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

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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

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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 PGI₂:TxA₂ 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, Bhuspesh 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
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 ISO CAPNIC 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 (PGI₂) 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 I₂ (PGI₂) results PRE-IH (AM) and POST-IH (PM) .................................102

Figure 4.3 Thromboxane A₂ (TxA₂) results PRE-IH (AM) and POST-IH (PM) .........................103

Figure 4.4 Prostaglandin ratios (PGI₂/TxA₂) i.e. vasodilator (PGI₂) vs vasoconstrictor (TxA₂) ......................................................................................................................................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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Arachidonic Acid</td>
</tr>
<tr>
<td>ABP</td>
<td>Arterial Blood Pressure</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin-Converting–Enzyme</td>
</tr>
<tr>
<td>AIHS</td>
<td>Alberta Innovates – Health Solutions</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>bpm</td>
<td>Beats Per Minute</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>Calcium</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral Blood Flow</td>
</tr>
<tr>
<td>CHREB</td>
<td>Conjoint Health Research Ethics Board</td>
</tr>
<tr>
<td>CIH</td>
<td>Chronic Intermittent Hypoxia</td>
</tr>
<tr>
<td>CIHR</td>
<td>The Canadian Institutes of Health Research</td>
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<tr>
<td>CLBX</td>
<td>Celecoxib</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac Output</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>COX-1</td>
<td>Cyclooxygenase-1</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>COX-3</td>
<td>Cyclooxygenase-3</td>
</tr>
<tr>
<td>COXIBs</td>
<td>Selectively Cyclooxygenase-2 Inhibitors</td>
</tr>
<tr>
<td>CPAP</td>
<td>Continuous Positive Airway Pressure</td>
</tr>
<tr>
<td>CVC</td>
<td>Cerebrovascular Conductance</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Diseases</td>
</tr>
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<td>CVR</td>
<td>Cerebrovascular Resistance</td>
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<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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</tr>
<tr>
<td>DEF</td>
<td>Dynamic End-tidal Forcing</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ED</td>
<td>Endothelial Dysfunction</td>
</tr>
<tr>
<td>EDCF</td>
<td>Endothelium-derived Contracting Factor</td>
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<td>EDHF</td>
<td>Endothelium-derived Hyperpolarizing Factor</td>
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<td>Endothelium-derived Relaxing Factor</td>
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<td>EIA</td>
<td>Enzyme Immunoassay</td>
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<td>ET-1</td>
<td>Endothelin-1</td>
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<tr>
<td>GLM</td>
<td>General linear Model</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
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<td>IH</td>
<td>Intermittent Hypoxia</td>
</tr>
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<td>INDO</td>
<td>Indomethacin</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalized Ratio</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid Chromatography-Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>LOX</td>
<td>Lipoxygenase</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Blood Pressure</td>
</tr>
<tr>
<td>MBP</td>
<td>Mean Arterial Blood Pressure</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle Cerebral Artery</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimeter of Mercury</td>
</tr>
<tr>
<td>MSK</td>
<td>Musculoskeletal Disorders</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Non-steroidal Anti-inflammatory Drugs</td>
</tr>
<tr>
<td>NSERC</td>
<td>Natural Sciences and Engineering Research Council of Canada</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OSA</td>
<td>Obstructive Sleep Apnea</td>
</tr>
<tr>
<td>P</td>
<td>Power (Total power of the Doppler spectrum averaged over the Cardiac cycle)</td>
</tr>
<tr>
<td>P&lt;sub&gt;A&lt;sub&gt;C&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Partial Pressure of Arterial Carbon Dioxide</td>
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<td>P&lt;sub&gt;A&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Partial Pressure of Arterial Oxygen</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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</tr>
<tr>
<td>PETCO₂</td>
<td>End-tidal Partial Pressure of Carbon Dioxide</td>
</tr>
<tr>
<td>PETO₂</td>
<td>End-tidal Partial Pressure of Oxygen</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PGD₂</td>
<td>Prostaglandin D₂</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>Prostaglandin F₂α</td>
</tr>
<tr>
<td>PGHS</td>
<td>Prostaglandin H Synthase</td>
</tr>
<tr>
<td>PGI₂</td>
<td>Prostaglandin I₂ (i.e., Prostacyclin)</td>
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<tr>
<td>PGI₂M</td>
<td>Prostaglandin I₂ Metabolite</td>
</tr>
<tr>
<td>PLBO</td>
<td>Placebo</td>
</tr>
<tr>
<td>RDI</td>
<td>Respiratory Disturbance Index</td>
</tr>
<tr>
<td>RM</td>
<td>Repeated Measures</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SaO₂</td>
<td>Arterial Oxyhemoglobin Saturation</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SDB</td>
<td>Sleep Disordered Breathing</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke Volume</td>
</tr>
<tr>
<td>SNA</td>
<td>Sympathetic Nerve Activity</td>
</tr>
<tr>
<td>TCD</td>
<td>Transcranial Doppler Ultrasound</td>
</tr>
<tr>
<td>TxA₂</td>
<td>Thromboxane A₂</td>
</tr>
<tr>
<td>VSM</td>
<td>Vascular Smooth Muscle</td>
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<tr>
<td>∇P</td>
<td>Peak Blood Velocity of the Doppler Waveform Averaged Over the Cardiac Cycle</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
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 that 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 I₂ (PGI₂), also known as Prostacyclin, and Prostaglandin E₂ (PGE₂) are vasodilatory while Thromboxane A₂ (TxA₂) and Prostaglandin F₂α (PGF₂α) are vasoconstrictors. Similarly, PGI₂ is a platelet anti-agregant and TxA₂ 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 Cyclooxygenase-1 (COX-1) which primarily forms TxA₂ (Clarke et al., 1991) and Cyclooxygenase-2 (COX-2) which predominantly leads to the formation of PGI₂ and PGE₂ (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$_2$), Thromboxane 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
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$_2$, TxA$_2$ and PGF$_{2a}$ (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). PGI₂ and PGE₂ are mainly mediated through COX-2 while TxA₂ and PGF₂α are formed through COX-1 (Smith & Langenbach, 2001; Smith et al., 2000) in the PG cascade. PGI₂ and PGE₂ increase renal medullary blood flow which drives pressure natriuresis and diuresis (Fries & Grosser, 2005). Most of these PGs (e.g. PGI₂, PGE₂, TxA₂, PGF₂α) 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 TxA₂ and lower production of PGI₂ (Wang et al., 2008) showing vasoconstrictor component being more active. Studies using animal models have revealed that PGI₂ 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 PGI₂ while TxA₂ 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 PGI₂ and TxA₂ is implicated in the delicate balance of cardiovascular risk. It is assumed COX inhibition causes PGI₂ and PGE₂ inhibition while TxA₂ 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 TxA2/PGL2/PGE2 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., PGE2 and PGI2), 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 (PGI2 and PGE2) (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 TxA2.
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 PGI₂ and PGE₂ 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\textsubscript{2}, PGE\textsubscript{2}, PGF\textsubscript{2\alpha}, PGD\textsubscript{2}, and TxA\textsubscript{2} (White & Hagen, 1982). Similar to the peripheral vasculature, TxA\textsubscript{2} and PGF\textsubscript{2\alpha} are mainly cerebral vasoconstrictors in adults while PGI\textsubscript{2} causes cerebral vasodilatation (Chemtob et al., 1996). The effect of PGE\textsubscript{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\textsubscript{2} and PGE\textsubscript{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 PGI2 and vasoconstrictor TxA2 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 downstreamPGs 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.clinicaltrials.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 m²), obesity (BMI > 35), sleep apnea (RDI > 10 evenets/hr and an overnight mean SaO₂ < 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, r² = 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 $\sim 4$ 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 $\text{PETO}_2$ (End-tidal Partial Pressure of Oxygen) and $\text{PETCO}_2$, (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 ($\text{PETCO}_2 = +1 \text{ Torr above resting levels PETO}_2 = +88 \text{ Torr}$) for 4 min. At the end of the 4 min, subjects performed a 2 min isocapnic hypoxia stage ($\text{PETCO}_2 = +2$, $\text{PETO}_2 = +50 \text{ Torr}$), 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 ($\text{PETCO}_2 = +5$ and $\text{PETCO}_2 = +8 \text{ Torr}$).

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 \( \text{PO}_2 \) and \( \text{PCO}_2 \) 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 \( \text{O}_2 \) and \( \text{CO}_2 \) 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 the temporomandibular 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 ($\overline{VP}$), 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 ($\overline{P} \times$ 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.,...
With an assumption of constant MCA vessel diameter, it is acceptable to consider \( \nabla P \) 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 (SaO\(_2\)) was estimated from PET\(_2\)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 PETO2 and PETCO2, 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 PETO2 and PETCO2 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 (.flf), 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 waveform 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 $\text{PETO}_2$, $\text{PETCO}_2$, 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 $\text{PO}_2$ and $\text{PCO}_2$ 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 ($\text{SaO}_2$) 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 (PETO₂ = 88.0 Torr and PETCO₂ = +1.0 Torr above rest) followed by six cycles of IH comprised of 90 seconds of isocapnic hypoxia (PETO₂ = 45.0 Torr and PETCO₂ = +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 PETO₂ = 45.0 Torr) and one-minute of normoxia (peak PETO₂ = 88.0 Torr). The PETCO₂ was maintained isocapnic by adding 100% CO₂ 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 PETO₂ = 45.0 Torr. Periods of normoxia (PETO₂ = 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 PETO$_2$ = 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 PO$_2$ and PCO$_2$. 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.

\[
\text{BP gain} = \frac{(\text{BP}_{\text{isocapnic-hypoxia}} - \text{BP}_{\text{isocapnic-euoxia}})}{\% \Delta \text{Arterial oxygen saturation}}
\]

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 $\text{PET}_{\text{CO}_2}$ 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.  \[ \text{CVC} = \overline{V_p}/\text{MAP} \]

B.  \[ \text{CVR} = \text{MAP}/\overline{V_p} \]

Where:

- CVC = cerebrovascular conductance (cm × sec$^{-1}$ × mmHg$^{-1}$)
- CVR = cerebrovascular resistance (mmHg × sec × cm$^{-1}$)
- MAP = mean arterial pressure (mmHg)
- $\overline{V_p}$ = peak flow velocity of the MCA (cm × 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. CO₂, O₂ 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 PETO₂ = +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 = \((\text{CVR}_{\text{isocapnic-hypoxia}} - \text{CVR}_{\text{isocapnic-euoxia}}) / (\% \Delta \text{Arterial oxygen saturation})\)

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 \((\text{mmol of Creatinine} *1000)/88.402\)
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.

\[
\text{PGcorrected (ng/mmoL of Cr/mL)} = \frac{\text{PG (pg/mL)}}{\text{Creatinine (mmoL/L)/1000}}
\]

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

\[
\text{Creatinine (mg/dL) = [Creatinine (mmoL/L) * 1000]} / (88.402)
\]

Then,

\[
\text{PGcorrected (ng/mg of Cr/dL)} = \frac{\text{[PG (pg/mL) * 100]}}{\text{[Creatinine (mg/dL)] /1000}}
\]

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·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., Po₂ and PCO₂), oximetry measures, electrolytes (Na⁺, K⁺, Cl⁻, Ca²⁺), 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 I₂), **Figure 4.3** (Thromboxane A₂), **Figure 4.4** ratios (PGI₂/TxA₂) and **Figure 4.5** changes in PGs (subtracting morning values from evening values). After four days of drug ingestion, the morning urinary PGI₂M i.e. PGI₂ metabolites (two active metabolites of PGI₂: 6k-PGF₁α and 2,3-d-6k-PGF₁α) 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, PGI₂M remained significantly lower with indomethacin (p = 0.004) compared to placebo, while there was just a pattern (not significant) for PGI₂M with celecoxib to remain lower than PLBO (p = 0.062). The PGI₂M 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 TXB₂ (11-dehydro Thromboxane B₂ 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$_2$/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$_2$/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, \( \text{PETCO}_2 \) 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 \( \text{PETCO}_2 \) and increase in \( \text{PETO}_2 \) but they were not statistically significant within the intervention. There was a significant decrease in \( \text{PETCO}_2 \) 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 \( \text{PETO}_2 \) with placebo and celecoxib, but not with indomethacin \((\text{PLBO}, p = 0.035; \text{INDO}, p = 0.337; \text{CLBX}, p < 0.001)\) 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 \((\text{INDO vs PLBO}, p = 0.01; \text{INDO vs CLBX}, p = 0.039)\) but not with celecoxib \((\text{CLBX vs PLBO}, p = 0.458)\). 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, \( Vp \))

Air breathing CBF (\( Vp \)) 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 ($V_p$) 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 $V_p$ 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., $V_p$ 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 $P_{ET\text{CO}_2}$ and $P_{ET\text{O}_2}$ 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 $P_{ET\text{CO}_2}$ 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 $P_{ET\text{O}_2}$ 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 $P_{ET\text{CO}_2}$ and $P_{ET\text{O}_2}$ 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 ($\bar{V}_p$) was significantly lower with indomethacin (p = 0.024), but ($\bar{V}_p$) 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 PETO$_2$ with their corresponding saturations were consistent in the morning and afternoon. The PETCO$_2$ was maintained constant (isocapnic) throughout the euoxic-hypoxic cycles (Figure 4.8, Panel 2). Before and after IH exposure, the PETCO$_2$ and PETO$_2$ along with
blood oxygen saturation (SpO₂) 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 (PCO₂) 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 (PO₂) 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.
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 PGI₂:TxA₂ 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$_2$M) 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., TxA₂) the stable metabolite 11-dehydro TXB₂ 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 TxA₂ significantly compared to placebo. In the long term uses of celecoxib, this uninhibited TxA₂ may have played crucial role in raising BP with celecoxib (Grosser et al., 2010; FitzGerald, 2004). After IH exposure, indomethacin maintained lower urinary TxA₂ concentrations compared to placebo and celecoxib, but celecoxib left TxA₂ uninhibited even after IH exposure which raises blood pressure. Within the drug comparisons (i.e. before and after IH exposure, AM vs PM), the TxA₂ 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 PGI₂ and TxA₂ (i.e. ratio of the stable metabolites of PGI₂ and TXA₂) is used to reflect the balance between the vasodilator PGI₂ and the vasoconstrictor TXA₂. 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 PGI₂:TXA₂ 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 PGI₂:TXA₂ 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 PGI₂. Hence, IH exposure non-statistically upregulated PGI₂ as compared to TxA₂ across the protocols. The change in the PGI₂:TXA₂ 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 PETCO₂. Indomethacin significantly lowered PETCO₂ 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 PETCO₂ to decrease and PETO₂ to increase, reflecting enhanced resting ventilation. However a comparison of pre-IH and post-IH PETCO₂, 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 PETO₂ in respective protocols, there was significant increase with placebo (p = 0.035) and celecoxib (p < 0.001). IH is expected to increase PETO₂ in placebo (Pialoux et al., 2009; Foster et al., 2009) and the reported increase in PETO₂ 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$_2$M 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 PGI₂ and TxA₂ but slightly increased with IH exposure in the afternoon. The changes in ratio PGI₂:TXA₂ 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 PETCO₂ was clamped at +1 Torr and PETO₂ 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.,
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 on 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 PGI₂ and TxA₂ 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.
Table 4.1 General and sleep characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.8±5.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.6±8.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.6±12.1</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.9±2.5</td>
</tr>
<tr>
<td>Neck Circumference (cm)</td>
<td>39.2±3.2</td>
</tr>
<tr>
<td>Abdominal Circumference (cm)</td>
<td>88.7±8.6</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>96.5±7.5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>88.7±9.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119.1±11.1</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>73.5±10.6</td>
</tr>
<tr>
<td>Pulse Rate (beats/min)</td>
<td>60.8±7.1</td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian (11), Asian (1)</td>
</tr>
<tr>
<td>Sex</td>
<td>Males</td>
</tr>
<tr>
<td>Sleep parameters¹</td>
<td></td>
</tr>
<tr>
<td>Monitoring (hours)</td>
<td>6.4±1.2</td>
</tr>
<tr>
<td>Total Time on Probe (hours)</td>
<td>6.4±1.4</td>
</tr>
<tr>
<td>RDI (Total)</td>
<td>1.8±1.1</td>
</tr>
<tr>
<td>RDI (Supine)</td>
<td>2.2±1.9</td>
</tr>
<tr>
<td>RDI (Lateral)</td>
<td>1.6±1.2</td>
</tr>
<tr>
<td>Mean SaO² (%)</td>
<td>94.8±1.1</td>
</tr>
<tr>
<td>Lowest SaO² (%)</td>
<td>89.2±4.6</td>
</tr>
<tr>
<td>Time in SaO² &lt; 90 %</td>
<td>0.0±0.1</td>
</tr>
</tbody>
</table>

¹Determined 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, SaO² = blood oxygen saturation (recorded with pulse oximetry)
### Table 4.2 Venous blood results (ABL800, Screening)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.4±0.1</td>
</tr>
<tr>
<td>Anion Gap (mmol/L)</td>
<td>9.0±2.2</td>
</tr>
<tr>
<td>cH (mmol/L)</td>
<td>45.3±4.8</td>
</tr>
<tr>
<td>Anion Gap Kc (mmol/L)</td>
<td>13.0±2.2</td>
</tr>
<tr>
<td>cHCO₃ P (mmol/L)</td>
<td>27.1±1.9</td>
</tr>
<tr>
<td>cHCO₃ P st (mmol/L)</td>
<td>23.8±1.6</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>51.1±6.5</td>
</tr>
<tr>
<td>PO₂ mmHg</td>
<td>29.1±11.0</td>
</tr>
<tr>
<td>p50c (mmHg)</td>
<td>30.2±2.6</td>
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<tr>
<td>SO₂ (%)</td>
<td>44.7±18.7</td>
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<tr>
<td>cHct (%)</td>
<td>48.9±2.7</td>
</tr>
<tr>
<td>ctHb (g/dL)</td>
<td>16.0±0.9</td>
</tr>
<tr>
<td>FCOHb (%)</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>FO₂Hb (%)</td>
<td>44.1±18.3</td>
</tr>
<tr>
<td>cCrea (µmol/L)</td>
<td>75.0±15.1</td>
</tr>
<tr>
<td>Creatinine Serum (CLS: mg/dL)</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td>cGlu (mmol/L)</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>cLac (mmol/L)</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>ctBil (mg/L)</td>
<td>3.6±6.7</td>
</tr>
<tr>
<td>cNa (mmol/L)</td>
<td>139.5±1.8</td>
</tr>
<tr>
<td>cK (mmol/L)</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>cCa (mEq/L)</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>cCl (mmol/L)</td>
<td>103.4±2.2</td>
</tr>
<tr>
<td>GFR (mL/min/173cm²)</td>
<td>117.8±14.0</td>
</tr>
</tbody>
</table>

**Table 4.2** Venous blood results (ABL800, Screening)

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

**Abbreviations:** c = concentration
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>156.3±6.3</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>RBC (10^12/L)</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>92.1±2.6</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>342.8±6.2</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13.1±0.7</td>
</tr>
<tr>
<td>Platelet Count (10^9/L)</td>
<td>225.8±35.3</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>5.9±1.7</td>
</tr>
<tr>
<td>Neutrophil (10^9/L)</td>
<td>3.3±1.4</td>
</tr>
<tr>
<td>Lymphocyte (10^9/L)</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Monocyte (10^9/L)</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Eosinophil (10^9/L)</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Basophil (10^9/L)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>INR</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>72.7±18.0</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18.7±4.4</td>
</tr>
<tr>
<td>APT (U/L)</td>
<td>23.3±4.8</td>
</tr>
<tr>
<td>Creatinine (Serum :µmol/L)</td>
<td>84.6±12.6</td>
</tr>
<tr>
<td>Creatinine (Serum: mg/dL)</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Na (Urine:mmol/L)</td>
<td>64.3±30.3</td>
</tr>
<tr>
<td>Creatinine (Urine: mmol/L)</td>
<td>7.8±5.4</td>
</tr>
<tr>
<td>GFR (mL/min/173cm²)</td>
<td>107.9±14.5</td>
</tr>
</tbody>
</table>

**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>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>pH</td>
<td>5.7±0.8</td>
</tr>
<tr>
<td>Leukocytes (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Nitrite (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Protein (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glucose (N/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Ketones (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Urobilinogen (N/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Bilirubin (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Blood (-/+ )</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1.0±0.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PLACEBO</th>
<th>INDOMETHACIN</th>
<th>CELECOXIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>AM 7.4±0.0 PM 7.4±0.0</td>
<td>AM 7.4±0.0 PM 7.4±0.0</td>
<td>AM 7.4±0.0 PM 7.4±0.1</td>
</tr>
<tr>
<td>Anion Gap (mmol/L)</td>
<td>8.3±1.9 AM 7.5±1.9 PM 7.7±2.5</td>
<td>8.0±2.3 AM 8.2±2.6 PM 9.1±2.5</td>
<td></td>
</tr>
<tr>
<td>cH (mmol/L)</td>
<td>40.2±1.6 AM 38.4±0.8 PM 39.9±1.1</td>
<td>38.1±0.8 AM 40.8±1.7 PM 40.4±5.6</td>
<td></td>
</tr>
<tr>
<td>AnionGap_Kc (mmol/L)</td>
<td>12.3±1.8 AM 11.9±1.8 PM 12.3±2.1</td>
<td>12.4±2.6 PM 13.5±2.5</td>
<td></td>
</tr>
<tr>
<td>cHCO₃⁻ (mmol/L)</td>
<td>23.4±1.3 AM 24.9±1.4 PM 23.1±1.0</td>
<td>24.3±1.3 AM 22.8±1.7 PM 23.8±3.5</td>
<td></td>
</tr>
<tr>
<td>cHCO₃⁻ P_st (mmol/L)</td>
<td>23.7±1.1 AM 25.1±1.0 PM 23.5±1.0</td>
<td>24.7±0.8 AM 23.2±1.4 PM 24.1±3.1</td>
<td></td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>38.9±1.8 AM 39.5±2.2 PM 37.9±2.8</td>
<td>38.4±2.0 AM 39.1±3.0</td>
<td></td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>73.3±4.8 AM 70.9±4.0 PM 75.0±5.0</td>
<td>73.7±6.3 AM 76.9±5.7 PM 69.2±7.5</td>
<td></td>
</tr>
<tr>
<td>p50c (mmHg)</td>
<td>23.8±1.3 AM 24.2±1.0 PM 23.9±1.0</td>
<td>24.0±1.1 AM 24.1±1.4 PM 24.9±2.0</td>
<td></td>
</tr>
<tr>
<td>SO₂ (%)</td>
<td>95.5±1.0 AM 95.0±1.2 PM 95.8±1.3</td>
<td>96.0±1.1 AM 94.4±1.8</td>
<td></td>
</tr>
<tr>
<td>pO₂_Aa_est (mmHg)</td>
<td>12.7±4.1 AM 14.6±4.2 PM 12.9±5.9</td>
<td>13.7±4.5 AM 9.6±4.0 PM 16.5±5.1</td>
<td></td>
</tr>
<tr>
<td>cHCT (%)</td>
<td>48.1±2.5 AM 52.3±3.3 PM 47.6±2.1</td>
<td>49.9±4.0 AM 48.4±3.4 PM 50.2±6.4</td>
<td></td>
</tr>
<tr>
<td>ctHb (g/dL)</td>
<td>15.5±0.8 AM 17.1±1.1 PM 15.6±0.7</td>
<td>16.4±1.3 AM 15.8±1.1 PM 16.4±2.1</td>
<td></td>
</tr>
<tr>
<td>FCOHb (%)</td>
<td>0.9±0.2 AM 1.0±0.2 PM 1.2±0.8</td>
<td>1.1±0.3 AM 0.9±0.1 PM 0.9±0.2</td>
<td></td>
</tr>
<tr>
<td>FO₂Hb (%)</td>
<td>94.1±0.9 AM 93.5±1.2 PM 94.1±1.2</td>
<td>94.1±1.3 AM 94.6±1.1 PM 93.0±1.7</td>
<td></td>
</tr>
<tr>
<td>cCrea (µmoL/L)</td>
<td>75.1±18.0 AM 73.9±20.7 PM 76.0±15.6</td>
<td>74.3±14.8 AM 73.1±14.1 PM 68.8±12.7</td>
<td></td>
</tr>
<tr>
<td>cGlu (mmol/L)</td>
<td>9.5±11.6 AM 5.6±1.0 PM 6.2±1.4</td>
<td>5.9±0.8 AM 5.9±1.0 PM 5.9±0.9</td>
<td></td>
</tr>
<tr>
<td>cLac (mmol/L)</td>
<td>1.7±0.5 AM 1.4±0.3 PM 1.6±0.8</td>
<td>1.3±0.3 AM 1.7±0.6 PM 1.5±0.4</td>
<td></td>
</tr>
<tr>
<td>ctBil_mg_L</td>
<td>2.3±3.8 AM 2.3±4.3 PM 2.3±4.3</td>
<td>2.5±4.3 AM 1.3±2.3 PM 3.3±4.8</td>
<td></td>
</tr>
<tr>
<td>cNa (mmol/L)</td>
<td>139.9±2.4 AM 140.8±2.5 PM 140.2±3.6</td>
<td>141.3±2.5 AM 139.7±1.7 PM 141.4±2.3</td>
<td></td>
</tr>
<tr>
<td>cK (mmol/L)</td>
<td>4.0±0.2 AM 4.4±0.3 PM 4.2±0.2</td>
<td>4.4±0.3 AM 4.2±0.3 PM 4.4±0.2</td>
<td></td>
</tr>
<tr>
<td>cCa (mEq/L)</td>
<td>2.5±0.1 AM 2.4±0.1 PM 2.4±0.1</td>
<td>2.4±0.1 AM 2.5±0.1 PM 2.5±0.1</td>
<td></td>
</tr>
<tr>
<td>cCl (mmol/L)</td>
<td>108.1±1.8 AM 108.4±1.4 PM 109.3±2.9</td>
<td>109.2±2.3 AM 108.8±2.7 PM 108.6±2.2</td>
<td></td>
</tr>
</tbody>
</table>

**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


Footnotes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) pathways; Prostaglandins (PG) are formed by specific isomerases from the COX product PGH₂. They act through G protein transmembrane receptors (IP, TPₐ, TPₜ, DP₁, DP₂, EP₁, EP₂, EP₃, EP₄, FP₂, FP₂); Abbreviations: AA = arachidonic acid, COX = cyclooxygenase, PGH₂ = Prostaglandin H₂, PGs = prostaglandins, IP = prostaglandin receptors, COX-1 = cyclooxygenase-1, COX-2 = cyclooxygenase-2; PGI₂ = Prostaglandin I₂, TxA₂ = Thromboxane A₂, PGD₂ = Prostaglandin D₂, PGE₂ = Prostaglandin E₂, PGF₂a = Prostaglandin F₂a; 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


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: PETO$_2$ = partial pressure of end-tidal oxygen; PETCO$_2$ = partial pressure of end-tidal carbon dioxide; Panel 1, RED: acute intermittent hypoxia challenge; Panel 2, BLACK: isocapnia at +1 Torr PETCO$_2$
**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 (PGI₂/TxA₂) i.e. vasodilator (PGI₂) vs vasoconstrictor (TxA₂)

Footnotes: The balancing ratio of vasodilator (PGI₂) to vasoconstrictor (TxA₂); the values represent means ± SD; Abbreviations: PGI₂ = prostaglandin I₂ (prostacyclin), TxA₂ – thromboxane A₂. 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 $\text{PETCO}_2$ and Panel 1b Isocapnic baseline $\text{PETCO}_2$ (+1 Torr); Panel 2a Air breathing $\text{PETO}_2$ and Panel 2b. Isocapnic baseline $\text{PETO}_2$ (Euoxia: +88 Torr); Panel 3a. Air breathing CBF ($V_p$) and Panel 3b. $V_p$ during isocapnic ($\text{PETCO}_2$: +1 Torr, $\text{PETO}_2$: +88 Torr); the values represent means ± SD; the significance: *, p < 0.05 and **, p < 0.01;

Abbreviations: $\text{PETCO}_2$ = end-tidal carbon dioxide, $\text{PETO}_2$= end-tidal oxygen, CBF ($V_p$) = 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. $\text{PETO}_2$ Six Cycles of hypoxia-normoxia averaged into a single cycle. Euoxia: +88 Torr, Hypoxia: +45 Torr of 90 seconds each; Panel 2. Isocapnia ($\text{PETCO}_2$: +1 Torr maintained during the intermittent hypoxia cycles); Panel 3. Oxygen saturation; the values represent means ± SD; Abbreviations: SpO2 = blood oxygen saturation, $\text{PETO}_2$ = end-tidal oxygen, $\text{PETCO}_2$ = 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 $V_p$), **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 $\bar{V}_b$), 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


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


Ref Type: Report

Ref Type: Report

Ref Type: Report


### Appendix A: Pharmacodynamics and pharmacokinetics of the drugs

#### Pharmacodynamics and pharmacokinetics of Indomethacin and Celecoxib

<table>
<thead>
<tr>
<th>Properties</th>
<th>Indomethacin</th>
<th>Celecoxib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of action</td>
<td>~30 minutes</td>
<td>1 Hour (Malmstrom et al., 1999)</td>
</tr>
<tr>
<td>Duration</td>
<td>4-6 hours</td>
<td>5-7 Hours (Malmstrom et al., 1999)</td>
</tr>
<tr>
<td>Absorption</td>
<td>Oral: Immediate release: Prompt and extensive; Extended release: 90% over 12 hours</td>
<td>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 &amp; Keating, 2007).</td>
</tr>
<tr>
<td>Distribution</td>
<td>Vd: 0.34-1.57 L/kg; crosses blood brain barrier</td>
<td>Vd (apparent): ~400 L</td>
</tr>
</tbody>
</table>
## Pharmacodynamics and pharmacokinetics of Indomethacin and Celecoxib

<table>
<thead>
<tr>
<th>Properties</th>
<th><strong>Indomethacin</strong></th>
<th><strong>Celecoxib</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein binding</td>
<td>99%</td>
<td>~97% primarily to albumin</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Hepatic; significant enterohepatic recirculation</td>
<td>Hepatic via CYP2C9 (Hinz et al., 2007); forms inactive metabolites</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>100%</td>
<td>Absolute: Unknown</td>
</tr>
<tr>
<td>Half-life elimination</td>
<td>4.5 hours; prolonged in neonates</td>
<td>~11 hours (fasted)</td>
</tr>
<tr>
<td>Time to peak</td>
<td>Oral: Immediate release: 2 hours</td>
<td>~3 hours</td>
</tr>
<tr>
<td>Excretion</td>
<td>Urine (60%, primarily as glucuronide conjugates); feces (33%, primarily as metabolites)</td>
<td>Feces (~57% as metabolites, &lt; 3% as unchanged drug); urine (27% as metabolites, &lt; 3% as unchanged drug)</td>
</tr>
</tbody>
</table>
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)
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.
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, 1 tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain’s; Baked, 2 slices.
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, 1 tbsp, coffee black, 2 cups.
12:30pm, Pepperoni Pizza, McCain’s; Baked, 2 slices.
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.

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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!
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: 210-4520 (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
"MALE VOLUNTEERS REQUIRED TO TAKE PART IN AN EXPERIMENT ON BLOOD PRESSURE REGULATION"

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 - Ethic ID: 23121
Health Canada Clinical Trial Application Protocol ID: UC-MMHAF-COX-IH-2010001
Health Canada Clinical Trial Control Number: 138344
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
   o Protocol Overview/Participant Flow
   o 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: ____________________________________
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 through 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.
**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; PETO$_2$ - end-tidal partial pressure of O$_2$. 

**Protocol Overview / Participant Flow**
**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.
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? ____________________________

Have you had an opportunity to ask questions and discuss this study described in the attached consent form? ________________

Have you received satisfactory answers to all of your questions concerning the study described in the attached consent form? ________________

Have you received enough information about the study described in the attached consent form? ________________

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? ________________

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? ________________

Signature: ____________________________ Date: ____________________________

Name (in block letters): _______________________________________________

Ethics ID: 23121
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. _____/_____/_____ |
| | | YYYY MM DD |

| First Name: ______________________ | Last Name: __________________________ |

| Sex: (circle) | Male | Female | Self Reported Race: ____________________ |

**Contact Info:**

| Phone #: _________________________ | Okay to leave message: □ Yes □ No |

| E-mail: __________________________ |

175
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\textsuperscript{1}, Andrew E. Beaudin\textsuperscript{1}, Sofia B. Ahmed\textsuperscript{2,5}, and Marc J. Poulin\textsuperscript{1,3,4,5,6}.

Department of Physiology and Pharmacology\textsuperscript{1}, Medicine\textsuperscript{2}, and Clinical Neurosciences\textsuperscript{3}; Hotchkiss Brain Institute\textsuperscript{4} and the Libin Cardiovascular Institute of Alberta\textsuperscript{5}, Faculties of Medicine and Kinesiology\textsuperscript{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
# MEDICAL HISTORY

## Part 1: CURRENT / RECENT Medical History

<table>
<thead>
<tr>
<th>Any Current Major Medical Complaints?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Please Elaborate (if any):*

<table>
<thead>
<tr>
<th>CURRENT Medication(s) &amp; Dosage? (including OTC medication, herbal medications and vitamins)</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Reason for Medication:*

<table>
<thead>
<tr>
<th>CURRENT Recreational Drug Use?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Type & Daily amount: ____________________________
__________________________________________

### Constitutional symptoms?

<table>
<thead>
<tr>
<th>a. Fever?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Notes: ________________________________

<table>
<thead>
<tr>
<th>b. Night Sweats?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Notes: ________________________________

<table>
<thead>
<tr>
<th>c. Weight loss?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Notes: ________________________________

<table>
<thead>
<tr>
<th>d. Weight gain?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Notes: ________________________________

<table>
<thead>
<tr>
<th>e. Fatigue / Weakness?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
</table>

*Notes: ________________________________

## Part 2: Medical History

<table>
<thead>
<tr>
<th>PAST Chronic Medication(s) use &amp; Dosage?</th>
<th>When taken? (mm/yyyy to mm/yyyy):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reason for Medication:*

*__________________________________________
__________________________________________
__________________________________________

<table>
<thead>
<tr>
<th>History of coronary disease?</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ex. MI, CHF, CABG, By-pass Surgery, etc)</td>
<td>Notes: __________________________</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------------</td>
<td></td>
</tr>
<tr>
<td>Chest pain / Discomfort? (Ex. Angina)</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of hypertension?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of stroke or TIA?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Headaches?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Migraines?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Problem sleeping? (Ex. Insomnia)</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Snoring?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of witnessed apnea during sleep?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of nocturnal choking?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Previous diagnosis of OSA?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of lung disease?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Shortness of breath / Wheezing</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal disorders?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Genitourinary complaints?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal complaints?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of easy bruising?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Difficulty stopping bleeding?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>History of peptic ulcers or bleeding in the bowels?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Allergy / allergic reactions to sulfonamides (“sulfa” drugs)?</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>Smoking?</td>
<td>□ Yes □ Quit □ No</td>
<td></td>
</tr>
</tbody>
</table>

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:*

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

*Estimated Glomerular Filtration Rate: __________ mL·min⁻¹·1.73m⁻² (Calculated from serum Creatinine sample analyzed)*
# PHYSICAL EXAMINATION

## Part A – Visual Inspection

<table>
<thead>
<tr>
<th>General appearance (Ex. Face)</th>
<th>Trauma (Ex. minor bruises, major bleeding, painkiller)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Yes ☐ No</td>
</tr>
</tbody>
</table>

Notes: ______________________

## Part B - Measurements

### Physical Characteristics
- Height: _______________cm
- Weight: _______________kg
- BMI: ____________kg·m$^2$

### Circumferences
- Neck: ____________cm
- Abdomen: ____________cm
- Hip: ____________cm

### Temperature:
- ____________°C

### Blood Pressure & Heart Rate

<table>
<thead>
<tr>
<th>SBP: _____mmHg</th>
<th>DBP: _____mmHg</th>
<th>MAP: _____mmHg</th>
<th>HR: _____bpm</th>
<th>Average: ____mmHg</th>
<th>____mmHg</th>
<th>____mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP: _____mmHg</td>
<td>DBP: _____mmHg</td>
<td>MAP: _____mmHg</td>
<td>HR: _____bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP: _____mmHg</td>
<td>DBP: _____mmHg</td>
<td>MAP: _____mmHg</td>
<td>HR: _____bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

### Head & Neck

<table>
<thead>
<tr>
<th>Lymphadenopathy?</th>
<th>□ Yes ☐ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notes: ______________________</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral / Nasal Ulcers?</th>
<th>□ Yes ☐ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notes: ______________________</td>
<td></td>
</tr>
</tbody>
</table>
### Cardiovascular System

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal Heart Sounds?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Murmur?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotid Bruit?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Edema?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Respiration

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crackles on auscultation?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheezing?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Abdomen

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent bowel sounds?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderness?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatosplenomegaly?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascites?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Neurological

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weakness?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diminished sensation?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal reflexes?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Dermatological**

<table>
<thead>
<tr>
<th></th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematomas?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin abnormality?</td>
<td>□ Yes</td>
<td>□ No</td>
</tr>
<tr>
<td>Notes:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**12-Lead ECG**

<table>
<thead>
<tr>
<th></th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormalities?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assessed By: ______________________ (Print Name)
Signature: ____________ Date: __________

Completed by: ___________________________ (initial here)
<table>
<thead>
<tr>
<th>Test</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Specific Gravity</td>
<td>1.000 1.005 1.010 1.015 1.020 1.025 1.030</td>
</tr>
<tr>
<td>2. pH</td>
<td>5 6 7 8 9</td>
</tr>
<tr>
<td>3. Leukocytes</td>
<td>neg. +1 +2 +3</td>
</tr>
<tr>
<td>5. Protein</td>
<td>neg. +1 +2 +3</td>
</tr>
<tr>
<td>6. Glucose</td>
<td>norm. +1 +2 +3 +4</td>
</tr>
<tr>
<td>7. Ketones</td>
<td>neg. +1 +2 +3</td>
</tr>
<tr>
<td>8. Urobilinogen</td>
<td>norm. +1 +2 +3 +4</td>
</tr>
<tr>
<td>9. Bilirubin</td>
<td>neg. +1 +2 +3</td>
</tr>
<tr>
<td>10. Blood</td>
<td>neg. +1 +2 +3 +4</td>
</tr>
<tr>
<td>11. Hemoglobin</td>
<td>+1 +2 +3 +4</td>
</tr>
</tbody>
</table>
## COX-IH Study Exclusion Criteria Checklist

<table>
<thead>
<tr>
<th>Exclusion Areas</th>
<th>Criterion Value</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Younger than 19 OR Older than 45?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>BMI</td>
<td>&gt; 35 kg·m⁻²?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>≥ 140 / 90 (SBP / DBP)</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>12-Lead ECG</td>
<td>Abnormalities?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Living in Calgary</td>
<td>Less than 1 year?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Smoking</td>
<td>Within the past year?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Taking Regularly?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Drug Allergies</td>
<td>Allergic to Sulphonamides?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Medical Questionnaire</td>
<td>Positive (i.e., Yes) response to any aspect of questionnaire?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Urinary Protein Excretion</td>
<td>&gt; 150 mg·24h⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Sodium Concentration</td>
<td>&lt; 20 mEq·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td><strong>Complete Blood Count (CBC)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Outside range of: 137 to 180 g·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>WBC Count</td>
<td>Outside range of: 4.0 to 11.0 × 10⁹ cells·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mean Corpuscle Count (MCV)</td>
<td>Outside range of: 82 to 100 fl</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>150 to 400 × 10⁹ cells·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Liver Function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaline Transaminase (ALT)</td>
<td>Outside range of: 1 to 60 IU·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Aspartate Transaminase (AST)</td>
<td>Outside range of: 8 to 40 IU·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Outside range of: 30 to 130 IU·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>International Normalized Ratio (INR)</td>
<td>Outside range of: 0.9 to 1.1</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Glucose (Fasting)</td>
<td>&gt; 7.0 mmol·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Estimated Glomerular Filtration Rate (GFR) (Calculated from serum creatinine level)</td>
<td>≤ 60 mL·min⁻¹·1.73m⁻²</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Potassium (K⁺)</td>
<td>Outside range of: 3.3 to 5.1 mmol·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Sodium (Na⁺)</td>
<td>Outside range of: 133 to 145 mmol·L⁻¹</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Respiratory Disturbance Index (RDI) | > 10 events·h⁻¹ | □ Yes | □ No
---|---|---|---
Mean SaO₂ 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: _______________________

185
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

<table>
<thead>
<tr>
<th>Identifications</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient ID</td>
<td></td>
</tr>
<tr>
<td>Patient Last Name</td>
<td></td>
</tr>
<tr>
<td>Patient First Name</td>
<td></td>
</tr>
<tr>
<td>Sample type</td>
<td>Capillary</td>
</tr>
<tr>
<td>T</td>
<td>37.0 °C</td>
</tr>
</tbody>
</table>

### Blood Gas Values

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.365</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCO₂</td>
<td>40.5</td>
<td>mmHg</td>
<td></td>
</tr>
<tr>
<td>pO₂</td>
<td>73.1</td>
<td>mmHg</td>
<td></td>
</tr>
</tbody>
</table>

### Oximetry Values

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>cHb</td>
<td>15.2</td>
<td>g/dL</td>
<td></td>
</tr>
<tr>
<td>aO₂</td>
<td>95.9</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>FO₂Hb</td>
<td>94.7</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>FCO₂Hb</td>
<td>0.8</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>FaHb</td>
<td>4.0</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>FMetHb</td>
<td>0.5</td>
<td>%</td>
<td></td>
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</tbody>
</table>

### Electrolyte Values

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>cK⁺</td>
<td>4.4</td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>cCl⁻</td>
<td>95</td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>cCa²⁺</td>
<td>2.72</td>
<td>meq/L</td>
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</table>

### Metabolite Values

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<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGlu</td>
<td>8.3</td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>cLac</td>
<td>1.5</td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>cCree</td>
<td>88</td>
<td>μmol/L</td>
<td></td>
</tr>
<tr>
<td>cBil</td>
<td>0</td>
<td>mg/L</td>
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</tr>
</tbody>
</table>

### Temperature Corrected Values

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(T)</td>
<td>7.365</td>
<td></td>
</tr>
<tr>
<td>pCO₂(T)</td>
<td>40.5</td>
<td>mmHg</td>
</tr>
<tr>
<td>pO₂(T)</td>
<td>73.1</td>
<td>mmHg</td>
</tr>
</tbody>
</table>

### Oxygen Status

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Vo₂ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>cO₂c</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>pO₂c</td>
<td>23.04</td>
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</table>

### Acid Base Status

<table>
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<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBase</td>
<td>-2.0</td>
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</tr>
<tr>
<td>cHCO₃⁻(P₄S₄)₃</td>
<td>22.6</td>
<td>mmol/L</td>
</tr>
</tbody>
</table>

**Notes:**
- 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

Thaw 8 samples in water/ice bucket from -80°C to 4°C

Pre chill centrifuge (4°C), mix individual samples thoroughly and centrifuge them

Add 5mL of supernatant from each sample to 50 mL Falcon tube [1 mL Pipette x 5 for each sample ]

Mix 50 mL Falcon tube gently and thoroughly

Aliquot 1.2 mL into microcentrifuge tubes [i.e. 600 mL Pipette x2 for each microcentrifuge = 1.2 mL] . A total of 25 samples.

5mL of urine to CLS for creatinine analysis

Store samples in -80°C freezer and bring to Kathy’s lab on Mon, June 18

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