The Effect of Menopause on Acid-Base Regulation and the Chemoreflex Control of Breathing During Wakefulness

By

Megan E. Preston

A thesis submitted to the School of Kinesiology and Health Studies in conformity with the requirements for the degree of Master of Science

Queen’s University

Kingston, Ontario, Canada

September, 2007

Copyright © Megan E. Preston, 2007
ABSTRACT

Acid-base regulation, as reflected by hydrogen ion concentration ([H⁺]), and the chemoreflex control of breathing were examined in healthy pre- (PRE; n=20) and postmenopausal (POST; n=15) women of a comparable age (45 ± 2.7 vs. 52 ± 1.8 years). [H⁺] behaviour was examined in both groups at rest and during exercise above the ventilatory threshold using Stewart’s (119) physicochemical approach to acid-base analysis. Ventilatory chemoreflex characteristics were assessed using Duffin’s (33) modified rebreathing protocol that includes 5 min of prior hyperventilation and maintenance of either hyperoxic (150 mmHg) or hypoxic (50 mmHg) iso-oxia. As expected, the ovarian hormones progesterone ([P₄]) and estrogen ([E₂]) were significantly lower in the POST group. [H⁺] was unaffected by menopausal status at rest or during exercise. At rest the POST group exhibited significantly higher PaCO₂ and [SID] values relative to the PRE group. In general, the acidifying effects of increased PaCO₂ were offset by the alkalizing effect of increases in [SID] (or vice versa) in the POST group such that [H⁺] did not differ between PRE and POST groups. The central ventilatory chemoreflex also differed between groups with the POST group exhibiting a significantly higher threshold and a lower sensitivity in the response to CO₂ relative to the PRE group. [P₄] was found to partially account for the significant group differences in acid-base and central ventilatory chemoreflex control characteristics supporting the role of [P₄] as an important determinant of acid-base status and the chemical control of ventilation in healthy women. Findings of the current study may have potential relevance in understanding the increased occurrence of various health conditions such as osteoporosis and sleep disordered breathing in females following the onset of menopause.
CO-AUTHORSHIP

Chapter 3 Manuscript: The effect of menopause on acid-base regulation at rest and during exercise above the ventilatory threshold

This manuscript was written in its entirety by Megan Preston with constructive criticism and feedback provided by Drs. Ian Janssen and John Fisher.

Chapter 4 Manuscript: The effect of menopause on the chemoreflex control of breathing during wakefulness

This manuscript was written in its entirety by Megan Preston with constructive criticism and feedback provided by Drs. Ian Janssen and John Fisher, as well as Dennis Jensen, Ph.D. Candidate in the Clinical Exercise Physiology Laboratory.
FINANCIAL SUPPORT

Financial support for this study was provided by the Ontario Thoracic Society (Block Term Grant). Megan Preston was also a recipient of an Ontario Graduate Scholarship in 2005-2006.
ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisors Drs. Ian Janssen and John Fisher, for their endless guidance and support throughout this entire project. I am indebted to you both for selflessly accepting me as one of your graduate students following the passing of my late supervisor, Dr. Larry Wolfe. Thank you for all of your invaluable insight and expertise – I couldn’t have done this without you.

I would also like to acknowledge the contribution of my late supervisor, Dr. Larry Wolfe. Although my time spent working with Larry was limited the amount I learned under his supervision is everlasting. I hope that he would have been proud of the work herein.

Special thanks to all of those who took the time out of their busy schedules to help make this project as successful as it was. Thanks to Dr. Don Jennings who helped me bring a little old school to a new school project. Don, it was a privilege working with you. To the fantastic nurses who helped me out – Sarah McLennan, Diana Lindsay, and Penny Lowe – you ladies are incredible. Thank you all for making testing run as smoothly as it did – you could squeeze blood out of a rock. Special thanks also goes out to Chris Scovill who can fix anything and who did in fact fix everything, and of course Angie Maltby, who bends over backwards to help out all of us grad students.

To my lab mates - Dennis, Lindsay, Tracey, and Graeme – thank you all for being an endless source of support. I think it is safe to say that we have been through quite a lot together and there isn’t another group of individuals with whom I would have rather gone through it all. You guys are incredible.

Last but not least, thank you to all of my family and friends who were with me every step of the way. You made the bad times bearable and the good times the best! My sincerest thanks to you all!
# TABLE OF CONTENTS

**ABSTRACT** ................................................................................................................................................. i  
**COAUTHORSHIP** ........................................................................................................................................... ii  
**FINANCIAL SUPPORT** ................................................................................................................................. iii  
**ACKNOWLEDGEMENTS** ............................................................................................................................... iv  
**TABLE OF CONTENTS** ................................................................................................................................. v  
**LIST OF TABLES** .......................................................................................................................................... vii  
**LIST OF FIGURES** ......................................................................................................................................... viii  
**GLOSSARY OF TERMS AND ABBREVIATIONS** ......................................................................................... ix  
**CHAPTER 1  GENERAL INTRODUCTION** ................................................................................................. 1  
**CHAPTER 2  REVIEW OF THE LITERATURE** ............................................................................................ 4  
  2. 1. Introduction ............................................................................................................................................... 4  
  2. 2. Overview of the female reproductive years ......................................................................................... 5  
    2. 2. 1. The normal menstrual cycle ............................................................................................................ 5  
    2. 2. 2. Pregnancy ........................................................................................................................................ 6  
    2. 2. 3. Perimenopause ................................................................................................................................. 6  
    2. 2. 4. Menopause ...................................................................................................................................... 7  
  2. 3. Acid-base regulation ............................................................................................................................... 7  
    2. 3. 1. An overview of acid-base regulation according to the Alphastat hypothesis............................... 7  
    2. 3. 2. Stewart’s physicochemical approach to acid-base regulation ....................................................... 11  
    2. 3. 3. Effect of the menstrual cycle and pregnancy on acid-base regulation .......................................... 12  
    2. 3. 4. Effect of menopause on acid-base regulation ............................................................................... 13  
  2. 4. The chemical control of ventilation ..................................................................................................... 17  
    2. 4. 1. Overview of respiratory control .................................................................................................... 17  
    2. 4. 2. Central chemoreceptors and the control of ventilation ................................................................. 20  
    2. 4. 3. Peripheral chemoreceptors and the control of ventilation ............................................................ 21  
    2. 4. 4. Measuring chemoreflex characteristics ......................................................................................... 21  
    2. 4. 5. Acid-base regulation and the control of ventilation .................................................................. 25  
    2. 4. 6. [SID] and plasma osmolality in the control of ventilation ............................................................. 26  
    2. 4. 7. Ovarian steroids and ventilation .................................................................................................... 27
LIST OF TABLES

CHAPTER 3  MANUSCRIPT: Effect of Menopause on Acid-Base Regulation at Rest and During Exercise Above the Ventilatory Threshold

Table 3.1. General subject characteristics
Table 3.2. Measures of dynamic lung function
Table 3.3. Cardiorespiratory variables at rest and during exercise at 110% TVENT
Table 3.4. Plasma biochemistry variables at rest and during exercise at 110% TVENT
Table 3.5. Correlations (r values) between [H+] and its predictors at rest in pre- and postmenopausal women
Table 3.6. Correlations (r values) between [H+] and its predictors during exercise at 110% TVENT in pre- and postmenopausal women
Table 3.7. Adjusted group means for PaCO2, [SID] and plasma osmolality at rest and during exercise at 110% TVENT

CHAPTER 4  MANUSCRIPT: Effect of Menopause on the Chemoreflex Control of Breathing During Wakefulness

Table 4.1. General subject characteristics
Table 4.2. Dynamic lung function and resting cardiorespiratory variables
Table 4.3. Resting plasma biochemistry including [H+] and its independent determinants
Table 4.4. Ventilatory responses to carbon dioxide under hyperoxic and hypoxic rebreathing conditions
Table 4.5. Correlations (r values) between chemoreflex characteristics and blood biochemistry measurements in pre- and postmenopausal groups
Table 4.6. Adjusted group means for the significant ventilatory chemoreflex characteristics under hyperoxic and hypoxic rebreathing conditions
LIST OF FIGURES

CHAPTER 2  Review of the Literature

Figure 2.1. Integration of the theoretical approaches to acid-base regulation and the control of ventilation in a mammalian system

Figure 2.2. Feedback control loop for the chemical and behavioural control of ventilation

Figure 2.3. Representative plot of the central ventilatory chemoreflex response to CO$_2$ that has been added to the wakefulness drive to breathe

CHAPTER 3  MANUSCRIPT: Effect of Menopause on Acid-Base Regulation at Rest and During Exercise Above the Ventilatory Threshold

Figure 3.1. Outline of Exercise Study Protocol

Figure 3.2. Differences in the unadjusted (black bars) and estimated adjusted (grey bars) means of PaCO$_2$, [SID], and plasma osmolality at rest and during exercise at 110% $T_{VENT}$ in pre- and postmenopausal groups.

CHAPTER 4  MANUSCRIPT: Effect of Menopause on the Chemoreflex Control of Breathing During Wakefulness

Figure 4.1. Mean chemoreflex responses in the pre- and postmenopausal groups

Figure 4.2. Differences in the unadjusted (black bars) and estimated adjusted (grey bars) means for the rebreathing characteristics that differed significantly between pre- and postmenopausal groups under hyperoxic and hypoxic rebreathing conditions.
GLOSSARY OF TERMS AND ABBREVIATIONS

[A⁻]  Ionized weak acid concentration

[ALB]  Albumin concentration

[A_{TOT}]  Total weak acid concentration

[Cl⁻]  Chloride ion concentration

[CO₃^{2-}]  Carbonate ion concentration

[GLOB]  Globulin concentration

[H⁺]  Hydrogen ion concentration

[HA]  Un-ionized weak acid concentration

[HCO₃⁻]  Bicarbonate ion concentration

[K⁺]  Potassium ion concentration

[La⁻]  Lactate ion concentration

[Mg^{2+}]  Magnesium ion concentration

[Na⁺]  Sodium ion concentration

[OH⁻]  Hydroxyl ion concentration

[P_{TOT}]  Total inorganic phosphate concentration

[SID]  The strong ion difference: The sum of the strong cations minus the sum of the strong anions. Calculated as ([Na⁺] + [K⁺] + 2[Ca^{2+}]) – ([Cl⁻] + [La⁻])

[TP]  Total protein concentration

ANG II  Angiotensin II

ARG  Arginine Vasopressin

BMI  Body mass index

CNS  Central nervous system

CO₂  Carbon dioxide

CSF  Cerebrospinal fluid

CVO  Circumventricular organ
<table>
<thead>
<tr>
<th>symbol</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_2$</td>
<td>Estrogen</td>
</tr>
<tr>
<td>$[E_2]$</td>
<td>Estrogen concentration</td>
</tr>
<tr>
<td>$f$</td>
<td>Breathing frequency (breaths/minute)</td>
</tr>
<tr>
<td>$FEV_1$</td>
<td>Forced expired volume in 1 second (litres)</td>
</tr>
<tr>
<td>$FP$</td>
<td>Follicular phase</td>
</tr>
<tr>
<td>$FSH$</td>
<td>Follicle stimulation hormone</td>
</tr>
<tr>
<td>$FVC$</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>$H^+$</td>
<td>Hydrogen ion</td>
</tr>
<tr>
<td>$[H^+]_{CSF}$</td>
<td>Hydrogen ion concentration of the cerebrospinal fluid</td>
</tr>
<tr>
<td>$HCO_3^-$</td>
<td>Bicarbonate ion</td>
</tr>
<tr>
<td>$HR$</td>
<td>Heart rate (beats per minute)</td>
</tr>
<tr>
<td>$HRR$</td>
<td>Heart rate reserve</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>Partial pressures of carbon dioxide above normal levels</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>Partial pressures of oxygen above normal levels</td>
</tr>
<tr>
<td>Hypocapnia</td>
<td>Partial pressures of carbon dioxide below normal levels</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Partial pressures of oxygen below normal levels</td>
</tr>
<tr>
<td>Iso-oxia</td>
<td>Constant partial pressure of oxygen</td>
</tr>
<tr>
<td>$LP$</td>
<td>Luteal phase</td>
</tr>
<tr>
<td>$LH$</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>$MPA$</td>
<td>Medroxyprogesterone acetate</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles per litre</td>
</tr>
<tr>
<td>Osmolality</td>
<td>Moles of solute per kilogram of water</td>
</tr>
<tr>
<td>$P_4$</td>
<td>Progesterone</td>
</tr>
<tr>
<td>$[P_4]$</td>
<td>Progesterone concentration</td>
</tr>
<tr>
<td>$P_{aCO_2}$</td>
<td>Partial pressure of carbon dioxide in arterial blood</td>
</tr>
<tr>
<td>$P_{aO_2}$</td>
<td>Partial pressure of oxygen in arterial blood</td>
</tr>
</tbody>
</table>
PCO$_2$  Partial pressure of carbon dioxide
P$_{ET}$CO$_2$  End tidal partial pressure of carbon dioxide
P$_{ET}$O$_2$  End tidal partial pressure of oxygen
pH  The measure of a solutions acidity or alkalinity. pH = -log [H$^+$]
pK  Dissociation constant
pmol/L  Picomoles per litre
PO$_2$  Partial pressure of oxygen
POST  Postmenopausal group
PRE  Premenopausal group
RAAS  Renin-angiotensin-aldosterone system
SaO$_2$  Arterial blood oxygen saturation
SD  Standard deviation
[SID]$_{CSF}$  Strong ion difference of the cerebrospinal fluid
[SID]$_a$  Strong ion difference of arterial blood
V$_{CO2}$  Production of carbon dioxide (litres per minute)
V$_E$  Minute ventilation
V$_{EB}$  Subthreshold minute ventilation
V$_{ES}$  Sensitivity of the chemoreflex response to carbon dioxide
V$_{O2}$  Oxygen consumption (litres per minute)
VRT$^{CO2}$  Ventilatory recruitment threshold for carbon dioxide
V$_T$  Tidal volume (millilitres)
Menopause is a significant transition in a woman's life that is marked by the cessation of menses due the permanent loss of ovarian follicular function (40). In industrialized societies, menopause occurs naturally in woman at around 50 years of age (40, 71, 101), although it can be surgically induced at younger ages via the removal of the ovaries. Currently there are no known biological markers that signal the onset of menopause and as such, postmenopausal status can only be identified retrospectively and is usually acknowledged following a year without a menstrual period (85, 94).

Menopause is associated with several distinct physiological changes. Amongst these changes is the reduction in circulating levels of gestational hormones in the blood, specifically estrogen and progesterone. The effects of these two hormones extend far beyond their roles in reproduction, influencing other important physiological processes such as ventilation and metabolism. Alterations to these two processes are of particular importance as they contribute to overall acid-base status, as reflected by the pH of arterial blood. Deviations in pH outside of a normal physiological range can greatly affect normal physiologic function.

The respiratory system plays a key role in regulating acid-base balance. More specifically, the respiratory chemoreflexes (central and peripheral) help regulate systemic pH by modulating the arterial partial pressure of carbon dioxide. Both the central and peripheral chemoreflexes are a part of a classical feedback control system that modifies ventilation in response to varying partial pressures of carbon dioxide (PCO₂) and oxygen (PO₂). In addition to the central and peripheral chemoreflex drives to breathe, ventilation is also affected by the state of arousal, which represents a non-chemoreflex drive to breathe. In 1988, Duffin and McAvoy (32) developed a modified version of Read’s rebreathing protocol (96) to examine and measure the ventilatory chemoreflex characteristics. Unlike Read’s technique, the modified rebreathing protocol
allows for the measurement of both the chemoreflex (central and peripheral) and non-chemoreflex drives to breathe in addition to the identification of the ventilatory recruitment threshold for carbon dioxide (i.e. the $\text{PCO}_2$ at which discharge from the chemoreceptors stimulates ventilation).

Other systems such as the renal system work in conjunction with the respiratory system to regulate whole-body acid-base balance, such that pH is maintained within physiological limits (119). In 1981, Peter Stewart (119) introduced a physicochemical approach to assess acid-base behaviour. Although his approach remains controversial, it has been validated in numerous animal (90) and human (23, 42, 60, 93) studies as an accurate method of quantifying acid-base changes. Previous research in healthy women has applied Stewart’s approach to examine the effect of changes in estrogen and progesterone during the menstrual cycle and pregnancy on the control of ventilation and changes in acid-base regulation (42, 57, 93, 115). However, none have addressed how such regulation may be affected by the physiological influences accompanying menopause, where the hormonal milieu is remarkably different.

This thesis will address acid-base behaviour and the chemoreflex drives to breathe in the unique pre- and postmenopausal female subpopulation whose demographic impact on health care is increasing, and for whom natural hormone levels are reduced. More specifically, it will provide insight as to how the unique, menopausal changes in estrogen and progesterone influence ventilatory control and acid-base balance and compare them to premenopausal women of a similar age. Implications of this work are by no means limited to the goals outlined by this thesis. In addition to extending our understanding of acid-base and ventilatory control, they may be relevant to research related to the accelerated bone loss or increased occurrence of sleep disordered breathing observed in postmenopausal women as both disorders may be linked to alterations in either control mechanisms.
Objectives

The effect of menopause on acid-base regulation and the chemoreflex control of breathing remain unclear. The purpose of the present study was to examine the chemoreflex control of ventilation in addition to acid-base status in pre- and postmenopausal women of similar age using modern approaches. The specific study objectives were to:

1. To determine whether hydrogen ion concentration ([H⁺]) varies between pre- and postmenopausal women at rest and during exercise above the ventilatory threshold (TVENT, the point at which ventilation begins to rise exponentially), and if so, whether this difference is explained in part, by Stewart’s physiochemical determinants of [H⁺] including the partial pressure of carbon dioxide (PaCO₂), total weak acid concentration ([ATOT]), and the strong ion difference ([SID]).

2. To examine whether there are differences in the ventilatory recruitment threshold in addition to central and peripheral chemoreflex sensitivity in the ventilatory response to CO₂ in pre- and postmenopausal women.

Hypotheses

1. [H⁺] will be higher in postmenopausal women compared to premenopausal women at rest and at 110% TVENT and that this difference will be explained in part, by differences in PaCO₂, [ATOT], and [SID].

2. Ventilatory chemoreflex sensitivity (central and peripheral) will be lower and the ventilatory recruitment threshold will be higher in postmenopausal women relative to premenopausal women and that this variability in chemoreceptor sensitivity and threshold will be explained by differences in circulating estrogen ([E₂]) and progesterone ([P₄]), plasma osmolality, and [SID] between subject groups.
2.1 Introduction

In all organisms, the ability to preserve an internal homeostatic environment is critical in ensuring proper systemic functioning in order to sustain life. Acid-base regulation and the control of ventilation are both key components in maintaining a homeostatic environment. Both of these systems are inherently linked to one another and work in conjunction with each other to manage the continuous challenge imposed upon a physiological system by metabolism, dietary intake and environmental stressors (i.e. temperature) (119). The regulation of acid-base status is essential in conserving protein charge state and structure, which is critical to the proper functioning of enzymes, ion channels, cellular receptors and cell structure.

As acid-base regulation and ventilatory control are central to physiologic functioning, examination of these particular processes is of utmost importance. Previous work has examined the effect of fluctuating ovarian hormones (specifically progesterone and estrogen) in addition to varying exercise intensities on acid-base regulation and the control of ventilation in healthy pregnant (43, 55, 57, 129) and eumenorrheic women, who were either free from (43, 93, 115) or taking oral contraceptives (81). Currently, there is a lack of information regarding the effect of menopause on acid-base regulation and the control of breathing. As menopause is an inevitable transition for all women who reach middle age, it is important that the effect of ovarian hormonal withdrawal on acid-base regulation and the control of breathing be examined in a menopausal population. Information of this nature is of particular importance in this subset of females as it may shed light on potential causes for the increased manifestation and development of various health conditions such as osteoporosis and sleep disordered breathing, which are commonly diagnosed following menopause.
The following review of literature discusses the effect of estrogen (\([E_2]\)) and progesterone (\([P_4]\)) on the various constituents and components of acid-base regulation and the chemical control of ventilation. The information herein will provide the necessary background information and rationale for the importance of my thesis which examines the effect of menopause on acid-base regulation and the chemoreflex control of ventilation.

2.2 Overview of the female reproductive years

2.2.1 The normal menstrual cycle

The female reproductive cycle is on average 28 days in length and can be divided into two phases: the follicular phase (days 1-14) and the luteal phase (days 15-28) (112). Both phases are distinctly different in their hormonal profiles and reproductive processes. The follicular phase is marked by the onset of menses and extends up until ovulation (112). During the follicular phase, \(E_2\) levels increase in response to the secretion of follicle-stimulating hormone. The increased release of \(E_2\) stimulates the continued release of follicle-stimulating hormone and luteinizing hormone whose levels peak around the 14th day of the menstrual cycle stimulating ovulation. The luteal phase commences following ovulation and lasts up until the first day of the subsequent menstrual period (112). Throughout the luteal phase, both \(P_4\) and to a lesser extent \(E_2\), are continuously secreted by the corpus luteum (100). During the luteal phase, levels of circulating \(P_4\) surpass those of \(E_2\) such that it replaces \(E_2\) as the dominant hormone (100). At the end of the luteal phase and in the absence of fertilization, \(P_4\) and \(E_2\) levels drop due to the degeneration of the corpus luteum (100). The falling levels of \(P_4\) and \(E_2\) subsequently lead to hemorrhagic changes in the uterine lining resulting in the onset of menstruation (100).
2. 2. 2. Pregnancy

In the event of fertilization, pregnancy induced secretion of human chorionic gonadotropin prevents the degeneration of the corpus luteum (112) thereby disrupting the continuation of the menstrual cycle. The corpus luteum continues to secrete P₄ and E₂ up until the 7th to 10th week of pregnancy at which point the placenta takes over (the luteal-placenta shift) (128). Throughout pregnancy, levels of circulating P₄ and E₂ continue to rise reaching levels several hundred times higher than those observed during the normal menstrual cycle (112). The increased secretions of both hormones are critical in the physiologic adaptations of the maternal system that are essential in maintaining pregnancy. Following parturition or termination of the pregnancy, P₄ and E₂ levels decrease, removing the feedback inhibition of gonadotropin releasing hormone, follicle stimulating hormone, and luteinizing hormone, which stimulates follicular growth and the commencement of the follicular phase of the menstrual cycle (100).

2. 2. 3. Perimenopause

Perimenopause is the term used to describe the transition period between regular, premenopausal menstruation and the final menstrual cycle (104). During this period of time, reproductive hormone levels (E₂, P₄, follicle-stimulating hormone and luteinizing hormone) fluctuate due to alterations in ovarian function resulting in menstrual irregularity with possible increases in episodic amenorrhea eventually culminating in menopause (70). The beginning of the perimenopausal period is identified once menstrual cycle length begins to differ from its established premenopausal cyclic pattern (70). The inception of perimenopause occurs naturally around 47 years of age and lasts on average approximately 4 years (71). Women who are smokers have been shown to commence perimenopause earlier and have a shorter menopausal transition (71). Older
women also tend to experience a shorter perimenopausal period (70, 125). Approximately 10% of women do not experience a lengthy perimenopause but instead cease menstruation abruptly (<6 months) (71).

2. 2. 4. Menopause

Menopause is marked by the cessation of menses due the permanent loss of ovarian follicular function (40). In industrialized societies, menopause occurs naturally around the age of 51 (40, 71), although it can be surgically induced at younger ages via removal of the ovaries alone or in combination with the uterus (70). Currently there are no known biological markers that indicate the onset of menopause. As such, menopause, defined as the absence of menses for 12 consecutive months, (85) can only be diagnosed retrospectively.

Menopause is a state of multiple hormone deficiencies. Plasma levels of circulating E2 and P4 are drastically reduced due to the reduction in the number of primordial follicles remaining in the ovaries. As E2 and luteinizing hormone form a negative feedback loop, menopausal reductions in the amount of E2 secreted by the ovaries results in elevated levels of both luteinizing hormone and follicle-stimulating hormone (104). The impact of menopausal reductions in circulating E2 and P4 are widespread having both physiological and psychological consequences that include, but are not limited to, alterations in cardiovascular and skeletal health, tissue and organ function, metabolism, mood and cognition.

2. 3. Acid-base Regulation

2. 3. 1. An overview of Acid-base regulation according to the Alphastat hypothesis

The regulation of hydrogen ion concentration ([H+]) is central to maintaining a homeostatic environment within an organism. Perturbations in [H+] that deviate outside
an organism’s normal physiologic range have adverse consequences on cellular
structure and systemic function. Traditionally, [H+] behaviour is described in relation to
the partial pressure of carbon dioxide (PCO2) and bicarbonate concentration ([HCO3–]) of
a system in which PCO2 and [HCO3–] represent the respiratory and metabolic
components, respectively, of acid-base status (109). Unlike the traditional approach in
which the maintenance of [H+] is recognized as the primary purpose of acid-base
regulation, the alphastat hypothesis (79, 97) proposes the maintenance of protein
charge state, and thus protein conformation and function, as being the primary goal of
acid-base regulation.

According to the alphastat hypothesis, the charge state of a protein is dependent
upon the ionization constants (pK) of its titratable groups in addition to the pH of its
surrounding environment (96). The pKs of amino acids vary, ranging from a pH of 2 to a
pH of 11 (18) and can be altered by changes in temperature, osmolality, and strong ion
concentrations (52). With respect to a biological system, protein charge state is
dependent upon the protonation of the imidazole-histidine residues present in proteins
as the pK of this particular moiety falls within the normal physiological range of pH (97).
As such, within a biological system, [H+] is regulated in response to changes in
temperature, osmolality and/or strong ion concentrations, in order to conserve and/or
offset changes in pK of the imidazole-histadine groups such that protein charge state
and ultimately protein function are maintained (52, 97). Amongst its importance to
numerous physiologic processes, the conservation of a protein’s charge state is critical
in maintaining cellular integrity and proper enzymatic function.

Although the alphastat hypothesis provides an understanding as to why the
regulation of [H+] is important within a physiologic system, it does not describe how [H+] is
regulated. Stewart’s (119, 121) physicochemical approach to acid-base balance fits
nicely with the alphastat hypothesis by providing a framework to explain how [H+] is
regulated within a physiologic system such that protein integrity is conserved. Figure 2.1 depicts the integration of the alphastat hypothesis and Stewart’s physicochemical approach to acid-base regulation in addition to Jennings’ hypothesis (51, 52) for the chemical control of ventilation which is discussed in section 2.4.6. of this review.
Figure 2.1. Integration of the theoretical approaches to acid-base regulation and the control of ventilation in a mammalian system. H⁺, hydrogen ion; SID, strong ion difference; AᵣTOTAL, total weak acid; PaCO₂, arterial partial pressure of CO₂; TP, total protein; ALB, albumin; GLOB, globulin; PiTOTAL, total inorganic phosphate; Na⁺, sodium; Ca²⁺, calcium; K⁺, potassium; Mg²⁺, magnesium; Cl⁻, chloride; La⁻, lactate; pK, dissociation constant; AVP, arginine vasopressin; ANG II, angiotensin II. Square brackets indicate concentrations.
2.3.2. Stewart’s Physicochemical Approach to Acid-Base Regulation

In 1981, Peter Stewart introduced a unique physicochemical approach to understand, describe, and predict the behaviour of H+ within a physiological aqueous solution (119). His approach is built upon the concepts of the existence of strong and weak electrolytes and the classification of systemic variables as being either dependent or independent as well as three fundamental laws of physics: the conservation of mass, the conservation of charge, and the law of mass action (119-121).

Briefly, Stewart’s approach states that in any physiological aqueous solution, [H+] is a dependent variable whose concentration is determined by three independent variables: the strong ion difference ([SID]), the partial pressure of carbon dioxide (PCO2), and the total weak acid concentration ([ATOT]). [SID] represents the differences between the sum of all cation concentrations (i.e. [Na+], [K+], [Ca2+]) minus the sum of all anion concentrations (i.e. [Cl−], [La−]). [ATOT] represents the total weak acid concentration, which in human plasma corresponds to the total concentration of circulating protein (i.e. albumin, [ALB]; globulin, [GLOB] and total phosphate, [PiTOT]). An alteration in any of Stewart’s independent variables results in a concomitant, compensatory response in the other independent variables such that changes in [H+] are minimized (119, 121).

Alterations in the independent variables also affects the concentrations of five additional dependent variables which include bicarbonate ([HCO3−]), carbonate ion ([CO32−]), unionized weak acid ([HA]), ionized weak acid ([A−], and hydroxyl ion ([OH−]) (119-121).

Various physiologic systems and processes serve to alter Stewart’s three independent variables. In general, ventilation is well matched to the metabolic production of CO2 (24, 25) such that PaCO2 is maintained at around 40 mmHg, with the exception of pregnancy in which PaCO2 is lower (55, 65). PaCO2 can be rapidly altered via changes in metabolic rate (i.e. exercise) and changes in ventilation (121, 130). Within an aqueous environment, changes in PCO2 parallel changes in [H+] such that increases in
PCO₂ are associated with increases in [H⁺]. Unlike PaCO₂, alterations in [SID] occur slowly and are determined by metabolic rate, diet, ion absorption, and excretion by the gastrointestinal tract and kidneys (121). Within solution, [SID] has an inverse relationship to [H⁺] such that decreases in [SID] serve to increase [H⁺]. Changes in [ATOT] occur over the span of days and are determined primarily by metabolism of its constituents by the liver (121). Although [ATOT] does not deviate significantly within a cellular compartment (as most cell membranes are impermeable to proteins), changes in [ATOT] parallel changes in [H⁺] (119, 121). As [ATOT] remains relatively constant within a cellular compartment, [H⁺] in addition to Stewart's five other dependent variables, are primarily affected by alterations in [SID] and PCO₂ (119, 121).

Stewart’s mechanistic approach to acid-base analysis has been validated as an accurate method by which to estimate and quantify [H⁺] behaviour in animals (90), critical care patients (23, 132), and healthy men (59-61, 127) as well as in pregnant and normally menstruating female populations (42, 43, 57, 93).

2. 3. 3. Effect of the Menstrual Cycle and Pregnancy on Acid-Base Regulation

Stewart’s physicochemical approach has been utilized by several studies to examine acid-base regulation in pregnant and regularly menstruating women. Pregnancy is associated with a reduction in [H⁺] due to decreases in resting PaCO₂ and [ATOT] (21, 42, 128, 129). The alkalizing effect of reductions in PaCO₂ and [ATOT] are partially offset by the acidifying effect of reduced [SID] such that the net effect is a reduction in [H⁺] during pregnancy relative to the non-pregnant state (21, 42, 57, 128). Changes in the three independent variables have been partially attributed to the dramatic increases in circulating ovarian hormone levels associated with pregnancy (42, 133). [H⁺] is also lower during exercise above and below the ventilatory threshold in
pregnant women when compared to non-pregnant controls, although both groups exhibit similar increases in [H\(^+\)] during the transition from rest to exercise (21, 42, 57).

Similar observations have been noted using Stewart’s physicochemical approach to examine [H\(^+\)] behaviour across the phases of the menstrual cycle at rest and during exercise. Much like pregnancy, the luteal phase of the menstrual cycle is associated with hormonally stimulated reductions in resting PaCO\(_2\) and [A\(_{TOT}\)] when compared to the follicular phase (93). However, unlike pregnancy, the alkalizing effect of reductions in [A\(_{TOT}\)] and PaCO\(_2\) are completely offset by the acidifying effect of reductions in [SID] such that [H\(^+\)] does not differ across the menstrual cycle (93, 115). Phasic differences in [SID], PaCO\(_2\) and [A\(_{TOT}\)] persist during exercise above and below the ventilatory anaerobic threshold although differences are less pronounced when compared to resting values (93).

As evidenced from the above information, the application of Stewart’s approach is particularly advantageous when examining acid-base regulation across the different phases of the menstrual cycle. Although [H\(^+\)] is maintained across the menstrual cycle, the independent variables that dictate [H\(^+\)] behaviour and thus the mechanisms behind maintaining [H\(^+\)] are significantly different across the follicular and luteal phases. Furthermore, pregnancy presents its own unique mechanism for the regulation of [H\(^+\)]. Taken together, the above examples highlight the importance of a mechanistic approach in understanding the elaborate nature of [H\(^+\)] behaviour and its regulation across different populations.

2. 3. 4. Effect of Menopause on Acid-Base Regulation

Despite its importance, relatively few studies have examined systemic acid-base status following the menopausal transition. Of the few studies available, none have utilized Stewart’s physicochemical approach to examine [H\(^+\)] behaviour.
As discussed above, research examining acid-base regulation across the phases of the menstrual cycle and pregnancy has highlighted the impact of varying hormone levels on the independent determinants of [H⁺]. As menopause is associated with considerable reductions in circulating ovarian hormone levels, it is reasonable to infer that menopause would be associated with changes in Stewart’s independent variables that would in theory, oppose those seen during pregnancy or the luteal phase of the menstrual cycle.

With respect to PaCO₂, menopausal reductions in P₄ should translate into increases in PaCO₂ due to reductions in V̇E (4). The administration of synthetic P₄ alone or in combination with E₂ has been shown to significantly reduce PaCO₂ in healthy postmenopausal women (86, 99). According to Stewart, increases in PaCO₂ would serve to increase [H⁺], thereby rendering a system more acidic.

Research examining menopausal changes in [ATOT] and its components (albumin, globulin and inorganic phosphate) is varied. As both pregnancy and the luteal phase of the menstrual cycle are associated with reductions in [ATOT] it is reasonable to speculate that menopause would be associated with an increase in [ATOT]. Indeed, Hodgkinson and colleagues (47) observed that postmenopausal women exhibit higher plasma [ALB], [PiTOT], and total protein concentrations relative to premenopausal controls and that concentrations of each variable are reduced in the same group of postmenopausal women following a month of estrogen replacement therapy. When comparing pre- and postmenopausal women, Sokoll and Dawson-Hughes (116) noted that the postmenopausal group had higher serum [GLOB], but unlike Hodgkinson et al. (47) found no difference in [ALB] between pre- and postmenopausal women (116). Conversely, Marshall et al. (68) did not observe significant differences in plasma [ALB], [GLOB] or total protein concentrations when comparing pre- and postmenopausal
women. However, these researchers also found that administration of $E_2$ in postmenopausal women served to decrease $[ALB]$ (68).

To further complicate matters, Orr-Walker et al. (86) did not find that $[ALB]$ differed significantly between postmenopausal controls and menopausal women following three months of hormone replacement therapy with either $E_2$ or $P_4$ alone or in combination. However, the aforementioned study utilized a cross sectional design in addition to a smaller sample size than those utilized in the longitudinal studies conducted by Hodgkinson et al. (47) and Marshall et al. (68) ($n = 10$, $n = 100$, $n = 90$ for the three studies respectively). As such, it is possible that significant group differences in plasma $[ALB]$ were not noted following hormone replacement therapy due to insufficient statistical power.

To summarize, information regarding changes in $[ATOT]$ following the onset of menopause is conflicting. The majority of information suggests that total protein, $[ALB]$, and $[GLOB]$ increases slightly following menopause. Applying Stewart’s approach, increases in $[ATOT]$ would serve to increase $[H^+]$ thereby rendering a system more acidic (119, 121).

It is also expected that $[SID]$ would increase following the onset of menopause. Previous studies have shown that the concentrations of sodium ($[Na^+]$), total and ionized calcium ($[Ca^{2+}]$), and magnesium ($[Mg^{2+}]$), are elevated in postmenopausal women (45, 47, 68, 116). Slight changes have also been noted in anion concentrations following menopause such that lactate ($[La^-]$) and bicarbonate ($[HCO_3^-]$) are significantly increased and chloride ($[Cl^-]$) is reduced in post- relative to premenopausal women (45-47). Hormone replacement therapy has been shown to reverse the menopausal differences in certain ion concentrations. Plasma concentrations of $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ in otherwise healthy postmenopausal women were significantly reduced following one month of estrogen replacement therapy (45, 47, 68). In addition, Orr-Walker et al. (86) noted
significant reductions in \([\text{HCO}_3^-]\) and \([\text{Ca}^{2+}]\) following three months of treatment with MPA alone and in combination with E_2 although significant changes in the concentrations of Na^+, K^+ or Cl^- were not noted following any of the hormonal interventions.

Despite the amount of information available, none of the above studies have included measures of [SID]. Using the mean plasma electrolyte concentrations in pre-(ages 30-45 years) and postmenopausal women (ages 50-55 years) from previous studies (46, 68) to calculate [SID] (119), it appears that [SID] is increased following the onset of menopause. Applying Stewart’s approach, an increase in [SID] would serve to decrease [H^+] and thus would have an alkalizing effect.

In terms of an overall change in pH associated with menopause, the available literature provides no definitive answers. Some studies suggest that menopause is accompanied by increases (44, 46, 116) or decreases in pH (38, 39). Examination of the effect of hormone replacement therapy on pH in postmenopausal women is also somewhat inconclusive. Orr-Walker et al. (86) found that pH increased significantly in postmenopausal women following three months of combined MPA and E_2 therapy although a trend towards an increased pH was evident following a week of treatment (p=0.056). Conversely, Regenstiener et al. (99) observed no effect of treatment with either MPA or E_2 alone or in combination on pH in postmenopausal women following a week of treatment.

Taken together, the theoretical application of Stewart’s physicochemical approach to the available information would suggest that [H^+] either does not change or is slightly increased following menopause. Simplistically, the acidifying effect of postulated menopausal increases in PaCO_2 and [A_{TOT}] would be partially or completely offset by the alkalizing effect of postulated increases in [SID] such that [H^+] remains constant or is slightly increased following menopause.
2.4. The Chemical Control of Ventilation

2.4.1. Overview of Respiratory Control

The primary function of the respiratory system is gas exchange. The lung is the site where O₂ and CO₂ are exchanged between the blood and the external environment such that arterial partial pressures of O₂ (PaO₂) and CO₂ (PaCO₂) are maintained within normal limits (130). Ventilation is tightly coupled to an individual’s metabolic demand (24, 25). Ventilation also plays a central role in acid-base balance by regulating [H⁺] through its effects on alveolar ventilation and PCO₂ (30, 121).

In general, the respiratory control system can be thought of as a classical feedback system that incorporates a central controller, effectors, and sensors (19, 24). This classical feedback loop is illustrated in Figure 2.2. Chemoreceptors are key sensors involved in the control of ventilation. Proprioceptors and mechanoreceptors (i.e., stretch, irritant and c-fiber receptors, as well as muscle spindles and tendon organs) are also important in the control of ventilation however their roles will not be discussed in this review. Chemoreceptors are nerve terminals that are capable of detecting chemical changes in their surrounding environment (19, 92, 102). Two groups of chemoreceptors are thought to be involved in the control of ventilation: the central chemoreceptors, which are located in the central nervous system (CNS), and the peripheral chemoreceptors, that are located in the common carotid arteries (19). Information regarding chemical changes is transmitted from the chemoreceptors to the medullary respiratory centers located within the brain (central controller)(19). Chemoreceptor-induced changes in efferent neuronal outputs project to the respiratory muscles (effectors) that affect ventilation.

The control pathway described above extending from the stimuli sensed by peripheral and central ventilatory chemoreceptors to the CNS and respiratory muscles is
termed a chemoreflex (31). Both the central and peripheral ventilatory chemoreceptors have separate chemoreflex effects that are additive in nature and form the overall chemoreflex drive to breathe (31).

Although ventilation is under the reflex control of the chemoreflexes, it is also subject to behavioural control, which is voluntary and can temporarily override the metabolic drives to breathe (84, 88, 102, 113). Behavioural drives to breathe, otherwise known as nonchemoreflex drives to breathe, are independent of the chemoreflexes and are dependent on the “state” of an individual (31, 88). The “wakefulness” drive to breathe is an example of a nonchemoreflex drive to breathe that is withdrawn during sleep (due to the removal of a wakeful state) (31, 35). Overall, the wakefulness drive to breathe accounts for approximately 30-35% of the overall drive to breathe in a resting human (66).
Figure 2.2. Feedback control loop for the chemical and behavioural control of ventilation. Information regarding chemical changes in the environment is monitored by sensors. Information gathered by the sensors is transmitted to the central controller where a response to the stimuli is organized. The response is then sent down efferent neurons to the effectors that adjust their output thereby and alter ventilation. The chemoreflex control of breathing can be temporarily overridden by behavioural drives to breath.
2.4.2. Central Chemoreceptors and the Control of Ventilation

The central chemoreceptors are most commonly cited as being located on the ventral surface of the medulla, although recent research reports that they are more widespread throughout the brainstem (80, 102). The central chemoreceptors respond to changes in [H⁺] within the cerebrospinal fluid ([H⁺]_{CSF}) (29, 80) such that increases in [H⁺] stimulate ventilation (130). As the cerebrospinal fluid is separated from the circulating blood by a blood-brain barrier that is impermeable to polar ions, H⁺ cannot diffuse freely across the blood-brain barrier to affect ventilation directly. Conversely, [H⁺]_{CSF} is altered by PaCO₂, which easily diffuses across the blood-brain barrier. As PaCO₂ rises, the increase in cerebrospinal fluid PCO₂ and the presence of carbonic anhydrase results in a hydration reaction that liberates H⁺ via the following chemical reaction:

Carbonic Anhydrase

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- 
\]

The increase in [H⁺]_{CSF} as a result of increased PaCO₂ subsequently stimulates ventilation (19, 121). As the cerebrospinal fluid has a limited amount of protein available to help buffer excess [H⁺]_{CSF} (122), it experiences a greater change in pH for a given change in PaCO₂ in comparison to arterial blood (74, 130). As a result, relatively small changes in PaCO₂ can elicit large ventilatory responses via the central chemoreflex drive to breath. Subsequently stimulation of ventilation via the central chemoreflex can rapidly reduce PaCO₂ levels such that the cerebrospinal fluid pH is quickly returned to its normal value (19, 121). Overall, the central chemoreflex accounts for approximately 45-50% of the overall drive to breathe in resting humans (66).
2. 4. 3. Peripheral Chemoreceptors and the Control of Ventilation

The peripheral chemoreceptors are found in the carotid bodies that are located in the bifurcation of the common carotid arteries (12, 19). These receptors respond to increases in PaCO2 (via its effect on arterial [H\(^+\)]) as well as reductions in PaO2 (12, 29). Contrary to the “classical model” of the peripheral chemoreflex, Duffin’s laboratory has suggested that PaO2 does not effect ventilation independently of CO2 but rather exerts its effect on ventilation by altering the peripheral chemoreflex response to CO2 (31). More specifically, they propose that decreases in PO2 serve to increase the sensitivity and decrease the threshold of the peripheral chemoreflex response to CO2 (31). Overall, the peripheral chemoreflex accounts for approximately 30-35% of the overall drive to breath in resting humans (66).

2. 4. 4. Measuring Chemoreflex Characteristics

As previously discussed, the model of ventilatory control describes resting ventilation (\(\dot{V}_E\)) and PaCO2 as being determined by chemoreflex and non-chemoreflex drives to breathe and their intersection with the metabolic hyperbola (relationship between PaCO2 and alveolar ventilation whose shape is dependent upon the metabolic production of CO2)(24, 29). Both the central and peripheral chemoreflexes are described in terms of their response to CO2. Each chemoreflex has a ventilatory recruitment threshold for CO2 (VRT\(_{CO2}\)), which represents the PCO2 at which output from the chemoreceptors results in increases in ventilation (29). Ventilation below this threshold is termed subthreshold ventilation and represents the behavioural or nonchemoreflex drives to breathe (\(\dot{V}_{ES}\)) (29). The slope of the linear relationship between \(\dot{V}_E\) and PCO2 above the VRT\(_{CO2}\) is representative of the receptors chemosensitivity to CO2 (\(\dot{V}_{ES}\)) (29, 96). The drive to breathe produced by both chemoreceptors (central and peripheral) is
additive in nature, and their combined sensitivity to CO₂ above the VRTCO₂ is dependent on PO₂ (29, 31).

Read (96) was the first to introduce the rebreathing method as an effective technique to evaluate the central chemoreflex response to CO₂. During Read’s procedure subjects rebreathe from a bag containing a hyperoxic gas mixture with a PCO₂ that is close to the PCO₂ of mixed venous blood (96). Throughout rebreathing, changes in ventilation and PCO₂ are evaluated in order to determine the sensitivity of the central chemoreceptors to changes in CO₂. Although Read’s (96) technique is effective in measuring central chemoreflex sensitivity, it does not allow for the evaluation of the peripheral chemoreflex due to the use of a hyperoxic gas mixture. As the sensitivity of the peripheral chemoreflex is altered by PO₂, its relative contribution to the overall ventilatory response to CO₂ is negated during hyperoxia (29). In addition, Read’s (96) rebreathing technique is incapable of measuring subthreshold ventilation and determines VRTCO₂ by extrapolating the linear relation between \( \dot{V}_E \) and PaCO₂ to the x-axis (33).

In 1987, Duffin introduced a modified rebreathing protocol to assess the ventilatory chemoreflexes that has several methodological and interpretive advantages over Read’s (96) rebreathing technique. Specifically, Duffin’s technique (29, 33), allows for the examination of both the central and peripheral chemoreflex response to CO₂, in addition to the direct measurement of VRTCO₂ and \( \dot{V}_E \). The modified rebreathing protocol utilizes prior hyperventilation of room air to lower the body’s CO₂ stores thereby allowing for the direct measurement of the chemoreceptors’ VRTCO₂ as rebreathing progresses from hypocapnic to hypercapnic PCO₂ levels (33). A deep and deliberate breathing pattern is used during hyperventilation, which avoids the development of a slow term potentiation effect (a prolonged respiratory neural after-discharge following hyperventilation) (36) that could influence an individual’s ventilatory response (75). Prior
reductions in CO₂ below the VRT₃₂ also permit \( \dot{V}_E \) to be measured. Following the hyperventilation procedure, subjects rebreathe either a hyperoxic or hypoxic gas mixture in order to assess the central chemoreflex and the combined central and peripheral chemoreflex response to CO₂, respectively (see below).

Duffin’s protocol incorporates an initial 3 deep breaths at the start of rebreathing to rapidly equilibrate the PCO₂ in the rebreathing bag, the lungs, and arterial blood with the mixed venous blood (29). As the subject and the rebreathing bag form a closed system, increases in \( P_{ET}CO₂ \) are indicative of the metabolic production of CO₂. As \( P_{ET}CO₂ \) gradually increases in the rebreathing bag it will eventually reach a level that surpass the VRT₃₂ (29). Once the VRT₃₂ is exceeded, ventilation begins to increase linearly with increases in \( P_{ET}CO₂ \). The slope of the relationship between ventilation and \( P_{ET}CO₂ \) is indicative of the chemoreceptors’ sensitivity to CO₂ such that a steeper slope is representative of a greater sensitivity (29, 96). Figure 2.3 shows an example of the central chemoreflex response to PCO₂ that has been added to the wakefulness drive to breathe.

Measurement of the peripheral chemoreflex is more complex than the central component. This reflects the fact that hyperoxic conditions can be used to silence the peripheral chemoreceptor response to CO₂ in order to measure the central ventilatory chemoreflex, whereas there is no method available to eliminate the central chemoreflex response to CO₂ under normoxic or hypoxic conditions. The peripheral chemoreflex response to CO₂ can be teased out by conducting two rebreathing tests; the first under hyperoxic conditions in which only the central chemoreceptors are responsible for the
Figure 3. Representative plot of the central ventilatory chemoreflex response to CO₂ that has been added to the wakefulness drive to breathe. $\dot{V}_E$, wakefulness drive to breathe; VRTCO₂, ventilatory recruitment threshold for CO₂; $V_{ES}$, sensitivity of the chemoreflex response to CO₂; $\dot{V}_E$, minute ventilation; $P_{ETCO₂}$, end-tidal CO₂ tension; BTPS, body temperature pressure saturated.
observed response, and the second under hypoxic conditions that allows for the evaluation of the combined response of the central and peripheral chemoreflex drives to breathe (29). As the two chemoreflex drives to breathe are additive, subtraction of the responses under both rebreathing conditions allows for the separation of the peripheral chemoreflex contribution to the ventilatory response to CO₂.

2.4.5 Acid-base Regulation and the Control of Ventilation

Levels of CO₂ are tightly linked to [H⁺], which confers a central role in acid-base regulation. In general, the respiratory system is capable of providing an immediate response to disruptions in acid-base balance. Regulation of [H⁺] via ventilatory alterations to PaCO₂ occurs rapidly (within seconds to minutes), whereas regulation of [H⁺] by the renal system is slow acting, taking anywhere from several hours to days to have an effect (100).

It is important to consider the role of acid-base regulation when modeling the chemoreflexes and the chemical control of ventilation. As PaCO₂ exerts its effects on the chemoreceptors via a direct effect on [H⁺], it should be emphasized that PaCO₂ represents the measured stimulus for ventilation, as opposed to [H⁺] which is assumed to be the primary stimulus of chemoreceptors (30, 95, 102). It is however important to note that some central ventilatory chemoreceptors may respond also to bicarbonate or molecular CO₂ (102, 103).

Using a steady-state modeling approach to examine the effect of changes in acid-base status on the chemoreflex control of breathing, Duffin (30) recently noted that acid-base disturbances such as metabolic acidosis or alkalosis, primarily affects the chemoreflex control of ventilation by altering the VRTCO₂. In other words, changes in the relationship between PaCO₂ and [H⁺] may alter the ventilatory response to CO₂ in terms of the measured stimulus (PaCO₂) despite little to no change in the actual stimulus, [H⁺]
These modeling-based predictions are supported by Jennings and colleagues (51, 52), who observed changes in the VRTCO₂ in response to dietary alterations in SID. More specifically, it was noted that changes in the threshold for PaCO₂ offset the effect of changes in SID, such that [H⁺] did not change (51, 52).

2.4.6. [SID] and Plasma Osmolality in the Control of Ventilation

SID and plasma osmolality have been implicated in the control of alveolar ventilation and PaCO₂ (51, 52). Indeed, Jennings noted that ventilatory adaptations following dietary-induced metabolic acidosis in dogs were strongly predicted by alterations in the cerebrospinal fluid and arterial blood [SID] ([SID]₇₉ and [SID]ₐ, respectively) (48). More specifically, reductions in SID (which act to increase [H⁺]) were associated with a reduced VRTCO₂ (1) and a lower PaCO₂ (which acts to decrease [H⁺]) (51). The reduced PaCO₂ subsequently offset the acidic changes due to SID such that [H⁺] remained unchanged (51). Similar findings have been noted in human studies, where changes in [SID]₇₉ were accompanied by compensatory changes in ventilation that regulated PaCO₂ and resulted in a constant [H⁺]₇₉ (52).

Plasma osmolality has also been shown to influence ventilation in both human and animal models such that reductions in osmolality are associated with the stimulation of ventilation and lowering of PaCO₂ (as reviewed in (1, 43, 51)). Osmolality appears to affect ventilation independently of [SID] and may exert its effect on respiratory control centrally via circumventricular organs present in the hypothalamus (52).

Circumventricular organs are sensory organs located within the brain that lack a blood-brain barrier (52). Stimulation of the circumventricular organs affects fluid and electrolyte homeostasis by influencing drinking behaviour, salt appetite, and the release of arginine vasopressin (52).
2.4.7. Ovarian Steroids and Ventilation

In addition to their importance as reproductive hormones, both P₄ and E₂ play significant roles in the control of ventilation. P₄ is a potent respiratory stimulant (16, 41, 106), which is thought to act (in part) centrally via estrogen-dependent progesterone receptors present in the hypothalamus (4, 16). There is evidence to suggest that E₂ serves to up-regulate the number of progesterone receptors in the hypothalamus by increasing the amount of progesterone receptor mRNA within the cells of the hypothalamus (5). Both [E₂] and [P₄] are capable of acting on sites within the brain as both sex steroids are highly lipid soluble and can diffuse easily across the blood-brain barrier (9). In addition to their presence in the CNS, progesterone receptors have been identified in both the trachea and lungs, which suggests that P₄ might also act locally to affect breathing (106).

Although the effect of P₄ on ventilation is well recognized and documented in females (discussed below), it is important to acknowledge that ventilatory effects are also present in males. The administration of medroxyprogesterone acetate (MPA), an exogenous form of progesterone, stimulated increases in resting \( \dot{V}_E \) and reductions in PaCO₂ (15, 114), as well as increasing the hypercapnic ventilatory response (HCVR) in males (15). Tatsumi et al. (124) found that administration of a synthetic P₄ in conjunction with E₂ increased the ventilatory response to CO₂ in male rats, whereas administration of either hormone alone did not. Similar findings were noted in male cats where combined E₂ and P₄ treatment resulted in increased ventilation and hypoxic ventilatory response (41). In addition to highlighting ventilatory adaptations in response to administration of synthetic ovarian hormones in males, these studies also provide support of the importance of E₂ in mediating the effect of P₄ on ventilation.
2. 4. 8. Effect of the Menstrual Cycle and Pregnancy on Ventilatory Control

Both human pregnancy and the luteal phase of the menstrual cycle are characterized in part, by hormonally induced adaptations in ventilation. With respect to the menstrual cycle, the transition from the follicular phase to the luteal phase is associated with increases in $\dot{V}_E$ and subsequent reductions in PaCO$_2$ (93, 108, 115). Research addressing changes in the chemoreflex drives to breathe during the different phases of the menstrual cycle is inconclusive, with several studies identifying significant increases in both central and peripheral chemoreflex sensitivity to CO$_2$ and hypoxia respectively during the luteal phase (28, 34, 108), while others have not (10, 98, 115, 123). Observations of phasic changes in the threshold for CO$_2$ have also been conflicting with some researchers identifying a reduced threshold during the luteal phase (123) while others have observed no change (34, 108, 115). Currently there appears to be no phasic differences in the nonchemoreflex drives to breathe (115). Of the studies that have examined phasic differences in the ventilatory response to CO$_2$, Slatkovska et al. (115) is the only one to have employed Duffin’s (33) modified rebreathing technique. The remaining studies utilized older techniques such as Read’s rebreathing technique and progressive isocapnic hypoxia, to assess the ventilatory chemoreflex characteristics. As older rebreathing protocols are not as robust as Duffin’s (33) modified approach, findings from these earlier studies should be interpreted carefully.

During human pregnancy, significant alterations to ventilation are evident by the 7$^{th}$ week of gestation (129). Pregnancy is associated with increases in $\dot{V}_E$ and significant reductions in PaCO$_2$, both of which are tightly correlated with $P_a$ (42, 43, 55, 129). When compared to postpartum results, pregnancy is associated with increases in both peripheral and central chemoreflex sensitivity as well as reductions in the threshold of CO$_2$ (55, 77).
Decreases in plasma osmolality and [SID] serve to stimulate ventilation (51, 52). During pregnancy, both plasma osmolality (42, 43) and [SID] (42, 43, 57) are significantly reduced due in part to the expansion of the maternal blood volume. These effects are consistent with Jennings’ findings and would predict the stimulation of ventilation and the associated pregnancy-induced respiratory alkalosis that is observed. Similarly, changes in ventilation reported during the luteal phase of the menstrual cycle occur in conjunction with reductions in [SID] (93, 115) and plasma osmolality (93), findings that are also consistent with studies of the effects of [SID] and osmolality using a canine model (51). Indeed, Heenan and Wolfe (43) identified significant correlations between PaCO2 with [SID], plasma osmolality and [P4] within pooled data from pregnant and non-pregnant subjects which lends additional support for the role of [SID] and plasma osmolality in the control of ventilation.

2. 4. 9. Effect of Menopause on Ventilatory Control

In contrast to the pregnant and non-pregnant state, relatively little is known regarding the chemical drives to breathe following the onset of menopause, when the hormonal milieu is drastically different. It is reasonable to infer that ventilation would be greatly affected due to the removal of P4 as a respiratory stimulant. The removal of P4 would translate into an increased PaCO2 due to reductions in \( \dot{V}_E \) (4). Research examining the effect of hormone replacement therapy has shown that treatment with MPA alone or in combination with conjugated E2 significantly decreased PaCO2 levels in healthy postmenopausal women (86, 99), as well as in postmenopausal women with respiratory insufficiencies (105).

To date, very few studies have examined the effect of menopause on the ventilatory response to CO2 and hypoxia. Regensteiner et al. (99) noted an increase in the hypercapnic and hypoxic ventilatory responses in surgically induced menopausal
women following treatment with either MPA alone or in combination with E₂. In another study, when compared to younger, premenopausal women tested in the luteal phase of their menstrual cycle (i.e. when hormone levels are high), postmenopausal women exhibited a significantly lower peripheral chemoreflex sensitivity to hypercapnia, while no difference was observed in central chemoreflex sensitivity (26). Currently no information is available regarding potential menopausal changes in the non-chemoreflex drives to breathe and the ventilatory recruitment threshold for CO₂.

The increased occurrence of sleep disordered breathing following the menopausal transition suggests that changes in the hormonal milieu may impact on ventilatory control. Cross-sectional studies have shown that postmenopausal women exhibit nearly four times the prevalence of obstructive sleep apnea relative to their premenopausal counterparts (13) suggesting a potential protective effect of female sex hormones against the development of obstructive sleep apnea (82, 89, 91). Hormone replacement therapy involving P₄ either alone or in combination with E₂ has been shown to be effective in reducing the amount of sleep disordered breathing episodes in postmenopausal women (67, 89, 110), although this is not necessarily a universal finding (14, 22). Of the above mentioned studies the effect of hormone replacement therapy was examined in non-obese women (BMI <26 kg/m²) (14, 67, 89) and in women following adjustment of known determinants of the disorder including BMI and neck circumference (110). Given the present research findings, reductions in ovarian hormones alone do not appear to account for the increased occurrence of sleep disordered breathing amongst postmenopausal women.

Currently limited information exists regarding the potential effects of changes in plasma osmolality and [SID] on the control of ventilation following the onset of menopause. Findings from previous studies examining acid-base status in postmenopausal women suggest that [SID] may increase with menopause (described
previously). Based on Jennings’ work, increases in [SID] would serve to suppress ventilation (51), which in addition to reductions in progesterone and estrogen would serve to increase PaCO₂. Furthermore, no information could be found regarding the effect of menopause on plasma osmolality and its potential effect on ventilation within a menopausal population.

2.5. Summary

It is known that changes in circulating ovarian hormone levels, specifically P₄ and E₂, can greatly affect acid-base regulation and ventilatory control. Although a tremendous amount of attention has been given to understanding the effect of increasing E₂ and P₄ on acid-base regulation and the control of breathing across the phases of the menstrual cycle and during pregnancy, limited information is available regarding the effect of natural reductions in these particular hormones on acid-base status and ventilatory control following menopause. The available literature suggests that menopause is associated with changes in ventilation as well as alterations in plasma electrolyte and protein concentrations. As [H⁺] is determined by PaCO₂, [SID], and [A_TOT], alterations in the individual constituents of each independent variable could potentially affect acid-base status if their change is not compensated for by changes in the other independent variables.

The current study set out to examine the postulated differences in acid-base regulation and ventilatory control between healthy pre- and postmenopausal women of the similar age. Findings from this study will not only extend our current understanding of the effect of endogenous ovarian hormone levels on the chemical control of ventilation and acid-base regulation, but will also provide a base of knowledge regarding the normal menopausal homeostatic environment upon which other physiological and/or pathological perturbations are imposed.
CHAPTER 3 MANUSCRIPT: The Effect of Menopause on Acid-Base
Regulation at Rest and during Exercise above the Ventilatory Threshold
The effect of menopause on acid-base regulation at rest and during exercise above the ventilatory threshold ($T_{VENT}$) was examined in healthy pre- (PRE; n=20) and postmenopausal (POST; n=16) women of a comparable age (45± 2.7 vs. 52± 1.8 years). Stewart's independent predictors of $[H^+]$ (the strong ion difference [SID], arterial partial pressure of CO$_2$ (PaCO$_2$), and total weak acid concentration, [$A_{TOT}$]), plasma osmolality, estrogen ($[E_2]$), and progesterone ($[P_4]$) were assessed using arterialized venous blood samples collected at rest and during steady state exercise performed at 110% $T_{VENT}$. $[H^+]$ did not differ either at rest or during exercise in PRE and POST groups. At rest, the POST group exhibited significantly higher PaCO$_2$ and [SID] relative to the PRE. In general, the acidifying effect of increases in PaCO2 is offset by the alkalizing effect of increases in [SID] (or vice versa) in the POST group such that $[H^+]$ did not differ between groups. Significant PRE vs. POST group differences were not observed in the independent predictors of $[H^+]$ during exercise. Plasma osmolality was higher in the POST vs. the PRE under both resting and exercise testing conditions. Statistical adjustment for $[E_2]$ and $[P_4]$ reduced the magnitude of the differences in PaCO$_2$, [SID], and osmolality between PRE and POST groups. These findings suggest that although $[H^+]$ itself did not differ in PRE- and POST women, menopausal status does affect the independent determinants of $[H^+]$. Furthermore, menopausal differences in the independent determinants of $[H^+]$ and osmolality are partially accounted for by differences in $[P_4]$ and $[E_2]$, supporting the roles of these hormones as important determinants of acid-base status in healthy women.

**Keywords:** Menopause, acid-base regulation, physicochemical model, progesterone, estrogen
INTRODUCTION

Menopause is a significant physiological transition in females that marks the shift from reproductive capability to the permanent loss of ovarian follicular function. In addition to the cessation of menses, menopause is associated with alterations in several physiological processes and an increase in the development of numerous pathologies such as osteoporosis and sleep apnea (13, 40, 104). The increased occurrence of such pathologies following menopause is thought to be due in part to the direct effect of natural reductions in circulating estrogen (E2) and progesterone (P4) (2, 64, 106). Withdrawal of these two hormones may also indirectly influence systemic function and the development of disease via their effects on systemic acid-base regulation (27).

The regulation of hydrogen ion concentration ([H⁺]) is important for the maintenance of protein conformation and thus physiologic function (97). The acid-base composition of blood can be altered by fluctuations in E₂ and P₄ present in normally menstruating and pregnant women (4, 43, 93, 133). The application of Stewart’s (119) physicochemical approach to acid-base analysis within the aforementioned female subpopulations has provided a mechanistic approach to the interpretation of [H⁺] homeostasis within dynamic physiological systems. Briefly, Stewart (119) showed that [H⁺] is a dependent variable whose value is determined by three independent variables: the strong ion difference [SID], total weak acid concentration [ATOT], and the partial pressure of CO₂ (PCO₂). Alterations in any of the independent variables elicit concomitant changes in the others such that fluctuations and disturbances in [H⁺] are minimized (121).

Relatively little is known regarding the effect of menopausal reductions in P₄ and E₂ on acid-base status. An understanding of how [H⁺] is effected following menopause is of particular importance as it may impact on the manifestation and development of various health related disorders such as osteoporosis and sleep disordered breathing,
that are commonly diagnosed postmenopausally. Some (45, 47, 116) but not all (68), studies have noted significant differences in plasma electrolyte and protein concentrations in postmenopausal women relative to younger, premenopausal women. The effect of hormone replacement therapy on acid-base status in postmenopausal women is also varied with some (45, 47, 86) but not all (86, 99) studies observing significant changes in measures of acid-base status following hormone administration. However, these studies utilized conventional approaches to the interpretation of acid-base balance and not all have made comparisons to premenopausal women of a comparable age.

We addressed the hypothesis that menopause exerts an effect on acid-base status at rest and during exercise above the ventilatory threshold. We predicted that menopausal alterations in \([H^+]\) between healthy pre- and postmenopausal women will be attributable in part to group differences in the independent predictors of \([H^+]\) as well as circulating levels of \([P_4]\) and \([E_2]\). To test these hypotheses, acid-base status was evaluated in pre- and postmenopausal women of a similar age who were examined at rest and during steady state exercise above the ventilatory threshold. Relationships between \([H^+]\), Stewart's three independent variables, and circulating levels of \([E_2]\) and \([P_4]\) were examined at rest and during exercise to explore their potential roles in acid-base regulation within the two groups.
METHODS

Subjects.

Subjects were two groups of healthy, non-smoking, physically active premenopausal (PRE, n=20) and postmenopausal (POST, n=16) women between 42 and 54 years of age. Potential subjects were recruited from Kingston, Ontario and surrounding areas via media advertisements, posted announcements, and word of mouth. Volunteers were excluded if they were perimenopausal; taking any form of medication, including oral contraceptives and hormone replacement therapy, in the six months prior to participation; had a history of cardiorespiratory, metabolic, hematological, and/or eating disorder(s); were born at or had recently come back from a trip at high altitude; or if menopause was surgically induced. Premenopausal women were eumenorrheic and reported no menstrual cycle irregularities or disturbances prior to and throughout participation in the study. Postmenopausal status was defined as the absence of menses for a minimum of 1 year (85). Pre- and postmenopausal status was confirmed during testing via measurement of resting plasma estradiol ([E₂]) and progesterone ([P₄]).

Prior to participation, subjects completed the revised Physical Activity Readiness Questionnaire (PAR-Q, available at: www.csep.ca/forms.asp) and obtained medical clearance from their family physician. Written, informed consent was obtained from all subjects. The study protocol was approved by the Health Sciences Research Ethics Board at Queen’s University.
**Study Design.**

Subjects participated in testing on two separate occasions separated by a minimum of three days. Participants were asked to abstain from caffeine and strenuous physical activity on the day of testing.

During the first laboratory visit, basic physical characteristics were measured and included height, body mass, and resting blood pressure. Body mass index (BMI) was calculated as body mass (kg) divided by body height (m²). Pulmonary function measurements including peak flow, forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV₁) were obtained using a turbine spirometer (Pneumoscan, model S301). The best of 3 attempts was used for analysis. The FEV₁/FVC ratio was calculated from the measured values. Measures of pulmonary function were compared to percent predicted values calculated from equations obtained from Knudson et al. (58) and Morris et al. (78).

There were no restrictions placed on menstrual cycle phase for the first laboratory visit and testing session. However, premenopausal participants were tested in the follicular phase of their menstrual cycle during their second testing session. Subjects were tested during the follicular phase when estrogen and progesterone levels are low to ensure that differences observed between groups could be attributable to menopausal status as opposed to basal hormone status.

Menstrual cycle phase status was determined using the first day of the last menstrual cycle and the average length of a minimum of three previous menstrual cycles. The luteal phase (LP) was assumed to be 14 days for all subjects (20). The follicular phase (FP) was calculated by subtracting 14 days from the length of an average menstrual cycle for each participant. Menstrual cycle status was confirmed via measurements of resting plasma [E₂] and [P₄].
Core body temperature was measured in a subset of the participants (PRE n=8, POST n=9) during the second laboratory visit using an ingestible temperature sensor (CorTemp™, HQ Inc., Palmetto, Florida). Sensors were calibrated prior to ingestion using a cooling water bath and comparing recorded temperatures to those of a standard glass thermometer. Differences in readings were adjusted for by applying an appropriate correction factor. For example, if CorTemp™ readings were consistently $x$ °C higher than temperatures recorded using the standard thermometer, core temperature data collected during testing was adjusted to account for that $x$ °C difference. The CorTemp™ sensor was ingested a minimum of 2 h prior to testing to ensure passage of the sensor into the gastrointestinal tract. Throughout testing, temperature readings were recorded every 30s by the ambulatory CorTemp™ data recorder. Mean temperature data obtained during the testing session was used to verify that blood gas tensions were calculated using an appropriate core body temperature (see below).

**Exercise Testing and Blood Sample Collection Protocol**

Subjects performed two exercise tests on a Sensor Medics (Model 800s) constant work rate cycle ergometer. The testing protocol is outlined in Figure 3.1. Throughout testing, heart rate (HR) was monitored using both a Marquette Max-1 electrocardiograph (GE Medical Systems, Chicago, Illinois) and Polar Vantage heart rate monitor (Polar Electro Inc., Lake Success, New York).

During the first testing session, a submaximal exercise test was used to determine the subject’s ventilatory threshold ($T_{vent}$) using the V-slope method (8). The exercise protocol involved 5 min of resting data collection followed by a 4 min warm-up at 20 W. The warm-up was followed by a 10 W/min ramp increase in work rate until a HR corresponding to 80% of the subject’s heart rate reserve (HRR) was achieved (42, 43, 93). HRR was calculated as $HR_{rest} + 0.8(HR_{max} - HR_{rest})$. 
Breath-by-breath alveolar gas exchange was measured during exercise testing using a computerized system that allows for the integration of a respiratory mass spectrometer (Perkin-Elmer, MGA 1100) and a volume turbine (VMM-1100) (49). Metabolic and respiratory variables were calculated using the algorithm of Beaver et al. (7).

Participants completed their second exercise test during their second visit to the laboratory. Prior to the exercise test, participants completed 10 min of resting breath-by-breath ventilatory data collection. Before data collection was initiated, an indwelling catheter was inserted into a dorsal hand vein situated as far from the thumb as possible to allow for the collection of resting blood samples. The catheterized hand and forearm were then heated gently for 5 min in a Plexiglas box by circulating warm air to promote vasodilation and “arterialization” of the venous blood (37, 72). Resting data collection began once “arterialization” was achieved (confirmed by a PCO$_2$ > 40 mmHg and a PO$_2$ > 65 mmHg). After the third minute of resting data collection, the catheterized hand and forearm were reheated using circulating warm air for 3 min, after which “arterialized” blood samples were collected for analysis of PO$_2$, PCO$_2$, [E$_2$], [P$_4$], plasma osmolality, and electrolytes.

Following resting data collection, subjects completed another exercise test on the cycle ergometer, which consisted of a 3 min warm-up at 0 W followed by a ramp increase in work rate from 0 W to a wattage corresponding to 110% $T_{VENT}$. This work rate was sustained for 9 min. After the third minute of exercise, the catheterized hand and forearm were reheated using circulating warm air for 3 min, after which “arterialized” blood samples were collected for analysis of PO$_2$, PCO$_2$, plasma osmolality, and electrolytes. The exercise component was followed by an additional 10 min of resting data collection.
**Biochemical Analysis**

Blood samples used for the determination of arterIALIZED blood gas tensions (PaO$_2$ and PaCO$_2$), bicarbonate concentration ([HCO$_3^-$]), and hydrogen ion concentration ([$H^+$]) were collected using a syringe containing lyophilized heparin and were analyzed immediately upon collection using a Radiometer ABL-5 acid-base analyzer at a standard temperature of 37°C (confirmed via core temperature measurements; PRE, 37.1 ± 0.2 °C and POST, 37.1 ± 0.3 °C). Quality control checks were conducted prior to testing using four control samples to ensure the accuracy of measurements as well as proper analyzer function.

Remaining lyophilized blood was centrifuged for 10 min at 2500 rpm and the plasma separated and stored at -80°C for later analysis of total protein ([TP]), albumin ([ALB]), total phosphate ([Pi$_{TOT}$]) and electrolytes (sodium, [Na$^+$]; potassium, [K$^+$]; calcium, [Ca$^{2+}$]; chloride, [Cl$^-$]) at the Kingston General Hospital Core laboratory. [TP] was measured using the Biuret method. [ABL] was determined using a conventional dye-binding technique. [Pi$_{TOT}$] was determined using a phosphomolybdate complex. Plasma [Na$^+$], [K$^+$], [Ca$^{2+}$], and [Cl$^-$] were measured using ion-selective electrodes. The inter-assay coefficients of variability were less than 3% for [ALB], [Pi$_{TOT}$] and [TP]; less than 2% for [Ca$^{2+}$] and [K$^+$]; and less than 1% for [Na$^+$] and [Cl$^-$].

Blood samples for the determination of plasma osmolality were collected using an S-Monovette syringe containing lithium-heparin (an anticoagulant). Blood samples were centrifuged for 10 min at 2500 rpm, and the plasma separated for analysis. Plasma osmolality was measured using an automated analyzer (Precision Systems, “Osmette A”, Natick, Massachusetts) that utilizes a freezing point depression technique. Prior to running samples, the analyzer was calibrated using 100 and 500 mOsmol/Kg/H$_2$O standard solutions.
Blood samples for the determination of plasma lactate concentration ([La⁻]) were collected in vacutainers containing potassium oxalate (an antiglycolytic agent) and sodium fluoride (an anticoagulant). Upon collection, samples were centrifuged for 10 min at 2500 rpm and the plasma separated and frozen for later analysis using an automated analyzer (Model 2300, Yellow Springs Instruments, Yellow Springs, Ohio). Prior to running samples, the analyzer was calibrated using a 15 mmol/L standard lactate solution to ensure its proper functioning.

[SID] was calculated as ([Na⁺] + [K⁺] + 2 [Ca²⁺]) – ([Cl⁻] + [La⁻]) (61). [Ca²⁺] was calculated from [Ca²⁺_{tot}] using the equation [0.02*(43-[ABL])]+[Ca²⁺_{tot}]*0.469. [ATOT] was calculated from [TP] (g/L) and converted to mEq/L using a conversion factor of 0.243 (61).

Blood samples for the measurement of [E₂] and [P₄] were collected at rest in vacutainers containing no additives. Samples were allowed to clot for ~1 h on ice before undergoing centrifugation for 10 min at 2500 rpm. The serum from each sample was frozen and stored at -80°C for later analysis by radioimmunoassay at the Kingston General Hospital Core laboratory. The inter-assay coefficient of variation was <5% for both the [E₂] and [P₄] assays.

**Minimum Sample Size Collection**

A conventional power calculation formula for the comparison of two independent populations of unequal sizes was used to estimate the minimum sample size for this study assuming 80% power and confidence level of P < 0.05. The outcome variables considered most important in this study were V̇E, PaCO₂ and [H⁺]. Standard deviations from Preston et al. (93) were used to calculate sample sizes capable of detecting a between group difference of 1.00 L/min, 1.50 mmHg and 1.25 nEq/L for V̇E, PCO₂ and
respectively. The resulting estimates were 9, 11, and 9 subjects per group for $\dot{V}_e$, $\text{PCO}_2$ and $[\text{H}^+]$ respectively. As such, a minimum sample size of 15 subjects per group was considered sufficient for statistical analyses.

**Statistical Analysis**

Statistical analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, Illinois). Results were considered significant if $P<0.05$. Independent t-tests were used to identify differences between the PRE and POST groups in general physical characteristics, measures of dynamic lung function, cardiorespiratory variables and plasma biochemistry.

Measured $[\text{H}^+]$ and Stewart’s independent predictors of $[\text{H}^+]$ ([SID], $[\text{A}_\text{TOT}]$ and $\text{PaCO}_2$) measured at rest and during exercise were compared between the PRE and POST groups using independent t-tests. A general linear model with repeated measures was used to identify significant group, condition (i.e., rest or exercise), and group*condition interactions for all the biochemical and cardiorespiratory measures taken.

A Pearson product-moment correlation grid was used to identify significant associations between $[\text{H}^+]$ and its physiochemical determinants and postulated covariates such as circulating $[\text{E}_2]$ and $[\text{P}_4]$ levels and plasma osmolality. As the nature of the relation between $[\text{H}^+]$ and the other variables was not influenced by menopausal status (e.g., no interaction effect), the correlations were run and are presented within the entire sample of women. Stewart’s three independent variables were entered into a stepwise linear regression model to determine their strength as predictors of $[\text{H}^+]$ within the study group as a whole under each condition (rest and exercise). $[\text{P}_4]$ and $[\text{E}_2]$ were also entered into a stepwise linear regression model to determine whether they were
important determinants of Stewart’s independent variables and plasma osmolality within the study group as a whole under both resting and exercise conditions.

A general linear model, which included the independent hormonal predictors ([P₄] and/or [E₂]) of Stewart’s independent variables and plasma osmolality as covariates, was used to determine whether differences in [SID], PaCO₂, [A_Total] and plasma osmolality between the PRE and POST groups remained after controlling for the known hormonal predictors of these variables.

The mean age of the PRE and POST groups differed significantly (~ 7 years) thus we had to consider whether age or menopausal status was accounting for the observed differences between the two groups. When comparing group vs. age, menopausal status served as a better predictor of the biochemical and cardiorespiratory variables such that after accounting for menopausal status age did not improve the predictability of any of the variables. As such, it was concluded that the observed differences between groups were driven for the most part by menopausal status and as a result, age was not controlled for the remaining analyses.
RESULTS

Subject Characteristics

General subject characteristics for the 20 PRE and 16 POST subjects are displayed in Table 3.1. PRE and POST groups differed significantly in age (45.0 ± 2.7 vs. 52.3 ± 1.8 yrs) but in none of the remaining physical characteristics. On average, PRE subjects were tested during the fifth day (5.5 ± 2.9 days, range 1-10 days; follicular phase) of their menstrual cycle, while the POST subjects had not experienced a menstrual cycle for at least one year (1.8 ± 1.1 years, range 1-5 years) prior to testing. As expected, $[E_2]$ and $[P_4]$ were significantly higher in the PRE group.

Measures of dynamic lung function are shown in Table 3.2. Menopausal status did not have an effect on measures of dynamic lung function which included peak flow, forced vital capacity (FVC), forced expired volume in one second, and the $FEV_1/FVC$ ratio. Percent predicted values for FVC were significantly higher in the POST group relative to the PRE (99 ± 14.9 vs. 89.5 ± 9.7%). Percent predicted values for the remaining lung function measurements did not differ between the two groups.

Cardiorespiratory Variables at Rest and During Exercise at 110% $T_{VENT}$

The cardiorespiratory measurements collected at rest and during exercise are displayed in Table 3.3. A significantly lower resting tidal volume ($V_T$) was observed in the POST group relative to the PRE, although the difference was no longer significant when $V_T$ was corrected for body weight. The POST group also exhibited a higher breathing frequency at rest relative to the PRE group. No differences were observed between groups for minute ventilation ($\dot{V}_E$). Menopausal status did not affect any of the remaining cardiorespiratory measures at rest.
No significant between group differences were noted for any of the cardiorespiratory variables during exercise at 110% $T_{\text{VENT}}$. There was a significant effect of condition (rest vs. exercise) such that all of the cardiorespiratory variables were significantly higher during exercise than at rest with the exception of $\dot{V}_{\text{E}}/\dot{V}O_2$ and $\dot{V}_{\text{E}}/\dot{V}CO_2$ for both PRE and POST groups. There were no group*condition interactions indicating that the changes in cardiorespiratory variables from rest to exercise were not different in PRE and POST women.

**Plasma Biochemistry Variables at Rest and During Exercise at 110% $T_{\text{VENT}}$**

At rest, no significant between group differences were noted for measured [H$^+$] due to the opposing effects of two of Stewart’s independent predictors of [H$^+$], PaCO$_2$ and [SID], which were both significantly higher in the POST relative to the PRE group (Table 3.4). Although plasma [SID] was higher in the POST group, significant between-group differences were not observed in the individual electrolytes (Table 3.4). The POST group also exhibited significantly higher levels of plasma [HCO$_3^-$] and plasma osmolality relative to the PRE group (Table 3.4). No significant between-group differences were observed for [$A_{\text{TOT}}$], Stewart’s third independent predictor of [H$^+$] (Table 3.4). Plasma levels of albumin and phosphate were also unaffected by menopausal status (Table 3.4).

Exercising plasma biochemistry measurements were unavailable for one of the POST subjects. During exercise no significant differences were noted between PRE and POST groups for measured [H$^+$] or any of Stewart’s independent predictors of [H$^+$]. [Ca$^{2+}$] and [La$^-$] were significantly higher in the POST group during exercise, however, the changes in both ions offset each other such that [SID] did not differ between groups. No significant between group differences were noted for the remaining electrolytes or plasma [HCO$_3^-$] (Table 3.4). During exercise the POST group exhibited a significantly
higher plasma osmolality relative to the PRE group. Plasma levels of albumin and phosphate were also unaffected by menopausal status during exercise (Table 3.4).

With the exception of PaCO₂, there was a significant effect of testing condition such that all of the biochemical variables were higher during exercise (Table 3.4). Significant group differences were observed in [SID] such that under both rest and exercise the POST group had a higher [SID] relative to the PRE group (Table 3.4). Significant group differences were observed in [K⁺], [Ca²⁺], [Cl⁻] and [La⁻] when collapsed across condition such that the POST group had higher [K⁺], [Ca²⁺], and [La⁻] in addition to lower [Cl⁻] relative to the PRE group (Table 3.4). When collapsed across condition, a significant group effect was also noted for PaCO₂, plasma osmolality, and [HCO₃⁻] such that the POST group exhibited higher values for all three variables in comparison to the PRE group. A group*condition interaction was only observed for [Ca²⁺], [La⁻], and plasma osmolality indicating that the magnitude of the difference in these variables across testing conditions was influenced by menopausal status, while the other biochemical variables were not (Table 3.4).

**Relationships Between Biochemical Variables at Rest and During Exercise at 110% TVENT**

*Biochemical relationships at rest.*

PaCO₂, [SID], and [A_TOT] were entered into a stepwise multiple regression model to determine their significance as predictors of [H⁺]. At rest, 77.5% of the variance in [H⁺] was predicted by PaCO₂ and [SID]. All of Stewart’s independent predictors of [H⁺] were significantly correlated with each other (Table 3.5). Both resting PaCO₂ and [SID] were found to be significantly correlated with [P₄] (Table 3.5). Resting plasma osmolality was significantly correlated with [E₂] while plasma [HCO₃⁻] was significantly correlated to measured [H⁺], PaCO₂ and [SID] (Table 3.5).
Biochemical relationships during exercise at 110% $T_{VENT}$

The significant correlation between measured [H⁺] and PaCO₂ remained during exercise (Table 3.6). PaCO₂ and [SID] were significantly correlated with each other ($r = 0.57$). PaCO₂, [SID], and [ATOT] were entered into a stepwise multiple regression model to determine their significance as predictors of [H⁺]. During exercise at 110% $T_{VENT}$, 69.2% of the variance in [H⁺] was predicted by PaCO₂, [SID], and [ATOT]. Plasma osmolality was found to be significantly correlated with [E₂], [P₄], and [ATOT] during exercise, while plasma [HCO₃⁻] was significantly correlated with PaCO₂ and [SID] (Table 3.6).

The final set of analyses considered whether the significant differences in PaCO₂, [SID], and plasma osmolality in the PRE and POST groups were accounted for by [P₄] and [E₂]. Table 3.7 lists the adjusted group means (adjusted for significant independent predictors) for PaCO₂, [SID], and plasma osmolality under both testing conditions. In all cases, adjusting for [P₄] and [E₂] reduced the magnitude of the difference in PaCO₂, [SID], and plasma osmolality between the PRE and POST groups. This is further illustrated in Figure 3.2. Significant between-group differences remained for plasma osmolality during exercise at 110% $T_{VENT}$ ($p=0.03$) following adjustment for [E₂] although significant group differences no longer existed for plasma osmolality at rest following adjustment of [E₂] ($p=0.057$) or for PaCO₂ and [SID] at rest following adjustment for P₄ ($p=0.076$ and $p=0.057$ for PaCO₂ and [SID], respectively).
DISCUSSION

Our goal was to examine whether \([H^+]\) differed between pre- and postmenopausal women of a similar age, and if so, whether differences were accounted for by variations in ovarian hormone levels. We assessed acid-base regulation and \([H^+]\) at rest and during the challenge imposed on \([H^+]\) homeostasis by exercise at 110% of \(T_{\text{VENT}}\). We found that menopausal status did not affect \([H^+]\) either at rest or during exercise although it did influence the independent predictors of \([H^+]\). This suggests that acid-base status is preserved following the menopausal transition although the maintenance of \([H^+]\) in postmenopausal women is achieved via larger, offsetting changes in \(\text{PaCO}_2\) and [SID] relative to premenopausal women. \([E_2]\) and \([P_4]\) accounted for the pre- vs. postmenopausal differences in the independent predictors of \([H^+]\) providing support for their hypothesized role as important variables in acid-base regulation.

Our findings shed new light on the factors influencing acid-base status in this subpopulation of women and provide new insight into the regulation of \([H^+]\) following menopause. In addition, the findings are of relevance to those interested in sex differences in the regulation of \([H^+]\) and the impact of aging and menopause.

**Effect of Menopause on Acid-Base Status**

This is the first study to employ Stewart’s physicochemical approach to compare acid-base regulation in pre- and postmenopausal women of a similar age. Menopausal status did not have a significant effect on \([H^+]\) at rest or in response to exercise at 110% \(T_{\text{VENT}}\). However, as \([H^+]\) is dependent upon the behaviour of three independent variables (119), the absence of change in \([H^+]\) between the groups does not rule out the possibility of postmenopausal alterations in [SID], \(\text{PaCO}_2\), and \([A_{\text{TOT}}]\). Indeed, at rest, the postmenopausal group exhibited a significantly higher \(\text{PaCO}_2\) in comparison to
premenopausal women. PaCO₂ decreased slightly in both groups during steady state exercise however unlike at rest, mean values did not differ significantly between groups.

The increased resting PaCO₂ in the postmenopausal women is likely due in part to the removal of P₄ (in conjunction with E₂) as a respiratory stimulant (4). P₄ appears to partially exert its effect on ventilation centrally via a P₄ receptor-mediated mechanism (4, 6), while E₂ indirectly affects ventilation by up-regulating the number of P₄ receptors present in the hypothalamus (4). The latter would suggest that lower levels of E₂ and P₄ in postmenopausal relative to premenopausal women translate into a lower number of P₄ receptors available to bind substrate in addition to the reduced stimulation of ventilation by P₄. Both of these alterations could in turn, partially explain the postmenopausal increases in PaCO₂ observed in the present study. Indeed, at rest, there was a negative correlation between PaCO₂ and [P₄]. This finding is supported by observations made during pregnancy and menstrual cycle variations in which higher levels of circulating P₄ and E₂ are associated with reductions in PaCO₂ (43, 55, 93). Furthermore, we found that adjusting for [P₄] as a significant predictor of PaCO₂ decreased the magnitude of the difference in PaCO₂ between pre- and postmenopausal women, providing further evidence that [P₄] is an important determinant of PaCO₂.

In addition to PaCO₂, plasma osmolality and [SID] have been implicated in ventilatory control such that reductions in either are associated with the stimulation of ventilation and a decrease in PaCO₂ (51, 52). The mean data of our study are consistent with previous observations in a canine model such that the higher plasma osmolality and [SID] observed in the POST group may have contributed to the increase in PaCO₂ we observed. Furthermore, [SID] and PaCO₂ were found to be positively correlated with each other under both conditions which support a relationship between the two variables. However, as the effect of [SID] on the control of ventilation was not directly examined here and because osmolality and PaCO₂ were not correlated, the direct effect
of [SID] and osmolality on ventilatory control and PaCO₂ cannot be confirmed and requires further research. Unlike [SID], a significant association between PaCO₂ and osmolality was not observed under either condition.

The significantly higher postmenopausal resting [SID] was noted in the absence of a significant group difference in the individual measured ions. Indeed, the difference appears to be the result of several minor, non-statistically significant different shifts in cation and anion concentrations (see Table 3.4). This finding contradicts the observations of others in which menopause has been associated with significant changes in ion concentrations relative to premenopausal women (45-47, 68). However, it is important to note that the previous studies mentioned did not control for menstrual cycle phase in their premenopausal groups nor did they ensure that the observed differences were attributable to menopausal status and not strictly an effect of aging. The significant pre- vs. postmenopausal differences in [SID] did not persist during exercise. This finding may be accounted for by the fact that the postmenopausal women experienced a greater increase in [La⁻] during exercise.

As with PaCO₂, [SID] was negatively correlated with [P₄]. Current evidence suggests that renin-angiotensin-aldosterone system (RAAS) activation and the release of arginine vasopressin are stimulated by increases in P₄ and E₂ (83, 118). Activation of the RAAS and increased secretion of arginine vasopressin serves to increase plasma volume, which in turn would affect plasma osmolality and [SID]. As such, the higher resting osmolality and [SID] in the POST group may be attributable in part to reduced activity of the RAAS in response to lower [E₂] and [P₄]. Indeed, adjusting for [P₄] as a significant predictor of [SID] reduced the magnitude of the difference in [SID] between pre- and postmenopausal women such that the group means were no longer significantly different.
Similarly, plasma osmolality was found to be negatively associated with [E₂] under both resting and exercise conditions. Controlling for [E₂] as a significant predictor of plasma osmolality reduced the magnitude of the difference between pre- and postmenopausal groups such that the group means no longer differed significantly from each other. These findings support the postulated role of [E₂] in determining plasma osmolality.

Finally, in this study, [ATOT] did not vary by menopausal status either at rest or during exercise which is consistent with the findings of previous studies in which significant differences were not observed in the individual constituents of [ATOT] (i.e. [ALB], P iTOT) between pre- and postmenopausal women (45, 68, 116). However, when comparing pre- and postmenopausal women, Hodgkinson et al. (47) noted significant increases in total protein and plasma albumin concentrations in postmenopausal women. The observed menopausal differences in total protein concentration may be due to the larger sample size utilized by the aforementioned study (PRE n= 100, POST n=164). However it is important to note that with the exception of the current study, none of the above mentioned studies examining total protein concentrations between pre- and postmenopausal women controlled for age or menstrual cycle phase status in their premenopausal groups.

Although [H⁺] did not differ significantly between pre- and postmenopausal women either at rest or during exercise, it is important to highlight that the independent predictors of [H⁺] were affected by menopausal status such that postmenopausal women exhibited significantly higher PaCO₂ and [SID] relative to the premenopausal group. At rest, the acidifying effects of increased PaCO₂ in the postmenopausal women were offset by the alkalizing effect of an elevated [SID] (or vice versa), such that [H⁺] remained consistent between groups. Pre- and postmenopausal women also differed in the magnitude of their response to the perturbation in [H⁺] homeostasis imposed by exercise.
above $T_{\text{VENT}}$. The postmenopausal women exhibited a trend towards greater reductions in $\text{PaCO}_2$ and $[\text{SID}]$ relative to the premenopausal women such that $[\text{H}^+]$, yet again, remained constant across the groups.

Findings from the current study may have particular relevance in understanding the increased manifestation and development of various health conditions such as osteoporosis and sleep disordered breathing in females following menopause. With respect to bone health, both metabolic and respiratory acidosis have been implicated as causal mechanisms in the release of bone mineral content and bone loss in humans (27, 63). As acid-base status has been identified as an important regulator of bone remodelling (3, 62, 73), it is possible that menopausal alterations in acid-base status may promote bone catabolism. Findings from the current study suggest that the accelerated bone loss following menopause is not attributable to menopausal differences in acid-base status as $[\text{H}^+]$ did not differ between pre- and postmenopausal groups. However, as this study did not incorporate measures of bone metabolism, additional research must be conducted to discount the role of menopausal alterations in acid-base status in the development of osteoporosis.

The current findings may also contribute to our understanding of sleep disordered breathing in postmenopausal women as acid-base regulation and ventilation are inherently linked to one another. Although this study did not examine the control of ventilation in postmenopausal women, the identification of significant pre- and postmenopausal differences in $\text{PaCO}_2$ are highly suggestive of menopausal differences in the control of ventilation. This finding highlights the importance of future research in examining the effect of menopause on the control of ventilation as clinically it has important implications in eventually understanding the development of sleep disordered breathing following menopause.
Study Limitations

Despite its usefulness as a clinical tool, Stewart's (119, 121) physicochemical approach to acid-base analysis is not without measurement challenges. The accuracy of [SID] can be affected by the cumulative measurement error of the individual strong ions used to calculate [SID]. Slight deviations in [SID] can create further errors when used to calculate [H+] using Stewart's equation (119, 121). In the current study we used Stewart's approach to assist in the explanation of the mechanisms contributing to the hydrogen ion concentration that was measured independently, which reduces concerns regarding cumulative measurement error.

A key limitation of the current study is the cross-sectional design. Although a longitudinal design would have been ideal, the time required to complete such a study was impractical. The effects of the cross-sectional design were minimized given that the pre- and postmenopausal women were similar with respect to age, physical characteristics, lung function measures, resting cardiorespiratory characteristics and physical fitness. Furthermore, because menopause and age are linked to one another, we cannot completely dismiss that the differences between pre- and postmenopausal women were in part accounted for by the slight differences in age in these two groups of women.

Finally, as with most laboratory based studies, the study sample was relatively small and was not designed to be representative of the population. Previous studies have identified differences in the same primary outcome variables that we studied in other female subpopulations using identical methods and similar sample sizes (42, 81, 93). These findings support our use of similar methodologies to detect differences between pre- and postmenopausal women.
Summary

In summary, [H⁺] did not differ between pre- and postmenopausal women, although menopausal status did affect the independent determinants of [H⁺], specifically PaCO₂ and [SID]. In particular, the acidifying effect of increases in PaCO₂ and the alkalizing effect of increases in [SID] in postmenopausal women offset each other such that [H⁺] did not differ from that of the premenopausal women. Differences in PaCO₂ and [SID] appear to be due in part to the withdrawal of [E₂] and [P₄] following menopause. As acid-base regulation is central in preserving the proper functioning of a physiological system, the implications of this work extend beyond understanding the effect of ovarian hormones on acid-base status and have potential relevance in comprehending the manifestation and development of various health conditions following menopause such as osteoporosis and sleep disordered breathing.
### Table 3.1. General subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>45.0 ± 2.7</td>
<td>52.3 ± 1.8 **</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.0 ± 5.7</td>
<td>161.8 ± 7.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.0 ± 14.7</td>
<td>64.4 ± 8.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9 ± 4.7</td>
<td>24.6 ± 2.8</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118.2 ± 10.8</td>
<td>117 ± 8.7</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>71.5 ± 7.8</td>
<td>75.0 ± 6.5</td>
</tr>
<tr>
<td>[P₄], nmol/L</td>
<td>6.89 ± 9.9</td>
<td>1.03 ± 0.8*</td>
</tr>
<tr>
<td>[E₂], pmol/L</td>
<td>318.8 ± 255.3</td>
<td>67.0 ± 47.4**</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index; SPB, systolic blood pressure; DBP, diastolic blood pressure; P₄, progesterone; E₂, estrogen. Square brackets indicate concentrations. *Significantly different from premenopausal group (P<0.05). **Significantly different from premenopausal group (P<0.01).
Table 3.2. Measures of dynamic lung function.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Flow, L/sec</td>
<td>5.4 ± 0.8</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>% of predicted peak flow</td>
<td>86.2 ± 12.4</td>
<td>91.7 ± 14.3</td>
</tr>
<tr>
<td>FVC, L</td>
<td>3.2 ± 0.4</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>% of predicted FVC</td>
<td>89.5 ± 9.7</td>
<td>99.0 ± 14.9*</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>2.6 ± 0.4</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>% of predicted FEV₁</td>
<td>97.2 ± 11.0</td>
<td>103.4 ± 15.4</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>83.4 ± 7.0</td>
<td>79.1 ± 9.5</td>
</tr>
<tr>
<td>% of predicted FEV₁/FVC</td>
<td>108.8 ± 9.5</td>
<td>105.1 ± 12.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. FVC, forced vital capacity; FEV₁, forced expired volume in one second; FEV₁/FVC, ratio of FEV₁ to FVC. *Significantly different from premenopausal group (P<0.05).
Table 3.3. Cardiorespiratory variables at rest and exercise at 110% $T_{VENT}$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PREMENOPAUSAL (n=20)</th>
<th>POSTMENOPAUSAL (n=16)</th>
<th>110% $T_{VENT}$</th>
<th>PREMENOPAUSAL (n=20)</th>
<th>POSTMENOPAUSAL (n=15)</th>
<th>P for Group</th>
<th>P for Condition</th>
<th>P Group * Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_E$, L/min</td>
<td>8.0 ± 1.7</td>
<td>8.4 ± 4.4</td>
<td>46.0 ± 7.8</td>
<td>44.9 ± 7.4</td>
<td>0.802</td>
<td>0.000</td>
<td>0.563</td>
<td></td>
</tr>
<tr>
<td>$V_T$, mL</td>
<td>638.9 ± 228.0</td>
<td>507.1 ± 107.5*</td>
<td>1707.2 ± 244.8</td>
<td>1587.4 ± 300.6</td>
<td>0.053</td>
<td>0.000</td>
<td>0.908</td>
<td></td>
</tr>
<tr>
<td>$V_T$/kg, mL/kg</td>
<td>8.8 ± 4.3</td>
<td>7.9 ± 1.7</td>
<td>26.3 ± 4.1</td>
<td>24.8 ± 4.2</td>
<td>0.076</td>
<td>0.000</td>
<td>0.850</td>
<td></td>
</tr>
<tr>
<td>$f$, breaths/min</td>
<td>13.5 ± 3.2</td>
<td>16.7 ± 4.8*</td>
<td>27.4 ± 3.9</td>
<td>29.1 ± 5.1</td>
<td>0.084</td>
<td>0.000</td>
<td>0.272</td>
<td></td>
</tr>
<tr>
<td>$V_O2$, mL/min</td>
<td>295.1 ± 39.7</td>
<td>299.4 ± 69.6</td>
<td>1458.8 ± 227.2</td>
<td>1354.7 ± 200.8</td>
<td>0.239</td>
<td>0.000</td>
<td>0.134</td>
<td></td>
</tr>
<tr>
<td>$V_{CO2}$, mL/min</td>
<td>253.0 ± 31.7</td>
<td>264.0 ± 54.7</td>
<td>1474.8 ± 240.5</td>
<td>1430.6 ± 240.2</td>
<td>0.703</td>
<td>0.000</td>
<td>0.507</td>
<td></td>
</tr>
<tr>
<td>$V_E/V_O2$</td>
<td>28.5 ± 8.3</td>
<td>34.6 ± 33.7</td>
<td>32.1 ± 3.5</td>
<td>33.8 ± 5.6</td>
<td>0.383</td>
<td>0.776</td>
<td>0.501</td>
<td></td>
</tr>
<tr>
<td>$V_E/V_{CO2}$</td>
<td>32.4 ± 6.2</td>
<td>38.7 ± 25.6</td>
<td>31.7 ± 3.6</td>
<td>32.2 ± 6.7</td>
<td>0.330</td>
<td>0.174</td>
<td>0.273</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. $V_E$, minute ventilation; $V_T$, tidal volume; $V_T$/kg, tidal volume normalized for body weight; $f$, breathing frequency; $V_O2$, oxygen uptake; $V_{CO2}$, carbon dioxide output; $V_E/V_O2$, ventilatory equivalent for oxygen; $V_E/V_{CO2}$, ventilatory equivalent for carbon dioxide. *Significantly different from premenopausal group within condition (P<0.05).
Table 3.4. Plasma biochemistry variables at rest and exercise at 110% TVENT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PREMENOPAUSAL (n=20)</th>
<th>POSTMENOPAUSAL (n=16)</th>
<th>110% TVENT (n=20)</th>
<th>POSTMENOPAUSAL (n=16)</th>
<th>P for Group</th>
<th>P for Condition</th>
<th>P Group * Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured [H+] nEq/L</td>
<td>38.4 ± 2.0</td>
<td>38.8 ± 2.5</td>
<td>43.3 ± 2.8</td>
<td>44.1 ± 3.4</td>
<td>0.402</td>
<td>0.000</td>
<td>0.690</td>
</tr>
<tr>
<td>PₐCO₂, mmHg</td>
<td>37.7 ± 3.9</td>
<td>41.2 ± 4.2 *</td>
<td>37.2 ± 3.6</td>
<td>39.6 ± 5.4</td>
<td>0.019</td>
<td>0.182</td>
<td>0.424</td>
</tr>
<tr>
<td>[SID], mEq/L</td>
<td>37.0 ± 1.4</td>
<td>38.6 ± 1.9 **</td>
<td>35.2 ± 1.2</td>
<td>36.0 ± 1.9</td>
<td>0.017</td>
<td>0.000</td>
<td>0.112</td>
</tr>
<tr>
<td>[Na⁺], mmol/L</td>
<td>136.9 ± 1.2</td>
<td>136.9 ± 2.0</td>
<td>139.4 ± 1.6</td>
<td>140.1 ± 1.6</td>
<td>0.553</td>
<td>0.000</td>
<td>0.059</td>
</tr>
<tr>
<td>[K⁺], mmol/L</td>
<td>4.0 ± 0.41</td>
<td>4.4 ± 1.10</td>
<td>4.7 ± 0.57</td>
<td>4.97 ± 0.46</td>
<td>0.039</td>
<td>0.000</td>
<td>0.615</td>
</tr>
<tr>
<td>[Ca²⁺], mmol/L</td>
<td>1.09 ± 0.03</td>
<td>1.11 ± 0.03</td>
<td>1.11 ± 0.04</td>
<td>1.17 ± 0.08**</td>
<td>0.013</td>
<td>0.000</td>
<td>0.028</td>
</tr>
<tr>
<td>[Cl⁻], mmol/L</td>
<td>105.1 ± 1.85</td>
<td>103.9 ± 1.77</td>
<td>105.9 ± 2.0</td>
<td>104.7 ± 1.63</td>
<td>0.015</td>
<td>0.011</td>
<td>0.766</td>
</tr>
<tr>
<td>[La⁻], mmol/L</td>
<td>1.02 ± 0.56</td>
<td>1.0 ± 0.45</td>
<td>5.19 ± 1.54</td>
<td>6.8 ± 1.59**</td>
<td>0.009</td>
<td>0.000</td>
<td>0.005</td>
</tr>
<tr>
<td>[ATOT], mEq/L</td>
<td>16.4 ± 0.6</td>
<td>16.5 ± 0.7</td>
<td>17.7 ± 0.9</td>
<td>18.0 ± 0.6</td>
<td>0.357</td>
<td>0.000</td>
<td>0.382</td>
</tr>
<tr>
<td>[PiTOT], mmol/L</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>0.110</td>
<td>0.000</td>
<td>0.962</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>41.7 ± 1.6</td>
<td>42.1 ± 2.4</td>
<td>44.3 ± 2.2</td>
<td>44.2 ± 4.3</td>
<td>0.892</td>
<td>0.000</td>
<td>0.760</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg/H₂O</td>
<td>280.2 ± 3.6</td>
<td>284.1 ± 3.2**</td>
<td>287.3 ± 4.4</td>
<td>291.7 ± 3.5**</td>
<td>0.002</td>
<td>0.000</td>
<td>0.312</td>
</tr>
<tr>
<td>[HCO₃⁻], mmol/L</td>
<td>23.7 ± 1.4</td>
<td>25.5 ± 1.4**</td>
<td>20.8 ± 1.1</td>
<td>21.6 ± 2.3</td>
<td>0.009</td>
<td>0.000</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Values are means ± SD. H⁺, hydrogen ion; PaCO₂, arterial partial pressure of CO₂; SID, strong ion difference; Na⁺, sodium, K⁺, potassium; Ca²⁺, calcium; Cl⁻, chloride; La⁻, lactate; ATOT, total weak acid; PiTOT, total phosphate; HCO₃⁻, bicarbonate. Square brackets indicate concentrations.

*Significantly different from premenopausal group within condition (P < 0.05). **Significantly different from premenopausal group within condition (P < 0.01).
Table 3.5. Correlations (r values) between [H⁺] and its predictors at rest in pre- and postmenopausal women.

<table>
<thead>
<tr>
<th></th>
<th>[HCO₃⁻]</th>
<th>PₐCO₂</th>
<th>[SID]</th>
<th>[A_TOT]</th>
<th>[P₄]</th>
<th>[E₂]</th>
<th>Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured [H⁺], nEq/L</td>
<td>0.408*</td>
<td>0.803*</td>
<td>0.162</td>
<td>0.277</td>
<td>-0.172</td>
<td>-0.034</td>
<td>0.271</td>
</tr>
<tr>
<td>[HCO₃⁻], mmol/L</td>
<td>1</td>
<td>0.851**</td>
<td>0.781**</td>
<td>0.227</td>
<td>-0.423</td>
<td>-0.244</td>
<td>0.178</td>
</tr>
<tr>
<td>PₐCO₂, mmHg</td>
<td>1</td>
<td>0.610**</td>
<td>0.350*</td>
<td>-0.390*</td>
<td>-0.141</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>[SID], mEq/L</td>
<td>1</td>
<td>0.380*</td>
<td>-0.447**</td>
<td>-0.273</td>
<td>0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[A_TOT], mEq/L</td>
<td>1</td>
<td></td>
<td>-0.131</td>
<td>-0.091</td>
<td></td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>[P₄], nmol/L</td>
<td>1</td>
<td>0.323</td>
<td></td>
<td>-0.305</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[E₂], pmol/L</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>-0.464**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolality, mosmol/kg/H₂O</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H⁺, hydrogen ion; PₐCO₂, arterial partial pressure of carbon dioxide; SID, strong ion difference; A_TOT, total weak acid; P₄, progesterone; E₂, estradiol; HCO₃⁻, bicarbonate. Square brackets indicate concentration. *Significant correlation (P<0.05). **Significant correlation (P<0.01).
Table 3.6. Correlations (r values) between [H+] and its predictors during exercise at 110% T_{VENT} in pre- and postmenopausal women.

<table>
<thead>
<tr>
<th></th>
<th>[HCO₃⁻]</th>
<th>P_aCO₂</th>
<th>[SID]</th>
<th>[A_TOT]</th>
<th>[P₄]</th>
<th>[E₂]</th>
<th>Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H⁺], nEq/L</td>
<td>0.253</td>
<td>0.773**</td>
<td>0.230</td>
<td>0.263</td>
<td>-0.267</td>
<td>-0.056</td>
<td>0.291</td>
</tr>
<tr>
<td>[HCO₃⁻], mmol/L</td>
<td>1</td>
<td>0.771**</td>
<td>0.632**</td>
<td>0.054</td>
<td>-0.220</td>
<td>-0.180</td>
<td>0.166</td>
</tr>
<tr>
<td>P_aCO₂, mmHg</td>
<td>1</td>
<td>0.571**</td>
<td>0.163</td>
<td>-0.277</td>
<td>-0.182</td>
<td>0.240</td>
<td></td>
</tr>
<tr>
<td>[SID], mEq/L</td>
<td>1</td>
<td>0.298</td>
<td>-0.312</td>
<td>-0.054</td>
<td>0.058</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[A_TOT], mEq/L</td>
<td>1</td>
<td></td>
<td>-0.216</td>
<td>-0.015</td>
<td>0.339*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[P₄], nmol/L</td>
<td>1</td>
<td>0.323</td>
<td></td>
<td>-0.348*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[E₂], pmol/L</td>
<td>1</td>
<td></td>
<td></td>
<td>-0.349*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolality, mosmol/kg/H₂O</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T_{VENT}, ventilatory threshold; H⁺, hydrogen ion; P_aCO₂, arterial partial pressure of carbon dioxide; SID, strong ion difference; A_TOT, total weak acid; P₄, progesterone; E₂, estradiol; HCO₃⁻, bicarbonate. Square brackets indicate concentration. *Significant correlation (P<0.05). **Significant correlation (P<0.01).
Table 3.7. Adjusted group means for PaCO₂, [SID] and plasma osmolality at rest and during exercise at 110% T\text{VENT}.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PREMENOPAUSAL (n=20)</th>
<th>POSTMENOPAUSAL (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting P\textsubscript{s}CO₂, mmHg</td>
<td>38.1 ± 4.1 \textsuperscript{a}</td>
<td>40.7 ± 4.1 \textsuperscript{a}</td>
</tr>
<tr>
<td>Resting [SID], mEq/L</td>
<td>37.2 ± 1.6 \textsuperscript{a}</td>
<td>38.3 ± 1.6 \textsuperscript{a}</td>
</tr>
<tr>
<td>Resting Osmolality, mosmol/kg/H\textsubscript{2}O</td>
<td>280.7 ± 3.7 \textsuperscript{b}</td>
<td>283.4 ± 3.8 \textsuperscript{b}</td>
</tr>
<tr>
<td>Exercising Osmolality, mosmol/kg/H\textsubscript{2}O</td>
<td>287.5 ± 4.4 \textsuperscript{b}</td>
<td>291.3 ± 4.6 \textsuperscript{b} *</td>
</tr>
</tbody>
</table>

Values are adjusted means ± SD. Values were adjusted for the independent predictors of each biochemical variable. Symbols represent means adjusted for: \textsuperscript{a} Progesterone; \textsuperscript{b} Estrogen. PaCO\textsubscript{2}, partial pressure of CO\textsubscript{2}; [SID], strong ion difference. Square brackets indicate concentration. *Significantly different from premenopausal group (P<0.05).
FIGURE LEGEND

Figure 3.1. Exercise protocol for the first and second laboratory visits. The first laboratory visit consisted of a progressive submaximal exercise test to 80% of the subject’s heart rate reserve (HRR). During the second laboratory visit, participants completed 10 min of resting breath-by-breath data collection followed by a steady state exercise test at a work rate corresponding to 110% of the anaerobic ventilatory threshold ($T_{VENT}$).

Figure 3.2. Differences in the unadjusted (black bars) and adjusted means (grey bars) of PaCO$_2$, [SID] and plasma osmolality at rest and during exercise at 110% $T_{VENT}$ in pre- and postmenopausal groups. In all cases, adjusting for either progesterone or estrogen as significant predictors (described in the text) reduced the magnitude of the differences in all of the biochemical variables between the PRE and POST groups for $A$, resting PaCO$_2$, $B$, resting [SID], and $C$, plasma osmolality at rest and during exercise at 110% $T_{VENT}$. Significant group differences remained following adjustment for significant predictors for plasma osmolality during exercise at 110% $T_{VENT}$. PRE, premenopausal; POST, postmenopausal; PaCO$_2$, partial pressure of CO$_2$; [SID], the strong ion difference; $T_{VENT}$, ventilatory threshold. Square brackets indicate concentration.
Figure 3.1

**FIRST LABORATORY VISIT**

- **PROGRESSIVE SUBMAX EXERCISE**
  - 0 min: Start resting data collection
  - 5 min: Begin warm up at 20 Watts
  - 9 min: Begin ramp increase
  - Increase work rate by 5 watts every 30s up to 80% HRR
  - X min: Stop data at 80% HRR

**SECOND LABORATORY VISIT**

- **REST**
  - 1 min: Start data collection
  - 3 min: Start arterialization
  - 6 min: Blood collection
  - 10 min: Stop data collection

- **EXERCISE**
  - 0 min: Warm up at 0 Watts
  - 3 min: Begin ramp increase to 110% TVENT
  - 7 min: Start arterialization
  - 10 min: Blood collection
  - 12 min: Begin resting data collection
  - 22 min: Stop data collection
Figure 3.2.

A

Difference in PaCO₂ between PRE & POST

0
1
2
3
4
5

0
1
2
3
4
5

Rest

B

Difference in [SID] between PRE & POST

0
0.5
1
1.5
2
2.5
3
3.5
4

0
1
2
3
4
5

Rest

C

Difference in plasma osmolality between PRE & POST

0
2
4
6
8
10

0
2
4
6
8
10

Rest

110% T_{VENT}
CHAPTER 4  MANUSCRIPT: THE EFFECT OF MENOPAUSE ON THE CHEMOREFLEX CONTROL OF BREATHING DURING WAKEFULNESS
ABSTRACT

The effect of menopause on ventilatory control was examined in two groups of healthy pre- (PRE; n=20) and postmenopausal (POST; n=15) women. Central (CC) and peripheral ventilatory chemoreflex control characteristics were assessed using a modified CO₂ rebreathing protocol that includes prior hyperventilation and the maintenance of either hyperoxic (150 mmHg) or hypoxic (50 mmHg) iso-oxia. Arterialized blood samples were collected during 10 min of resting breath-by-breath data collection for the determination of arterial PCO₂ (PaCO₂), strong ion difference ([SID]), progesterone ([P₄]) and estradiol ([E₂]). The POST group exhibited higher PaCO₂ that was associated with a higher threshold (VRTCO₂) and lower sensitivity (VE_S) in the CC response to CO₂ relative to the PRE. The POST had significantly lower [P₄] and [E₂] as well as a higher [SID] relative to the PRE. Pooled group data showed significant correlations between [P₄] with PaCO₂, [SID], CC VE_S, and VRTCO₂ under hyperoxic and hypoxic conditions. Significant correlations were also noted between PaCO₂ with hyperoxic and hypoxic VRTCO₂ as well as between [SID] with hyperoxic and hypoxic VRTCO₂ and VE_S. Within the pooled data, PaCO₂ predicted VRTCO₂ under both rebreathing conditions while VE_S was predicted by [P₄] under the hyperoxic conditions. Adjusting for predictors reduced the magnitude of the difference in the rebreathing characteristics that differed significantly between groups such that only VRTCO₂ under hyperoxic conditions remained significantly different between groups (p=0.037) while VRTCO₂ under hypoxic conditions and VE_S under hyperoxic conditions did not. These data suggest that menopause alters the CC control of breathing and that these changes are partially accounted for by menopausal alterations in PaCO₂ and [P₄] supporting the roles of these variables as important determinants of ventilatory control in healthy PRE and POST women.
Keywords: Menopause, ventilatory control, chemoreflex, chemical control of breathing, progesterone, the strong ion difference
Introduction

Circulating levels of ovarian hormones influence the chemical control of ventilation. Progesterone (P_4) is a respiratory stimulant (16, 41, 56, 108) that is believed to exert its effect in part, centrally on the hypothalamus via an estrogen-dependent, progesterone receptor-mediated mechanism (4, 16).

Increased levels of P_4 and estrogen (E_2) such as those seen during pregnancy and to a lesser extent, the luteal phase (LP) of the menstrual cycle, are associated with increases in minute ventilation (\dot{V}_E) and reductions in the arterial partial pressure of carbon dioxide (PaCO_2) (42, 93, 108, 115, 133). It has been speculated that the hyperventilation observed during pregnancy and in the LP of the menstrual cycle are due in part to the effect of elevated ovarian hormone levels on the ventilatory chemoreflex drives to breathe (133). Pregnancy has been associated with an increase in both central and peripheral chemoreflex sensitivity (55, 77), in addition to a reduction in the threshold of the central ventilatory chemoreflex response to CO_2 (55). Examination of the ventilatory chemoreflex response to CO_2 across the phases of the menstrual cycle have been inconclusive with some studies (28, 69, 108) observing an increase in both central and peripheral chemoreflex sensitivity, while others have not (10, 98, 115, 123).

In addition to the established effect of ovarian hormones on ventilation, it has been suggested that the strong ion difference ([SID]; the difference between the concentration of strong cations minus the concentration of strong anions) and plasma osmolality play an important role in the chemical control of ventilation (51, 52). Jennings (52) showed that changes in arterial and cerebrospinal fluid [SID] in both humans and animals consistently predict ventilatory regulation of PaCO_2, such that decreases in either [SID] or plasma osmolality are associated with a stimulation of ventilation. Both [SID] and plasma osmolality are significantly reduced during pregnancy (42, 43, 57) and
the LP of the menstrual cycle (93). Based on Jennings’ hypotheses, these changes should result in a stimulation of ventilation, which is observed experimentally in both cases. Thus, several human and animal models indicate that changes in [SID], plasma osmolality, and circulating [P₄] predict PaCO₂ and changes in ventilation.

In contrast to the pregnant and non-pregnant state, relatively little is known regarding the chemical drive(s) to breathe following the onset of menopause, when the hormonal milieu is drastically altered. Although it is reasonable to infer that ventilatory control by the chemoreflexes would be affected due to the removal of P₄ (in conjunction with E₂) as a respiratory stimulant, the effects of potential changes in [SID] and plasma osmolality are unknown and could potentially offset those induced by hormonal changes. Research examining the effect of hormone replacement therapy has shown that treatment with medroxyprogesterone acetate alone or in combination with conjugated E₂ significantly decreased PaCO₂ levels in healthy postmenopausal women (86), as well as in postmenopausal women with respiratory insufficiency (105) suggesting that P₄ and E₂ may partially explain the ventilatory adaptations to menopause.

To the best of our knowledge, no study has examined the effect of menopause on the chemoreflex control of breathing and its obvious but often neglected relationship with acid-base regulation. The present study addressed the hypothesis that menopause would significantly alter the ventilatory chemoreflex drives to breathe and that the changes would be correlated with alterations in circulating hormone levels and plasma [SID]. Central and peripheral chemoreflex characteristics in pre- and postmenopausal women of a similar age were measured. Relationships between the measured chemoreflex characteristics, circulating levels of E₂ and P₄, resting blood gases, [SID] and plasma osmolality were examined to explore their potential roles in the chemical control of ventilation within the two groups. We predicted that postmenopausal women would exhibit a higher ventilatory recruitment threshold in addition to a decrease in
ventilatory chemoreflex sensitivity (both central and peripheral) relative to premenopausal women and that differences between groups would be explained in part by differences in circulating P₄ and E₂, [SID] and plasma osmolality.
METHODS

Subjects

Subjects were two groups of healthy, non-smoking, physically active premenopausal (PRE, n=20) and postmenopausal (POST, n=15) women between 42 and 54 years of age. Potential subjects were recruited from Kingston, Ontario and surrounding areas via media advertisements, posted announcements, and word of mouth. Volunteers were excluded if they were perimenopausal; taking any form of medication, including oral contraceptive and hormone replacement therapy, in the six months prior to participation; had a history of cardiorespiratory, metabolic, haematological, and/or eating disorder(s); were born at or had recently come back from a trip at high altitude; or if menopause was surgically induced. PRE women were eumenorrheic and reported no menstrual cycle irregularities or disturbances prior to and throughout participation in the study. POST status was defined as the absence of menses for a minimum of 1 year (85). PRE and POST status was confirmed during testing via measurement of resting plasma \([E_2] \text{ and } [P_4]\).

Prior to participation, subjects completed the revised Physical Activity Readiness Questionnaire (PAR-Q, available at: www.csep.ca/forms.asp) and obtained medical clearance from their family physician. Written, informed consent was obtained from all subjects. The study protocol was approved by the Health Sciences Research Ethics Board at Queen's University.

Study Design

Subjects participated in testing on two separate occasions separated by a minimum of three days. Participants were asked to abstain from caffeine and strenuous physical activity on the day of testing.
During the first laboratory visit, basic physical characteristics were measured and included height, body mass, and resting blood pressure. Body mass index (BMI) was calculated as body mass (kg) divided by body height (m²). Pulmonary function measurements, including peak flow, forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV₁), were obtained using a turbine spirometer (Pneumoscan, model S301). The best of 3 attempts was used for analysis. The FEV₁/FVC ratio was calculated from the measured values. Measures of pulmonary function were compared to, and percent predicted values calculated from equations obtained from Knudson et al. (58) and Morris et al. (78). During the first testing session participants completed a familiarization hyperoxic CO₂ rebreathing procedure (see below).

During the second laboratory visit, participants completed hyperoxic and hypoxic CO₂ rebreathing procedures that were separated by a 45 min rest interval. The order of the hyperoxic and hypoxic rebreathing trials was randomized between subjects. Rebreathing was followed by a 1.5 h break after which participants completed 10 min of resting, breath-by-breath ventilatory data collection (described below). Arterialized blood samples were collected during the 6th min of resting, breath-by-breath data collection for biochemical analysis (37, 72).

Core body temperature was measured in a subset of the participants (PRE, n=8; POST, n=9) during the second laboratory visit using an ingestible temperature sensor (CorTemp™, HQ Inc., Palmetto, Florida). Sensors were calibrated prior to ingestion using a cooling water bath and comparing recorded temperatures to those of a standard glass thermometer. Differences in readings were adjusted for by applying an appropriate correction factor. For example, if CorTemp™ readings were consistently $x$ °C higher than temperatures recorded using the standard thermometer, core temperature data collected during testing was adjusted to account for that $x$ °C difference. The CorTemp™ sensor was ingested a minimum of 2 h prior to testing to ensure passage of the sensor.
into the gastrointestinal tract. Throughout testing, temperature readings were recorded every 30 s by the ambulatory CorTemp™ data recorder. Mean temperature data obtained during the testing session was used to verify that blood gas tensions were calculated using an appropriate core body temperature (see below).

There were no restrictions placed on menstrual cycle phase for the first laboratory visit and testing session. However, PRE participants were tested in the follicular phase of their menstrual cycle during their second laboratory visit and testing session. Subjects were tested during the follicular phase when [E₂] and [P₄] are low to ensure that differences observed between groups could be attributable to menopausal status as opposed to hormone fluctuations.

Menstrual cycle phase status was determined using the first day of the last menstrual cycle and the average length of a minimum of three previous menstrual cycles. The luteal phase was assumed to be 14 days for all subjects (20). The follicular phase was calculated by subtracting 14 days from the length of an average menstrual cycle for each participant. Menstrual cycle status was confirmed via measurements of resting plasma [E₂] and [P₄].

**Rebreathing Procedure**

Central and peripheral ventilatory chemoreflex control characteristics were assessed using a modified version of Read’s (96) rebreathing procedure that includes 5 min of prior hyperventilation and maintenance of iso-oxia (33, 54). Prior to rebreathing, participants voluntarily hyperventilated room air for 5 min in order to lower body CO₂ stores below 23 mmHg using a slow, deep and deliberate breathing pattern to avoid short-term potentiation effects (36). Following hyperventilation, participants were switched at the end of expiration from breathing room air to a rebreathing bag containing either a hyperoxic (24% O₂, 6% CO₂, N₂ balanced) or hypoxic (4.5% O₂, 6% CO₂, N₂
balanced) gas mixture. Completion of the modified rebreathing protocol under hyperoxic conditions was used to assess central chemoreflex sensitivity, whereas completion of the rebreathing protocol under hypoxic conditions was used to assess peripheral chemoreflex sensitivity.

Rebreathing began with 4 deep breaths to allow for the rapid equilibration of the PCO$_2$ in the rebreathing bag, the lungs, and arterial blood to that of the mixed venous blood. Equilibration was verified by a plateau in end tidal CO$_2$ (P$_{ET}$CO$_2$) and was a prerequisite for continuation of the test. Following equilibration, participants were instructed to breathe as they felt the need.

Throughout rebreathing iso-oxia was maintained at either a hyperoxic (150 mmHg) or hypoxic (50 mmHg) end tidal O$_2$ (P$_{ET}$O$_2$), while P$_{ET}$CO$_2$ was allowed to rise within the rebreathing bag. Arterial blood O$_2$ saturation (Sa$_{O2}$) and heart rate were monitored continuously throughout rebreathing using an ear oximeter (OXI, Radiometer Copenhagen, Copenhagen, Denmark). Rebreathing was terminated if minute ventilation (V$_E$) exceeded 100 L/min, P$_{ET}$CO$_2$ exceeded 55 mmHg, Sa$_{O2}$ fell below 70%, and/or subject discomfort.

**Rebreathing Apparatus**

The modified rebreathing apparatus, data acquisition, and analysis software has been previously described in detail (54). Briefly, participants wore nose clips while breathing through a mouthpiece connected to a wide-bore T-valve (Hans Rudolph 2100a) that allows switching between breathing room air and gas contained in the rebreathing bag. During rebreathing, breath-by-breath changes in V$_E$, tidal volume (V$_T$), and respiratory rate (f) were measured using a bidirectional volume turbine (Alpha Technologies, VMM-1100), while P$_{ET}$O$_2$ and P$_{ET}$CO$_2$ were measured by a respiratory
mass spectrometer (Perkin-Elmer, MGA 1100). Iso-oxia was maintained via a computer
controlled flow of 100% O₂ to the bag side of the T-valve. Custom data acquisition
software (courtesy Dr. J. Duffin, University of Toronto; written using Labview, National
Instruments Austin, Texas) was used to calculate $\dot{V}_E$, $V_T$, inspiratory and expiratory times,
$P_{ET}CO_2$, and $P_{ET}O_2$ on a breath-by-breath basis. Prior to each rebreathing session, the
rebreathing system was calibrated using gases of known CO₂ and O₂ concentrations
and a standardized volume syringe.

Analysis of breath-by-breath data was accomplished using a spreadsheet
(Microsoft, Excel) specifically designed for this purpose. Data from the initial equilibration
at the start of rebreathing as well as outlying data points resulting from sighs, swallows
and aberrant breaths were excluded prior to the breath-by-breath analysis. The $\dot{V}_E$
responses to hyperoxic and hypoxic rebreathing tests were fitted to a model consisting
of the sum of two segments separated by a breakpoint (Duffin et al., (33); briefly
described in Appendix B). The $P_{ET}CO_2$ at which $\dot{V}_E$ increased in a linear fashion with
increases in $P_{ET}CO_2$ was identified as the ventilatory recruitment threshold for CO₂
($VRTCO_2$; Appendix B, panel B). The $\dot{V}_E$ preceding the $VRTCO_2$ was used as an estimate
of subthreshold ventilation ($\dot{V}_{E_b}$), which represents the non-chemoreflex drive to breathe
(Appendix B, panel B). The slope of the linear relationship between $\dot{V}_E$ and $P_{ET}CO_2$
(expressed in L/min/mmHg) above the $VRTCO_2$ was used as an estimate of chemoreflex
sensitivity ($\dot{V}_{ES}$; Appendix B, panel B) (75). In general, it is assumed that the $VRTCO_2$ and
$\dot{V}_{ES}$ measured under hyperoxic conditions originate from the central chemoreflex alone,
whereas the same measures under hypoxic conditions are a result of the summation of
both the central and peripheral chemoreflex drives to breathe (33). Additional
assumptions of the model have been described in detail previously by Duffin et al. (33).
Resting Ventilatory and Blood Biochemistry Data Collection

Completion of both rebreathing trials was followed by a 1.5 h rest interval after which participants completed 10 min of resting breath-by-breath ventilatory data collection. Prior to data collection, an indwelling catheter was inserted into a dorsal hand vein situated as far from the thumb as possible to allow for the collection of resting blood samples. The catheterized hand and forearm were then heated gently for 5 min in a Plexiglas box by circulating warm air to promote vasodilation and “arterialization” of the venous blood (37, 72). Resting data collection began once “arterialization” was achieved (confirmed by a PCO\(_2\) greater than 40mmHg and a PO\(_2\) greater than 65 mmHg). Breath-by-breath alveolar gas exchange was measured using a computerized system that allows for the integration of a respiratory mass spectrometer (Perkin-Elmer, MGA 1100) and a volume turbine (VMM-1100) (49). Metabolic and respiratory variables were calculated using the algorithm of Beaver et al. (7).

After the 3\(^{rd}\) min of resting data collection, the catheterized hand and forearm were reheated using circulating warm air for 3 min, after which arterialized blood samples were collected for analysis of PaO\(_2\), PaCO\(_2\), \([E_2]\), \([P_4]\), plasma osmolality, and electrolytes.

Throughout testing heart rate was monitored using both a Marquette Max-1 electrocardiograph (GE Medical Systems, Chicago, Illinois) and Polar Vantage heart rate monitor (Polar Electro Inc., Lake Success, New York).

Biochemical Analysis

Blood samples used for the determination of arterial blood gas tensions (PaO\(_2\) and PaCO\(_2\)), bicarbonate concentration ([HCO\(_3^-\)]), and hydrogen ion concentration ([H\(^+\)]) were collected using a syringe containing lyophilized heparin and were analyzed immediately upon collection using a Radiometer ABL-5 acid-base analyzer at a standard
temperature of 37°C (confirmed via core temperature measurements; PRE, 37.1 ± 0.2 °C and POST, 37.1 ± 0.3 °C). Quality control checks were conducted prior to testing using four control samples to ensure the accuracy of measurements as well as proper functioning of the analyzer.

Remaining lyophilized blood was centrifuged for 10 min at 2500 rpm and the plasma separated and stored at -80°C for later analysis of total protein ([TP]), albumin ([ALB]), total phosphate ([PiTOT]), and electrolytes (sodium, [Na⁺]; potassium, [K⁺]; calcium, [Ca²⁺]; chloride, [Cl⁻]) at the Kingston General Hospital Core laboratory. [TP] was measured using the Biuret method. [ABL] was determined using a conventional dye-binding technique. [PiTOT] was determined using a phosphomolybdic acid complex. Plasma [Na⁺], [K⁺], [Ca²⁺], and [Cl⁻] were measured using ion-selective electrodes. The inter-assay coefficients of variability were less than 3% for [ALB], [PiTOT] and [TP], less than 2% for [Ca²⁺] and [K⁺], and less than 1% for [Na⁺] and [Cl⁻].

Blood samples for the determination of plasma osmolality were collected using a S-Monovette syringe containing lithium-heparin (an anticoagulant). Blood samples were centrifuged for 10 min at 2500 rpm, and the plasma separated for analysis. Plasma osmolality was measured using an automated analyzer (Precision Systems, “Osmette A”, Natick, Massachusetts) that utilizes a freezing point depression technique. Prior to running samples, the analyzer was calibrated using 100 and 500 mOsmol/Kg/H₂O standard solutions.

Blood samples for the determination of plasma lactate concentration ([La⁻]) were collected in vacutainers containing potassium oxalate (an antiglycolytic agent) and sodium fluoride (an anticoagulant). Upon collection, samples were centrifuged for 10 min at 2500 rpm and the plasma separated and frozen for later analysis using an automated analyzer (Model 2300, Yellow Springs Instruments, Yellow Springs, Ohio). Prior to
running samples, the analyzer was calibrated using a 15 mmol/L standard lactate solution to ensure its proper functioning.

\[
[\text{SID}] = ([\text{Na}^+] + [\text{K}^+] + 2 [\text{Ca}^{2+}]) - ([\text{Cl}^-] + [\text{La}^-]).
\]

\[
[\text{Ca}^{2+}] \text{ was calculated from } [\text{Ca}^{2+}_{\text{tot}}] \text{ using the equation } 0.02(43-[\text{ABL}]) + [\text{Ca}^{2+}_{\text{tot}}] \times 0.469. \]

\[
[\text{ATOT}] \text{ was calculated from } [\text{TP}] (\text{g/L}) \text{ and converted to mEq/L using a conversion factor of } 0.243.
\]

Blood samples for the measurement of [E₂] and [P₄] were collected at rest in vacutainers containing no additives. Samples were allowed to clot for ~1 h on ice before undergoing centrifugation for 10 min at 2500 rpm. The serum from each sample was frozen and stored at -80°C for later analysis by radioimmunoassay at the Kingston General Hospital Core laboratory. The inter-assay coefficient of variation was <5% for both the [E₂] and [P₄] assays.

**Minimum Sample Size Collection**

A conventional power calculation formula for the comparison of two independent populations of unequal sizes was used to estimate the minimum sample size for this study assuming 80% power and a P<0.05. The outcome variables considered most important in this study were VRTCO₂ and \( \dot{\text{V}}_{\text{E}} \) of both the central and peripheral ventilatory chemoreflexes. Standard deviations from Jensen et al. (54) were used to calculate sample sizes capable of detecting a between group difference of 1.00 L/min/mmHg in \( \dot{\text{V}}_{\text{E}} \) and 2 mmHg difference in VRTCO₂. The resulting estimates were 7 and 9 subjects per group for central and peripheral \( \dot{\text{V}}_{\text{E}} \) respectively, and 8 subjects per group for VRTCO₂. As such, a minimum sample size of 15 subjects per group was considered sufficient for statistical analyses.
**Statistical Analysis**

Statistical analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, Illinois). Results were considered significant if $P<0.05$. Independent t-tests were used to identify differences between the PRE and POST groups for the general physical characteristics, resting cardiorespiratory variables, and plasma biochemistry.

Rebreathing data ($\dot{V}_E$, $\dot{V}_{ES}$, VRTCO$_2$, central and peripheral chemoreflex contributions to $\dot{V}_{ES}$) within each of the hyperoxic and hypoxic conditions was compared in PRE and POST groups using independent t-tests. A general linear model with repeated measures was used to identify significant group, condition (i.e., rebreathing trial), and group*condition interactions for each of the measured ventilatory chemoreflex characteristics.

A Pearson product-moment correlation grid was used to identify significant associations between central and peripheral chemoreflex characteristics and postulated modulators of ventilation such as circulating [E$_2$] and [P$_4$] levels, [SID] and plasma osmolality across the study sample as a whole. As the nature of the relation between [H$^+$] and the other variables was not influenced by menopausal status (e.g., no interaction effect), the correlations were run and are presented within the entire sample of women. Significant correlates were entered into a stepwise linear regression model to determine the independent predictors of each of the central and peripheral chemoreflex characteristic measures.

A general linear model, which included the independent predictors of the central and peripheral chemoreflex characteristic measures as covariates, was used to determine whether differences in central and peripheral chemoreflex characteristics between the PRE and POST groups remained after controlling for the known predictors of these characteristics.
Because the mean age of the PRE and POST groups differed significantly (~7 years), consideration was given to whether age or menopausal status accounted for the observed differences between the two groups. When comparing group vs. age, menopausal status served as a better predictor of the biochemical and rebreathing variables such that after accounting for menopausal status age did not improve the predictability of any of the variables. As such, it was concluded that the observed differences between groups were driven by menopausal status and as a result, age was not controlled for the remaining analyses.
RESULTS

Subject Characteristics

General subject characteristics for the 20 PRE and 15 POST subjects are displayed in Table 4.1. PRE and POST groups differed significantly in age (45.3 ± 3.2 yrs vs. 52.1 ± 1.8 yrs) but in none of the remaining physical characteristics. On average, PRE subjects were tested during the fifth day (5.3 ± 3.0 days, range 1-10 days; follicular phase) of their menstrual cycle while POST subjects had not experienced a menstrual cycle for at least one year (1.8 ± 1.2 years, range 1-5 years) prior to testing. As expected, [E₂] and [P₄] were significantly higher in the PRE group.

Dynamic lung function and resting cardiorespiratory variables are shown in Table 4.2. Menopausal status had no effect on spirometric measures of pulmonary function. Percent predicted values for all four of the lung functions measured did not differ between PRE and POST groups. Compared with PRE, resting tidal volume (Vₜ) was significantly lower (by ~140 mL or 22%) in the POST group; however, this difference disappeared when Vₜ was corrected for body weight. The POST group also exhibited a higher breathing frequency relative to the PRE group. The net effect of the abovementioned changes in breathing pattern was no difference in V̇ₚ. Menopausal status did not affect any of the remaining resting cardiorespiratory measures.

Resting plasma biochemistry variables were unavailable for one of the PRE subjects. No significant between-group differences were noted for [H⁺] despite significant differences in two of Stewart’s independent predictors of [H⁺], PaCO₂ and [SID], which were both higher in the POST relative to the PRE group (Table 4.3). Although [SID] was higher in the POST group, significant between-group differences were not observed in the individual electrolytes (Table 4.3). Both PaCO₂ and [SID] were
found to be significantly correlated with $[P_d]$ ($r = -0.39$ and $r = -0.46$ for PaCO$_2$ and [SID] respectively).

The POST group exhibited significantly higher levels of [HCO$_3^-$] and plasma osmolality relative to the PRE group (Table 4.3). Plasma osmolality was significantly correlated with $[E_2]$ ($r = -0.45$). No significant between-group differences were observed for $[A_{TOT}]$, Stewart’s third independent predictor of $[H^+]$ (Table 4.3). Plasma levels of albumin and phosphate were also unaffected by menopausal status (Table 4.3).

**Ventilatory Chemoreflex Characteristics**

The ventilatory responses to carbon dioxide under hyperoxic and hypoxic rebreathing conditions are displayed in Table 4.4 and illustrated in Figure 4.1. Complete rebreathing data was only available for 17 PRE (or 81%) and 14 POST (or 93%) subjects. Despite familiarization of subjects to the rebreathing procedure, discomfort and anxiety during rebreathing was cited as the main reason for the participants’ inability to complete rebreathing under both hyperoxic and hypoxic conditions.

The ventilatory response to hypocapnia (i.e., subthreshold ventilation, $\dot{V}_E$) did not differ between groups under either the hyperoxic or hypoxic rebreathing condition. However, significant effects of rebreathing condition existed such that $\dot{V}_E$ was higher during the hypoxic rebreathing trial (Table 4.4). No significant group*condition interaction was observed, indicating that the magnitude of the difference in $\dot{V}_E$ across hyperoxic and hypoxic conditions was not influenced by menopausal status.

In accordance with our original hypothesis, the VRT$\text{CO}_2$ was significantly higher in POST vs. PRE group under both hyperoxic and hypoxic rebreathing conditions (Table 4.4). Significant effects of rebreathing condition also existed such that VRT$\text{CO}_2$ was
higher in the hyperoxic condition. No significant group*condition interaction was observed.

Central, but not peripheral \( \dot{V}E_s \) was significantly lower in the POST vs. PRE group. In other words, the slope of the ventilatory response to progressive hypercapnia was consistently reduced in the POST vs. PRE women under hyperoxic, but not hypoxic, rebreathing conditions. There was no difference in the relative contribution of the peripheral chemoreflex to the ventilatory response to hypoxic \( CO_2 \) rebreathing (calculated as the mean difference in the hypoxic and hyperoxic \( \dot{V}E_s \); peripheral contribution of 0.55 \( \pm \) 2.3 L/min/mmHg (or 10%) and 0.69 \( \pm \) 0.9 L/min/mmHg (or 21%) for the PRE and POST groups, respectively (\( P=0.823 \)). Taken together, these data suggest that the effect of menopause on ventilatory control by the chemoreflexes is expressed primarily by changes in central (but not peripheral) ventilatory chemoreflex responsiveness. Significant effects of rebreathing condition also existed such that \( \dot{V}E_s \) was lower in the hyperoxic condition (Table 4.4). No significant group*condition interaction was observed.

Additional analyses were performed to determine whether the measured biochemical variables (Table 4.3) accounted for the differences in the significant \( CO_2 \) rebreathing responses in PRE and POST women noted in Table 4.4. The first step in that process was to determine which biochemical variables were correlated to the significantly different ventilatory chemoreflex characteristics (\( \dot{V}E_b \), VRT\( CO_2 \) or \( \dot{V}E_s \)) as shown in Table 4.5. The significant correlates for each rebreathing characteristic under hyperoxic and/or hypoxic rebreathing conditions were then entered into a stepwise linear regression model to determine the independent predictors of the respective rebreathing characteristic. VRT\( CO_2 \) was predicted by \( PaCO_2 \) under hyperoxic and hypoxic rebreathing conditions while \( \dot{V}E_s \) was predicted by \( [P_a] \) under the hyperoxic trial.
The final set of analyses considered whether the differences in rebreathing responses in the PRE and POST groups were accounted for by the biochemical variables that were identified as significant independent predictors of the chemoreflex characteristics. Table 4.6 lists the adjusted group means (adjusted for significant independent predictors) for the VRTCO2 under hyperoxic and hypoxic conditions as well as \( \dot{V}_E \) under hyperoxic conditions. In all cases, adjusting for the significant predictors reduced the magnitude of the differences in VRTCO2 and \( \dot{V}_E \) between the PRE and POST groups. This is illustrated in Figure 4.2. Significant between-group differences remained in the VRTCO2 under hyperoxic conditions (\( p=0.037 \)) after adjusting for PaCO2 although significant group differences did not remain in the VRTCO2 under hypoxic conditions following adjustment of for PaCO2 (\( p=0.108 \)) or \( \dot{V}_E \) under hyperoxic conditions following adjustment for [P4] (\( p=0.141 \)).
DISCUSSION

We found that significant differences existed in the ventilatory status and chemoreflex control of breathing in postmenopausal women. Our findings shed new light on the factors influencing chemoreflex status in this population of women and provide insight into the respiratory set point upon which other physiological or pathological perturbations are imposed. Our findings are of relevance to those interested in sex differences in the control of breathing and the impact of aging and menopause.

The main findings of our study were: 1) a significantly higher PaCO₂ in the POST vs. the PRE group that was associated with 2) a higher VRTCO₂ and decreased ñES of the central ventilatory chemoreflex response to CO₂ in the POST vs. PRE group, and 3) adjusting for PaCO₂ and [P₄] as predictors of the chemoreflex control characteristics reduced the magnitude of the difference between groups supporting their role in the chemoreflex control of breathing.

Effect of Menopause on the Chemoreflex Control of Breathing

As predicted, the POST group exhibited a significantly higher PaCO₂ vs. the PRE group. As PRE and POST groups did not differ in metabolic rate or in peripheral and nonchemoreflex drives to breathe, group differences in PaCO₂ are likely explained by changes in the central ventilatory chemoreflex alone. Indeed the POST group had a significantly higher central VRTCO₂ and a reduced ñES in comparison to the PRE group.

It is widely held that P₄ and E₂ can affect ventilatory drive (4) such that higher levels of circulating P₄ and E₂ are associated with reductions in PaCO₂ (43, 55, 93). This statement is supported by both the mean and correlative data of the current study in which the POST group had significantly lower levels of P₄ and E₂ in association with higher PaCO₂ values relative to the PRE group.
P₄ appears to exert its effect (in part) centrally via an E₂-dependent, P₄ receptor-mediated mechanism (4, 6) while E₂ seems to up-regulate the number of P₄ receptors in the hypothalamus by increasing the amount of P₄ receptor mRNA (4). Thus, the reduced E₂ observed in our POST women would be predicted to decrease the amount of P₄ receptors available to bind substrate. This in addition to lower circulating levels of P₄ would be expected to reduce ventilation following the onset of menopause. However significant group differences in ventilation were not observed in the present study. As such, postmenopausal reductions in [P₄] and [E₂] may be associated with increases in PaCO₂ due to their direct or indirect effect on the central chemoreflex drive to breathe.

Numerous studies have highlighted an association between ovarian hormone levels with V˙ES and VRTCO₂. With respect to V˙ES, increases in endogenous or synthetic P₄ and E₂ have been shown to significantly increase central V˙ES (16, 41, 55, 99, 108). Our data suggest this relation also holds true for postmenopausal reductions in P₄ and E₂, which are associated with a reduced V˙ES. Indeed, this conclusion is further strengthened by our pooled group data, which revealed a significant positive correlation between V˙ES and [P₄].

Similar to the correlation observed for V˙ES, VRTCO₂ was negatively correlated to [P₄] within the pooled group data. This appears to be a robust relationship as it has also been observed in pooled data from pregnant and non-pregnant women (55). There is also a trend for VRTCO₂ to be lower during the LP of the menstrual cycle when [P₄] is elevated (115). Taken together, the above data suggests that the chemical control of ventilation, specifically the threshold and sensitivity of the central ventilatory chemoreflex response to CO₂, is altered during menopause and that these changes are in part due to the removal of P₄ and E₂ as important respiratory stimulants.
Menopausal status did not have an effect on [H\(^+\)] due to the offsetting effects of increases in both PaCO\(_2\) and [SID] in the POST group. Applying Stewart's approach to acid-base regulation, an increase in [H\(^+\)] due to an elevation in PaCO\(_2\) would be offset by a decrease in [H\(^+\)] due to the small yet significant increase in [SID] (or vice versa) within the POST group such that [H\(^+\)] does not differ between groups. As menopause exerts an effect on the independent predictors of [H\(^+\)] thereby altering the relationship between each independent predictor and [H\(^+\)], one might expect that menopausal differences would also exist within the chemoreflex control of ventilation due to the differing relationship between [H\(^+\)] and PaCO\(_2\). It has been suggested that disturbances in acid-base status may alter the ventilatory chemoreflexes by changing the relationship between PaCO\(_2\) and [H\(^+\)], which represent the measured and the ‘actual’ stimulus of the chemoreceptors respectfully (30). Our study would support this hypothesis.

Strong correlations were noted between [SID] and the VRT\(_{CO2}\) and \(\dot{V}_E\) of the central chemoreflex. Group differences in VRT\(_{CO2}\) and [SID] are consistent with the physiological regulatory responses observed by Anderson and Jennings (1), where dietary reductions in [SID] in a canine model (simulating metabolic acidosis), were accompanied by compensatory reductions in the VRT\(_{CO2}\), such that PaCO\(_2\) was reduced and [H\(^+\)] was unchanged. In our study, it is likely that postmenopausal increases in [SID] are offset by increases in PaCO\(_2\) due to remodelling of the central chemoreflex, such that the VRT\(_{CO2}\) is increased in the POST vs. PRE women despite the absence of change in [H\(^+\)]. Using a mathematical, steady state modeling approach to describe the chemoreflex control of breathing, Duffin (30) noted that consistent with our experimental observations, simulated increases in [SID] (metabolic alkalosis) primarily affected the chemoreflex control of ventilation by altering the VRT\(_{CO2}\), without any change to the threshold in terms of [H\(^+\)].
As changes in sensitivity have not been associated with [SID] changes alone (1) it is possible that the relationship between [SID] and \(\dot{V}_E\) may be due in part to the effect of \([P_4]\) on [SID]. This is supported by the correlation between [SID] and \([P_4]\) within the pooled group data. A similar correlation was observed in pregnant and non-pregnant females (43). Current evidence suggests that the renin-angiotensin-aldosterone system (RAAS) is activated in response to rises in \(P_4\) and \(E_2\) (83). Activation of the RAAS would decrease plasma osmolality, subsequently lowering [SID] as well. The lower \(E_2\) and \(P_4\) that we observed in the POST vs. the PRE group may have altered the RAAS subsequently changing plasma osmolality and [SID]. Reductions in osmolality have also been shown to stimulate \(\dot{V}_E\) (51). Although significant relationships were not noted between osmolality and measures of \(\dot{V}_E\), the POST group did exhibit significantly higher plasma osmolalities relative to the PRE, which may have reduced \(\dot{V}_E\) (51) and contribute to the higher \(PaCO_2\) observed in the POST.

**Critique of Methods**

A critique of Duffin’s modified rebreathing protocol has been described previously (54, 75, 76). Duffin’s (33) protocol, which is based on Read’s original rebreathing technique, has many methodological and interpretive advantages over other techniques used to assess the ventilatory chemoreflexes, including Read’s (96) rebreathing technique, steady state or end-tidal CO\(_2\) forcing (11), and progressive isocapnic hypoxia (126). Advantages (33) include estimation of \(\dot{V}_{E_{02}}\) and the direct measurement of the \(VRT_{CO_2}\) vs. extrapolation of the linear relation between \(\dot{V}_E\) and \(PaCO_2\) to the x-axis routinely used by other methods (25).

However, results obtained using Duffin’s (33) protocol may be influenced by a short-term potentiation or neural after-discharge effect (36). Such an effect could
influence the estimate of $\dot{V}E_b$ due to the inclusion of prior hyperventilation (33). This is unlikely in our study, as this effect rarely occurs in healthy individuals following hypocapnic hyperventilation (76), and because subjects used a deep and deliberate breathing pattern during hyperventilation to avoid the occurrence of such an effect (36). As $\dot{V}E_b$ is also influenced by an individual’s state of arousal and anxiety (111), attempts were made to reduce anxiety/arousal by including a familiarization rebreathing trial and controlling lab conditions (i.e. noise, lighting) during testing.

Although there is variability in the measurement of the hypercapnic and hypoxic ventilatory response to CO$_2$ (107, 134) our laboratory recently reported within-subject, between-day coefficients of variations (CVs) of 4 and 3% for hyperoxic and hypoxic $\text{VRT}_{\text{CO}_2}$ respectively and CVs of 21 and 23% for hyperoxic and hypoxic $\dot{V}E_s$ (115). Thus the modified rebreathing technique is a robust method with respect to detecting physiological changes in $\text{VRT}_{\text{CO}_2}$ and $\dot{V}E_s$.

Previous studies from our laboratory have identified differences in the primary outcome variables of our study in other female subgroups using identical methods and similar sample sizes (55, 81, 93, 115). As such we are confident that the methods used by this study were sensitive enough to detect differences between our pre- and postmenopausal groups.

A design limitation of the current study is the cross-sectional nature of the subject population. Although a longitudinal design would be ideal to test our hypotheses, the time required to complete such a study is impractical. We attempted to ameliorate the effects of the cross-sectional design by matching groups for physical characteristics, lung function, resting cardiorespiratory measures and physical fitness. In addition, both groups were similar with respect to age, which is a clear strength of our study compared to a design using younger premenopausal women (26). Given that menopause is an
age-linked biological phenomenon it would be exceedingly difficult to completely match our groups for age and indeed, could induce another bias due to the required pre-selection of women who are premenopausal beyond the age normally reported. As age affects ventilatory mechanics (117) amongst several other biochemical factors, we ensured via statistical means, that the observed group differences in the current study were driven primarily by menopausal status as opposed to age. However, because menopause and age are linked to one another, we cannot completely dismiss that the differences between pre- and postmenopausal women were in part accounted for by the slight differences in age in these two groups of women.

**Conclusion**

In summary, we assessed chemoreflex and nonchemoreflex drives to breathe in pre- and postmenopausal women of the same relative age. Relative to premenopausal women, postmenopausal women exhibited a higher resting PaCO₂ that was attributable to increases in the threshold and reduced sensitivity of the central ventilatory chemoreflex. The menopausal changes in the central ventilatory chemoreflex occurred in the absence of any change of [H⁺] and appear to be due, at least in part, to changes in P₄, PaCO₂ and [SID]. As we are currently living in an aging society, research examining the impact of menopause on female physiology is exceedingly important and is of particular relevance. Findings from the current study extend beyond our understanding of acid-base regulation and CO₂ control in postmenopausal women, having potentially relevant implications in understanding the increased occurrence of sleep apnea following the onset menopause.
Table 4.1. General subject characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>45.3 ± 3.2</td>
<td>52.1 ± 1.8**</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.4 ± 5.9</td>
<td>162.2 ± 8.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.2 ± 14.3</td>
<td>64.3 ± 8.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8 ± 4.6</td>
<td>24.5 ± 2.9</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118.5 ± 10.6</td>
<td>118.3 ± 8.0</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>72.1 ± 8.0</td>
<td>75.3 ± 6.6</td>
</tr>
<tr>
<td>[P₄], nmol/L</td>
<td>6.67 ± 9.7</td>
<td>1.1 ± 0.8 *</td>
</tr>
<tr>
<td>[E₂], pmol/L</td>
<td>306.1 ± 255.6</td>
<td>67.3 ± 49.1**</td>
</tr>
</tbody>
</table>

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; P₄, progesterone; E₂, estradiol. Square brackets indicate concentrations. *Significantly different from premenopausal group (P< 0.05). **Significantly different from premenopausal group (P<0.01).
Table 4.2. Dynamic lung function and resting cardiorespiratory variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak flow, L/sec</td>
<td>5.4 ± 0.8</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>% of predicted peak flow</td>
<td>85.9 ± 12.2</td>
<td>91.3 ± 14.6</td>
</tr>
<tr>
<td>FVC, L</td>
<td>3.2 ± 0.4</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>% of predicted FVC</td>
<td>89.9 ± 9.6</td>
<td>98.2 ± 15.1</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>% of predicted FEV₁</td>
<td>97.2 ± 10.8</td>
<td>103.3 ± 16.0</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>83.0 ± 7.1</td>
<td>79.6 ± 9.6</td>
</tr>
<tr>
<td>% of predicted FEV₁/FVC</td>
<td>108.4 ± 9.4</td>
<td>105.8 ± 12.4</td>
</tr>
<tr>
<td>(\dot{V}_E), L/min</td>
<td>8.0 ± 1.7</td>
<td>8.5 ± 4.5</td>
</tr>
<tr>
<td>(V_t), mL</td>
<td>638.9 ± 228.0</td>
<td>498.7 ± 105.8*</td>
</tr>
<tr>
<td>(V_t/kg), mL/kg</td>
<td>8.4 ± 4.6</td>
<td>7.93 ± 1.7</td>
</tr>
<tr>
<td>(f), breaths/min</td>
<td>13.5 ± 3.2</td>
<td>17.0 ± 4.9*</td>
</tr>
<tr>
<td>(\dot{V}_O₂), mL/min</td>
<td>295.1 ± 39.7</td>
<td>298.1 ± 71.6</td>
</tr>
<tr>
<td>(\dot{V}_CO₂), mL/min</td>
<td>253.0 ± 31.7</td>
<td>262.6 ± 56.3</td>
</tr>
<tr>
<td>(\dot{V}_E/\dot{V}_O₂)</td>
<td>28.5 ± 8.3</td>
<td>34.6 ± 34.9</td>
</tr>
<tr>
<td>(\dot{V}_E/\dot{V}_CO₂)</td>
<td>32.4 ± 6.2</td>
<td>34.1 ± 18.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. FVC, forced vital capacity; FEV₁, forced expired volume in one second; \(\dot{V}_E\), minute ventilation; \(V_t\), tidal volume; \(V_t/kg\), tidal volume normalized for body weight; \(f\), breathing frequency; \(\dot{V}_O₂\), oxygen uptake; \(\dot{V}_CO₂\), carbon dioxide output; \(\dot{V}_E/\dot{V}_O₂\), ventilatory equivalent for oxygen; \(\dot{V}_E/\dot{V}_CO₂\), ventilatory equivalent for carbon dioxide. *Significantly different from premenopausal group (\(P<0.05\)).
Table 4.3. Resting plasma biochemistry including [H⁺] and its independent determinants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H⁺], nEq/L</td>
<td>38.4 ± 2.0</td>
<td>38.7 ± 2.6</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>37.7 ± 3.9</td>
<td>41.2 ± 4.3*</td>
</tr>
<tr>
<td>[SID], mEq/L</td>
<td>37.0 ± 1.4</td>
<td>38.7 ± 1.9**</td>
</tr>
<tr>
<td>[Na⁺], mmol/L</td>
<td>136.9 ± 1.2</td>
<td>136.9 ± 2.1</td>
</tr>
<tr>
<td>[K⁺], mmol/L</td>
<td>4.0 ± 0.4</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>[Ca²⁺], mmol/L</td>
<td>1.09 ± 0.0</td>
<td>1.11 ± 0.0</td>
</tr>
<tr>
<td>[Cl⁻], mmol/L</td>
<td>105.1 ± 1.9</td>
<td>103.9 ± 1.8</td>
</tr>
<tr>
<td>[LA⁻], mmol/L</td>
<td>1.0 ± 0.6</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>[ATOT], mEq/L</td>
<td>16.4 ± 0.6</td>
<td>16.5 ± 0.7</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>41.7 ± 1.6</td>
<td>42.3 ± 2.3</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg/H₂O</td>
<td>280.2 ± 3.6</td>
<td>283.9 ± 3.2**</td>
</tr>
<tr>
<td>[HCO₃⁻], mmol/L</td>
<td>23.7 ± 1.4</td>
<td>25.5 ± 1.5**</td>
</tr>
</tbody>
</table>

Values are means ± SD. H⁺, hydrogen ion; PaCO₂, arterial partial pressure of carbon dioxide; SID, strong ion difference; Na⁺, sodium; K⁺, potassium; Ca²⁺, calcium; Cl⁻, chloride; La⁻, lactate; ATOT, total weak acid; HCO₃⁻, bicarbonate. Square brackets indicate concentrations. *Significantly different from premenopausal group (P<0.05), **Significantly different from premenopausal group (P<0.01).
Table 4.4. Ventilatory responses to carbon dioxide under hyperoxic and hypoxic rebreathing conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperoxic Rebreathing Response</th>
<th>Hypoxic Rebreathing Response</th>
<th>P for Group</th>
<th>P for Condition</th>
<th>P Group* Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premenopausal (n=20)</td>
<td>Postmenopausal (n=15)</td>
<td>Premenopausal (n=17)</td>
<td>Postmenopausal (n=14)</td>
<td></td>
</tr>
<tr>
<td>$\dot{V}_{EB}$, L/min</td>
<td>10.6 ± 5.2</td>
<td>10.2 ± 5.5</td>
<td>14.1 ± 5.4</td>
<td>11.4 ± 4.5</td>
<td>0.483</td>
</tr>
<tr>
<td>VRT$CO_2$, mmHg</td>
<td>45.7 ± 3.4</td>
<td>49.2 ± 3.0**</td>
<td>42.2 ± 3.1</td>
<td>45.1 ± 3.2*</td>
<td>0.005</td>
</tr>
<tr>
<td>$\dot{V}_{ES}$, L/min/mmHg</td>
<td>3.3 ± 1.6</td>
<td>2.2 ± 0.8*</td>
<td>3.9 ± 2.1</td>
<td>2.9 ± 0.9</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Values are means ± SD. $\dot{V}_{EB}$, basal ventilation; VRT$CO_2$, the ventilatory recruitment threshold for CO$_2$; $\dot{V}_{ES}$, ventilatory chemoreflex sensitivity to CO$_2$. Condition is referring to the rebreathing trial (hyperoxic or hypoxic). *Significantly different from premenopausal group within condition (P< 0.05). **Significantly different from premenopausal group within condition (P< 0.01).
Table 4.5. Correlations (r values) between chemoreflex characteristics and blood biochemistry measurements in pre- and postmenopausal groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperoxic Rebreathing Responses</th>
<th>Hypoxic Rebreathing Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \dot{V}_B ) L/min</td>
<td>VRT( \text{CO}_2 ) mmHg</td>
</tr>
<tr>
<td>[H(^+)], nEq/L</td>
<td>-0.45**</td>
<td>0.30</td>
</tr>
<tr>
<td>PaCO(_2), mmHg</td>
<td>-0.36*</td>
<td>0.67**</td>
</tr>
<tr>
<td>[SID], nEq/L</td>
<td>-0.17</td>
<td>0.53**</td>
</tr>
<tr>
<td>[ATOT], mEq/L</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>[P(_4)], nmol/L</td>
<td>0.02</td>
<td>-0.37*</td>
</tr>
<tr>
<td>[E(_2)], pmol/L</td>
<td>-0.00</td>
<td>-0.17</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH(_2)O</td>
<td>-0.01</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\( \dot{V}_B \), basal minute ventilation; VRT\( \text{CO}_2 \), ventilatory recruitment threshold for carbon dioxide; \( \dot{V}_E \), ventilatory chemoreflex sensitivity to CO\(_2\); H\(^+\), hydrogen ion; PaCO\(_2\), arterial partial pressure of carbon dioxide; SID, strong ion difference; ATOT, total weak acid; P\(_4\), progesterone; E\(_2\), estradiol. Square brackets indicate concentration. Bolded values indicate significant correlations. *Significant correlation (P<0.05). **Significant correlation (P<0.01).
Table 4.6. Estimated adjusted group means for the significant ventilatory chemoreflex characteristics under hyperoxic and hypoxic rebreathing conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxic VRTCO2, mmHg</td>
<td>46.2 ± 2.7 a</td>
<td>48.3 ± 2.7 a **</td>
</tr>
<tr>
<td>Hypoxic VRTCO2, mmHg</td>
<td>42.6 ± 3.0 a</td>
<td>44.5 ± 3.0 a</td>
</tr>
<tr>
<td>Hyperoxic $\dot{V}E_S$, L/min/mmHg</td>
<td>3.1 ± 1.4 b</td>
<td>2.4 ± 1.4 b</td>
</tr>
</tbody>
</table>

Values are adjusted means ± SD. Values were adjusted for the independent predictors of the ventilatory characteristics. Symbols represent means adjusted for: a, arterial partial pressure of carbon dioxide; b, progesterone; VRTCO2, the ventilatory recruitment threshold for CO2; $\dot{V}E_S$, ventilatory chemoreflex sensitivity to CO2. *Significantly different from premenopausal group (P<0.05).
FIGURE LEGEND

Figure 4.1. Mean chemoreflex responses in the pre- and postmenopausal groups.

A, PRE (dashed line) and POST (solid line) group chemoreflex responses under hyperoxic conditions. Subthreshold ventilations ($\tilde{V}_E$) are similar in both groups although the PRE group exhibits a lower ventilatory recruitment threshold for CO$_2$ (VRT$_{CO_2}$) and a greater ventilatory sensitivity ($\tilde{V}_E$S) relative to the POST group. B, PRE and POST group chemoreflex responses under hypoxic conditions. Basal ventilation is approximately 3L/min greater in the PRE group relative to the POST. Again, the PRE group exhibits a lower ventilatory recruitment threshold and sensitivity when compared to the POST group. PET$_{CO_2}$, end tidal partial pressure of CO$_2$; BTPS, body temperature pressure saturated.

Figure 4.2. Differences in the unadjusted (black bars) and estimated adjusted means (grey bars) for the rebreathing characteristics that differed significantly between pre- and postmenopausal groups under hyperoxic and hypoxic rebreathing conditions. In all cases, adjusting for the significant predictors (described in the text) reduced the magnitude of the difference in the rebreathing characteristics between the PRE and POST groups for A, VRT$_{CO_2}$ under hyperoxic conditions, B, $\tilde{V}_E$S under hyperoxic conditions and C, VRT$_{CO_2}$ under hypoxic conditions. Significant group differences only persisted in the VRT$_{CO_2}$ under hyperoxic conditions once adjusting for its significant predictor (p=0.04).
Figure 4.1.

(A) Hyperoxic

(B) Hypoxic
Figure 4.2.

A. Differences in \( \Delta \text{VRTCO}_2 \) between PRE & POST for Hyperoxic condition.

B. Differences in \( \Delta V \text{E} \) between PRE & POST for Hyperoxic condition.

C. Differences in \( \Delta \text{VRTCO}_2 \) between PRE & POST for Hypoxic condition.
CHAPTER 5  GENERAL DISCUSSION

5.1. Clinical Implications

Our findings show that although \([H^+]\) is not altered by menopausal status, the mechanism by which \([H^+]\) is regulated pre- and postmenopausally is inherently different. Although the exact mechanism of how acid-base regulation is altered following menopause cannot be identified here, it appears that the menopausal withdrawal of \(P_4\) and \(E_2\) is partially responsible for alterations in the independent predictors of \([H^+]\). Postmenopausally, it would seem that the acidifying effects of increases in \(\text{PaCO}_2\) are offset by the alkalizing effect of increases in \([\text{SID}]\) (or vice versa) such that \([H^+]\) does not differ from premenopausal values.

Furthermore, the central chemoreflex drive to breathe is altered following menopause such that postmenopausal women exhibit a higher threshold and a lower sensitivity in the response to \(CO_2\) relative to premenopausal women of a similar age. Again, the current study cannot identify whether menopausal differences in the central ventilatory chemoreflex are the caused by the observed increased \(\text{PaCO}_2\) following menopause or an adaptation in response to a menopausal change in \([\text{SID}]\) (or for that matter, a change in another variable not measured in the current study).

In addition to providing valuable insight into how acid-base regulation and the chemical control of breathing differ between healthy pre- and postmenopausal women, our findings may also have particular relevance in other clinical fields and areas of research including:

1. Understanding the accelerated bone loss and development of osteopenia and osteoporosis commonly observed in females following menopause: The skeleton is the primary source of stored \(CO_2\) (87) and serves as an important buffer system for excess \([H^+]\) when the kidneys and lungs are unable to cope with the additional \([H^+]\) (17, 73). Both metabolic and respiratory acidosis have been
implicated as causal mechanisms in the release of bone mineral content and bone loss in humans (27, 63). As acid-base status has been determined as an important regulator of the bone remodelling process (3, 62, 73), understanding how acid-base status is regulated following menopause might provide invaluable insight into potential causal mechanisms for the bone loss observed in postmenopausal females. Results from the current study would suggest that differences in [H+] between pre- and postmenopausal women are not responsible for the accelerated bone loss following menopause as [H+] did not differ between groups. Although our findings do not support the role of menopausal differences in [H+] as a potential cause of bone loss, measures of bone metabolism were not examined and compared to measures of acid-base status herein and as such, this area warrants further examination.

2. Understanding the increased occurrence of sleep disordered breathing following menopause: In the current study, the ventilatory recruitment threshold for CO₂ (VRTCO₂) differed significantly between the pre- and postmenopausal groups by approximately 4 mmHg with a similar group difference in resting PaCO₂. As the wakefulness drive to breath did not differ between groups, the change in ventilation from wakefulness to a sleeping state should theoretically be the same in both pre- and postmenopausal women (i.e. sleep induced hypoventilation and hypercapnia should be the same). Relative to premenopausal women, the higher VRTCO₂ in menopausal women would in theory, increase their predisposition to experiencing an apneic event due to the fact that they are operating on the flatter part of the metabolic hyperbola (i.e. the relationship between ventilation and PaCO₂ at a given CO₂ production) where relatively small increases in ventilation can lower PaCO₂ below the apneic threshold (131). However, the reduced sensitivity of the central ventilatory chemoreflex response to CO₂ in
postmenopausal women may serve to stabilize ventilation during sleep by decreasing the amount of recurring apneas following an initial apneic event as the central chemoreceptors are less sensitive to CO₂ and as such are less susceptible to inducing a ventilatory overshoot of the apneic threshold.

5.2. Limitations of the Current Study

The limitations of the current study are as follows:

1. The cross-sectional design: Although there are inherent drawbacks of conducting a cross-sectional study as opposed to a longitudinal study, the time required to complete a longitudinal study for a M.Sc. thesis was impractical. Attempts were made to rectify this shortcoming by recruiting pre- and postmenopausal participants with similar physical characteristics, lung function measures, resting cardiorespiratory measures, and physical fitness.

2. The statistically significant differences in age between groups: It is known that age affects lung structure and ventilatory mechanics (50, 117). However, given that menopause is an age-related phenomenon, it was not possible to match the pre- and postmenopausal groups for age. Furthermore, matching for age might have induced another bias due to the required pre-selection of women who are premenopausal beyond the age normally reported (~51 years; (70)). To account for this potential confounder, we verified via statistical means, that the observed group differences were driven by menopausal status and were not strictly age-related.

3. We did not tightly regulate or control dietary intake or fluid consumption. Inter-individual differences in salt and fluid intake may have potentially influenced plasma osmolality and [SID] measures.
5.3. Future Directions

As with all areas of research, the directions of future research in this particular field are numerous and extend from the examination of processes at a molecular level to examination of systemic functioning in clinical populations. In general, future research should be directed towards eliminating the limitations of the current study and should build upon the current findings. Possible directions of study include:

1. Conducting a similar study in which the limitations of the present study are better addressed.

2. Measurements of circulating levels of arginine vasopressing (AVP) and angiotensin II are collected and assessed in both groups to further explore the potential influence of endogenous ovarian hormone levels on RAAS activation and AVP release. Measurements of these particular hormones would provide additional insight into the [SID] and plasma osmolalities observed within both groups in addition to being potentially relevant to understanding differences in the chemical control of ventilation between pre- and postmenopausal women as per Jennings’ hypothesis (51, 53).

3. Compare and contrast the chemoreflex and non-chemoreflex drives to breathe between postmenopausal women and other healthy (i.e. males similar in age) and diseased populations (i.e. similarly aged women with varying degrees of respiratory insufficiency) to see whether differences exist in the chemical control of breathing. Such studies would also benefit from the incorporation of Borg measurements to identify and compare potential differences in breathing discomfort between groups.

4. Examine acid-base regulation and bone metabolism during the menopausal transition to see whether alterations in acid-base regulation parallel the dramatic increase in bone metabolism observed in females in the first few years following
the onset of menopause. A similar study could be conducted with respect to the development of sleep disordered breathing following the onset of menopause.

5. Examine the effect of hormone replacement therapy (progesterone and estrogen alone and in combination) in all of the aforementioned study suggestions.
REFERENCES:


49. Hughson RL, Northey DR, Xing HC, Dietrich BH, and Cochrane JE. Alignment of ventilation and gas fraction for breath-by-breath respiratory gas


56. **Jordan AS, Catcheside PG, Orr RS, O'Donoghue FJ, Saunders NA, and McEvoy RD.** Ventilatory decline after hypoxia and hypercapnia is not


63. **Lemann J, Litzow JR, and Lennon EJ.** The Effects of Chronic Acid Loads in Normal Man: Further Evidence for the Participation of Bone Mineral in


82. **Netzer NC, Eliasson AH, and Strohl KP.** Women with sleep apnea have lower levels of sex hormones. *Sleep and Breathing* 7: 25-29, 2003.


87. **Pasquale SM, Messier AA, Shea ML, and Schaefer KE.** Bone CO2-titration curves in acute hubercapnia obtained with a modified titration technique.


105. **Saaresranta T, Irjala K, Polo-Kantola P, and Polo O.**


108. **Schoene RB, Robertson HT, Pierson DJ, and Peterson AP.**


Appendix A1. Significant correlations for biochemical variables in pre- (closed diamonds) and postmenopausal (open diamonds) groups at rest. R values represent pooled data from both groups.
Appendix A1. Significant correlations for biochemical variables in pre- (closed diamonds) and postmenopausal (open diamonds) groups at rest. R values represent pooled data from both groups.
APPENDIX A2

CHAPTER 3 – Acid-base Manuscript Significant Correlations during Exercise at 110% $T_{VENT}$
Appendix A2. Significant correlations for measured biochemical variables in pre-(closed diamonds) and postmenopausal (open diamonds) groups during exercise at 110% of $T_{VENT}$. R values represent pooled data from both groups.
Appendix A2. Correlations between $\left[HCO_3^-\right]$ and $[\text{SID}]$ in pre- (closed diamonds) and postmenopausal (open diamonds) groups during exercise at 110% $T_{\text{VENT}}$. A significant correlation existed within the postmenopausal ($p=0.000$) but not in the premenopausal ($p=0.491$) group.
APPENDIX B

Description of CO$_2$ Rebreathing Analysis with a Representative Plot
Rebreathing Data Analysis:

Breath-by-breath data accumulated from each rebreathing trail was analyzed using a spreadsheet designed specifically for this purpose (Microsoft Excel). Prior to analysis, data from the initial equilibration at the start of rebreathing in addition to outlying data points resulting from aberrant breaths, sighs and swallows were excluded from analysis. Breath-by-breath measurements of $P_{ET}$ CO$_2$ were plotted against time and fitted with a least-squares regression line whose slope is dependent upon the metabolic production of CO$_2$. Panel A displays a representative PCO$_2$ vs. time plot from one of the study’s premenopausal subjects. The equation of the regression line shown in panel A allows for the prediction of $P_{ET}$CO$_2$ vs. time, thereby minimizing interbreath variability due to measurement. $\dot{V}_E$ was then plotted against $P_{ET}$CO$_2$ values, as shown for the same premenopausal woman in Panel B of the figure.

The $\dot{V}_E$ vs. $P_{ET}$CO$_2$ plot was analyzed by fitting a model made up of the sum of two segments separated by a breakpoint. Both segments are fitted through an iterative process whereby the breakpoint and other parameters (i.e. exponential fit coefficients) are varied to obtain an optimal fit to the observed data by minimizing the sum of squares (Levenberg-Marquardt algorithm) using commercial software (courtesy Dr. J. Duffin, University of Toronto; written using Labview, National Instruments Austin, Texas). The first segment of the $\dot{V}_E$ vs. $P_{ET}$CO$_2$ plot displayed in panel B shows a slight exponential decline to a subthreshold $\dot{V}_E$ ($\dot{V}_{EB}$). Fitting the exponential decline accounts for any short-term potentiation induced by 5 minutes of prior hyperventilation. The second segment of the $\dot{V}_E$ vs. $P_{ET}$CO$_2$ plot is a linear segment that extends up from the breakpoint. This segment shows the linear increase in $\dot{V}_E$ with increasing $P_{ET}$CO$_2$ above the breakpoint.

In addition to fitting each segment, the curve-fitting program simultaneously calculates the exponential constants, basal values, thresholds and slopes that describe
the two segments of the rebreathing response. The first segment of the rebreathing response shown in panel B depicts the representative subject's \( \dot{V}_{EB} \) and represents their non-chemoreflex drive to breathe. The breakpoint separating the two segments is taken as a measure of the chemoreflex threshold for \( \dot{V}_E \) to progressive increases in CO\(_2\) during a rebreathing condition (i.e., under hyperoxic or hypoxic conditions). This breakpoint is termed the ventilatory recruitment threshold for CO\(_2\) (VRT\(_{CO2}\)). The slope of the linear segment following the VRT\(_{CO2}\) is taken as the chemoreflex sensitivity to CO\(_2\) (VRT\(_S\)).
Appendix B. Respiratory responses of a modified rebreathing test under hyperoxic conditions in a representative premenopausal subject. $\dot{V}_B$, subthreshold ventilation; $\text{VRTCO}_2$, ventilatory recruitment threshold for CO$_2$; $\dot{V}_S$, ventilatory chemoreflex sensitivity to CO$_2$; BTPS, body temperature pressure saturated; $\dot{V}_E$, minute ventilation; $P_{\text{ETCO}_2}$, end tidal partial pressure of CO$_2$. 
APPENDIX C

CHAPTER 4 – Ventilatory Chemoreflex Manuscript Significant Correlations
Appendix C. Significant correlations between chemoreflex characteristics and blood biochemistry measurements in pre- (closed diamonds) and postmenopausal (open diamonds) subjects. R values were derived from pooled data.
Appendix C. Significant correlations between chemoreflex characteristics and blood biochemistry measurements in pre- (closed diamonds) and postmenopausal (open diamonds) subjects. R values were derived from pooled data.
Appendix C. Significant correlations between blood biochemistry measurements in pre- (closed diamonds) and postmenopausal (open diamonds) subjects. R values were derived from pooled data.
APPENDIX D

Examples of Statistical Tests Used
Independent T-Test: Peak Flow PRE vs. POST

Group Statistics

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>PkFlwT2</td>
<td>1</td>
<td>21</td>
<td>5.352</td>
<td>.8177</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>5.353</td>
<td>.6885</td>
</tr>
</tbody>
</table>

Independent Samples Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Levene's Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
<td>t</td>
</tr>
<tr>
<td>PkFlwT2</td>
<td>.409</td>
<td>.527</td>
<td>-.004</td>
</tr>
<tr>
<td></td>
<td>Equal variances assumed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equal variances not assumed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Pearson Correlation

Correlations

<table>
<thead>
<tr>
<th>Group</th>
<th>PCO2Rest</th>
<th>SIDRest</th>
<th>P4</th>
<th>VEBHyp</th>
<th>VRTC02Hyp</th>
<th>VESHyp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group Pearson Correlation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO2Rest</td>
<td>.399**</td>
<td>.463**</td>
<td>-.357*</td>
<td>-.039</td>
<td>.486**</td>
<td>-.370*</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.018</td>
<td>.005</td>
<td>.033</td>
<td>.826</td>
<td>.003</td>
<td>.029</td>
</tr>
<tr>
<td>N</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>SIDRest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO2Rest</td>
<td>.399**</td>
<td>.622**</td>
<td>-.385*</td>
<td>-.358*</td>
<td>.667**</td>
<td>-.282</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.018</td>
<td>.000</td>
<td>.022</td>
<td>.038</td>
<td>.000</td>
<td>.106</td>
</tr>
<tr>
<td>N</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>P4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEBHyp</td>
<td>-.357*</td>
<td>-.385*</td>
<td>-.456**</td>
<td>1</td>
<td>.330</td>
<td>.001</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.033</td>
<td>.022</td>
<td>.006</td>
<td>.931</td>
<td>.030</td>
<td>.015</td>
</tr>
<tr>
<td>N</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>VRTC02Hyp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEBHyp</td>
<td>-.357*</td>
<td>-.358*</td>
<td>-.172</td>
<td>.015</td>
<td>-1.00</td>
<td>.179</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.033</td>
<td>.022</td>
<td>.006</td>
<td>.931</td>
<td>.030</td>
<td>.015</td>
</tr>
<tr>
<td>N</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>VESHyp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRTC02Hyp</td>
<td>.486**</td>
<td>.667**</td>
<td>.528**</td>
<td>-.367*</td>
<td>-.100</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.003</td>
<td>.000</td>
<td>.001</td>
<td>.030</td>
<td>.566</td>
<td>.113</td>
</tr>
<tr>
<td>N</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

*: Correlation is significant at the 0.05 level (2-tailed).

**: Correlation is significant at the 0.01 level (2-tailed).
Linear Stepwise Regression: VRTCO2 hyper with PaCO₂, [SID], and [P₄] entered as possible predictors

[DataSt1] C:\Documents and Settings\graeme\My Documents\Megan\MScThesis\Analysis\REB Analysis Spreadsheet (10_04_07).sav

### Variables Entered/Removed

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCO2Rest</td>
<td></td>
<td>Stepwise (Criteria: Probability of F-to-enter &lt;= .050, Probability of F-to-remove &gt;= .100).</td>
</tr>
</tbody>
</table>

a. Dependent Variable: VRTCO2hyper

### Model Summary

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.667a</td>
<td>.445</td>
<td>.428</td>
<td>2.73548</td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), PCO2Rest

### ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>192.057</td>
<td>1</td>
<td>192.057</td>
<td>25.666</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>239.452</td>
<td>32</td>
<td>7.483</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>431.509</td>
<td>33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), PCO2Rest  
b. Dependent Variable: VRTCO2hyper

### Coefficients

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>25.934</td>
<td>4.212</td>
<td>6.158</td>
</tr>
<tr>
<td></td>
<td>PCO2Rest</td>
<td>.542</td>
<td>.107</td>
<td>.667</td>
</tr>
</tbody>
</table>

a. Dependent Variable: VRTCO2hyper

### Excluded Variables

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta In</th>
<th>t</th>
<th>Sig.</th>
<th>Partial Correlation</th>
<th>Collinearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIDRest</td>
<td>.183a</td>
<td>1.088</td>
<td>.285</td>
<td>.192</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>-.128a</td>
<td>-.897</td>
<td>.377</td>
<td>-.159</td>
</tr>
</tbody>
</table>

a. Predictors in the Model: (Constant), PCO2Rest  
b. Dependent Variable: VRTCO2hyper
Univariate Analysis of Variance: VRTCO2 hyper without PaCO2 entered as a covariate

[DataSet1] C:\Documents and Settings\graeme\My Documents\Megan\MScThesis\Analysis\REB Analysis Spreadsheet (10_04_07).sav

Between-Subjects Factors

<table>
<thead>
<tr>
<th></th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>104.511a</td>
<td>1</td>
<td>104.511</td>
<td>10.188</td>
<td>.003</td>
<td>.236</td>
</tr>
<tr>
<td>Intercept</td>
<td>77309.650</td>
<td>1</td>
<td>77309.650</td>
<td>7536.615</td>
<td>.000</td>
<td>.996</td>
</tr>
<tr>
<td>Group</td>
<td>104.511</td>
<td>1</td>
<td>104.511</td>
<td>10.188</td>
<td>.003</td>
<td>.236</td>
</tr>
<tr>
<td>Error</td>
<td>338.510</td>
<td>33</td>
<td>10.258</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>78536.410</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>443.020</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .236 (Adjusted R Squared = .213)

Estimated Marginal Means

Group

<table>
<thead>
<tr>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable: VRTCO2hyper</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Mean Std. Error 95% Confidence Interval</td>
</tr>
<tr>
<td>Lower Bound Upper Bound</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

Pairwise Comparisons

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval for Differencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>-3.492a</td>
<td>1.094</td>
<td>.003</td>
<td>-5.718 -1.266</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3.492a</td>
<td>1.094</td>
<td>.003</td>
<td>1.266 5.718</td>
</tr>
</tbody>
</table>

Based on estimated marginal means

a. The mean difference is significant at the .05 level.

Univariate Tests

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrast</td>
<td>104.511</td>
<td>1</td>
<td>10.188</td>
<td>.003</td>
<td>.236</td>
</tr>
<tr>
<td>Error</td>
<td>338.510</td>
<td>33</td>
<td>10.258</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The F tests the effect of Group. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
Univariate Analysis of Variance: VRTCO₂ hyper with PaCO₂ entered as a covariate, Adjusted means

**Between-Subjects Factors**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

**Tests of Between-Subjects Effects**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>223.836*</td>
<td>2</td>
<td>111.918</td>
<td>16.706</td>
<td>0.000</td>
<td>0.519</td>
</tr>
<tr>
<td>Intercept</td>
<td>311.619</td>
<td>1</td>
<td>311.619</td>
<td>46.516</td>
<td>0.000</td>
<td>0.600</td>
</tr>
<tr>
<td>PCO2Rest</td>
<td>106.174</td>
<td>1</td>
<td>106.174</td>
<td>15.849</td>
<td>0.000</td>
<td>0.338</td>
</tr>
<tr>
<td>Group</td>
<td>31.779</td>
<td>1</td>
<td>31.779</td>
<td>4.744</td>
<td>0.037</td>
<td>0.133</td>
</tr>
<tr>
<td>Error</td>
<td>207.673</td>
<td>31</td>
<td>6.699</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75976.073</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>431.509</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .519 (Adjusted R Squared = .488)

**Estimated Marginal Means**

**Group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>1</td>
<td>46.195</td>
<td>.620</td>
<td>44.930</td>
</tr>
<tr>
<td>2</td>
<td>48.332</td>
<td>.705</td>
<td>46.893</td>
</tr>
</tbody>
</table>

a. Covariates appearing in the model are evaluated at the following values: PCO2Rest = 39.132.

**Pairwise Comparisons**

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig. a</th>
<th>95% Confidence Interval for Difference a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>-2.137*</td>
<td>.981</td>
<td>.037</td>
<td>-4.138</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2.137*</td>
<td>.981</td>
<td>.037</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Based on estimated marginal means

*: The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

**Univariate Tests**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrast Error</td>
<td>31.779</td>
<td>1</td>
<td>31.779</td>
<td>4.744</td>
<td>.037</td>
<td>.133</td>
</tr>
<tr>
<td>Error</td>
<td>207.673</td>
<td>31</td>
<td>6.699</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The F tests the effect of Group. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
Comparing the Effect of Age vs. Group

As menopause is an age related phenomenon it was necessary to ensure that the premenopausal vs. postmenopausal differences in the study outcome variables were due to menopausal status and not age pre se. To begin, a Pearson product-moment correlation grid was used to identify significant associations between group (premenopausal or postmenopausal) and age with the key study variables within the entire sample. Next, variables that were significantly correlated with age and group were predicted using a stepwise linear regression model to see if age and/or group served as independent predictors of the variable being examined. In all cases, group alone was a significant predictor of the study variables in the stepwise regression analyses. To further explore the potential effect of age on the premenopausal vs. postmenopausal differences in the key study variables, a general linear model was run for each variable with and without age entered as a covariate to determine whether statistical pre- vs. postmenopausal differences in the variables remained after controlling for the effect of age. Statistical significance remained for all variables. Given these findings it was felt that the observed differences were due to menopausal status and not to an effect of age.
## Correlations

### [DataSet1] C:\Documents and Settings\graeme\My Documents\Megan\MScThesis\Analysis\REB Analysis Spreadsheet (04_04_07).sav

<table>
<thead>
<tr>
<th>Group</th>
<th>Pearson Correlation</th>
<th>Age</th>
<th>PCO2Rest</th>
<th>OsmoRest</th>
<th>SIDRest</th>
<th>E2</th>
<th>P4</th>
<th>VEBHyper</th>
<th>VRTCO2Hyper</th>
<th>VESHyper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>0.786**</td>
<td>0.395*</td>
<td>0.473**</td>
<td>0.463**</td>
<td>-0.521**</td>
<td>-0.357*</td>
<td>-0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>0.018</td>
<td>0.004</td>
<td>0.005</td>
<td>0.001</td>
<td>0.033</td>
<td>0.828</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Age</td>
<td>Pearson Correlation</td>
<td>.786**</td>
<td>1</td>
<td>0.395*</td>
<td>0.397*</td>
<td>0.462**</td>
<td>-0.502**</td>
<td>-0.379*</td>
<td>0.000</td>
<td>.410*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>0.019</td>
<td>0.018</td>
<td>0.005</td>
<td>0.002</td>
<td>0.023</td>
<td>0.999</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>PCO2Rest</td>
<td>Pearson Correlation</td>
<td>.399*</td>
<td>0.398*</td>
<td>1</td>
<td>0.205</td>
<td>0.622**</td>
<td>-0.133</td>
<td>-0.385*</td>
<td>-0.358*</td>
<td>-0.667*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.018</td>
<td>0.019</td>
<td>0.237</td>
<td>0.006</td>
<td>0.446</td>
<td>0.022</td>
<td>0.038</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>OsmoRest</td>
<td>Pearson Correlation</td>
<td>.473**</td>
<td>0.397*</td>
<td>2.05</td>
<td>1</td>
<td>0.139</td>
<td>-0.454**</td>
<td>-0.296</td>
<td>-0.012</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
<td>0.018</td>
<td>0.237</td>
<td>0.425</td>
<td>0.066</td>
<td>0.085</td>
<td>0.945</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>SIDRest</td>
<td>Pearson Correlation</td>
<td>.463**</td>
<td>.462**</td>
<td>0.622**</td>
<td>0.139</td>
<td>1</td>
<td>-0.284</td>
<td>-0.456**</td>
<td>-0.172</td>
<td>-0.528**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
<td>0.006</td>
<td>0.245</td>
<td>0.085</td>
<td>0.066</td>
<td>0.006</td>
<td>0.330</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>E2</td>
<td>Pearson Correlation</td>
<td>.521**</td>
<td>.502**</td>
<td>.133</td>
<td>.454**</td>
<td>.284</td>
<td>1</td>
<td>.320</td>
<td>.003</td>
<td>.171</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.002</td>
<td>0.446</td>
<td>0.006</td>
<td>0.098</td>
<td>0.057</td>
<td>0.988</td>
<td>0.326</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>P4</td>
<td>Pearson Correlation</td>
<td>.357*</td>
<td>.379*</td>
<td>.385*</td>
<td>.296</td>
<td>.456**</td>
<td>.320</td>
<td>1</td>
<td>.015</td>
<td>.367*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.033</td>
<td>0.023</td>
<td>0.022</td>
<td>0.085</td>
<td>0.006</td>
<td>0.057</td>
<td>0.931</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>VEBHyper</td>
<td>Pearson Correlation</td>
<td>-.039</td>
<td>.000</td>
<td>.358*</td>
<td>-.012</td>
<td>-.172</td>
<td>-.003</td>
<td>.015</td>
<td>1</td>
<td>-.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.826</td>
<td>.999</td>
<td>.038</td>
<td>.945</td>
<td>.330</td>
<td>.988</td>
<td>.931</td>
<td>.566</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>VRTCO2Hyper</td>
<td>Pearson Correlation</td>
<td>.486**</td>
<td>.410*</td>
<td>.667**</td>
<td>.204</td>
<td>.528**</td>
<td>-.171</td>
<td>-.367*</td>
<td>-.100</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.003</td>
<td>.015</td>
<td>.000</td>
<td>.246</td>
<td>.001</td>
<td>.328</td>
<td>.030</td>
<td>.566</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>VESHyper</td>
<td>Pearson Correlation</td>
<td>-.370</td>
<td>-.462**</td>
<td>-.282</td>
<td>-.075</td>
<td>-.370*</td>
<td>.289</td>
<td>.407*</td>
<td>.179</td>
<td>-.273</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.029</td>
<td>.005</td>
<td>.106</td>
<td>.672</td>
<td>.031</td>
<td>.093</td>
<td>.015</td>
<td>.303</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).
Regression: Resting PaCO₂ with Group and Age

[DataSet1] C:\Documents and Settings\graeme\My Documents\Megan\MScThesis\Analysis\REB Analysis Spreadsheet (04_04_07).sav

Variables Entered/Removed

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group</td>
<td></td>
<td>Stepwise (Criteria: Probability of F-to-enter &lt;= .050, Probability of F-to-remove &gt;= .100).</td>
</tr>
</tbody>
</table>

a. Dependent Variable: PCO2Rest

Model Summary

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.399a</td>
<td>.159</td>
<td>.134</td>
<td>4.0879</td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), Group

ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>104.501</td>
<td>1</td>
<td>104.501</td>
<td>6.253</td>
</tr>
<tr>
<td>Residual</td>
<td>551.471</td>
<td>33</td>
<td>16.711</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>655.971</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), Group
b. Dependent Variable: PCO2Rest

c. Predictors: (Constant), Group

Coefficients

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>34.183</td>
<td>2.111</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>3.492</td>
<td>1.396</td>
<td>.399</td>
<td>2.501</td>
</tr>
</tbody>
</table>

a. Dependent Variable: PCO2Rest

Excluded Variables

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta In</th>
<th>t</th>
<th>Sig.</th>
<th>Partial Correlation</th>
<th>Collinearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>.204a</td>
<td>.695</td>
<td>.492</td>
<td>.122</td>
</tr>
</tbody>
</table>

a. Predictors in the Model: (Constant), Group
b. Dependent Variable: PCO2Rest
Univariate Analysis of Variance: Resting PaCO$_2$ with Group as fixed factor

Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>104.501</td>
<td>1</td>
<td>104.501</td>
<td>6.253</td>
<td>.018</td>
</tr>
<tr>
<td>Intercept</td>
<td>53280.072</td>
<td>1</td>
<td>53280.072</td>
<td>3188.278</td>
<td>.000</td>
</tr>
<tr>
<td>Group</td>
<td>104.501</td>
<td>1</td>
<td>104.501</td>
<td>6.253</td>
<td>.018</td>
</tr>
<tr>
<td>Error</td>
<td>551.471</td>
<td>33</td>
<td>16.711</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54360.000</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>655.971</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Univariate Analysis of Variance: Resting PaCO$_2$ with group as fixed factor and age as a covariate

Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>112.704</td>
<td>2</td>
<td>56.352</td>
<td>3.319</td>
<td>.049</td>
</tr>
<tr>
<td>Intercept</td>
<td>68.583</td>
<td>1</td>
<td>68.583</td>
<td>4.040</td>
<td>.053</td>
</tr>
<tr>
<td>Age</td>
<td>8.203</td>
<td>1</td>
<td>8.203</td>
<td>.483</td>
<td>.492</td>
</tr>
<tr>
<td>Group</td>
<td>10.190</td>
<td>1</td>
<td>10.190</td>
<td>.600</td>
<td>.444</td>
</tr>
<tr>
<td>Error</td>
<td>543.268</td>
<td>32</td>
<td>16.977</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54360.000</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>655.971</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .159 (Adjusted R Squared = .134)
APPENDIX E

Study Consent Form
CONSENT FORM FOR A RESEARCH PROJECT
ENTITLED:

“Effects of Menopause on Acid-Base Regulation and Respiratory Chemoreflex Sensitivity.”

PURPOSE OF THE STUDY

You are being invited to participate in a research project which will study the changes in breathing, blood acidity and bone health that occur during menopause.

Menopause marks the change from the child-bearing to the non-child-bearing time of a woman’s life. It signifies the end of her period and involves a decrease in the production of hormones produced by ovaries (progesterone and estrogen).

High levels of progesterone and estrogen cause increases in breathing and reductions in the acidity of blood during pregnancy. However, the effects of low levels of these reproductive hormones on blood acidity of blood and breathing
have not been studied during human menopause. Also, the ways in which these hormones cause changes in breathing and acidity are poorly understood.

Recent research has identified blood acidity due to protein-rich diet as an important factor that can lead to bone loss in women as they age. However, the influence of blood acidity on bone health as a result of changes in reproductive hormones have not been studied during human menopause.

To better understand the interactions between reproductive hormones, breathing, blood acidity, and bone health that occur during the menopause, we will study 15 healthy postmenopausal and 15 healthy premenopausal women. Each woman will undergo special tests to measure breathing sensitivity, and an exercise test involving measurement of blood acidity during rest and light and heavy exercise on a stationary cycle. Blood tests will also be conducted to measure indicators of bone formation and breakdown.

The study is being conducted by Dr. Ian Janssen of the School of Physical and Health Education, Dr. John Fisher, Department of Physiology, Queen’s University and Dr. Gregory A.L. Davies, from the Department of Obstetrics and Gynaecology at Kingston General Hospital.

**PROCEDURES AND MEASUREMENTS**

If you decide to enter this study you will participate in two laboratory visits over a period of approximately one month. The following tests will be conducted during the corresponding laboratory visits:

**What Happens Prior to Laboratory Visits**

1. Completion of a medical history questionnaire by you and your family physician. This questionnaire determines whether it is safe for you to participate in physical activity.

2. Completion of a general health questionnaire concerning your physical activity, general health, and lifestyle habits and your menstrual cycle status (i.e. time of your list period).

3. Introduction and completion of a Menstrual Cycle Log (pre-menopausal women) and Three-Day Physical Activity Record Form (pre-menopausal and postmenopausal women).

4. **Familiarization with and completion of all of the above forms will take approximately 1-2 hours of your time.**
What Happens at Laboratory Visit 1


2. Basic physical and body measurements (height and weight) and measurement of blood pressure.

3. Basic lung physical measurement. This brief test (approximately 2 minutes) will involve breathing into a mouthpiece, with your nose sealed with a nose piece, as quickly and forcefully as you can on three consecutive trials.

4. This visit will involve an exercise test on an upright stationary cycle with gradual increases in pedaling resistance until a specific heart rate is reached. This exercise test will allow us to determine the exercise level where you begin to accumulate the chemicals in your blood that lead to fatigue for the next laboratory visit. Just before and during the test you will breathe room air through a special mouthpiece and your nose will be sealed with a nosepiece. The test will be stopped at a heart rate that represents high physical exertion but poses no threat to your health or on the appearance of any signs or symptoms indicating that the test should be stopped for your safety (i.e. lightheadness, confusion, dizziness, abnormal skin color changes, or nausea). This exercise test will take approximately 30 minutes.

5. During this laboratory visit you will be also asked to perform hyperoxic familiarization rebreathing test (explained below) which will take approximately 30 minutes.

6. **The first laboratory visit will last approximately 3 hours and will include 1.5-hour rest break between the exercise and rebreathing tests.**

What Happens at Laboratory Visit 2

1. Core body temperature will be measured using an ingestible body temperature sensor, which transmits your temperature to a small, computerized temperature monitoring system. You will be required to visit the laboratory on the morning of the second testing session for administration of the temperature sensor at least 2 hours prior to exercise testing to ensure passage of the capsule from the stomach into the gastrointestinal tract. The sensor is contained in a small capsule (about the size of a vitamin pill) and you will ingest the capsule with water, similar to how you would take any pill. During this brief visit, you will receive a
tutorial on how to operate the small temperature monitoring system (about the size of a cellular phone). You will be asked to carry this sensor with you and record your body temperature at 30-minute intervals throughout the day until you go to bed. During exercise testing, core temperature will be recorded every 30 seconds by a study investigator. The temperature sensor will remain in your gastrointestinal tract for approximately 18-30 hours before the entire capsule is excreted in the feces. It is important to note, that ingestion of the sensor is not mandatory and will only be administered to willing participants.

2. Basic lung physical measurement. This brief test (approximately 2 minutes) will involve breathing into a mouthpiece, with your nose sealed with a nosepiece, as quickly and forcefully as you can on three consecutive trials.

3. Urine sample collection for determination of bone activity.

4. During this visit you will be asked to perform two tests that involve rebreathing of air from a bag that contains either an above normal oxygen concentration or air containing below normal oxygen concentration, respectively. Before each test, you will be asked to hyperventilate by taking deep and deliberate breaths of room air for 5 minutes (hyperventilation component). At the end of hyperventilation you will be asked to take a series of deep breaths in and out. At the end of the last full breath out, you will be switched to breathing in and out of a bag that contains a special mixture of oxygen and carbon dioxide (either a low oxygen and low carbon dioxide mixture or a high oxygen and low carbon dioxide mixture). This part of the test is called the rebreathing component. After you have been switched to the rebreathing bag, you will be asked to relax and breathe normally. As you continue to breathe in and out of the bag, the buildup of carbon dioxide will stimulate you to increase the amount of breathing that you are doing.

The concentration of oxygen and carbon dioxide that you breathe in and out, as well as, your pulse rate and oxygen concentration of your blood will be monitored at all times. There will be a rest period of approximately forty-five minutes between the two rebreathing tests and the total time for the two tests and the rest period between will be approximately 1.5 hours.

5. This visit will further involve pedaling a stationary cycle at a pedaling resistance that will be fairly heavy. You will start with a 10 minute rest period, followed by a 3 minute warm-up with a low pedaling resistance. The exercise period will be approximately 6 minutes and will be followed by a 10 minute rest recovery. During this exercise test (including rest, warm-up, and recovery) you will be required to wear a mouth piece and a nose clip to prevent you from breathing the room air through your nose.
while allowing you to breathe through your mouth using the special mouthpiece.

Small blood samples (total of 5 tablespoons or less) will be obtained before the exercise test and near the end of exercise using a special needle apparatus (venous catheter) inserted in the back of your hand prior to the test. Before exercise test we will ask you to soak your hand in a container of warm water. Your forearm will also be gently heated using a hairdryer prior to each blood draw. The entire exercise component (including blood taking) will last approximately 45 minutes.

6. The second laboratory visit will last approximately 4 hours and will include 1.5-hour rest break between the exercise and the rebreathing tests.

**RISKS**

1. *Exercise Test*: Strenuous exercise is associated with a risk of transient abnormal blood pressure responses or disturbances in heart rhythm. Exercise can also trigger a heart attack or other serious heart problems in persons with heart disease. This is rare in normally active healthy women. If at any time it becomes more uncomfortable than you like, this procedure will be discontinued.

2. *Ingestible Body Temperature Sensor*: There are no known health risks associated with ingestion of a core temperature sensor in healthy individuals. However, as a precautionary measure ingestion of a temperature sensor is not recommended for individuals who have a history of esophageal and/or gastrointestinal tract disorders. Therefore, individuals with these medical problems will not be permitted to enrol in the study.

3. *Blood Taking*: Procedures to take blood involve a risk of bruising of your arm, a small risk of infectious disease, and a risk of pain and discomfort. Other risks may include temporary light-headedness. Precautions will be taken to avoid these difficulties. If at any time it becomes more uncomfortable than you like, this procedure will be discontinued.

4. *Hyperventilation Component*: Major discomfort is unusual during breathing tests. However, some breathing discomfort (shortness of breath) may be experienced, and some people experience headache and/or a sense of dizziness when performing these tests -- these feelings
are usually temporary. If at any time it becomes more uncomfortable than you like, this procedure will be discontinued.

5. **Rebreathing Component:** The increased breathing associated with this test may result in temporary discomfort such as dizziness, light-headedness and fainting. It is expected that you will experience a heightened desire to breathe and a feeling of breathlessness as the test proceeds. During one of the rebreathing tests (hypoxic) the concentration of oxygen in your blood may fall. This will be monitored at all times and a critical end-point (70%) will be established to ensure your safety. If at any time it becomes more uncomfortable than you like, this procedure will be discontinued.

### BENEFITS

From participation in this study you will gain a greater understanding your reproductive physiology and menopause, as well as your physical fitness and the status of your bone activity. Results form the study may improve the understanding of how postmenopausal women develop osteoporosis and they may aid in the future treatment of this condition. There are no other direct benefits available to you from participating in this study.

### EXCLUSIONS

Postmenopausal volunteers:
You will not be considered for this study if your last period occurred within past six months. For safety reasons, you will not be considered for the study if you are physically inactive. Also, for safety reasons, you will be excluded from the study if you are a smoker, if you take hormone replacement therapy and/or other medications, if you are more than 54 and less than 42 years old, born at high altitude, have recently returned from a trip at high altitude, have a history of cardiorespiratory, esophageal, or gastrointestinal disease, or if you have any signs and symptoms which suggest that it may be dangerous for you to participate (see below).

Pre-menopausal volunteers:
You will not be considered for this study if you have any signs of menstrual cycle disturbances (i.e. irregular period; lack of period for 3 months or more in the past 12 months). For safety reasons, you will not be considered for the study if you are physically inactive. Also, for safety reasons, you will be excluded from the study if you are a smoker, if you take oral contraceptives and/or other medications, if you are more than 54 and less than 42 years old, born at high altitude, have recently returned from a trip at high altitude, have a history of cardiorespiratory, esophageal, or gastrointestinal disease, or if you have any signs and symptoms which suggest that it may be dangerous for you to participate (see below).
SAFETY PRECAUTIONS

Safety precautions for the study will include the following:

1. All subjects who enter the study will be healthy pre- and postmenopausal women (ages 42-54), who are nonsmokers, have no history of cardiorespiratory disease and have been normally active during the six-month period prior to involvement in the study.

2. Before entering the study, you will obtain medical clearance from your family physician using a standard form employed at our laboratory to be sure it is safe for you to participate. You will not be allowed to enter the study if anything is found which indicates that it is dangerous to participate.

3. You will be asked to consume a standard meal (350 kcal, 40% carbohydrate, 40% fat, 20% protein) 1-2 h prior to each testing session to minimize the chance of low blood sugar.

4. You will be asked to abstain from caffeine intake and strenuous physical activity on the day of testing.

5. Hypoxic and hyperoxic testing will be separated by a minimum of 45 minutes.

6. Rebreathing and exercise testing will be separated by a minimum of 1-2 hours.

7. Warm-up and cool-down procedures will be used to promote your comfort and safety.

8. Blood taking will be performed by an experienced nurse specialist.

9. Testing personnel will be trained to deal with cardiovascular healthy emergencies (eg. problems with your heart). The likelihood of such emergencies is very low based on past experiences in our laboratory.

10. If you develop health problems or if any information becomes available to suggest that it is dangerous to continue the study, you will be withdrawn from further participation by Dr. G. A. L. Davies (medical monitor of the study).
CONFIDENTIALITY

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you. Your data will be kept in locked files which are available only to the investigators and research assistants who will perform statistical analyses of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular governmental functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Physical and Health Education at Queen’s University.

FREEDOM TO WITHDRAW FROM THE STUDY

Your participation in this study is voluntary. As a participant you should understand that you are free to refuse to participate in any component of the study and may withdraw your consent to participate without prejudice or consequence to any other interactions that you may have with the investigators of the study. It should be understood that your participation in the research project has no bearing on any academic component of your academic program if you are a student. You should also be aware that the investigator(s) may terminate your participation at any time, regardless of your wishes, if you develop a health condition that would make it potentially unsafe for you to continue.

LIABILITY

In the event that you are injured as a result of the study procedures, medical care will be provided to you until resolution of the medical problem. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

PAYMENT

You will not receive payment or financial compensation from involvement in this study. You will be reimbursed for any costs related to obtaining medical clearance that are not covered by health insurance and/or any parking costs related to the laboratory tests.
SUBJECT STATEMENT AND SIGNATURE SECTION

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

Ian Janssen, Ph.D.
533-6000 ext. 78631
Room PEC 214, Physical Education Centre,
Queen's University, Kingston, Ontario. K7L 3N6

OR

Gregory A.L. Davies, M.D., FRCS(C),
548-6072
(Medical Monitor)
Department of Obstetrics and Gynaecology, K.G.H.
76 Stuart Street, Kingston, Ontario. K7L 3N6

OR

Janice M. Deakin, Ph.D.
533-6111
(Department Head)
Room 225, Physical Education Centre,
Queen's University, Kingston, Ontario. K7L 3N6

If I have questions concerning research subject’s rights, I will contact:

Dr. Albert F. Clark, Chair,
533-6081
Office of Research Services
Fleming Hall, Jemmett Wing 301,
Queen's University, Kingston, Ontario. K7L 3N6
By signing this consent form, I am indicating that I agree to participate in this study.

Subject Name (please print)    Name of Witness (please print)

Permanent Address of Subject    Signature of Witness

Signature of Subject    Date

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

_________________________________       __________________________________
Ian Janssen, Ph.D.      Date
To the study participant: Please answer all questions in sections A and B of this form. Have your family doctor fill out section C.

To the physician: Please fill out section C of this form. Completing this form may not require a medical re-evaluation of your patient. If the results of recent tests are readily available that might prove useful to study personnel while dealing with the participant, please include that information in this questionnaire.
SECTION A: PATIENT INFORMATION (please print)

NAME

___________________________________________________________________________

ADDRESS

____________________________________________________________________________

TELEPHONE

HOME:___________________________OTHER:____________________________

BIRTHDATE (DD/MM/YY)____________________ DATE (DD/MM/YY)________________

SECTION B: MEDICAL HISTORY
Below is a physical activity pre-participation screening questionnaire developed from the Canadian Society of Exercise Physiology Physical Activity Readiness Questionnaire. Please assess your health needs by marking answering the following questions:

YES  NO

A. Has your doctor ever said you have heart trouble?    ____  ____

B. Do you get pains in your chest?      ____  ____

C. Do you often feel faint or experience dizziness?   ____  ____

D. Do you have any of the following heart disease risk factors?
   ▪ High blood pressure                      ____  ____
   ▪ High blood cholesterol                   ____  ____
   ▪ Diabetes                                 ____  ____
   ▪ Close blood relative who had heart disease before 55 (brother or father) or age 65 (mother or sister)  ____  ____
   ▪ You smoke                                ____  ____

E. Is there a good reason, not mentioned above why you should avoid exercise?  ____  ____

F. Do you have or ever had problems with any of the following?
   ▪ Heart Or blood vessels                   ____  ____
   ▪ Nerve or brain                          ____  ____
   ▪ Breathing or lungs                      ____  ____
   ▪ Hormones, thyroid, or diabetes          ____  ____
- Muscles, joints, or bones
- Other (please list) ___________________________________________

G. Please list any serious injuries suffered, or surgeries you have had?
________________________________________________
________________________________________________
________________________________________________
________________________________________________
________________________________________________

H. If you have had surgery, was any metal (e.g., pins or screws) left in your body?
________________________________________________
________________________________________________
________________________________________________
________________________________________________
________________________________________________

I. Are you presently taking any medications? If yes, please list.
________________________________________________
________________________________________________
________________________________________________
________________________________________________
________________________________________________

J. Are you presently undergoing physiotherapy, or any other sort of treatment? If yes, please list.
________________________________________________
________________________________________________
________________________________________________
________________________________________________
________________________________________________
SECTION C: MEDICAL REFERRAL

1. Review of Systems
   a) Cardiovascular _____________________________________________
   b) Respiratory _______________________________________________
   c) Neurological_______________________________________________
   d) Gastrointestinal___________________________________________
   e) Genitourinary_______________________________________________
   f) Endocrine___________________________________________________
   g) Musculoskeletal____________________________________________
   h) Skin _______________________________________________________
   i) Gynecological______________________________________________

2. Physical Examination
   Blood Pressure_____________________ Pulse: _____________________
   Cardiovascular:_______________________________________________
   Respiratory:___________________________________________________
   Head and Neck:_______________________________________________
   MSK:________________________________________________________
   Abdomen:_____________________________________________________
   12-lead ECG:_________________________________________________
   Neurological:_________________________________________________
3. Laboratory Findings (not mandatory) 

Date of Test(s): _________

Hb ______________ WBC ______________ Plts _________________

Total Cholesterol ____________ HDL ____________ Chol/HDL ____________

LDL _____________ Triglycerides _____________ Uric Acid _____________

TSH _________ Glucose____________

Fasting or random 75g OGTT @ 120 min ____________

4. Additional Abnormalities

List other abnormalities of which you are aware:

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

5. Current Medications and Doses

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________
6. Physical Activity Recommendation

On the basis of your knowledge and present medical evaluation of the applicant, you would recommend (mark the appropriate answer):

_______ Participation in the exercise tests (described in the Study Details Section) supervised by a physical education graduate*

_______ Participation in the exercise tests is not recommended

* Trained in CPR and has prior work experience in the health/fitness industry

Physician’s Name:
________________________________________________________________________________________

Physician’s Signature:
________________________________________________________________________________________

Date:
________________________________________________________________________________________

Phone Number:
________________________________________________________________________________________

Address:
________________________________________________________________________________________

Thank you very much for your help. We hope that this study and its results will be beneficial to you and your patient.
APPENDIX G

General Health and Fitness Questionnaire
## GENERAL HEALTH-FITNESS QUESTIONNAIRE

Date______________________  Subject No._____________________

### PHYSICAL ACTIVITY/EXERCISE TRAINING HISTORY

List only regular fitness/recreational activities:

<table>
<thead>
<tr>
<th>INTENSITY</th>
<th>FREQUENCY (d/wk)</th>
<th>TIME (min/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>20-40</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

- Heavy
- Medium
- Light

Does your regular occupation (job/home) activity involve:

- Heavy lifting
- Frequent walking/stair climbing
- Occasional walking (>once/hr)
- Prolonged standing
- Mainly sitting

### NUTRITION AND EATING BEHAVIOUR HISTORY

Are you presently on diet?  

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

What type of diet (e.g. protein)?

How long have you been on diet?

When was the last time you dieted?  
(dd/mm/yy)

Supplements (e.g. vitamins)  
Yes (list):

Have you ever had an eating disorder?  

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

What type of eating disorder?  
(e.g. anorexia)

Start date-End date of your Eating disorder (mm/yy)?

### MENSTRUAL HISTORY --  □ POSTMENOPAUSAL

Approximate date of your last menstruation:
**MENSTRUAL HISTORY -- □ PREMENOPAUSAL**

<table>
<thead>
<tr>
<th>Have you been menstruating regularly in the past 6-months?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

If you have irregular menstrual cycles, please describe and state duration of your irregularity:

<table>
<thead>
<tr>
<th>Irregular cycle length (e.g. 38-23-30 days etc.):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed cycles (e.g. no period for &gt;3 months):</td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
</tr>
</tbody>
</table>

**HORMONE THERAPY HISTORY**

<table>
<thead>
<tr>
<th>Have you been taking any of the following within the past 6 months:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral contraceptives:</td>
</tr>
<tr>
<td>Hormone replacement therapy:</td>
</tr>
</tbody>
</table>

**SMOKING HISTORY**

<table>
<thead>
<tr>
<th>Have you ever smoked cigarettes, cigars, or pipe?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start date-End date (mm/yy):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How many per day?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HIGH ALTITUDE HISTORY**

<table>
<thead>
<tr>
<th>Were you born at high altitude?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Have returned from a trip at high altitude?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>Start date-End date</td>
<td></td>
</tr>
</tbody>
</table>

**EMERGENCY CONTACT PERSON**

<table>
<thead>
<tr>
<th>Name:</th>
<th>Last</th>
<th>First</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telephone:</td>
<td></td>
<td>Relationship:</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX H

Menstrual Cycle History Questionnaire
Menstrual Cycle History Questionnaire:
Premenopausal Subjects

Date____________________________   Subject No.________________

PARTICIPANT INSTRUCTIONS: Indicate the Start Date, End Date, and Duration of your prior and present menstrual cycles (based on your personal menstrual log or based on your memory). Menstrual cycle starts on the first day of your menstrual bleeding and finishes on the last day before your next menstrual bleeding. For the purposes of this form, Start Date refers to the first day of your menstrual bleeding and End Date refers to the last day prior to your menstrual bleeding of the next menstrual cycle. Duration refers to the number of days between and including the Start Date and the End Date.

<table>
<thead>
<tr>
<th>Present menstrual cycle*</th>
<th>Start Date (dd/mm/yy)</th>
<th>End Date (dd/mm/yy)</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past menstrual cycle #1 (most recent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past menstrual cycle #2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past menstrual cycle #3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average Length: ____________________________

Confirmation
EML  [E]   [P]

Exercise Test 1: MC Phase_______________ Date__________ dd/mm/yy
Exercise Test 2: MC Phase_______________ Date__________ dd/mm/yy

Target Phases

Early Follicular Phase = EF to MF
OR
Mid-Luteal Phase = ML

168