University of Calgary

PRISM Repository **https://prism.ucalgary.ca**

The Vault Open Theses and Dissertations

2013-04-23

Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans

Pun, Matiram

Pun, M. (2013). Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans (Master's thesis, University of Calgary, Calgary, Canada). Retrieved from https://prism.ucalgary.ca. doi:10.11575/PRISM/25809

http://hdl.handle.net/11023/617

Downloaded from PRISM Repository, University of Calgary

UNIVERSITY OF CALGARY

Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans

by

Matiram Pun

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

APRIL, 2013

© Matiram Pun 2013

Abstract

Background: Intermittent hypoxia (IH) simulating obstructive sleep apnea raises blood pressure (BP) and impairs cerebral blood flow response. The pathophysiology of intermittent hypoxia-mediated increase in BP involves multiple mechanisms but the role of cyclooxygenase (COX) catalyzed vasoactive prostaglandins (PG) is unclear.

Methods: A placebo controlled double-blind randomized cross-over trial was designed using nonselective COX inhibitor indomethacin (50 mg tid/po), selectively COX-2 inhibitor celecoxib (200 mg bid/po) comparing with placebo. Healthy males ingested either of drugs for 4 days and physiological measurements were taken on 5th day with an acute isocapnic-hypoxia challenge pre- and post 6 hrs of IH exposure. Urinary PGs were assayed pre- and post- IH exposure.

Results: After 4 days of drug, INDO increased BP compared to PLBO and CLBX; and lowered CBF compared to PLBO (air and baseline breathing). Mean arterial pressure gain with INDO increased followed by CLBX in response to acute isocapnic hypoxia and it was driven by increased gains in both systolic BP and diastolic BP (statistically not significant). CBF gain was blunted with CLBX while it was similar between INDO and PLBO although they were not statistically significant. With 6 hrs of IH (post-IH), CBF gain remained blunted with CLBX but was augmented with INDO (statistically not significant). CVC gain was lower with CLBX (statistically not significant). Both drugs lowered vasodilator and vasoconstrictor PGs compared to PLBO. Pre-IH PGI_2:TxA_2 was higher with INDO compared to PLBO ($p < 0.001$) and CLBX ($p < 0.001$).

Conclusion: Indomethacin perturbs cardio- and cerebrovascular homeostasis in more robust manner as compared to placebo and celecoxib after 4 days of ingestion.

Acknowledgements

Thanks to everyone who helped me with this. I would like to thank the following people, whose contributions have made this project possible. Dr. Marc J. Poulin, my supervisor, for his patience, guidance and support. Because of him, I have been able to achieve my graduate education goals. Dr. Sofia B Ahmed, my co-supervisor, for her patience, guidance, support and for her during all the experiments and interventions. Dr Donna M Slater, for an expert inputs on Enzyme Immunoassays (EIAs) and for serving on my supervisory committee. Dr. Patrick J Hanly, for his wonderful support in screening subjects going through their sleep records (Remmer's Sleep Recordings) and for serving on my supervisory committee. Prof Dr Katherine Wynne-Edwards and her team (Dr Andrea De Souza and Ms Lea Bond) for their expert help in Sample Extraction/Purification, EIA and Prostaglandin Analysis. Dr Buddha Basnyat, the president of Mountain Medicine Society of Nepal (MMSN), for his continued support, encouragement and guidance in the research field. Dr Sanju Lama, senior colleague from the program for her encouragement and support.

I would like to thank Dr Grant R Gordon, assistant professor, from Hotchkiss Brain Institute of University of Calgary, Calgary for agreeing to serve as my external examiner with the Department of Medical Science.

Mr Andrew E Beaudin, a doctoral student and my collaborator in the project, for his involvement, patience, and working extremely hard to round up the project to the better direction. I would also like to thank him for kindly giving his time and knowledge to my

training. Dr Craig D Steinback , Dr Margarret H Davenport, Ms Sara Hartmann, Mr Bradley Hansen, Ms Linda Brigan, Ms Heather Tows, Ms Cindy Lee, Ms Brenda Green and Christina Yang for their continued support and assistance, and for helping create an enjoyable place to study. And finally, to all of the volunteers that gave their time and commitment to the study.

I would like to extend my thanks to the landlords (Johnson family) at Calgary: Arta Johnson and Kelvin Johnson who have provided space, warmth and interaction. It was like a family and I enjoyed every moment of interaction and stay. I cannot forget my Nepalese friends whom I met during this period: Aditya Gurung, Anubhuti Parajuli, Anup Dhital, Bhupesh Khadka and family, Dhurba Tripathi and family, Haris Pandey, Narendra Adhikari and family, Noorma Shrestha, Paras Mandal, Ram Chandra Adhikari, Sarbajit Gurung, Surendra Adhikari and Suresh Mulmi at University of Calgary. They made my stay at Calgary memorable and provided a wonderful community for sports, marking Nepalese festivals (in small circle) and sharing the moments. Best of Luck to you all in all future endeavours!

The research contained in this thesis was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Alberta Innovates – Health Solutions (AIHS) and The Canadian Institutes of Health Research (CIHR). My personal support was provided by the University of Calgary and William H. Davies Medical Research Scholarships (2011).

I would like to dedicate this thesis to my late parents, brothers, sister-in-law and other family members for their immense support, encouragement and patience. I would like to remember my past and current mentors who have been instrumental for me to be here and shape this thesis. Then I would dedicate to my colleagues working in this project and friends who have been part and parcel of life in- and outside academia. Finally, the volunteers for the study to be as subject to go through all the protocols. I appreciate their time and commitment to this project. Without their involvement and adherence, I would not have been able to make this project complete.

Thank you!

Table of Contents

Abstract ii
Acknowledgements iii
Dedication v
Table of Contents vi
List of Tables x
List of Figures and Illustrations xi
List of Symbols, Abbreviations and Nomenclature xiii
Epigraph xvi

CHAPTER ONE: INTRODUCTION 1

1.1 INTRODUCTION 1

1.2 REVIEW OF LITERATURE 6
1.2.1 Literature search 6
1.2.2 Intermittent hypoxia and hypertension 6
1.2.3 Intermittent hypoxia and cerebral blood flow 7
1.2.4 Cyclooxygenase (COX) enzymes 8
1.2.5 Research challenges in COX inhibition and prostaglandins 14
1.2.6 COX inhibitors, hypertension and cardiovascular risk 15
1.2.7 Intermittent hypoxia, vascular inflammation and prostaglandin 17
1.2.8 Hypoxia induced vascular expression of prostaglandins 18
1.2.9 Prostaglandins, intermittent and systemic hypertension 20
1.2.10 Vascular function in intermittent hypoxia and COX inhibition 21
1.2.11 Prostaglandins, cerebral blood flow and intermittent hypoxia 24

1.3 RATIONALES 27
1.3.1 Rationale of the study 27
1.3.2 Rationale of the drug choice and regimen 29

1.4 OBJECTIVES 31

1.5 HYPOTHESES 31

1.6 EXPECTED OUTCOMES 32

CHAPTER TWO: RESEARCH METHOD 33

2.1 ETHICS AND APPROVALS 33

2.2 STUDY POPULATION 33

2.3 SAMPLE SIZE CALCULATION: STATISTICAL DESIGN OF THE STUDY.....34

2.4 EXPERIMENTAL COMMITMENT FOR THE STUDY VOLUNTEERS.....35

2.5 INITIAL CONTACT, LAB TOUR AND RECRUITMENT.....36

2.6 SCREENING VISIT.....36

2.7 FAMILIARIZATION TEST (LLOYD-CUNNINGHAM PROTOCOL).....37

2.8 EXPERIMENTAL SET UP AND TERMINATION OF EXPERIMENT.....38

2.9 THE EXPERIMENTAL PROTOCOLS.....38
2.9.1 Drug ingestion, home blood pressure monitoring and dietary chart.....38
2.9.2 Protocols: indomethacin, celecoxib and placebo.....39

2.10 EXPERIMENTAL TECHNIQUES.....40
2.10.1 Dynamic end-tidal forcing (DEF) system.....40
2.10.2 Respiratory measurements.....41
2.10.3 Transcranial Doppler (TCD) ultrasound.....42
2.10.4 Arterial oxyhemoglobin saturation.....43
2.10.5 Blood pressure monitoring.....43

2.11 DATA ACQUISITION SOFTWARE.....44
2.11.1 Chamber program.....44
2.11.2 BreatheM program.....44
2.11.3 LabCharts: PowerLab.....44

2.12 DATA COLLECTION.....45
2.12.1 Baseline measurements.....45
2.12.2 Acute tests: Morning and afternoon experiments.....46
2.12.3 Intermittent hypoxia exposure: Six hours chamber protocol.....46

2.13 BLOOD AND URINE COLLECTION AND STORAGE.....47

2.14 MANAGEMENT AND COPYRIGHT OF THE DATA.....48

CHAPTER THREE: DATA ANALYSIS.....49

3.1 ACUTE HYPOXIC EXPOSURE DATA.....49

3.2 OUTCOME VARIABLES OF THE STUDY.....49

3.3 ANALYSIS OF THE DATA.....49
3.3.1 Exhale program.....50
3.3.2 Average program.....50

3.3.3 Modelling of the data 51

3.4 CARDIOVASCULAR RESPONSES: BLOOD PRESSURE GAINS 51

3.5 CEREBROVASCULAR CONDUCTANCE AND RESISTANCE 52

3.6 CEREBROVASCULAR RESPONSES 53

3.7 CAPILLARY BLOOD SAMPLES 54

3.8 URINARY ANALYSIS 54
3.8.1 Urinary sodium, creatinine and proteins (CLS Analysis).....54
3.8.2 Sample extraction and urinary prostaglandin measurements 55

3.9 STATISTICAL INTERPRETATION OF THE DATA 57

CHAPTER FOUR: RESULTS 58

4.1 SUBJECTS 58

4.2 SCREENING 58
4.2.1 Venous blood samples 58
4.2.2 Urine samples 59
4.2.3 Sleep disordered breathing.....59

4.3 HOME BLOOD PRESSURE MONITORING 59

4.4 URINARY PROSTAGLANDINS.....60

4.5 ACUTE TESTS 62
4.5.1 Air breathing 62
4.5.2 Isocapnic euoxic baseline 64
4.5.3 Acute isocapnic hypoxic challenge.....66

4.6 CAPILLARY BLOOD SAMPLES 69

CHAPTER FIVE: DISCUSSION.....71

5.1 MAJOR FINDINGS 71

5.2 HOME BLOOD PRESSURE MONITORING 72

5.3 PROSTAGLANDIN RESPONSES.....73

5.4 ACUTE TESTS: AIR BREATHING 75

5.5 ACUTE TESTS: ISOCAPNIC EUOXIC BASELINE.....80

5.6 ACUTE ISOCAPNIC HYPOXIA CHALLENGE.....82

BIBLIOGRAPHY.....114

APPENDICES.....144
Appendix A: Pharmacodynamics and pharmacokinetics of the drugs 144
Appendix B: Take home package.....146
Appendix C: Flyers.....162
Appendix D: Familiarization package and informed consent.....164
Appendix E: Screening package.....175
Appendix F: Representative LabChart from 6 hours of chamber IH exposure 186
Appendix G: Representative radiometer results 187
Appendix H. Urinary prostaglandin analysis algorithms.....188
Appendix I. Solid phase extraction (SPE) protocol.....189
Appendix J. Mean sample preparation of urine.....190

List of Tables

Table 4.1 General and sleep characteristics of the subjects …….……89

Table 4.2 Venous blood results (ABL800, Screening) ……...…….90

Table 4.3 Venous blood results (CLS, Screening).....91

Table 4.4 Urinary dipstick results.....92

Table 4.8 Capillary blood sample results during experimental session 93

List of Figures and Illustrations

Figure 1.1 Prostaglandin action in paracrine and autocrine pathways 94

Figure 1.2 The Prostaglandin pathway 95

Figure 1.3 Prostacyclin (PGI_2) productions and its action on target cells in the vasculature …….....96

Figure 1.4 The vascular and endothelial prostaglandin synthesis in the brain 97

Figure 2.1 Experimental protocol over the days of drug ingestion leading to experimental day interventions 98

Figure 2.2 Study flow chart showing the subject recruitment, screening and drug interventions …….....99

Figure 2.3 Acute isocapnic hypoxia testing protocol 100

Figure 4.1 Home blood pressure monitoring at home and experimental day morning BP ….....101

Figure 4.2 Prostaglandin I_2 (PGI_2) results PRE-IH (AM) and POST-IH (PM) 102

Figure 4.3 Thromboxane A_2 (TxA_2) results PRE-IH (AM) and POST-IH (PM) 103

Figure 4.4 Prostaglandin ratios (PGI_2/TxA_2) i.e. vasodilator (PGI_2) vs vasoconstrictor (TxA_2) …….....104

Figure 4.5 Prostaglandin changes from PRE-IH (AM) to POST-IH (PM) 105

Figure 4.6 End-tidal gases and CBF responses to air breathing and isocapnic euoxia (baseline) …….....106

Figure 4.7 Blood pressure response to air breathing and isocapnic euoxia baseline ….107

Figure 4.8 End-tidal gases during acute test at AM (PRE-IH) and PM (POST-IH) Gain in cerebral blood flow during acute hypoxic test 108

Figure 4.9 BP responses during acute test at AM (PRE-IH) and PM (POST-IH) 109

Figure 4.10 Gain BP responses to acute test at AM (PRE-IH) and PM (POST-IH) 110

Figure 4.11 CBF responses to during acute test at AM (PRE-IH) and PM (POST-IH) ……..111

Figure 4.12 Gain CBF responses to acute test at AM (PRE-IH) and PM (POST-IH) ...112

Figure 4.13 Diagrammatic summary of the study 113

List of Symbols, Abbreviations and Nomenclature

AA	Arachidonic Acid
ABP	Arterial Blood Pressure
ACE	Angiotensin-Converting–Enzyme
AIHS	Alberta Innovates – Health Solutions
ANOVA	Analysis of Variance
BMI	Body Mass Index
bpm	Beats Per Minute
Ca^{2+}	Calcium
CBF	Cerebral Blood Flow
CHREB	Conjoint Health Research Ethics Board
CIH	Chronic Intermittent Hypoxia
CIHR	The Canadian Institutes of Health Research
CLBX	Celecoxib
CO	Cardiac Output
CO_2	Carbon Dioxide
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
COX-3	Cyclooxygenase-3
COXIBs	Selectively Cyclooxygenase-2 Inhibitors
CPAP	Continuous Positive Airway Pressure
CVC	Cerebrovascular Conductance
CVD	Cardiovascular Diseases
CVR	Cerebrovascular Resistance
DBP	Diastolic Blood Pressure

DEF	Dynamic End-tidal Forcing
ECG	Electrocardiogram
ED	Endothelial Dysfunction
EDCF	Endothelium-derived Contracting Factor
EDHF	Endothelium-derived Hyperpolarizing Factor
EDRF	Endothelium-derived Relaxing Factor
EIA	Enzyme Immunoassay
ET-1	Endothelin-1
GLM	General linear Model
HR	Heart Rate
IH	Intermittent Hypoxia
INDO	Indomethacin
INR	International Normalized Ratio
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOX	Lipoxygenase
MAP	Mean Arterial Blood Pressure
MBP	Mean Arterial Blood Pressure
MCA	Middle Cerebral Artery
mmHg	Millimeter of Mercury
MSK	Musculoskeletal Disorders
NO	Nitric Oxide
NSAIDs	Non-steroidal Anti-inflammatory Drugs
NSERC	Natural Sciences and Engineering Research Council of Canada
O_2	Oxygen
OSA	Obstructive Sleep Apnea
$\overline{P}$	Power (Total power of the Doppler spectrum averaged over the Cardiac cycle)
$P_{A_{CO_2}}$	Partial Pressure of Arterial Carbon Dioxide
$P_{A_{O_2}}$	Partial Pressure of Arterial Oxygen

$P_{ET}CO_2$	End-tidal Partial Pressure of Carbon Dioxide
$P_{ET}O_2$	End-tidal Partial Pressure of Oxygen
PG	Prostaglandin
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGHS	Prostaglandin H Synthase
PGI_2	Prostaglandin I_2 (i.e., Prostacyclin)
PGI_2M	Prostaglandin I_2 Metabolite
PLBO	Placebo
RDI	Respiratory Disturbance Index
RM	Repeated Measures
ROS	Reactive Oxygen Species
SaO_2	Arterial Oxyhemoglobin Saturation
SBP	Systolic Blood Pressure
SDB	Sleep Disordered Breathing
SV	Stroke Volume
SNA	Sympathetic Nerve Activity
TCD	Transcranial Doppler Ultrasound
TxA_2	Thromboxane A_2
VSM	Vascular Smooth Muscle
$\overline{V}_P$	Peak Blood Velocity of the Doppler Waveform Averaged Over the Cardiac Cycle
WHO	World Health Organization

Epigraph

"The woods are lovely, dark and deep.

But I have promises to keep,

And miles to go before I sleep,

And miles to go before I sleep."

- **ROBERT FROST**

CHAPTER ONE: INTRODUCTION

1.1 Introduction

Intermittent hypoxia (IH) is defined as repeated episodes of hypoxia interspersed with episodes of normoxia (Neubauer, 2001) leading to periods of oxygen desaturation and reoxygenation in the blood. Chronic IH (CIH) exposure is thought to play an important role in the pathophysiology of obstructive sleep apnea (OSA). OSA is a chronic medical condition characterized by repeated episodes of apnea during sleep, which are characteristically associated with oscillations in oxyhemoglobin saturation (SaO_2) (Zamarron *et al.*, 1999). Therefore, OSA patients are exposed to CIH which is thought to be the underlying mechanism that links OSA with an increased risk of cardiovascular disease (CVD) (Punjabi *et al.*, 2008).

Epidemiological studies have shown OSA to be an independent risk factor for the development of hypertension (Morrell *et al.*, 2000;Nieto *et al.*, 2000;Peppard *et al.*, 2000). In some cohort studies involving hypertensive patients, the prevalence of hypertension among OSA patients has been found to be as high as 50% (Pack & Gislason, 2003) though this is confounded by many factors such as age, sex, body mass index (BMI), alcohol consumption and smoking (Parish & Somers, 2004). In the general population, hypertension is one of the most important modifiable risk factors for the development of CVD. In 2000, hypertension was estimated to affect 26% of the adult population, and it is projected to rise up to 29% by 2025, which will contribute to increased cardiovascular morbidity and mortality (Kearney *et al.*, 2005). The World Health Organization (WHO) has indicated hypertension is the leading risk factor for

death, is predicting a hypertension epidemic, and is, therefore, advocating prevention and treatment programs (WHO, 2009).

The prevalence of OSA differs among different populations (Somers *et al.*, 2008;Punjabi, 2008) and the studies estimate that ~17% of the North American adult population may have OSA (Young *et al.*, 1993;Young *et al.*, 2005). The prevalence of OSA is even higher in specific high-risk patient populations, such as those with congestive heart failure (Prevalence: 32%) (Sin *et al.*, 1999), end-stage renal disease (Prevalence: 60%) (Kraus & Hamburger, 1997;Hanly & Pierratos, 2001), and stroke (Prevalence: 68%) (Yaggi *et al.*, 2005), and may exacerbate the considerable cardiovascular morbidity and mortality associated with these conditions.

While the mechanisms by which OSA leads to hypertension are unclear, exposure to IH has been shown to increase both systolic and diastolic blood pressure (SBP and DBP) in healthy human subjects (Foster *et al.*, 2010a) and animal studies (Gonzalez Bosc *et al.*, 2009). Hence, CIH is considered as the principal triggering factor for hypertension among OSA patients.

The role of prostaglandins (PGs) in hypertension has been widely studied with the general consensus that they play a role in the regulation of blood pressure (BP) (Wilson *et al.*, 1990;Colina-Chourio *et al.*, 2000;Helmersson-Karlqvist *et al.*, 2012). It is possible that there is a physiological alteration in the balance of vasodilatory and vasoconstrictor

PGs during IH mediated increase BP. Specifically, the balance between the concentrations of the vasodilatory molecule prostacyclin and the vasoconstrictor molecule thromboxane could be crucial. Prostacyclin also works as platelet anti-aggregation while Thromboxane promotes aggregation. Hence, the increased incidence of hypertension among OSA patients (Somers *et al.*, 2008;Young *et al.*, 2002) and hypercoagulability (Guardiola *et al.*, 2001) involve PGs.

Structurally, PGs are fatty acid-derived, they work as hormones (both endocrine and paracrine) and are short-lived (**Figure 1.1**). The terminal vascular PGs formed by Cyclooxygenase (COX) Enzymes catalyzing membrane bound Phospholipids play important roles in regulating vascular homeostasis and maintaining platelet function. There are oppositely acting vascular PGs; Prostaglandin I_2 (PGI_2), also known as Prostacyclin, and Prostaglandin E_2 (PGE_2) are vasodilatory while Thromboxane A_2 (TxA_2) and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are vasoconstrictors. Similarly, PGI_2 is a platelet anti-aggregant and TxA_2 causes platelet aggregation. Therefore, the entire downstream PGs work in an intricate balance to maintain vascular homeostasis and platelet function. The two isomerases of COX are Cyclooxygenae-1 (COX-1) which primarily forms TxA_2 (Clarke *et al.*, 1991) and Cyclooxygenae-2 (COX-2) which predominantly leads to the formation of PGI_2 and PGE_2 (Ricciotti *et al.*, 2013;Fitzgerald *et al.*, 1981;Yu *et al.*, 2012;Cannon & Cannon, 2012). Here, unopposed activity of prothrombotic cyclooxygenase-1 (COX-1)-mediated thromboxane has been implicated as a cause of the

increase in arterial blood pressure (ABP) among selective cyclooxygenase-2 (COX-2) non-steroidal anti-inflammatory drugs (NSAIDs) users (Justice & Carruthers, 2004).

The inhibition of PGs with NSAIDs is the first-line treatment for musculoskeletal (MSK) disorders (Kean & Buchanan, 2005;Risser *et al.*, 2009), a condition affecting millions (approximately 10-50%) of the world's population as estimated by WHO (WHO, 2003a;WHO, 2003b). There are more than 30 million people taking NSAIDs daily and 12 million Americans are treated with both NSAIDs and antihypertensive drugs simultaneously (Cheng & Harris, 2003). NSAIDs work by inhibiting the COX enzyme in the inflammation cascade where PGs upregulation and increased activity occurs (Vo *et al.*, 2009). Indomethacin is a commonly used nonselective COX inhibitor (Rainsford, 2007) and celecoxib is the world's most commonly prescribed selective COX-2 inhibitor (O'Connor & Lysz, 2008) as pain medication. Both types of NSAIDs (COX-1 and COX-2 inhibitors which are also known as COXIBs) have been associated with an increase in ABP, reflecting the inhibition of the vasodilatory activity of PGs (Johnson *et al.*, 1994;Aw *et al.*, 2005;Pope *et al.*, 1993). Evaluation of the contribution of PGs to the hypoxia-mediated increase in ABP is of immense interest and clinical importance given that reducing DBP by as little as 5-6 mmHg can reduce the risk of stroke by 33-50% and the risk of coronary heart disease by 4-22% (Collins *et al.*, 1990a;MacMahon *et al.*, 1990;Collins *et al.*, 1990b).

The studies involving nonselective COX inhibitors especially Indomethacin have consistently shown that they reduce cerebral blood flow (CBF) (Xie *et al.*, 2006;Fan *et*

al., 2011;Fan *et al.*, 2010;Barnes *et al.*, 2012b) suggesting that PGs play important roles in CBF. Similarly, Aspirin which is an irreversible COX-1 inhibitor is used in ischaemic stroke to thin out blood and improve cerebral perfusion by inhibiting TxA2 activity (Warden *et al.*, 2012;Patrono & Roth, 1996;Catella-Lawson, 2001). PGs also appear to work in tandem with other vascular mediators (e.g. nitric oxide) of endothelium in the cerebral vasculature (Andresen *et al.*, 2006;Capone *et al.*, 2010). Therefore, there is ample indirect evidence from human studies that PGs play vital roles in CBF. Previous studies among OSA patients have reported decreased CBF vascular reactivity and sensitivity (Urbano *et al.*, 2008;Foster *et al.*, 2007;Morgan *et al.*, 2010) but they have not explored the roles of COX inhibitors. It is known that there is endothelial dysfunction (ED) in OSA patients. The activation of endothelin in OSA patients could possibly involve the COX pathway as well (Durgan & Bryan, 2012;Capone *et al.*, 2012). Therefore, the roles of vasoactive PGs in the brain among CIH exposed individuals could be an important pathway involved in CBF regulation. From a previous study among healthy individuals undergoing six hours of IH, there was a significant increase in ABP (Foster *et al.*, 2010b). This same study also showed that there was a blunted diurnal decrease in the CBF in response to hypoxia (Foster *et al.*, 2010b). The IH mediated increase in ABP in this human model is an experimental verification of the some increase ABP in OSA patients who suffer from CIH. The roles of COX enzymes in both healthy and patient models of CIH have not been explored. Hence, using the nonselective COX inhibitor Indomethacin and the selectively COX-2 inhibitor Celecoxib (comparing with placebo), the roles of PGs can be teased out.

Therefore, the aim of the proposed study is to determine the effects of nonselective and selective COX inhibition on the ABP and CBF responses to acute isocapnic hypoxia in healthy human volunteers before and after six hours of exposure to IH.

1.2 Review of literature

1.2.1 Literature search

This literature review was compiled using the online life-science journal database PubMed. Keywords in the search used were cyclooxygenase, cyclooxygenase inhibition, cyclooxygenase inhibitors, COX inhibition, prostaglandins, eicosanoids, arachidonic acid, COX inhibitors, COX-1, COX-2, hypertension, obstructive sleep apnea (OSA), blood pressure, cerebral blood flow (CBF) and IH. Both synonyms and truncations of these words were used in conjunction with multiple key word combinations to achieve an overall review of the pertinent literature. The relevant and related literatures of the articles found and cited were also reviewed.

1.2.2 Intermittent hypoxia and hypertension

OSA is a chronic medical condition characterized as described above (**Section 1.1**) by repetitive closure of the upper airway due to pharyngeal collapse during sleep leading to sleep fragmentation, excessive daytime sleepiness, fatigue, hypoxemia (White, 2005;Ryan & Bradley, 2005;Flemons, 2002;Patil *et al.*, 2007;Dempsey *et al.*, 2010) and suffering from CIH throughout life unless treated. Hence, CIH is the central characteristic of OSA and is linked to an increased incidence of obesity, hypertension and other

cardiovascular and cerebrovascular morbidity (Wolf *et al.*, 2007;Levy *et al.*, 2009;Foster *et al.*, 2007;Reichmuth *et al.*, 2009;Kent *et al.*, 2011).

Patients with OSA often have hypertension (Nieto *et al.*, 2000;Davies *et al.*, 2000a), defined clinically as SBP above 140 mmHg and DBP above 90 mmHg (World Health Organization, 2003). CIH seems to be the main triggering factor (Foster *et al.*, 2010b;Atkeson *et al.*, 2003;Somers *et al.*, 2008;Patt *et al.*, 2010;Kent *et al.*, 2011;Fletcher, 2003;Hedner *et al.*, 1995;Dempsey *et al.*, 2010). Both OSA and hypertension are independently and strongly associated with cardiovascular diseases (Punjabi *et al.*, 2008;Pack & Gislason, 2003). While the pathophysiology of hypertension among OSA patients seems to involve multiple factors such as ED, increased reactive oxygen species (ROS), decreased nitric oxide (NO) production, increased sympathetic nerve activity (SNA), low grade systemic inflammation and angiotensin-II (ang-II) production, the role of PGs remains unclear (Krieger *et al.*, 1991a;Kimura *et al.*, 1998a).

1.2.3 Intermittent hypoxia and cerebral blood flow

OSA patients have a decreased CBF response to hypercapnia and hypoxia (Foster *et al.*, 2007;Urbano *et al.*, 2008;Reichmuth *et al.*, 2009). It has also been shown that the hypercapnic vasodilation in the cerebral circulation is blunted among OSA individuals (Reichmuth *et al.*, 2009;Morgan *et al.*, 2010) and it is restored with successful CPAP therapy (Reichmuth *et al.*, 2009). Data from animal model of OSA indicate that CIH increases ROS production in cerebral blood vessels and neurons with the suggestion that it may reduce the production of nitric oxide and PGs (Capone *et al.*, 2012) although this

interaction has not been specifically investigated. Hence, the cerebrovascular consequences of OSA involve blunted cerebrovascular regulation involving increased ROS production, alterations in neurovascular coupling and possibly changes in NO and PG productions (Durgan & Bryan, 2012).

1.2.4 Cyclooxygenase (COX) enzymes

The cyclooxygenase (COX) isoenzyme includes COX-1, COX-2 and COX-3. COX-1 and COX-2 are widely expressed (Rouzer & Marnett, 2009;Dubois *et al.*, 1998;Smith *et al.*, 2000;Vane *et al.*, 1998) and have been well studied but little is known about COX-3 (Berenbaum, 2004;Schwab *et al.*, 2003). COX-1 is usually constitutively expressed in gut mucosa (protection), platelet (aggregation) and regulation of renal hemodynamic and renal perfusion (Botting, 2006) and vascular homeostasis (Siegle *et al.*, 1998). COX-1 is often referred as a 'housekeeping' enzyme for its protective nature. COX-2 is primarily an inducible isoform and is expressed in responses to the pain, inflammation, fever, trauma, or stress (Gajraj, 2003). COX-2 is also expressed in cancer tissues (tumor hypoxia) e.g. colorectal cancer (Elder *et al.*, 2000), brain tumor (Karim *et al.*, 2005a), breast cancer (Howe & Dannenberg, 2003;Singh & Lucci, 2002;Davies *et al.*, 2002) contributing in tumor angiogenesis and progression (Karim *et al.*, 2005b;Iniguez *et al.*, 2003).

However, COX-2 is physiologically expressed as well in kidney and brain (Lipsky, 1999). COX-2 derived PGs play crucial role to maintain renal perfusion synergistically with locally produced vasodilator NO. They often counteract the vasoconstrictor effects

of Angiotensin-II or renal sympathetic activity (Lopez *et al.*, 2003). Animal studies have consistently reported vital roles of both COX-1 and COX-2 in maintaining CBF and vascular homeostasis in the brain (Liu *et al.*, 2012c;Matsuura *et al.*, 2009;Stefanovic *et al.*, 2006). In humans, several studies with nonselective COX inhibitor (inhibiting both COX-1 and COX-2; Indomethacin) have shown the involvement of these enzymes in CBF regulation (Xie *et al.*, 2006;Fan *et al.*, 2011;Fan *et al.*, 2010;Barnes *et al.*, 2012b). To our knowledge, the COX-2 has not yet been explored in human brain and blood flow both directly or indirectly.

1.2.4.1 Prostaglandins

Prostaglandins are a group of short-lived, fatty acid-derived local hormones expressed in pain, inflammation and stress; vasodilation and vasoconstriction (blood vessels); smooth muscle contraction, ovulation and immune response (blood vessels, duct smooth muscles, airways and gut mucosa) and other physiological processes (e.g. normal platelet function and leukocytes) (Evett *et al.*, 1993;Grosser *et al.*, 2010). The activation of phospholipase A_2 (PLA_2) initiates eicosanoid biosynthesis in mammalian cells. This is followed by the release of arachidonic acid (AA) from cell membrane phospholipids in response to the interaction of a stimulus with a receptor within the cell membrane. Next, prostaglandin endoperoxide synthase enzyme (PGH synthase) catalyzes the AA into different PGs. The different PGs are Prostaglandin I_2 which is also called Prostacyclin (PGI_2), Prostaglandin E_2 (PGE_2), Thromboaxane A_2 (TxA_2) Prostaglandin D_2 (PGD_2) and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Claria, 2003). The PGH synthase is also known as the COX enzyme (DeWitt *et*

al., 1993) and is expressed in different organs of human body (Gryglewski, 2008). The COX-PG pathway is illustrated in **Figure 1.2** (Fries & Grosser, 2005).

The in vivo, ex-vivo, and human studies exploring specific roles of COX pathways and downstream PGs along with their receptors (Narumiya *et al.*, 1999;Woodward *et al.*, 2011) have highlighted pathophysiological roles of PGs (Vane, 1971;Jadhav *et al.*, 2009;Potter *et al.*, 2011;Liu *et al.*, 2012a;Liu *et al.*, 2012b;Niwa *et al.*, 2001). The PGs have their own multiple receptors e.g. PGI_2 (IPs), PGE_2 (EPs), TxA_2 (TP), PD_2 (DPs) to act through (Narumiya *et al.*, 1999;Woodward *et al.*, 2011;Liu *et al.*, 2012a). They are involved in normal vascular regulation and cellular responses; and disease pathology (Davidge, 2001;Ricciotti & FitzGerald, 2011). The human models of COX pathway studies have, so far, been limited to a few nonselective COX inhibitors in brain and ventilatory responses (Xie *et al.*, 2006;Fan *et al.*, 2011;Barnes *et al.*, 2012b;Fan *et al.*, 2010), exercise and cardiovascular responses (Markwald *et al.*, 2011;Crecelius *et al.*, 2011a;Crecelius *et al.*, 2011b;Barnes *et al.*, 2012a) and other systemic vascular functions (Morales *et al.*, 2012;Casey & Joyner, 2011;Barnes *et al.*, 2012a). The studies looking into brain blood flow and blood pressure have not yet looked into downstream prostaglandins either.

1.2.4.2 Prostaglandins, blood pressure and cardiovascular homeostasis

It has long been shown that PGs play an important role in the regulation of renal vascular function and BP. The most important PGs are PGI_2, PGE_2, TxA2 and $PGF_{2\alpha}$ (Ponnuchamy & Khalil, 2009). Most of the terminal PGs are produced from a common

substrates of AA by catalyzing of both COX-1 and COX-2 (Caughey *et al.*, 2001). PGI_2 and PGE_2 are mainly mediated through COX-2 while TxA_2 and $PGF_{2\alpha}$ are formed through COX-1 (Smith & Langenbach, 2001;Smith *et al.*, 2000) in the PG cascade. PGI_2 and PGE_2 increase renal medullary blood flow which drives pressure natriuresis and diuresis (Fries & Grosser, 2005). Most of these PGs (e.g. PGI_2, PGE_2, TxA_2, $PGF_{2\alpha}$) play an important role in the regulation of normal vascular tone and BP (Sellers & Stallone, 2008).

It is presumed that the endothelium-derived vasoconstrictor PGs, mainly through the COX-1 pathway, contribute to the ED that occurs with aging and spontaneous hypertension (Vanhoutte, 2009). In a study, postoperative hypertension has been associated with the relative increase of TxA_2 and lower production of PGI_2 (Wang *et al.*, 2008) showing vasoconstrictor component being more active. Studies using animal models have revealed that PGI_2 suppression leads to the elevation of BP, acceleration of atherogenesis and modulation of the vascular response to hemodynamic stress (Rudic *et al.*, 2005). COX-2 knockout mice have shown increased thrombotic activity (Seta *et al.*, 2009).

Vasodilation, platelet clumping inhibition, disruption of previously aggregated platelets are attributed to PGI_2 while TxA_2 is a potent platelet aggregating agent, causing vasoconstriction and promoting the proliferation of vascular smooth muscle cells (VSMCs) (Funk & Fitzgerald, 2007). Therefore, the complex interplay of opposing

components PGI_2 and TxA_2 is implicated in the delicate balance of cardiovascular risk. It is assumed COX inhibition causes PGI_2 and PGE_2 inhibition while TxA_2 remains unopposed especially during COX-2 selective inhibition leading to increased cardiovascular risk (McAdam *et al.*, 1999;Catella-Lawson *et al.*, 1999;Moncada & Vane, 1981;Cheng *et al.*, 2002;Yu *et al.*, 2012).

Recent studies reveal that even traditional NSAIDs (i.e. nonselective COX inhibitors) are associated increased cardiovascular toxicity. Acetaminophen has been found to raise BP among patients with coronary artery disease (Sudano *et al.*, 2010;White & Campbell, 2010), and a large population-based case-control study reported the use of NSAIDs increase the risk of atrial fibrillation or flutter and it is more so with COX-2 inhibitors (Morten *et al.*, 2011;Jerry, 2011).

1.2.4.3 Prostaglandins in the brain: Neurovascular expression

The regulation of the blood flow to the brain is complex and involves multiple factors. As the brain is a vital organ required for life, the need for teasing out the intricacy of this regulation is a paramount importance while we attempt to deal with various insults to it such as stroke, small vessel disease, concussion and head injury. A variety of chemical stimuli have been used to study cerebral blood flow and the molecules involved in its regulation. Most terminal PGs are actively involved in central and peripheral blood flow regulation. As described earlier, PGs are constitutively produced to maintain normal physiology and also induced during stress, inflammation and injury. It is even more challenging in the case of brain when considering the involvement of PGs since the

source of PGs in the brain that act on the smooth muscle cell of the cerebral vasculature involves circulating platelets, endothelial cells and neurons.

The constitutive COX pathways appear to differ depending upon the organ and site of expression. For example, COX-2 is physiologically expressed in kidney to maintain homeostasis while COX-1 is constitutively expressed in gut mucosa to protect from stress and inflammation (Claria, 2003;Dubois *et al.*, 1998). Whereas neurons and their terminals seem to express COX-1 constitutively as shown from the animal study tissue preparations (Garcia-Bueno *et al.*, 2009;Capone *et al.*, 2010) while it is understood COX-2 is physiologically expressed in brain blood vessel endothelium (Andresen *et al.*, 2006). The final activity would probably be the same for all terminal PGs. However, it is not well known which one is predominant in the regulation of blood flow to the brain: circulating platelets' TxA2, endothelial $TxA_2/PGI_2/PGE_2$ or neural PGs. A carefully designed study using nonselective *versus* selective (COX-2) inhibitors could enable us to tease out the PGs active in blood vessels (quantification of major vasodilator PG vs major vasoconstrictor PG). The effect of neural PGs can be assessed but the direct quantification and assessment will be difficult.

Cerebrovascular reactivity is altered in states where neurovascular coupling is profoundly disrupted in many disease conditions e.g. ischemic stroke, Alzheimer disease, and hypertension which compromises brain function (Girouard & Iadecola, 2006). Neurovascular coupling refers to the communication between neurons and the

vasculature at the local level in the brain. The relationship between local neural activity and adjacent blood vessels leads to subsequent changes/controls in CBF. Hence, one could use transcranial Doppler ultrasound (TCD) to measure CBF and compute vasoreactivity to reflect the condition of neurovascular coupling.

In addition, the time taken by COX inhibitors to reach the neurons may be longer than that during which they act on platelets and endothelium. Hence, peak plasma steady-state values could be the ideal point to measure physiological responses. Hence, it can be assumed the peak plasma level might when there is an effective concentration within neural cells to cause its desired effect. Therefore, the best way to study the COX inhibition and explore the pathway along with terminal PGs among human subjects would be to use selective and nonselective COX inhibitors in an elegantly designed experiment and quantify PGs. The variables measured and PGs quantified will provide a picture of the physiological roles of COX enzyme and COX inhibitors' effect on them.

1.2.5 Research challenges in COX inhibition and prostaglandins

While COX inhibiting drugs are utilized extensively, challenges still remain to fully understand the specific cellular, tissue and vascular pathways through which these drugs act. The phenotypic expressions of these enzymes (inhibiting and activating) can provide important insight about physiological and clinical implication. The challenge is that the end products of the COX pathways (i.e., terminal PGs) act through autocrine, paracrine, and even endocrine manners to maintain homeostasis (Kobayashi & Narumiya, 2002;Banu *et al.*, 2003;Ruan *et al.*, 2011;Yuhki *et al.*, 2011) as illustrated in **Figure 1.1**.

The difficulty is even more prominent while the activities of COX pathways continue ex-vivo such as during blood sampling. This is especially important with respect to platelets (i.e., COX-1 activity affecting TxA_2). Secondly, the breakdown of PGs occurs immediately to different metabolites (Mitchell, 1992). Another body fluid that could be used is urine, but the problem is same as of blood i.e. breakdown. Urine sampling and quantification is confounded with the fact that kidneys produce PGs to maintain blood flow, salt and water balance. Hence, urinary PGs do not necessarily reflect circulating PGs.

It is also not clear whether measured metabolites represent actual circulating PGs that play an important role as vascular mediators in the vascular beds (Vane & Corin, 2003) as illustrated in **Figure 1.3**. However, there are verified methodologies available to measure stable metabolites of specific PGs in urine and the techniques seem to be the best way to quantify the PGs thus far. Therefore, a novel experiment with proper design of drug intervention with placebo control, the urinary PG quantification should reflect vascular and physiological expression (Tsikas *et al.*, 2012;Wennmalm & Fitzgerald, 1988;Tsikas *et al.*, 2003).

1.2.6 COX inhibitors, hypertension and cardiovascular risk

Population studies and the WHO estimate that 10-50% of individuals suffer from rheumatic disorders (arthritis and musculoskeletal disorders) (WHO, 2003b;Woolf & Pfleger, 2003;Brooks, 2006;Brooks, 2006). Most of them suffer from severe pain and require NSAIDs for long term management (Kean & Buchanan, 2005). NSAIDs are one

of the most frequently prescribed drugs around the world and about 30 million people are exposed to them every day (Aalykke & Lauritsen, 2001). Between 35 and 70 million prescriptions are written each year in US (Abdul-Hadi *et al.*, 2009) and the burden could be underestimated as many NSAIDs are available without a prescription (Grootendorst *et al.*, 2005).

NSAIDs have been shown to elevate SBP and DBP (Sowers *et al.*, 2005;Ahmed *et al.*, 2009), destabilize controlled hypertension, contribute to resistant hypertension (Moser & Setaro, 2006) and interfere with the BP lowering effect of all antihypertensive drug classes except calcium channel blockers (Sarafidis & Bakris, 2008). However the mechanism of action for NSAIDs to cause a rise in blood pressure and interference with antihypertensive medications are still not clear. It is known that NSAIDs inhibit the production of local renal vasodilator PGs (i.e., PGE_2 and PGI_2), thereby decreasing renal blood flow which leads to sodium and fluid retention (Sarafidis & Bakris, 2008), which ultimately contributes to increasing BP. Data regarding the impact of COX-2 selective inhibitors are conflicting (Lipsky *et al.*, 2000;Bannwarth & Lipsky, 2001). The therapeutic action of antihypertensive agents like angiotensin-converting–enzyme (ACE) inhibitors, loop diuretics depend on the availability of these PGs (PGI_2 and PGE_2) (Fierro-Carrion & Ram, 1997;Whelton *et al.*, 2001). Hence, NSAIDs appear to reduce the efficacy of these antihypertensive agents due to the reduction of PGs that occur with their use. Therefore, the cardiovascular risks of COX inhibitors are clearly based on the

increased incidence of hypertension and augmented thrombotic potentials (Seta *et al.*, 2009;Cheng *et al.*, 2002;Stichtenoth *et al.*, 2005;Yu *et al.*, 2012).

1.2.7 Intermittent hypoxia, vascular inflammation and prostaglandin

The cardiovascular risks among OSA patients have been well documented. OSA typically have usually high BMI, have higher circulating levels of cholesterol, increased risk of diabetes and hypertension, all of which are risk factors for cardiovascular morbidity and mortality. OSA, along with other forms of SDB (e.g. central sleep apnea, Cheyne-Stokes breathing), include pathophysiological mechanisms that result in CIH and subsequent cardiovascular consequences. The cardiovascular consequences of these conditions, along with hypertension, are related to ED, increased SNA, activation of renin-angiotensin system (RAS) and triggering an inflammatory cascade (Foster *et al.*, 2010b;Atkeson *et al.*, 2003;Somers *et al.*, 2008;Patt *et al.*, 2010;Kent *et al.*, 2011;Fletcher, 2003;Hedner *et al.*, 1995;Dempsey *et al.*, 2010). The vascular level of inflammation triggered by CIH could well be the central point of cerebrovascular and other cardiovascular consequences among OSA individuals (Jelic *et al.*, 2008;Jelic & Le Jemtel, 2008a;Kasasbeh *et al.*, 2006;Miller & Cappuccio, 2007). The inflammatory cascade in the blood vessels could be mediated by COX pathway to form vascular PGs to maintain balance in the blood vessels especially among platelets, endothelium and VSMCs. It has reported that OSA patients have hypercoagulability (Guardiola *et al.*, 2001) which might be mediated by activation of platelet TxA_2.

In hypoxia mediated vascular inflammation, the role of COX pathway is not well elucidated although it is well known that COX-2 is over-expressed (Fredenburgh *et al.*, 2008). The vasodilator PGs (e.g. PGI_2 and PGE_2 are mainly formed through this pathway and, have been shown to promote vascular leakage, angiogenesis and vasodilation in tumor hypoxia environment (Kundu & Surh, 2008). At the same time, the effect of IH on the formation of TxA_2, which are mainly produced by platelets (via COX-1) and have potent vasoconstrictor activity, is unknown. If CIH increases TxA_2 activity, it could well increase the risk of hypertension and thrombotic events. The cellular level of inflammation in the endothelium and VSMCs could involve PGs, NO and endothelium-derived relaxing and constricting factors (EDRFs and EDCFs) to increase vascular tone or relax it. Hence, it is possible that endothelial PGs play an important role in vascular tone while platelet TxA_2 formation is more involved in thrombotic activity. Previous research has strongly correlated vascular inflammatory responses with OSA and an augmented COX-2 expression (Li *et al.*, 2003;Jelic *et al.*, 2008;Jelic & Le Jemtel, 2008b). Hence, IH mediated vascular inflammation appears to involve COX pathway and terminal PGs which interact with nitric oxide to maintain vascular tone.

1.2.8 Hypoxia induced vascular expression of prostaglandins

The mechanism of IH induced hypertension among animal models and experimental human models simulating OSA have revealed there are multiple mechanisms involved from central and peripheral nervous system (increased SNA and catecholamine secretion, decreased baroreflex and enhanced chemoreflex sensitivity) to vascular factors e.g. increased productions of ROS, angiotensin II (ang II) and edothelin-1 (ET-1) (Gonzalez

Bosc *et al.*, 2009;Kanagy, 2009;Fletcher, 2001;Fletcher *et al.*, 2002;Prabhakar *et al.*, 2001;Foster *et al.*, 2010a).

Animal studies have shown increased tissue expression of COX-2 under IH exposure in the brain (Li *et al.*, 2003;Kheirandish *et al.*, 2005) but it is not clear whether there is a consequent increased production of vasodilator PGI_2 or vasoconstrictor TxA_2. Jelic et al. have shown that COX-2 expression is reduced in vascular endothelial cells among OSA patients following CPAP treatment (Jelic *et al.*, 2008). The urinary PG metabolites among OSA patients have shown either decreased production of dilatory versus constrictor PGs (Krieger *et al.*, 1991a) or a compensatory increase in dilatory PGs (Kimura *et al.*, 1998a). Hence, the involvement of these PGs remains unresolved.

A study using knock-out mice showed that hypoxia-induced pulmonary hypertension and vascular remodeling are exacerbated among the COX-2 deficient group where there is enhanced ET-1 receptor expression and increased pulmonary artery VMSCs hypertrophy (Fredenburgh *et al.*, 2008). The deletion of the COX-2 gene has been found to exacerbate pulmonary hypertension, enhance sensitivity to TxA_2, and induce intravascular thrombosis in response to hypoxia suggesting that PGs modulate the pulmonary response to hypoxia (Cathcart *et al.*, 2008;Pidgeon *et al.*, 2004). Similarly, in the pulmonary vasculature of newborn piglets, there is an increased production of vasoconstrictor PGs during the early phase of chronic hypoxia-induced pulmonary hypertension (Fike *et al.*, 2003). A study among neonatal hypoxemic respiratory failure human patients has found a

predominance of vasoconstrictor activity as the basis of pulmonary hypertension (Sood *et al.*, 2007). This is why PGI_2 analogues (e.g. Iloprost, Treprostinil) have been used to treat pulmonary artery hypertension (as a vasodilator) (Badesch *et al.*, 2004). Hence, it is consistent that hypoxia induces COX-2 expression in pulmonary artery VSMCs during hypoxic exposure and it seems vasoconstrictor PGs play predominant roles. To date, there are no studies on PGs involvement in systemic hypertension, and vascular physiology under IH exposure simulating OSA patients among healthy human subjects.

1.2.9 Prostaglandins, intermittent and systemic hypertension

Prostaglandins play a predominant role in the regulation of renal blood flow and systemic ABP (Wilson *et al.*, 1990;Colina-Chourio *et al.*, 2000). However, the exact molecular mechanism of PGs involvement in BP regulation is complex and the effects of COX inhibition are difficult to predict because the ultimate effect of inhibition depends upon on the interplay of vasodilator (PGE_2 and PGI_2) and vasoconstrictor (TxA_2 and $PGF_{2\alpha}$) groups (Nasjletti, 1998). In general, PGs play a crucial role in vascular endothelial function, renal blood flow homeostasis and, therefore, systemic BP regulation (Vanhoutte, 1989;Davidge, 2001;He & MacGregor, 2003;Francois *et al.*, 2004;Francois *et al.*, 2008;Anderson *et al.*, 1976).

With respect to IH and OSA, Krieger et al (Krieger *et al.*, 1991a) measured urinary excretion of 6-keto-$PGF_{1\alpha}$ and thromboxane TxB_2 (stable metabolites of PGI_2 and of TxA_2, respectively) in a group of OSA patients with systemic hypertension and found a

decrease in the PGI_2 to thromboxane ratio indicating a shift towards potential vasoconstriction. In addition, Kimura H et al (Kimura *et al.*, 1998a) found an elevated PGI_2 to TxA_2 ratio (indicating higher amounts of PGI_2 (i.e., vasodilator) than Tx A_2 (i.e., vasoconstrictor)) compared to healthy, age-matched controls that was decreased following 3 days of effective treatment of OSA with nasal CPAP. These findings suggest the production of PGs is shifted toward vasodilatation in untreated OSA patients, potentially reflecting a compensatory increase in the vasodilatory PGI_2. Therefore, it is still unclear whether PGI_2 is involved as a compensatory mechanism to the vasoconstriction caused by OSA or if enhanced TxA_2 is an active component of OSA induced hypertension.

To our knowledge, there have been no studies investigating the role of PGs in IH-induced systemic hypertension in healthy human volunteers exposed to IH similar to that experienced by patients with severe OSA. Given the roles PGs play in vascular regulation in brain, cardiovascular homeostasis and vascular inflammation/stress/injury; it can be assumed that they play major roles in IH induced vascular perturbation. Considering the need and widespread use of COX inhibitors, studies teasing out downstream vascular PGs will be of physiological and pathophysiological significance.

1.2.10 Vascular function in intermittent hypoxia and COX inhibition

Most of the cardiovascular consequences among OSA patients could be explained on the basis of ED. The ED is the central part of other disease conditions e.g. hypertension, diabetes, obesity, dyslipidemia which often coexist with OSA. CIH is associated with

hypertension and a previous study from our lab using a healthy, human model mimicking 6 hours of IH exposure associated with severe OSA increases mean ABP (MAP) (Foster *et al.*, 2010a). The ED involves disarray among a variety of vasodilators and vasoconstrictor molecules that maintain vascular homeostasis. Among them NO and PGs play vital roles (Moncada, 2006). The manipulation of PGs formation via COX inhibitors (both NSAIDs and COXIBs) has been associated with cardiovascular toxicity (Trelle *et al.*, 2011;Fosslien, 2005;Funk & Fitzgerald, 2007;Cannon & Cannon, 2012). Considering COX is both constitutively expressed and induced in many disease conditions (e.g., inflammation, stress, injury), an organ specific vascular characterization and a molecular basis of COX expression might be necessary to explain the basis of toxicity. The most plausible explanation of cardiovascular toxicity of COX-2 inhibitors is based on imbalance between vasodilator-vasoconstrictor PGs (FitzGerald, 2004;Yu *et al.*, 2012) which postulates raising BP and increasing thrombotic potentials. This much emphasized imbalance theory between vasoconstrictor-vasodilator PG production emphasizes in causing hypertension and increasing thrombotic events (Cheng *et al.*, 2002;Yu *et al.*, 2012;Grosser *et al.*, 2010;Grosser *et al.*, 2006;Stichtenoth *et al.*, 2005). A perturbation in homeostatic balance does happen and this has deleterious effect on the cardiovascular system which can lead to vascular diseases such as hypertension, stroke, atherosclerosis or myocardial infarction although most of the literature on this explanatory theory come from animal studies (Flavahan, 2007). Moreover, the cardiovascular side effects of these medications may be explained by the fact that these drugs raise BP, increase thrombotic events, retain salt and water and impair kidney function. A more holistic approach to

COX-inhibitor toxicity will need further studies and exploration beyond the imbalance theory as has been proposed in a cardio-renal concept (Whelton *et al.*, 2001;DeMaria & Weir, 2003;Hinz *et al.*, 2007).

Human physiological studies could help explain the remaining gaps between animal and human studies and help with the clinical setbacks these drugs (i.e., NSAID and COXIBS) have suffered due to unexpected adverse events. By using a very controlled and sophisticated technology, experiments can be designed that mimic OSA within healthy individuals. Having mentioned the deleterious outcomes of OSA (i.e., CIH) and showing that our model of the IH experienced each night by patients with severe OSA raises blood pressure and blunts the CBF response to both IH (Foster *et al.*, 2007;Reichmuth *et al.*, 2009) and carbon dioxide (Reichmuth *et al.*, 2009;Urbano *et al.*, 2008), the next step would be to look into different mediators that play an important role in the cardiovascular system imposed by IH exposure. The role of COX pathways and terminal PGs among OSA patients and IH is largely unknown (Fosslien, 2005). From cancer studies, COX-2 has been shown to be augmented and modulate angiogenesis in response to hypoxia resulting from vascular inflammation (Wang *et al.*, 2007;Turini & Dubois, 2002;Salvado *et al.*, 2012). This gives an indication that PGI_2 and PGE_2 are more widely expressed in tumor hypoxia and angiogenesis. Animal studies mimicking OSA (i.e., CIH induction) have reported that there is increased COX-2 expression and PG production (Li *et al.*, 2003;Nacher *et al.*, 2009). The evidence is further consolidated with a large population based studies among SDB population with increased cancer incidence (Nieto *et al.*, 2012)

and mortality (Martinez-Garcia *et al.*, 2012). The hypoxia and inflammation are the central part of the tumor pathology and IH has been shown to increase cancer progression in animal model of OSA (Almendros *et al.*, 2012). The centre of this pathophysiology could be augmented vascular COX expression.

Interestingly, there are no studies looking into the effect of CIH among healthy individuals on COX pathway and PG production. Using pharmaceuticals that either selectively inhibits specific COX isomerases or are nonselective for COX isomerases could help to tease out the intricate pathway involved in vascular homeostasis. Using the drugs that inhibit COX pathway that are already known for their cardiovascular toxicity, from clinical population, would make a study even more robust. The identification of terminal PGs in this study will help unravel the vascular mechanism that COX pathway plays under the effect of COX inhibiting drugs, CIH and placebo.

1.2.11 Prostaglandins, cerebral blood flow and intermittent hypoxia

Prostaglandin synthesis by the cerebral vasculature is qualitatively similar to that of other blood vessels in the body with the most abundantly produced PGs in the brain include PGI_2, PGE_2, $PGF_{2\alpha}$, PGD_2, and TxA_2 (White & Hagen, 1982). Similar to the peripheral vasculature, TxA_2 and $PGF_{2\alpha}$ are mainly cerebral vasoconstrictors in adults while PGI_2 causes cerebral vasodilatation (Chemtob *et al.*, 1996). The effect of PGE_2 produced by brain endothelium is mainly vasodilatory action (Andresen *et al.*, 2006) but its effect differs with maturity and age (Chemtob *et al.*, 1996). The vasodilating (PGI_2 and PGE_2)

and vasoconstricting ($PGF_{2\alpha}$ and TxA_2) mediators in the brain from the PG pathway are illustrated in **Figure 1.4** (Andresen *et al.*, 2006).

Therefore, COX inhibitors may have an important influence in the cerebral circulation. The microvessels, mainly capillaries, synthesize predominantly PGI_2 (White & Hagen, 1982) which is needed for hypercapnia-mediated cerebral vasodilatation to initiate (Pickard *et al.*, 1980). Indomethacin, a nonselective COX inhibitor, has been found to reduce resting CBF by approximately 25% while preserving the reactivity of the cerebrovascular circulation to CO_2 (Pickles *et al.*, 1984). However, more recent and well controlled studies have consistently reported that the CBF reactivity to hypercapnia is blunted by ~ 20-25% among young healthy individuals(Xie *et al.*, 2006;Fan *et al.*, 2011) and the indomethacin effect seems equally robust among older individuals as well (Barnes *et al.*, 2012b). Indomethacin also decreases intracranial pressure, increases cerebral perfusion pressure (Puppo *et al.*, 2007) and regulates vascular tone by acting on the endothelium despite its poor intake in the brain (Upton *et al.*, 2008).

So far these cerebrovascular sensitivity and reactivity studies in human have been carried out with the administration of nonselective COX inhibitors (almost exclusively indomethacin) (Xie *et al.*, 2009a;Xie *et al.*, 2006;Fan *et al.*, 2011;Barnes *et al.*, 2012b) but there are no investigations comparing with selective COX-2 inhibition. Furthermore, the roles of PGs are indirect as none of these studies have actually measured them. Therefore, there is a need of further studies to tease out the roles of specific COX

enzymes and terminal PGs. Designing a study with nonselective COX inhibitors vs selective COX inhibitors and measuring PGs in body fluid could be an ideal setup. Hence, the effects of indomethacin (a nonselective COX inhibitor) and celecoxib (a selective COX-2 inhibitor) on the CBF response during and following IH exposure among healthy human volunteer subjects have been pursued.

1.3 Rationales

1.3.1 Rationale of the study

IH is observed in many SDB conditions such as OSA, Cheyne–Stokes respiration, central sleep apnea and obesity related hypoventilation (Levy *et al.*, 2008). Similar kinds of IH exposure occurs among Andean workers (*e.g.*, miners and observatory workers (Richalet *et al.*, 2002)) and hypoxic tent training among athletes to improve performance (Wehrlin *et al.*, 2006).

IH exposure is a central component of the pathophysiology of hypertension among OSA patients. However, the complete mechanisms underlying hypertension among OSA patients is still unclear. Therefore, IH exposure simulating that experienced by patients with OSA could provide a better understanding of vascular consequences in terms of pathophysiology and disease management.

To date, there have been no studies examining the roles of the PG pathway in the IH-mediated increase in ABP and blunted CBF response. Hence, the use of an experimental human model of IH with COX inhibition (both nonselective and selective) may enhance our understanding of the complex pathophysiology of IH-induced hypertension and alterations in CBF regulation. Understanding the roles of the vasodilator PGI_2 and vasoconstrictor TxA_2 in BP and CBF regulation following IH exposure is an important milestone in comprehending hypertension and cerebrovascular physiology and could possibly benefit OSA patients.

The effects of COX inhibitors (especially a nonselective Indomethacin) have been used to studying CBF physiology. It is has been uniformly reported that high doses of Indomethacin (100mg or 1.2mg/kg body weight) reduce CBF after 90 minutes (Xie *et al.*, 2006;Xie *et al.*, 2009b;Fan *et al.*, 2011;Barnes *et al.*, 2012b). Unfortunately, important questions remain unanswered such, what happens to the terminal PGs and to COX-1 or COX-2 pathway responsible. Hence, crucial physiological questions remain unanswered from the abovementioned studies.

It is well known that platelets produce COX-1 and this is where Aspirin irreversibly acts to inhibit the production of TxA_2 (effective platelet anti-aggregation). The endothelium produces both COX-1 and COX-2 working in tandem with platelets to maintain vascular tone and platelet function. NSAID toxicity, especially in long term use, that may lead to cardiovascular morbidity and mortality appears to involve both COX-1 and COX-2 inhibition. The COX-2 enzyme is expressed to maintain normal homeostasis, during hypoxia, inflammation, injury, plaque formation, and cancer angiogenesis. Most of these mechanisms are based on ex-vivo and animal studies while the clinical data suggest the potential for toxicity as the drugs are in use. Hence, a properly designed human study is long warranted in this field (Knights *et al.*, 2010).

The NSAID medications used in the present study are currently in use among the clinical population. As of 2009, Celecoxib is the only FDA approved selectively COX-2 inhibitors in North America (Hinz *et al.*, 2007;Marnett, 2009) as others were either

banned or voluntarily withdrawn from the market (Melnikova, 2005). The use of these medications was 'off-label' among healthy populations under IH exposure. The dose and duration of the drugs is based on their clinical uses regimen for acute inflammation treatment. It can range from acute inflammation, injury, migraine, sports to a long term use populations likes of osteoarthritis (OA), rheumatoid arthritis (RA) and aging population. The study designed was to look into multiple effects on the outcome measures under the effect of these drugs. The outcome measures will not only enable us to look into the traditional theory of COX inhibition and CV toxicity of imbalance in PGs but also into the cerebral effects.

1.3.2 Rationale of the drug choice and regimen

A relatively large dose of nonselective COX inhibitor (indomethacin) has been used to study cerebrovascular, cardiovascular and ventilatory response studies in humans (Xie *et al.*, 2009a;Xie *et al.*, 2006;Fan *et al.*, 2011;Barnes *et al.*, 2012b). There are no studies so far comparing indomethacin with selective COX-2 inhibition. The discussion of the findings have uniformly implicated the responses are due to the inhibited downstream PGs formation without actually measuring them. Therefore, there is a need of further studies to tease out role specific COX enzymes and quantification of terminal PGs. Hence, we decided to use indomethacin (nonselective COX inhibitor), celecoxib (COX-2 selective inhibitor) and compare them with placebo.

Indomethacin, the nonselective COX inhibitor, has a serum half-life of 3.5 – 5.5 hours (Adams *et al.*, 1982) and 200mg/day is the recommended maximum daily dose for acute

pain, inflammation and injury. Therefore, 50 mg oral dose three times a day for five days was chosen. The serum half-life of celecoxib, a selective COX-2 inhibitor, is 11 – 11.5 hours (Qin *et al.*, 2008;Sauter *et al.*, 2008) and dosage used in anti-inflammatory treatments are 100mg oral once daily to 400mg oral twice daily (Mitchell & Warner, 2006) reaching maximum of 800mg/day. Therefore, considering a half-life ~ 12 hours and maximum drug dosage, 200mg oral twice a day (Stichtenoth *et al.*, 2005;Schwartz *et al.*, 2004) for five days (Stichtenoth *et al.*, 2005) was chosen. A visually matched placebo was given on the third session in a similar dosage regimen. The last dose was spared on fifth experimental day since data would be collected by then. The pharmacodynamics and pharmacokinetics of these drugs have been presented as table in a detailed manner (**Appendix A**). The similar dosage regimens used in a previous study has revealed changes in urinary PGs (Stichtenoth *et al.*, 2005). Similarly, six hours of IH exposure among healthy human subjects has shown increase in ABP (Foster *et al.*, 2010a). Hence in this experimental design, the abovementioned dosages should have significant physiological changes in PGs as well as BP response during 6 hour IH exposure.

1.4 Objectives

Objective 1: To measure and compare MAP, SBP and DBP in indomethacin, celecoxib and placebo phases under IH exposure in healthy men.

Objective 2: To measure middle cerebral artery blood velocity in indomethacin, celecoxib and placebo phases during isocapnic hypoxia exposure in healthy men.

Objective 3: To quantify urinary terminal prostaglandins among indomethacin, celecoxib and placebo phases in healthy men.

1.5 Hypotheses

Hypothesis 1: Both nonselective inhibitors that target COX-1 and COX-2 and a selective inhibitor for COX-2 inhibitors will exacerbate the IH-mediated increase in blood pressure among healthy volunteers.

Hypothesis 2: There will be a reduction in basal cerebral blood flow with both indomethacin and celecoxib in response to IH compared to the placebo phase. The reduction in basal CBF in the celecoxib phase will be greater compared to the reduction in the indomethacin phase.

Hypothesis 3: The increase in BP and decrease in CBF will be mediated by differential inhibition of downstream vasoactive prostaglandins mediated by COX-1 and COX-2 enzymes.

1.6 Expected outcomes

1) There would be an increase in MAP in indomethacin, celecoxib and placebo phases under IH exposure. Further, the increase in MAP would be higher during the indomethacin and celecoxib phases as compared to the placebo phase.

2) The increase in MAP in the celecoxib phase would be higher than the increase in MAP in the indomethacin and placebo phases in response to IH.

3) There would be a decreased cerebral blood flow response to IH exposure in indomethacin, celecoxib and placebo phases and the decrease in CBF will be more pronounced among indomethacin and celecoxib compared to placebo. The decrease in cerebral blood flow response would be more prominent among celecoxib phase as compared to indomethacin and placebo phase.

4) There would be a decrease in urinary vasodilatory terminal prostaglandins but increase of vasoconstrictor terminal prostaglandins in both indomethacin and celecoxib phases. The vasodilator prostaglandins will decrease more in celecoxib phase than indomethacin phase.

CHAPTER TWO: RESEARCH METHOD

2.1 Ethics and approvals

The study was approved by University of Calgary Research Ethics board 'Conjoint Health Research Ethics Board (CHREB) at the University of Calgary; Ethics Protocol # 23121'. A clinical trial application was approved by Health Canada ('Health Canada Clinical Trial Application: PROTOCOL#UC-MMHAP-COX-IH-2010001, 9427-U0206-66C') for the off-label use of pharmacological agents (Celecoxib, Indomethacin and Placebo). The study has been entered into the Clinical Trial Registry system with an Identifier: NCT01280006 (www.clinicaltrails.gov).

2.2 Study population

Healthy male volunteers that met the inclusion/exclusion criteria were invited to participate. Subjects were included if they were between 19 and 45 years, were free from cardiovascular and respiratory disease, and, at the time of the study, had resided within Calgary, AB (elevation: 1100m) for at least one year.

Potential subjects were excluded if they had been diagnosed or had a history of cardio-pulmonary or renal disease (urinary protein excretion > 150mg/24hr; estimated glomerular filtration rate < 90 mL/min/1.73 m^2), obesity (BMI > 35), sleep apnea (RDI > 10 evenets/hr and an overnight mean SaO_2 < 90 %) confirmed by at home cardio-pulmonary monitoring during sleep (Remmers Sleep Recorder, SagaTech Electronics IncCanada), had smoked within the past year, or if they were hypertensive (systolic > 140 mmHg; diastolic > 90 mmHg). In addition, volunteers were excluded if they had bleeding

disorders, history of upper gastrointestinal bleeding, peptic ulcer disease, inflammatory bowel disease, severe liver impairment or active liver disease, hyperkalemia, allergy to sulfonamides (i.e., "sulfa" group of drugs (Chamberlin & Silverman, 2009)), gastritis, collagen vascular diseases or any current inflammatory condition/musculoskeletal (MSK) disorders or were taking NSAIDs. Potential subjects were also excluded if they were taking any medications including recreational drugs and chronic alcohol consumption, had abnormal urinary protein excretion (>150mg), or if they were diabetic (fasting blood glucose level ≥7mmol during an initial screening visit.

These criteria were used to ensure all subjects were healthy and did not have any medical conditions that may affect cardio-respiratory control. Females were excluded because previous research in animals have reported females may be protected from the hypertension and tachycardia induced by IH (Hinojosa-Laborde & Mifflin, 2005).

2.3 Sample size calculation: statistical design of the study

A previous study from our lab using a similar model of IH demonstrated that there is an increase in MAP of approximately 8 mmHg (Foster *et al.*, 2010a) among healthy male subjects. In this study, six hours of IH exposure was significantly correlated with MAP (correlation coefficient, $r^2 = 0.744$). Based on the literature that NSAIDs (i.e. both nonselective COX-2 inhibitors and selective COX inhibitors) raise BP. Hence, the samples size was computed considering the difference in the change of MAP in response to 6 hour of IH exposure with placebo (considering baseline MAP for placebo is ~ 90 mmHg during isocapnic IH) and the effect of COX inhibitors. Therefore in an estimation

of sample size using 80% power and two tailed tests with an $\alpha = 0.05$ level of significance, it was determined that 10 subjects were required to detect a ~ 4 mmHg increase in MAP. Hence, considering a 20% dropout rate, 12 healthy male volunteers meeting the inclusion criteria were recruited.

2.4 Experimental commitment for the study volunteers

Subjects attended the Laboratory of Human Cerebrovascular Physiology at the University of Calgary on four separate occasions to complete one familiarization session and three experimental protocols as shown below (**Figure 2.1**). Each protocol was separated by at least four days to allow wash-out of medications between experimental protocols, occurred at the same time-of-day, and was administered in a randomized, double blind, cross-over fashion (**Figure 2.2**). For each of the three experimental protocols subjects were asked to be consistent with their dietary pattern (habit). This information was recorded in a 'Take Home Package' (**Appendix B**) for each experimental protocol (indomethacin, celecoxib and placebo). On the morning of each experimental session the subjects provided a urine sample so that their baseline level of urinary PG and sodium excretion could be assessed. Controlling the participants' diet and measuring the level of urinary sodium ensured that salt balance was similar for each day of testing to avoid the interaction of higher dietary sodium intake and its effect on blood pressure (Sacks *et al.*, 2001;Vollmer *et al.*, 2001).

2.5 Initial contact, lab tour and recruitment

Subjects were recruited through poster advertisements and flyers at the University of Calgary (**Appendix C**), an advertisement in the University of Calgary's Graduate Student Association's (GSA) newsletter, and by word of mouth. Posters were placed throughout the University of Calgary and Foothills Medical Center campuses. Interested participants who telephoned or emailed their interest in participating in the study were provided with an overview of the study protocol. Potential subjects were offered a lab tour to introduce them to the equipment and to provide them with additional information regarding the study. Subsequently, the potential subject completed a medical history, anthropometry and physical examination including vital signs, electrocardiogram (ECG), and laboratory screening (complete blood count, international normalized ratio (INR) for coagulation status, glucose, liver enzymes, serum creatinine and urinalysis). After the screening session, if eligible, potential subjects were asked to provide written informed consent (**Appendix D**). All subjects were counselled to avoid the use of drugs or alcohol during the study. If a prescription medication was necessary for health-related reasons during the protocol, then the suitability of continuing in the study was reviewed by the study investigators (along with the Physician on-call/cover for the study) and discussed with the participant.

2.6 Screening visit

The screening visit was scheduled in the morning after 12 hours fast, during which potential subjects were asked to refrain from alcohol, caffeine, heavy exercise on the prior day. The volunteers provided informed written consent. They also filled in the

medical questionnaire from familiarization package. Then the potential participant provided ~ 80 mL of mid-stream urine which was tested for proteinuria (dipstick) using Chemstrip®10 (Roche Diagnostics, 201 Boul. Armand-Frappier, Laval, H7V 4A2, Quebec, Canada) while 10 mL was sent to Calgary Laboratory Services (CLS), Foothills Medical Center, Calgary and the remaining volume was stored at -80 °C for future PG analysis. A venous blood was drawn from anti-cubital fossa of the non-dominant hand for screening markers [e.g. liver, kidney and haematology parameters] and fasting glucose (ABL800, Radiometer Medical ApS, DK-2700, Brønshøj, Denmark). The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease Study Equation (Levey *et al.*, 2007). Next, anthropometry, a 12-lead ECG, brachial BP, and a physical examination were performed as explained in screening package (**APPENDIX E**).

2.7 Familiarization test (Lloyd-Cunningham protocol)

This session familiarized subject with breathing apparatus used to administer the acute hypoxic and hypercapnic challenges during the 3 experimental visits described in detail in Section 2.8.1. The subject was instrumented and comfortably rested while breathing through a mouthpiece with their nose occluded. Based upon a 10 min mean of resting air breathing $P_{ET_{O_2}}$ (End-tidal Partial Pressure of Oxygen) and $P_{ET_{CO_2}}$, (End-tidal Partial Pressure of Carbon dioxide), subjects subsequently underwent three different hypercapnic stages each incorporating the same (number of) hypoxic challenges. This test is based upon the Lloyd-Cunningham Test (Lloyd *et al.*, 1958). First, the subject was exposed to

isocapnic euoxia ($P_{ET_{CO_2}}$ = +1 Torr above resting levels $P_{ET_{O_2}}$ = +88 Torr) for 4 min. At the end of the 4 min, subjects performed a 2 min isocapnic hypoxia stage ($P_{ET_{CO_2}}$ = +2, $P_{ET_{O_2}}$ = + 50 Torr), making the total stage duration 6 min. This hypercapnic euoxia and hypoxia cycle of 6 min is then repeated for two more stages of increasing hypercapnia ($P_{ET_{CO_2}}$ = +5 and $P_{ET_{CO_2}}$ = +8 Torr).

2.8 Experimental set up and termination of experiment

The experimental set up was extensively described at lab tour and during the familiarization session (**Section 2.4**). After the familiarization session, the subjects started medication in a double-blind randomized fashion (**Figure 2.2**). After four days of medication, the subjects arrived in the lab at 8.00 AM on the fifth day (Experimental Day). Urine, venous blood (for serum and plasma), and capillary blood samples were taken. On each experimental day, a physician was on-call to be consulted with if there were any health issue. The subject could pull out of the study at any point if felt that they did not want to continue. Additionally, we (study administrators) could have ended an experiment if we felt there was any serious adverse drug reaction or concern on participants' health due to our experimental intervention.

2.9 The experimental protocols

2.9.1 Drug ingestion, home blood pressure monitoring and dietary chart

Each session the subjects were given a medication package, take home package (**Appendix B**) and home blood pressure monitor (Omron Healthcare Inc., Bannockburn,

Illinois, USA). They were instructed to take medicines as per the protocol and record their blood pressure in the morning, afternoon and evening everyday. The take home package is the catalogue for the dietary chart and blood pressure monitoring. The subjects were instructed to keep a normal diet as per their lifestyle and habit. They were asked to record the entire dietary intake, BP and any uncomfortable/signs/symptom they noticed. If there was any concern they would reach physician on-call via pager or any investigators via phone and email. The protocols of each arm of the study have been described in the following sections.

2.9.2 Protocols: indomethacin, celecoxib and placebo

Subjects were randomly pre-treated with either of three drugs (indomethacin, celecoxib or placebo) three times daily for five days and the fifth day was the experimental day. The doses of indomethacin (nonselective COX inhibitor) chosen was 50 mg to be taken with food at 6 hours apart for four days (8:00 a.m., 2:00 p.m., and 8:00 p.m.) and two dosages (50 mg each) on fifth experimental day (8:00 a.m. and 2:00 p.m.). The second last dose was taken at least one hour prior to baseline measurements and the last dose was taken during IH exposure (2:00 p.m). The dose of celecoxib was 200 mg, twice daily 12 hours apart after food for four days (8:00 a.m. and 8:00 p.m.) and a single morning dose on the fifth experimental day (8:00 a.m.). In order to keep the drug ingestion schedule double blinded, a middle placebo pill (to be taken at 2:00 p.m. was included that was visually matching to remaining capsules. Placebo, in visually similar looking capsules as indomethacin and celecoxib, was given for three times daily for four consecutive days with the last placebo tablet being taken at least one hour prior to the beginning of baseline

measurements as per the protocol of indomethacin and celecoxib. Indomethacin (Apotex Inc), Celecoxib (Pfizer Canada Inc.) and Placebo (100 mg lactose; DIN 00501190) were provided by the Research Pharmacy at the Foothills Medical Centre.

The volunteers were referred to physician if they reported of having easy bruises, bleeding disorders and significant gastrointestinal upset leading to vomiting blood, blood in the stool or melena (black tarry stool). The subjects were instructed to see a physician if their SBP rose to above 140 mmHg or if there was any increase in BP associated with symptoms of high BP such as headache, blurring of vision, dizziness or fainting. The subject was referred to the physician if there was SBP increase of 10 mmHg from the baseline while he/she was under medication).

2.10 Experimental techniques

2.10.1 Dynamic end-tidal forcing (DEF) system

The technique of dynamic end-tidal forcing (DEF) (BreatheM version 2.40, University Laboratory of Physiology, Oxford, UK) enabled us to control each subject's end-tidal gases on a breath-by-breath basis accurately, regardless of ventilatory rate(Poulin *et al.*, 1998). Subjects breathed through mouth-piece with nose-clips on. The Chamber program (Chamber V2.43, University Laboratory of Physiology, Oxford, UK) was used for the baseline measurements (**2.11.1 Chamber program**). The baseline end-tidal gases were used to build a study protocol of isocapnic IH which is called Forcing Function File. The forcing function file was loaded into BreatheM program (**2.11.2 BreatheM program**) which runs the DEF function.

A turbine device and volume transducer (VMM-400, Interface Associates) was used to measure respiratory volumes. A pneumotachograph and differential pressure transducer (RSS100-HR, Hans Rudolf, Kansas City, MO) were used for measuring respiratory flow direction and timing information. An experimental controlling computer would generate the inspired P_{O_2} and P_{CO_2} predicted to give the desired end-tidal partial pressures by using a fast gas-mixing system (Robbins *et al.*, 1982b)). Hence, the controlling computer would continuously receive feedback of the measured end-tidal partial pressures on a breath-by-breath basis as the experiment progressed. These measured end-tidal values would then be compared with the desired values, and the computer adjusted the initial predicted inspired gas mixture by using an integral proportional feedback algorithm based on the deviations of the measured end-tidal values from the desired end-tidal values (Ainslie & Poulin, 2004a;Poulin *et al.*, 1998;Poulin *et al.*, 1996;Robbins *et al.*, 1982a). This algorithm and system is called DEF system which is basically based on the forcing function built on the desired stimulus.

2.10.2 Respiratory measurements

Respiratory measurements for the testing were obtained through a mouthpiece, with the nose occluded by a nose clip. The mass spectrometer analyzed the inspired and expired gases for fractional concentrations of O_2 and CO_2 at a rate of 20 ml•min^{-1}. Inspiratory and expiratory timings and flow were measured using a pneumotachograph and differential transducer (RSS100-HR, Hand-Rudolph Inc., Kansas City, MO, USA). A turbine device that included an electronic processing module, a photodetector pick-up assembly, and a

volume cartridge (VMM-400, Interface Associates, Laguna Niguel, CA, USA) measured respiratory volumes.

2.10.3 Transcranial Doppler (TCD) ultrasound

The blood flow to the brain was measured with transcranial Doppler ultrasound (TCD). The velocity of the blood to the brain was measured in the right middle cerebral artery via the temporal window just above temperomandibular joint(Aaslid *et al.*, 1982) using a 2-MHz pulsed Doppler ultrasound system (Multigon Industries, Inc. One Odell Plaza, Yonkers, NY 10701, USA, www.multigon.com). Initially, ultrasound gel (Aquasonic 100, Parker Laboratories INC., Fairfield, NJ) was applied to the Doppler probe before it was placed on the right temporal window. In order to find the optimal signal power and quality, numerous manipulations of the depth, angle, and position were attempted. Once the optimal signal was found and landmarked, the probe was affixed with a snug fitting headband (marc600, Spencer Technologies, Seattle, Washington, USA).

Three separate signals were obtained from the TCD every 10 ms, (i.e., collected at 100 Hz). One signal was derived from the maximum frequency shift of the Doppler spectra ($\overline{V}_P$), indicative of the maximum velocity of blood moving through the MCA (Poulin & Robbins, 1996). The total power ($\overline{P}$) of the Doppler signal, which is an index of cross-sectional area(Poulin & Robbins, 1996). The final signal was the intensity-weighted mean frequency of the Doppler spectrum (VIWM). In certain cases, the product of $\overline{P}$ and VIWM ($\overline{P}$ × VIWM) can be used as an index of blood flow changes because it takes into account any potential changes occurring in the MCA cross-sectional area (Poulin *et al.*,

1998). With an assumption of constant MCA vessel diameter, it is acceptable to consider $\overline{V}_P$ as an index of changes in CBF (Poulin & Robbins, 1996;Poulin *et al.*, 1998).

2.10.4 Arterial oxyhemoglobin saturation

Using finger pulse-oximetry, arterial oxyhemoglobin saturation (SaO_2) was monitored continuously and non-invasively (Model 3900, Datex Ohmeda Inc., Madison, WI) during the experimental sessions. The probe was placed on the left index finger. For the analysis and reporting, the arterial oxyhemoglobin saturation (Sa_{O_2}) was estimated from $P_{ET_{O_2}}$ by using Severinghaus equation (Severinghaus, 1979). A capillary blood sample (200 µl) was taken before both acute tests in the morning and afternoon (following the 6 hr IH exposure) from a small puncture on the finger and analyzed for blood gases and electrolytes (Radiometer ABL800 (Radiometer Medical ApS, DK-2700, Brønshøj, Denmark).

2.10.5 Blood pressure monitoring

Arterial blood pressure was recorded continuously throughout the testing using the non-invasive technique of photoplethysmography (Portapres or Finometer, TNO TPD Biomedical Instrumentation, Amsterdam, Netherlands). Since the cuff was placed at a level below the heart, a hydrostatic height correction was required was placed at the level of the heart to correct for this height difference. Caution was taken to account for the position of the finger, and movement and placement of the Portapres/Finometer cuff (Imholz *et al.*, 1998). The brachial BP was recorded during experiments and it was used to calibrate the Finometer values.

2.11 Data acquisition software

2.11.1 Chamber program

The Chamber program was used to determine resting values of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$, which were later used with the technique of dynamic end-tidal forcing. Specifically, the collection of this data was assembled into a breath-by-breath respiratory data file (.bbc). ECG data were sampled and 1000Hz and all other respiratory and cardiovascular data were sampled at a rate of 100 Hz. A 10 minute average of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ values were obtained at rest.

2.11.2 BreatheM program

During three experimental testing days of acute tests (both AM and PM), physiological data were collected using the BreatheM software program. For each experimental test, 6 files were produced. The uncalibrated respiratory and cardiovascular data was collected every 10ms into a raw file (.raw). The other files contained breath-by-breath respiratory data (.bbm), ECG data (.frw), beat-by-beat data (.hbb), a forcing log file (.ffl), and a settings file (.ini). Forcing function files used in conjunction with BreatheM were created for isocapnic intermittent hypoxia. ECG data were sampled and 1000 Hz, and all other data were sampled at a rate of 100 Hz.

2.11.3 LabCharts: PowerLab

The data acquired during experimental procedures were displayed in a continuous wave form pattern in a separate computer juxtaposed to main experimental computer in which

we controlled the parameters. The equipment (PowerLab/16SP and LabChart V6.13 software, ADInstruments, Colorado Springs, CO, USA) was used for both acute tests and IH chamber exposure. The visual display could help experimenter to assess visually and easily to the values obtained in the database.

2.12 Data collection

2.12.1 Baseline measurements

Resting measurements of P_{ETO_2}, P_{ETCO_2}, BP, heart rate (HR), and CBF was taken using dedicated computer software (Chamber V2.43, University Laboratory of Physiology, Oxford, UK) for 10 minutes while breathing room air sitting comfortably in experimental chair with all instrumentation. The respired gases were sampled continuously at a rate of 20 ml/min and analyzed for P_{O_2} and P_{CO_2} by mass spectrometry (AMIS 2000, Innovision, Odense, Denmark). BP by photoplethysmography (Portapress, TPD Biomedical Instrumentation, Amsterdam, Netherlands), HR by three-lead ECG (Micromon, 7142B monitor, Kontron Medical, UK), and arterial oxyhemoglobin saturation (SaO_2) by pulse oximetry (3900 Datex-Ohmeda, Louisville, CO, USA), and CBF velocity by TCD ultrasound (TC22, SciMed, Bristol, England) were continuously measured. BP was also be assessed every 3 minutes by an automated arm cuff placed on the right arm (Dinamap; Johnson and Johnson Medical, Inc., New Brunswick, NJ) and used to calibrate the values obtained by finger pulse photoplethysmography. All cardiovascular parameters were determined beat-by-beat basis (every 10 ms) by specifically designed computer software (Chamber V2.43, University Laboratory of Physiology, Oxford, UK).

2.12.2 Acute tests: Morning and afternoon experiments

The experimental procedures carried out before and after six hours IH exposure has been termed 'Acute Tests'. These tests were run with dedicated software and design. The system is called DEF system (as explained in the **Section 2.10.1**). The protocol included isocapnic IH stages. The acute isocapnic IH testing protocol was carried out as described in a previous study(Foster *et al.*, 2010a). Following baseline measurements, the protocol would begin with a 5-minute period of isocapnic euoxia ($P_{ET_{O_2}}$ = 88.0 Torr and $P_{ET_{CO_2}}$ = +1.0 Torr above rest) followed by six cycles of IH comprised of 90 seconds of isocapnic hypoxia ($P_{ET_{O_2}}$ = 45.0 Torr and $P_{ET_{CO_2}}$ = +1.0 Torr above rest) and 90 seconds of isocapnic euoxia.

2.12.3 Intermittent hypoxia exposure: Six hours chamber protocol

For IH exposure, subjects underwent 6 hours of continuous cycles of one-minute of hypoxia (nadir $P_{ET_{O_2}}$ = 45.0 Torr) and one-minute of normoxia (peak $P_{ET_{O_2}}$ = 88.0 Torr). The $P_{ET_{CO_2}}$ was maintained isocapnic by adding 100% CO_2 to the mouth of the subject during periods of hypoxia as described previously (Foster *et al.*, 2010a). IH was delivered by using a purpose-built chamber (Howard *et al.*, 1995;Poulin *et al.*, 2002). The gas composition in the chamber was altered by either adding nitrogen or oxygen, and by adding or removing carbon dioxide. To create hypoxia, the chamber was maintained at a gas composition resulting in a $P_{ET_{O_2}}$ = 45.0 Torr. Periods of normoxia ($P_{ET_{O_2}}$ = 88.0 Torr) were constituted by delivering 100% oxygen to the subject's inspiration through a

facemask (mirage NV Series 2, Resmed, New South Wales, Australia) connected to a two-way non-rebreathing valve (2600 Series, Hans Rudolph, Kansas, USA) and a 25cm long section of wide bore tubing. When oxygen flow was stopped, the subject would simply breathe the air composition of the chamber which resulted in a $P_{ET_{O_2}}$ = 45.0 Torr. A computer controlled gas solenoid valve was used to turn the flow of oxygen through the tubing on and off at one minute intervals. During IH exposure, respired gas was sampled from a nasal cannula and analyzed by a dual oxygen and carbon dioxide analyzer (NormocapOxy, Datex-Ohmeda, Louisville, CO, USA) for P_{O_2} and P_{CO_2}. A representative LabChart graph of six hour IH exposure from a subject has been presented here (**APPENDIX F**).

2.13 Blood and urine collection and storage

Venous blood was drawn from anti-cubital fossa of the non-dominant hand before morning and afternoon acute tests. The blood was centrifuged and serum or plasma were aliquoted into 500 µL tubes and stored in -80°C until they were assayed. Urine was collected at four time points on the experimental day. The first sample of the day was collected in the morning when subject arrived in the lab before IH exposure. Then the second sample was collected at the end of the IH exposure (6 hours later) as shown in **Figure 2.1**. The urine samples were aliquoted in 10 mL vials and stored in the – 80 °C refrigerator until they were assayed.

2.14 Management and copyright of the data

Prior to data collection and analysis, all subjects were assigned identification numbers. Any information that could identify subjects was kept confidential. Electronic experimental data files were immediately backed up to a password protected network share at the University of Calgary for permanent storage. Hardcopy of experimental notes and laboratory results were stored in individual folders for each subject stored safely in the lab safety file cabinet of designated study area. The biological fluids and samples (blood and urine) collected in this study (blood was centrifuged for serum and plasma) were aliquoted and stored in -80°C until they were assayed. Other data, such as screening questionnaires, and consent forms were collected and stored in paper form in the respective individuals' folder. Access to any confidential information, or to any of the stored data, is only accessible to Prof. Dr. Marc J Poulin (Principal Investigator), Mr Andrew Beaudin or Matiram Pun (trainees). Any original paper copies of the data are stored in compliance with the University of Calgary's Policy Statement on Ethical Conduct for Research Involving Humans(Serrano-Duenas, 2007).

CHAPTER THREE: DATA ANALYSIS

3.1 Acute hypoxic exposure data

The data collected before and after 6 Hrs of IH is called acute data (**Section 2.12.1** and **Section 2.12.2**). This is the physiological response under the effect of drug intervention, isocapnic IH stimulation. The vascular variables (cardiovascular and cerebrovascular) have been reported.

3.2 Outcome variables of the study

Our primary outcome variable is arterial pressure including the change in systolic, diastolic, and mean arterial BPs that occurs across an exposure to IH. Co-primary outcome variables are the changes in the urine components of the terminal PGs (e.g. PGI_2, and TxA_2)**.** Our secondary outcomes are the vascular measurements of CBF and the indices of cerebrovascular function (cerebrovascular resistance and conductance).

3.3 Analysis of the data

The two most important dedicated programs were used to acquire the data. They are Chamber Program and BreatheM Program. The data output from these programs were in .txt files and these data were analyzed by specially designed programs called Exhale and Average to generate our variables. Then these data were transported to MS Excel (Microsoft Office 2007) and adjusted. The raw and individual plots were generated by using Sigma Plot (V11 and V12, Systat Software Inc., Chicago, IL 60606, USA). Hence, the software programs were used to extract the data and then processed to the format in

which we could perform statistical analysis. After generating all individual data in MS excel, they were analysed using Matlab (Version 7.4.0.287, MathWorks, Inc., MA, USA) and SPSS (Version 19, Chicago, IL, USA).

3.3.1 Exhale program

This program extracted the data from raw files collected from the experiment. Using the calibration file (.ini), the EXHALE program enabled the conversion of the uncalibrated raw file from BreatheM into meaningful breath-by-breath and beat-by-beat data for respiratory and cardiovascular data, respectively. A total of 5 files were formed from EXHALE (.bb1, .bb2, .bb3, .bb4, .bbt). For this study, the .bbt and .bb1 files were used in the analysis. The primary variables of the study BP data and the CBF indices were stored in the .bbt file. In the .bb1 file, pertinent ventilatory, event marker, and HR data were found.

3.3.2 Average program

The AVERAGE analysis program was used to average the EXHALE data files into a series of time bins used to examine the physiological responses for each protocol. For this study, the .mbt and .mb1 files, which contain cardiovascular and respiratory cardiovascular data, were used for further analyses. Custom templates were used to generate consecutive 15 sec and 30 sec average templates (tmp) were used.

3.3.3 Modelling of the data

Data (cerebrovascular and cardiovascular responses) from each cycle of hypoxia and euoxia were interpolated at a 1s interval, overlaid, and averaged together to create a single 3-minute cycle of euoxia and hypoxia using specifically designed software created in Matlab (V7.4.0.287, MathWorks, Inc., MA, USA). This process significantly improved the signal-to-noise ratio of the collected data.

3.4 Cardiovascular responses: Blood pressure gains

The DBP, SBP, and MAP have been reported. The BP recorded during experiments was extracted using EXHALE software program (a described previously in **Section 3.3.1**) for each heart beat. The BP obtained from EXHALE was calibrated to the BP obtained from the brachial BP cuff which is considered to be the most standard. The brachial BP was measured intermittently during the experiment by automated BP cuff (Dinamap; Johnson and Johnson Medical, Inc., New Brunswick, NJ). The home BP responses during drug ingestion period (five days) have been reported as well. The BP responses (gains) to hypoxic were expressed as the percentage changes in BP per percentage desaturation or end-tidal carbon dioxide.

$$\text{BP gain} = (BP_{\text{isocapnic-hypoxia}} - BP_{\text{isocapnic-euoxia}}) / (\% \Delta\text{Arterial oxygen saturation})$$

The gains for SBP and DBP were calculated in similar manner.

3.5 Cerebrovascular conductance and resistance

The CBF can be best calculated and explained using cerebrovascular conductance (CVC) and resistance (CVR). These are calculated variables that take into account the potential impact of changes in MAP on CBF(Foster *et al.*, 2010a). Evidence suggests blood pressure should be considered during an analysis of CBF because the mechanisms of their response to $P_{ET_{CO_2}}$ appear related(Claassen *et al.*, 2007). Here, CVC was reported because it is likely more relevant in situations where changes in flow are dominant(Wayne, 1989). CVR is more pertinent in studies where changes in vascular tone have a greater effect on MAP, than they do on blood flow(Wayne, 1989). CVC is determined by indexing cerebral blood flow against MAP. CVR is calculated by indexing MAP against CBF. For these calculations, CBF was used as ($\overline{V}_P$) the index of blood flow, expressed as a percentage of baseline ($\overline{V}_P$). The equations are as follows:

A. $CVC = \overline{V}_P/MAP$

B. $CVR = MAP/\overline{V}_P$

Where:

CVC = cerebrovascular conductance ($cm \times sec^{-1} \times mmHg^{-1}$)

CVR = cerebrovascular resistance ($mmHg \times sec \times cm^{-1}$)

MAP = mean arterial pressure (mmHg)

$\overline{V}_P$ = peak flow velocity of the MCA ($cm \times sec^{-1}$) averaged over each heart beat, expressed as a percentage of baseline $\overline{V}_P$

3.6 Cerebrovascular responses

The MCA brain blood flow measurement in the study was used as an index for CBF measurements (Poulin & Robbins, 1996;Vantanajal *et al.*, 2007). TCD (details in **Section 2.10.3 Transcranial Doppler ultrasound**) was used for this purpose and it provides non-invasively beat-by-beat measurement of blood flow velocity and can reflect a change in flow assuming a constant vessel diameter of MCA. Here, cerebrovascular gains from the TCD output have been calculated.

Cerebrovascular gain is a parameter that reflects the capacity of blood vessels in the brain to dilate and this is considered to be one of the most important markers for brain vascular reserve. The use of TCD to measure the CBF under the different brain vascular stimuli (e.g. CO_2, O_2 and drugs) is a non-invasive and quantitative means to estimate cerebrovascular reactivity in humans. It can be useful addition to the baseline blood flow measurement and help in physiological studies and also in clinical settings. The maximal vasodilatory range or reactivity to $P_{ET}O_2$ = +45 Torr was determined by the change in MCA flow velocity per change in arterial oxygen desaturation as following:

$$\text{CBF gain} = (\text{CBF}_{\text{isocapnic-hypoxia}} - \text{CBF}_{\text{isocapnic-euoxia}}) / (\%\ \Delta\text{Arterial oxygen saturation})$$

And for conductance and resistance as:

$$\text{CVC gain} = (\text{CVC}_{\text{isocapnic-hypoxia}} - \text{CVC}_{\text{isocapnic-euoxia}}) / (\%\ \Delta\text{Arterial oxygen saturation})$$

CVR gain = ($CVR_{isocapnic\text{-}hypoxia}$ - $CVR_{isocapnic\text{-}euoxia}$) / (% ΔArterial oxygen saturation)

3.7 Capillary blood samples

A capillary blood sample (200 µl) was taken in the morning immediately before the acute isocapnic hypoxia measurements and repeated in the afternoon following the 6 hr IH exposure and before acute isocapnic hypoxia measurements as shown in. These samples were taken from a small puncture (using spring lancet; BD Microtainer® Contact-Activated Lancet, Franklin Lakes, NJ USA 07417**)** on the finger and analyzed for blood gases and electrolytes. The analysis was done in the Radiometer ABL800 (Radiometer Medical ApS, DK-2700, Brønshøj, Denmark). The analysis output file has been scanned and presented as an example (**APPENDIX G**).

3.8 Urinary analysis

3.8.1 Urinary sodium, creatinine and proteins (CLS Analysis)

The urine sample was sent to CLS from each experimental session for the analysis of Sodium (Na^+), Creatinine (Cr), Microalbumin (µAlb) and Protein (Pr). Creatinine was used for the correction of PG analysed. Sodium (Na^+) and protein (Pr) excretion was analysed for the salt intake and renal status. The creatinine given as mmoL/L was converted to mg/dL with the following calculation:

Creatinine (mg/dL) = (mmoL of Creatinine/88.402)*1000

OR (mmoL of Creatinine *1000)/88.402

The constant 88.402 is for µmol/L but our unit given is mmoL/L. Hence, to convert this denominator for mmoL/L is calculated as: 1 mmoL/L = 88.402/1000

3.8.2 Sample extraction and urinary prostaglandin measurements

These urine samples from before and after IH exposure and during 6 hours of IH exposure were analyzed for urine concentrations of stable metabolites of terminal PGs using EIA methods (Cayman Chemicals Inc., Ann Arbor, Michigan, USA). Before looking into PG, the urine samples were purified/extracted for the removal of other interference biomarkers. The algorithms of urinary sample extraction of PG analysis has been illustrated in the **Appendix H**.

The urine sample was thawed in slurry ice water (i.e. maintained 0 C and taken through the required and recommended protocols of extraction. The different PGs required different types of extraction depending upon the type of the PG we were looking for. A Solid Phase Extraction (SPE) Protocol was utilized for the sample extraction as per the recommendations of Cayman Chemicals Inc. (their EIA kit was used to analyze PGs). We have utilized commercially available SPE cartridges (C-18 Column) for the purpose from Waters (Oasis Sample Extraction Products; Oasis®HLB 6cc/150ng /Extraction Cartridges) and used as vacuum assisted SPE method **(Appendix I)**. The resultant purified or extracted sample was used for the Assays (EIA). The extraction and EIA was carried out in collaboration with Dr Katherine Wynne-Edwards.

The extracted urine sample was analyzed by commercially available kits (Cayman Chemicals, Ann Arbor, MI, USA) and specific immunoassays (Krieger *et al.*, 1991a;Kimura *et al.*, 1998a;Tsikas, 1998) by VersaMax™ Absorbance Microplate Reader (Molecular Devices Corporation, 1311 Orleans Drive, Sunnyvale, CA 94089 USA) using software SoftMax® Pro, Software Molecular Devices (Molecular Devices Corporation; 1311 Orleans Drive Sunnyvale, CA, USA ; Version 5 for Mac® in Mac Computer). The raw results were analyzed as per the recommendation of respective kits from the template (computer spreadsheet available for data analysis) Cayman Inc. suggested (www.caymanchem.com/analysis/eia). The PGs assayed and analysed was corrected with creatinine.

PGcorrected (ng/mmoL of Cr/mL) = [PG (pg/mL)] / [Creatinine (mmoL/L)/1000]

OR, to express PG in ng/mg of Cr/dL, first the creatinine given in mmoL/L was converted to mg/dL as following:

Creatinine (mg/dL) = [Creatinine (mmoL/L) * 1000] / (88.402)

Then,

PGcorrected (ng/mg of Cr/dL) = [PG (pg/mL) * 100] / [Creatinine (mg/dL)] /1000

NB: PG in pg/mL is multiplied by 100 to convert into mg/dL (as 1 dL = 100 mL) and then outcome of PG is pg/mg of Cr/dL which is divided by 1000 to convert into ng/mg of Cr/dL (as 1000 pg = 1 ng).

At first, we validated the SPE method for the extraction of urine sample creating a mean sample of eight participants from the morning sample of placebo and evening sample of Indomethacin group **(Appendix J)**. Then we ran EIA analysis for PG to the extracted sample with centrifuged mean sample. The values were similar. Therefore we analysed rest of the urine sample with the protocol of centrifuge, aliquot and analysis.

3.9 Statistical interpretation of the data

All data are expressed as means ± SD. Descriptive statistics were computed for all variables, including measures of central tendency (means, medians, percentiles) and dispersion (standard deviations, ranges). The aims of this study were to assess by comparing primary and secondary outcome variables within individuals statistically, using general linear model (GLM) repeated-measures analyses of variance (ANOVA) (SPSS version 19.0 & 20.0, Chicago, IL, USA). A probability value of $p < 0.05$ is considered statistically significant. Independent t-tests were used to compare group means involving single data points. Repeated measures ANOVAs were used to compare differences over multiple data points.

CHAPTER FOUR: RESULTS

4.1 Subjects

Twelve male subjects (11 Caucasian, 1 Asian) passed the study selection criteria and were included in the study. They had an average age of 25.8±5.1 yr, height of 180.6±8.0 cm, weight of 81.6±12.1 kg, BMI of 24.9±2.5 $kg \cdot m^{-2}$ and were all normotensive with a normal 12-lead electrocardiogram (**Table 4.1)**. All subjects signed a written informed consent prior to participating in the study.

4.2 Screening

4.2.1 Venous blood samples

The venous blood samples were analyzed immediately after drawing from antecubital vein with the blood gas analyzer (i.e., Radiometer ABL 837) are shown in **Table 4.2**. Parameters included acid-base status, blood gases (i.e., P_{O_2} and P_{CO_2}), oximetry measures, electrolytes (Na^+, K^+, Cl^+, Ca^{2+}), and metabolic values (glucose, lactate, creatinine, bilirubin).

The venous blood samples from antecubital vein were also analyzed on the very day by Calgary Laboratory Services (CLS) and the results are shown in **Table 4.3**. Samples were analyzed for the following parameters: blood counts (Red Blood Cells: RBC, White Blood Cells: WBC and differentials, platelets) and volumes (Mean Corpuscular Volume: MCV, Mean Corpuscular Haemoglobin Concentration: MCHC, and Red Cell Distribution Width: RDW), liver function (Alkaline Phosphatase: ALP, Alanine Transaminase: ALT and Abnormal Prothrombin: APT), kidney function (Creatinine and

calculated GFR) and electrolytes and INR (international normalized ratio). The ranges were all within normal ranges.

4.2.2 Urine samples

Urinary dipstick test results and urine sodium and creatinine values are shown in **Table 4.4**. Subjects did not have proteinuria or haematuria and had normal sodium and creatinine values.

4.2.3 Sleep disordered breathing

Data used to confirm the lack of sleep disordered breathing are shown in **Table 4.1** Parameters included were: total monitoring time, total time with probe on and respiratory disturbance index (RDI: total, lateral and supine), mean SaO_2 (94.8±1.1), minimum SaO_2 (89.2±4.6) and total time with $SaO_2 < 90$.

4.3 Home blood pressure monitoring

Changes in morning blood pressure on the four days preceding each experimental day and the morning blood pressure on the experimental day prior to the acute hypoxic test are shown in **Figure 4.1**. In the placebo condition, blood pressures (i.e., SBP, DBP, and MAP) were similar across all 4 days prior to the experimental day. In contrast, there were patterns of changes in blood pressures with Indomethacin while in the Celecoxib condition. On the morning of the experimental days (i.e., Day 5) MAP and DBP were significantly higher with indomethacin compared to placebo (MAP, 89.76±8.10 vs 83.53±7.34, $p = 0.01$ and DBP, 72.75±8.88 vs 65.917±8.70, $p = 0.005$) and celecoxib

(MAP, 89.76±8.10 vs 84.97±6.61,p = 0.039 and DBP, 72.75±8.88 vs 67.86±7.39, p = 0.033) while they were similar between celecoxib and placebo (MAP, 84.97±6.61 vs 83.53±7.34, p = 0.458 and DBP, 67.86±7.39 vs 65.917±8.70, p = 0.314). Similarly, SBP was significantly elevated with indomethacin compared to placebo (123.73±10.99 vs 117.17±12.41, p = 0.048) but it did increase significantly compared to Celecoxib (123.73±10.99 vs 119.19±10.74, p = 0.432).

4.4 Urinary prostaglandins

The prostaglandin results have been presented in the **Figure 4.2** (Prostaglandin I_2), **Figure 4.3** (Thromboxane A_2), **Figure 4.4** ratios (PGI_2/TxA_2) and **Figure 4.5** changes in PGs (subtracting morning values from evening values). After four days of drug ingestion, the morning urinary PGI_2M i.e. PGI_2 metabolites (two active metabolites of PGI_2: 6k-$PGF_{1\alpha}$ and 2,3-d-6k-$PGF_{1\alpha}$) was significantly lower with indomethacin ($p < 0.001$) and celecoxib (p = 0.007) compared to placebo but there was no difference between indomethacin or celecoxib (p = 0.783) (**Figure 4.2**). After six hours of IH exposure, PGI_2M remained significantly lower with indomethacin (p = 0.004) compared to placebo, while there was just a pattern (not significant) for PGI_2M with celecoxib to remain lower than PLBO (p = 0.062). The PGI_2M levels, within the drug groups before and after IH exposure, did not significantly increase in the afternoon (PLBO, p = 0.988; INDO, p = 0.779 and CLBX, p = 0.73).

Compared to placebo and celecoxib, four days of indomethacin significantly lowered morning urinary 11-dehydro TXB_2 (11-dehydro Thromboxane B_2 is the active metabolite

of TxA_2) ($p < 0.001$) (**Figure 4.3**). Between placebo and celecoxib, there was no significant difference ($p = 0.83$). After six hours of IH exposure, the 11-dehydro TXB_2 remained decreased significantly with indomethacin as compared to placebo ($p < 0.001$) and celecoxib ($p < 0.001$) but not with celecoxib compared to placebo ($p = 0.937$). The pre-IH and post-IH comparison within the drug intervention, 11-dehydro TXB_2 did not decrease significantly with placebo and celecoxib groups and did not increase significantly with indomethacin (PLBO, $p = 0.979$; INDO, $p = 0.914$ and CLBX, $p = 0.94$).

The morning ratio of PGI_2M/11-dehydro TXB_2 (**Figure 4.4**) was significantly higher with indomethacin compared to placebo ($p < 0.001$) and celecoxib ($p < 0.001$) while the no change with celecoxib as compared to placebo ($p = 0.796$). After IH exposure, 11-PGI_2M/dehydro TXB_2 remained significantly increased with indomethacin as compared to placebo ($p < 0.001$) and celecoxib ($p < 0.001$) and again there was no significant change with celecoxib (vs PLBO, $p = 0.825$).

The pre-IH and post-IH changes in the ratios with the interventions (PLBO, INDO and CLBX) were not statistically significant (PLBO, $p = 0.638$; INDO, $p = 0.81$ and CLBX, $p = 0.954$). The pre-IH and post-IH changes i.e. subtracting AM values (before six hours of IH exposure) from PM (after six hours of IH exposure) were also compared and they were not significant. The results are presented in the **Figure 4.5**.

4.5 Acute tests

4.5.1 Air breathing

Respiratory measures

In the morning of the experimental days (i.e., day 5), compared to placebo, $P_{ET_{CO_2}}$ was significantly lower during air breathing at rest with indomethacin ($p = 0.019$), but not with celecoxib ($p = 0.169$). After 6 h of IH, there was a decrease in $P_{ET_{CO_2}}$ and increase in $P_{ET_{O_2}}$ but they were not statistically significant within the intervention. There was a significant decrease in $P_{ET_{CO_2}}$ with the placebo ($p = 0.023$), but not with indomethacin ($p = 0.816$) or celecoxib ($p = 0.068$) in pre-IH and post-IH comparison within drug protocols. In addition, there was a corresponding significant increase in $P_{ET_{O_2}}$ with placebo and celecoxib, but not with indomethacin (PLBO, $p = 0.035$; INDO, $p = 0.337$; CLBX, $p < 0.001$) in the afternoon as compared to morning. The summary of these results have been presented in the **Figure 4.6A**.

Blood pressure (BP)

The morning air breathing MAP after four days of drug ingestion was increased with indomethacin and celecoxib compared to placebo (**Figure 4.7A**). There is significant increase with indomethacin compared to placebo and celecoxib (INDO vs PLBO, $p = 0.01$; INDO vs CLBX, $p = 0.039$) but not with celecoxib (CLBX vs PLBO, $p = 0.458$). In comparison to placebo, the morning SBP was significantly higher with indomethacin ($p = 0.048$). Morning DBP was significantly higher with indomethacin compared to

placebo ($p = 0.005$) and celecoxib ($p = 0.033$) but not with celecoxib compared to placebo ($p = 0.314$).

The six hours of IH exposure increased MAP significantly during afternoon air breathing with indomethacin compared to placebo (INDO vs PLBO, $p < 0.001$) and celecoxib (INDO vs CLBX, $p < 0.001$) while celecoxib did not significantly increase compared to placebo (CLBX vs PLBO, $p = 0.75$). The pre-IH and post-IH exposure comparisons in MAP increases were not significant within the interventions (PLBO, $p = 0.61$; INDO, $p = 0.275$; CLBX, $p = 0.873$). Air breathing morning SBP was significantly higher with indomethacin compared to placebo ($p = 0.048$) but within the group comparison (pre-IH vs post-IH) was not significantly higher. DBP was significantly higher in the morning during air breathing in indomethacin group as compared to placebo ($p = 0.005$) and celecoxib ($p = 0.033$) but not with celecoxib as compared to placebo ($p = 0.314$). After six hours of IH exposure, blood pressure did not significant increase among drug interventions INDO vs PLBO ($p = 0.48$), INDO vs CLBX ($p = 0.38$) and CLBX vs PLBO ($p = 0.779$). The increase in DBP after IH exposure was not statistically significant either (PLBO, $p = 0.694$; INDO, $p = 0.726$; CLBX, $p = 0.86$). The summary of all the BP results have been presented in **Figure 4.7A**

Cerebral blood flow (CBF, $\bar{V}_P$)

Air breathing CBF ($\bar{V}_P$) after four days of drug ingestion as compared to placebo was significantly decreased with indomethacin (INDO vs PLBO, $p = 0.025$) but not with

celecoxib (CLBX vs PLBO, p = 0.248) (**Figure 4.6A, Panel 3a**). CBF ($\bar{V}P$) change was more with indomethacin compared to celecoxib but not significantly reduced (INDO vs CLBX, p = 0.194). The six hours IH exposure did not alter resting air breathing $\bar{V}P$ within the protocols (PLBO, p = 0.599; INDO, p = 0.728 and CLBX, p = 0.332) and the differences observed prior to the IH exposure was maintained (i.e., $\bar{V}P$ was lower during indomethacin and celecoxib compared to placebo although statistically not significant). The decrease across the protocols were consistent after six hours of IH exposure in the afternoon but not statistically significant (INDO vs PLBO, p = 0.987; CLBX vs PLBO, p = 0.198 and INDO vs CLBX, p = 0.168).

4.5.2 Isocapnic euoxic baseline

Respiratory measures

Baseline PET_{CO_2} and PET_{O_2} values are illustrated in **Figure 4.6B, Panel 1b** and **Panel 2b**. Prior to IH exposure the end-tidal oxygen and carbon dioxide were stabilized (i.e. baseline. The baseline PET_{CO_2} across the protocols were placebo (AM: 38.8±1.5 and PM: 38.9 ± 1.56), indomethacin (AM: 37.53±2.1 and PM: 38.0±1.9) and celecoxib (AM: 38.3±2.2 and PM: 38.2±2.1), The baseline PET_{O_2} were placebo (88.0±0.7 and PM: 87.8±0.7), indomethacin (AM: 88.2±0.6 and PM: 88.1±0.6) and celecoxib (AM: 87.8±0.5 and PM: 88.2 ±0.4). There were no differences in PET_{CO_2} and PET_{O_2} between the three drug conditions and pre-IH and post-IH acute tests of the day.

Blood pressure (BP)

The baseline BP results have been presented in **Figure 4.7B**. Morning baseline MAP was increased approaching statistically significant with indomethacin (INDO vs PLBO, p = 0.057) and not significantly with celecoxib as compared to placebo (CLBX vs PLBO, p = 0.293). The baseline SBP with indomethacin and celecoxib, compared to placebo, were not statistically increased (INDO vs PLBO, p = 0.132; CLBX vs PLBO, p = 0.3). The increase in baseline DBP approached towards statistically significantly with indomethacin (INDO vs PLBO, p = 0.067) but was not significant with celecoxib (CLBX vs PLBO, p = 0.24) compared to placebo.

After six hours of IH exposure, afternoon baseline MAP was significantly higher with indomethacin compared to placebo (INDO vs PLBO, p = 0.007) and celecoxib INDO vs CLBX, p = 0.042) but not with celecoxib (CLBX vs PLBO, p = 0.313). SPB remained elevated with indomethacin and celecoxib with no statistical significance (INDO vs PLBO, p = 0.062; INDO vs CLBX, p = 0.48 and CLBX vs PLBO, p = 0.155). DBP was significantly elevated with indomethacin compared to placebo (INDO vs PLBO, p = 0.007) and celecoxib (INDO vs CLBX, p = 0.008) but it was not significantly elevated with celecoxib compared to placebo (CLBX vs PLBO, p = 0.817).

The pre-IH and post-IH comparison, indomethacin and celecoxib were not significantly different (PLBO, p = 0.913; INDO, p = 0.774 and CLBX, p = 0.878). The SBP was not significantly (PLBO, p = 0.973; INDO, p = 0.977 and CLBX, p = 0.889). DPB with

indomethacin or with celecoxib was not significantly different (PLBO, p = 0.974; INDO, p = 0.815 and CLBX, p = 0.851).

Cerebral blood flow (CBF)

There baseline cerebral flow has been presented in **Figure 4.6B, Panel 3b**. In comparison to PLBO, morning (i.e., pre-IH) baseline ($\overline{V}P$) was significantly lower with indomethacin (p = 0.024), but ($\overline{V}P$) with celecoxib was similar with placebo (p = 0.163). After IH exposure, the CBF with indomethacin and celecoxib as compared to placebo were not statistically different (INDO vs PLBO, p = 0.528; CLBX vs PLBO, p = 0.061 and INDO vs CLBX, p = 0.149). The six hours of IH exposure did not significantly decrease CBF within drug groups (AM vs PM) with placebo (p = 0.721) and celecoxib (p = 0.723). Similarly, the CBF response with INDO compared to morning (Pre-IH vs Post-IH) was not statistically significant (p = 0.743).

4.5.3 Acute isocapnic hypoxic challenge

Respiratory measures

The overlay of the 6 cycles of the hypoxic test for end-tidals and arterial oxygen saturation are shown in the **Figure 4.8**. The euoxic and hypoxic controls of $P_{ET_{O_2}}$ with their corresponding saturations were consistent in the morning and afternoon. The $P_{ET_{CO_2}}$ was maintained constant (isocapnic) throughout the euoxic-hypoxic cycles (**Figure 4.8, Panel 2**). Before and after IH exposure, the $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ along with

blood oxygen saturation (SpO_2) were similar to Pre-IH values (illustrated in the **Figure 4.8**) within the drug protocols.

Blood pressure

The blood pressure responses to the acute hypoxic tests before and after IH exposure are presented in the **Figure 4.9** over the cycles of 90 seconds of euoxia to 90 seconds of isocapnic hypoxia (total of 180 seconds plot). Under the acute hypoxic challenge test in the morning after four days of drug ingestion; there were patterns of changes and they have been calculated as gains. The gain of the responses (**Figure 4.10**) are not statistically different MAP: INDO (vs PLBO, p = 0.998) and CLBX (vs PLBO, p = 0.995); SBP: INDO (vs PLBO, p = 0.99) and CLBX (vs PLBO, p = 0.99) and DBP: INDO (vs PLBO, p = 0.994) and CLBX (vs PLBO, p = 0.988).

Six hours of IH exposure, only DBP was significantly higher with indomethacin (but not with celecoxib) compared to placebo (p = 0.049) but the gain of responses (**Figure 4.10**) were not statistically significant MAP: INDO (vs PLBO, p = 0.947) and CLBX (vs PLBO, p = 0.983); SBP: INDO (vs PLBO, p = 0.999) and CLBX (vs PLBO, p = 0.998) and DBP: INDO (vs PLBO, p = 0.771) and CLBX (vs PLBO, p = 0.847).

The acute hypoxic challenge after IH exposure increased BP within drugs (pre-IH and post-IH comparison of indomethacin, celecoxib and placebo) but the gain of responses (**Figure 4.10**) were not statistically significant i.e. MAP (PLBO, p = 0.959; INDO, p =

0.481 and CLBX, p = 0.808), SBP (PLBO, p = 0.995; INDO, p = 0.998 and CLBX, p = 0.998) and DBP (PLBO, p = 0.768; INDO, p = 0.093 and CLBX, p = 0.499).

Cerebral blood flow

The CBF results have been presented in the **Figure 4.11** is the cycle of 180 seconds (containing first 90 seconds of euoxia and next 90 seconds of isocapnic hypoxia) during acute isocapnic hypoxic challenge. The patterns of changes in CBF during acute intermittent hypoxia challenge in the morning and afternoon have been illustrated in the **Figure 4.11 (Panel 1)**. The gain of the responses (**Figure 4.12**) were not statistically significant with the drugs as compared to placebo e.g. INDO (vs PLBO, p = 0.990), CLBX (vs PLBO, p = 0.973) and INDO vs CLBX (p = 0.962). After six hours of IH exposure, the hypoxic challenge reduced CBF and the change was similar with the drugs (celecoxib reducing most effectively). The gains of the responses (**Figure 4.12, Panel 1**) with the drugs as compared to placebo were not statistically significant: INDO (vs PLBO, p = 0.955), CLBX (vs PLBO, p = 0.923) and INDO vs CLBX (p = 0.421). The pre-IH and post-IH comparison within drug protocols, CBF was decreased with celecoxib and placebo but increased with indomethacin but the response gains were not statistically significant: PLBO (p = 0.964), INDO (p = 0.935) and CLBX (p = 0.988).

The responses of changes in cerebrovascular conductance during morning hypoxic challenge with indomethacin and celecoxib compared to placebo are presented in **Figure 4.11 (Panel 2)**. The response gains were not significantly different INDO (vs PLBO, p =

0.981), CLBX (vs PLBO, p = 0.999) and INDO vs CLBX (p = 0.997). After six hours of IH exposure, afternoon conductance gains with indomethacin and celecoxib were not significantly different INDO (vs PLBO, p = 0.998), CLBX (vs PLBO, p = 0.995) and INDO vs CLBX (p = 0.931) compared to placebo. Pre-IH and post-IH comparison, gains of conductance with celecoxib and placebo were not statistically different PLBO (p = 0.954), INDO (p = 0.997) and CLBX (p = 0.999).

The cerebrovascular resistance gains in the morning hypoxic test with indomethacin and celecoxib as compared to placebo were not significant INDO (vs PLBO, p = 0.958), CLBX (vs PLBO, p = 1) and INDO (vs CLBX, p = 0.974) (**Figure 4.12, Panel 3**). The resistance gains after IH exposure were with celecoxib and indomethacin as compared to placebo were not statistically different NDO (vs PLBO, p = 0.681), CLBX (vs PLBO, p = 1) and INDO vs CLBX (p = 0.67). The pre-IH and post-IH CVR were comparisons compared to the morning were not statistically different PLBO (p = 1), INDO (p = 0.77) and CLBX (p = 0.988).

4.6 Capillary blood samples

The pH changes across the protocols in the afternoon as compared to morning were not significantly (PLBO, p = 0.538; INDO, p = 0.317 and CLBX, p = 0.992). The partial pressure of carbon dioxide (P_{CO_2}) changes with indomethacin group and other two protocols were not statistically different (PLBO, p = 0.988; INDO, p = 0.935 and CLBX, p = 0.938). The partial pressure of oxygen (P_{O_2}) was significantly decreased with celecoxib (CLBX, p = 0.012) but not in the placebo and indomethacin groups (PLBO, p =

0.767 and INDO, p = 0.902). Hematocrit changes across the protocols was significantly increased afternoon compared to morning with placebo (PLBO, p = 0.021) but not with others (INDO, p = 0.574 and CLBX, p = 0.678). Creatinine (cCrea) changes in the afternoon in all three protocols were not significantly decreased (PLBO, p = 0.281; INDO, p = 0.657 and CLBX, p = 0.869). Chloride was fairly stable (PLBO, p = 0.989; INDO, p = 0.989 and CLBX, p = 0.971). Sodium was significantly higher in CLBX group (CLBX, p = 0.04) but not in others (PLBO, p = 0.219 and INDO, p = 0.282) while potassium was significantly higher in placebo (PLBO, p = 0.003) but not in others (INDO, p = 0.479 and CLBX, p = 0.38) after six hours of IH exposure. The results have been summarized in the **Table 4.5**.

CHAPTER FIVE: DISCUSSION

5.1 Major findings

This is the first study comparing nonselective COX inhibition and selective COX-2 inhibition on cardiovascular and cerebrovascular responses to hypoxia before and after a 6 hrs of IH exposure with quantification of downstream prostanoids formed by the catalyzing of AA via COX enzymes. The major findings are, firstly, indomethacin significantly increased morning BP among healthy male volunteers after four days of drug ingestion compared to placebo and celecoxib. Secondly, after four days of ingestion, compared to placebo, indomethacin significantly lowered CBF during air breathing and isocapnic baseline. Third, both indomethacin and celecoxib lowered vasodilator prostanoids significantly compared to placebo while only indomethacin significantly lowered vasoconstrictor (compared to placebo) after four days of drug ingestion. The morning ratio of PGI_2:TxA_2 was significantly higher (i.e. increased vasodilatory shift) in the indomethacin group compared to placebo ($p < 0.001$) and celecoxib ($p < 0.001$). Fourth, the acute isocapnic hypoxic challenge after four days of drug ingestion did not have significant changes in the gain of BP responses but after 6 hrs of IH exposure. Fifth, the gain of CBF response with four days of drug ingestion appeared blunted with celecoxib but was similar between indomethacin and placebo. When exposed to 6 hrs of IH, the CBF gain remained as of the morning with celecoxib while appeared augmented with indomethacin (not significant changes). Finally, the pattern of changes (not significant changes) in CVC gains were lower with celecoxib and further lowered with IH exposure while CVR gain was lower with indomethacin and was further lowered with

IH exposure suggesting that nonselective COX-inhibitors affect flow while selective COX-2 inhibitors affect vascular tone more.

5.2 Home blood pressure monitoring

The blood pressure was monitored at home during the period of drug ingestion. Indomethacin increased the blood pressure while BP has fluctuated over the days with celecoxib. The MAP increased linearly with indomethacin over the drug ingestion days and it was driven by increase in both SBP and DBP. Meanwhile, celecoxib had a fluctuating response in BP and had an increased response on the morning of the fifth day. It was hypothesized that both COX inhibitors would raise blood pressure after four days of drug ingestion (Stichtenoth *et al.*, 2005). Indomethacin raised BP more than celecoxib compared to placebo contrary to our hypothesis that celecoxib would raise BP more. Nonselective COX inhibitors do have cardiovascular side effects and indomethacin is considered one of the bad old drugs including other nonselective COX inhibitors e.g. diclofenac (McGettigan & Henry, 2013;Schmidt *et al.*, 2011;Sudano *et al.*, 2010;White & Campbell, 2010). A number of epidemiological population studies and meta-analyses have enlisted nonselective COX inhibitors (e.g. indomethacin, ibuprofen, diclofenac) as showing consistently higher associated cardiovascular toxicity compared to placebo (McGettigan & Henry, 2013;Reddy & Roy, 2013;Schmidt *et al.*, 2011;Jerry, 2011;FitzGerald, 2004;Sudano *et al.*, 2010). Indomethacin raised air breathing MAP, SBP and DBP significantly compared to placebo (**Figure 4.7A**), but only MAP and DBP compared to celecoxib. Celecoxib did not raise BP significantly compared to placebo but it caused fluctuations (destabilized) in BP over the 4 days of ingestion. It has recently

been reported that fluctuations in BP may be equal to or even more detrimental to cardiovascular outcome with a sustained elevations in BP (Tzeng *et al.*, 2012;Rothwell, 2013). The day-to-day variability of BP seems to have more robust cardiovascular adverse events (Parati *et al.*, 2013;Mancia, 2012;Tzeng *et al.*, 2012;Rothwell, 2013). In this study, COX inhibitors have affected the blood pressure physiology and they have either raised or destabilized it. In this context, Indomethacin seems worse than celecoxib. Therefore, although only nonselective COX inhibitor (indomethacin) produced a sustained elevation of BP, the increased variability observed with celecoxib may also be as important for the adverse outcome in cardiovascular health.

5.3 Prostaglandin responses

Both indomethacin and celecoxib significantly reduced morning vasodilator prostaglandin i.e. PGI_2 (PGI_2M) after four days of drug ingestion. However, there were no significant difference in the suppression of PGI_2 between the nonselective COX inhibitor Indomethacin and the selective COX-2 inhibitor celecoxib. After 6 hours of isocapnic IH, PGI_2 concentration still remained lower with indomethacin compared to both placebo and celecoxib. Surprisingly, PGI_2 appeared to increase (not significant) from the pre-IH values within the two drug conditions. From cancer biology, it is not entirely surprising to see hypoxic upregulation of PGI_2 (Iniguez *et al.*, 2003) but to our knowledge this is the first study to show that IH exposure among healthy individuals under intervention of nonselective, selective and placebo conditions raise PGI_2 although it is not statistically significant.

With respect to the major vasoconstrictor PG (i.e., TxA_2) the stable metabolite11-dehydro TXB_2 was significantly lowered by indomethacin compared to placebo after four days. But, as hypothesized and observed in the previous studies (Rossoni *et al.*, 2002;Skarke *et al.*, 2012), celecoxib did not lower TxA_2 significantly compared to placebo. In the long term uses of celecoxib, this uninhibited TxA_2 may have played crucial role in raising BP with celecoxib (Grosser *et al.*, 2010;FitzGerald, 2004). After IH exposure, indomethacin maintained lower urinary TxA_2 concentrations compared to placebo and celecoxib, but celecoxib left TxA_2 uninhibited even after IH exposure which raises blood pressure. Within the drug comparisons (i.e. before and after IH exposure, AM vs PM), the TxA_2 appeared to decrease with celecoxib and placebo to our surprise but it appeared to increase with indomethacin although the changes were not statistically significant.

The ratio between PGI_2 and TxA_2 (i.e. ratio of the stable metabolites of PGI_2 and TXA_2) is used to reflect the balance between the vasodilator PGI_2 and the vasoconstrictor TXA_2. The ratio was significantly higher in indomethacin as compared to placebo and celecoxib groups but there was no change with celecoxib as compared to placebo. Hence, indomethacin tipped the balance towards vasodilation while celecoxib maintained ratio towards vasoconstriction. The presence of an increased PGI_2:TXA_2 ratio with indomethacin appears to be in contrast to the fact that blood pressure was elevated with indomethacin, and perhaps reflects a compensatory increase in vasodilator response. This has been observed in hypertensive OSA patients (Kimura *et al.*, 1998b), but is not a consistent finding (Krieger *et al.*, 1991b). Hence, there is no clear consensus (or enough

literature) on which direction the ratio of vasodilator to vasoconstrictor shifts among OSA patients or in subjects exposed IH. After six hours of IH exposure, the PGI_2:TXA_2 ratio was significantly lower with indomethacin compared to placebo and celecoxib. Furthermore, celecoxib did not make any significant change in the ratio as compared to placebo. Within the drug comparison (i.e. pre-IH and post-IH exposure), the ratio appeared slightly stimulated across the protocols showing that the IH exposure has stimulatory effect on vasodilatory aspect which somehow supports the concept of compensatory expression of PGI_2. Hence, IH exposure non-statistically upregulated PGI_2 as compared to TxA_2 across the protocols. The change in the PGI_2:TXA_2 ratio after IH (i.e. post-IH values minus pre-IH) was also not significantly changed across the protocols.

5.4 Acute tests: Air breathing

Respiratory measures

After four days of drugs, both indomethacin and celecoxib reduced air breathing $P_{ET_{CO_2}}$. Indomethacin significantly lowered $P_{ET_{CO_2}}$ compared to placebo ($p = 0.02$) and this may be a mechanism by which indomethacin has been shown to enhance OSA severity (Bugess *et al.*, 2010) via destabilizing ventilation. However, capillary pH was not changed significantly and therefore, this needs further exploration. After six hours of IH exposure, there were statistically not significant changes for $P_{ET_{CO_2}}$ to decrease and $P_{ET_{O_2}}$ to increase, reflecting enhanced resting ventilation. However a comparison of pre-IH and post-IH $P_{ET_{CO_2}}$, the significant drop with placebo ($p = 0.023$) but not with

indomethacin and celecoxib indicate that the IH exposure model utilized works (Pialoux *et al.*, 2009;Foster *et al.*, 2009) but the drugs were interacting differently. Again the pre- and post-IH $P_{ET}O_2$ in respective protocols, there was significant increase with placebo ($p = 0.035$) and celecoxib ($p < 0.001$). IH is expected to increase $P_{ET}O_2$ in placebo (Pialoux *et al.*, 2009;Foster *et al.*, 2009) and the reported increase in $P_{ET}O_2$ with celecoxib may be a result of the sulfonamide moiety of celecoxib (Dogne *et al.*, 2007) although this has not been exclusively studied in hypoxic exposure among health humans.

Blood pressure

COX inhibitors have been shown to raise blood pressure with long term use (FitzGerald, 2004;Gislason *et al.*, 2006;Mukherjee *et al.*, 2001;Garcia Rodriguez *et al.*, 2008) with COX-2 inhibitors having the greatest effect. In the present study, with four days of drug ingestion, indomethacin increased morning air breathing MAP significantly compared to placebo and celecoxib. Surprisingly, although celecoxib increased MAP too, it was not significant. The increase in MAP with indomethacin was mainly driven by an increase in SBP, although DBP was also significantly increased with indomethacin as compared to placebo and celecoxib. Hence, it contrast to long term ingestion by patient populations (FitzGerald, 2004;Gislason *et al.*, 2006;Mukherjee *et al.*, 2001;Garcia Rodriguez *et al.*, 2008), it appears that with short duration ingestion by healthy individuals, indomethacin raised BP more than celecoxib.

After six hours of IH exposure, the air breathing MAP remained significantly higher with indomethacin as compared to placebo and celecoxib. There is no literature where healthy individuals were exposed to IH as to simulate OSA with COX inhibitors on board. Since, IH increases MAP (Foster *et al.*, 2010b), it was expected the COX inhibitors would act to enhance any IH-induced increase in BP. From the past clinical and physiological studies, COX-2 inhibitor was expected to increases BP most (Cannon & Cannon, 2012;FitzGerald, 2004). Surprisingly, unlike the nonselective COX inhibitor indomethacin, celecoxib (selective COX-2 inhibitor) did not raise resting BP significantly as compared to placebo. Moreover, pre- and post-IH blood pressures were not significantly different. The BP with celecoxib does not increase significantly compared to placebo despite celecoxib suppressing PGI_2M and TxA_2 being uninhibited. It could be carbonic anhydrase inhibition of celecoxib that may have offset the COX-2 inhibiting as well as IH effect on BP (Dogne *et al.*, 2007). Secondly; celecoxib (other COX-2 inhibitors) may take longer duration to affect BP significantly. It is possible that the drug affects more with particular age group with other conditions e.g. clinical population (rheumatoid/osteo-arthritis). It is also important to note that celecoxib is not as cardiovascular toxic as other COX-2 inhibitors (e.g. Rofecoxib) (Wolfe *et al.*, 2004) and this is the reason why celecoxib is the only available drug in North America. Finally, it is possible that vasodilatory PGs could have significantly produced by COX-1 pathway even in cardiovascular system (here blocked by indomethacin) (Kirkby *et al.*, 2012) and contributed to increase BP although controversies remain (Flavahan, 2007;Mitchell & Warner, 2006;Ricciotti *et al.*, 2013;Mitchell & Warner, 2013).

The morning air breathing SBP was significantly higher with indomethacin as compared to placebo and so was DBP (with indomethacin as compared to both placebo and celecoxib). Hence, the increase in MAP is with indomethacin was driven by both SBP and DBP. The post-IH BP appeared to increase among the protocols but they were not statistically different. Here, the IH and drug interaction was also not significant. It was expected celecoxib to increase post-IH BP most compared to indomethacin and placebo. This is because celecoxib being COX-2 inhibitors would raise BP on itself and exposing subjects to IH who are taking celecoxib, drug-IH interaction was expected to increase BP further.

Cerebral blood flow

After four days of drug ingestion, indomethacin significantly lowered the air breathing CBF as compared to placebo. CBF was also lower with celecoxib compared to placebo but the decrease was not significant. Between the two drug conditions, indomethacin lowered CBF more but it was not statistically significant. Hence, four days of clinically relevant dosage of indomethacin and celecoxib lowers the air breathing CBF. This could be a unique feature of indomethacin (the nonselective COX inhibitor) along with PG mediated. The indomethacin has changed air breathing end-tidals as described in the first paragraph of this section and the changes we have seen in CBF could possibly due to changes in ventilation. Previous literatures using double the dose of it have shown that indomethacin significantly lowers CBF within 90 minutes (Xie *et al.*, 2006;Fan *et al.*,

2010;Barnes *et al.*, 2012b). The unique feature here, we have used indomethacin over the days of clinical regimen (relevant dose and duration) and have compared with COX-2 inhibitor. The robust effect of indomethacin has also been utilized to lower ICP in traumatic brain injury and intensive care unit patients (Godoy *et al.*, 2012;Puppo *et al.*, 2007;Rasmussen, 2005).

After six hours of IH exposure, air breathing CBF tended to decrease more with indomethacin and celecoxib compared to placebo with the most significant drop occurring with celecoxib. This supports our hypothesis that COX-2 inhibitors should drop CBF most by inhibiting vasodilatory PG although the decreases were not statistically significant. When compared pre- and post-IH within the drug intervention, the CBF was decreased with placebo and celecoxib after six hours of IH exposure. To our surprise, the post-IH CBF appeared to increase slightly (not significant) with indomethacin compared to the morning (pre-IH CBF) although it remained lowered than that of placebo. Although these changes were only the patterns (i.e. they were not statistically significant), these findings are in line with the hypothesis that indomethacin decreased both PGI_2 and TxA_2 but slightly increased with IH exposure in the afternoon. The changes in ratio PGI_2:TXA_2 were not significantly increased in post-IH compared to pre-IH concentration all across the protocols although there appeared to be slightly stimulated indicating that there was increased vasodilatory shift with IH exposure as seen in previous study with OSA patients (Kimura *et al.*, 1998b).

5.5 Acute tests: Isocapnic euoxic baseline

Blood pressure

Similar to air breathing, when $P_{ET_{CO_2}}$ was clamped at +1 Torr and $P_{ET_{O_2}}$ at +88 Torr, morning MAP was elevated with indomethacin as compared to placebo ($p = 0.057$) while MAP did not significantly increase with celecoxib compared to placebo. Both systolic and diastolic BPs were increased with both the drugs compared to placebo without statistical significance. Indomethacin tended to increase BP higher than celecoxib. After six hours of IH, the afternoon baseline MAP was significantly elevated with indomethacin as compared to placebo ($p = 0.007$) and celecoxib ($p = 0.042$). Indomethacin has consistently affected the raised blood pressure while not celecoxib i.e. the higher baseline BP was maintained higher with indomethacin compared placebo and celecoxib post-IH as well. Celecoxib did not elevate post-IH MAP significantly as compared to placebo i.e no interaction between celecoxib and IH exposure. And the elevation in MAP was mainly driven by significant elevation of DBP with indomethacin as compared to placebo and celecoxib. SBP was elevated with both drugs indomethacin and celecoxib but they were not statistically significant. Celecoxib raised DBP as compared to placebo but not significantly. Hence, celecoxib raised baseline blood pressure but not as significantly as indomethacin. This was contrary to our hypothesis that celecoxib would elevated BP more with IH exposure. As seen in previous studies COX-2 inhibitors raising BP in clinical population over long duration (FitzGerald, 2004), we could not see substantial increase in BP with celecoxib here although there was statistically non-significant increase. It could be different interaction between the drug

and IH exposure or it might be a shorter duration of drug intervention as compared to other clinical studies where patients take drugs days to weeks and even months (Bombardier *et al.*, 2000;Curfman *et al.*, 2005;Lamberts *et al.*,). The changes in BP within the drug intervention protocols (pre- and post-IH) were not significantly different.

Cerebral blood flow

The acute effects of indomethacin on CBF were measured within 90 min and high doses of indomethacin (either 100 mg per oral or 1.2 mg/kg body weight) showing that there is significantly decrease in CBF (Xie *et al.*, 2006;Fan *et al.*, 2010;Wennmalm *et al.*, 1981). However, although after four days of the clinical dose of indomethacin used in our study resting morning CBF was significantly lower with indomethacin as compared to placebo, it was not of the same magnitude typically observed after a single high dose acute ingestion of indomethacin but it could be dose response (Pickard, 1981) and physiological balance over the days and the findings is actually similar to some of the previous literature (Amano & Meyer, 1982). The responses to isocapnic hypoxia after ingestion of high dose of indomethacin were not significantly different (Fan *et al.*, 2011). Nonselective COX inhibitor has a robust effect on the CBF after four days of drug ingestion compared to selectively COX-2 inhibitor celecoxib. But selectively COX-2 inhibition did not reduce CBF significantly. It indicates that CBF could be mainly regulated by downstream PGs produced by COX-1 pathway (Niwa *et al.*, 2001;Gordon *et al.*, 2008;Gordon *et al.*, 2011). After IH exposure, the afternoon CBF was decreased with both indomethacin and celecoxib compared to placebo but not significantly. Interestingly,

the decrease was more pronounce in case of celecoxib followed by indomethacin compared to placebo. When the CBF was compared within drug intervention i.e. pre- and post-IH exposure, it was decreased with placebo and celecoxib but appeared to increase with indomethacin but the changes were not statistically significant. It is interesting that the significant reduction in CBF with indomethacin was slightly elevated in the afternoon. The PG ratio has shifted to vasodilatory side for indomethacin while vasoconstrictive side with celecoxib (as proposed). Hence, it could be compensatory production of PGI_2 with indomethacin or it might be COX-1 pathway contributing predominantly in CBF regulation

5.6 Acute isocapnic hypoxia challenge

Blood pressure responses

The healthy human model of OSA to expose subjects for six hours with isocapnic IH increases the resting BP and the gain of the responses to acute isocapnic hypoxic challenge (Foster *et al.*, 2010b;Foster *et al.*, 2009). The MAP gains were similar across the protocols between placebo, indomethacin or celecoxib during pre-IH exposure (i.e. morning MAP gains). The four days of ingestion either of selective or nonselective COX-inhibitors did not have any significant effects on MAP response gains to acute hypoxic challenge. Since, the gain of responses with the drugs (both indomethacin and celecoxib) were similar to placebo group, either of COX inhibition (COX-2 or both COX-1 and COX-2) might not have significant effect on normal healthy individuals while they take shorter duration of medications when they were challenged with acute isocapnic hypoxia. When the subjects exposed to 6 hrs of IH, MAP response gains were highest with

indomethacin followed by celecoxib and then placebo although they did not reach statistically significant compared to placebo (**Figure 4.10, Panel 1.**). The MAP gains were contributed by both SBP and DBP gains. The changes in DBP gains were larger compared to SBP gains and the pattern was again indomethacin > celecoxib > placebo while SBP followed the pattern of celecoxib > indomethacin > placebo (**Figure 4.10, Panel 2 & 3.**). Hence, it is clear that the individuals who are COX inhibitors and get exposed to IH (mimicking OSA or have OSA) have increased MAP gain of responses especially to nonselective COX inhibitor (indomethacin). Given the public burden of NSAIDs (Aalykke & Lauritsen, 2001;Abdul-Hadi *et al.*, 2009;Grootendorst *et al.*, 2005), with this finding, patients having sleep disordered breathing should be careful of COX inhibitors (Bugess *et al.*, 2010). It is important to note that COX inhibitors increase cardiovascular risk and so do IH exposure. The gain of BP responses pre-IH and post-IH were increased all across the drug protocols with indomethacin affecting the most. They were not statistically significant although DBP gain (pre-IH vs post-IH) with indomethacin approached to significance ($p = 0.09$). The PG changes across the protocols pre-IH to post-IH follow the pattern in BP gain of responses. Indomethacin has highest impact on PGI_2 (**Figure 4.2**) and even higher in TxA_2 (**Figure 4.3.**). Hence, the post-IH increase in PGs (especially vasoconstrictor TxA_2) seems to have contributed increase in BP gains. The increase in gain of responses in BP with COX inhibitors among healthy individuals with just 6 hrs of IH exposure could be greater significance general population given the use of NSAIDs (Aalykke & Lauritsen, 2001;Abdul-Hadi *et al.*,

2009;Grootendorst *et al.*, 2005) and OSA prevalence (Young *et al.*, 1993;Young *et al.*, 2005;Somers *et al.*, 2008;Punjabi, 2008).

Cerebrovascular responses

There is reduction in absolute CBF with acute high dose of nonselective COX inhibitor (indomethacin) (Xie *et al.*, 2006;Fan *et al.*, 2010;Barnes *et al.*, 2012b). To date, the effect of COX inhibition n CBF is limited to nonselective especially to indomethacin. The gain of the CBF responses increases with hypoxic challenge (Ainslie & Poulin, 2004b;Beaudin *et al.*, 2011;Steinback & Poulin, 2008). The CBF gain after four days COX inhibition was lowest with selectively COX-2 inhibitors (statistically not significant) while indomethacin and placebo were similar. The six hours of IH exposure increased the gains across the protocols and it was highest in indomethacin while remained lowest in celecoxib (statistically not significant) as in the morning. Post-IH CBF response gain was higher with indomethacin and negligible with celecoxib compared to pre-IH (**Figure 4.12, Panel 1.**). The blunted response with celecoxib was not responding with IH exposure as much as with indomethacin and placebo. Interestingly, the suppressed PGI_2 and TxA_2 have been stimulated with IH exposure with indomethacin. Hence, indomethacin suppresses both vasoactive PGs and they stimulated with IH exposure which might be responsible for the increased CBF response gain. This was speculated in the previous studies (Barnes *et al.*, 2012b;Barnes *et al.*, 2013) but to our knowledge we are measuring for the first time in humans. Earlier study among OSA patients has also reported that there is blunted CBF response to hypoxia and it is restored

with successful CPAP therapy (Foster *et al.*, 2007). Therefore, in terms of CBF reactivity to hypoxic challenge before and after IH exposure, COX-2 inhibitor seems to have blunted it more than nonselective. This is warrants further with patients taking COX-2 inhibitors especially when a recent meta-analysis further highlights that OSA is associated with increased risk of stroke (Loke *et al.*, 2012).

The cerebrovascular conductance reactivity (CVC gains) where the changes in flow is dominant was lowest with selectively COX-2 inhibitor after four days of ingestion while nonselective COX-inhibitors did not affect much compared to placebo. Six hours of IH exposure, the gains with celecoxib were further reduced while it was slightly stimulated with indomethacin group i.e. the flow affected with COX-2 inhibition. The pattern followed same with pre-IH and post-IH within drugs i.e. slightly elevated in indomethacin but appeared to decrease with placebo and celecoxib (again statistically not significant). Although there is bigger individual variability across the protocols (which could be due to genetic basis) (McGettigan *et al.*, 2011), it is clear that COX-inhibitors have effects in CVC gains with IH exposure. The cerebrovascular resistance (CVR), where vascular tone has greater effect on MAP, was decreased most with indomethacin after four days of ingestion and it was further decreased after 6 hrs of IH exposure. The CVR with celecoxib was comparable to placebo. Hence, the nonselective COX-inhibitor affects on flow while COX-2 inhibitors affects more on vascular tone. As speculated previously COX pathway plays important role in cerebrovascular reactivity (Barnes *et al.*, 2013) and it seems COX-1 and COX-2 have distinct roles along with other multiple

mechanisms are certainly involved in OSA associated cerebrovascular consequences (Durgan & Bryan, 2012;Dempsey *et al.*, 2010).

Interpretations

These findings support current guidelines suggesting minimal cardiovascular risks associated with short-term, low-dose use of celecoxib in young to middle-aged adults (Wenner *et al.*, 2011). The Padol-Hunt hypothesis (Padol & Hunt, 2010) of inflammation and the concept of 'The gun must be loaded for COX-2 inhibitors to pull the trigger and cause cardiovascular toxicity' (Rainsford, 2010) seems close to these findings. This study also brings out the aspects of nonselective COX inhibitors which seem to cause a robust increase in BP and decrease in CBF. To effectively summarize and interpret the study, a clinical dose and regimen of nonselective COX-inhibitors are equally harmful in the cardiovascular and cerebrovascular homeostasis perturbation while interaction with six hours of intermittent hypoxia appears inconclusive (**Figure 13**, diagrammatic summary of the study). The involvement of COX-2 (with a representative drug, Celecoxib) appeared to be mild effect. In an attempt of decrease gastrointestinal toxicity (Wallace, 2002;Wallace, 2008), the pursuit of COX-2 inhibitors seems to have overlooked the cardiovascular toxicity of nonselective COX inhibitors. The population based epidemiological data coming lately have exposed it (Trelle *et al.*, 2011;McGettigan & Henry, 2013;McGettigan & Henry, 2011;Schjerning Olsen *et al.*, 2013) and the physiological data here support this.

Perspective

This study was designed to have clinical application and relevance. The dosages selected were in the clinical range that most general population and healthy individuals take when there is pain, inflammation and injury. Usually when there is trauma, headache or injuries (e.g. sports); they typically take few days to weeks (Hertel, 1997;Jones & Lamdin, 2010;Schnitzer, 2006;Pardutz & Schoenen, 2010;Holland *et al.*, 2012). Hence, the physiological changes in this range with clinically relevant dosage were studied. The 6 hrs of IH exposure with 10% desaturation is robust stimulus and simulates moderate OSA. COX inhibitors are the commonly used, very frequently prescribed and also available over the counter. Because they are related to cardiovascular toxicity, the implications of these findings are extremely important. Therefore, the outcome of the study can be directly correlated with human subjects or patients who take or need to take these drugs.

Limitations of the study

The study was on healthy human subjects and 6 hrs IH exposure mimicking OSA was during the day while subjects were awake. The IH exposure was only one day (6 hrs) while OSA subjects usually have history of weeks to months and even years of IH exposure prior to diagnosis. The regimen for COX inhibitors was 5 days while many subjects who have cardiovascular toxicity take for a longer duration. The prostaglandins were analysed from the urines which is the best so far available methods (Fitzgerald *et al.*, 1981;Cheng *et al.*, 2002;Yu *et al.*, 2012;Clarke *et al.*, 1991) but may not absolutely

reflect the circulating vascular level of PGs (Mitchell & Warner, 2006;Kirkby *et al.*, 2012). The CBF measurement was with TCD which assumes MCA diameter constant across the population.

Future directions

The future avenues of cardio- and cerebrovascular physiology should integrate patient population having cardio- and cerebrovascular diseases and are under treatment with various medications e.g. cyclooxygenase inhibitors. The patients with sleep disordered breathing who have been using COX-inhibitors for long duration will be interesting to look at. In fact, many of those COX-inhibitor users are ambulatory and can be very well be subjected to the hypoxia and hypercapnia challenge. The population groups using different COX-inhibitors with preferential isomerases inhibition in different dosages over different time periods with other comorbidities will help to tease out disease pathology. In healthy human subjects, it will be interesting to look at acute effects of these selective vs nonselective COX-inhibitors and possibly other prostaglandin isomerases.

TABLES

Table 4.1 General and sleep characteristics of the subjects	
Characteristics	**Mean**
Age (y)	25.8±5.1
Height (cm)	180.6±8.0
Weight (kg)	81.6±12.1
BMI (kg/m^2)	24.9±2.5
Neck Circumference (cm)	39.2±3.2
Abdominal Circumference (cm)	88.7±8.6
Hip Circumference (cm)	96.5±7.5
MAP (mmHg)	88.7±9.4
SBP (mmHg)	119.1±11.1
DBP (mmHg)	73.5±10.6
Pulse Rate (beats/min)	60.8±7.1
Race	Caucasian (11), Asian (1)
Sex	Males
Sleep parameters[1]	
Monitoring (hours)	6.4±1.2
Total Time on Probe (hours)	6.4±1.4
RDI (Total)	1.8±1.1
RDI (Supine)	2.2±1.9
RDI (Lateral)	1.6±1.2
Mean SaO_2 (%)	94.8±1.1
Lowest SaO_2 (%)	89.2±4.6
Time in $SaO_2 < 90$ %	0.0±0.1

Table 4.1 General and sleep characteristics of the subjects

[1]Determined by Remmer's sleep recorder (i.e SnoreSat), the values represent means ± SD, Abbreviations: BMI = body mass index, MAP = mean arterial blood pressure, SBP = systolic blood pressure, DBP = diastolic blood pressure, RDI = respiratory disturbance index, SaO_2 = blood oxygen saturation (recorded with pulse oximetry)

Table 4.2 Venous blood results (ABL800, Screening)	
Parameters	**Mean**
pH	7.4±0.1
Anion Gap (mmol/L)	9.0±2.2
cH (mmol/L)	45.3±4.8
Anion Gap Kc (mmol/L)	13.0±2.2
$cHCO_3$ P (mmol/L)	27.1±1.9
$cHCO_3$ P st (mmol/L)	23.8±1.6
P_{CO_2} (mmHg)	51.1±6.5
P_{O_2} mmHg	29.1±11.0
p50c (mmHg)	30.2±2.6
SO_2 (%)	44.7±18.7
cHct (%)	48.93±2.7
ctHb (g/dL)	16.0±0.9
FCOHb (%)	0.7±0.2
FO_2Hb (%)	44.1±18.3
cCrea (µmol/L)	75.0±15.1
Creatinine Serum (CLS: mg/dL)	0.9±0.9
cGlu (mmol/L)	5.0±0.3
cLac (mmol/L)	1.1±0.3
ctBil (mg/L)	3.6±6.7
cNa (mmol/L)	139.5±1.8
cK (mmol/L)	4.0±0.2
cCa (mEq/L)	2.4±0.1
cCl (mmol/L)	103.4±2.2
GFR (mL/min/173cm^{-2})	117.8±14.0

Table 4.2 Venous blood results (ABL800, Screening)

Venous blood analysed with ABL800 during screening, the values represent means ± SD,

Abbreviations: c = concentration

Table 4.3 Venous blood results (CLS, Screening)	
Parameters	**Mean**
Hemoglobin (g/L)	156.3±6.3
Hematocrit (L/L)	0.5±0.0
RBC (10^12/L)	5.0±0.3
MCV (fL)	92.1±2.6
MCHC (g/L)	342.8±6.2
RDW (%)	13.1±0.7
Platelet Count (10^9/L)	225.8±35.3
WBC (10^9/L)	5.9±1.7
Neutrophil (10^9/L)	3.3±1.4
Lymphocyte (10^9/L)	1.9±0.4
Monocyte (10^9/L)	0.5±0.1
Eosiniphil (10^9/L)	0.2±0.1
Basophil (10^9/L)	0.0±0.0
INR	1.0±0.0
ALP (U/L)	72.7±18.0
ALT (U/L)	18.7±4.4
APT (U/L)	23.3±4.8
Creatinine (Serum :µmol/L)	84.6±12.6
Creatinine (Serum: mg/dL)	1.0±0.1
Na (Urine:mmol/L)	64.3±30.3
Creatinine (Urine: mmol/L)	7.8±5.4
GFR (mL/min/173cm^2)	107.9±14.5

Table 4.3 Venous blood results (CLS, Screening)

Abbreviations: RBC = Red Blood Cells, WBC = White Blood Cells, MCV = Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW = Red Cell Distribution Width, ALP = Alkaline Phosphatase, ALT = Alanine Transaminase, APT = Abnormal Prothrombin, GFR = Glomerular Filtration Rate, INR = International Normalized Ratio, the values represent means ± SD

Table 4.4 Urinary dipstick results	
Parameters	**Mean**
Specific Gravity	1.0±0.0
p^H	5.7±0.8
Leukocytes (-/+)	0.0±0.0
Nitrite (-/+)	0.0±0.0
Protein (-/+)	0.0±0.0
Glucose (N/+)	0.0±0.0
Ketones (-/+)	0.0±0.0
Urobilinogen (N/+)	0.0±0.0
Bilirubin (-/+)	0.0±0.0
Blood (-/+)	0.0±0.0
Hemoglobin	1.0±0.0

Table 4.4 Urinary dipstick results

Urinary dipstick test was done in the lab immediately after collecting urine; the values represent means ± SD

Table 4.5 Capillary blood sample results during experimental sessions						
	PLACEBO		**INDOMETHACIN**		**CELECOXIB**	
Parameters	AM	PM	AM	PM	AM	PM
pH	7.4±0.0	7.4±0.0	7.4±0.0	7.4±0.0	7.4±0.0	7.4±0.1
Anion Gap (mmoL/L)	8.3±1.9	7.5±1.9	7.7±2.5	8.0±2.3	8.2±2.6	9.1±2.5
cH (mmoL/L)	40.2±1.6	38.4±0.8	39.9±1.1	38.1±0.8	40.8±1.7	40.4±5.6
AnionGap_Kc (mmoL/L)	12.3±1.8	11.9±1.8	11.9±2.4	12.3±2.1	12.4±2.6	13.5±2.5
$cHCO_3$_P (mmoL/L)	23.4±1.3	24.9±1.4	23.1±1.0	24.3±1.3	22.8±1.7	23.8±3.5
$cHCO_3$_P_st (mmoL/L)	23.7±1.1	25.1±1.0	23.5±1.0	24.7±0.8	23.2±1.4	24.1±3.1
pCO_2 (mmHg)	38.9±1.8	39.5±2.2	38.1±1.8	37.9±2.8	38.4±2.0	39.1±3.0
PO2 (mmHg)	73.3±4.8	70.9±4.0	75.0±5.0	73.7±6.3	76.9±5.7	69.2±7.5
p50c (mmHg)	23.8±1.3	24.2±1.0	23.9±1.0	24.0±1.1	24.1±1.4	24.9±2.0
SO_2 (%)	95.5±1.0	95.0±1.2	95.8±0.7	95.6±1.3	96.0±1.1	94.4±1.8
pO_2_A_a_est (mmHg)	12.7±4.1	14.6±4.2	12.9±5.9	13.7±4.5	9.6±4.0	16.5±5.1
cHCT (%)	48.1±2.5	52.3±3.3	47.6±2.1	49.9±4.0	48.4±3.4	50.2±6.4
ctHb (g/dL)	15.5±0.8	17.1±1.1	15.6±0.7	16.4±1.3	15.8±1.1	16.4±2.1
FCOHb (%)	0.9±0.2	1.0±0.2	1.2±0.8	1.1±0.3	0.9±0.1	0.9±0.2
FO2Hb (%)	94.1±0.9	93.5±1.2	94.1±1.2	94.1±1.3	94.6±1.1	93.0±1.7
cCrea (µmoL/L)	75.1±18.0	73.9±20.7	76.0±15.6	74.3±14.8	73.1±14.1	68.8±12.7
cGlu (mmoL/L)	9.5±11.6	5.6±1.0	6.2±1.4	5.9±0.8	5.9±1.0	5.9±0.9
cLac (mmoL/L)	1.7±0.5	1.4±0.3	1.6±0.8	1.3±0.3	1.7±0.6	1.5±0.4
ctBil_mg_L	2.3±3.8	2.3±4.3	2.3±4.3	2.5±4.3	1.3±2.3	3.3±4.8
cNa (mmoL/L)	139.9±2.4	140.8±2.5	140.2±3.6	141.3±2.5	139.7±1.7	141.4±2.3
cK (mmoL/L)	4.0±0.2	4.4±0.3	4.2±0.2	4.4±0.3	4.2±0.3	4.4±0.2
cCa (mEq/L)	2.5±0.1	2.4±0.1	2.4±0.1	2.4±0.1	2.5±0.1	2.5±0.1
cCl (mmoL/L)	108.1±1.8	108.4±1.4	109.3±2.9	109.2±2.3	108.8±2.7	108.6±2.2

Table 4.5 Capillary blood sample results during experimental sessions

Capillary blood sample results during experimental sessions collected during morning (AM) and afternoon (PM) immediately before acute tests, the values represent means ± SD, **Abbreviations:** c = Concentration, others refer to abbreviation table

FIGURES AND ILLUSTRATIONS

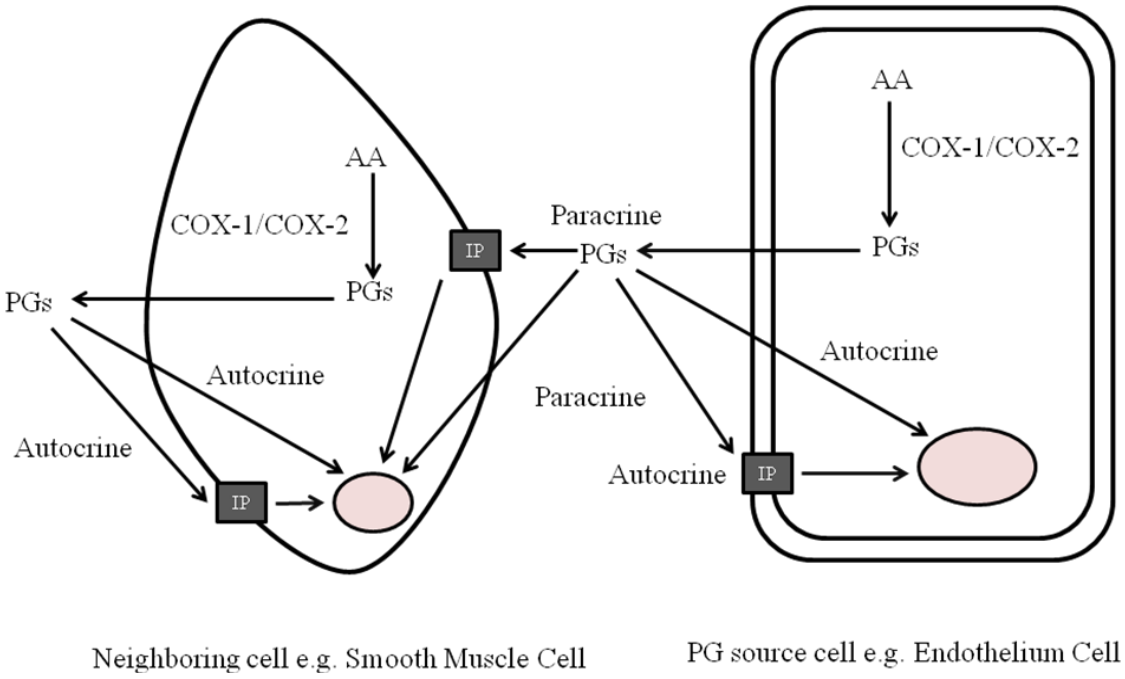


Figure 1.1 Prostaglandin actions in paracrine and autocrine pathways

Redrawn and modified from Dubois et al (Dubois *et al.*, 1998). The schematic diagram of potential mechanisms involved in the cyclooxygenase-mediated regulation via paracrine and autocrine pathways (translated into vascular wall)

Footnotes: The schematic diagram of potential mechanisms involved in the cyclooxygenase-mediated regulation via paracrine and autocrine pathways (translated into vascular wall); **Abbreviations:** AA = arachidonic acid, COX = cyclooxygenase, PGs = prostaglandins, IP = prostaglandin receptors, COX-1 = cyclooxygenase-1, COX-2 = cyclooxygenase-2

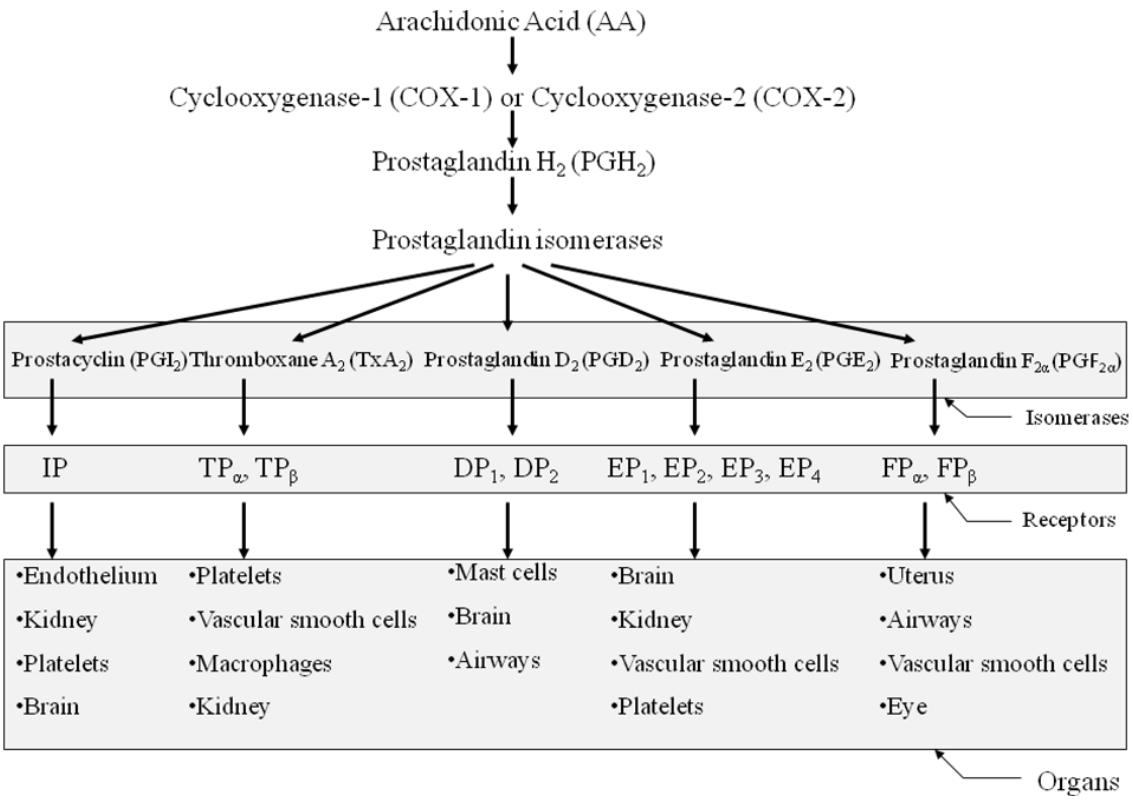


Figure 1.2 Prostaglandin pathway

Adopted from 'Fries, S. et al. Hematology 2005;2005:445-451. (Fries & Grosser, 2005)

Footnotes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) pathways; Prostaglandins (PG) are formed by specific isomerases from the COX product PGH_2. They act through G protein transmembrane receptors (IP, TP_α, TP_β, DP_1, DP_2, EP_1, EP_2, EP_3, EP_4, FP_α, FP_β); **Abbreviations:** AA = arachidonic acid, COX = cyclooxygenase, PGH_2 = Prostaglandin H_2, PGs = prostaglandins, IP = prostaglandin receptors, COX-1 = cyclooxygenase-1, COX-2 = cyclooxygenase-2; PGI_2 = Prostaglandin I_2, TxA_2 = Thromboxane A_2, PGD_2 = Prostaglandin D_2, PGE_2 = Prostaglandin E_2, $PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$; IP, TP, DP, EP and FP = respective PG receptors

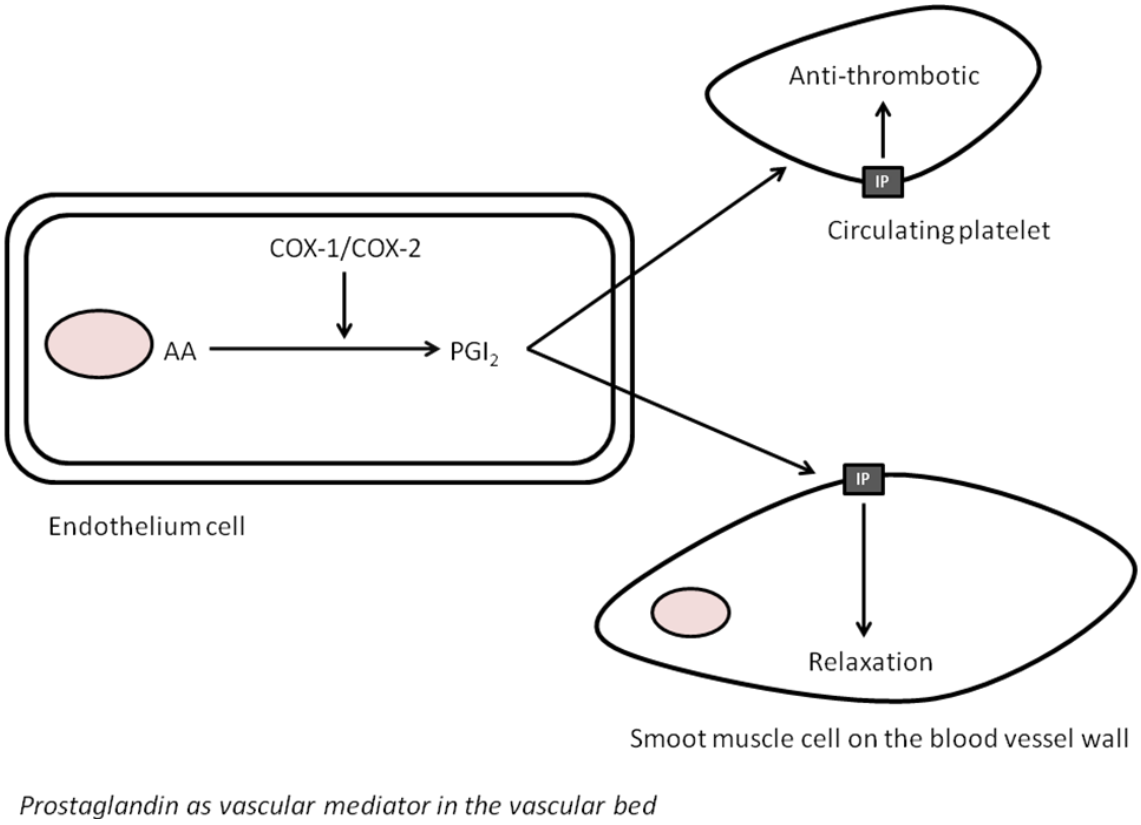


Figure 1.3 Prostacyclin (PGI_2) productions and its action on target cells in the vasculature

Redrawn and modified from Vane J and Corin, RE (Vane & Corin, 2003)

Footnotes: The schematic diagram of Prostacyclin (PGI_2) production and its target cells in the vascular bed; **Abbreviations:** AA = arachidonic acid, PGI_2 = Prostaglandin I_2, IP = prostaglandin receptors, COX-1 = cyclooxygenase-1, COX-2 = cyclooxygenase-2

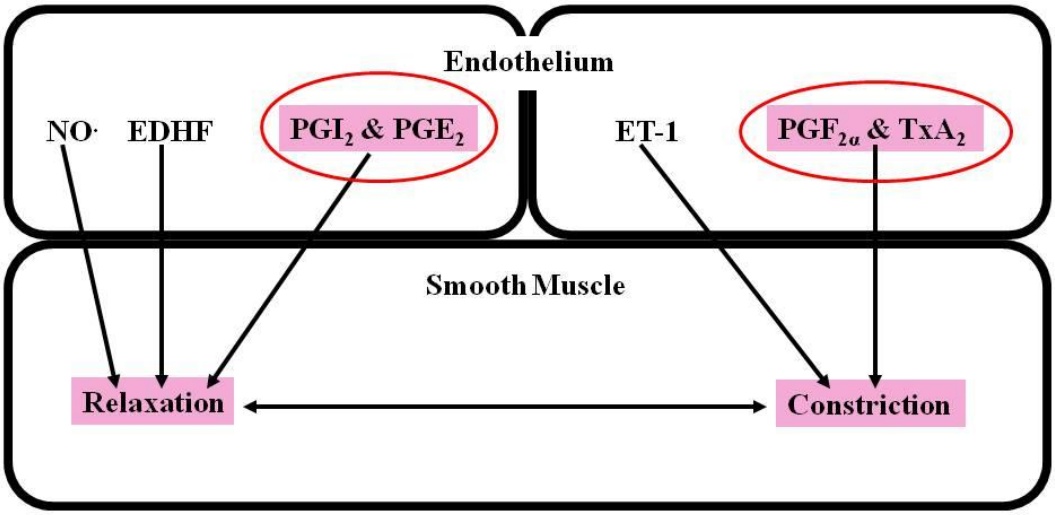


Figure 1.4 The vascular and endothelial prostaglandin synthesis in the brain

Redrawn from 'Andresen, J. et al. J Appl Physiol 100: 318-327 2006 (Andresen *et al.*, 2006)

Footnotes: The endothelium generates a variety of signals (e.g. prostaglandins) that influence cerebrovascular tone under normal conditions and during disease. The terminal prostaglandins that promote vascular smooth muscle (VSMCs) relaxation are PGI_2 and PGE_2 while $PGF_{2\alpha}$ and TxA_2 contribute in constriction in the brain.

Abbreviations: NO = nitric oxide, EDHF = endothelium-derived hyperpolarization factor, PGI_2 = prostacyclin, PGE_2 = prostaglandin E_2, (ET)-1 = endothelin, TxA_2 = thromboxane A_2, and $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$)

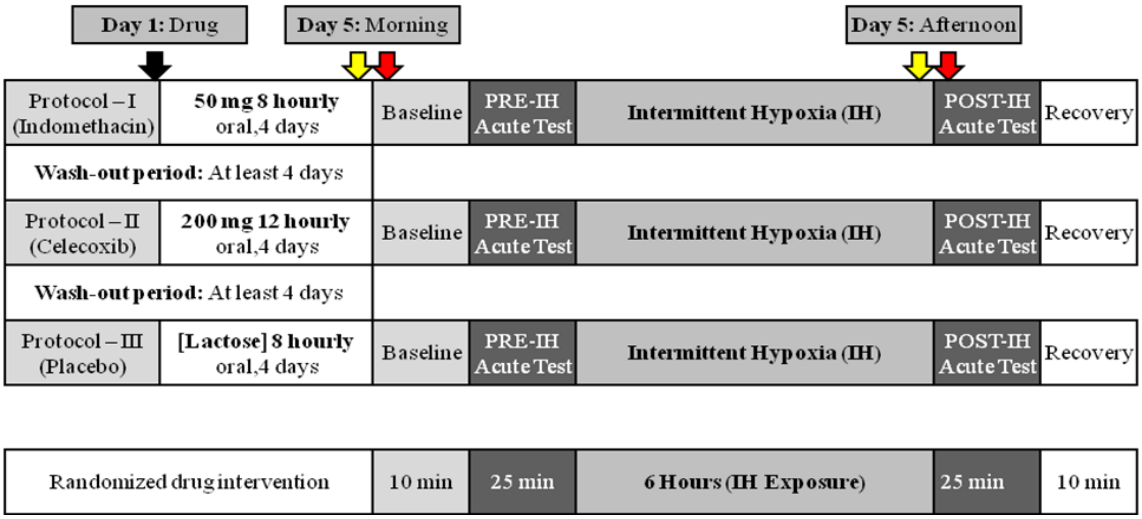


Figure 2.1 Experimental protocol over the days of drug ingestion leading to experimental day interventions

Footnotes: The subjects will go through three phases of intervention indomethacin, celecoxib and placebo. The completion of all three phases for one subject will take about four weeks. Red solid arrows represent time point of blood draw and capillary blood sampling. Yellow solid arrows point the time at which urine was collected during the experimental period. **Legends: BLACK** arrow = Starting of the drug ingestion, **YELLOW** arrow = Urine collection, **RED** arrow = Blood collection

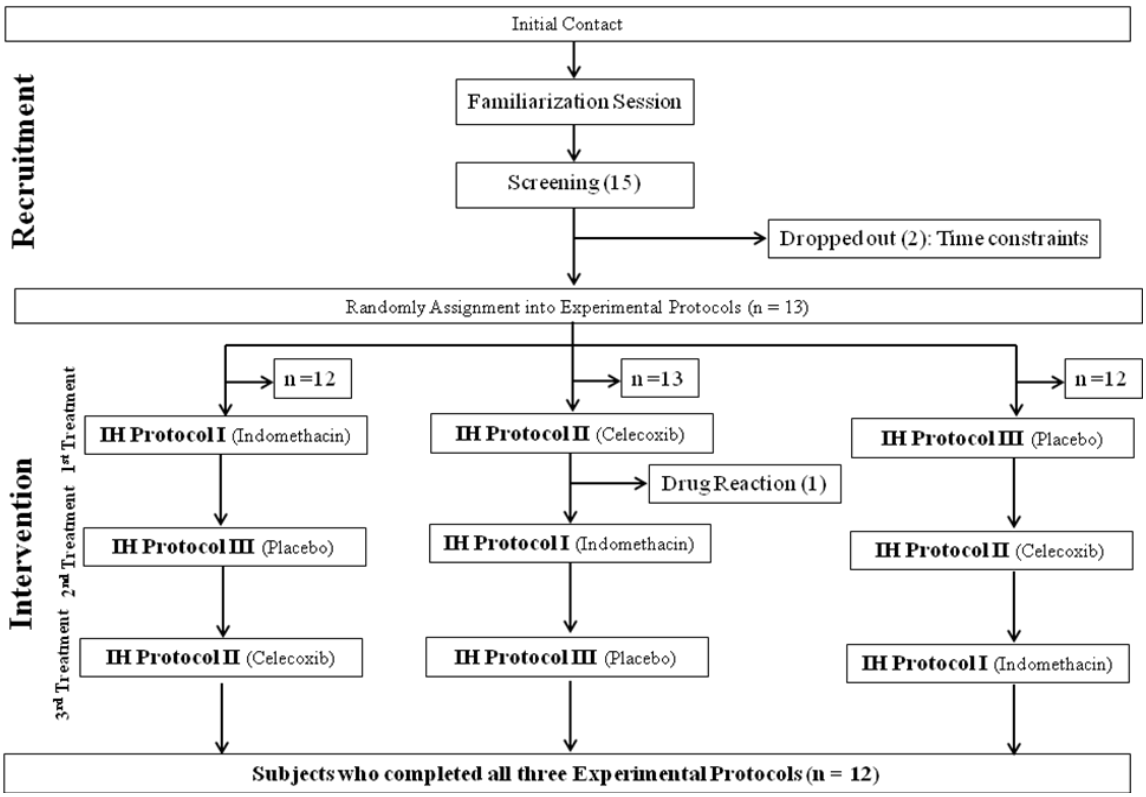


Figure 2.2 Study flow chart showing the subject recruitment, screening and drug interventions

Footnotes: Subjects went through as shown by the arrows and enter into randomly assigned drug intervention protocols (I, II, III); **Abbreviations:** COX = cyclooxygenase, IH = intermittent hypoxia, n = number of subjects, n = number of subjects undergoing interventions

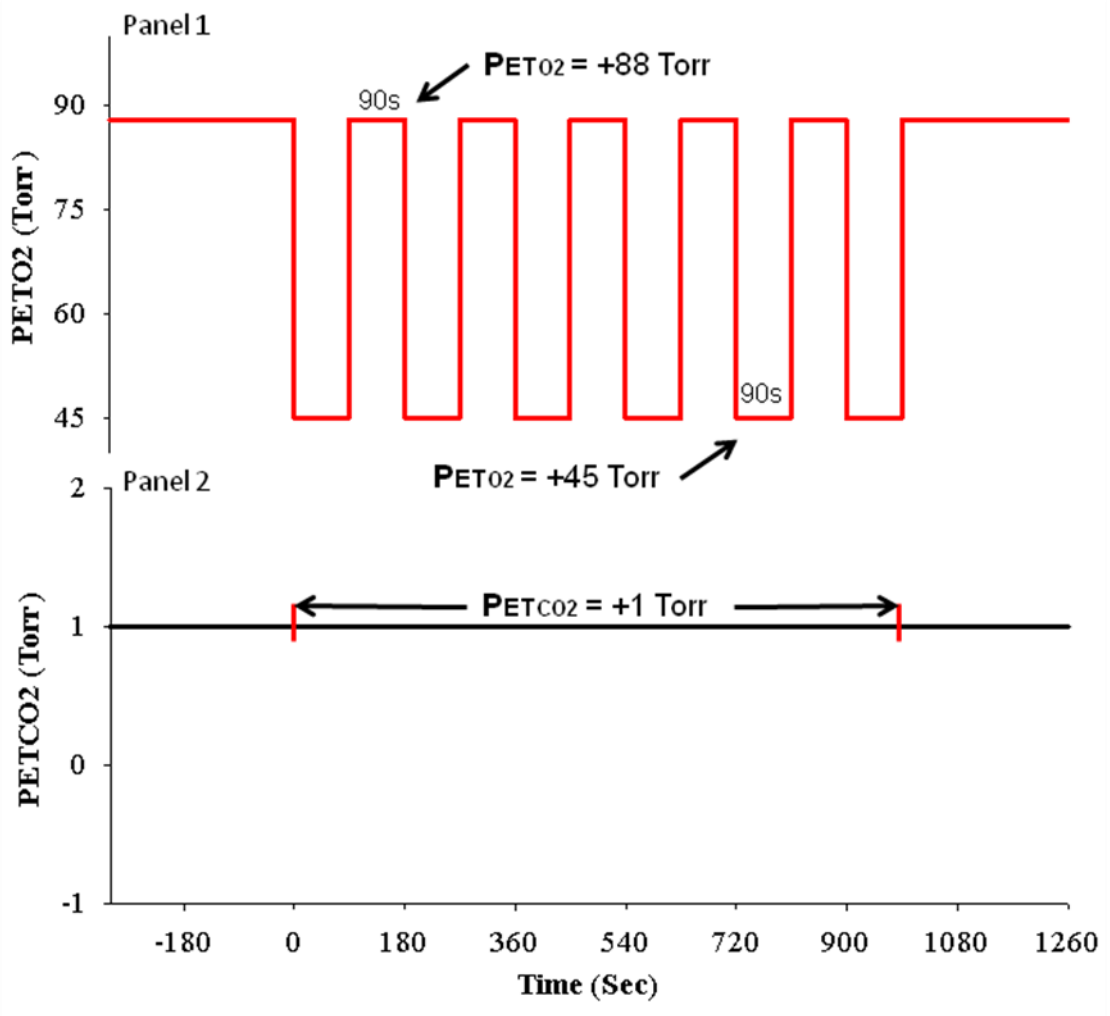


Figure 2.3 Acute isocapnic hypoxia testing protocol

Footnotes: Acute isocapnic hypoxic challenge test protocol in the morning (PRE-IH) and afternoon (POST-IH), it contains six cycles of hypoxia with isocapnia (= +1 Torr from baseline); **Abbreviations:** $P_{ET_{O_2}}$ = partial pressure of end-tidal oxygen; $P_{ET_{CO_2}}$ = partial pressure of end-tidal carbon dioxide; **Panel 1**, **RED:** acute intermittent hypoxia challenge; **Panel 2**, **BLACK:** isocapnia at +1 Torr $P_{ET_{CO_2}}$

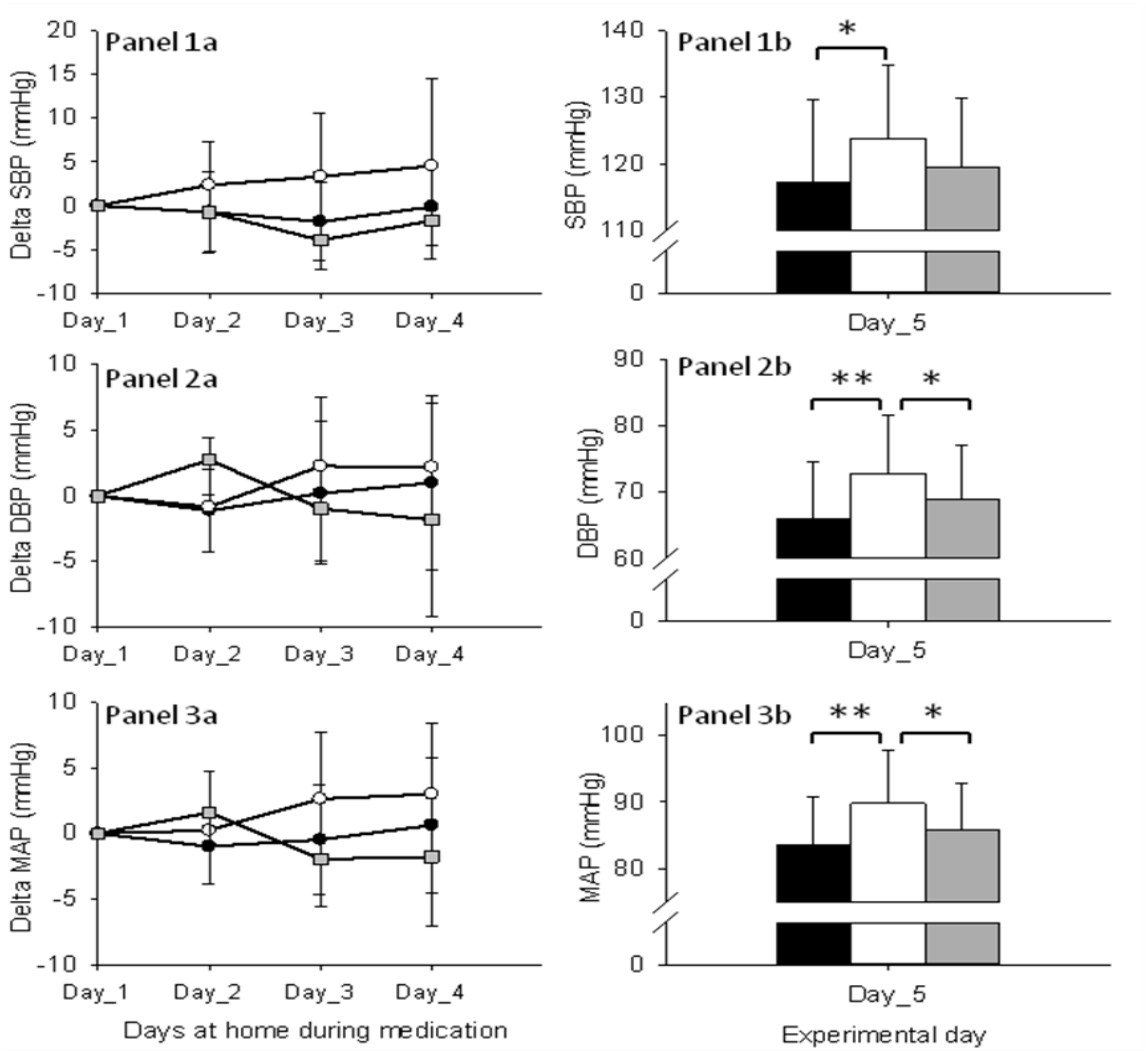


Figure 4.1 Home blood pressure monitoring at home and experimental day morning BP

Footnotes: The Delta BP (**Panel 1**) presented were subtracted from Day_1 Day_2, Day_3, Day_4 to Day_1; **Panel 2**: Fifth day absolute morning BP; the values presented represent as means ± SD; the significance: *, $p < 0.05$ and **, $p < 0.01$; **Abbreviations:** SBP = Systolic blood pressure, DBP = Diastolic blood pressure, MAP = Mean arterial blood pressure; **Legends: Panel a**, **SOLID** circle = placebo, **OPEN** circle = indomethacin, **GREY** square = celecoxib. **Panel b**, **SOLID** bar = placebo, **OPEN** bar = indomethacin, **GREY** bar = celecoxib

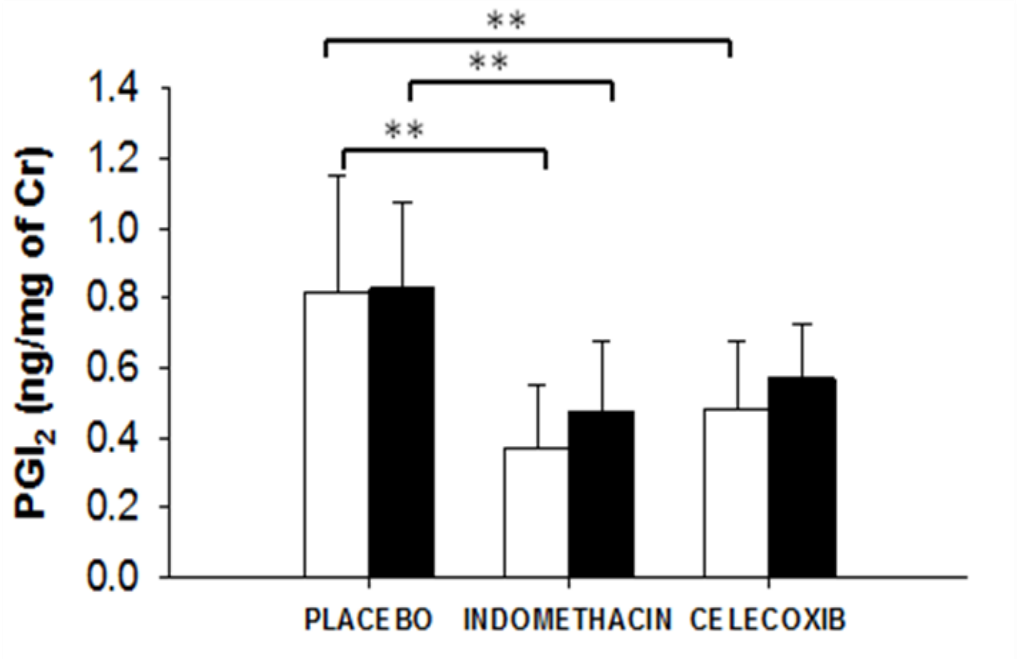


Figure 4.2 Prostaglandin I_2 (PGI_2) results PRE-IH (AM) and POST-IH (PM)

Footnotes: Prostaglandin I_2 (PGI_2) results as assayed by enzyme-immunoassay (EIA) method; the values represent means ± SD; the significance: *, $p < 0.05$ and **, $p < 0.01$; **Abbreviations:** PGI_2 = prostaglandin I_2 (prostacyclin). **OPEN** bars = AM (PRE-IH) and **SOLID** bars = PM (POST-IH)

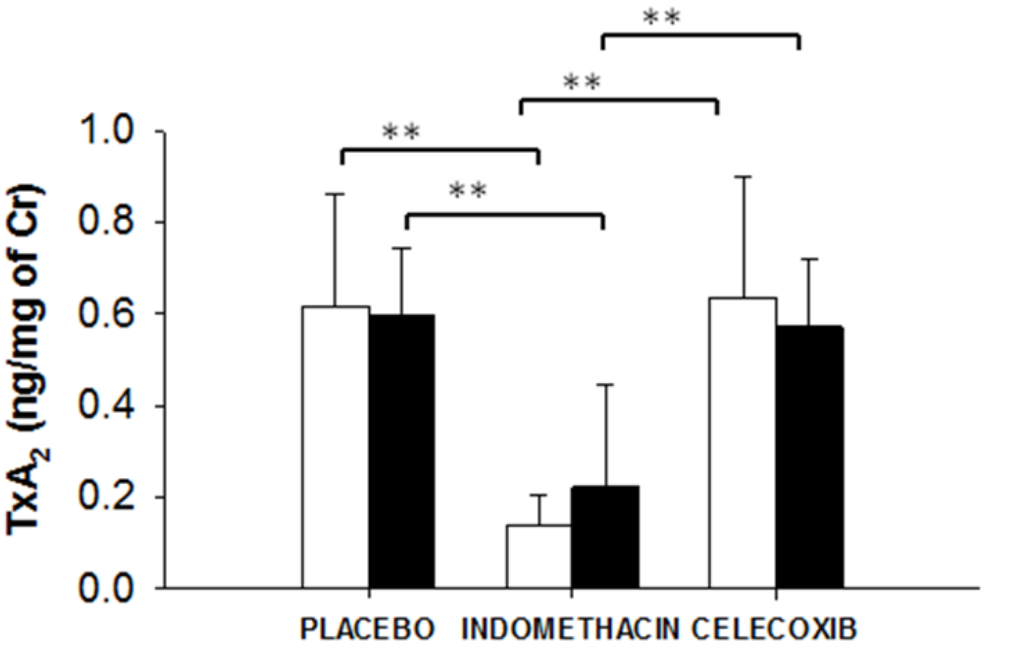


Figure 4.3 Thromboxane A_2 (TxA_2) results PRE-IH (AM) and POST-IH (PM)

Footnotes: Thromboxane A_2 (TxA_2) results as assayed by enzyme-immunoassay (EIA) method; the values represent means ± SD; the significance: *, $p < 0.05$ and **, $p < 0.01$; **Abbreviations:** TxA_2 = thromboxane A_2, **OPEN** bars = AM (PRE-IH) and **SOLID** bars = PM (POST-IH)

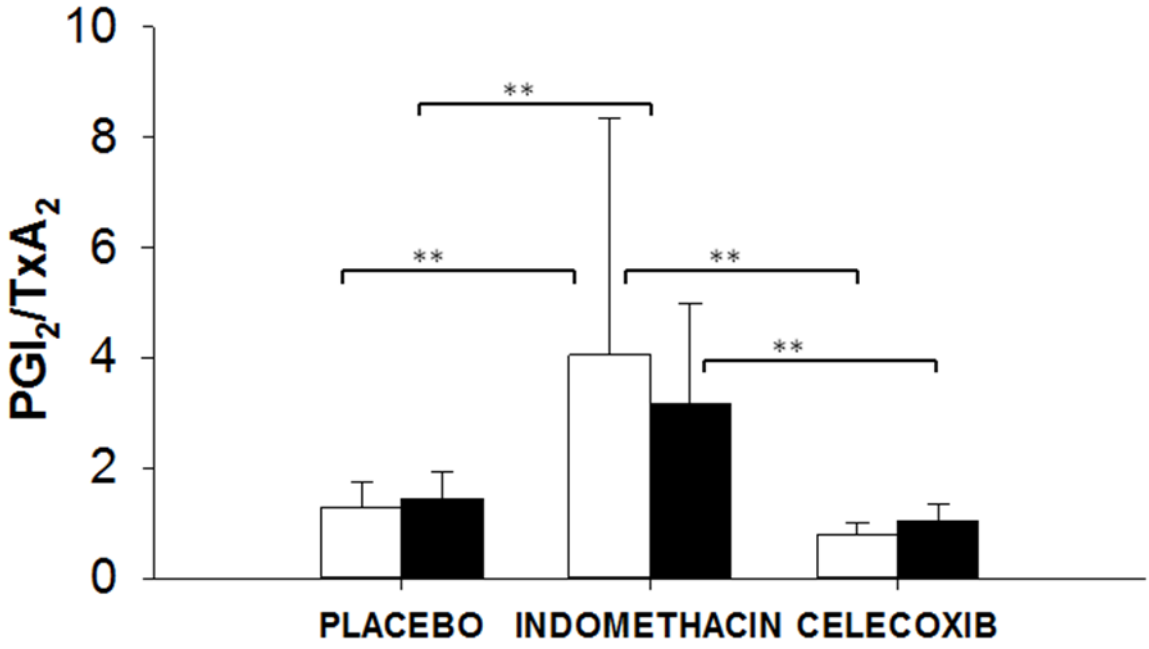


Figure 4.4 Prostaglandin ratios (PGI_2/TxA_2) i.e. vasodilator (PGI_2) vs vasoconstrictor (TxA_2)

Footnotes: The balancing ratio of vasodilator (PGI_2) to vasoconstrictor (TxA_2); the values represent means ± SD; **Abbreviations:** PGI_2 = prostaglandin I_2 (prostacyclin), TxA_2 – thromboxane A_2. The significance: *, $p < 0.05$ and **, $p < 0.01$; **OPEN** bars = AM (PRE-IH) and **SOLID** bars = PM (POST-IH)

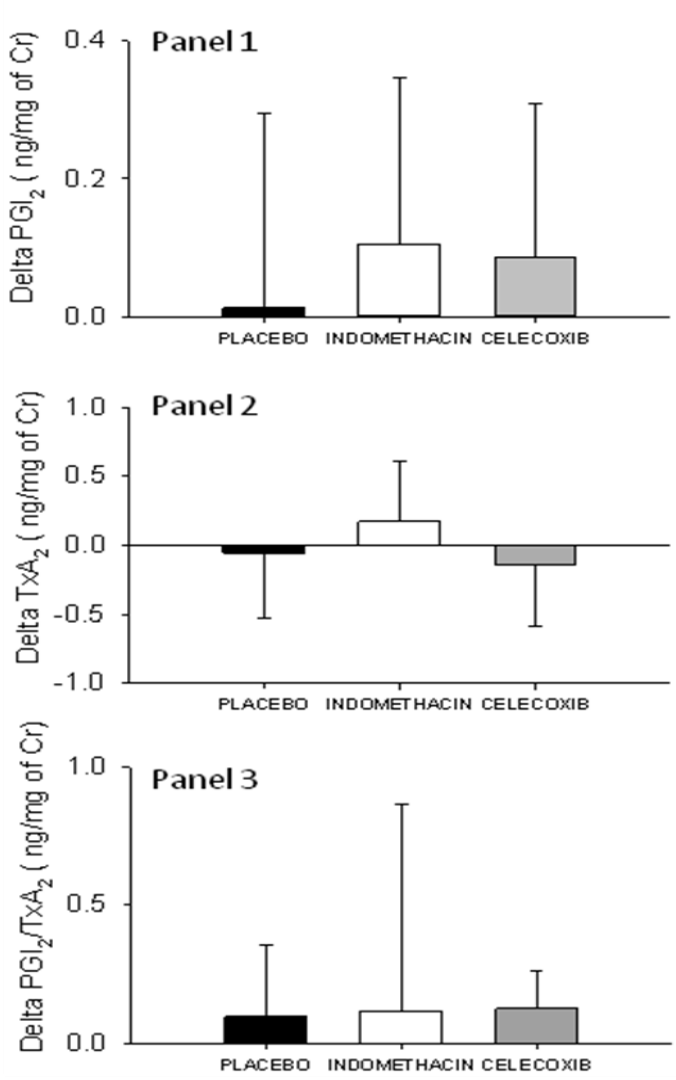


Figure 4.5 Prostaglandin changes from PRE-IH (AM) to POST-IH (PM)

Footnotes: The prostaglandin (PG) changes i.e. morning values subtracted from evening values (Δ values); the values represent means ± SD; **Abbreviations:** PGI_2 = prostaglandin I_2 (prostacyclin), TxA_2 = thromboxane A_2. **SOLID** bars = placebo, **OPEN** bars = indomethacin and **GREY** bars = celecoxib

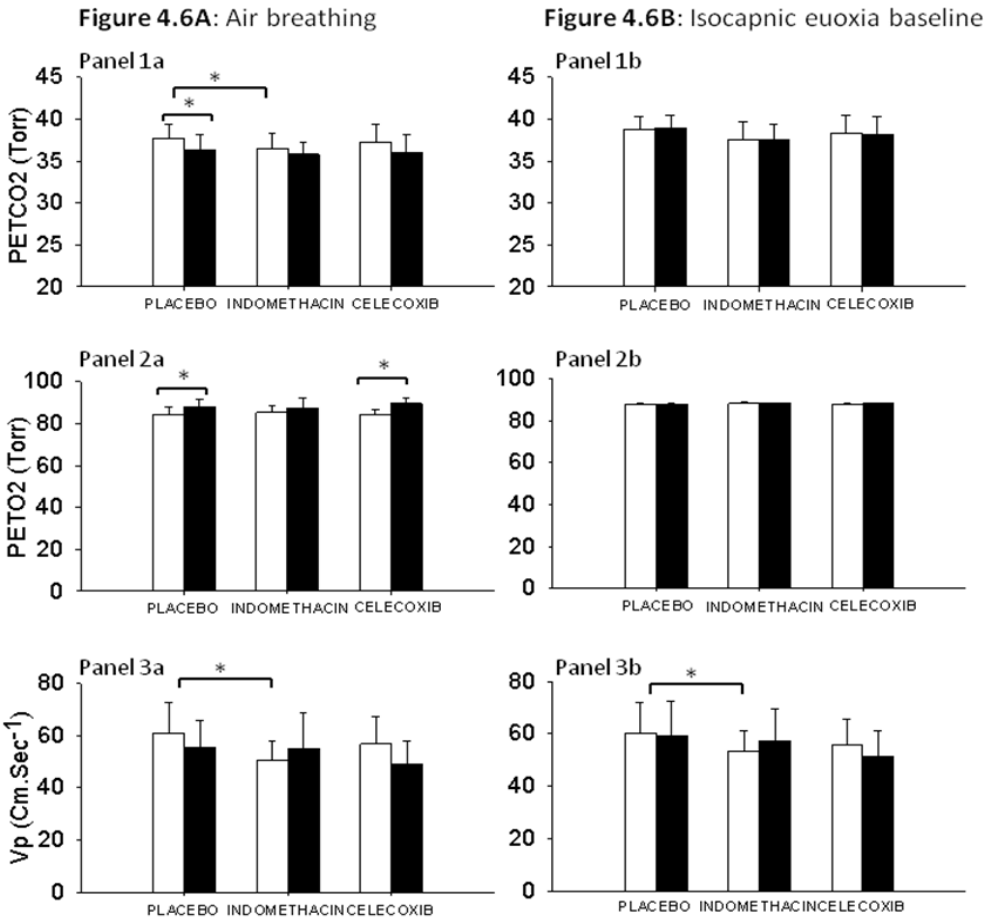


Figure 4.6 End-tidal gases and CBF responses to air breathing and isocapnic euoxia (baseline)

Footnotes: Figure 4.6A: Air breathing, **Figure 4.6B**: Isocapnic baseline; **Panel 1a.** Air breathing $\mathbf{P_{ET_{CO_2}}}$ and **Panel 1b** Isocapnic baseline $P_{ET_{CO_2}}$ (+1 Torr)**; Panel 2a** Air breathing $P_{ET_{O_2}}$ and **Panel 2b**. Isocapnic baseline $P_{ET_{O_2}}$ (Euoxia: +88 Torr); **Panel 3a.** Air breathing CBF ($\bar{V}P$) and **Panel 3b.** $\bar{V}P$ during isocapnic ($P_{ET_{CO_2}}$: +1 Torr, $P_{ET_{O_2}}$: +88 Torr); the values represent means ± SD; the significance: *, $p < 0.05$ and **, $p < 0.01$; **Abbreviations:** $P_{ET_{CO_2}}$ = end-tidal carbon dioxide, $P_{ET_{O_2}}$= end-tidal oxygen, CBF ($\bar{V}P$) = cerebral blood flow, **Legends:** In **Panels 1, 2** and **3**; **OPEN** bars = AM (PRE-IH) and **SOLID** bars = PM (POST-IH)

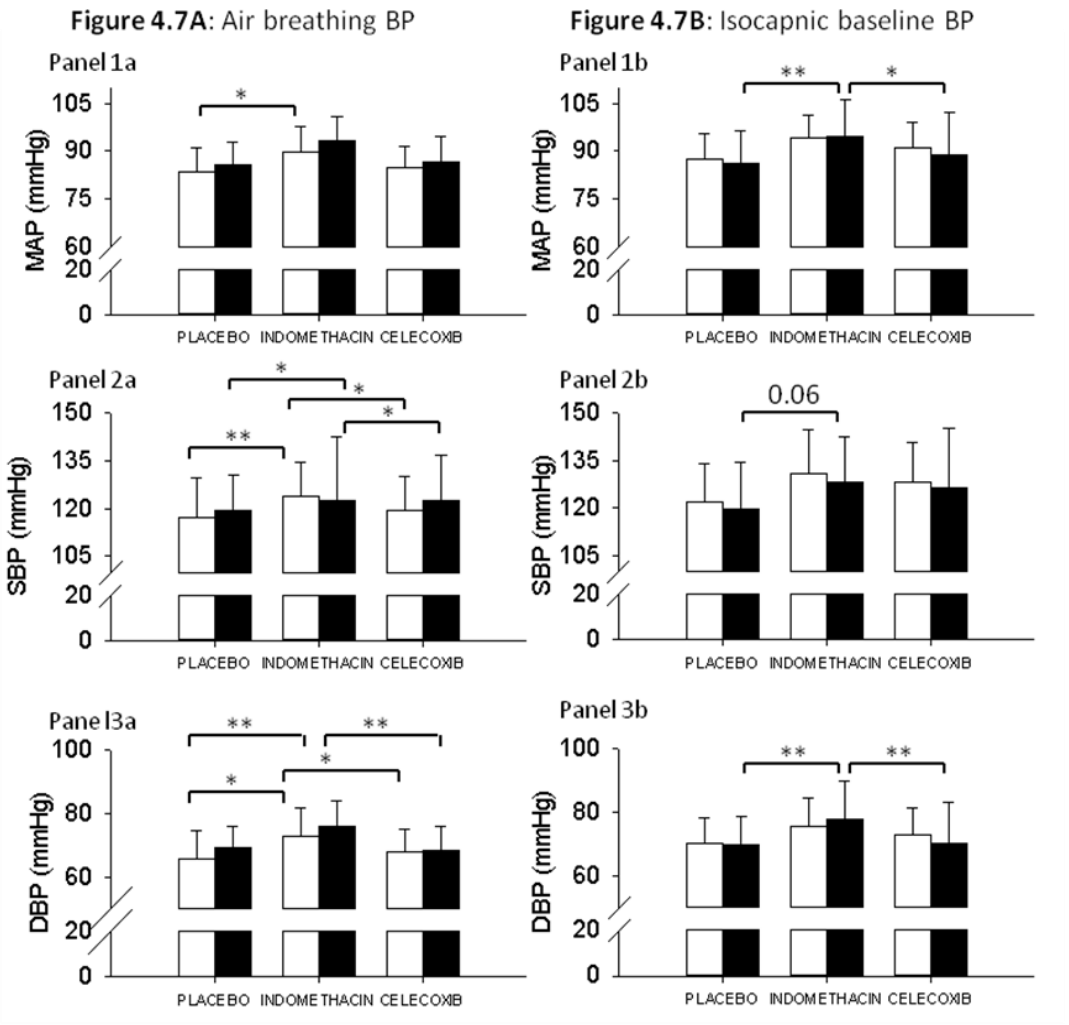


Figure 4.7 Blood pressure responses to air breathing and isocapnic euoxia baseline

Footnotes: Figure 4.7A: Air breathing BP, **Figure 4.7B:** Isocapnic baseline BP; The values represent means ± SD; the significance: *, $p < 0.05$ and **, $p < 0.01$; **Abbreviations:** SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial blood pressure

Legends: In **Panels 1, 2 and 3; OPEN** bars = AM (PRE-IH) and **SOLID** bars = PM (POST-IH)

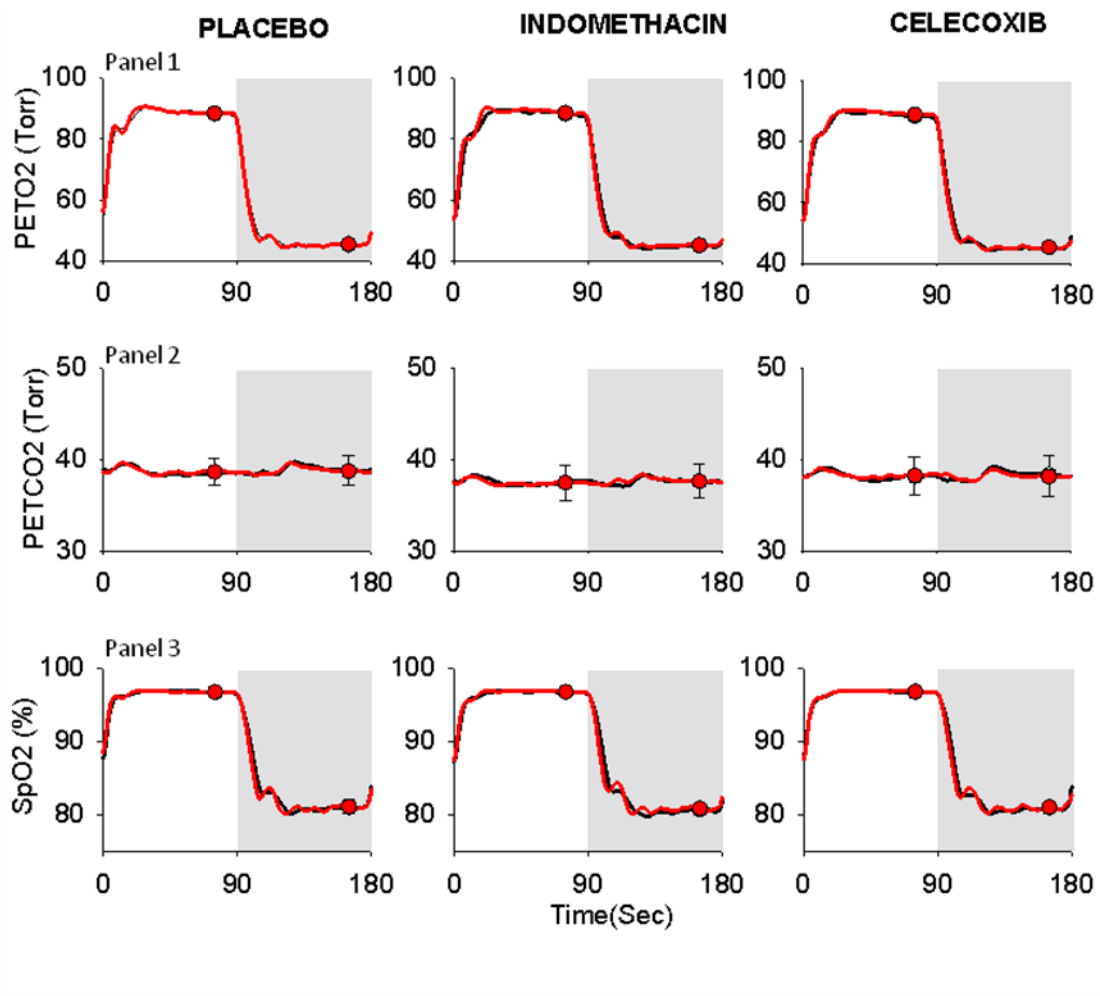


Figure 4.8 End-tidal gases during acute hypoxic test at AM (PRE-IH) and PM (POST-IH)

Footnotes: Panel 1. PET_{O_2} Six Cycles of hypoxia-normoxia averaged into a single cycle. Euoxia: +88 Torr, Hypoxia: +45 Torr of 90 seconds each; **Panel 2.** Isocapnia (PET_{CO_2}**:** +1 Torr maintained during the intermittent hypoxia cycles); **Panel 3.** Oxygen saturation; the values represent means ± SD; **Abbreviations**: SpO2 = blood oxygen saturation, PET_{O_2} = end-tidal oxygen, PET_{CO_2} = end-tidal carbon dioxide; **Legends:** Shaded Part = hypoxia cycle, **BLACK** = AM (PRE-IH), **RED** = PM (POST-IH)

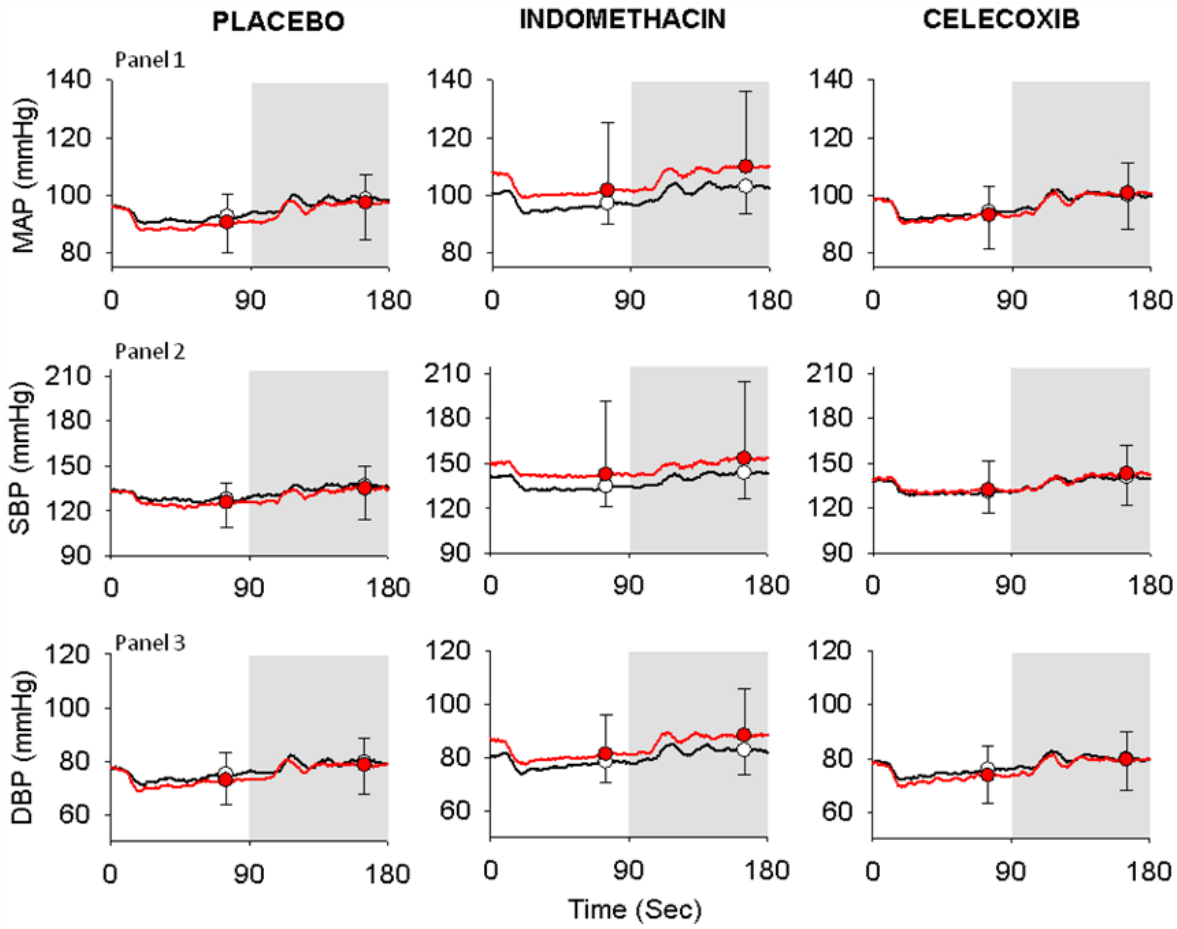


Figure 4.9 Blood pressure responses during acute hypoxic test at AM (PRE-IH) and PM (POST-IH)

Footnotes: Panel 1. Mean arterial blood pressure (MAP), **Panel 2** Systolic blood pressure (SBP) and **Panel 3** Diastolic blood pressure (DBP) during acute hypoxic test cycles; the values represent means ± SD; **Legends:** Shaded Part = Hypoxia Cycle, **BLACK** = AM (PRE-IH), **RED** = PM (POST-IH)

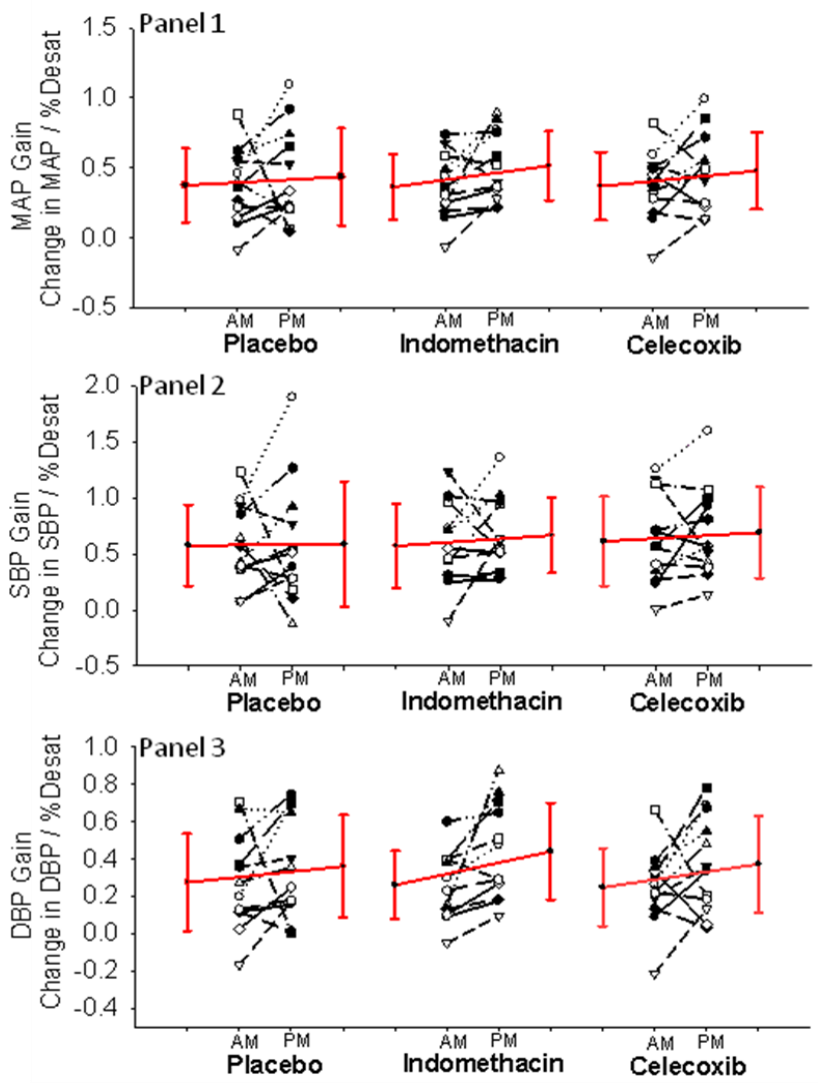


Figure 4.10 Gain in BP responses to acute hypoxic test at AM (PRE-IH) and PM (POST-IH)

Footnotes: Gains are the changes in BP/% blood oxygen desaturation (%Desat), **Panel 1.** Gain in mean arterial blood pressure (MAP), **Panel 2.** Gain in systolic blood pressure (SBP) and **Panel 3.** Gain in diastolic blood pressure (DBP); the values at AM (PRE-IH) and PM (POST-IH) represent mean ± SD

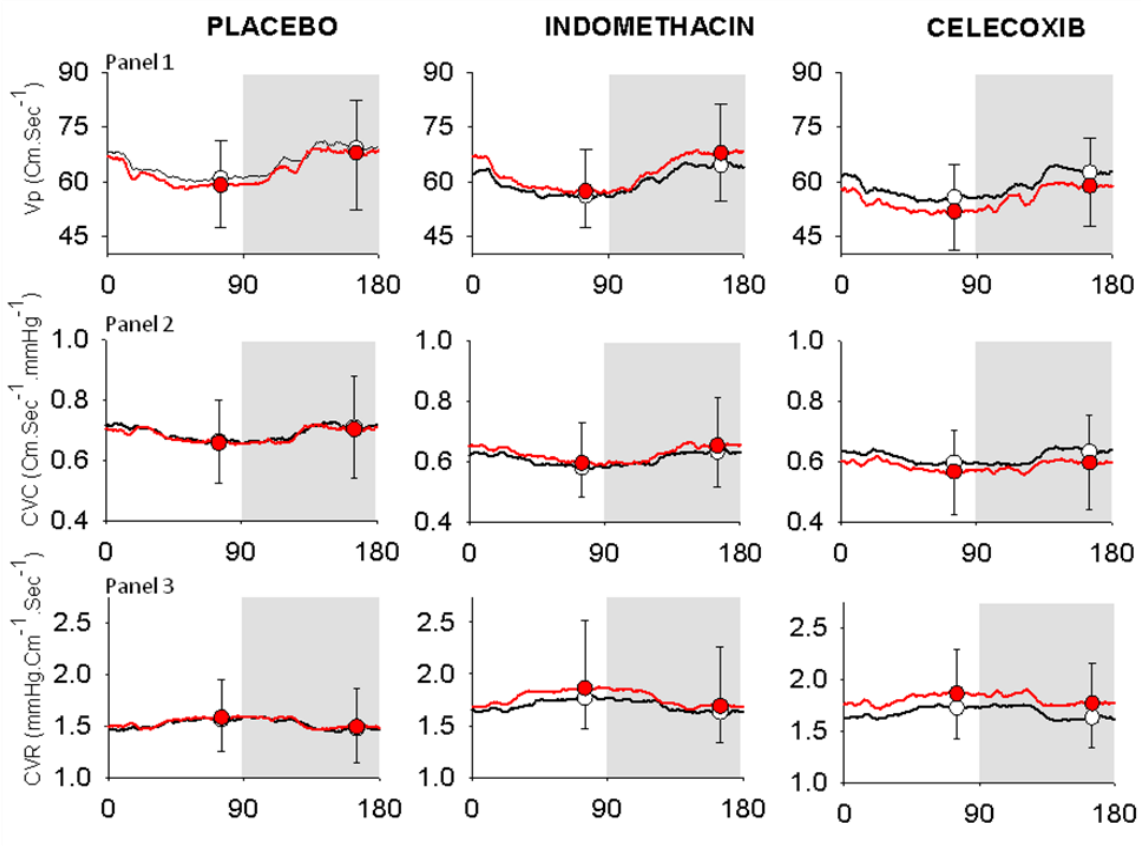


Figure 4.11 CBF responses during acute hypoxic test at AM (PRE-IH) and PM (POST-IH)

Footnotes: **Panel 1**. Cerebral blood flow (CBF or $\bar{V}P$), **Panel 2** Cerebrovascular conductance (CVC) and **Panel 3** Cerebrovascular resistance (CVR) during acute hypoxic test cycles; the values represent means ± SD; **Legends:** Shaded Part = Hypoxia Cycle, **BLACK** = AM (PRE-IH), **RED** = PM (POST-IH)

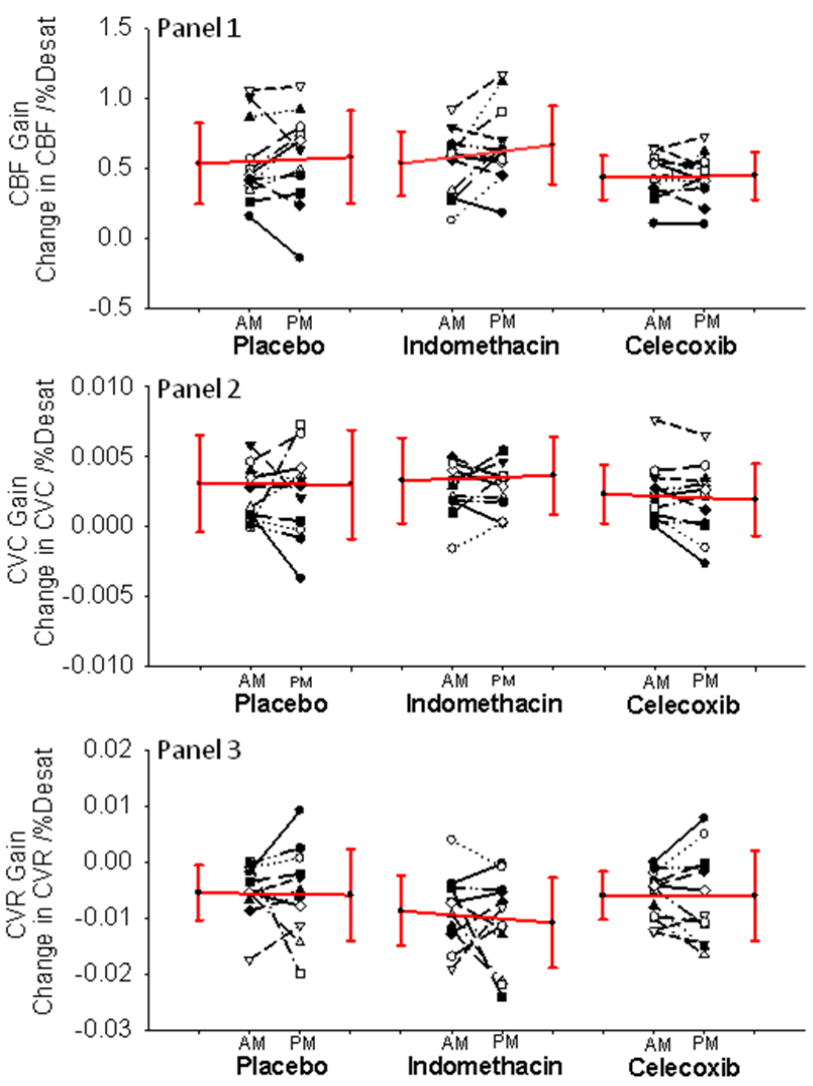


Figure 4.12 Gain in CBF responses to acute hypoxic test at AM (PRE-IH) and PM (POST-IH)

Footnotes: Gains are the change in cerebral blood flow (CBF or $\overline{V}P$), cerebrovascular conductance (CVC) or cerebrovascular resistance (CVR)/% blood oxygen desaturation (%Desat), **Panel 1.** Gain in CBF, **Panel 2.** Gain in CVC and **Panel 3.** Gain in CVR; the values at represent mean ± SD

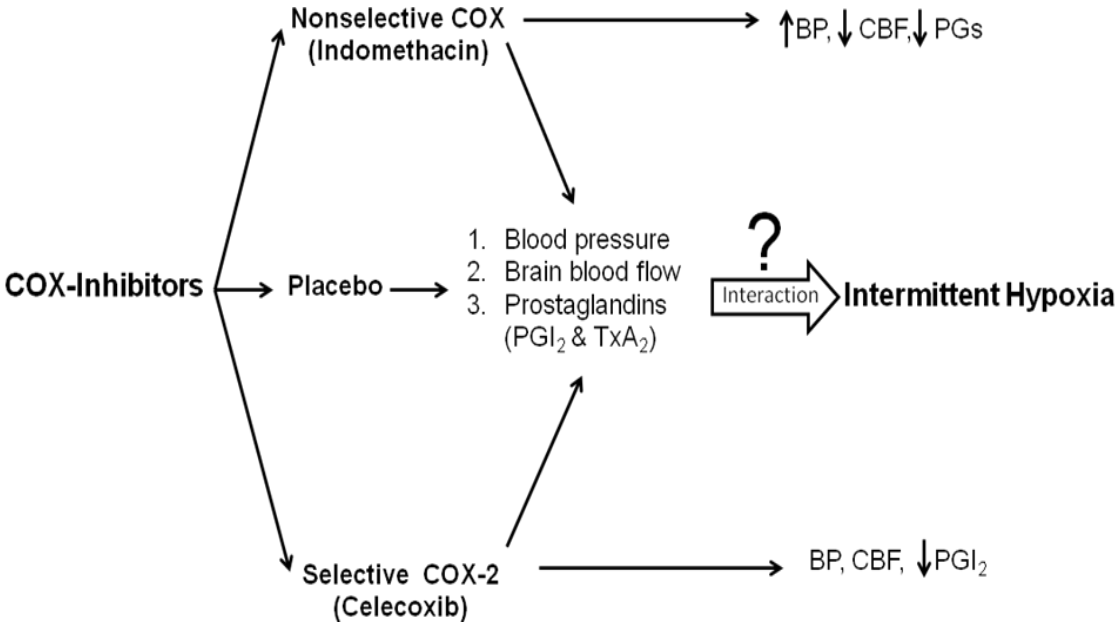


Figure 4.13 Diagrammatic summary of the study

Footnotes: The diagrammatic summary of the study taking COX inhibitors (nonselective COX inhibitor, Indomethacin and COX-2 selective inhibitor, Celecoxib) comparing them with placebo; the physiological variables and effects of drugs including the interaction (effect of) with intermittent hypoxia exposure; **Abbreviations:** COX = cyclooxygenase, CBF = cerebral blood flow, BP = blood pressure, PGs = prostaglandins, PGI2 = Prostaglandin I2, TxA2 = Thromboxane A2, COX-2 = cyclooxygenase-2, Upward arrow = indicating increased the physiological variables and Downward arrow = indicating decreased the physiological variables

BIBLIOGRAPHY

Aalykke C & Lauritsen K (2001). Epidemiology of NSAID-related gastroduodenal mucosal injury. *Best Pract Res Clin Gastroenterol* **15**, 705-722.

Aaslid R, Markwalder TM, & Nornes H (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57**, 769-774.

Abdul-Hadi O, Parvizi J, Austin MS, Viscusi E, & Einhorn T (2009). Nonsteroidal Anti-Inflammatory Drugs in Orthopaedics. *J Bone Joint Surg Am* **91**, 2020-2027.

Adams KR, Halliday LD, Sibeon RG, Baber N, Littler T, & Orme ML (1982). A clinical and pharmacokinetic study of indomethacin in standard and slow release formulations. *Br J Clin Pharmacol* **14**, 286-289.

Ahmed MI, Pisoni R, & Calhoun DA (2009). Current options for the treatment of resistant hypertension. *Expert Rev Cardiovasc Ther* **7**, 1385-1393.

Ainslie PN & Poulin MJ (2004a). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* **97**, 149-159.

Ainslie PN & Poulin MJ (2004b). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* **97**, 149-159.

Almendros I, Montserrat JM, Ramirez J, Torres M, Duran-Cantolla J, Navajas D, & Farre R (2012). Intermittent hypoxia enhances cancer progression in a mouse model of sleep apnoea. *Eur Respir J* **39**, 215-217.

Amano T & Meyer JS (1982). Prostaglandin Inhibition and Cerebrovascular Control in Patients with Headache. *Headache: The Journal of Head and Face Pain* **22**, 52-59.

Anderson RJ, Berl T, McDonald KM, & Schrier RW (1976). Prostaglandins: effects on blood pressure, renal blood flow, sodium and water excretion. *Kidney Int* **10**, 205-215.

Andresen J, Shafi NI, & Bryan RM, Jr. (2006). Endothelial influences on cerebrovascular tone. *J Appl Physiol* **100**, 318-327.

Atkeson A, Yeh SY, Malhotra A, & Jelic S (2003). Endothelial Function in Obstructive Sleep Apnea. *Progress in Cardiovascular Diseases* **51**, 351-362.

Aw TJ, Haas SJ, Liew D, & Krum H (2005). Meta-analysis of Cyclooxygenase-2 Inhibitors and Their Effects on Blood Pressure. *Arch Intern Med* **165**, 490-496.

Badesch DB, McLaughlin VV, Delcroix M, Vizza C, Olschewski H, Sitbon O, & Barst RJ (2004). Prostanoid therapy for pulmonary arterial hypertension. *Journal of the American College of Cardiology* **43**, S56-S61.

Bannwarth B & Lipsky PE (2001). Are COX-2 Inhibitors as Effective as Conventional NSAIDs in Acute Pain States? *Arch Intern Med* **161**, 127-128.

Banu SK, Arosh JA, Chapdelaine P, & Fortier MA (2003). Molecular cloning and spatio-temporal expression of the prostaglandin transporter: A basis for the action of prostaglandins in the bovine reproductive system. *Proceedings of the National Academy of Sciences* **100**, 11747-11752.

Barnes JN, Casey DP, Hines CN, Nicholson WT, & Joyner MJ (2012a). Cyclooxygenase inhibition augments central blood pressure and aortic wave reflection in aging humans. *American Journal of Physiology - Heart and Circulatory Physiology* **302**, H2629-H2634.

Barnes JN, Schmidt JE, Nicholson WT, & Joyner MJ (2012b). Cyclooxygenase inhibition abolishes age-related differences in cerebral vasodilator responses to hypercapnia. *J Appl Physiol* **112**, 1884-1890.

Barnes JN, Taylor JL, Kluck BN, Johnson CP, & Joyner MJ (2013). Cerebrovascular Reactivity is Associated with Maximal Aerobic Capacity in Healthy Older Adults. *J Appl Physiol*.

Beaudin AE, Brugniaux JV, Vohringer M, Flewitt J, Green JD, Friedrich MG, & Poulin MJ (2011). Cerebral and myocardial blood flow responses to hypercapnia and hypoxia in humans. *American Journal of Physiology - Heart and Circulatory Physiology* **301**, H1678-H1686.

Berenbaum F (2004). COX-3: fact or fancy? *Joint Bone Spine* **71**, 451-453.

Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, & Schnitzer TJ (2000). Comparison of Upper Gastrointestinal Toxicity of Rofecoxib and Naproxen in Patients with Rheumatoid Arthritis. *New England Journal of Medicine* **343**, 1520-1528.

Botting RM (2006). Inhibitors of cyclooxygenases: mechanisms, selectivity and uses. *J Physiol Pharmacol* **57 Suppl 5**, 113-124.

Brooks P (2006). The burden of musculoskeletal diseaseΓÇöa global perspective. *Clinical Rheumatology* **25**, 778-781.

Bugess KR, Fan JL, Peebles K, Thomas K, Lucas S, Lucas R, Dawson A, Swart M, Shepherd K, & Ainslie P (2010). Exacerbation of Obstructive Sleep Apnea by Oral Indomethacin. *Chest* **137**, 707-710.

Cannon CP & Cannon PJ (2012). COX-2 Inhibitors and Cardiovascular Risk. *Science* **336**, 1386-1387.

Capone C, Faraco G, Anrather J, Zhou P, & Iadecola C (2010). Cyclooxygenase 1-Derived Prostaglandin E2 and EP1 Receptors Are Required for the Cerebrovascular Dysfunction Induced by Angiotensin II. *Hypertension* **55**, 911-917.

Capone C, Faraco G, Coleman C, Young CN, Pickel VM, Anrather J, Davisson RL, & Iadecola C (2012). Endothelin 1-Dependent Neurovascular Dysfunction in Chronic Intermittent Hypoxia. *Hypertension* **60**, 106-113.

Casey DP & Joyner MJ (2011). Prostaglandins do not contribute to the nitric oxide-mediated compensatory vasodilation in hypoperfused exercising muscle. *American Journal of Physiology - Heart and Circulatory Physiology* **301**, H261-H268.

Catella-Lawson F (2001). Vascular biology of thrombosis. *Neurology* **57**, S5-S7.

Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, & FitzGerald GA (1999). Effects of Specific Inhibition of Cyclooxygenase-2 on Sodium Balance, Hemodynamics, and Vasoactive Eicosanoids. *Journal of Pharmacology and Experimental Therapeutics* **289**, 735-741.

Cathcart MC, Tamosiuniene R, Chen G, Neilan TG, Bradford A, O'Byrne KJ, Fitzgerald DJ, & Pidgeon GP (2008). Cyclooxygenase-2-Linked Attenuation of Hypoxia-Induced Pulmonary Hypertension and Intravascular Thrombosis. *Journal of Pharmacology and Experimental Therapeutics* **326**, 51-58.

Caughey GE, Cleland LG, Penglis PS, Gamble JR, & James MJ (2001). Roles of Cyclooxygenase (COX)-1 and COX-2 in Prostanoid Production by Human Endothelial Cells: Selective Up-Regulation of Prostacyclin Synthesis by COX-2. *J Immunol* **167**, 2831-2838.

Chamberlin KW & Silverman AR (2009). Celecoxib-Associated Anaphylaxis. *Ann Pharmacother* **43**, 777-781.

Chemtob S, Li DY, Abran D, Hardy P, Peri K, & Varma DR (1996). The role of prostaglandin receptors in regulating cerebral blood flow in the perinatal period. *Acta Paediatr* **85**, 517-524.

Cheng HF & Harris RC (2003). Does cyclooxygenase-2 affect blood pressure? *Curr Hypertens Rep* **5**, 87-92.

Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, & FitzGerald GA (2002). Role of Prostacyclin in the Cardiovascular Response to Thromboxane A2. *Science* **296**, 539-541.

Claassen JAHR, Zhang R, Fu Q, Witkowski S, & Levine BD (2007). Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol* **102**, 870-877.

Claria J (2003). Cyclooxygenase-2 biology. *Curr Pharm Des* **9**, 2177-2190.

Clarke RJ, Mayo G, Price P, & FitzGerald GA (1991). Suppression of Thromboxane A2 but Not of Systemic Prostacyclin by Controlled-Release Aspirin. *New England Journal of Medicine* **325**, 1137-1141.

Colina-Chourio JA, Godoy-Godoy N, & Avila-Hernandez RM (2000). Role of prostaglandins in hypertension. *J Hum Hypertens* **14 Suppl 1**, S16-S19.

Collins R, Peto R, Godwin J, & MacMahon S (1990a). Blood pressure and coronary heart disease. *Lancet* **336**, 370-371.

Collins R, Peto R, MacMahon S, Godwin J, Qizilbash N, Collins R, MacMahon S, Hebert P, Eberlein KA, Taylor JO, Hennekens CH, Fiebach NH, Qizilbash N, & Hennekens CH (1990b). Blood pressure, stroke, and coronary heart disease: Part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *The Lancet* **335**, 827-838.

Crecelius AR, Kirby BS, Richards JC, Garcia LJ, Voyles WF, Larson DG, Luckasen GJ, & Dinenno FA (2011a). Mechanisms of ATP-mediated vasodilation in humans: modest role for nitric oxide and vasodilating prostaglandins. *American Journal of Physiology - Heart and Circulatory Physiology* **301**, H1302-H1310.

Crecelius AR, Kirby BS, Voyles WF, & Dinenno FA (2011b). Augmented skeletal muscle hyperaemia during hypoxic exercise in humans is blunted by combined inhibition of nitric oxide and vasodilating prostaglandins. *The Journal of Physiology* **589**, 3671-3683.

Curfman GD, Morrissey S, & Drazen JM (2005). 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. *New England Journal of Medicine* **353**, 2813-2814.

Davidge ST (2001). Prostaglandin H Synthase and Vascular Function. *Circ Res* **89**, 650-660.

Davies CWH, Crosby JH, Mullins RL, Barbour C, Davies RJO, & Stradling JR (2000a). Case-control study of 24 hour ambulatory blood pressure in patients with obstructive sleep apnoea and normal matched control subjects. *Thorax* **55**, 736-740.

Davies G, Martin LA, Sacks N, & Dowsett M (2002). Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. *Ann Oncol* **13**, 669-678.

Davies NM, McLachlan AJ, Day RO, & Williams KM (2000b). Clinical Pharmacokinetics and Pharmacodynamics of Celecoxib. *Clinical Pharmacokinetics* **38**, 225.

DeMaria AN & Weir MR (2003). Coxibs-Beyond the GI Tract: Renal and Cardiovascular Issues. *Journal of Pain and Symptom Management* **25**, 41-49.

Dempsey JA, Veasey SC, Morgan BJ, & O'Donnell CP (2010). Pathophysiology of Sleep Apnea. *Physiol Rev* **90**, 47-112.

DeWitt DL, Meade EA, & Smith WL (1993). PGH synthase isoenzyme selectivity: The potential for safer nonsteroidal antiinflammatory drugs. *The American Journal of Medicine* **95**, S40-S44.

Dogne JM, Thiry A, Pratico D, Masereel B, & Supuran CT (2007). Dual carbonic anhydrase--cyclooxygenase-2 inhibitors. *Curr Top Med Chem* **7**, 885-891.

Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, De Putte AV, & Lipsky PE (1998). Cyclooxygenase in biology and disease. *FASEB J* **12**, 1063-1073.

Durgan DJ & Bryan RM (2012). Cerebrovascular Consequences of Obstructive Sleep Apnea. *Journal of the American Heart Association* **1**.

Elder DJ, Halton DE, Crew TE, & Paraskeva C (2000). Apoptosis induction and cyclooxygenase-2 regulation in human colorectal adenoma and carcinoma cell lines by the cyclooxygenase-2-selective non-steroidal anti-inflammatory drug NS-398. *Int J Cancer* **86**, 553-560.

Evett GE, Xie W, Chipman JG, Robertson DL, & Simmons DL (1993). Prostaglandin G/H synthase isoenzyme 2 expression in fibroblasts: regulation by dexamethasone, mitogens, and oncogenes. *Arch Biochem Biophys* **306**, 169-177.

Fan JL, Bugess KR, Thomas KN, Peebles KC, Lucas SJE, Lucas RAI, Cotter JD, & Ainslie PN (2010). Influence of indomethacin on ventilatory and cerebrovascular responsiveness to CO2 and breathing stability: the influence of Pco2 gradients. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **298**, R1648-R1658.

Fan JL, Burgess K, Thomas K, Peebles K, Lucas S, Lucas R, Cotter J, & Ainslie P (2011). Influence of indomethacin on the ventilatory and cerebrovascular responsiveness to hypoxia. *European Journal of Applied Physiology* **111**, 601-610.

Fierro-Carrion GA & Ram CV (1997). Nonsteroidal anti-inflammatory drugs (NSAIDs) and blood pressure. *Am J Cardiol* **80**, 775-776.

Fike CD, Kaplowitz MR, & Pfister SL (2003). Arachidonic acid metabolites and an early stage of pulmonary hypertension in chronically hypoxic newborn pigs. *Am J Physiol Lung Cell Mol Physiol* **284**, L316-L323.

Fitzgerald GA, Brash AR, Falardeau P, & Oates JA (1981). Estimated rate of prostacyclin secretion into the circulation of normal man. *J Clin Invest* **68**, 1272-1276.

FitzGerald GA (2004). Coxibs and Cardiovascular Disease. *New England Journal of Medicine* **351**, 1709-1711.

Flavahan NA (2007). Balancing prostanoid activity in the human vascular system. *Trends in Pharmacological Sciences* **28**, 106-110.

Flemons WW (2002). Obstructive Sleep Apnea. *N Engl J Med* **347**, 498-504.

Fletcher EC (2003). Sympathetic over activity in the etiology of hypertension of obstructive sleep apnea. *Sleep* **26**, 15-19.

Fletcher EC (2001). Physiological and Genomic Consequences of Intermittent Hypoxia: Invited Review: Physiological consequences of intermittent hypoxia: systemic blood pressure. *J Appl Physiol* **90**, 1600-1605.

Fletcher EC, Orolinova N, & Bader M (2002). Blood pressure response to chronic episodic hypoxia: the renin-angiotensin system. *J Appl Physiol* **92**, 627-633.

Fosslien E (2005). Cardiovascular Complications of Non-Steroidal Anti-Inflammatory Drugs. *Annals of Clinical & Laboratory Science* **35**, 347-385.

Foster GE, Brugniaux JV, Pialoux V, Duggan CTC, Hanly PJ, Ahmed SB, & Poulin MJ (2009). Cardiovascular and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia in healthy humans. *The Journal of Physiology* **587**, 3287-3299.

Foster GE, Hanly PJ, Ahmed SB, Beaudin AE, Pialoux V, & Poulin MJ (2010a). Intermittent Hypoxia Increases Arterial Blood Pressure in Humans Through a Renin-Angiotensin System-Dependent Mechanism. *Hypertension* **56**, 369-377.

Foster GE, Hanly PJ, Ahmed SB, Beaudin AE, Pialoux V, & Poulin MJ (2010b). Intermittent Hypoxia Increases Arterial Blood Pressure in Humans Through a Renin-Angiotensin System-Dependent Mechanism. *Hypertension* **56**, 369-377.

Foster GE, Hanly PJ, Ostrowski M, & Poulin MJ (2007). Effects of Continuous Positive Airway Pressure on Cerebral Vascular Response to Hypoxia in Patients with Obstructive Sleep Apnea. *Am J Respir Crit Care Med* **175**, 720-725.

Frampton JE & Keating GM (2007). Celecoxib: A Review of its Use in the Management of Arthritis and Acute Pain. *Drugs* **67**.

Francois H, Athirakul K, Mao L, Rockman H, & Coffman TM (2004). Role for Thromboxane Receptors in Angiotensin-II-Induced Hypertension. *Hypertension* **43**, 364-369.

Francois H, Makhanova N, Ruiz P, Ellison J, Mao L, Rockman HA, & Coffman TM (2008). A role for the thromboxane receptor in L-NAME hypertension. *Am J Physiol Renal Physiol* **295**, F1096-F1102.

Fredenburgh LE, Liang OD, Macias AA, Polte TR, Liu X, Riascos DF, Chung SW, Schissel SL, Ingber DE, Mitsialis SA, Kourembanas S, & Perrella MA (2008). Absence of Cyclooxygenase-2 Exacerbates Hypoxia-Induced Pulmonary Hypertension and Enhances Contractility of Vascular Smooth Muscle Cells. *Circulation* **117**, 2114-2122.

Fries S & Grosser T (2005). The Cardiovascular Pharmacology of COX-2 Inhibition. *Hematology* **2005**, 445-451.

Funk CD & Fitzgerald GA (2007). COX-2 inhibitors and cardiovascular risk. *J Cardiovasc Pharmacol* **50**, 470-479.

Gajraj NM (2003). Cyclooxygenase-2 inhibitors. *Anesth Analg* **96**, 1720-1738.

Garcia Rodriguez LA, Tacconelli S, & Patrignani P (2008). Role of Dose Potency in the Prediction of Risk of Myocardial Infarction Associated With Nonsteroidal Anti-Inflammatory Drugs in the General Population. *Journal of the American College of Cardiology* **52**, 1628-1636.

Garcia-Bueno B, Serrats J, & Sawchenko PE (2009). Cerebrovascular Cyclooxygenase-1 Expression, Regulation, and Role in Hypothalamic-Pituitary-Adrenal Axis Activation by Inflammatory Stimuli. *The Journal of Neuroscience* **29**, 12970-12981.

Girouard H & Iadecola C (2006). Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *J Appl Physiol* **100**, 328-335.

Gislason GH, Jacobsen S, Rasmussen JN, Rasmussen S, Buch P, Friberg J, Schramm TK, Abildstrom SZ, Kober L, Madsen M, & Torp-Pedersen C (2006). Risk of Death or Reinfarction Associated With the Use of Selective Cyclooxygenase-2 Inhibitors and Nonselective Nonsteroidal Antiinflammatory Drugs After Acute Myocardial Infarction. *Circulation* **113**, 2906-2913.

Godoy DA, Rabinstein AA, Biestro A, Ainslie PN, & Di NM (2012). Effects of indomethacin test on intracranial pressure and cerebral hemodynamics in patients with refractory intracranial hypertension: a feasibility study. *Neurosurgery* **71**, 245-257.

Gonzalez Bosc LV, Resta T, Walker B, & Kanagy NL (2009). Mechanisms of intermittent hypoxia-induced hypertension. *J Cell Mol Med*.

Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, & MacVicar BA (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* **456**, 745-749.

Gordon GRJ, Howarth C, & MacVicar BA (2011). Bidirectional control of arteriole diameter by astrocytes. *Experimental Physiology* **96**, 393-399.

Grootendorst PV, Marshall JK, Holbrook AM, Dolovich LR, O'Brien BJ, & Levy AR (2005). The Impact of Reference Pricing of Nonsteroidal Anti-Inflammatory Agents on the Use and Costs of Analgesic Drugs. *Health Services Research* **40**, 1297-1317.

Grosser T, Fries S, & Fitzgerald GA (2006). Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest* **116**, 4-15.

Grosser T, Yu Y, & FitzGerald GA (2010). Emotion Recollected in Tranquility: Lessons Learned from the COX-2 Saga. *Annu Rev Med* **61**, 17-33.

Gryglewski RJ (2008). Prostacyclin among prostanoids. *Pharmacol Rep* **60**, 3-11.

Guardiola JJ, Matheson PJ, Clavijo LC, Wilson MA, & Fletcher EC (2001). Hypercoagulability in patients with obstructive sleep apnea. *Sleep Medicine* **2**, 517-523.

Hanly PJ & Pierratos A (2001). Improvement of sleep apnea in patients with chronic renal failure who undergo nocturnal hemodialysis. *N Engl J Med* **344**, 102-107.

He FJ & MacGregor GA (2003). Review: Salt, blood pressure and the renin-angiotensin system. *Journal of Renin-Angiotensin-Aldosterone System* **4**, 11-16.

Hedner J, Darpo B, Ejnell H, Carlson J, & Caidahl K (1995). Reduction in sympathetic activity after long-term CPAP treatment in sleep apnoea: cardiovascular implications. *Eur Respir J* **8**, 222-229.

Helmersson-Karlqvist J, Bjorklund-Bodegard K, Larsson A, & Basu S (2012). 24-Hour ambulatory blood pressure associates inversely with prostaglandin F(2alpha), interleukin-6 and F(2)-isoprostane formation in a Swedish population of older men. *Int J Clin Exp Med* **5**, 145-153.

Hertel J (1997). The role of nonsteroidal anti-inflammatory drugs in the treatment of acute soft tissue injuries. *J Athl Train* **32**, 350-358.

Hinojosa-Laborde C & Mifflin SW (2005). Sex differences in blood pressure response to intermittent hypoxia in rats. *Hypertension* **46**, 1016-1021.

Hinz B, Renner B, & Brune K (2007). Drug Insight: cyclo-oxygenase-2 inhibitors[mdash]a critical appraisal. *Nat Clin Pract Rheum* **3**, 552-560.

Holland S, Silberstein SD, Freitag F, Dodick DW, Argoff C, & Ashman E (2012). Evidence-based guideline update: NSAIDs and other complementary treatments for episodic migraine prevention in adults: report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. *Neurology* **78**, 1346-1353.

Howard LS, Barson RA, Howse BP, McGill TR, McIntyre ME, O'Connor DF, & Robbins PA (1995). Chamber for controlling end-tidal gas tensions over sustained periods in humans. *J Appl Physiol* **78**, 1088-1091.

Howe LR & Dannenberg AJ (2003). COX-2 inhibitors for the prevention of breast cancer. *J Mammary Gland Biol Neoplasia* **8**, 31-43.

Imholz BPM, Wieling W, van Montfrans GA, & Wesseling KH (1998). Fifteen years experience with finger arterial pressure monitoring. *Cardiovasc Res* **38**, 605-616.

Iniguez MA, Rodriguez A, Volpert OV, Fresno M, & Redondo JM (2003). Cyclooxygenase-2: a therapeutic target in angiogenesis. *Trends in Molecular Medicine* **9**, 73-78.

Jadhav V, Jabre A, Chen MF, & Lee TJ-F (2009). Presynaptic Prostaglandin E2 EP1-Receptor Facilitation of Cerebral Nitrergic Neurogenic Vasodilation. *Stroke* **40**, 261-269.

Jelic S & Le Jemtel TH (2008a). Inflammation, Oxidative Stress, and the Vascular Endothelium in Obstructive Sleep Apnea. *Trends in Cardiovascular Medicine* **18**, 253-260.

Jelic S & Le Jemtel TH (2008b). Inflammation, Oxidative Stress, and the Vascular Endothelium in Obstructive Sleep Apnea. *Trends in Cardiovascular Medicine* **18**, 253-260.

Jelic S, Padeletti M, Kawut SM, Higgins C, Canfield SM, Onat D, Colombo PC, Basner RC, Factor P, & LeJemtel TH (2008). Inflammation, Oxidative Stress, and Repair Capacity of the Vascular Endothelium in Obstructive Sleep Apnea. *Circulation* **117**, 2270-2278.

Jerry HG (2011). NSAIDs and atrial fibrillation. *BMJ* **343**.

Johnson AG, Nguyen TV, & Day RO (1994). Do Nonsteroidal Anti-inflammatory Drugs Affect Blood Pressure? A Meta-Analysis. *Annals of Internal Medicine* **121**, 289-300.

Jones P & Lamdin R (2010). Oral Cyclo-Oxygenase 2 Inhibitors versus Other Oral Analgesics for Acute Soft Tissue Injury: Systematic Review and Meta-Analysis. *Clin Drug Investig* **30**, 419-437.

Justice E & Carruthers DM (2004). Cardiovascular risk and COX-2 inhibition in rheumatological practice. *J Hum Hypertens* **19**, 1-5.

Kanagy NL (2009). Vascular effects of intermittent hypoxia. *ILAR J* **50**, 282-288.

Karim A, Fowler M, Jones L, Patwardhan R, Vannemreddy P, Mccarthy K, & Nanda A (2005a). Cyclooxygenase-2 expression in brain metastases. *Anticancer Res* **25**, 2969-2971.

Karim A, Fowler M, Jones L, Patwardhan R, Vannemreddy P, Mccarthy K, & Nanda A (2005b). Cyclooxygenase-2 expression in brain metastases. *Anticancer Res* **25**, 2969-2971.

Kasasbeh E, Chi DS, & Krishnaswamy G (2006). Inflammatory aspects of sleep apnea and their cardiovascular consequences. *South Med J* **99**, 58-67.

Kean WF & Buchanan WW (2005). The use of NSAIDs in rheumatic disorders 2005: a global perspective. *Inflammopharmacology* **13**, 343-370.

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, & He J (2005). Global burden of hypertension: analysis of worldwide data. *Lancet* **365**, 217-223.

Kent BD, Ryan S, & McNicholas WT (2011). Obstructive sleep apnea and inflammation: Relationship to cardiovascular co-morbidity. *Respiratory Physiology & Neurobiology* **178**, 475-481.

Kheirandish L, Row BW, Li RC, Brittian KR, & Gozal D (2005). Apolipoprotein E-deficient mice exhibit increased vulnerability to intermittent hypoxia-induced spatial learning deficits. *Sleep* **28**, 1412-1417.

Kimura H, Niijima M, Abe Y, Edo H, Sakabe H, Kojima A, Hasako K, Masuyama S, Tatsumi K, & Kuriyama T (1998a). Compensatory excretion of prostacyclin and thromboxane metabolites in obstructive sleep apnea syndrome. *Intern Med* **37**, 127-133.

Kimura H, Niijima M, Abe Y, Edo H, Sakabe H, Kojima A, Hasako K, Masuyama S, Tatsumi K, & Kuriyama T (1998b). Compensatory excretion of prostacyclin and thromboxane metabolites in obstructive sleep apnea syndrome. *Intern Med* **37**, 127-133.

Kirkby NS, Lundberg MH, Harrington LS, Leadbeater PDM, Milne GL, Potter CMF, Al-Yamani M, Adeyemi O, Warner TD, & Mitchell JA (2012). Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. *Proceedings of the National Academy of Sciences* **109**, 17597-17602.

Knights KM, Mangoni AA, & Miners JO (2010). Defining the COX inhibitor selectivity of NSAIDs: implications for understanding toxicity. *Expert Review of Clinical Pharmacology* **3**, 769.

Kobayashi T & Narumiya S (2002). Function of prostanoid receptors: studies on knockout mice. *Prostaglandins & Other Lipid Mediators* **68–69**, 557-573.

Kraus MA & Hamburger RJ (1997). Sleep apnea in renal failure. *Adv Perit Dial* **13**, 88-92.

Krieger J, Benzoni D, Sforza E, & Sassard J (1991a). Urinary excretion of prostanoids during sleep in obstructive sleep apnoea patients. *Clin Exp Pharmacol Physiol* **18**, 551-555.

Krieger J, Benzoni D, Sforza E, & Sassard J (1991b). Urinary excretion of prostanoids during sleep in obstructive sleep apnoea patients. *Clin Exp Pharmacol Physiol* **18**, 551-555.

Kundu JK & Surh YJ (2008). Inflammation: Gearing the journey to cancer. *Mutation Research/Reviews in Mutation Research* **659**, 15-30.

Lamberts M, Fosb++l EL, Olsen AM, Hansen ML, Folke F, Kristensen SL, Olesen JB, Hansen PR, K++ber L, Torp-Pedersen C, & Gislason GH Ongoing treatment with non-steroidal anti-inflammatory drugs at time of admission is associated with poorer prognosis in patients with first-time acute myocardial infarction. *International Journal of Cardiology*.

Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, & Van LF (2007). Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* **53**, 766-772.

Levy P, Bonsignore MR, & Eckel J (2009). Sleep, sleep-disordered breathing and metabolic consequences. *Eur Respir J* **34**, 243-260.

Levy P, Pepin JL, Arnaud C, Tamisier R, Borel JC, Dematteis M, Godin-Ribuot D, & Ribuot C (2008). Intermittent hypoxia and sleep-disordered breathing: current concepts and perspectives. *Eur Respir J* **32**, 1082-1095.

Li RC, Row BW, Gozal E, Kheirandish L, Fan Q, Brittian KR, Guo SZ, Sachleben LR, Jr., & Gozal D (2003). Cyclooxygenase 2 and Intermittent Hypoxia-induced Spatial Deficits in the Rat. *Am J Respir Crit Care Med* **168**, 469-475.

Lipsky PE (1999). The clinical potential of cyclooxygenase-2-specific inhibitors. *Am J Med* **106**, 51S-57S.

Lipsky PE, Brooks P, Crofford LJ, DuBois R, Graham D, Simon LS, van de Putte LBA, & Abramson SB (2000). Unresolved Issues in the Role of Cyclooxygenase-2 in Normal Physiologic Processes and Disease. *Arch Intern Med* **160**, 913-920.

Liu B, Luo W, Zhang Y, Li H, Zhang J, Tan XR, & Zhou Y (2012a). Concomitant activation of functionally opposing prostacyclin and thromboxane prostanoid receptors by cyclo-oxygenase-1-mediated prostacyclin synthesis in mouse arteries. *Experimental Physiology* **97**, 895-904.

Liu B, Luo W, Zhang Y, Li H, Zhu N, Huang D, & Zhou Y (2012b). Involvement of cyclo-oxygenase-1-mediated prostacyclin synthesis in the vasoconstrictor activity evoked by ACh in mouse arteries. *Experimental Physiology* **97**, 277-289.

Liu X, Li C, Falck JR, Harder DR, & Koehler RC (2012c). Relative contribution of cyclooxygenases, epoxyeicosatrienoic acids, and pH to the cerebral blood flow response to vibrissal stimulation. *American Journal of Physiology - Heart and Circulatory Physiology* **302**, H1075-H1085.

Lloyd BB, Jukes MGM, & Cunningham DJC (1958). The relation between alveolar oxygen pressure and the respiratory response to carbon dioxide in man. *Experimental Physiology* **43**, 214-227.

Loke YK, Brown JW, Kwok CS, Niruban A, & Myint PK (2012). Association of Obstructive Sleep Apnea With Risk of Serious Cardiovascular Events: A Systematic Review and Meta-Analysis. *Circulation: Cardiovascular Quality and Outcomes* **5**, 720-728.

Lopez R, Llinas MT, Roig F, & Salazar FJ (2003). Role of nitric oxide and cyclooxygenase-2 in regulating the renal hemodynamic response to norepinephrine. *Am J Physiol Regul Integr Comp Physiol* **284**, R488-R493.

MacMahon S, Peto R, Collins R, Godwin J, MacMahon S, Cutler J, Sorlie P, Abbott R, Collins R, Neaton J, Abbott R, Dyer A, & Stamler J (1990). Blood pressure, stroke, and coronary heart disease: Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *The Lancet* **335**, 765-774.

Malmstrom K, Daniels S, Kotey P, Seidenberg BC, & Desjardins PJ (1999). Comparison of rofecoxib and celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental pain: A randomized, placebo- and active-comparator-controlled clinical trial. *Clinical Therapeutics* **21**, 1653-1663.

Mancia G (2012). Short- and Long-Term Blood Pressure Variability: Present and Future. *Hypertension* **60**, 512-517.

Markwald RR, Kirby BS, Crecelius AR, Carlson RE, Voyles WF, & Dinenno FA (2011). Combined inhibition of nitric oxide and vasodilating prostaglandins abolishes forearm vasodilatation to systemic hypoxia in healthy humans. *The Journal of Physiology* **589**, 1979-1990.

Marnett LJ (2009). The COXIB Experience: A Look in the Rearview Mirror. *Annu Rev Pharmacol Toxicol* **49**, 265-290.

Martinez-Garcia M, Martinez-Alonso M, Duran-Cantolla J, Marin J, Gonzalez M, de la Pena M, Masdeu M, del Campo F, Farre R, Barbe F, Montserrat J, & Campos-Rodriguez F (2012). Association Between Sleep Apnea And Cancer Incidence. Longitudinal Study Of 8,900 Patients From The Multicenter Spanish Cohort. *Am J Respir Crit Care Med* **185(MeetingAbstracts)**.

Matsuura T, Takuwa H, Bakalova R, Obata T, & Kanno I (2009). Effect of cyclooxygenase-2 on the regulation of cerebral blood flow during neuronal activation in the rat. *Neuroscience Research* **65**, 64-70.

McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, & Fitzgerald GA (1999). Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 272-277.

McGettigan P & Henry D (2011). Cardiovascular risk with non-steroidal anti-inflammatory drugs: systematic review of population-based controlled observational studies. *PLoS Med* **8**, e1001098.

McGettigan P & Henry D (2013). Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. *PLoS Med* **10**, e1001388.

McGettigan P, Lincz LF, Attia J, McElduff P, Bissett L, Peel R, Stokes B, Hancock S, Henderson K, Seldon M, & Henry D (2011). The risk of coronary thrombosis with cyclo-oxygenase-2 inhibitors does not vary with polymorphisms in two regions of the cyclo-oxygenase-2 gene. *Br J Clin Pharmacol* **72**, 707-714.

Melnikova I (2005). Future of COX2 inhibitors. *Nat Rev Drug Discov* **4**, 453-454.

Miller MA & Cappuccio FP (2007). Inflammation, sleep, obesity and cardiovascular disease. *Curr Vasc Pharmacol* **5**, 93-102.

Mitchell JA & Warner TD (2006). COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. *Nat Rev Drug Discov* **5**, 75-86.

Mitchell JA & Warner TD (2013). Reply to Ricciotti et al.: Evidence for vascular COX isoforms. *Proceedings of the National Academy of Sciences* **110**, E184.

Mitchell MD (1992). Biochemistry of the prostaglandins. *Bailli+¿re's Clinical Obstetrics and Gynaecology* **6**, 687-706.

Moncada S (2006). Adventures in vascular biology: a tale of two mediators. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361**, 735-759.

Moncada S & Vane JR (1981). Prostacyclin and the vascular endothelium. *Bull Eur Physiopathol Respir* **17**, 687-701.

Morales A, Gao W, Lu J, Xing J, & Li J (2012). Muscle cyclo-oxygenase-2 pathway contributes to the exaggerated muscle mechanoreflex in rats with congestive heart failure. *Experimental Physiology* **97**, 943-954.

Morgan BJ, Reichmuth KJ, Peppard PE, Finn L, Barczi SR, Young T, & Nieto FJ (2010). Effects of Sleep-disordered Breathing on Cerebrovascular Regulation. *Am J Respir Crit Care Med* **182**, 1445-1452.

Morrell MJ, Finn L, Kim H, Peppard PE, Badr MS, & Young T (2000). Sleep fragmentation, awake blood pressure, and sleep-disordered breathing in a population-based study. *Am J Respir Crit Care Med* **162**, 2091-2096.

Morten S, Christian FC, Frank M, Kenneth JR, & Henrik TS (2011). Non-steroidal anti-inflammatory drug use and risk of atrial fibrillation or flutter: population based case-control study. *BMJ* **343**.

Moser M & Setaro JF (2006). Resistant or Difficult-to-Control Hypertension. *N Engl J Med* **355**, 385-392.

Mukherjee D, Nissen SE, & Topol EJ (2001). Risk of Cardiovascular Events Associated With Selective COX-2 Inhibitors. *JAMA: The Journal of the American Medical Association* **286**, 954-959.

Nacher M, Farre R, Montserrat JM, Torres M, Navajas D, Bulbena O, & Serrano-Mollar A (2009). Biological consequences of oxygen desaturation and respiratory effort in an acute animal model of obstructive sleep apnea (OSA). *Sleep Medicine* **10**, 892-897.

Narumiya S, Sugimoto Y, & Ushikubi F (1999). Prostanoid Receptors: Structures, Properties, and Functions. *Physiol Rev* **79**, 1193-1226.

Nasjletti A (1998). The Role of Eicosanoids in Angiotensin-Dependent Hypertension. *Hypertension* **31**, 194-200.

Neubauer JA (2001). Physiological and Genomic Consequences of Intermittent Hypoxia: Invited Review: Physiological and pathophysiological responses to intermittent hypoxia. *J Appl Physiol* **90**, 1593-1599.

Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'Agostino RB, Newman AB, Lebowitz MD, & Pickering TG (2000). Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. *JAMA* **283**, 1829-1836.

Nieto FJ, Peppard PE, Young T, Finn L, Hla KM, & Farre R (2012). Sleep-disordered Breathing and Cancer Mortality. *Am J Respir Crit Care Med* **186**, 190-194.

Niwa K, Haensel C, Ross ME, & Iadecola C (2001). Cyclooxygenase-1 Participates in Selected Vasodilator Responses of the Cerebral Circulation. *Circ Res* **88**, 600-608.

O'Connor JP & Lysz T (2008). Celecoxib, NSAIDs and the skeleton. *Drugs Today (Barc)* **44**, 693-709.

Pack AI & Gislason T (2003). Obstructive Sleep Apnea and Cardiovascular Disease: A Perspective and Future Directions. *Progress in Cardiovascular Diseases* **51**, 434-451.

Padol IT & Hunt RH (2010). Association of myocardial infarctions with COX-2 inhibition may be related to immunomodulation towards a Th1 response resulting in atheromatous plaque instability: an evidence-based interpretation. *Rheumatology* **49**, 837-843.

Parati G, Ochoa JE, Lombardi C, & Bilo G (2013). Assessment and management of blood-pressure variability. *Nat Rev Cardiol* **10**, 143-155.

Pardutz A & Schoenen J (2010). NSAIDs in the Acute Treatment of Migraine: A Review of Clinical and Experimental Data. *Pharmaceuticals* **3**, 1966-1987.

Parish JM & Somers VK (2004). Obstructive Sleep Apnea and Cardiovascular Disease. *Mayo Clinic Proceedings* **79**, 1036-1046.

Patil SP, Schneider H, Schwartz AR, & Smith PL (2007). Adult obstructive sleep apnea: pathophysiology and diagnosis. *Chest* **132**, 325-337.

Patrono C & Roth GJ (1996). Aspirin in Ischemic Cerebrovascular Disease. *Stroke* **27**, 756-760.

Patt BT, Jarjoura D, Haddad DN, Sen CK, Roy S, Flavahan NA, & Khayat RN (2010). Endothelial Dysfunction in the Microcirculation of Patients with Obstructive Sleep Apnea. *Am J Respir Crit Care Med* **182**, 1540-1545.

Peppard PE, Young T, Palta M, & Skatrud J (2000). Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med* **342**, 1378-1384.

Pialoux V, Hanly PJ, Foster GE, Brugniaux JV, Beaudin AE, Hartmann SE, Pun M, Duggan CT, & Poulin MJ (2009). Effects of Exposure to Intermittent Hypoxia on Oxidative Stress and Acute Hypoxic Ventilatory Response in Humans. *Am J Respir Crit Care Med* **180**, 1002-1009.

Pickard J, Tamura A, Stewart M, McGeorge A, & Fitch W (1980). Prostacyclin, indomethacin and the cerebral circulation. *Brain Res* **197**, 425-431.

Pickard JD (1981). Role of Prostaglandins and Arachidonic Acid Derivatives in the Coupling of Cerebral Blood Flow to Cerebral Metabolism. *J Cereb Blood Flow Metab* **1**, 361-384.

Pickles H, Brown MM, Thomas M, Hewazy AH, Redmond S, Zilkha E, & Marshall J (1984). Effect of indomethacin on cerebral blood flow, carbon dioxide reactivity and the response to epoprostenol (prostacyclin) infusion in man. *J Neurol Neurosurg Psychiatry* **47**, 51-55.

Pidgeon GP, Tamosiuniene R, Chen G, Leonard I, Belton O, Bradford A, & Fitzgerald DJ (2004). Intravascular Thrombosis After Hypoxia-Induced Pulmonary Hypertension: Regulation by Cyclooxygenase-2. *Circulation* **110**, 2701-2707.

Ponnuchamy B & Khalil RA (2009). Cellular mediators of renal vascular dysfunction in hypertension. *Am J Physiol Regul Integr Comp Physiol* **296**, R1001-R1018.

Pope JE, Anderson JJ, & Felson DT (1993). A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. *Arch Intern Med* **153**, 477-484.

Potter CMF, Lundberg MH, Harrington LS, Warboys CM, Warner TD, Berson RE, Moshkov AV, Gorelik J, Weinberg PD, & Mitchell JA (2011). Role of Shear Stress in Endothelial Cell Morphology and Expression of Cyclooxygenase Isoforms. *Arterioscler Thromb Vasc Biol* **31**, 384-391.

Poulin MJ, Fatemian M, Tansley JG, O'Connor DF, & Robbins PA (2002). Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans. *Exp Physiol* **87**, 633-642.

Poulin MJ, Liang PJ, & Robbins PA (1996). Dynamics of the cerebral blood flow response to step changes in end-tidal PCO2 and PO2 in humans. *J Appl Physiol* **81**, 1084-1095.

Poulin MJ, Liang PJ, & Robbins PA (1998). Fast and slow components of cerebral blood flow response to step decreases in end-tidal PCO2 in humans. *J Appl Physiol* **85**, 388-397.

Poulin MJ & Robbins PA (1996). Indexes of Flow and Cross-sectional Area of the Middle Cerebral Artery Using Doppler Ultrasound During Hypoxia and Hypercapnia in Humans. *Stroke* **27**, 2244-2250.

Prabhakar NR, Fields RD, Baker T, & Fletcher EC (2001). Intermittent hypoxia: cell to system. *Am J Physiol Lung Cell Mol Physiol* **281**, L524-L528.

Punjabi NM, Newman AB, Young TB, Resnick HE, & Sanders MH (2008). Sleep-disordered breathing and cardiovascular disease: an outcome-based definition of hypopneas. *Am J Respir Crit Care Med* **177**, 1150-1155.

Punjabi NM (2008). The Epidemiology of Adult Obstructive Sleep Apnea. *Proc Am Thorac Soc* **5**, 136-143.

Puppo C, Lopez L, Farina G, Caragna E, Moraes L, Iturralde A, & Biestro A (2007). Indomethacin and cerebral autoregulation in severe head injured patients: a transcranial Doppler study. *Acta Neurochir (Wien)* **149**, 139-149.

Qin W, Zhu W, Hewett J, Rottinghaus G, Chen YC, Flynn J, Kliethermes B, Mannello F, & Sauter E (2008). uPA is upregulated by high dose celecoxib in women at increased risk of developing breast cancer. *BMC Cancer* **8**, 298.

Rainsford KD (2007). Anti-inflammatory drugs in the 21st century. *Subcell Biochem* **42**, 3-27.

Rainsford KD (2010). Cardiovascular adverse reactions from NSAIDs are more than COX-2 inhibition alone. *Rheumatology* **49**, 834-836.

Rasmussen M (2005). Treatment of elevated intracranial pressure with indomethacin: Friend or foe? *Acta Anaesthesiologica Scandinavica* **49**, 341-350.

Reddy KS & Roy A (2013). Cardiovascular Risk of NSAIDs: Time to Translate Knowledge into Practice. *PLoS Med* **10**, e1001389.

Reichmuth KJ, Dopp JM, Barczi SR, Skatrud JB, Wojdyla P, Hayes D, Jr., & Morgan BJ (2009). Impaired Vascular Regulation in Patients with Obstructive Sleep Apnea: Effects of Continuous Positive Airway Pressure Treatment. *Am J Respir Crit Care Med* **180**, 1143-1150.

Ricciotti E & FitzGerald GA (2011). Prostaglandins and Inflammation. *Arterioscler Thromb Vasc Biol* **31**, 986-1000.

Ricciotti E, Yu Y, Grosser T, & FitzGerald GA (2013). COX-2, the dominant source of prostacyclin. *Proceedings of the National Academy of Sciences* **110**, E183.

Richalet JP, Donoso MV, Jimenez D, Antezana AM, Hudson C, Cortes G, Osorio J, & Leon A (2002). Chilean miners commuting from sea level to 4500 m: a prospective study. *High Alt Med Biol* **3**, 159-166.

Risser A, Donovan D, Heintzman J, & Page T (2009). NSAID prescribing precautions. *Am Fam Physician* **80**, 1371-1378.

Robbins PA, Swanson GD, & Howson MG (1982a). A prediction-correction scheme for forcing alveolar gases along certain time courses. *J Appl Physiol* **52**, 1353-1357.

Robbins PA, Swanson GD, Micco AJ, & Schubert WP (1982b). A fast gas-mixing system for breath-to-breath respiratory control studies. *J Appl Physiol* **52**, 1358-1362.

Rossoni G, Muscara MN, Cirino G, & Wallace JL (2002). Inhibition of cyclo-oxygenase-2 exacerbates ischaemia-induced acute myocardial dysfunction in the rabbit. *British Journal of Pharmacology* **135**, 1540-1546.

Rothwell PM (2013). Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *The Lancet* **375**, 938-948.

Rouzer CA & Marnett LJ (2009). Cyclooxygenases: structural and functional insights. *J Lipid Res* **50**, S29-S34.

Ruan YC, Zhou W, & Chan HC (2011). Regulation of Smooth Muscle Contraction by the Epithelium: Role of Prostaglandins. *Physiology* **26**, 156-170.

Rudic RD, Brinster D, Cheng Y, Fries S, Song WL, Austin S, Coffman TM, & FitzGerald GA (2005). COX-2-Derived Prostacyclin Modulates Vascular Remodeling. *Circ Res* **96**, 1240-1247.

Ryan CM & Bradley TD (2005). Pathogenesis of obstructive sleep apnea. *J Appl Physiol* **99**, 2440-2450.

Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N, Lin PH, & The DASH-Sodium Collaborative Research Group (2001). Effects on Blood Pressure of Reduced Dietary Sodium and the Dietary Approaches to Stop Hypertension (DASH) Diet. *N Engl J Med* **344**, 3-10.

Salvado MD, Alfranca A, Haeggstrom JZ, & Redondo JM (2012). Prostanoids in tumor angiogenesis: therapeutic intervention beyond COX-2. *Trends in Molecular Medicine* **18**, 233-243.

Sarafidis PA & Bakris GL (2008). Resistant hypertension: an overview of evaluation and treatment. *J Am Coll Cardiol* **52**, 1749-1757.

Sauter ER, Qin W, Hewett JE, Ruhlen RL, Flynn JT, Rottinghaus G, & Chen YC (2008). Celecoxib concentration predicts decrease in prostaglandin E2 concentrations in nipple aspirate fluid from high risk women. *BMC Cancer* **8**, 49.

Schjerning Olsen AM, Fosbol EL, Lindhardsen J, Andersson C, Folke F, Nielsen MB, Kober L, Hansen PR, Torp-Pedersen C, & Gislason GH (2013). Cause-Specific Cardiovascular Risk Associated with Nonsteroidal Anti-Inflammatory Drugs among Myocardial Infarction Patients - A Nationwide Study. *PLoS One* **8**, e54309.

Schmidt M, Christiansen CF, Mehnert F, Rothman KJ, & Sorensen HT (2011). Non-steroidal anti-inflammatory drug use and risk of atrial fibrillation or flutter: population based case-control study. *BMJ* **343**, d3450.

Schnitzer T (2006). Update on guidelines for the treatment of chronic musculoskeletal pain. *Clin Rheumatol* **25**, 22-29.

Schwab JM, Schluesener HJ, Meyermann R, & Serhan CN (2003). COX-3 the enzyme and the concept: steps towards highly specialized pathways and precision therapeutics? *Prostaglandins, Leukotrienes and Essential Fatty Acids* **69**, 339-343.

Schwartz JI, Vandormael K, Thach C, Lasseter KC, Holmes GB, Miller JL, Hreniuk D, Hilliard D, Snyder KM, Gertz BJ, & Gottesdiener KM (2004). Effect of rofecoxib, etoricoxib, celecoxib, and naproxen on urinary excretion of prostanoids in elderly volunteers. *Clin Pharmacol Ther* **75**, 34.

Sellers MM & Stallone JN (2008). Sympathy for the devil: the role of thromboxane in the regulation of vascular tone and blood pressure. *Am J Physiol Heart Circ Physiol* **294**, H1978-H1986.

Serrano-Duenas M (2007). High-altitude headache. *Expert Rev Neurotherapeutics* **7**, 245-248.

Seta F, Chung AD, Turner PV, Mewburn JD, Yu Y, & Funk CD (2009). Renal and cardiovascular characterization of COX-2 knockdown mice. *Am J Physiol Regul Integr Comp Physiol* **296**, R1751-R1760.

Severinghaus JW (1979). Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol* **46**, 599-602.

Siegle I, Klein T, Backman JT, Saal JG, Nusing RM, & Fritz P (1998). Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. *Arthritis Rheum* **41**, 122-129.

Sin DD, Fitzgerald F, Parker JD, Newton G, Floras JS, & Bradley TD (1999). Risk factors for central and obstructive sleep apnea in 450 men and women with congestive heart failure. *Am J Respir Crit Care Med* **160**, 1101-1106.

Singh B & Lucci A (2002). Role of cyclooxygenase-2 in breast cancer. *J Surg Res* **108**, 173-179.

Skarke C, Alamuddin N, Lawson JA, Cen L, Propert KJ, & Fitzgerald GA (2012). Comparative Impact on Prostanoid Biosynthesis of Celecoxib and the Novel Nonsteroidal Anti-Inflammatory Drug CG100649. *Clin Pharmacol Ther* **91**, 986-993.

Smith WL, DeWitt DL, & Garavito RM (2000). Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* **69**, 145-182.

Smith WL & Langenbach R (2001). Why there are two cyclooxygenase isozymes. *J Clin Invest* **107**, 1491-1495.

Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, & Young T (2008). Sleep Apnea and Cardiovascular Disease. *Circulation* **118**, 1080-1111.

Sood BG, Delaney-Black V, Glibetic M, Aranda JV, Chen X, & Shankaran S (2007). PGE2/TXB2 imbalance in neonatal hypoxemic respiratory failure. *Acta Paediatr* **96**, 669-673.

Sowers JR, White WB, Pitt B, Whelton A, Simon LS, Winer N, Kivitz A, van IH, Brabant T, & Fort JG (2005). The Effects of cyclooxygenase-2 inhibitors and nonsteroidal anti-inflammatory therapy on 24-hour blood pressure in patients with hypertension, osteoarthritis, and type 2 diabetes mellitus. *Arch Intern Med* **165**, 161-168.

Stefanovic B, Bosetti F, & Silva AC (2006). Modulatory role of cyclooxygenase-2 in cerebrovascular coupling. *NeuroImage* **32**, 23-32.

Steinback CD & Poulin MJ (2008). Cardiovascular and cerebrovascular responses to acute isocapnic and poikilocapnic hypoxia in humans. *J Appl Physiol* **104**, 482-489.

Stichtenoth DO, Marhauer V, Tsikas D, Gutzki FM, & Frolich JC (2005). Effects of specific COX-2-inhibition on renin release and renal and systemic prostanoid synthesis in healthy volunteers. *Kidney Int* **68**, 2197-2207.

Sudano I, Flammer AJ, Periat D, Enseleit F, Hermann M, Wolfrum M, Hirt A, Kaiser P, Hurlimann D, Neidhart M, Gay S, Holzmeister J, Nussberger J, Mocharla P, Landmesser U, Haile SR, Corti R, Vanhoutte PM, Luscher TF, Noll G, & Ruschitzka F (2010). Acetaminophen Increases Blood Pressure in Patients With Coronary Artery Disease. *Circulation* **122**, 1789-1796.

Trelle S, Reichenbach S, Wandel S, Hildebrand P, Tschannen B, Villiger PM, Egger M, & Juni P (2011). Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. *BMJ* **342**, c7086.

Tsikas D (1998). Application of gas chromatography-mass spectrometry and gas chromatography-tandem mass spectrometry to assess in vivo synthesis of prostaglandins, thromboxane, leukotrienes, isoprostanes and related compounds in humans. *Journal of Chromatography B: Biomedical Sciences and Applications* **717**, 201-245.

Tsikas D, Schwedhelm E, Suchy MT, Niemann J, Gutzki FM, Erpenbeck VJ, Hohlfeld JM, Surdacki A, & Frolich JC (2003). Divergence in urinary 8-iso-PGF2a (iPF2a-III, 15-F2t-IsoP) levels from gas chromatography-tandem mass spectrometry quantification after thin-layer chromatography and immunoaffinity column chromatography reveals heterogeneity of 8-iso-PGF2a: Possible methodological, mechanistic and clinical implications. *Journal of Chromatography B* **794**, 237-255.

Tsikas D, Zoerner AA, Haufe S, Engeli S, Stichtenoth DO, & Jordan J (2012). Microdialysis of Prostaglandins, Thromboxane, and Other Eicosanoids: Recall Past Knowledge. *Hypertension*.

Turini ME & Dubois RN (2002). Cyclooxygenases-2: A Therapeutic Target. *Annu Rev Med* **53**, 35-57.

Tzeng YC, MacRae B, & Rickards C (2012). A Recipe for Reducing Blood Pressure Variability: Adding Blood Flow to the Mix. *Hypertension* **60**, e12.

Upton RN, Rasmussen M, Grant C, Martinez AM, Cold GE, & Ludbrook GL (2008). Pharmacokinetics and pharmacodynamics of indomethacin: effects on cerebral blood flow in anaesthetized sheep. *Clin Exp Pharmacol Physiol* **35**, 317-323.

Urbano F, Roux F, Schindler J, & Mohsenin V (2008). Impaired cerebral autoregulation in obstructive sleep apnea. *J Appl Physiol* **105**, 1852-1857.

Vane JR & Corin RE (2003). Prostacyclin: A Vascular Mediator. *European Journal of Vascular and Endovascular Surgery* **26**, 571-578.

Vane JR (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* **231**, 232-235.

Vane JR, Bakhle YS, & Botting RM (1998). Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* **38**, 97-120.

Vanhoutte PM (1989). Endothelium and control of vascular function. State of the Art lecture. *Hypertension* **13**, 658-667.

Vanhoutte PM (2009). COX-1 and Vascular Disease. *Clin Pharmacol Ther* **86**, 212-215.

Vantanajal JS, Ashmead JC, Anderson TJ, Hepple RT, & Poulin MJ (2007). Differential sensitivities of cerebral and brachial blood flow to hypercapnia in humans. *J Appl Physiol* **102**, 87-93.

Vo T, Rice ASC, & Dworkin RH (2009). Non-steroidal anti-inflammatory drugs for neuropathic pain: How do we explain continued widespread use? *Pain* **143**, 169-171.

Vollmer WM, Sacks FM, Ard J, Appel LJ, Bray GA, Simons-Morton DG, Conlin PR, Svetkey LP, Erlinger TP, Moore TJ, & Karanja N (2001). Effects of Diet and Sodium Intake on Blood Pressure: Subgroup Analysis of the DASH-Sodium Trial. *Annals of Internal Medicine* **135**, 1019-1028.

Wallace JL (2002). Selective cyclooxygenase-2 inhibitors: after the smoke has cleared. *Digestive and Liver Disease* **34**, 89-94.

Wallace JL (2008). Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn't the Stomach Digest Itself? *Physiol Rev* **88**, 1547-1565.

Wang MT, Honn K, & Nie D (2007). Cyclooxygenases, prostanoids, and tumor progression. *Cancer and Metastasis Reviews* **26**, 525-534.

Wang Z, Wang C, Zhang W, Wang L, & Lei T (2008). Changes of TXA2 and PGI2 during postoperative hypertensive crisis in patients with hypertensive intracerebral hemorrhage. *J Huazhong Univ Sci Technolog Med Sci* **28**, 87-89.

Warden BA, Willman AM, & Williams CD (2012). Antithrombotics for secondary prevention of noncardioembolic ischaemic stroke. *Nat Rev Neurol* **8**, 223-235.

Wayne L (1989). Resistance or conductance for expression of arterial vascular tone. *Microvascular Research* **37**, 230-236.

Wehrlin JP, Zuest P, Hallen J, & Marti B (2006). Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. *J Appl Physiol* **100**, 1938-1945.

Wenner MM, Edwards DG, Ray CA, Rose WC, Gardner TJ, Stillabower M, & Farquhar WB (2011). Celecoxib does not alter cardiovascular and renal function during dietary salt loading. *Clinical and Experimental Pharmacology and Physiology* **38**, 543-549.

Wennmalm A & Fitzgerald GA (1988). Excretion of prostacyclin and thromboxane A2 metabolites during leg exercise in humans. *American Journal of Physiology - Heart and Circulatory Physiology* **255**, H15-H18.

Wennmalm A, Eriksson S, & Wahren J (1981). Effect of indomethacin on basal and carbon dioxide stimulated cerebral blood flow in man. *Clinical Physiology* **1**, 227-234.

Whelton A, Fort JG, Puma JA, Normandin D, Bello AE, & Verburg KM (2001). Cyclooxygenase-2--specific inhibitors and cardiorenal function: a randomized, controlled trial of celecoxib and rofecoxib in older hypertensive osteoarthritis patients. *Am J Ther* **8**, 85-95.

White DP (2005). Pathogenesis of Obstructive and Central Sleep Apnea. *Am J Respir Crit Care Med* **172**, 1363-1370.

White RP & Hagen AA (1982). Cerebrovascular actions of prostaglandins. *Pharmacol Ther* **18**, 313-331.

White WB & Campbell P (2010). Blood Pressure Destabilization on Nonsteroidal Antiinflammatory Agents: acetaminophen exposed? *Circulation* **122**, 1779-1781.

WHO. Musculoskeletal conditions affect millions. WHO News releases 2003 . 2003a. 6-1-2010a.
Ref Type: Report

WHO. The Burden of Musculoskeletal conditions at the start of the New Millenium. Report of a WHO Scientific Group . 2003b. 6-1-2010b.
Ref Type: Report

WHO. The world health report 2002 - Reducing Risks, Promoting Healthy Life. WHO . 2009. 10-1-2010.
Ref Type: Report

Wilson TW, Kaushal RD, & Dubois M (1990). Prostaglandins, the kidney, and hypertension. *West J Med* **153**, 168-172.

Wolf J, Lewicka J, & Narkiewicz K (2007). Obstructive sleep apnea: An update on mechanisms and cardiovascular consequences. *Nutrition, Metabolism and Cardiovascular Diseases* **17**, 233-240.

Wolfe F, Zhao S, & Pettitt D (2004). Blood pressure destabilization and edema among 8538 users of celecoxib, rofecoxib, and nonselective nonsteroidal antiinflammatory drugs (NSAID) and nonusers of NSAID receiving ordinary clinical care. *The Journal of Rheumatology* **31**, 1143-1151.

Woodward DF, Jones RL, & Narumiya S (2011). International Union of Basic and Clinical Pharmacology. LXXXIII: Classification of Prostanoid Receptors, Updating 15 Years of Progress. *Pharmacological Reviews* **63**, 471-538.

Woolf AD & Pfleger B (2003). Burden of major musculoskeletal conditions. *Bulletin of the World Health Organization* **81**, 646-656.

World Health Organization ISoHWG (2003). 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. [Miscellaneous Article]. *Journal of Hypertension* **21**, 1983-1992.

Xie A, Skatrud JB, Barczi SR, Reichmuth K, Morgan BJ, Mont S, & Dempsey JA (2009a). Influence of cerebral blood flow on breathing stability. *J Appl Physiol* **106**, 850-856.

Xie A, Skatrud JB, Barczi SR, Reichmuth K, Morgan BJ, Mont S, & Dempsey JA (2009b). Influence of cerebral blood flow on breathing stability. *J Appl Physiol* **106**, 850-856.

Xie A, Skatrud JB, Morgan B, Chenuel B, Khayat R, Reichmuth K, Lin J, & Dempsey JA (2006). Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *The Journal of Physiology* **577**, 319-329.

Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, & Mohsenin V (2005). Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* **353**, 2034-2041.

Young T, Palta M, Dempsey J, Skatrud J, Weber S, & Badr S (1993). The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* **328**, 1230-1235.

Young T, Peppard PE, & Taheri S (2005). Excess weight and sleep-disordered breathing. *J Appl Physiol* **99**, 1592-1599.

Young T, Peppard PE, & Gottlieb DJ (2002). Epidemiology of Obstructive Sleep Apnea. *Am J Respir Crit Care Med* **165**, 1217-1239.

Yu Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z, Landesberg G, Crichton I, Wu W, Pure E, Funk CD, & FitzGerald GA (2012). Vascular COX-2 Modulates Blood Pressure and Thrombosis in Mice. *Science Translational Medicine* **4**, 132ra54.

Yuhki Ki, Kojima F, Kashiwagi H, Kawabe Ji, Fujino T, Narumiya S, & Ushikubi F (2011). Roles of prostanoids in the pathogenesis of cardiovascular diseases: Novel insights from knockout mouse studies. *Pharmacology & Therapeutics* **129**, 195-205.

Zamarron S, Romero PV, Rodriguez JR, & Gude F (1999). Oximetry spectral analysis in the diagnosis of obstructive sleep apnoea. *Clin Sci* **97**, 467-473.

APPENDICES

Appendix A: Pharmacodynamics and pharmacokinetics of the drugs

Pharmacodynamics and pharmacokinetics of Indomethacin and Celecoxib		
Properties	*Indomethacin*	*Celecoxib*
Onset of action	~30 minutes	1 Hour (Malmstrom *et al.*, 1999)
Duration	4-6 hours	5-7 Hours (Malmstrom *et al.*, 1999)
Absorption	Oral: Immediate release: Prompt and extensive; Extended release: 90% over 12 hours	Food delays absorption by 1-2 hrs but it augments the extent of absorption by 10-20%. Coadministration with Mg++ or Al++ containing antacids reduces the Cmax by about 37% and absorption by 10% (Davies *et al.*, 2000b). Steady state level is reached after 5 days of treatment (Frampton & Keating, 2007).
Distribution	Vd: 0.34-1.57 L/kg; crosses blood brain barrier	Vd (apparent): ~400 L

Pharmacodynamics and pharmacokinetics of Indomethacin and Celecoxib		
Properties	*Indomethacin*	*Celecoxib*
Protein binding	99%	~97% primarily to albumin
Metabolism	Hepatic; significant enterohepatic recirculation	Hepatic via CYP2C9 (Hinz *et al.*, 2007); forms inactive metabolites
Bioavailability	100%	Absolute: Unknown
Half-life elimination	4.5 hours; prolonged in neonates	~11 hours (fasted)
Time to peak	Oral: Immediate release: 2 hours	~3 hours
Excretion	Urine (60%, primarily as glucuronide conjugates); feces (33%, primarily as metabolites)	Feces (~57% as metabolites, < 3% as unchanged drug); urine (27% as metabolites, < 3% as unchanged drug)

Appendix B: Take home package

Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

SUBJECT ID#:

_____

INITIALS:

_____

SESSION:	1	2	3	(circle one)
PROTOCOL:	A	B	C	(circle one)

Protocol ____ Day -4

Subject Self Blood Pressure Measures:

IMPORTANT: *While taking cyclooxygenase inhibitors (indomethacin and celecoxib), there is a chance (very small) you may have a gastrointestinal upset and bleeding. The cyclooxygenase inhibitors also increase in blood pressure. If your systolic blood pressure (the top blood pressure number) increases above 140 mmHg or if you have any symptoms of increase in blood pressure associated with blurring of vision, headache, dizziness or fainting please page Dr. Sofia Ahmed by calling (403) 944-1110 and when prompted to dial #07224. Then dial your phone number after the tone.*

Morning (between 7am and 10am):

1. SBP:_____mmHg; DBP:_____mmHg
2. SBP:_____mmHg; DBP:_____mmHg
3. SBP:_____mmHg; DBP:_____mmHg

Afternoon (between 12pm and 4pm):

4. SBP:_____mmHg; DBP:_____mmHg

5. SBP:_____mmHg; DBP:_____mmHg

6. SBP:_____mmHg; DBP:_____mmHg

Evening (between 7pm and 10pm):

7. SBP:_____mmHg; DBP:_____mmHg

8. SBP:_____mmHg; DBP:_____mmHg

9. SBP:_____mmHg; DBP:_____mmHg

Diet Log:

Please list the time of meal/snack, the food and beverage item, and the description of item and the unit of measure. Please eat the same foods and amount of foods for each day of each Protocol.

Example: 7:30am, Toast, whole wheat bread, 2 slices, butter, 1tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain's; Baked, 2 slices.

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Protocol ____ Day -3

Subject Self Blood Pressure Measures:

IMPORTANT: *While taking cyclooxygenase inhibitors (indomethacin and celecoxib), there is a chance (very small) you may have a gastrointestinal upset and bleeding. The cyclooxygenase inhibitors also increase in blood pressure. If your systolic blood pressure (the top blood pressure number) increases above 140 mmHg or if you have any symptoms of increase in blood pressure associated with blurring of vision, headache, dizziness or fainting please page Dr. Sofia Ahmed by calling (403) 944-1110 and when prompted to dial #07224. Then dial your phone number after the tone.*

Morning (between 7am and 10am):

1. SBP:_____mmHg; DBP:_____mmHg
2. SBP:_____mmHg; DBP:_____mmHg
3. SBP:_____mmHg; DBP:_____mmHg

Afternoon (between 12pm and 4pm):

4. SBP:_____mmHg; DBP:_____mmHg

5. SBP:_____mmHg; DBP:_____mmHg

6. SBP:_____mmHg; DBP:_____mmHg

Evening (between 7pm and 10pm):

7. SBP:_____mmHg; DBP:_____mmHg

8. SBP:_____mmHg; DBP:_____mmHg

9. SBP:_____mmHg; DBP:_____mmHg

Diet Log:

Please list the time of meal/snack, the food and beverage item, and the description of item and the unit of measure. Please eat the same foods and amount of foods for each day of each Protocol.

Example: 7:30am, Toast, whole wheat bread, 2 slices, butter, 1tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain's; Baked, 2 slices.

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Protocol ____ Day -2

Subject Self Blood Pressure Measures:

IMPORTANT: *While taking cyclooxygenase inhibitors (indomethacin and celecoxib), there is a chance (very small) you may have a gastrointestinal upset and bleeding. The cyclooxygenase inhibitors also increase in blood pressure. If your systolic blood pressure (the top blood pressure number) increases above 140 mmHg or if you have any symptoms of increase in blood pressure associated with blurring of vision, headache, dizziness or fainting please page Dr. Sofia Ahmed by calling (403) 944-1110 and when prompted to dial #07224. Then dial your phone number after the tone.*

Morning (between 7am and 10am):

1. SBP:_____mmHg; DBP:_____mmHg
2. SBP:_____mmHg; DBP:_____mmHg
3. SBP:_____mmHg; DBP:_____mmHg

Afternoon (between 12pm and 4pm):

4. SBP:_____mmHg; DBP:_____mmHg

5. SBP:_____mmHg; DBP:_____mmHg

6. SBP:_____mmHg; DBP:_____mmHg

Evening (between 7pm and 10pm):

7. SBP:_____mmHg; DBP:_____mmHg

8. SBP:_____mmHg; DBP:_____mmHg

9. SBP:_____mmHg; DBP:_____mmHg

Diet Log:

Please list the time of meal/snack, the food and beverage item, and the description of item and the unit of measure. Please eat the same foods and amount of foods for each day of each Protocol.

Example: 7:30am, Toast, whole wheat bread, 2 slices, butter, 1tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain's; Baked, 2 slices.

Protocol ____ Day -1

Subject Self Blood Pressure Measures:

IMPORTANT: *While taking cyclooxygenase inhibitors (indomethacin and celecoxib), there is a chance (very small) you may have a gastrointestinal upset and bleeding. The cyclooxygenase inhibitors also increase in blood pressure. If your systolic blood pressure (the top blood pressure number) increases above 140 mmHg or if you have any symptoms of increase in blood pressure associated with blurring of vision, headache, dizziness or fainting please page Dr. Sofia Ahmed by calling (403) 944-1110 and when prompted to dial #07224. Then dial your phone number after the tone.*

Morning (between 7am and 10am):

1. SBP:_____mmHg; DBP:_____mmHg
2. SBP:_____mmHg; DBP:_____mmHg
3. SBP:_____mmHg; DBP:_____mmHg

Afternoon (between 12pm and 4pm):

4. SBP:_____mmHg; DBP:_____mmHg

5. SBP:_____mmHg; DBP:_____mmHg

6. SBP:_____mmHg; DBP:_____mmHg

Evening (between 7pm and 10pm):

7. SBP:_____mmHg; DBP:_____mmHg

8. SBP:_____mmHg; DBP:_____mmHg

9. SBP:_____mmHg; DBP:_____mmHg

Diet Log:

Please list the time of meal/snack, the food and beverage item, and the description of item and the unit of measure. Please eat the same foods and amount of foods for each day of each Protocol.

Example: 7:30am, Toast, whole wheat bread, 2 slices, butter, 1tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain's; Baked, 2 slices.

Protocol ___ – Experimental Day

Date: _____

IF Session #1 or #2:

Washout Period ≈ at least FOUR days following the IH Exposure experimental Session

IF Session #3:

Study Participation Completed!!

Thank You!

Appendix C: Flyers

Appendix C1_COX-IH - Advert [CHREB] 2010_08_17

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Marc J. Poulin MA, PhD, DPhil
Professor, AHFMR Senior Scholar
DEPARTMENTS of PHYSIOLOGY & PHARMACOLOGY and CLINICAL NEUROSCIENCES

Health Sciences Centre
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1

T 403.220.8372
F 403.210.8420
W www.ucalgary.ca/~poulin
E poulin@ucalgary.ca

MALE VOLUNTEERS REQUIRED TO TAKE PART IN AN EXPERIMENT ON BLOOD PRESSURE REGULATION

PLEASE CONTACT:

Matiram Pun, Andrew Beaudin or Dr. Marc Poulin
Heritage Medical Research Building, Room 209, 230 or 210
University of Calgary
3330 Hospital Drive N.W.
Calgary, Alberta
T2N 4N1
Tel: 220-4520 (Matiram Pun)
210-8925(Andrew Beaudin)
220-8372 (Dr. Poulin)
Email: mpun@ucalgary.ca
abeaudin@ucalgary.ca
poulin@ucalgary.ca

You should not take part if:

- **you are under 18**
- **you are above 45**
- **you have any medical condition or are taking any medication**

Ethics ID: 23121

CREATE THE FUTURE OF HEALTH — An innovative medical school committed to excellence and leadership in education, research and service to society.

Appendix C2_COX-IH - Flyer [HEALTH CANADA] 2010_08_17

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Investigator: Dr. Marc J. Poulin
Co-Investigators: Dr. Sofia B. Ahmed, Dr. Matiram Pun, and Andrew E. Beaudin.

"MALE **VOLUNTEERS REQUIRED TO TAKE PART IN AN EXPERIMENT ON BLOOD PRESSURE REGULATION"**

PLEASE CONTACT:
Matiram Pun, Andrew Beaudin, or Dr. Marc Poulin
Heritage Medical Research Building
Room 209, 230 or 210
University of Calgary
3330 Hospital Drive N.W.
Calgary, Alberta
T2N 1N4
Tel: 403.220-4520 (Matiram Pun)
403.210-8925(Andrew Beaudin)
403. 220-8372 (Dr. Marc Poulin)
Fax: 403.210.8420
Email: mpun@ucalgary.ca
abeaudin@ucalgary.ca
poulin@ucalgary.ca

You should not take part if:

- You are under 18 years of age
- You are over 45 years of age
- You have any medical condition or are taking any medication

What you need to do:

- Provide four full days of commitment in the laboratory over four weeks
- Ingest two different drugs and a placebo intervention for the four days prior to each laboratory visit
- Supply blood and urine samples
- Be exposed to 6 hours of hypoxia per visit to the laboratory while wearing a face mask
- Breathe through a mouthpiece for 35 minutes in the morning and afternoon during an acute hypoxia and hyperoxia test
- Have your blood pressure measured
- Maintain a dietary diary

What you will get:

- Free parking on each day you visit the laboratory (i.e., 4 days in total)
- Be seen by a physician if there are any health issues during the experiment
- Contribute to our understanding of how hypoxia influences blood pressure.

This study has been approved by:
The Conjoint Health Research Ethics Board - Ethics ID: 23121
Health Canada Clinical Trial Application Protocol ID: UC-MMHAP-COX-IH-2010001
Health Canada Clinical Trial Control Number: 138344

CREATE THE FUTURE OF HEALTH *An innovative medical school committed to excellence and leadership in education, research and service to society.*

Appendix D: Familiarization package and informed consent

Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

Dr. Matiram Pun, Andrew E. Beaudin, Dr. Sofia B Ahmed, and Dr. Marc Poulin

FAMILIARIZATION PACKAGE

Included in Package:

1. Participant Information Form.
2. Study Protocol Diagrams
    - Protocol Overview/Participant Flow
    - Generic Drug Intervention Schedule
3. Informed Consent.
4. Letter to General Practitioner (i.e., family doctor)

Please Note: The letter to your general practitioner will be sent if you agree to participate in the study and provide the name of your family doctor.

Date Provided: _____

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Tuesday, April 23, 2013

Marc J. Poulin MA, PhD, DPhil
Professor, AHFMR Senior Scholar
DEPARTMENTS of PHYSIOLOGY & PHARMACOLOGY and CLINICAL NEUROSCIENCES

Health Sciences Centre
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1

T 403.220.8372
F 403.210.8420
W www.ucalgary.ca/~poulinE
E poulin@ucalgary.ca

Research Project Title: Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

Investigator: Dr. Marc J. Poulin

Co-Investigators: Dr. Sofia B. Ahmed, Dr. Patrick J Hanly, Dr. Matiram Pun (MSc Student), and Andrew E Beaudin (PhD Student)

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more details about something mentioned here, or information not included here, you should feel free to ask. Please take time to read this carefully and to understand any accompanying information.

Background & Purpose

Low blood oxygen (hypoxia) occurs in both normal conditions (high-altitude) and conditions such as obstructive sleep apnea (OSA). While they sleep, people with OSA have episodes of normal blood oxygen levels (normoxia) leading to periods of alternating low and normal blood oxygen levels. OSA patients who continuously get exposed to intermittent fluctuations in blood oxygen levels tend to develop high blood pressure and have increased cardiovascular risk.

Recent research from healthy human volunteers and patient populations has revealed that intermittent low blood oxygen is associated with an increase in blood pressure, though the exact mechanism is not clear. Prostaglandins, a

hormone system that dilates blood vessels, may play a protective role. Prescribed and over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs) (a class of painkillers) block prostaglandins and can increase blood pressure. Therefore, we are asking you if you would help us with a study, the purpose of which is to determine the effects 6 hours of exposure to intermittent fluctuations in oxygen levels on blood pressure, respiration and blood flow in your brain while you are taking NSAIDs.

What would I have to do?

The study will require approximately 25 hours of your time over a four week period to complete. At the beginning and end of these experiments, we will ask you to wear a plastic head band which holds in position a small probe which is used to measure the speed of blood flow in one of the arteries in your brain. It does this by using very high frequency sound which you cannot hear and recording the echoes from the moving blood. We will also measure blood pressure non-invasively by wrapping a cuff around your finger. This cuff will allow us to measure blood pressure in a beat-by-beat manner. To measure heart rate, we will place three ECG electrodes on your chest. With regards to the blood gases, we will measure oxygen and carbon dioxide pressures non-invasively. Breathing will be performed through a facemask during these procedures. We will take a little amount of expired and inspired gases every breath by using a sophisticated computer-controlled respiratory system. Once the experimental setting is ready, we will make control measurements when you are simply sitting quietly whilst breathing normal (i.e. room) air, and then follow with our experimental protocol.

Exposure to intermittent fluctuations in oxygen levels will take place in a specially built chamber where we can lower and add oxygen. This room is equipped with conveniences for your comfort. We will observe you for 6 hours in this chamber. During this time we will monitor heart rate, blood pressure, and blood oxygen saturation. We will collect urine samples at the interval of 2 hours, 4 hours and 6 hours.

Before and after each 6-hour exposure you will participate in a short 45 minutes experiment to determine your acute respiratory and blood flow response to intermittent levels of low and high oxygen. The final portion of the acute test continues with high oxygen level (partial pressure = 300 Torr) for ten minutes. The first five minutes of high oxygen level acute test will have normal level of carbon dioxide while last five minutes the subject will be subjected to breathe more carbondioxide (partial pressure = + 9 Torr of baseline measurement). During these experiments you will be situated comfortably in a reclined chair and asked to breathe though a facemask which will cover both your mouth and nose. As you breathe we will vary the gas mixture you receive by reducing the amount of oxygen you receive and adding nitrogen and carbon dioxide. The gas mixture

you will be breathing is equivalent to air with added nitrogen. The variations in the amount of carbon dioxide, oxygen and nitrogen in the gas mixtures are not harmful or dangerous, although they may cause a sensation of breathlessness. Levels of oxygen considerably lower than those used in these experiments can cause loss of consciousness.

Each session to the laboratory will be separated by at least four days (the wash-out period of the drugs) and the type of protocol to occur will be randomized. During your first visit to the laboratory we will familiarize you with the experimental set-up, ask you to fill out a short questionnaire to ensure you meet the inclusion/exclusion criteria. We will acquire a blood sample so that we can determine the amount of glucose in your blood and ask you for a urine sample so we can determine if protein is present in your urine. We will also measure your, height, weight, and abdominal/neck circumference.

You will be required to go through randomly assigned three protocols of the study and they include indomethacin, celecoxib and placebo. In **Protocol I**, you will be required to take first drug by mouth three times a day for 4 days. On the 5th day, you will come to the laboratory and should take your second last pill one hour before your visit to the lab. The last pill will be taken during your stay inside chamber. The test will involve the 45-minute breathing test (described above) before and after 6 hours of room air breathing in our chamber. In **Protocol II**, you will be required to take second drug by mouth two times a day for 4 days. On the 5th day, you will come to the laboratory and should take your last pill one hour before your visit to the lab. On this day you will undergo two 45-minute breathing tests separated by a 6-hour exposure to intermittent low and high levels of oxygen. Finally, in **Protocol III**, you will be taking third drug which will be taken twice a day orally. The placebo study is same as drug protocols except that you will be taking pills that do not have any active ingredients in them. You will be assigned to follow each protocol in random order, so you will not be aware if you are taking medication or placebo. The drugs indomethacin and celecoxib are cyclooxygenase inhibitors. Indomethacin is nonselective cyclooxygenase inhibitor while celecoxib is selective cyclooxygenase-2 inhibitor. They are both used to reduce pain. You will be provided indomethacin 50mg pills for four days and celecoxib 200mg pills for four days. You will be taking your second last dose (for the drug three times day) and last dose (for the drug two times a day) one hour before coming to the laboratory for your testing session. We ask that you do not take any other medications while participating in this study as it may reduce the effect of the drug we are studying. During the follow-up days to both the placebo and drug protocols we will provide you with a blood pressure monitoring device and ask that you take your blood pressure at 8:00am, 12:00pm, 4:00pm, and 8:00pm.

We will ask that you control your diet on each testing day and the four days leading up to each experimental session. We ask that you eat the same food on these days and at the same time of day. We will provide you with a diary so that you can record your diet on these days. We will also ask you to provide us with a urine sample on the morning of each experimental session.

Blood samples will be taken throughout experimentation from the antecubital vein (on the front of the forearm at the elbow crease) and from a small puncture on your ear lobe/finger. Samples will be analyzed for hemoglobin concentration, circulating hormones, electrolytes and metabolites in the blood. Tenderness may be present at the point of the needle or small lancet insertion but precautions will be taken to avoid bruising, discomfort, and infection. The urine will be collected during 6 hours of intermittent hypoxia exposure at an interval of 2 hours, 4 hours and 6 hours.

If you agree to take part in this study, we would ask you to abstain from alcohol and caffeine-containing beverages, and not to engage in heavy exercise, for a period of 12 hours before the start of the experiment and 12 hours after the completion of the experiment.

We suggest that you keep this letter and show it to anyone concerned with your medical care. If you have any questions or problems, please contact us.

What are the risks?

These experiments should involve no discomfort to you. However, it is possible that some of the gas mixtures could give you a headache and make you feel tired and possibly a bit sick feeling. If you begin to feel at all unwell you should tell us and we will stop the experiment immediately.

As with all non-steroidal anti-inflammatory drugs (NSAIDs) (including commonly used over the counter NSAIDs such as Advil®, Motrin®, ibuprofen, etc.), CELEBREX or indomethacin may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, hypertension, and stroke, which can be fatal. This risk may increase with duration of use. Patients with cardiovascular disease or risk factors for cardiovascular disease may be at greater risk.

Similarly, as with all NSAIDs (including commonly used over the counter NSAIDs such as Advil®, Motrin®, ibuprofen, etc.), CELEBREX or indomethacin may cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients are at greater risk for serious gastrointestinal (GI) events.

As mentioned above, while taking cyclooxygenase inhibitors (indomethacin and celecoxib) there is a very small chance you may have a gastrointestinal upset, here we want to closely monitor an increase in blood pressure. If your systolic blood pressure (the top blood pressure number) increases above 140 mmHg or if you have any symptoms of increase in blood pressure associated with blurring of vision, dizziness or fainting we ask that you stop taking the drug and seek treatment from a physician. Dr. Sofia B. Ahmed can be reached by pager by calling 403-944-1110 and asking for pager 07224.

Will I benefit if I take part?

If you agree to participate in this study you will not benefit directly. However, the information we obtain from this study may help us provide better understanding of pathophysiology and treatments for patients with obstructive sleep apnea and hypertension.

Do I have to participate?

You are not required to participate in this study. If you agree to participate you are free to withdraw from the study at anytime.

Will I be paid for participating, or do I have to pay for anything?

You will not be required to pay for any expenses relating to the study. We will only reimburse you for expenses you incur for participating in this study.

Will my records be kept private?

Only the principal investigator, co-investigators, and research assistant(s) will have access to your information. The information will not be disclosed to anyone other than the project personnel. Any information collected from you will be linked to your identity only by a unique subject identifier. This information is only accessible within the laboratory and is password protected. The University of Calgary Conjoint Health Research Ethics Board is also able to access your records.

If I suffer a research-related injury, will I be compensated?

In the event that you suffer injury as a result of participating in this research no compensation will be provided for you by the University of Calgary, the Calgary Regional Health Authority, or the Researchers. You still have all your legal rights. Nothing said here about treatment or compensation in any way alters your right to recover damages.

Signatures

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from

the study at any time without jeopardizing your health care. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. If you have further questions concerning matters related to this research, please contact:

Dr. Marc J. Poulin
Room 210, Heritage Medical Research Building, 3330 Hospital Drive NW, Calgary AB, T2N 4N1
Telephone numbers: 403-220-8372 (Work) and 403-270-0793 (Home). Fax: (403) 210-8420
Email: poulin@ucalgary.ca

Dr. Sofia B. Ahmed
Room C210, 1402-29th Street NW, Calgary AB, T2N 2T9
Tel: 403-944-2745; Fax: 403-944-2876
Email: Sofia.Ahmed@calgaryhealthregion.ca

If you have any questions about your rights as a possible participant in this research, please contact: The Chair of the Conjoint Health Research Ethics Board at the Office of Medical Bioethics, 403-220-7990.

_____	_____
Participant's Signature	**Date**
_____	_____
Investigator and/or Delegate's Signature	**Date**
_____	_____
Witness' Signature	**Date**

A copy of this consent form has been given to you to keep for your records and reference.

Protocol Overview / Participant Flow

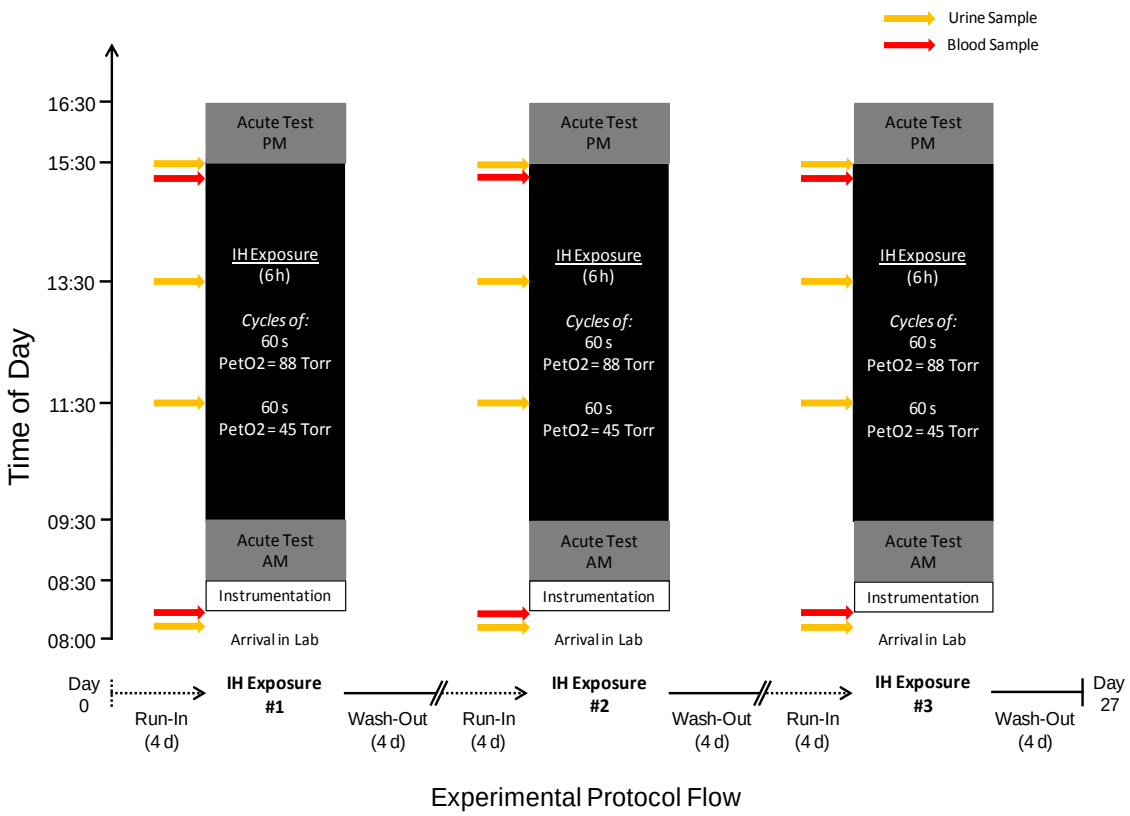


Figure A7.7.1: Participant flow through the three experimental protocols (I – Placebo, II - Indomethacin,III - Celecoxib). The protocol order is randomly assigned for each participant. Intermittent hypoxia (IH) exposure days consist of an acute hypoxia test in the morning (AM), exposure to 6 h of IH, and an acute hypoxia test in the afternoon (PM). Two blood samples are drawn throughout each day (red arrows) - one in the AM and a second in the PM. In addition, four urine samples are collected on each test day (orange arrows) – one in the AM and three during the IH exposure at 2, 4 and 6 h.

Abbreviation: Run-in – the 4 days preceding a testing session where the participant will be treated with either Indomethacin, Celecoxib, or Placebo; Wash-out – the days following the completion of each protocol (i.e., I, II or III) to allow elimination of prior medication from the body (at least 4 days); IH – intermittent hypoxia; PET_{O_2} - end-tidal partial pressure of O_2.

Generic Drug Ingestion Schedule

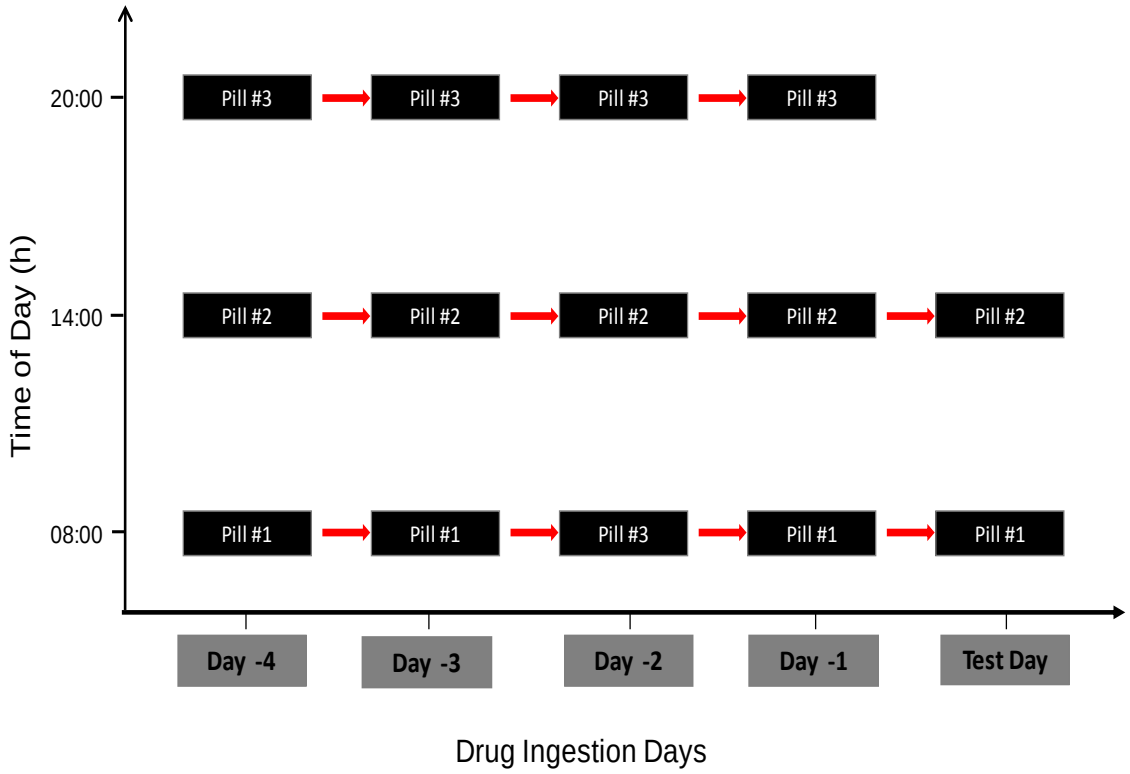


Figure A7.7.2: Drug ingestion schedule for the four days prior to the intermittent hypoxia exposure test days. Schedule is identical during all three protocols (i.e. placebo, indomethacin, and celecoxib).

Abbreviation: Day -4 – the fourth day prior to IH exposure; Day -3 – the third day prior to IH exposure; Day -2 – the second day prior to IH exposure; Day -1 – the day before IH exposure; Test Day – day on which the participant performs the AM and PM acute tests, separated by a 6 h exposure to IH. 08:00 = 8:00 am; 14:00 = 2:00 pm; and 22:00 = 10:00 pm.

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Tuesday, April 23, 2013

Marc J. Poulin MA, PhD, DPhil
Professor, AHFMR Senior Scholar
DEPARTMENTS of PHYSIOLOGY & PHARMACOLOGY and CLINICAL NEUROSCIENCES

Health Sciences Centre
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1

T 403.220.8372
F 403.210.8420
W www.ucalgary.ca/~poulinE
E poulin@ucalgary.ca

Research Project Title: Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

(The subject should complete the whole of this sheet.)

Have you read the attached consent form? Yes/No

Have you had an opportunity to ask questions and discuss this study described in the attached consent form? Yes/No

Have you received satisfactory answers to all of your questions concerning the study described in the attached consent form? Yes/No

Have you received enough information about the study described in the attached consent form? Yes/No

Are you content for the investigators to contact your GP to check that there are no medical reasons why you should not take part in the study? Yes/No

Who have you spoken to?

Dr./Mr./Ms./Mrs: _____

Do you understand that you are free to withdraw from the study:
- At any time;
- Without having to give a reason for withdrawing;
- Without affecting your future medical care? Yes/No

Signature: _____ Date: _____

Name (in block letters):_____

Ethics ID: 23121

Participant ID # & Initials: _____

Tuesday, April 23, 2013

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Marc J. Poulin MA, PhD, DPhil
Professor, AHFMR Senior Scholar
DEPARTMENTS of PHYSIOLOGY & PHARMACOLOGY and CLINICAL NEUROSCIENCES

Health Sciences Centre
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1

T 403.220.8372
F 403.210.8420
W www.ucalgary.ca/~poulinE
E poulin@ucalgary.ca

Research Project Title: Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

Investigators: Dr. Marc Poulin and Dr. Sofia B Ahmed.

Re: D.O.B.:

Dear:

Your patient has volunteered to take part in a study in which the respiratory, cardiovascular, and cerebral blood flow responses to intermittent hypoxia will be investigated.

The experiment involves subject exposure to three six-hour periods of intermittent low oxygen conditions (partial pressure = 45 Torr) in a purpose-built room. During intermittent low oxygen exposures the level of oxygen will fluctuate every 60 seconds between a partial pressure of 45 Torr and 88.0 Torr. The subject will also undergo an acute intermittent low oxygen test in the morning and afternoon before going into the chamber and after coming out of the chamber. During these acute tests, the level of oxygen will fluctuate every 90 seconds between a partial pressure of 45 Torr and 88.0 Torr for six cycles. The final portion of the acute test continues with high oxygen level (partial pressure = 300 Torr) for ten minutes. The first five minutes of high oxygen level acute test will have normal level of carbon dioxide while last five minutes the subject will be subjected to breathe more carbon dioxide (partial pressure = + 9 Torr of baseline measurement). During these exposures, experimental non-invasive measurements will be made of cerebral blood flow (using pulsed Doppler ultrasonography), mean arterial blood pressure (by using a finger arterial blood pressure monitoring system), heart rate (by ECG), and ventilation (by breathing from a mouthpiece). In addition, venous blood samples (10ml) will be taken from the antecubital vein and capillary blood samples (200µl) will be taken from a puncture of the ear lobe.

Your patient will be asked to come to the laboratory for 3 different study periods. One study period involves ingesting **indomethacin 50mg po q8h x 4 days**, the second study day involves ingesting **celecoxib 200mg po bid x 4 days**, and the third study day involves ingesting a **placebo medication x 4 days**. The study periods will be conducted in random order and the participant will be blinded as to which study period in which he is participating. There will be at least a four days of "wash-out" period between each study period. While we do not anticipate any adverse effects attributable to the study, there is a study physician (Dr. Ahmed) on call 24 hours/day in case of emergency.

There should be no long term side-effects of this experiment.

We would be grateful if you would let us know if there is any reason why this particular volunteer should not take part in this study. My telephone numbers are: Home (270-0793) and Work (220-8372).

Yours sincerely,

Dr. Marc J. Poulin
Ethics ID: 23121

Appendix E: Screening package

Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

Dr. Matiram Pun, Andrew E. Beaudin, Dr. Sofia B Ahmed, and Dr. Marc Poulin

SCREENING PACKAGE

Date: _____

Participant Information

ID #: _____

Age: _____D.O.B. _____/_____/____
MM DD YYYY

First Name: _____

Last Name:_____

Sex: (circle) Male Female

Self Reported Race:_____

Contact Info:

Phone #:_____

Okay to leave message: ☐ Yes ☐ No

E-mail: _____

Participant ID # & Initials: _____

Tuesday, April 23, 2013

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

Marc J. Poulin MA, PhD, DPhil
Professor, AHFMR Senior Scholar
DEPARTMENTS of PHYSIOLOGY & PHARMACOLOGY and CLINICAL NEUROSCIENCES

Health Sciences Centre
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1

T 403.220.8372
F 403.210.8420
W www.ucalgary.ca/~poulinE
E poulin@ucalgary.ca

Research Project Title: Effect of Selective and Nonselective Cyclooxygenase Enzyme Inhibition on Arterial Blood Pressure and Cerebral Blood Flow with Exposure to Intermittent Hypoxia in Humans.

GENERAL QUESTIONNAIRE

Investigators: Matiram Pun[1], Andrew E. Beaudin[1], Sofia B. Ahmed[2,5], and Marc J. Poulin[1,3,4,5,6]

Department of Physiology and Pharmacology[1], Medicine[2], and Clinical Neurosciences[3]; Hotchkiss Brain Institute[4] and the Libin Cardiovascular Institute of Alberta[5], Faculties of Medicine and Kinesiology[6], University of Calgary.

The following questionnaire is designed to assess suitability for the above study. Please read each question and indicate the appropriate response:

1) What is your date of birth? _____/_____/_____
MM / DD / YYYY

2) Has your doctor ever told you that you have cardiovascular, respiratory, sleep disorder, musculoskeletal, collagen vascular or kidney disease?

Yes No

3) Have you lived in Calgary for at least one year?

Yes No

4) Have you smoked in the last year?

Yes No

5) Are you currently taking any medications, including herbals, vitamins, supplements or over the counter medications such as ibuprofen, etc.?

Yes No

If yes, please list medications: _____

6) Are you allergic to any drugs known so far?

Yes No

Ethics ID: 23121

Participant ID # & Initials: _____

MEDICAL HISTORY

Part 1: CURRENT / RECENT Medical History

Any Current Major Medical Complaints?	☐ Yes ☐ No
Please Elaborate (if any):	
CURRENT Medication(s) & Dosage? *(including OTC medication, herbal medications and vitamins)* Reason for Medication:	
CURRENT Recreational Drug Use?	☐ Yes ☐ No Type & Daily amount:_____ _____

Constitutional symptoms?

a. Fever?	☐ Yes ☐ No Notes:_____
b. Night Sweats?	☐ Yes ☐ No Notes:_____
c. Weight loss?	☐ Yes ☐ No Notes:_____
d. Weight gain?	☐ Yes ☐ No Notes:_____
e. Fatigue / Weakness?	☐ Yes ☐ No Notes:_____

Part 2: Medical History

PAST Chronic Medication(s) use & Dosage?	When taken? (mm/yyyy to mm/yyyy): _____ _____ *Reason for Medication:* _____ _____
History of coronary disease?	□ Yes □ No

(Ex. MI, CHF, CABG, By-pass Surgery, etc)	Notes:_____
Chest pain / Discomfort? *(Ex. Angina)*	□ Yes □ No Notes:_____
History of hypertension?	□ Yes □ No Notes:_____
History of stroke or TIA?	□ Yes □ No Notes:_____
Headaches?	□ Yes □ No Notes:_____
Migraines?	□ Yes □ No Notes:_____
Problem sleeping? *(Ex. Insomnia)*	□ Yes □ No Notes:_____
Snoring?	□ Yes □ No Notes:_____
History of witnessed apnea during sleep?	□ Yes □ No Notes:_____
History of nocturnal choking?	□ Yes □ No Notes:_____
Previous diagnosis of OSA?	□ Yes □ No Notes:_____
History of lung disease?	□ Yes □ No Notes:_____
Shortness of breath / Wheezing	□ Yes □ No Notes:_____
Musculoskeletal disorders?	□ Yes □ No Notes:_____
Genitourinary complaints?	□ Yes □ No Notes:_____
Gastrointestinal complaints?	□ Yes □ No Notes:_____
History of easy bruising?	□ Yes □ No Notes:_____
Difficulty stopping bleeding?	□ Yes □ No Notes:_____
History of peptic ulcers or bleeding in the bowels?	□ Yes □ No Notes:_____
Allergy / allergic reactions to sulfonamides (“sulfa” drugs)?	□ Yes □ No Notes:_____
Smoking?	□ Yes □ Quit □ No
	If Yes, # of cigarettes / day: _____ *If Quit, # of years since you quit:* _____ *# of pack years prior to stopping:*_____
Alcohol consumption	□ Yes □ No Type & Daily amount:_____

Part 4: FAMILY Medical History

Please indicate whether any relatives (i.e., parents, siblings, grandparents, aunt or uncles) have ever been diagnosed with or suffer from any of the following:

Altitude Illness *(Ex. AMS, HAPE, HACE)*	□ Yes Family member? _____ Illness? _____ □ No
Sleep Disordered Breathing *(Ex. OSA, CSA, Cheyne-Stokes, etc)*	□ Yes Family member? _____ Disorder? _____ □ No
Hypertension / Preeclampsia	□ Yes Family member? _____ □ No
Heart disease and / or stroke	□ Yes Family member? _____ Disease? _____ □ No
Respiratory Disease(s) *(Ex. Asthma, COPD, etc)*	□ Yes Family member? _____ Disease? _____ □ No
Blood Disorder(s) *(Ex. Hemophilia, easy bruising, anemia, sickle cell disease, etc.)*	□ Yes Family member? _____ Disorder? _____ □ No
High cholesterol	□ Yes Family member? _____ □ No
Diabetes Mellitus *Type I or II?*	□ Yes Family member? _____ Type I or II? _____ □ No

Estimated Glomerular Filtration Rate: _____ $mL \cdot min^{-1} \cdot 1.73m^{-2}$ (Calculated from serum Creatinine sample analyzed)

Participant ID # & Initials: _____

PHYSICAL EXAMINATION

Part A – Visual Inspection

General appearance *(Ex. Face)*	
Trauma *(Ex. minor bruises, major bleeding, painkiller)*	☐ Yes ☐ No Notes:_____

Part B - Measurements

Physical Characteristics

Height: _____cm Weight: _____kg BMI: _____kg·m^2

Circumferences

Neck: _____cm Abdomen: _____cm Hip: _____cm

Temperature:

_____*ºC*

Blood Pressure & Heart Rate

SBP: _____mmHg DBP: _____mmHg MAP: _____mmHg HR: _____bpm

SBP: _____mmHg DBP: _____mmHg MAP: _____mmHg HR: _____bpm

SBP: _____mmHg DBP: _____mmHg MAP: _____mmHg HR: _____bpm

Average: _____mmHg _____mmHg _____mmHg _____bpm

Part C. Systemic Evaluation *(Inspection, Palpation, Auscultations and Percussion)*

Head & Neck

Lymphadenopathy?	☐ Yes ☐ No Notes:_____
Oral / Nasal Ulcers?	☐ Yes ☐ No Notes:_____

Participant ID # & Initials: _____

Cardiovascular System

Abnormal Heart Sounds?	☐ Yes ☐ No Notes:_____
Cardiac Murmur?	☐ Yes ☐ No Notes:_____
Carotid Bruit?	☐ Yes ☐ No Notes:_____
Peripheral Edema?	☐ Yes ☐ No Notes:_____

Respiration

Crackles on auscultation?	☐ Yes ☐ No Notes:_____
Wheezing?	☐ Yes ☐ No Notes:_____

Abdomen

Absent bowel sounds?	☐ Yes ☐ No Notes:_____
Tenderness?	☐ Yes ☐ No Notes:_____
Hepatosplenomegaly?	☐ Yes ☐ No Notes:_____
Ascites?	☐ Yes ☐ No Notes:_____

Neurological

Weakness?	☐ Yes ☐ No Notes:_____
Diminished sensation?	☐ Yes ☐ No Notes:_____
Abnormal reflexes?	☐ Yes ☐ No Notes:_____

Dermatological

Hematomas?	☐ Yes ☐ No Notes:_____
Skin abnormality?	☐ Yes ☐ No Notes:_____

12-Lead ECG

Abnormalities?	☐ Yes ☐ No Notes:_____ _____ Assessed By: _____(Print Name) Signature: _____Date: _____

Completed by: _____ (initial here)

Urinalysis Record Sheet

1. Specific Gravity	☐ 1.000	☐ 1.005	☐ 1.010	☐ 1.015	☐ 1.020	☐ 1.025	☐ 1.030
2. pH	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9		
3. Leukocytes	☐ neg.	☐ +1	☐ +2	☐ +3			
4. Nitrite	☐ neg.	☐ pos.					
5. Protein	☐ neg.	☐ +1	☐ +2	☐ +3			
6. Glucose	☐ norm.	☐ +1	☐ +2	☐ +3	☐ +4		
7. Ketones	☐ neg.	☐ +1	☐ +2	☐ +3			
8. Urobilinogen	☐ norm.	☐ +1	☐ +2	☐ +3	☐ +4		
9. Bilirubin	☐ neg.	☐ +1	☐ +2	☐ +3			
10. Blood	☐ neg.	☐ +1	☐ +2	☐ +3	☐ +4		
11. Hemoglobin	☐ +1	☐ +2	☐ +3	☐ +4			

COX-IH Study Exclusion Criteria Checklist

Exclusion Areas	Criterion Value		
Age (y)	**Younger than** 19 OR **Older than** 45?	☐ Yes	☐ No
BMI	> 35 $kg \cdot m^{-2}$?	☐ Yes	☐ No
Blood Pressure	≥ 140 / 90 (SBP / DBP)	☐ Yes	☐ No
12-Lead ECG	Abnormalities?	☐ Yes	☐ No
Living in Calgary	Less than 1 year?	☐ Yes	☐ No
Smoking	Within the past year?	☐ Yes	☐ No
NSAIDs	Taking Regularly?	☐ Yes	☐ No
Drug Allergies	Allergic to Sulphonamides?	☐ Yes	☐ No
Medical Questionnaire	Positive (i.e., Yes) response to any aspect of questionnaire?	☐ Yes	☐ No
Urinary Protein Excretion	> 150 $mg \cdot 24h^{-1}$	☐ Yes	☐ No
Sodium Concentration	< 20 $mEq \cdot L^{-1}$	☐ Yes	☐ No
Complete Blood Count (CBC)			
Hemoglobin	Outside range of: 137 to 180 $g \cdot L^{-1}$	☐ Yes	☐ No
WBC Count	Outside range of: 4.0 to 11.0 × 10^9 $cells \cdot L^{-1}$	☐ Yes	☐ No
Mean Corpuscle Count (MCV)	Outside range of: 82 to 100 fL	☐ Yes	☐ No
Platelet Count	150 to 400 × 10^9 $cells \cdot L^{-1}$	☐ Yes	☐ No
Liver Function			
Alaline Transaminase (ALT)	Outside range of: 1 to 60 $IU \cdot L^{-1}$	☐ Yes	☐ No
Aspartate Transaminase (AST)	Outside range of: 8 to 40 $IU \cdot L^{-1}$	☐ Yes	☐ No
Alkaline Phosphatase (ALP)	Outside range of: 30 to 130 $IU \cdot L^{-1}$	☐ Yes	☐ No
International Normalized Ratio (INR)	Outside range of: 0.9 to 1.1	☐ Yes	☐ No
Glucose (Fasting)	> 7.0 $mmol \cdot L^{-1}$	☐ Yes	☐ No
Estimated Glomerular Filtration Rate (GFR) (Calculated from **serum creatinine** level)	≤ 60 $mL \cdot min^{-1} \cdot 1.73m^{-2}$	☐ Yes	☐ No
Potassium (K^+)	Outside range of: 3.3 to 5.1 $mmol \cdot L^{-1}$	☐ Yes	☐ No
Sodium (Na^+)	Outside range of: 133 to 145 $mmol \cdot L^{-1}$	☐ Yes	☐ No

Respiratory Disturbance Index (RDI)	> 10 events·h^{-1}	☐ Yes ☐ No
Mean Sa_{O_2} during sleep?	< 90%	☐ Yes ☐ No

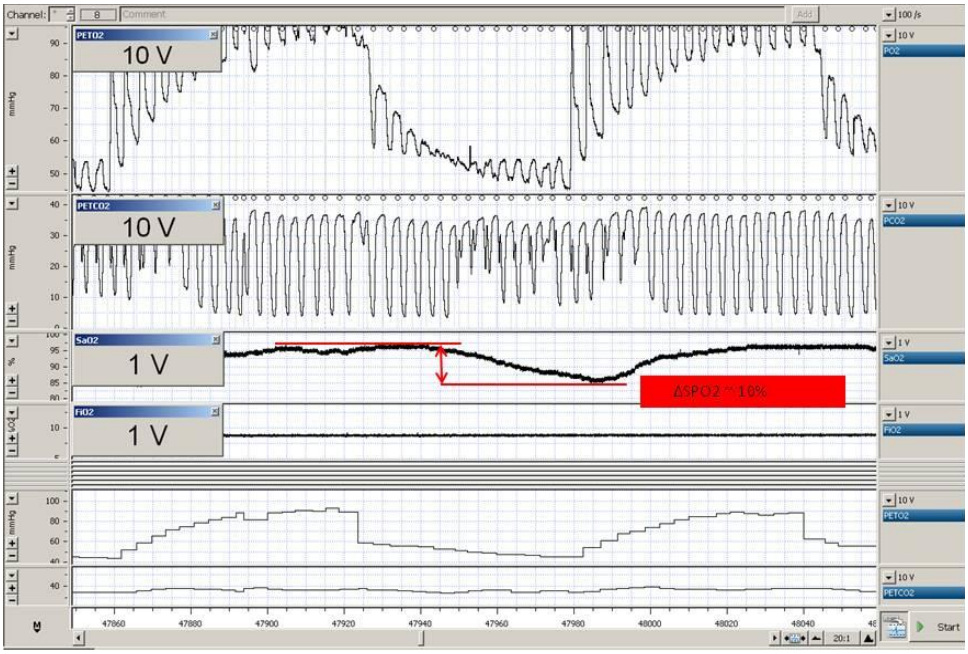
NOTE: To meet inclusion criteria of the COX-IH study, the answers to all of the following criteria must be "**No**".

Completed by: _____ (initial here)

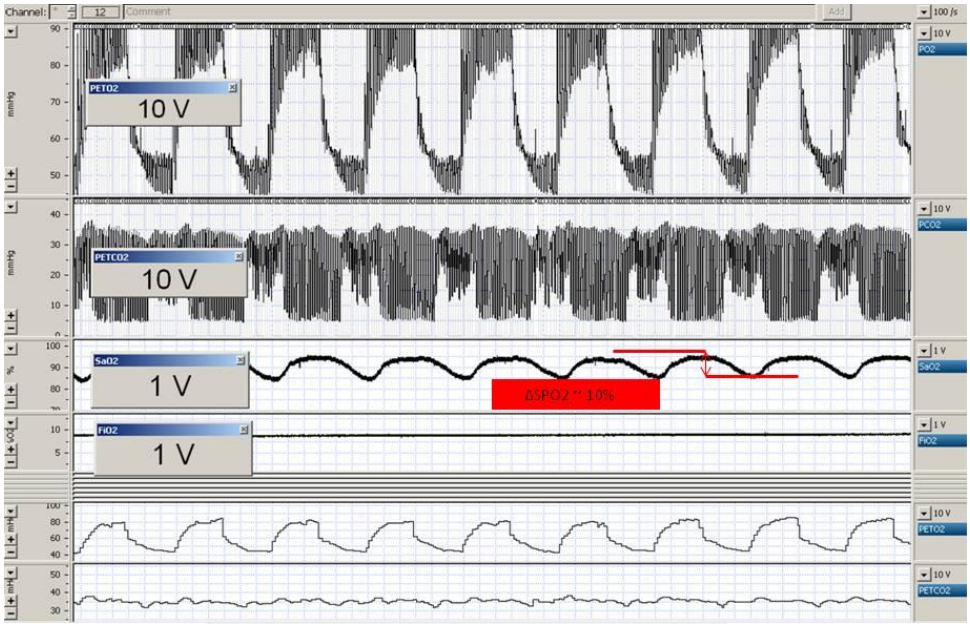
Date: _____

Appendix F: Representative LabChart from 6 hours of chamber IH exposure

Appendix F1. One cycle of hypoxia (enlarged)



Appendix F2. Multiple cycles of hypoxia



Appendix G: Representative radiometer results

RADIOMETER ABL800 FLEX

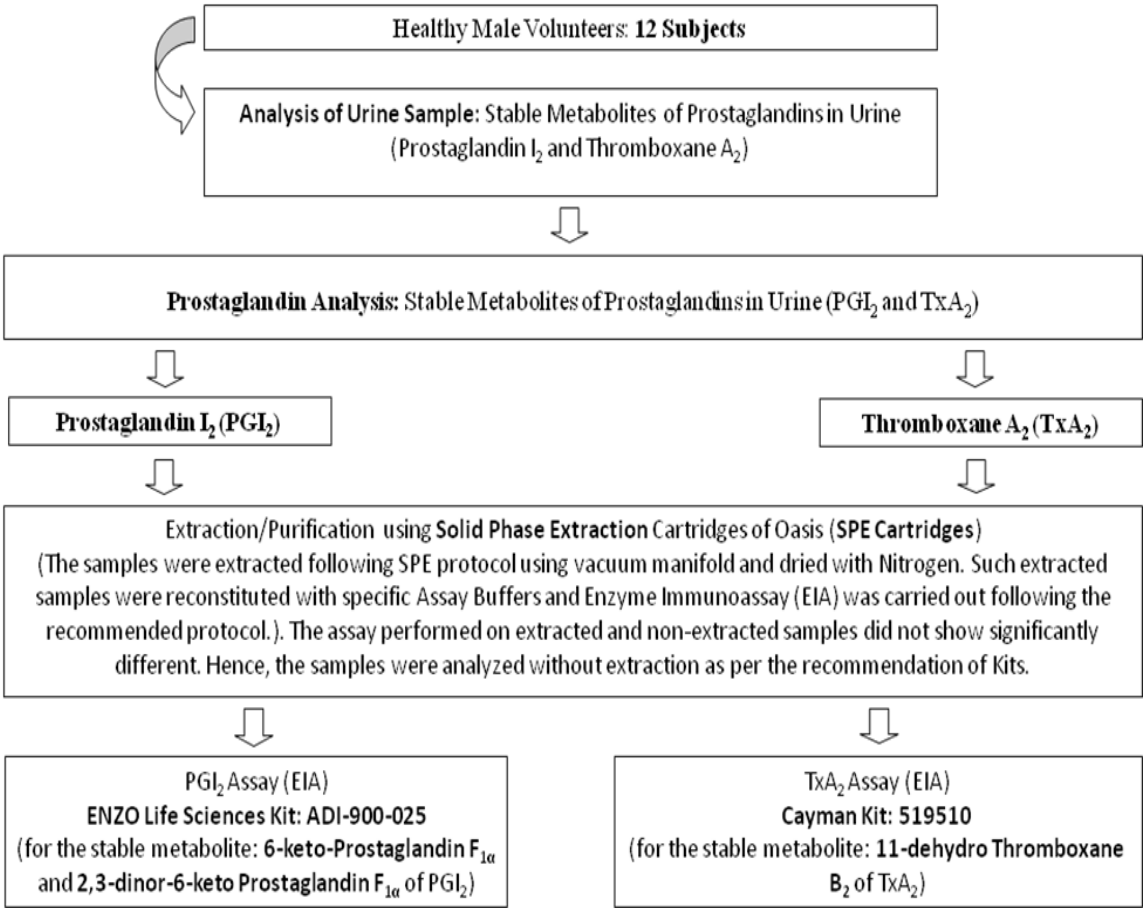
ABL837 The Poulin PATIENT REPORT	Capillary - C 125uL		01:37 PM Sample #		9/10/2010 43
Identifications					
Patient ID	249				
Patient Last Name					
Patient First Name					
Sample type	Capillary				
T	37.0 °C				
Blood Gas Values					
pH	7.365		[	-	]
pCO_2	40.5	mmHg	[	-	]
pO_2	73.1	mmHg	[	-	]
Oximetry Values					
ctHb	15.2	g/dL	[	-	]
sO_2	95.9	%	[	-	]
FO_2Hb	94.7	%	[	-	]
FCOHb	0.8	%	[	-	]
FHHb	4.0	%	[	-	]
FMetHb	0.5	%	[	-	]
Electrolyte Values					
cK^+	4.4	mmol/L	[	-	]
cNa^+	131	mmol/L	[	-	]
cCa^{2+}	2.72	meq/L	[	-	]
cCl^-	95	mmol/L	[	-	]
Metabolite Values					
cGlu	5.3	mmol/L	[	-	]
cLac	1.5	mmol/L	[	-	]
cCrea	65	µmol/L	[	-	]
ctBil	0	mg/L	[	-	]
Temperature Corrected Values					
pH(*T*)	7.365				
$pCO_2(T)$	40.5	mmHg			
$pO_2(T)$	73.1	mmHg			
Oxygen Status					
ctO_{2c}	20.2	Vol%			
$p50_c$	23.04	mmHg			
Acid Base Status					
$cBase(Ecf)_c$	-2.0	mmol/L			
$cHCO_3^-(P,st)_c$	22.6	mmol/L			

Notes

c Calculated value(s)
0902: Adaptive measuring mode applied

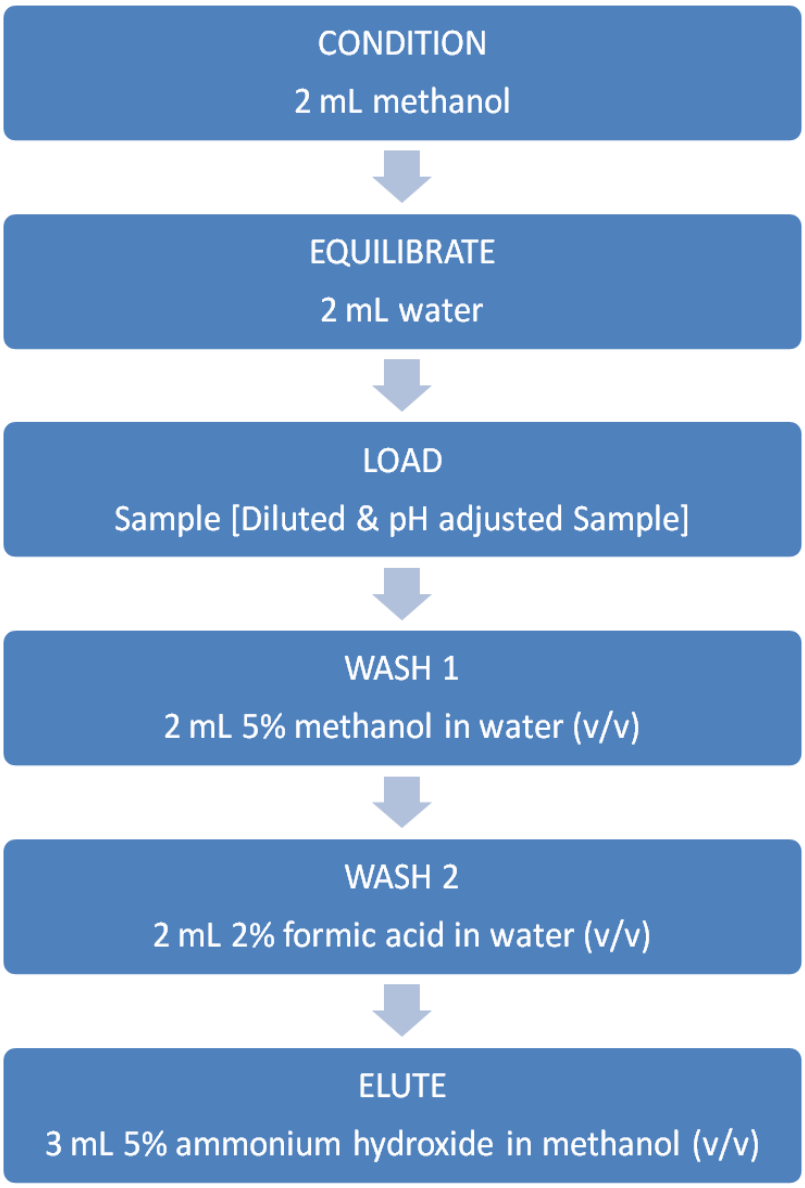
A representative Radiometer results of capillary blood sample of a subject

Appendix H. Urinary prostaglandin analysis algorithms



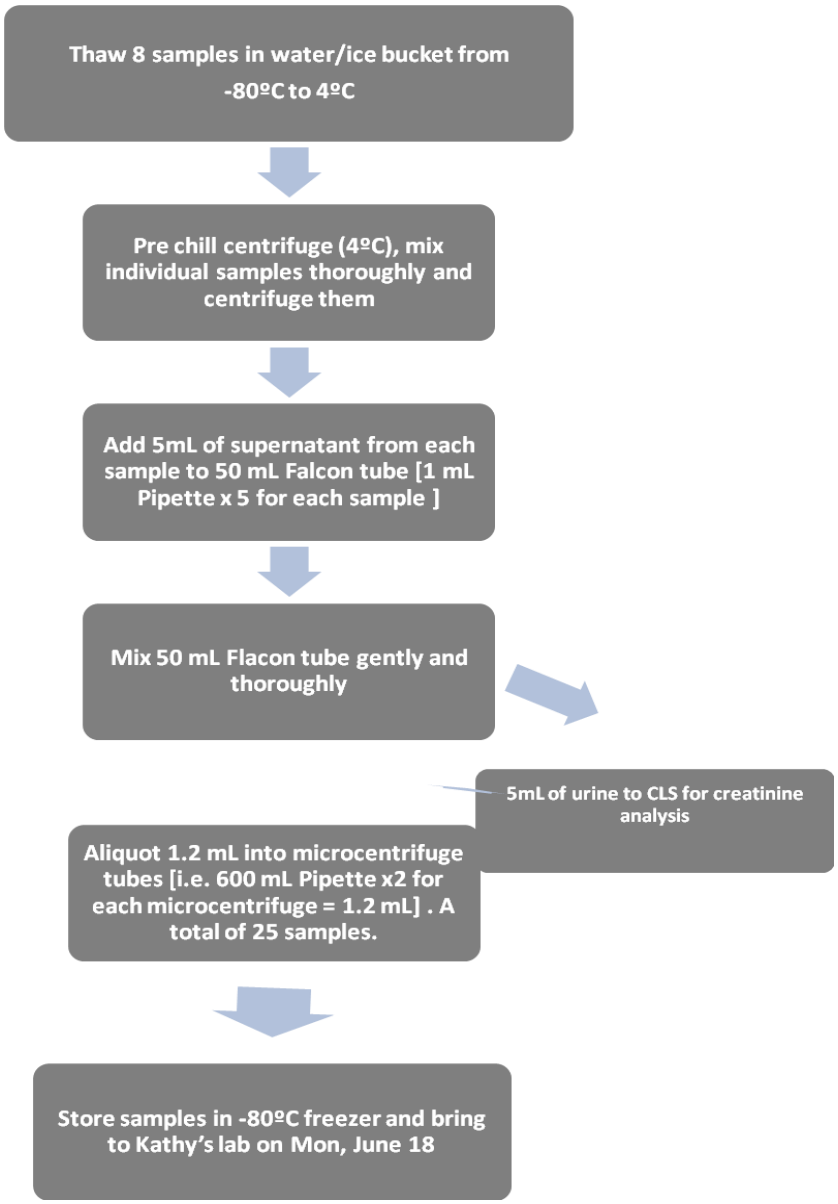
Urinary prostaglandin analysis flow chart

Appendix I. Solid phase extraction (SPE) protocol



Solid phase extraction (SPE) protocol for urinary prostaglandin metabolites

Appendix J. Mean sample preparation of urine



Flow chart for mean sample preparation of urine from the Protocol – Placebo (A) and Indomethacin (B)