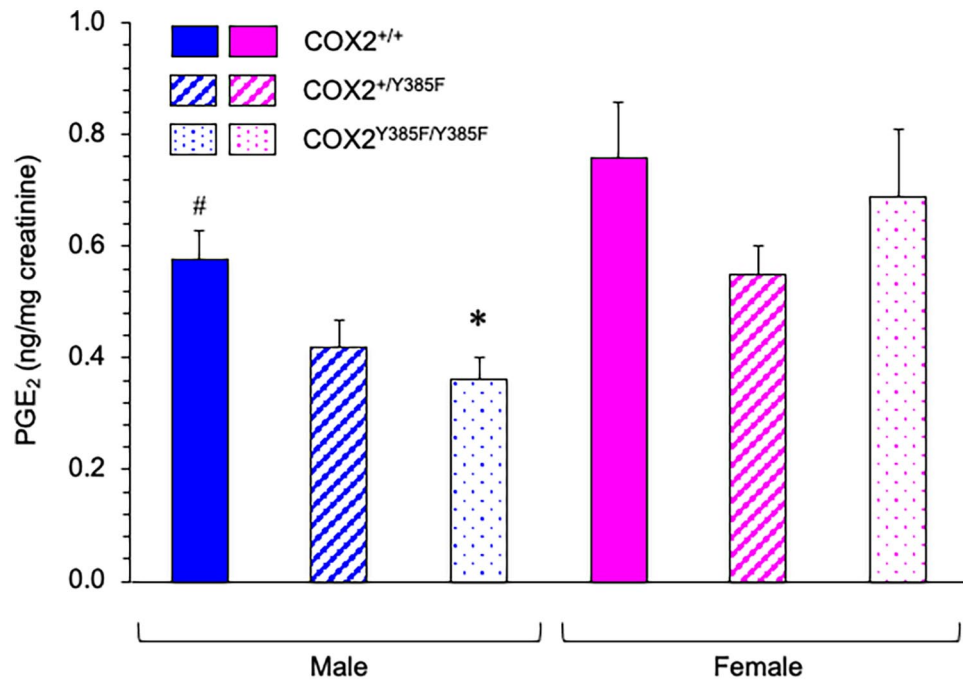


Figure 3. Urinary levels of PGE₂ were measured for male and female COX2^{+/+} (solid bar), COX2^{+/Y385F} (diagonal lines) and COX2^{Y385F/Y385F} (dots) mice and normalized to creatinine. For each group, n=8-12 mice; **P* < 0.05 compared to females of the same genotype. #*P* < 0.05 compared to male COX2^{+/Y385F} and COX2^{Y385F/Y385F} mice.

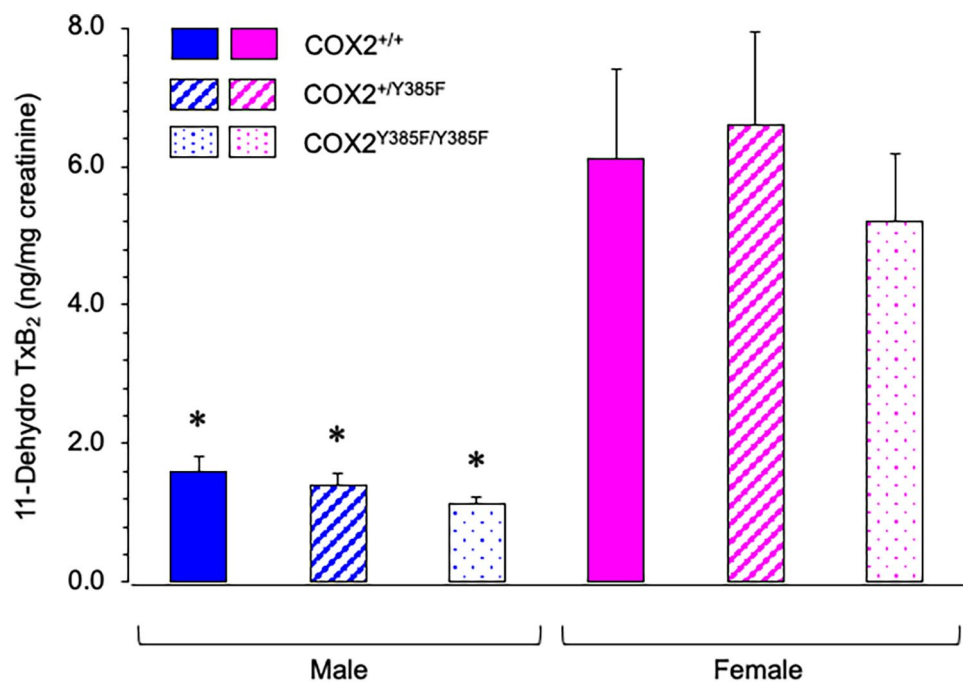


Figure 4. Urinary levels of 11-dehydro TxB₂ were measured for male and female COX2^{+/+} (solid), COX2^{+/Y385F} (diagonal lines) and COX2^{Y385F/Y385F} (dots) mice and normalized to creatinine. For each group, n=8-12 mice; **P* < 0.05 compared to females of the same genotype.

production.⁴⁵ Historically, sex and gender have been neglected in biochemical and pre-clinical research.^{46,47} For instance, while many chronic autoimmune diseases have been found to affect women to a greater extent than men, a survey of journal articles in this particular field from 2009 found that there was a strong bias toward the use of male animals.^{46,48} Furthermore,

it has been noted that in studies examining the role of different cell types in the complex progression of certain disease states, such as atherosclerosis, many studies, if not all, do not report the sex of the cells employed.⁴⁹ Thus, research should not only consider the sex of the animals (or human subjects) but also whether isolated cells are derived from males or females. The

sex-dependent differences in eicosanoid levels that have been observed in disease states indicate that males and females may require different therapeutic approaches.^{50,51} Furthermore, it is also important to consider that drugs may be metabolized differently by males and females with different sex-dependent side effects.^{46,52} A review of the sex differences in the pathophysiology and pharmacotherapy of inflammatory diseases related to eicosanoids has recently been reported.⁴⁵

Previous studies involving human neutrophils demonstrated that males produce higher levels of PGE₂ than females, indicating a sex-dependence that has consequences for the inflammatory response.⁵³ In contrast, in a separate study, peritoneal macrophages elicited from adult female rats released, in a dose-dependent manner, significantly more PGE₂ than macrophages from the male, upon stimulation, indicating gender differences in immune responses.⁵⁴ Herein, we demonstrate that LPS/IFN γ -stimulated macrophages from male and female mice produce different levels of PGE₂ that are COX2-dependent (Figure 1). Small differences between males and females within similar treatment groups for a particular genotype also indicated that the response is sex dependent.

Inhibition of NOS with L-NMMA significantly enhanced PGE₂ release in LPS/IFN γ -stimulated macrophages (compared to LPS/IFN γ -stimulated macrophages), but only in female COX2^{+/+} and COX2^{+/^{Y385F}} mice (Figure 1). It has previously been found that NO production negatively regulates COX2 expression in response to inflammatory stimuli in smooth muscle cells.³¹ Thus, inhibition of NO synthase was found to enhance LPS/IFN γ -mediated COX2 activity, although specific sex-dependent effects were not investigated.³¹ Furthermore, in our study, there was some indication that a loss of COX2 activity in male COX2^{Y385F/Y385F} control mice led to the production of slightly higher PGE₂ levels compared to COX2^{+/+} and COX2^{+/^{Y385F}} control mice, indicating that COX1 may be contributing to a greater extent in this case. In addition, it would be interesting to examine COX1, COX2, and NOS expression in macrophages obtained from male and female mice across all 3 genotypes in response to inflammatory stimuli.

It has previously been found that LPS increases both PGE₂ and NO production in macrophages, and that the release of both can be attenuated by pre-treatment with the COX2 selective inhibitor, celecoxib.⁵⁵ In our study, we also demonstrated that NO production by LPS/IFN γ -stimulated macrophages is COX2-dependent, as loss of COX2 impaired NO production by macrophages obtained from both male and female mice (Figure 2). While WT male macrophages produced a greater amount of NO than WT female macrophages upon LPS/IFN γ -stimulation, this sex-dependence was not observed in macrophages from male and female mice lacking COX2 functionality.

Urinary PGE₂ is mainly derived from the conversion of AA to PGH₂ (Scheme 1) by either COX1 or COX2 in the kidney. While COX2 is traditionally regarded as an inducible enzyme,

it is constitutively expressed at specific sites in the kidney. PGH₂ to PGE₂ conversion is catalyzed by different PGE synthases, of which cytosolic PGE synthase (cPGES) and microsomal PGE synthases type 1 (mPGES-1) occur in the kidney.⁵⁶ cPGES is believed to couple with COX1, whereas mPGES-1 is associated with COX2 and is upregulated together with COX2 in response to various pro-inflammatory stimuli.^{57,58} Prostanoid production in the kidney is important in the physiological control of vascular tone, renin release and blood pressure, and there is evidence that COX2-derived prostanoids regulate hemodynamics, as well as sodium and water reabsorption.⁵⁹ In contrast, elevated levels of COX2 and mPGE-1 in the kidney are associated with renal disease and cardiovascular risk, including hypertension and type 2 diabetes.⁶⁰ Thus a delicate balance of prostanoid production is required for healthy renal function, which is further made complicated by the existence of a sexual dimorphism in renal prostanoid production.⁴⁵ Deletion of mPGES-1 has been shown to reduce pain sensitivity,^{61,62} retard atherogenesis,⁶³ and fail to accelerate thrombogenesis, while suppressing PGE₂ production and redirecting prostaglandin precursors to prostacyclin (PGI₂) formation.⁶⁴ The results from such studies have prompted interest in developing drug targets of mPGES-1.⁶⁵

Our results (Figure 3) indicated that slightly higher levels of urinary PGE₂ were measured for females, but values only became significantly different between male and female COX2^{Y385F/Y385F} mice. While genotype did not appear to affect PGE₂ production in female mice, there were significant differences observed for male mice, indicating a dependence on COX2. A loss of COX2 activity in male mice significantly decreased their ability to synthesize urinary PGE₂. A similar trend in female animals producing more PGE₂ than their male counterparts has also been observed in other studies.^{28,45,66,67} In spontaneously hypertensive rats (SHR), urinary excretion of PGE₂ metabolites in female rats was higher than in males, indicating the presence of a sex-related difference in the renal prostanoid system.⁶⁶ Lower PGE₂ expression in males was correlated with lower expression of both mPGES-1 and COX2 in the renal inner medulla and outer medulla, respectively. Further studies demonstrated that PGE₂ production is testosterone-sensitive, rather than estrogen-regulated, as following orchietomy, PGE₂ metabolite excretion and mPGES-1 expression were both elevated in male rats.⁶⁶ Sullivan et al noted that since hypertensive males have a lower capacity than females to produce PGE₂, selective COX2 inhibition would further cause a sex-dependent loss of PGE₂ that could potentially cause severe kidney injury in males.⁶⁶ Our results (Figure 3) corroborate the finding that urinary PGE₂ production in males is COX2-dependent.

Interestingly, it was previously demonstrated that a urinary PGE₂ marker, known as tetranor-PGEM (11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid) was depressed in both male and female COX2^{Y385F} mice compared to wild-type mice but, in contrast to our results, significantly higher levels were

produced by males compared to females.⁶⁴ Tetranor-PGEM production was also suppressed in mice missing mPGES-1 and in low-density lipoprotein receptor knockout mice, but its production was again markedly higher in males than in females.^{64,65} Furthermore, an earlier study conducted with human subjects also found that males produced higher levels of urinary tetranor-PGEM than females, although this difference was diminished in children and in an older (45-80 years) age group, implying that other factors may contribute to its availability.⁶⁸ Previous literature indicates that tetranor-PGEM is a major metabolite of both PGE₁ and PGE₂, with PGE₁ arising from COX metabolism of dihomo- γ -linolenic acid, although the sex-dependence of its production is unclear.^{64,68} While the reason for the divergent results are not fully known, the different observations may be a consequence of analyzing a metabolite of both PGE₂ and PGE₁ (ie, tetranor-PGEM) in these previous studies^{64,65} rather than analyzing PGE₂ (as measured by enzyme-linked immunosorbent assay) in our present study, which is widely used elsewhere for determining biological levels of PGE₂.^{28,66,69} Furthermore, as remarked previously, our results concur with previous studies indicating that female animals produce more PGE₂ than their male counterparts.^{28,45,66,67} The contrasting results, however, clearly demonstrate that further work is necessary, not just in delineating the factors that contribute to the sex-dependence of prostanoid production, but also in establishing the best ways to analyze these markers.

In other studies using mice that are prone to immune-mediated nephritis (DBA/1 mice), female mice were found to be superior in producing higher urinary prostanoid levels as compared to their male counterparts, and this increase was correlated with higher COX2 and mPGES-1 mRNA expression in the kidneys of female mice.⁶⁷ Female mice also showed a greater abundance in COX1 mRNA expression compared to male mice, but this trend did not reach statistical significance. mPGES-1 was found to be partially involved in urinary PGE₂ production, since depletion of the *mPges1* gene resulted in an approximate 50% reduction in urinary PGE₂ in both sexes, indicating that other pathways also lead to PGE₂ synthesis. These results further highlight that there are significant sex-related differences in prostanoid formation that would need to be carefully considered when offering therapeutic approaches to the treatment of inflammation and pain.

Herein, we also demonstrated that female mice produced higher levels of urinary 11-dehydro-TxB₂ than males across all genotypes (Figure 4). Similar levels were observed for females across all genotypes and thus, did not appear to be COX2-dependent. While a sex-dependent difference in urinary 11-dehydro-TxB₂ production is apparent, this effect is not driven by COX2, but is likely COX1-derived. This finding is in accord with previous results demonstrating that COX1 contributes significantly to urinary thromboxane levels (measured as

2,3-dinor-TXB₂ metabolite) and that higher levels are found in female mice, indicating the presence of a sex-related difference in the renal prostanoid system.^{64,65} Urinary excretion of TxB₂ has also been noted to be higher for female versus male spontaneously hypertensive rats (SHR).⁶⁶ However, systemic TxB₂ did not reach statistical significance between males and females in SHR.⁶⁶ Interestingly, TxB₂ excretion was not affected by gonadectomy in either male or female SHR, indicating that sex-related hormones do not contribute to its levels.⁶⁶ While higher levels of renal TxA₂ might be expected to have a larger impact on blood pressure in females, it has been suggested that the concomitant increase in PGE₂ in females may work to offset any impact of increased TxA₂ on blood pressure, since female SHR have been shown to have a slower progression of renal injury compared with males.⁶⁶ However, the reason for and consequences of greater renal TxA₂ production in female SHR remain unknown.⁶⁶ A recent study, however, found that sex differences play a role in the factors responsible for the regulation of vascular tone and contraction of coronary arteries in male and female pigs, and explain a role for TxA₂ in females: TxA₂ in perivascular adipose tissue was found to mediate contraction in females, whereas males experienced greater sensitivity to PGF₂ α .⁷⁰ It is not yet known to what extent these sex differences are maintained under pathological conditions, such as obesity, although obesity is known to enhance the contractile responses of perivascular adipose tissue.

Conclusions

Herein, we demonstrate that the COX2 Y385F mutant mouse provides a model of selective COX2 inhibition and can be used to examine sex-dependent differences that occur in the production of COX-related metabolites. Furthermore, this model can be used to examine the effects of COX2 inhibition on other pathways, such as the NO pathway. The findings reported herein clearly demonstrate that sex-related differences in COX-related metabolites can be observed, but further studies unraveling the mechanisms and the impact of these differences need to be performed. Our findings also highlight the need for future investigations examining prostanoid biology to consider sex as a variable not just in animal models, but also at the cellular level, such that it is possible to develop safe and efficient therapies in the medical treatment of both males and females.

Declarations

Ethics approval and consent to participate

The animal protocol used in these studies was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Pace University.

Consent for publication

Not Applicable. This study did not involve human participants.

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Author contributions

Conceptualization – RKU; Data Curation – RKU, DMR, RSB, KDJ, SS, EM; Formal Analysis – RKU, RSB, KDJ, SS, EM; Funding Acquisition – RKU, RSB, KDJ, SS; Investigation – All authors; Methodology – RKU, WLB, DMR, SS, EM; Project Administration – RKU, WLB, DMR, EM; Resources – RKU; Supervision – RKU, DMR, EM; Validation – RKU; Visualization – RKU; Writing – original draft – RKU; Writing – review & editing – All authors.

Availability of data and materials

Not applicable.

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REFERENCES

- Picot D, Loll PJ, Garavito RM. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature*. 1994;367:243-249.
- Luong C, Miller A, Barnett J, Chow J, Ramesha C, Browner MF. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol*. 1996;3:927-933.
- Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem*. 2000;69:145-182.
- Michael Garavito R, Malkowski MG, DeWitt DL. The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins Other Lipid Mediat*. 2002;68-69:129-152.
- Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in ApcA716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell*. 1996;87:803-809.
- Baker CS, Hall RJ, Evans TJ, et al. Cyclooxygenase-2 is widely expressed in atherosclerotic lesions affecting native and transplanted human coronary arteries and colocalizes with inducible nitric oxide synthase and nitrotyrosine particularly in macrophages. *Arterioscler Thromb Vasc Biol*. 1999;19:646-655.
- Schönbeck U, Sukhova GK, Graber P, Coulter S, Libby P. Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions. *Am J Pathol*. 1999;155:1281-1291.
- Dietz R, Nastainczyk W, Ruf HH. Higher oxidation states of prostaglandin H synthase. Rapid electronic spectroscopy detected two spectral intermediates during the peroxidase reaction with prostaglandin G2. *Eur J Biochem*. 1988;171:321-328.
- Tsai AL, Palmer G, Kulmacz RJ. Prostaglandin H synthase. Kinetics of tyrosyl radical formation and of cyclooxygenase catalysis. *J Biol Chem*. 1992;267:17753-17759.
- Shimokawa T, Kulmacz RJ, DeWitt DL, Smith WL. Tyrosine 385 of prostaglandin endoperoxide synthase is required for cyclooxygenase catalysis. *J Biol Chem*. 1990;265:20073-20076.
- Hsi LC, Hoganson CW, Babcock GT, Garavito RM, Smith WL. An examination of the source of the tyrosyl radical in ovine prostaglandin endoperoxide synthase-1. *Biochem Biophys Res Commun*. 1995;207:652-660.
- Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*. 2001;294:1871-1875.
- Yu Y, Fan J, Chen XS, et al. Genetic model of selective COX2 inhibition reveals novel heterodimer signaling. *Nat Med*. 2006;12:699-704.
- Smith WL. The eicosanoids and their biochemical mechanisms of action. *Biochem J*. 1989;259:315-324.
- Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci U S A*. 1975;72:2994-2998.
- Huang RY, Chen GG. Cigarette smoking, cyclooxygenase-2 pathway and cancer. *Biochim Biophys Acta-Rev Cancer*. 2011;1815:158-169.
- Haeggström JZ, Rinaldo-Matthis A, Wheelock CE, Wetterholm A. Advances in eicosanoid research, novel therapeutic implications. *Biochem Biophys Res Commun*. 2010;396:135-139.
- Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *Br J Pharmacol*. 1999;128:1121-1132.
- Curfman GD, Morrissey S, Drazen JM. Expression of concern: Bombardier et al., "comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis," *N Engl J Med* 2000;343:1520-8. *N Engl J Med*. 2005;353:2813-2814.
- Moore N. Coronary risks associated with diclofenac and other NSAIDs: an Update. *Drug Safety*. 2020;43:301-318.
- Pirlamarla P, Bond RM. FDA labeling of NSAIDs: review of nonsteroidal anti-inflammatory drugs in cardiovascular disease. *Trends Cardiovasc Med*. 2016;26:675-680.
- Antman EM, Bennett JS, Daugherty A, Furberg C, Roberts H, Taubert KA. Use of nonsteroidal antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association. *Circulation*. 2007;115:1634-1642.
- Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J*. 2004;18:790-804.
- Langenbach R, Morham SG, Tian HF, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*. 1995;83:483-492.
- Morham SG, Langenbach R, Loftin CD, et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell*. 1995;83:473-482.
- Loftin CD, Trivedi DB, Tian HF, et al. Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. *Proc Natl Acad Sci U S A*. 2001;98:1059-1064.
- Yu Y, Funk CD. A novel genetic model of selective COX-2 inhibition: comparison with COX-2 null mice. *Prostaglandins Other Lipid Mediat*. 2007;82:77-84.
- Upmacis RK, Shen H, Benguigui LE, et al. Inducible nitric oxide synthase provides protection against injury-induced thrombosis in female mice. *Am J Physiol Heart Circ Physiol*. 2011;301:H617-H24.
- Lobo I. Genetics and statistical analysis. *Nature Education*. 2008;1:109.
- Alpdogan S, Clemens R, Hescheler J, Neumaier F, Schneider T. Non-Mendelian inheritance during inbreeding of Ca(v)3.2 and Ca(v)2.3 deficient mice. *Sci Rep*. 2020;10:15993.
- Lamon BD, Upmacis RK, Deeb RS, Koyuncu H, Hajjar DP. Inducible nitric oxide synthase gene deletion exaggerates MAPK-mediated cyclooxygenase-2 induction by inflammatory stimuli. *Am J Physiol Heart Circ Physiol*. 2010;299:H613-H23.
- Upmacis RK, Deeb RS, Hajjar DP. Reprint of "oxidative alterations of cyclooxygenase during atherogenesis" [Prostag. Oth. Lipid. M. 80 (2006) 1-14]. *Prostag Oth Lipid M*. 2007;82:1-XIV.
- Hajjar DP, Deeb RS, Upmacis RK. Complex "cross talk" involving nitric oxide metabolites: who's listening? *Curr Atheroscler Rep*. 2006;8:347-348.
- Trebino CE, Eskra JD, Wachtmann TS, Perez JR, Carty TJ, Audoly LP. Redirection of eicosanoid metabolism in mPGES-1-deficient macrophages. *J Biol Chem*. 2005;280:16579-16585.
- Diaz-Muñoz MD, Osmá-García IC, Cacheiro-Llaguno C, Fresno M, Iñiguez MA. Coordinated up-regulation of cyclooxygenase-2 and microsomal prostaglandin E synthase 1 transcription by nuclear factor kappa B and early growth response-1 in macrophages. *Cell Signal*. 2010;22:1427-1436.
- Catella F, Healy D, Lawson JA, FitzGerald GA. 11-dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. *Proc Natl Acad Sci U S A*. 1986;83:5861-5865.
- Blewett AJ, Varma D, Gilles T, Libonati JR, Jansen SA. Development and validation of a high-performance liquid chromatography-electrospray mass spectrometry method for the simultaneous determination of 23 eicosanoids. *J Pharm Biomed Anal*. 2008;46:653-662.
- Capra V, Bäck M, Barbieri SS, Camera M, Tremoli E, Rovati GE. Eicosanoids and their drugs in cardiovascular diseases: focus on atherosclerosis and stroke. *Med Res Rev*. 2013;33:364-438.
- Yang CC, Chang KW. Eicosanoids and HB-EGF/EGFR in cancer. *Cancer Metastasis Rev*. 2018;37:385-395.

40. Coras R, Kavanaugh A, Boyd T, et al. Pro- and anti-inflammatory eicosanoids in psoriatic arthritis. *Metabolomics*. 2019;15:65.
41. Zhou J, Chen L, Liu Z, Sang L, Li Y, Yuan D. Changes in erythrocyte polyunsaturated fatty acids and plasma eicosanoids level in patients with asthma. *Lipids Health Dis*. 2018;17:206.
42. Titz B, Luetlich K, Leroy P, et al. Alterations in serum polyunsaturated fatty acids and eicosanoids in patients with mild to moderate chronic obstructive pulmonary disease (COPD). *Int J Mol Sci*. 2016;17:1583.
43. Yagami T, Koma H, Yamamoto Y. Pathophysiological roles of cyclooxygenases and prostaglandins in the central nervous system. *Mol Neurobiol*. 2016;53:4754-4771.
44. Almer G, Guégan C, Teismann P, et al. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. *Ann Neurol*. 2001;49:176-185.
45. Pace S, Sautebin L, Werz O. Sex-biased eicosanoid biology: Impact for sex differences in inflammation and consequences for pharmacotherapy. *Biochem Pharmacol*. 2017;145:1-11.
46. Wald C, Wu C. Biomedical research of Mice and women: the bias in animal models. *Science*. 2010;327:1571-1572.
47. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16:626-638.
48. Whitacre CC. Sex differences in autoimmune disease. *Nat Immunol*. 2001;2:777-780.
49. Franconi F, Rosano G, Basili S, Montella A, Campesi I. Human cells involved in atherosclerosis have a sex. *Int J Cardiol*. 2017;228:983-1001.
50. Kim AM, Tingen CM, Woodruff TK. Sex bias in trials and treatment must end. *Nature*. 2010;465:688-689.
51. Check Hayden E. Sex bias blights drug studies. *Nature*. 2010;464:332-333.
52. Martin RM, Biswas PN, Freemantle SN, Pearce GL, Mann RD. Age and sex distribution of suspected adverse drug reactions to newly marketed drugs in general practice in England: analysis of 48 cohort studies. *Br J Clin Pharmacol*. 1998;46:505-511.
53. Pace S, Rossi A, Krauth V, et al. Sex differences in prostaglandin biosynthesis in neutrophils during acute inflammation. *Sci Rep*. 2017;7:3759.
54. Du JT, Vennos E, Ramey E, Ramwell PW. Sex differences in arachidonate cyclo-oxygenase products in elicited rat peritoneal macrophages. *Biochim Biophys Acta*. 1984;794:256-260.
55. Huang Y, Liu J, Wang LZ, Zhang WY, Zhu XZ. Neuroprotective effects of cyclooxygenase-2 inhibitor celecoxib against toxicity of LPS-stimulated macrophages toward motor neurons. *Acta Pharmacol Sin*. 2005;26:952-958.
56. Olesen ETB, Fenton RA. Is there a role for PGE2 in urinary concentration? *J Am Soc Nephrol*. 2013;24:169-178.
57. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. *Clin Immunol*. 2006;119:229-240.
58. Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacol Rev*. 2007;59:207-224.
59. Cheng HF, Harris RC. Cyclooxygenases, the kidney, and hypertension. *Hypertension*. 2004;43:525-530.
60. Nasrallah R, Hassouneh R, Hébert RL. PGE2, kidney disease, and cardiovascular risk: beyond Hypertension and Diabetes. *J Am Soc Nephrol*. 2016;27:666-676.
61. Trebino CE, Stock JL, Gibbons CP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc Natl Acad Sci U S A*. 2003;100:9044-9049.
62. Kamei D, Yamakawa K, Takegoshi Y, et al. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. *J Biol Chem*. 2004;279:33684-33695.
63. Wang M, Zukas AM, Hui Y, Ricciotti E, Puré E, FitzGerald GA. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. *Proc Natl Acad Sci U S A*. 2006;103:14507-14512.
64. Cheng Y, Wang M, Yu Y, Lawson J, Funk CD, FitzGerald GA. Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. *J Clin Invest*. 2006;116:1391-1399.
65. Tang SY, Monslow J, R Grant G, et al. Cardiovascular consequences of prostanoic I receptor deletion in microsomal prostaglandin E Synthase-1-Deficient hyperlipidemic mice. *Circulation*. 2016;134:328-338.
66. Sullivan JC, Sasser JM, Pollock DM, Pollock JS. Sexual dimorphism in renal production of prostanoids in spontaneously hypertensive rats. *Hypertension*. 2005;45:406-411.
67. Francois H, Facemire C, Kumar A, Audoly L, Koller B, Coffman T. Role of microsomal prostaglandin E synthase 1 in the kidney. *J Am Soc Nephrol*. 2007;18:1466-1475.
68. Seyberth HW, Sweetman BJ, Frolich JC, Oates JA. Quantification of the major urinary metabolite of the E prostaglandins by mass spectrometry: Evaluation of the method's application to clinical studies. *Prostaglandins*. 1976;11:381-397.
69. Kim K, Kim SJ. Association of leukotrienes and prostaglandins with splenic (18) F-FDG uptake in hepatobiliary cancer patients. *Hell J Nucl Med*. 2021;24:228-233.
70. Ahmad AA, Randall MD, Roberts RE. Sex differences in the role of phospholipase A(2)-dependent arachidonic acid pathway in the perivascular adipose tissue function in pigs. *J Physiol-London*. 2017;595:6623-6634.