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The Effect of Biological Sex on Arterial Stiffness and Renin-Angiotensin-Aldosterone System Activity in Response to COX-2 Inhibition

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Abstract

Background: Cardiovascular disease is the leading cause of death globally. Cyclooxygenase (COX)-derived prostaglandins play an important role in cardiovascular health regulation. Animal studies suggest a greater vascular dependence on prostaglandins in females, though whether this extends to humans is unknown. We aimed to assess the effect of COX-2 inhibition on blood pressure and arterial stiffness, validated markers of cardiovascular risk, in adults. ***Methods:*** Healthy premenopausal females and males were studied in high-salt balance before and after 14 days of daily oral celecoxib 200mg ingestion on two identical study days. Blood pressure and pulse-wave velocity (PWV) were measured at baseline and in response to an Angiotensin II (AngII) challenge, a validated marker of renin-angiotensin-aldosterone system activity. ***Results:*** Thirteen females (38±13 years) and 11 males (34±9 years) were studied. Pre-COX-2 inhibition, resting measures of SBP (p=0.2) and DBP (p=0.1) were similar between sexes. Post-COX-2 inhibition, resting SBP (p<0.001) and DBP were (p=0.02) significantly lower in females as compared to males. COX-2 inhibition was not associated with changes in arterial parameters by sex (ΔDBP:p=0.54; ΔPWV:p=0.55; females vs. males). COX-2 inhibition was associated with increased SBP (p=0.039 vs. pre-COX-2 inhibition), but no change in DBP (p=0.16) or PWV (p=0.52) response to AngII challenge in females. Measures did not differ in response to AngII pre- vs post-COX-2 inhibition in males (SBP:p=0.88; DBP:p=0.93; PWV:p=0.97). ***Conclusions:*** The effects of COX-2 inhibition on arterial function may differ by sex, though further studies are needed. Given the association between NSAIDs and cardiovascular risk, increased attention into sex-specific pathophysiology is warranted.

Keywords: Sex Differences, Arterial Stiffness, Cyclooxygenase, Renin-Angiotensin-Aldosterone System Activity, Cardiovascular Disease.

Introduction

Cardiovascular disease is the leading cause of death globally[1], and considerable interest has been focused on the potential of arterial hemodynamics in predicting cardiovascular-related events and death[2]. Nonsteroidal anti-inflammatory drugs (NSAIDs), and in particular selective cyclooxygenase (COX)-2 inhibitors, are associated with increased cardiovascular risk[3,4] yet are amongst the most commonly used medications worldwide[5,6], providing both anti-inflammatory and analgesic effects. Animal studies suggest that COX-2-derived vasodilatory prostaglandins play a more prominent role in arterial vasoregulation in females[7,8], an effect that may be mediated by estrogen[9,10] and testosterone[11]. COX-2 inhibition in humans with uncomplicated type 1 diabetes abolished sex differences in the renal hemodynamic response to Angiotensin II (AngII) challenge, suggesting an augmented female prostanoid-dependence and that prostaglandins may contribute to renin-angiotensin-aldosterone system (RAAS)-mediated sex differences[12]. However, whether this same phenomenon exists in the systemic vasculature in a healthy population is unknown.

As women are more likely to be prescribed and take over-the-counter NSAIDs[13], and are more likely than men to die of cardiovascular events such as myocardial infarction[14,15], with an intensified risk in younger women[16–18], an understanding of sex-specific changes in vascular hemodynamic and cardiovascular effects of prostaglandin inhibition is warranted. While any increase in arterial stiffness is associated with increased risk of a cardiovascular event, this association is stronger in younger adults[2]. Moreover, death from both ischemic heart disease and stroke increases progressively and linearly from systolic and diastolic blood pressure measures as low as 115 mmHg and 75 mmHg, respectively[19].

As such, changes in arterial hemodynamics as a consequence of COX-2 inhibition may lead to greater individual cardiovascular risk. We thus hypothesized that premenopausal females would demonstrate an increase in arterial stiffness and blood pressure in response to COX-2 inhibition compared with males, consistent with greater dependence on COX-2-derived prostaglandins in arterial function. We further hypothesized that COX-2 inhibition would augment the effect of AngII in females compared to males due to increased dependence on vasodilatory prostaglandins.

Material and Methods

Healthy participants were recruited from the Calgary area and inclusion criteria included: males and premenopausal females aged ≥18 years, who could comply with a high-salt diet to induce maximum RAAS suppression and provide written, informed consent. Exclusion criteria included cardiovascular, cerebrovascular, and kidney disease; hypertension (blood pressure [BP] >140/90 mmHg or use of anti-hypertensive medications); diabetes mellitus; current smoking; pregnancy; and chronic use of NSAIDs or exogenous hormone therapy. For female participants, use of hormonal contraception was an exclusion criteria due to the effect of exogenous estrogen on vascular function[20]. All participants underwent a medical history, physical examination, and laboratory screening. All demographic data was self-reported. This study was approved by the University of Calgary Conjoint Health Research Ethics Board (CHREB ID: REB14-1806). Written informed consent was obtained from all study subjects in accordance with the Declaration of Helsinki.

Study Day Protocol

Participants were instructed to consume >200 mmol sodium/day for 3 days before each study day to ensure maximal RAAS suppression[21] through a combination of increasing salt intake during meals and supplementation with high-salt soup broth packets. A 24-h urine collection or a 2nd morning spot urine sample was used to confirm a high-salt state before commencement of research study days[22]. Participants were studied in the supine position in a warm, quiet room after an 8h fast. All females were studied 14 days after the first day of uterine bleeding, which most closely aligns with the ovulation period. However, given the wide variation in duration of menstrual cycles[23], serum estradiol and progesterone were measured to confirm appropriate scheduling. At 8 am, an 18-gauge peripheral venous cannula was inserted into the antecubital vein of each arm (1 for infusion, 1 for blood sampling). After a 90-min equilibration period, blood pressure and arterial stiffness measurements were taken at baseline and in response to a graded infusion of AngII ($3\ ng \times kg^{-1} \times min^{-1} \times 30\ min$, $6\ ng \times kg^{-1} \times min^{-1} \times 30\ min$) as an index of RAAS activity[24]. Blood samples were collected at baseline and every 30 minutes until the end of the study.

COX-2 Inhibition

After completing the first study day, participants underwent COX-2 inhibition by ingesting celecoxib (200mg) daily for 14 consecutive days[12]. On day 14 of celecoxib ingestion, participants

underwent reassessment of blood pressure, arterial stiffness and RAAS activity in a study day identical to pre-COX-2 inhibition assessment. In female participants, the second study day was performed at the same timepoint of the menstrual cycle as the first study day.

Measurement of Blood Pressure and Arterial Stiffness

Participants were studied in the supine position using a standard BP cuff placed on the right arm. Systolic (SBP) and diastolic (DBP) blood pressure measurements were recorded every 15 minutes with an automatic recording device (Critikon DINAMAP ProCare Monitor; GE Medical Systems, Milwaukee, WI). The mean of two readings taken by the same registered nurse (D.Y.S.) were recorded. Arterial stiffness was determined through measurements of carotid-femoral pulse-wave velocity (PWV) through applanation tonometry using two non-invasive piezo-resistive pressure transducers (Millar Instruments, Houston, TX) and acquired (Sphygmacor Version 8,0, Atcor Medical) using both the right carotid and femoral arteries both at baseline (0min) and in response to the AngII infusion (60min) by one operator (D.Y.S). Sequential waveforms over a period of 10s were collected and automatically calculated as the pulse transit time as a function of distance travelled by the electrocardiogram-gated pulse between the two sites.

Statistical Analysis

Results are presented as mean ± standard deviation (SD) or percentage, as appropriate. Our primary outcomes were the changes (Δ) in arterial hemodynamics (SBP, DBP, PWV) at baseline and in response to AngII at 60 minutes (as a measure of arterial RAAS activity) pre- as compared to post-COX-2 inhibition therapy, stratified by sex. Secondary study outcomes were the changes in plasma renin activity and aldosterone at baseline and in response to AngII pre- and post-COX-2 inhibition, stratified by sex. If measurements (i.e., SBP, DBP, PWV) were unattainable from participants at one timepoint (e.g., 60min) during AngII infusion on the study day, the value from the other timepoint (e.g., 0min) was used to maximize statistical power. Pre- and post-COX-2 inhibition comparisons were made using the Student's paired t-tests and between sex comparisons were made using unpaired t-tests. All statistical analyses were performed using SPSS V.26.0 (SPSS, Chicago, IL, USA). Normality of each variable was confirmed with Shapiro-Wilk test of normality. The significance level was defined as $p<0.05$.

Results

Baseline Characteristics

Participants' baseline characteristics are displayed in **Table 1**. The majority of participants self-identified as white and this was not statistically different between females and males (p=0.3). All participants were normotensive, non-diabetic and in high-salt balance, indicative of a maximal RAAS suppression state. No sex differences were observed in age (p=0.4) or BMI (p=0.4). HDL was significantly higher in females compared to males (p=0.02) but all cholesterol values were within acceptable range[25].

Pre- vs Post-COX-2 Inhibition: Blood Pressure

Changes in resting measures of blood pressure are reported in **Table 2.** Pre-COX-2 inhibition, resting measures of SBP (p=0.2) and DBP (p=0.1) were similar between females and males. COX-2 inhibition was not associated with a significant within-sex change in SBP (female: p=0.2; male: p=0.2) or DBP (female*:* p=0.5; male: p=0.8). However, resting BP was lower in females compared to males post-COX-2 inhibition (SBP, p<0.001; DBP, p=0.02) with a trend towards a sex difference in resting SBP response to COX-2 inhibition (p=0.06; *Figure 1, Panel A*).

Pre- vs Post-COX-2 Inhibition: Arterial Stiffness

Changes in resting measures of PWV are reported in **Table 2**. Resting measures of PWV were similar between females and males both pre- and post-COX-2 inhibition (p=0.3 for both). COX-2 inhibition did not affect resting PWV in females (p=0.8), or males (p=0.5). The change in resting PWV was not different between sexes (change in resting PWV, p=0.5 females vs. males; *Figure 1, Panel C*).

Pre- vs Post-COX-2 Inhibition: Blood Pressure Response to Angiotensin II

Pre-COX-2 inhibition, all participants demonstrated an increase in SBP (females, p<0.001; males, p=0.02) and DBP (females, p<0.001; males, p=0.007) in response to the AngII challenge with no observed sex differences (ΔSBP, females vs males, p=0.7; ΔDBP females vs males, p=0.9). Compared to pre-COX-2 measures, COX-2 inhibition was associated with a significantly greater sensitivity of SBP to the AngII challenge in females (ΔSBP, p=0.039, ΔDBP, p=0.2), while COX-2 inhibition was not associated with changes in blood pressure sensitivity to AngII challenge in

males (ΔSBP, p=0.9; ΔDBP, p=0.9). The change in blood pressure response was not different between sexes (ΔSBP, p=0.1, *Figure 2, Panel A*; ΔDBP, p=0.3, *Figure 2, Panel B*; all values females vs. males response).

Pre- vs Post-COX-2 Inhibition: Arterial Stiffness Response to Angiotensin II

Pre-COX-2 inhibition, females demonstrated a greater numerical increase in PWV (p<0.001) than males (p=0.03) in response to the AngII challenge but this sex difference did not achieve statistical significance (p=0.5). COX-2 inhibition was not associated with changes in PWV sensitivity to AngII challenge in either females (p=0.5) and males (p=0.9) (ΔPWV, p=0.8 females vs. males; *Figure 2 Panel C*).

Pre- vs Post-COX-2 Inhibition: Circulating RAAS components

Pre-COX-2 inhibition, females had significantly lower renin levels (p=0.005) but similar aldosterone levels compared to males (p=0.1). Post-COX-2 inhibition, renin (p=0.015) and aldosterone (p=0.002) values did not change significantly in females, but both decreased in males. All participants demonstrated a decrease in renin values (females, p<0.001; males, p<0.001) and an increase in aldosterone levels (females, p<0.001; males, p=0.003) in response to the AngII challenge pre-COX-2 inhibition. However, a significant decrease in renin in females but not males (Δrenin, p=0.045; Δaldosterone, p=0.3; all values females vs. males response) was observed post-COX-2 inhibition. Compared to pre-COX-2 measures, COX-2 inhibition did not change the sensitivity of renin (p=0.2) or aldosterone to AngII challenge (p=0.2) in females. In males, COX-2 inhibition significantly blunted sensitivity of renin to AngII challenge (p=0.003), but had no effect on aldosterone sensitivity (p=0.9) (Δrenin, p=0.08; Δaldosterone, p=0.2; all values female vs. male response).

Discussion

In this exploratory study, we aimed to assess the effects of COX-2 inhibition on resting arterial hemodynamics and responsiveness to an AngII challenge in healthy, young females and males. Our key findings were: 1) increased SBP sensitivity to AngII in females but not males; and 2) significant blunting of renin response to AngII in response to COX-2 inhibition was observed in

males but not females. Our exploratory study suggests that COX-2 inhibition may have differential sex-based effects on SBP and RAAS activity.

In light of increasing evidence of sex-based differences in cardiovascular pathophysiology[26] which may lead to poorer clinical outcomes in younger women, this study adds to the growing literature highlighting sex-based differences in arterial function and the need for sex-stratification of study results. Despite being at an overall decreased risk of cardiovascular disease compared to age-matched men, young women are more likely to die following myocardial infarction[15,16]. Many traditional cardiovascular risk factors, which are now more prevalent in younger adults compared to previous generations, have a larger impact in young women as compared to young men[27,28], and the Framingham coronary risk score has actually increased in women aged 35 to 54 years of age[29,30]. Previous studies have suggested that use of NSAIDs, including COX-2 inhibitors, have shown mixed results in terms of their association with cardiovascular risk[31]; however, results were not stratified by sex. Based on post-marketing studies, females are greater consumers of COX-2 inhibitors (over 85%)[32–34]; however, a review of 28 rofecoxib clinical trials demonstrated that 80% of the trials did not describe efficacy results by sex, only one reported side effects by sex, and only 8% of the trials considered the influence of hormonal variation in the results[35].

The results of our study suggest that female SBP measures may be more sensitive to the effects of COX-2 inhibition compared to males. These findings are similar to the effects of COX-2 inhibition on kidney hemodynamics in individuals with type 1 diabetes, whereby females demonstrated greater increases filtration fraction and kidney vascular resistance with a decline in kidney blood flow compared to males[12]. Previous studies have highlighted that prostaglandin expression differs across vascular beds[36]; moreover, diabetes is known to alter prostaglandin production[37], which may also explain differing results between studies. Animal studies have demonstrated conflicting results of COX-2 inhibition on arterial hemodynamics. While female spontaneously hypertensive rats (SHR) have greater COX-2 expression in the renal medulla and enhanced urinary excretion of prostaglandin E_2 metabolites compared to male SHR, treatment with celecoxib did not significantly alter blood pressure or albuminuria in either female or male SHR[8].

A greater vasoconstrictor response to AngII challenge reflects lower baseline RAAS activity; conversely, a blunted response is consistent with baseline upregulation of RAAS activity [38]. Our results suggest that COX-2 inhibition was associated with a downregulation of RAAS activity in females and males. Sex-associated differences in the regulation of arterial function and morphology by the renin-angiotensin aldosterone system related to COX-2 have also been previously described [39]. In anesthetized female mice, treatment with COX-2 inhibition did not alter blood pressure compared to treatment with COX-1 inhibition or placebo. However, consistent with our findings in humans, infusion with AngII resulted in a significantly greater increase in blood pressure in the COX-2 treated mice compared to COX-1 inhibition placebo[40], although male mice were not included in this study. This suggests that COX-2 activity, at least in females, generates a vasodepressor response moderating the pressor effect of AngII. Estrogen binding on the estrogen receptor subtype alpha up-regulates the production of atheroprotective prostacyclin, PGI-2, through activation of COX-2[41]. Deletion of the PGI-2 receptor removed the atheroprotective effect of estrogen in ovariectomized female mice, suggesting that chronic treatment of premenopausal individuals with selective COX-2 inhibitors may increase cardiovascular risk in pre-menopausal females or post-menopausal females using menopausal hormone replacement therapy. Although exploratory in nature, this study provides a potential framework for future studies assessing sex-stratified arterial hemodynamic changes with NSAID use in humans.

This exploratory study has limitations. Firstly, healthy individuals were recruited for the present study, which may limit the generalizability of the results to other patient populations. However, inclusion of healthy individuals allows for minimization of confounding variables potentially affecting the primary outcome of interest. Secondly, the menstrual cycle[42] and exogenous hormone use (e.g., contraceptives)[43] impact the RAAS, but all female participants were studied 14 days after the start of their last menstrual period to standardize measurements. Our female participants were pre-menopausal, and our results may not be generalizable to the post-menopausal population. However, given that young women are more likely to die after myocardial infarction compared to age-matched men[17], the results of our study suggest a potential age- and sex-specific mechanism contributing to cardiovascular risk. Future studies including both pre- and post-menopausal females may parse out potential estrogen-mediated mechanisms influencing cardiovascular health, and may have implications in understanding cardiovascular risk associated with female sex-

specific pregnancy-associated complications, such as gestational hypertension and preeclampsia. Finally, while previous animal studies have highlighted sex-specific pharmacokinetic differences with exposure to COX-2 inhibition[44,45], it is unclear from our exploratory study if the observed outcomes were due to sex-based differences in pharmacokinetic and/or pharmacodynamic effects of COX-2 inhibition.

In summary and in light of reports linking long-term COX-2 inhibitor use with increased cardiovascular risk, this study not only provides insights into how this risk might occur, but how this risk may differ by sex in younger populations. NSAID use is increasing[46], particularly in females, and there is greater recognition of the cardiovascular risks associated with NSAID use[47]. Future studies exploring the impact of NSAID use across the lifespan, including the menopausal transition may reveal sex- and age-specific risk factors. Determining if sex differences in arterial hemodynamic measures are due to differential drug metabolism as a consequence of interindividual differences in *COX-2* gene expression may present opportunities for optimizing prescription patterns for females and males, while recognizing the intersection of sex with other factors including gender, and ethnicity/race on cardiovascular health. In this era of precision health, greater attention to how risk factors and interventions may impact cardiovascular risk differently by such intersections could result in improved cardiovascular health for all.

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Disclosures

The authors have no conflicts of interest to declare.

Contributors

C.L.R, S.M.D and S.B.A conceived the study design. D.Y.S collected the data. C.L.R cleaned the data, completed statistical analyses, and designed tables and figures. C.L.R wrote the first draft of

the manuscript. C.L.R, S.M.D, D.Y.S and S.B.A all read and approved the final version of the manuscript.

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Tables

Table 1. Anthropometric, Demographic and Blood Sampling Results for Females and Males Pre-COX-2 Inhibition

Variables	**Females**	**Males**
n	13	11
Age, yr	38 ± 13	34 ± 9
White, n (%)	9 (69%)	11 (100%)
BMI, kg/m^2	24.9 ± 3.9	26.2 ± 3.4
Trigylcerides, mmol/L	0.7 ± 0.4	1.1 ± 0.5
Total Cholesterol, mmol/L	4.2 ± 0.9	4.0 ± 0.6
HDL, mmol/L	1.5 ± 0.3*	1.2 ± 0.3
LDL, mmol/L	2.3 ± 0.7	2.3 ± 0.5
Fasting Glucose, mmol/L	4.7 ± 0.4	4.8 ± 0.4
Fasting Insulin, pmol/L	36.0 ± 1.0	50.7 ± 31.5
hs-CRP, mg/L	1.0 ± 1.1	0.8 ± 1.2
Est. 24-hour UNa, mmol/d	368 ± 127	461 ± 105

Notes: Day 1 values presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001 females significantly different from males. BMI: body mass index; HDL: high-density lipoproteins; hs-CRP: high sensitivity C-reactive protein; LDL: low-density lipoproteins; UNa: urinary sodium.

Table 2. Arterial Hemodynamics and RAAS Activity in Females and Males During Graded Angiotensin II Challenge Pre- and Post-COX-2 Inhibition.

	Day 1 (Pre-COX-2 Inhibition)			
	0 min		**60 min**	
Variables	**Females**	**Males**	**Females**	**Males**
SBP, mmHg	111 ± 11	118 ± 15	125 ± 17‡‡‡	131 ± 15‡
DBP, mmHg	65 ± 8	71 ± 9	77 ± 10‡‡‡	82 ± 12‡‡
PWV, m/s	7.6 ± 1.0	7.9 ± 1.0	9.2 ± 1.6‡‡‡	9.2 ± 1.7‡‡
Renin, ng/L/s	0.17 ± 0.11	0.34 ± 0.15**	0.06 ± 0.03‡‡‡	0.13 ± 0.07**‡‡‡
Aldosterone, pmol/L	142 ± 77	199 ± 91	327 ± 119‡‡‡	387 ± 225‡‡
	Day 14 (Post-COX-2 Inhibition)			
	0 min		**60 min**	
	Females	**Males**	**Females**	**Males**
SBP, mmHg	107 ± 8	123 ± 12***	134 ± 21‡‡‡	135 ± 12‡
DBP, mmHg	64 ± 8	72 ± 7*	81 ± 8‡‡‡	83 ± 10‡‡‡
PWV, m/s	7.4 ± 1.7	8.3 ± 2.0	9.1 ± 1.5‡‡	9.6 ± 1.6
Renin, ng/L/s	0.15 ± 0.14	0.21 ± 0.17†	0.08 ± 0.08‡	0.15 ± 0.11
Aldosterone, pmol/L	136 ± 81	108 ± 46††	397 ± 211‡‡‡	291 ± 144†‡‡‡

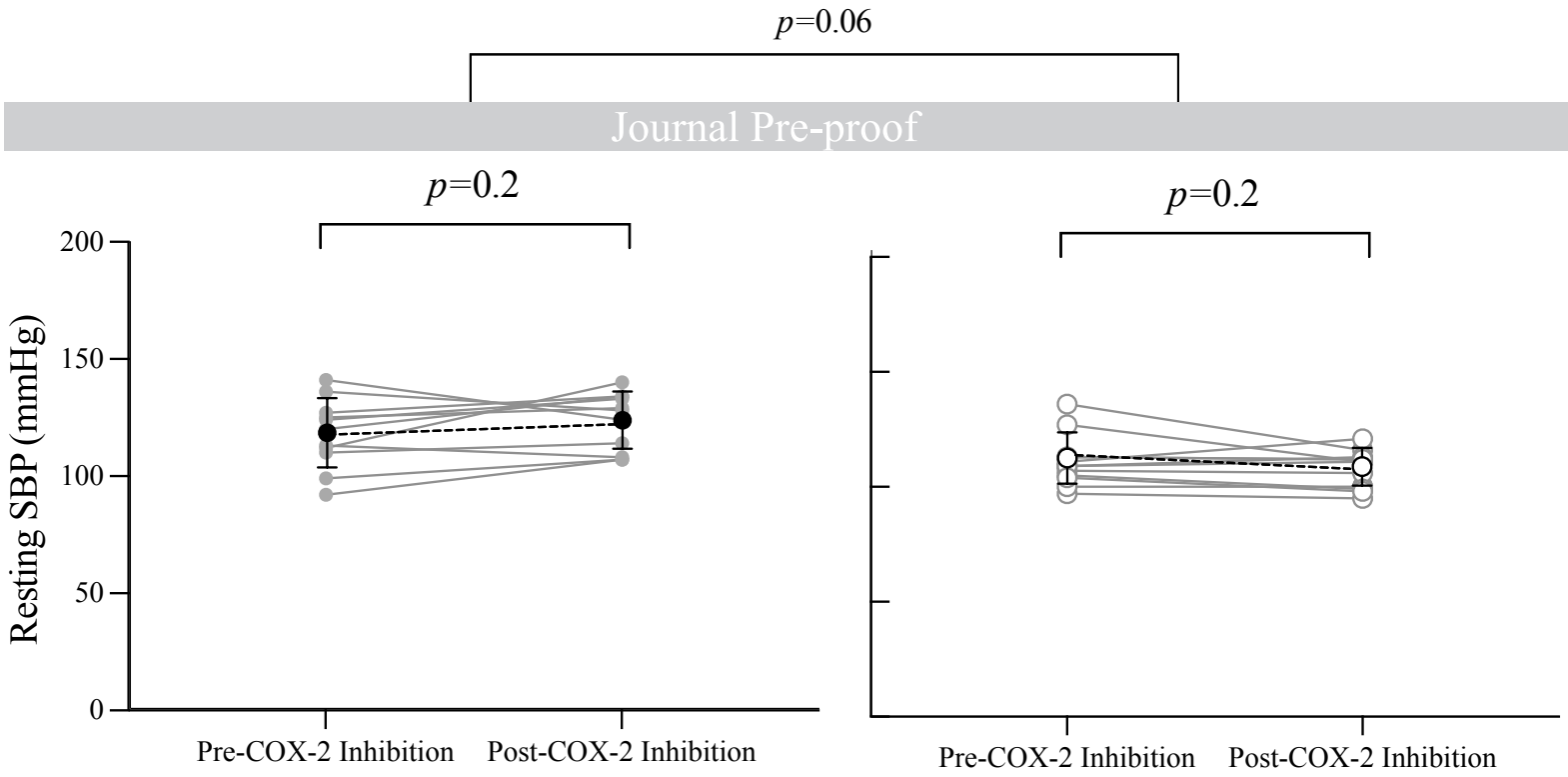
Notes: Values presented as mean ± SD. *p<0.05, ** p<0.01, ***p<0.001 significantly different females vs. males within same Day. †p<0.05, ††p<0.01, †††p<0.001 significantly different Day 1 vs. Day 14 within sex and timepoint. ‡p<0.05, ‡‡p<0.01, ‡‡‡p<0.001 significantly different from 0 min within sex and Day. DBP: diastolic blood pressure; PWV: Pulse-wave velocity; SBP: systolic blood pressure.

Figure Legends

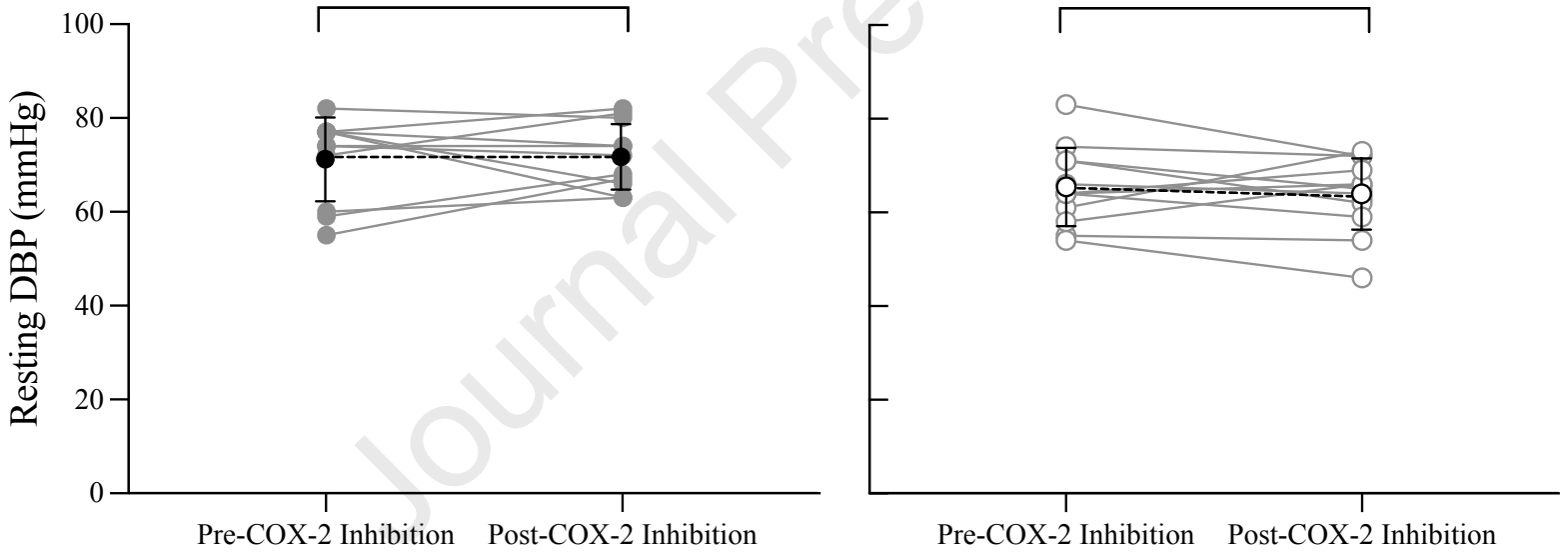
Figure 1. Change in resting systolic blood pressure (SBP, Panel A), diastolic blood pressure (DBP, Panel B) and pulse-wave velocity (PWV, Panel C) pre- and post- cyclooxygenase-2 (COX-2) inhibition in males (solid circles) and females (empty circles). Individual values presented in grey. Mean ± SD presented in black.

Figure 2. Change in systolic blood pressure (SBP, Panel A), diastolic blood pressure (DBP, Panel B) and pulse-wave velocity (PWV, Panel C) in response to a graded Angiotensin II (AngII) infusion both pre- and post- cyclooxygenase-2 (COX-2) inhibition in males (solid circles) and females (empty circles). Individual values presented in grey. Mean ± SD presented in black. *p<0.05.

A



B



C