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Role of adenylyl cyclase S674 in central and forearm vasomotor control

James Corkal
The University of Western Ontario

Supervisor
Dr. J. Kevin Shoemaker
The University of Western Ontario

Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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ROLE OF ADENYLYL CYCLASE S674 IN CENTRAL AND

FOREARM VASOMOTOR CONTROL

(Thesis Format: Monograph)

by

James Cameron <u>Corkal</u>

Graduate Program in Kinesiology

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

Abstract

This study examined the cardiac and vasomotor responses to submaximal handgrip exercise and beta-adrenergic control in carriers (n = 6) and non-carriers (n = 4) of a genetic variant of adenylyl cyclase 6 (AC S674). Rhythmic handgrip contractions (1 minute bout; 2 second contraction-relaxation period) were performed at three different intensities (20, 40, and 60% of maximal voluntary contraction force) to test the vasodilatory response to exercise. Additionally, two 5 minute infusions of isoproterenol (0.01 and 0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$ diluted in 5% dextrose) and one 10 minute infusion of propranolol (0.1 $mg \cdot kg^{-1}$ diluted in 0.9% saline) were used to examine beta-adrenergic mediated cardiovascular responses. Ascending aorta and brachial artery mean blood flow velocities (pulsed Doppler ultrasound) and brachial artery blood pressure (Finometer) were continuously measured during handgrip and pharmacological protocols. Vascular mechanics of the forearm were calculated using a three-element lumped windkessel model. At baseline, AC S674 carriers have decreased systemic vascular conductance and forearm vascular bed compliance, as well as increased pulse pressure. However, AC S674 carriers did not exhibit altered cardiac or vasomotor control during handgrip exercise, isoproterenol infusion, or propranolol infusion. These results indicate that expression of the dysfunctional genetic variant AC S674 has profound effects on systemic hemodynamics at rest. Chronic elevation in vascular contractile state may result in vascular stiffening and enhanced pulse pressures with detrimental long-term consequences for cardiovascular health.

Co-Authorship Statement

Dr. Rosemary Craen: Dr. Craen prepared and administered pharmacological agents.

Dr. Sophie Lalande: Dr. Lalande assisted with data collection and conducted echocardiograms on participants.

Dr. Peter Mack: Dr. Mack prepared and administered pharmacological agents.

Adam McIntyre: Mr. McIntyre performed all genomic DNA analysis and assisted with writing Section 3.5.4.

T. Dylan Olver: Mr. Olver assisted with data collection and performed all aortic ultrasound measurements. Mr. Olver also assisted with data analysis and the editing of this document.

Dr. Maxim Rachinsky: Dr. Rachinsky prepared and administered pharmacological agents.

Dr. J. Kevin Shoemaker: Dr. Shoemaker supervised study design, data analysis, and the writing of this document.

Acknowledgements

This is really hard for me to do. There are too many people to thank and not enough space to get to every one of you. However, I will try my best.

I have been a student at The University of Western Ontario for the better part of the last decade – and yes, I will still refer to it as UWO. It still feels strange wrapping up this document and ending my tenure as a student at UWO. I am also pretty sure there are some people who will be glad to finally get rid of me too. Well, University Hospital is not far from Thames Hall, so you are not entirely rid of me yet. How do you like them apples?

In all seriousness, I would not be writing this section if it were not for a special group of individuals who have helped me reach this point. First and foremost, I need to thank my family, especially my parents (Tom and Jan). In addition to being responsible for my existence, I would like to thank you for always believing in me and providing me with guidance, criticism, and inspiration. Amanda, you are a wonderful sister. Jack, sit. Good boy. I could not have done this without any of you.

To my UWO friends, where would I be without you? You are all wonderful and amazing. Thank you for being the best friends I could ever ask for. There is also no better group of lab mates than the NVRL team (Dylan, Mark, Chantelle, Torri, Tamara, Charlotte, Nicole, Maria, Danielle, Jacquie, Katelyn, Carly, Carolyn, Carol, and Arlene). There is no other group of people who I would rather eat copious amounts of cake and pizza with.

Last, but not least, I would like to thank Dr. Kevin Shoemaker. I cannot express how grateful I am for giving me the opportunity to come into your lab in 2010. You have provided me with all the necessary tools to be an effective – and hopefully successful – researcher. Your passion for research and teaching are second to none. It has truly been an honour to work under your supervision. Although I am not too sure what the lab is going to do without me, just kidding.

In the immortal words of Douglas Adams, "Don't panic."

Table of Contents

List of Abbreviations

AC   Adenylyl Cyclase

AC S674   Adenylyl Cyclase 6 genetic variant

ATP   Adenosine Triphosphate

BP   Blood Pressure (mmHg)

C   Compliance ($ml \cdot mm\ Hg^{-1} \cdot 100ml^{-1}$)

cAMP   cyclic Adenosine Monophosphate

CO   Cardiac Output CO ($l \cdot min^{-1} \cdot m^{2}$)

DBP   Diastolic Blood Pressure (mm Hg)

FBF   Forearm Blood Flow ($ml \cdot min^{-1} \cdot 100ml^{-1}$)

HR   Heart Rate ($beats \cdot min^{-1}$)

K   Viscoelasticity ($mm\ Hg \cdot ml^{-1} \cdot min^{-1}$)

L   Inertia ($mm\ Hg \cdot ml^{-1} \cdot min^{2}$)

MAP   Mean Arterial Pressure (mm Hg)

PP   Pulse Pressure (mm Hg)

R   Resistance ($mm\ Hg \cdot ml^{-1} \cdot min^{-1}$)

SBP   Systolic Blood Pressure (mm Hg)

SV   Stroke Volume ($ml \cdot min^{-1}$)

SVC   Systemic Vascular Conductance
($ml \cdot min^{-1} \cdot 100ml^{-1} \cdot mm\ Hg^{-1}$)

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Chapter 1

1. Introduction

The human cardiovascular system is a progression of vessels arranged in parallel and in series. Oxygenated blood is ejected from the left ventricle into the aorta and dispersed through arteries, arterioles and capillaries of the systemic circulation before venous return to the right atrium. The flow of blood to any tissue in the human body depends on perfusion pressure (i.e. mean arterial pressure) and vascular tone (i.e. resistance; Delp, 1998). Perfusion pressure is regulated by feedback control systems that rely on autonomic nerves and circulating hormones (Dampney, 2002). Vascular tone is influenced by sympathetic vasomotor nerve activity, circulating vasoactive hormones, and local factors such as metabolites and endothelial factors (Clifford, 2011). The fundamental purpose of cardiovascular control is to maintain homeostasis by matching blood flow to the metabolic demands of all tissues within the body.

Blood flow to contracting skeletal muscle increases rapidly at the onset of voluntary submaximal exercise to meet the metabolic requirements of that muscle. This exercise hyperemic response is achieved through an increase in cardiac output and vasodilation of the exercising skeletal muscle vascular bed (Dampney, 2002). Cardiac output increases due to elevations in heart rate and stroke volume (Åstrand, 1964; Hermansen, 1970; Eriksen, 1990; Rowell, 1993). However, despite over 100 years of research, summarized in several reviews (Saltin, 1998; Clifford, 2004; Joyner, 2007; Saltin, 2007, Clifford, 2011), considerable uncertainty remains regarding the vasodilatory mechanisms responsible for vasomotor control and blood pressure regulation during exercise.

Adenylyl cyclase (AC) is a G-protein-coupled enzyme that reduces intracellular calcium within vascular smooth muscle. Adenylyl cyclase catabolizes intracellular ATP into cyclic AMP (cAMP) via beta-adrenergic stimulation of the $G_{\alpha s}$ protein. Elevated levels of cAMP produce a decrease in intracellular calcium and subsequent relaxation of vascular smooth muscle. Recently, a dysfunctional variant of the adenylyl cyclase 6 isoform (AC S674) has been identified in humans (Gros, 2005) Expression of the AC S674 variant correlates with greater venous dilation in response to beta-adrenergic stimulation, and increased post-exercise hyperemia to volitional handgrip exercise (Gros, 2007; Hodges, 2010). Therefore, the enhanced vasodilatory capacity of

AC S674 carriers may provide further insight into the mechanisms responsible for exercise hyperemia.

Adenylyl cyclase is activated via β_1 (cardiac) and β_2 (vascular smooth muscle) receptor stimulation and therefore contributes to neurogenic vasomotor control. Vascular resistance, or its inverse of conductance, reflects the state of vascular contraction and alters blood flow to a vascular bed through changes in arterial diameter. However, the concept of vascular resistance involves steady state flow and does not account for the pulsatile nature of blood flow (Zamir, 2007). Recent evidence has suggested that compliance is an independent variable that deserves consideration when examining vasomotor regulation in skeletal muscle (Zamir, 2007; Frances, 2011). The study of vascular bed mechanics is integral to understanding vasomotor control and blood flow to skeletal muscle.

1.2 Purpose

The primary purpose of this study was to investigate the cardiac and vasomotor responses to beta-adrenergic control in carriers and non-carriers of the variant AC S674. The second purpose of this study was to examine the impact of the AC S674 variant to vasomotor control during submaximal handgrip exercise.

1.3 Hypothesis

This study tested the hypothesis that individuals who express the AC S674 variant will: 1) express increased vasodilatory and tachycardia response to beta-adrenergic activation, and; 2) have a greater vasodilatory response to handgrip exercise.

Chapter 2

2. Literature Review

2.1 Blood Pressure

Blood pressure at any location of the vasculature encompasses three components: 1) static pressure that is related to the volume of blood within the vascular system at zero flow; 2) hydrostatic pressure caused by the force of gravity and referred to as *pgh*, where *p* is fluid density, *g* is the acceleration due to gravity, and *h* is the height of the hydrostatic column, and; 3) dynamic pressure generated by the heart is equal to blood flow x resistance (Rowell, 1993). The last component alone is responsible for the movement of blood and is commonly referred to as 'blood pressure'.

2.1.2 Systemic Vasculature & Blood Pressure

The rhythmic beating of the left ventricle creates a pulsatile pressure waveform that is propagated throughout the systemic vasculature. This pressure waveform is regulated by arterial structure and mechanical components (i.e. elastin and collagen). Each region of the systemic vasculature serves a different function: 1) the large central elastic arteries (e.g. aorta, carotid etc.) act as a cushioning reservoir that stores blood during systole and eject blood to the periphery during diastole; 2) muscular arteries (e.g. brachial, femoral) serve as long conduits to transport blood to the periphery and organs, and; 3) high resistance arterioles that maintain mean arterial pressure and modulate steady state blood flow to vascular beds.

As the pressure waveform generated by the heart travels to the periphery, local systolic and pulse pressures increase. However, diastolic and mean pressures decrease when moving from central to peripheral vascular segments due to the progressive increase in arterial stiffness, and arterial branching that reduces overall vascular resistance (Nichols & O'Rourke, 2005). The total pressure waveform recorded at any location of the arterial tree is the sum of the forward wave produced by left ventricular ejection, and the backward traveling wave that is reflected at peripheral sites (Agabiti-Rosei, 2007). Potential wave reflection sites include branching points, areas of altered arterial elastance, and the high-resistance arterioles (Nichols & O'Rourke, 2005).

Thus, the pressure wave becomes increasingly altered as it moves farther away from the aorta in the circulation.

2.1.3 Blood Pressure and Blood Flow

The systemic arterial tree acts as a closed loop circuit consisting of thousands of cylindrical tubes. As such, many scientists began to apply the principles of fluid mechanics to better understand the relationship between blood pressure and flow. Jean Louis Poisseuille (1846) was the first hemodynamic scientist to establish that flow in a tube was related to the fourth power of the tube's internal radius. Poiseuille's Law is:

$$P_1 - P_2 = \frac{Q\mu L 8}{\pi r^4}$$

where $P_1 - P_2$ is the pressure change in the tube, Q is the volumetric flow rate, μ is the dynamic viscosity, L is the length of the tube, and r is the radius of the tube.

Additionally, Georg Simon Ohm (1827) stated that the current through a conductor between two points is directly proportional to the potential difference across two points. The inclusion of resistance to this principle ended with the development of Ohm's Law:

$$I = \frac{V}{R}$$

where I is the current through the conductor, V is the potential difference measured across the conductor, and R is the resistance of the conductor. Applied to the cardiovascular system, Ohm's law is transformed:

$$Q = \frac{P_1 - P_2}{R}$$

where Q is the volumetric flow rate of blood, $P_1 - P_2$ is the pressure drop across the artery, and R is the resistance to blood flow in the artery. Therefore, a direct relationship can be made between vascular contractile state and blood flow in the cardiovascular system.

Due to Poisseuille and Ohm's equations consisting of similar variables, Poisseuille's law can be altered to the following:

$$R = \frac{8\mu L}{\pi r^4}$$

where R is the resistance to blood flow in the artery, μ is the dynamic viscosity of blood, L is the length of the vessel, and r is the radius of the vessel. In fluid mechanics pressure is the driving

force behind flow. Blood flow will travel from areas of high pressure (arteries) to areas of low pressure (veins). Furthermore, changes in artery radius will create the largest flow responses in the vasculature. Dilation in an artery or vascular bed will reduce resistance thereby increasing blood flow.

Vascular conductance is the inverse of resistance and is defined as the ability for a fluid to flow through a conduit per unit pressure difference:

$$R = \frac{1}{VC}$$

where R is the resistance to blood flow in the artery, and VC is the conductance of blood through the artery. When substituted into Ohm's equation for blood flow, the resultant equation is:

$$VC = \frac{Q}{P_1 - P_2}$$

where VC is the conductance of blood through the artery, Q is the volumetric flow rate of blood, and $P_1 - P_2$ is the pressure drop across the artery. Similar to resistance, an increase in arterial diameter (thereby decreasing resistance and increasing flow) will produce a large change in vascular conductance.

The study of hemodynamics has elucidated the strong relationship between pressure and flow. Pressure is the driving force behind flow in the arterial system and especially through vascular beds. During exercise, muscular contractions squeeze blood out of the venous capacitance vessels thereby enhancing the arterial- venous ($P_1 - P_2$) pressure difference and along with elevated vascular conductance (through arterial vasodilation) drive flow through the vascular bed (Rowell, 1993).

2.2 Arterial Blood Flow

The physics that govern Poisseuille and Ohm's laws concern oscillatory flow through rigid tubes of constant diameters. However, arteries are viscoelastic tubes whose diameters fluctuate with pulsating pressures that are generated by pulsatile flows from the left ventricle (Sonesson, 1994). As such, the arterial system has been modeled in a number of different ways to study hemodynamics: tube models, anatomically based distribution models, and lumped models (Westerhof, 2009). The arterial Windkessel model was first proposed by Stephen Hales to account for the pulsatile nature of blood flow. The arteries act as a cushion that converts pulsatile flow from the heart into steady state flow through vascular beds. Thus, the Windkessel

model is a lumped model that describes the pressure-flow relationship of the whole arterial system from its entrance.

2.2.1 The Arterial Windkessel

The first arterial Windkessel model was developed by Otto Frank to determine the resistance (R, total peripheral resistance) and compliance (C) of the systemic vascular bed based on aortic measurements (Westerhof, 2009). However, it is now known that the pulsatile mechanics of flow through a vascular bed are additionally influenced by viscoelasticity (K) and inertance (L; Zamir 2007). Together, R (resistance due to viscous drag at the vessel wall); C, and K (due to viscoelastic opposition to stretch of the vessel wall); and L (inertia of the blood and vessel wall) compose a modified four element lumped Windkessel used to predict flow in a peripheral vascular bed (Zamir, 2007; Zamir, 2009; Frances, 2011).

2.2.2 The RCKL Model

Flow into a vascular bed relies on both steady state (R) and oscillatory (C, K, and L) components (Zamir, 2009). R is obtained by simultaneously measuring mean pressure and mean flow at the point of entry to a vascular bed (Zamir, 2009). Additionally, the values of C, K, and L are calculated from the simultaneous measurement of oscillatory pressure and flow at the vascular bed entry point (Zamir, 2009). For a given set of values (C, K, and L), the model predicts a flow wave that would be produced by a measured pressure wave during pulsatile flow (Zamir, 2007). The most accurate model can be determined by the lowest error value that is determined by the sum of the differences squared between the two waveforms at each point along the wave (Nielson, 2011). The process is automated using MATLAB software and techniques of fast Fourier transform (Brigham, 1988; Figure 2.1).
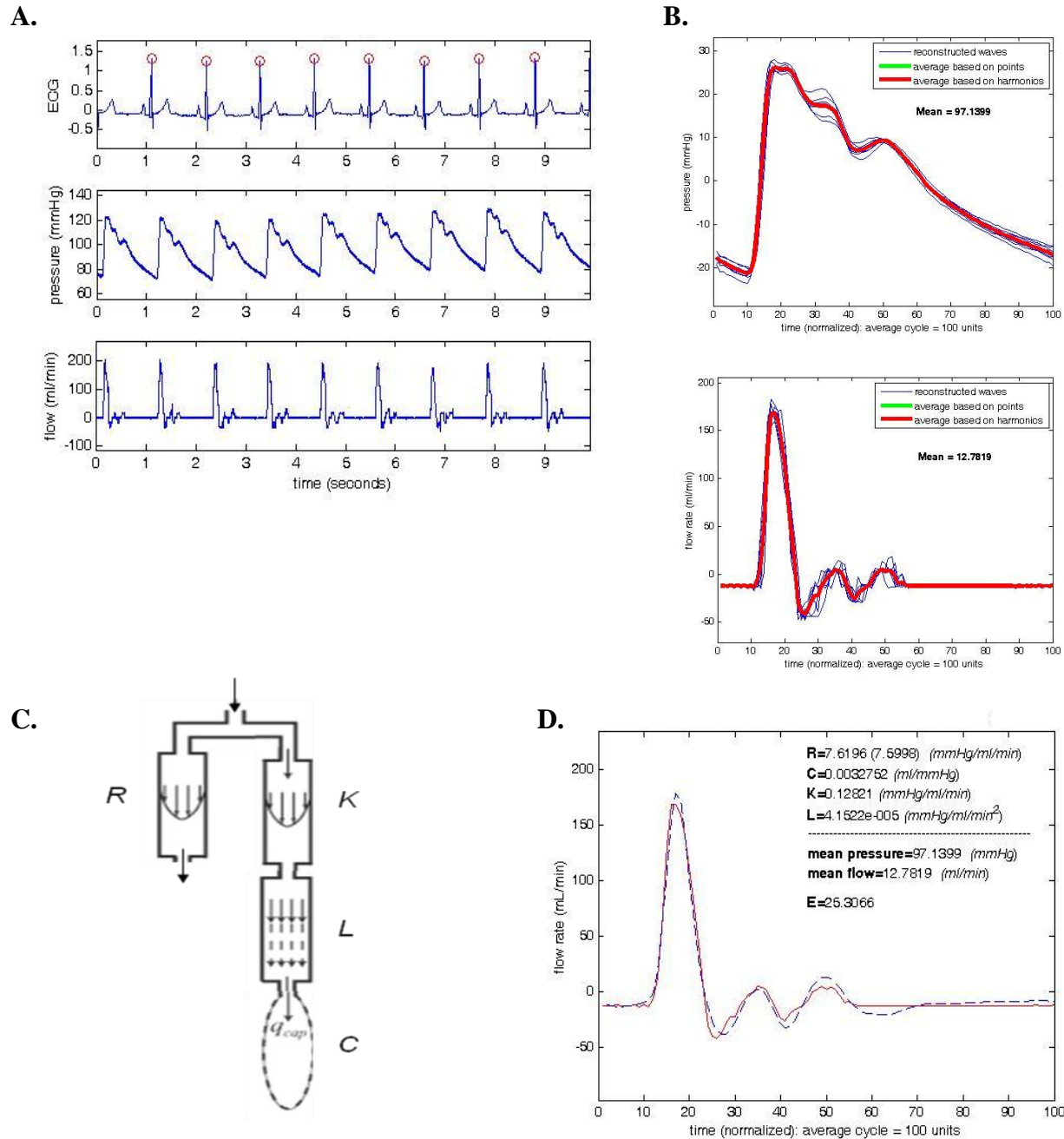
Figure 2.1 The RCKL Model **A.** ECG, pressure, and flow channels; **B.** Mean pressure waveform (top) and mean flow waveform (bottom); **C.** *R* (mean pressure and flow) is measured in parallel to *C*, *K*, and *L* (oscillatory pressure and flow) at the vascular bed point of entry; **D.** Predicted flow wave (dashed-line) and measured flow wave (solid) with values of R, C, K, and L displayed.

2.2.3 Vascular Bed Mechanics & Vasomotor Control

The steady state component of blood flow regulation to skeletal muscle vascular beds is controlled by vascular resistance (or its inverse of vascular conductance) and perfusion pressure. However, arterial compliance plays an integral role in the mechanics of pulsatile flow. Compliance reflects the elasticity of the arterial wall, but can be affected by vascular smooth muscle (VSM) contractile state and, therefore neural innervation (Grassi, 1995). For example, relaxation of VSM has been shown to cause increased compliance in the brachial artery and forearm (Fitchett, 1984; Safar, 1987). Recent research from our laboratory has confirmed the importance of compliance as a regulatory variable in vasomotor control. Specifically, Zamir (2007) examined the forearm vascular bed and reported increased compliance with arm elevation (relative to the heart), and decreased compliance as a result of lower body negative pressure or a cold pressor test. In a follow-up study conducted by Frances (2011), adrenergic activation via noradrenaline infusion decreased forearm vascular bed compliance. In addition to alpha adrenergic receptors, beta adrenergic receptors contribute a dilatory stimulus to VSM (Garovic, 2002; Eisenach, 2004). Beta-adrenergic control exerts its effect through the adenylyl cyclase enzyme. Very little research has been conducted on the role of this enzyme in either vasomotor responses to exercise or vascular mechanics.

2.3 Vasodilation and Hyperemia

Numerous reviews have examined the vasodilatory mechanisms responsible for exercise hyperemia (Saltin, 1998; Clifford, 2004; Joyner, 2007; Saltin, 2007; Clifford 2011). The classical view is that there is an interaction between numerous vasodilatory mechanisms (lactate, H^+ ions, oxygen, ATP and adenosine, nitric oxide, prostaglandins, and endothelial derived hyperpolarizing factor) released via skeletal muscle, endothelium, and erythrocytes that contribute to hyperemia. Essentially, vasodilation is the result of processes decreasing concentrations of intracellular calcium within vascular smooth muscle cells (Clifford, 2004). The enzyme adenylyl cyclase is a regulator of intracellular calcium within vascular smooth muscle (Figure 2.2). However, only one study to date, from our group, has examined the effects of adenylyl cyclase on exercise hyperemia in humans (Hodges, 2010).
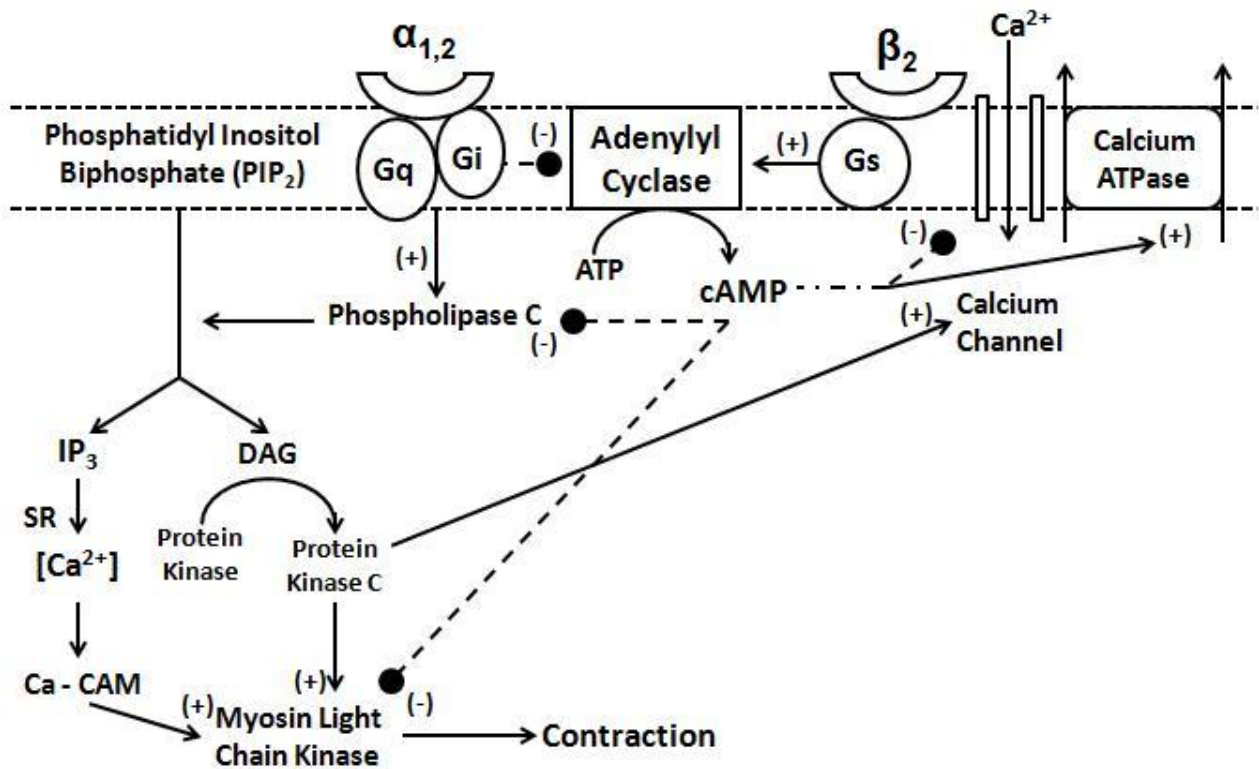
Figure 2.2 Adrenergic mediated pathways of adenylyl cyclase in a vascular smooth muscle cell. β_2 receptor activation activates adenylyl cyclase to convert ATP into cAMP producing a decrease in intracellular calcium, inhibition of Phopholipase C, relaxation of vascular smooth muscle, and a consequent arterial vasodilation. (DAG, diacylglycerol; IP_3, inositol triphosphate; SR, sarcoplasmic reticulum; reprinted with permission from K. Shoemaker).

2.3.1 Adenylyl Cyclase

Adenylyl cyclase (AC) is a ubiquitous enzyme that catalyses the conversion of intracellular ATP to cAMP. The adenylyl cyclases are large (1 080 – 1 248 amino acids) polypeptides that cross the plasma membrane 12 times in two cassettes of six transmembrane spanning domains; each individual cassette is followed by a large cytosolic domain (Figure 2.3; Cooper, 1995; Patel, 2001).
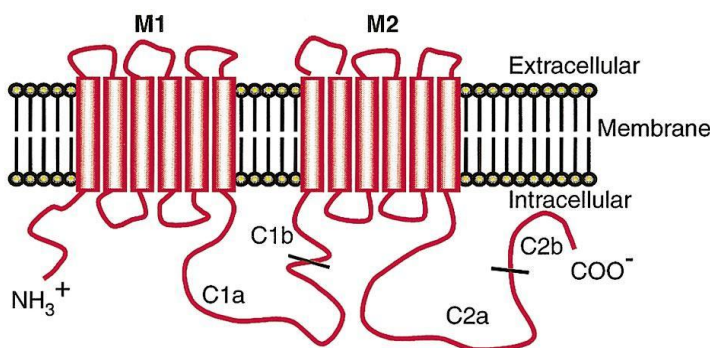
Figure 2.3 Schematic of the proposed structure for membrane bound adenylyl cyclase. The AC enzyme is composed of two transmembrane regions (M1 and M2) combined with two cytoplasmic domains (C1 and C2; Patel, 2001; reprinted with permission).

The structure of AC allows for extracellular (via α_i, α_s, and β_γ subunits of G protein-coupled receptors) and intracellular (via protein kinases, phosphatases, calcium, and calcium/calmodulin) interactions to occur (Cooper, 1995; Hanoune, 2001; Gros, 2005). The two cytosolic domains of AC constitute the catalytic site and are regulated by varying concentrations of ions such as calcium and magnesium (Hurley, 1999). Due to these many interactions, AC has been proposed to be the rate-limiting step in the G protein-coupled receptor-signaling cascade (Ostrom, 2012).

2.3.2 Adenylyl Cyclase Isoforms

Currently, there are nine membrane bound isoforms (AC1-9) and one soluble form of mammalian AC that have been characterized and separated into three different groups (Group 1: AC1, AC3, AC8; Group 2: AC2, AC4, AC7; and Group 3: AC5 and AC6; Defer, 2000; Hanoune, 2001; Patel, 2001). The additional soluble AC is the predominant form in mammalian sperm and is classified separately (Wuttke, 2001). All of the AC isoforms share a large sequence homology in the primary structure of the catalytic site. However, the catalytic sites of each isoform have a specific pattern of regulation by G proteins, calcium/calmodulin, and protein kinases (Defer, 2000; Hanoune, 2001; Ostrom, 2012). These distinct properties allow AC isoforms to play an interpretive role in signal transduction as opposed to a linear activation pathway of G protein-coupled receptors. Therefore, external signal integration from G protein-coupled receptors depends on the properties and relative level of isoform expression in tissues (Cooper, 1995; Defer, 2000). Although there are many isoforms and regulatory processes, the

resultant end product of all adenylyl cyclase isoforms is the same (i.e. an increase in intracellular cAMP; Ostrom, 2012).

2.3.3 Adenylyl Cyclase: Genetic Variants

Genetic variants have been described for a variety of G proteins and G protein-coupled receptors linked to adenylyl cyclase (Rana, 2001). Expression of these genetic variants leads to alterations in receptor-mediated activation of adenylyl cyclase in addition to alterations in downstream effector pathways. Currently, the identification of dysfunctional genetic variants has been limited to isoforms AC3, AC6 and AC9 (Ikoma, 2003; Small, 2003). The AC6 isoform is the predominant adenylyl cyclase in the human adult left ventricle and is a critical regulator in vascular smooth muscle (Wang, 2004; Gros, 2006). Furthermore, the expression of a genetic variant of AC6 that alters AC6 function could lead to modified vascular adenylyl cyclase-mediated effects (Gros, 2007).

2.3.4 Adenylyl Cyclase 6 Variant

The AC6 variant results from a single nucleotide polymorphism in intron 17 of the gene that codes for the normal AC6 isoform (Gros, 2005). This variant was originally discovered in a population of Japanese individuals (Ikoma, 2003). Further research concluded that a missense mutation occurred at amino acid 674 within the AC6 catalytic domain (Gros, 2005). This AC6 variant has a genotypic frequency of approximately 6% in Caucasians and is referred to as AC S674 (Gros, 2005).

Recent research has concluded that AC S674 is a hyperfunctional variant compared to the more common wild type AC A674. Gene transfer of AC S674 to rat vascular smooth muscle cells resulted in increased AC activity and arborization (Gros, 2007). Isolated mononuclear leukocyte cells from humans expressing AC S674 displayed significantly increased AC activity and AC-mediated cell retraction (Gros, 2007). Additionally, the expression of AC S674 in humans correlated with: 1) increased AC enzymatic activity and; 2) enhanced AC-mediated vasodilation to isoproterenol as measured by a dorsal vein linear variable differential transformer (Gros, 2007). In regards to blood flow control, human carriers of AC S674 expressed greater post-exercise forearm blood flow responses to isometric handgrip exercise (Hodges, 2010).

However, the direct effect of AC S674 stimulation or inhibition on human cardiac and vasomotor control remains to be elucidated.

2.3.5 Beta-Adrenergic Control

Adenylyl cyclase is stimulated when the heterotrimeric G protein, $G_{\alpha s}$, is activated by epinephrine or norepinephrine binding to β_1 or β_2 adrenergic receptors (Saucerman, 2003). The activation of β_1 and, to a lesser extent, β_2 receptors in the heart produces an overall increase in cardiac output. The increase in cardiac output is a combination of increased cardiac stimulation (β_1; positive inotropy, lusitropy, and chronotropy) and myocardial contractility (β_2; Katz, 2011). As mentioned previously (Figure 2.2), the activation of β_2 receptors in VSM produces a relaxation of smooth muscle and subsequent arterial vasodilation. Conversely, beta-adrenergic antagonists prevent epinephrine and norepinephrine from binding to β_1 and β_2 receptors. When this occurs, AC is deactivated and results in diminished physiological responses (i.e. decreased heart rate; Katz, 2011). Thus, beta-adrenergic mediated regulation of AC is imperative for both cardiac and vasomotor control.

2.4 Blood Flow Measurements

Blood flow to a vascular bed increases in proportion to the metabolic demands of the active muscle tissue (Andersen & Saltin, 1985). Measurement of arterial inflow is necessary to accurately assess the hyperemic response in exercising limbs. Regional limb blood flow measurements have previously been conducted via electromagnetic flow meters, plethysmography, intravascular indicators (e.g. thermodilution), Doppler ultrasound, and magnetic resonance imaging (Rådegran, 1999). Recently, Doppler ultrasound has become the preferred modality to measure blood flow to exercising limbs.

2.4.1 Doppler Ultrasound

Ultrasound is defined as a mechanical vibration with a frequency (f) above the range of human hearing. Ultrasound measurements calculate the Doppler frequency shift (Δf) that occurs as sound waves are reflected by erythrocytes (Gill, 1979). More simply defined, Δf is the transmitted frequency (f_T) minus the received frequency (f_R):

$$\Delta f = f_T - f_R$$

Thus, blood velocity can be described as positive (towards the transducer) or negative (away from the transducer). The Doppler equation is used to detect blood velocity:

$$\Delta f = \frac{2 \times f \times v \times cos\theta}{c}$$

where Δf is the change in frequency received by the transducer, v is the velocity of erythrocytes, θ is the angle between the axis of the beam and direction of flow (insonation angle), and c is the velocity of sound in blood (approximately 1540 m/s; Radegran, 1999; Wood, 2010). The insonation angle must be below 60° for accurate assessment of blood flow measurements. Angles greater than 60° will provide an amplified error when blood flow is calculated from the velocity profile.

The strength of Doppler ultrasound is that it provides a continuous, non-invasive estimate of beat-to-beat blood velocity (Shoemaker, 1996). Coupled with the cross-sectional area of the examined artery (e.g. brachial artery), limb blood flow can be calculated at rest, at onset of and during submaximal exercise, as well as post-exercise (Radegran, 1999). Additionally, Doppler ultrasound during rest and exercise has proven to be a reproducible and reliable modality to assessing blood flow control (Shoemaker, 1996).

2.5 Brachial Artery Pressure Measurements

The Finometer[TM] allows for the continuous, non-invasive collection of arterial pressure. Arterial pressures are obtained from a cuff that is placed around the intermediate phalanx of the middle finger. Specifically, the Finometer[TM] calculates arterial pressure via the volume clamp technique and the physiocal set-point criteria (Guelen, 2003; & Guelen, 2008). However, as mentioned previously, blood pressure and pulse shape vary within the arterial tree. Thus, a brachial pressure waveform must be remodeled from the pressure waveform obtained in the finger (Bogert, 2004). The reconstructed brachial artery pressures are sensitive and accurate representations of brachial artery pressures in a supine position (Guelen, 2003; Schutte, 2003; Guelen, 2008). Additionally, our laboratory has demonstrated the accuracy of the calculated brachial artery pressure waveform using handheld tonometry (Zamir, 2007).

Chapter 3

3. Methodology

3.1 Ethical Approval

Participants from Western University and the public volunteered to take part in this study after receiving written and verbal details of the experimental procedures and signing consent forms approved by The Human Subjects Research Ethics Board at Western University. Participants were made aware that they could withdraw from the study at any time without consequence.

3.2 Subjects

A total of 80 Caucasian adults between the ages of 18 and 30 were genotyped to determine whether they were carriers of the AC6 S674 allele. A 10 mL blood sample was drawn from the antecubital fossa with the participant in a supine position. The blood sample was put on ice and transported to the Blackburn Cardiovascular Genetics Laboratory, Western University for extraction of white blood cells and genotyping. Data on height, weight, and waist circumference were also obtained. Exclusion criterion included: previously reported cardiovascular, metabolic, or neurological disease; diabetes; administered prescribed medication that affects autonomic or cardiovascular function (with the exclusion of oral contraceptives); urinary or digestive problems; Raynaud's syndrome, or; pregnancy.

Of the 80 individuals screened, four (two males) participants were identified as heterozygous carriers for the AC6 S674 variant (henceforth referred to as the AC6 variant group). An additional six participants (three male) were age-matched from the pool of non-variant carriers and served as a control group. Oral contraceptive use was documented; however no standard of timing in regards to menstrual cycle was measured. There were no perceivable differences between males and females once data was normalized and were therefore pooled.

3.3 Genomic DNA Analysis

Genomic DNA was extracted from whole blood using the Puregene DNA isolation kit (Gentra Systems, QIAGEN Inc, Mississauga, ON, Canada). Genotyping was performed via

exon-specific polymerase chain reaction (PCR) amplifications in the AC6 gene (Gros, 2005). PCR amplifications were completed in 30µL mixtures containing 32pmol of each primer; 0.2mM of each dATP, dCTP, dGTP, DTTP, and; 1.5mM $MgCl_2$, 50mM KCl, 20mM Tris-HCl (pH 8.4), 1.0 units of Taq platinum DNA polymerase (Invitrogen; Life Technologies Corporation, Carlsbad, California). Thirty cycles were conducted that contained: denaturation at 95°C, annealing at 60°C ,and extension at 72°C for 30s each; followed by a final extension for 7mins at 72°C and cooling to 4°C. PCR products were purified with CIP/Exol (New England Biolabs, Pickering, Ontario) and sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California). Analysis of DNA sequences was measured by Seq Scape v2.6 (Applied Biosystems, Foster City, California).

3.4 Experimental Design

3.4.1 Hemodynamics

In the follow-up study to assess cardiovascular control of AC S674, all measurements were performed with participants in a quiet, darkened room with constant temperature. Participants reported to the laboratory after a three hour fast and having abstained from caffeine, alcohol, and exercise for a period of 12 hours. A venous catheter was inserted into a large vein in the antecubital fossa of the right arm for delivery of pharmacological agents. Heart rate was recorded via a three lead ECG (Pilot-9200; Colin Medical Instruments, San Antonio, Texas). Continuous blood pressure measures were obtained from the middle finger of the left hand via finger cuff plethysmography (FMS Finometer; Finapres Medical Systems, Arnheim, Netherlands). Finometer measurements of the middle finger were recalculated to produce brachial artery blood pressure waveforms of the left arm(Schutte, 2003; Bogert, 2004; Bogert & van Lieshout, 2005). Calculated brachial artery waveforms have previously been shown to accurately represent true waveforms as assessed by applanation tonometry(Zamir, 2007). Additionally, brachial blood pressure values were matched to manual sphygmomanometer measurements of the right arm for consistency.

3.4.2 Ultrasound

Ultrasound imaging of the ascending aorta and brachial arteries was performed simultaneously with participants in the supine position. Blood flow velocity of the ascending aorta (via the suprasternal notch) was assessed by pulsed-wave Doppler ultrasound (2MHz; Vingmed CFM 750; GE Medical Systems Canada, Mississauga, Canada). Brachial artery diameters of the right and left arm were obtained using two-dimensional ultrasound imaging (10MHz; B-Mode ultrasound: GE System 5; GE Healthcare), while pulsed-wave Doppler ultrasonography (4.7 MHz; GE System 5; GE Healthcare) measured mean blood flow velocities.

All analog signals of hemodynamic and ultrasound data were sampled at 1000Hz and collected online by the PowerLab data acquisition system (PowerLab 6; ADInstruments, Castle Hill, Australia). Participants lay supine for a period of 10 minutes before commencement of data collection.

3.4.3 Handgrip Exercise

Baseline measurements were recorded for 5minutes, followed by three 1minute handgrip bouts (at 20, 40, and 60% of maximum volitional contraction [MVC]) with 3 minutes between each bout. Measures of maximal voluntary contraction force were performed 10 minutes prior to the commencement of baseline measures. The 1 minute bouts of handgrip exercise consisted of a 2 second exercise-relaxation protocol (Figure 3.1). The order of 20 and 40% handgrips was assigned randomly determined by a coin flip while the 60% handgrip was always conducted last. The 60% handgrip protocol was performed last to minimize the effect of muscle fatigue on forearm blood flow. A standardized Borg scale was used to assess perceived exertion after each exercise bout.
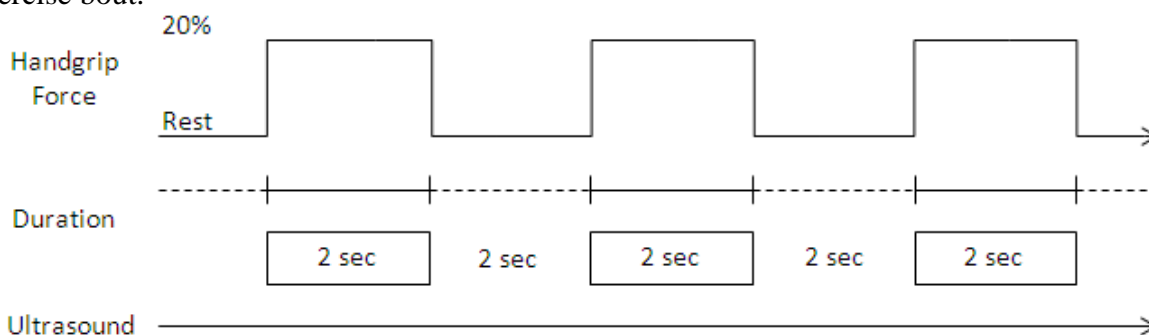

Figure 3.1 Handgrip exercise for 20% MVC indicates the 2 second intermittent contraction-relaxation protocol with Doppler ultrasound collected throughout.

3.4.4 Cardiac and Vasomotor Control

Previously, venous infusion of isoproterenol has elicited a greater venous dilation in carriers of the AC S674 variant (Gros, 207). To assess the function of AC S674 on cardiac and vasomotor control, isoproterenol and propranolol were infused separately through a venous catheter. Isoproterenol is a non-specific beta-adrenergic agonist and propranolol is a non-specific beta-adrenergic antagonist. As such, venous infusion of isoproterenol and propranolol allows for cardiac and vascular smooth muscle responses to occur. Baseline measurements were recorded for 1minute, followed by two 5 minute infusions of isoproterenol (0.01 and 0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$ diluted in 5% dextrose). Infusion was stopped, and heart rate and blood pressure were monitored until they returned to pre-isoproterenol baseline. Isoproterenol doses of 0.01 and 0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$ increase heart rate and stroke volume in health y individuals (Edgell, 2007) and these doses were used here to study both the cardiac and vascular response in both wild type and ACS674 variants. Isoproterenol infusion was followed by a single dose propranolol infusion (0.1 $mg \cdot kg^{-1}$ diluted in 0.9% saline) of 10 minutes. Measurements were collected for 1 minute pre and 5 minutes post-propranolol infusion. Due to the long half-life of propranolol, a single dose was infused to observe alterations to cardiac and vasomotor function from baseline. A separate saline trial (0.9% NaCl) was randomly inserted before, between, or after the isoproterenol infusions via random selection.

3.5 Data Analysis

3.5.1 Stroke Volume

Aortic diameters were measured (Vivid *i*; GE Healthcare, Mississauga, Canada) and analyzed using EchoPac Dimension software. Stroke volume was calculated as: $SV = SVV \times AA$, where *SVV* is the mean blood flow velocity in the ascending aorta, and *AA* is the aortic area. Cardiac output was calculated ($CO = HR \times SV$) and normalized to body surface area (BSA) using the Du Bois formula ($BSA = 0.007184 \times W^{0.425} \times H^{0.725}$). Subsequently, mean arterial pressure (*MAP = diastolic blood pressure* + 1/3 *pulse pressure*), and systemic vascular conductance (*SVC = CO/MAP*) were calculated.

3.5.2 Forearm Blood Flow

Brachial artery diameters were stored and measured digitally (EchoPac Dimension software). Three measurements were averaged to acquire a mean value during systole and diastole to determine brachial artery diameters. Brachial blood flow was calculated as: *Flow* (ml/min) $= v \times (\pi \times r^2) \times 60$, where v is the blood velocity, and r is the brachial artery radius ($r =$ (2/3diastolic diameter + 1/3 systolic diameter)/2) (Radegran, 1997). Blood flow values were averaged for 10 cardiac cycles at baseline and 2-3 cardiac cycles during the relaxation phases of handgrip exercise and again immediately post-exercise. Forearm vascular conductance (FVC) was calculated as limb blood flow divided by MAP. Forearm volume was calculated using the equation for a truncated cone ($V = h[C_1^2 + C_1C_2 + C_2^2] /12\pi$), where V is the volume of the forearm, C_1 is the circumference of the wrist, C_2 is the circumference of the widest part of the forearm at the elbow, and h is the distance between circumference measurements (Taylor, 2006). Thus, FBF and FVC were normalized to 100 ml of tissue.

3.5.3 Vascular Bed Mechanics

A modified three-element lumped Windkessel model was utilized to quantify forearm vascular bed mechanics. This model included determination of vascular compliance (C), viscoelasticity (K) and inertance (L). The value of R was obtained by simultaneously measuring mean pressure and mean flow at the point of entry to the vascular bed (23). The values of C, K, and L are calculated from simultaneous measurement of oscillatory pressure and flow at the vascular bed entry point (23). For a given set of values (C, K, and L), the model predicts a flow wave that would be produced by a measured pressure wave during pulsatile flow (22). Additionally, compliance was normalized to 100 ml of forearm tissue.

Ten consecutive cardiac cycles at baseline and during isoproterenol (5mins) and propranolol (5, 10, and 15mins) were analysed. Data (ECG, brachial pressure, and left arm brachial flow) were stored and saved in MATLAB format for RCKL program analysis. The blood pressure and flow waveforms were time-aligned to produce the estimated flow waveform with the lowest error.

3.5.4 Statistical Analysis

Baseline hemodynamic and vascular mechanic data were determined by comparing the mean values of the measured variables using an independent samples t-test. Additionally, the total change of forearm blood flow (ΔFBF) and forearm vascular conductance (ΔFVC) from rest to exercise termination was assessed using an independent samples t-test. Moreover, to assess the changes in forearm blood flow during rhythmic handgrip exercise (handgrip × group), a split-plot ANOVA was applied for each exercise intensity.

To determine the effect of isoproterenol (dose × group) on cardiac and vascular measurements, a split-plot (mixed design) analysis of variance (ANOVA) was used. A Greenhouse-Geisser correction was used to account for violations of sphericity when applicable. A significant interaction effect prompted the analysis of simple main effects to identify univariate differences between groups. The total response ($\Delta = 15$ minutes - baseline) in hemodynamic and vascular mechanic data during propranolol infusion was assessed using an independent samples t-test. The level of significance for statistical tests was set at $p < 0.05$. All data are presented as means ± standard deviation.

Chapter 4

4. Results

In three separate instances, participant data were excluded from the final analysis of cardiac and forearm vasomotor control. Firstly, one control participant failed to comply with the handgrip protocol. Data from this participant were not included in the final calculation of handgrip responses in the control group. Second, a separate control participant was excluded from the analysis of forearm vascular bed mechanics because the data were statistically an outlier. Third, a participant in the AC S674 group did not undergo the pharmacological protocol because of noticeable baseline bradycardia (33 bpm) causing the concern on the part of the anesthetist to infuse the pharmacologic agents.

4.1 Baseline Assessment

4.1.1 Anthropometric Measurements

There were no significant differences in anthropometric measurements between groups at baseline (Table 4.1). However, aortic diameter was significantly smaller in the AC S674 group.

Table 4.1 Anthropometric Measurements

	Control (n = 6)	AC S674 (n = 4)
Age (years)	25 ± 2	25 ± 4
Height (cm)	168 ± 6	177 ± 6
Weight (kg)	63 ± 12	69 ± 2
BMI (kg·m^2)	22.1 ± 3.2	22.3 ± 1.1
Waist Circumference (cm)	84 ± 7	86 ± 5
Aortic Diameter (cm)	2.8 ± 0.3	2.1 ± 0.2 *

*Significantly different from controls ($p < 0.05$). Values are mean $\pm$ standard deviation.

4.1.2 Hemodynamic Measurements

Hemodynamic measurements at baseline were not different during saline infusion. As such, non-saline values are expressed in Table 4.2. Stroke volume, cardiac output, DBP, MAP and SVC were decreased in the AC S674 variant group compared to controls ($p < 0.05$). Additionally, PP was increased in the AC S674 variant group compared to controls ($p < 0.05$).

Table 4.2 Baseline hemodynamic characteristics

	Control (n = 6)	AC S674 (n = 4)
HR (beats·min^{-1})	59 ± 5	48 ± 14
SV (ml·min^{-1})	112 ± 27	51 ± 13^t *
CO (L·min^{-1}·m^2)	3.96 ± 1.26	1.42 ± 0.21^t *
SBP (mm Hg)	114 ± 8	114 ± 3
DBP (mm Hg)	77 ± 4	62 ± 6 *
PP (mm Hg)	37 ± 7	52 ± 5 *
MAP (mm Hg)	89 ± 5	79 ± 4 *
SVC (L·min^{-1}·m^2)	0.045 ± 0.018	0.017 ± 0.002^t *
FBF (ml·min^{-1}·100ml^{-1})	2.21 ± 0.59	2.76 ± 0.83
FVC (ml·min^{-1}·100ml^{-1}·mm Hg^{-1})	0.0248 ± 0.007	0.0345 ± 0.010

HR, heart rate; SV, stroke volume; CO, cardiac output; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; SVC, systemic vascular conductance; FBF, forearm blood flow; FVC, forearm vascular conductance. ^{t} indicates sample size of three. Values are mean ± standard deviation.
*Significantly different from controls ($p < 0.05$).

4.1.3 Forearm Vascular Bed Mechanics

The values of resistance, viscoelasticity and inertia were not significant between the AC S674 variant group and controls. However, compliance was lower in the AC S674 variant group compared to controls ($p < 0.05$; Table 4.3).

Table 4.3 Baseline forearm vascular bed mechanics

	Control (n = 5)	AC S674 (n = 4)
R (mm Hg·ml^{-1}·min^{-1})	5.44 ± 1.46	4.55 ± 1.79
C (ml·mm Hg^{-1}·100ml^{-1})	$9.2 \times 10^{-4} \pm 2.0 \times 10^{-4}$	$6.8 \times 10^{-4} \pm 6.1 \times 10^{-5}$ *
K (mm Hg·ml^{-1}·min^{-1})	$4.9 \times 10^{-2} \pm 2.8 \times 10^{-2}$	$8.7 \times 10^{-2} \pm 5.1 \times 10^{-2}$
L (mm Hg·ml^{-1}·min^2)	$1.4 \times 10^{-5} \pm 1.1 \times 10^{-5}$	$1.6 \times 10^{-5} \pm 1.5 \times 10^{-5}$

R, resistance; C, compliance; K, viscoelasticity; L, inertia. Values are mean ± standard deviation.
*Significantly different from controls ($p < 0.05$).

4.2 Handgrip Exercise

4.2.1 Forearm Blood Flow

There was no significant group x handgrip interaction effect on FBF. FBF increased significantly in response to handgrip exercise. This main effect was observed for all levels of handgrip intensity (20% MVC: $p < 0.05$, Figure 4.1A; 40% MVC: $p < 0.05$, Figure 4.3 A; 60% MVC; $p < 0.05$, Figure 4.5 A).

The change in FBF from pre- to post-handgrip exercise was not significant between AC S674 and control groups at each handgrip intensity ($p =$ NS; Figures 4.2, 4.4, and 4.6).

4.2.2 Mean Arterial Pressure

There was no significant group x handgrip interaction effect on MAP. MAP remained constant throughout all levels of handgrip exercise ($p =$ NS). However, MAP was greater in controls throughout handgrip exercise at 20 and 40% MVC. This main effect of group was observed at 20 and 40% MVC (20% MVC: $p < 0.05$, Figure 4.1 B; 40% MVC: $p < 0.05$, Figure 4.3 B), but not 60% MVC ($p =$ NS; Figure 4.5 B).

4.2.2 Forearm Vascular Conductance

There was no significant group x handgrip interaction effect on FVC. FVC increased significantly in response to handgrip exercise. This main effect was observed for all levels of handgrip intensity (20% MVC: $p < 0.05$, Figure 4.1 C; 40% MVC: $p < 0.05$, Figure 4.3 C; 60% MVC: $p < 0.05$, Figure 4.5 C).

The change in FVC from pre- to post-handgrip exercise was not significant between AC S674 and control groups at each handgrip intensity ($p =$ NS; Figures 4.2, 4.4 and 4.6).

A.

B.

C.

Figure 4.1 Forearm blood flow (FBF), mean arterial pressure (MAP), and forearm vascular conductance (FVC) responses to 20% MVC handgrip exercise in control (n = 5) and AC S674 (n = 4). **A.** Main effect of handgrip on FBF ($p < 0.05$); **B.** Main effect of group on MAP ($p < 0.05$); **C.** Main effect of handgrip on FVC ($p < 0.05$). Values are mean ± standard deviation.

Figure 4.2 ΔFBF (top) and ΔFVC (bottom) to 20% handgrip exercise in control (n = 5) and AC S674 (n = 4). No significant differences occurred between groups for either FBF or FVC (*p* = NS). Values are mean ± standard deviation.

A.

B.

C.

Figure 4.3 Forearm blood flow (FBF), mean arterial pressure (MAP), and forearm vascular conductance (FVC) responses to 40% MVC handgrip exercise in control (n = 5) and AC S674 (n = 4). **A.** Main effect of handgrip on FBF ($p < 0.05$); **B.** Main effect of group on MAP ($p < 0.05$); **C.** Main effect of handgrip on FVC ($p < 0.05$). Values are mean ± standard deviation.

Figure 4.4 ΔFBF (top) and ΔFVC (bottom) to 40% handgrip exercise in control (n = 5) and AC S674 (n = 4). No significant differences occurred between groups for either FBF or FVC (*p* = NS). Values are mean ± standard deviation.
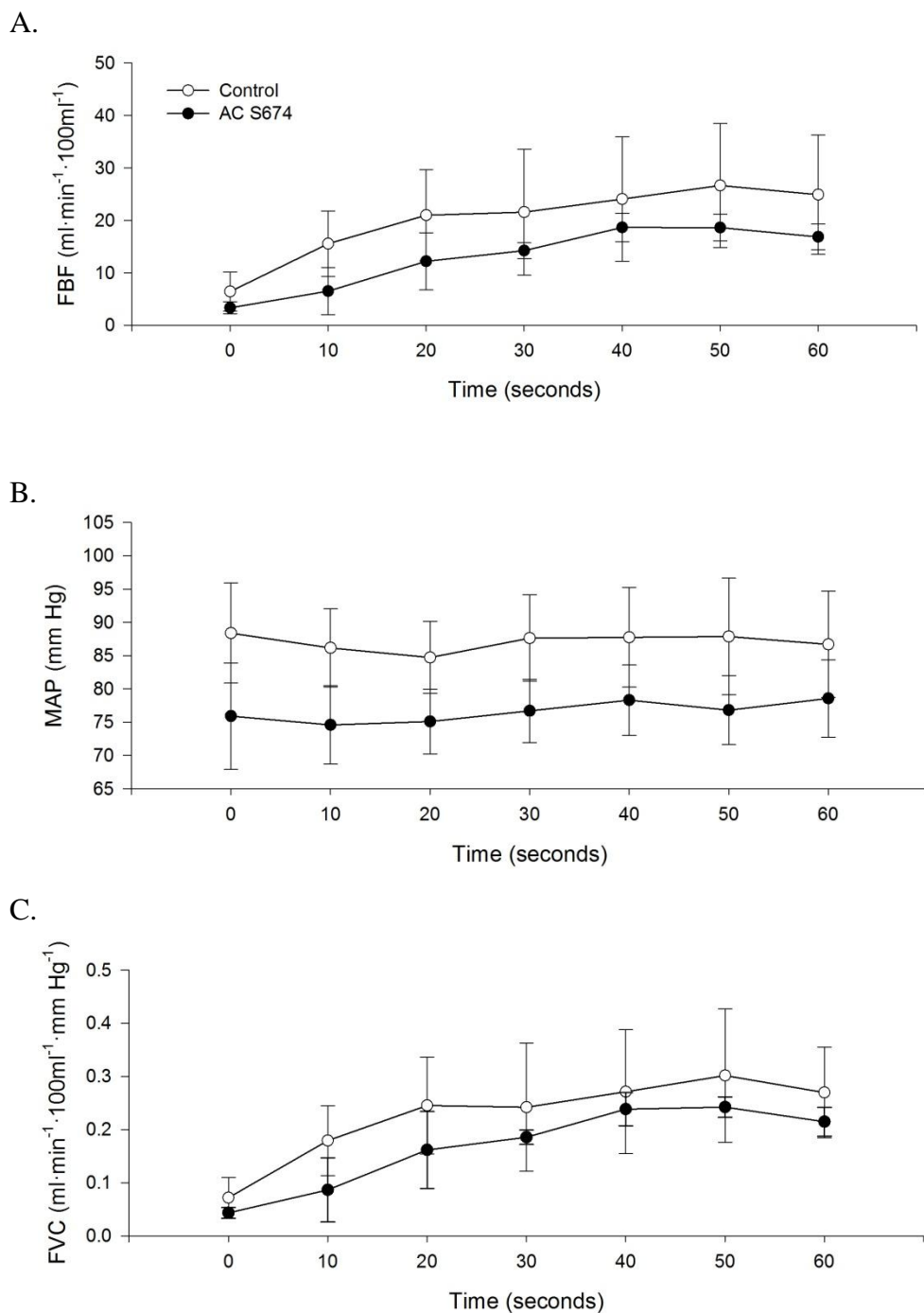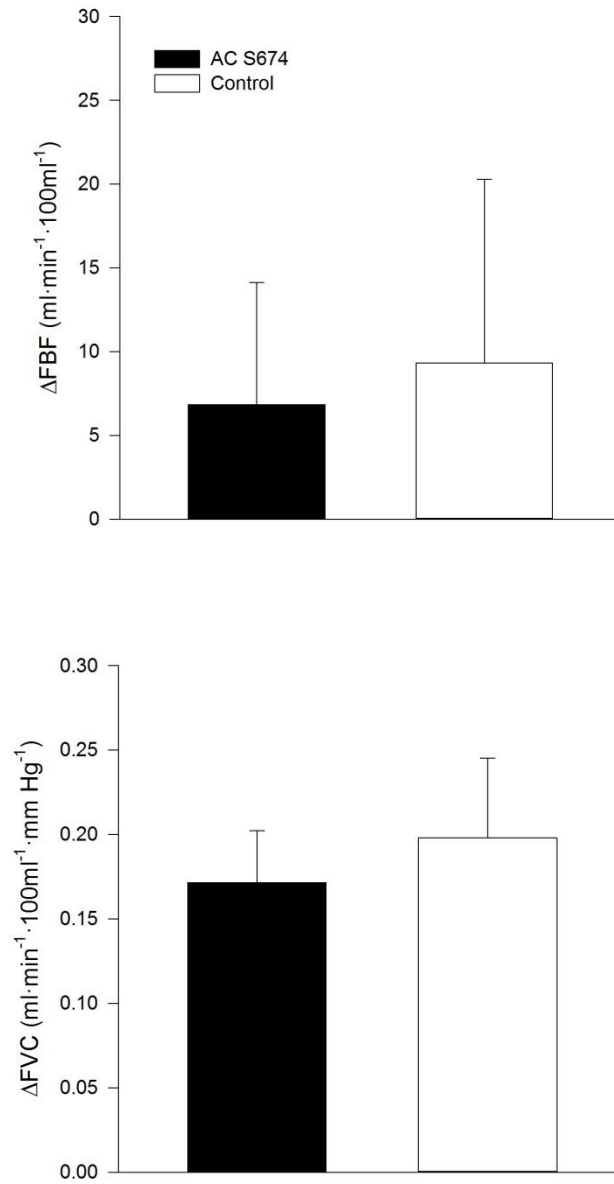
A.

B.

C.
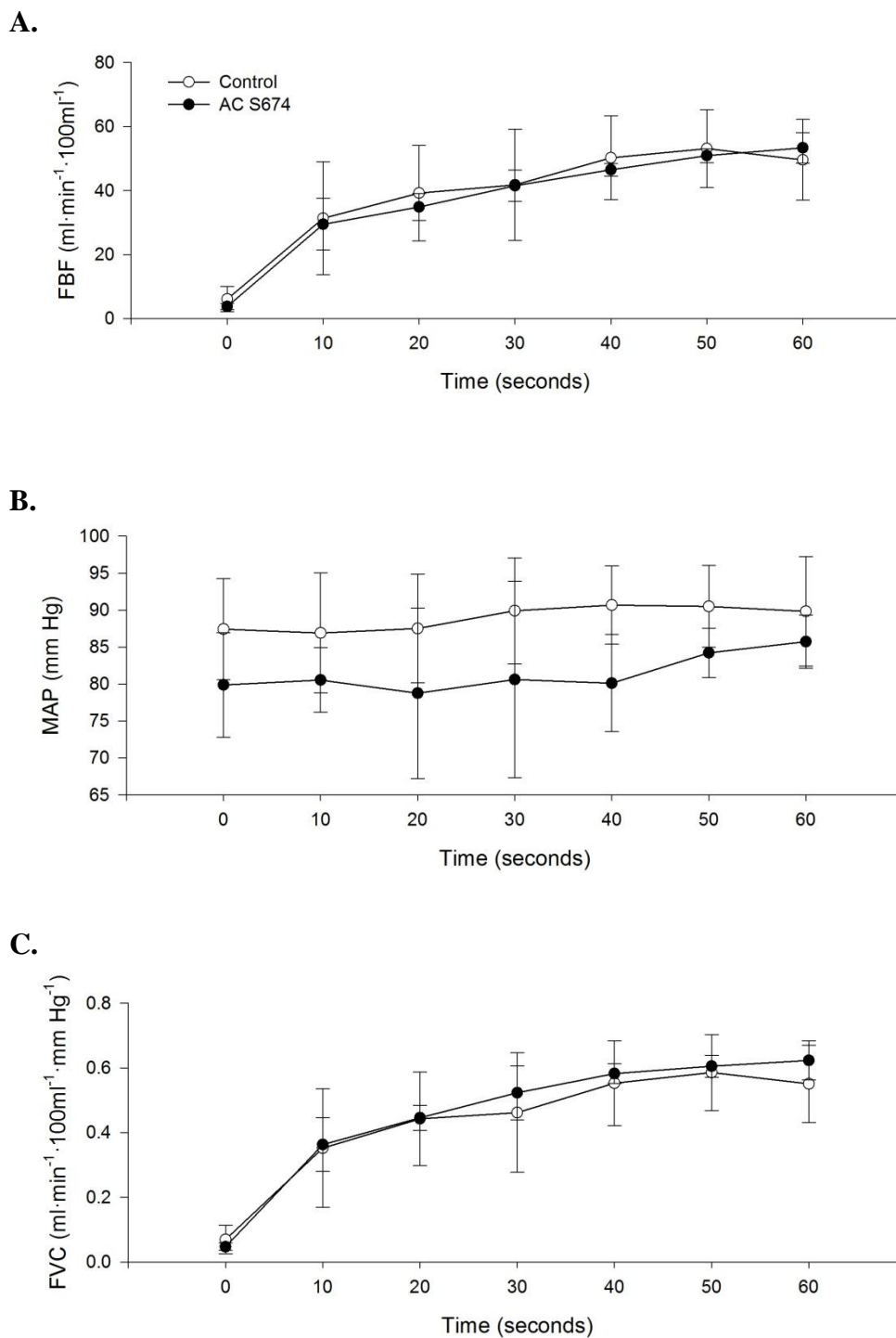
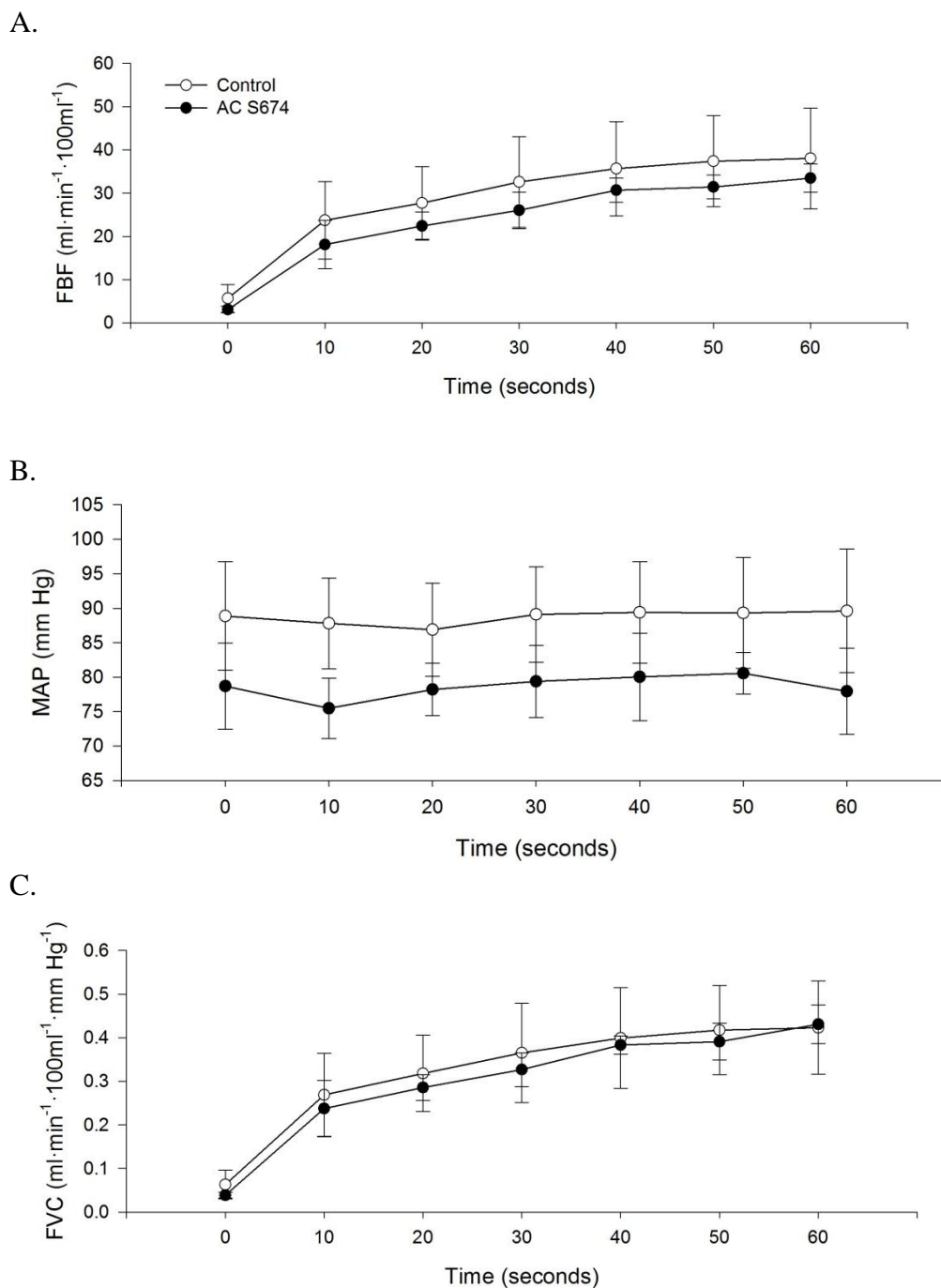**Figure 4.5** Forearm blood flow (FBF), mean arterial pressure (MAP), and forearm vascular conductance (FVC) responses to 60% MVC handgrip exercise in control (n = 5) and AC S674 (n = 4). **A.** Main effect of handgrip on FBF ($p < 0.05$); **B.** No significant interaction or main effects observed for MAP ($p =$ NS); **C.** Main effect of handgrip on FVC ($p < 0.05$). Values are mean ± standard deviation.
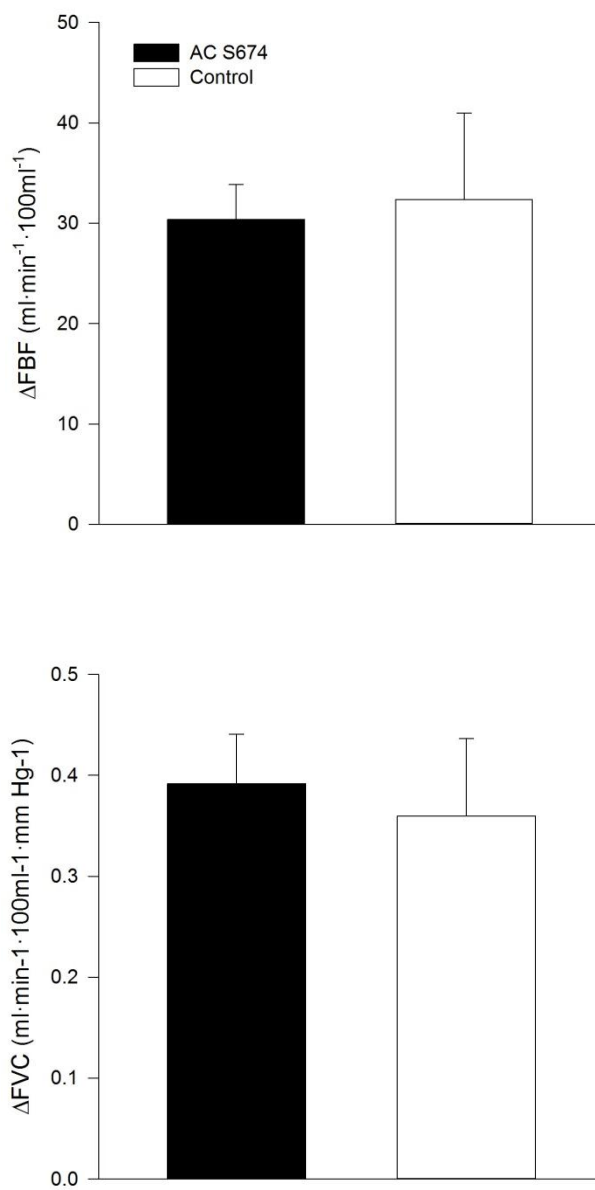
Figure 4.6 ΔFBF (top) and ΔFVC (bottom) to 60% handgrip exercise in control (n = 5) and AC S674(n = 4). No significant differences occurred between groups for either FBF or FVC (*p* = NS). Values are mean ± standard deviation.
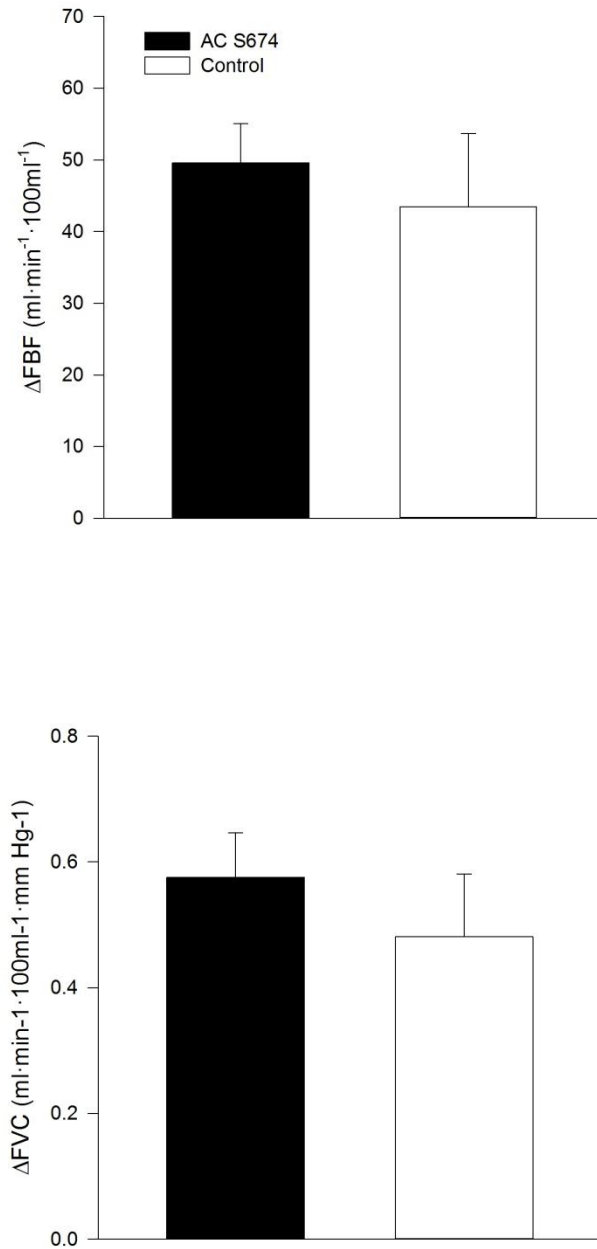
4.3 Cardiac and Vasomotor Control

4.3.1 Heart Rate

There was no significant group x dose interaction effect of isoproterenol on heart rate. However, there was a main effect of isoproterenol dose on HR. Specifically, HR increased across isoproterenol doses ($p < 0.05$; Figure 4.7 C).

The change in heart rate was not significantly different between AC S674 and control groups throughout propranolol infusion (Figure 4.14 A).

4.3.2 Stroke Volume

There was no significant group x dose interaction effect of isoproterenol on stroke volume. However, stroke volume was increased in controls compared to AC S674 ($p < 0.05$, Figure 4.8 C).

The change in stroke volume was not significantly different between AC S674 and control groups throughout propranolol infusion (Figure 4.14 B).

4.3.3 Cardiac Output

There was no significant group x dose interaction effect of isoproterenol on cardiac output. However, there was a main effect of isoproterenol dose on CO. Specifically, CO increased across isoproterenol doses ($p < 0.05$; Figure C). There was also a main effect of group on cardiac output across isoproterenol doses (controls > AC S674; $p < 0.05$, Figure 4.9 C).

The change in cardiac output was not significantly different between AC S674 and control groups throughout propranolol infusion (Figure 4.14 C).

4.3.4 Mean Arterial Pressure

There was no significant group x dose interaction effect of isoproterenol on mean arterial pressure. Additionally, mean arterial pressure was not significantly different across isoproterenol doses ($p = $ NS; Figure 4.10 C).

The change in mean arterial pressure was not significantly different between AC S674 and control groups throughout propranolol infusion (Figure 4.15 A).

4.3.5 Systemic Vascular Conductance

There was no significant group x dose interaction effect of isoproterenol on systemic vascular conductance. Additionally, systemic vascular conductance was not significantly different across isoproterenol doses (p = NS; Figure 4.11 C).

The change in systemic vascular conductance was not significantly different during propranolol infusion (p = NS; Figure 4.15 B)

4.3.6 Forearm Blood Flow

There was no significant group x dose interaction effect of isoproterenol on forearm blood flow. However, there was a main effect of isoproterenol dose on forearm blood flow. Specifically, forearm blood flow increased across isoproterenol doses ($p < 0.05$, Figure 4.12 C).

The change in forearm blood flow was not significantly different during propranolol infusion (p = NS; Figure 4.16 A).

4.3.7 Forearm Vascular Conductance

There was no significant group x dose interaction effect of isoproterenol on forearm vascular conductance. However, there was a main effect of isoproterenol dose on forearm vascular conductance. Specifically, forearm vascular conductance increased across isoproterenol doses ($p < 0.05$, Figure 4.13 C).

The change in forearm vascular conductance was not significantly different during propranolol infusion (p = NS; Figure 4.16 B).

4.3.8 Forearm Vascular Bed Mechanics

There were no significant group x dose interaction effect of isoproterenol on resistance, compliance, viscoelasticity, or inertia. However, resistance decreased across isoproterenol doses ($p < 0.05$, Figure 4.17 A). Conversely, compliance increased across isoproterenol doses ($p < 0.05$, Figure 4.17 B). Additionally, there was a main effect of group on compliance ($p < 0.05$, Figure 4.17 B). Specifically, compliance was lower in AC S674 compared to controls. Viscoelasticity was not significantly different between groups and across isoproterenol doses (Figure 4.17 C). There was a main effect of isoproterenol dose on inertia ($p < 0.05$, Figure 4.17 D). Propranolol infusion did not have a significant effect on ΔR, ΔC, ΔK, or ΔL (p = NS; Figure 4.18 and Figure 4.19).
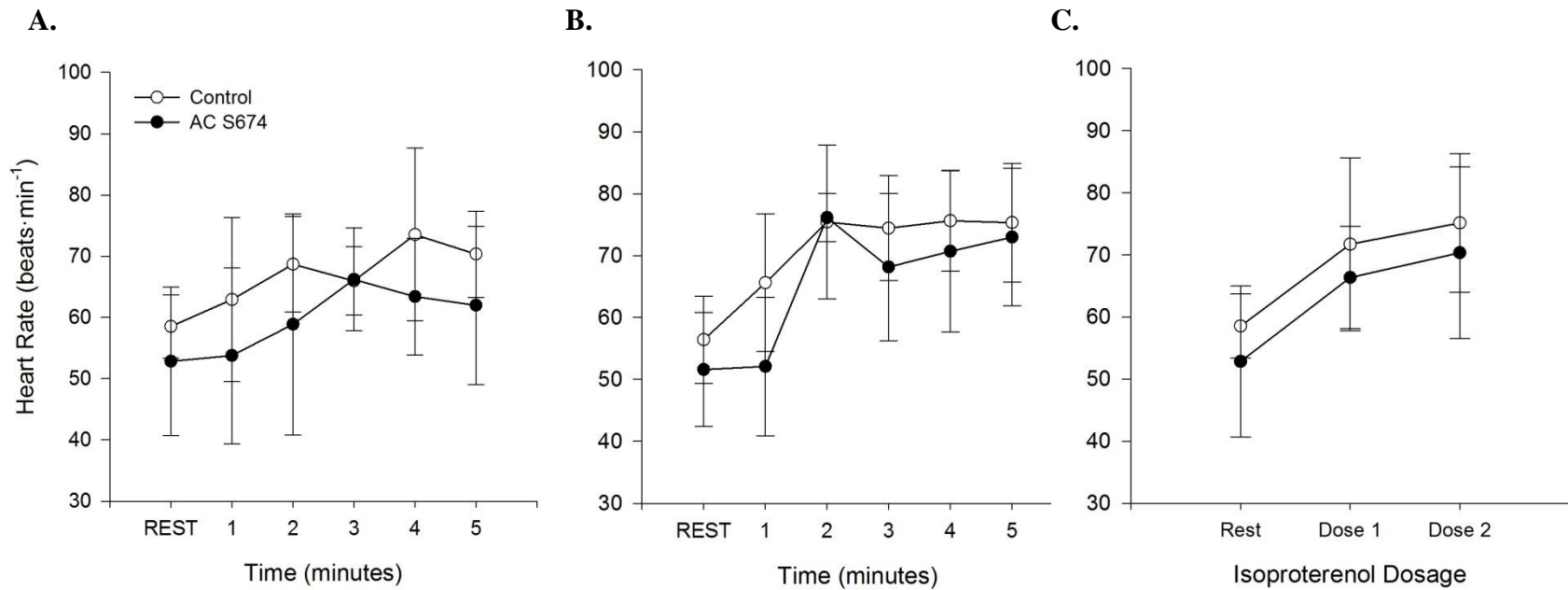
Figure 4.7 Heart rate (HR) responses to isoproterenol. **A.** HR response to first isoproterenol dose ($0.01\mu g \cdot kg^{-1}$); **B.** HR response to second isoproterenol dose ($0.02\mu g \cdot kg^{-1}$); **C.** Maximum HR values obtained during isoproterenol dose one and two. A main effect of isoproterenol dose on HR occurred (**C**; $p < 0.05$; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.

Figure 4.8 Stroke volume (SV) responses to isoproterenol. **A.** SV response to first isoproterenol dose ($0.01\mu g \cdot kg^{-1}$); **B.** SV response to second isoproterenol dose ($0.02\mu g \cdot kg^{-1}$); **C.** Maximum stroke volume values obtained during isoproterenol dose one and two. A main effect group on SV occurred (**C**; $p < 0.05$; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
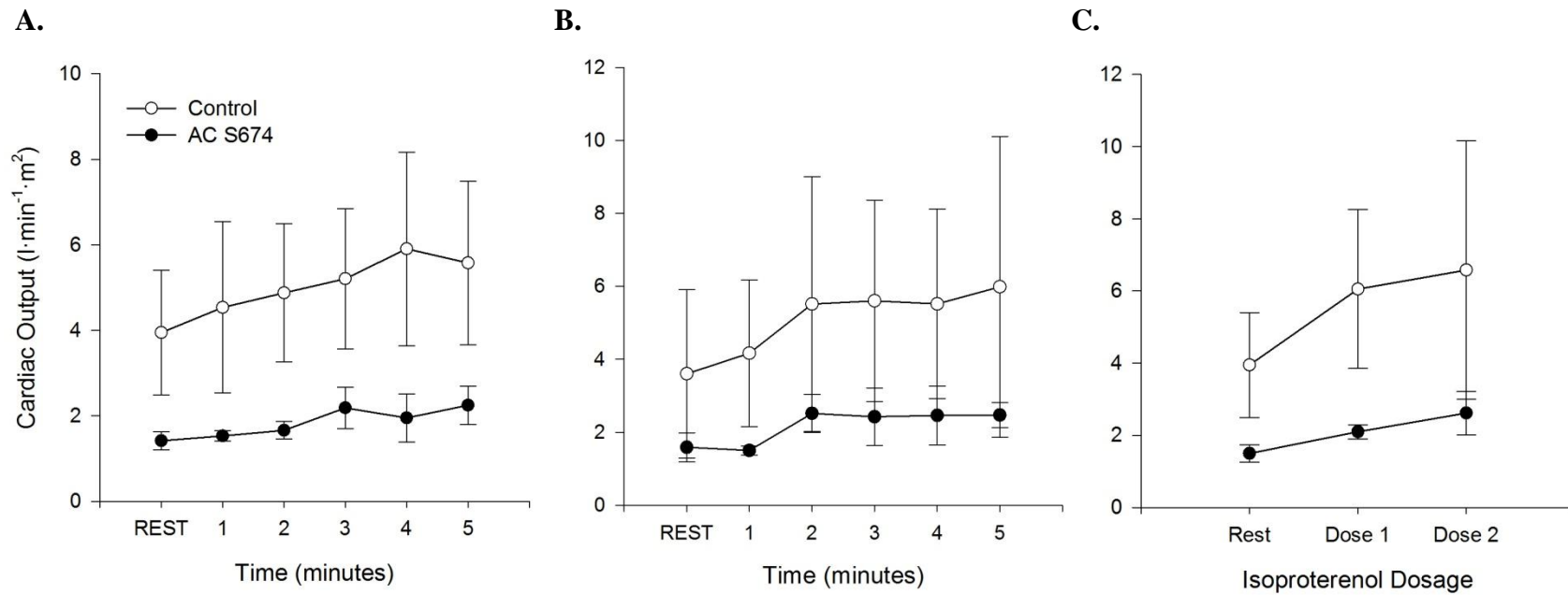
Figure 4.9 Cardiac output (CO) responses to isoproterenol. **A.** CO response to first isoproterenol dose (0.01µg·kg^{-1}); **B.** COresponse to second isoproterenol dose (0.02µg·kg^{-1}); **C.** Maximum CO values obtained during dose one and two. A main effect of isoproterenol dose on CO occurred (**C**; $p < 0.05$; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
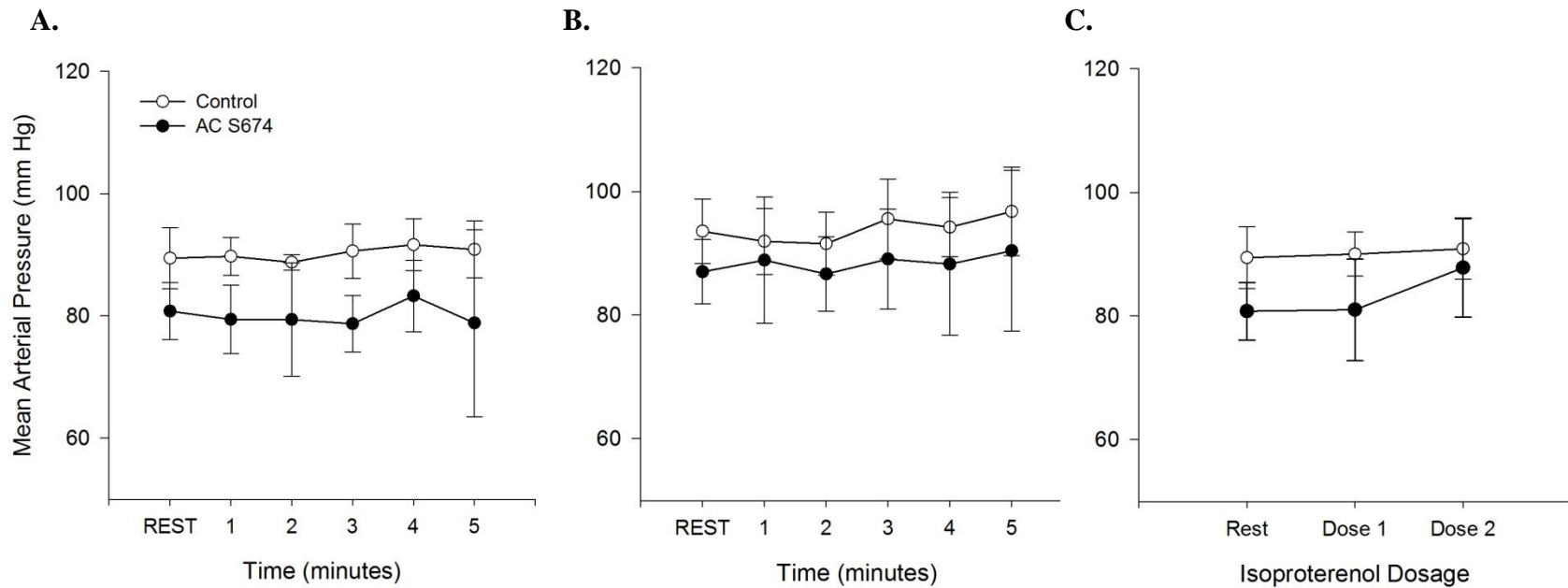
Figure 4.10 Mean arterial pressure (MAP) responses to isoproterenol. **A.** MAP response to first isoproterenol dose $(0.01\mu g \cdot kg^{-1})$; **B.** MAP response to second isoproterenol dose $(0.02\mu g \cdot kg^{-1})$; **C.** Maximum MAP values obtained during dose one and two. There was no effect of isoproterenol dose or group on MAP (**C**; p = NS; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
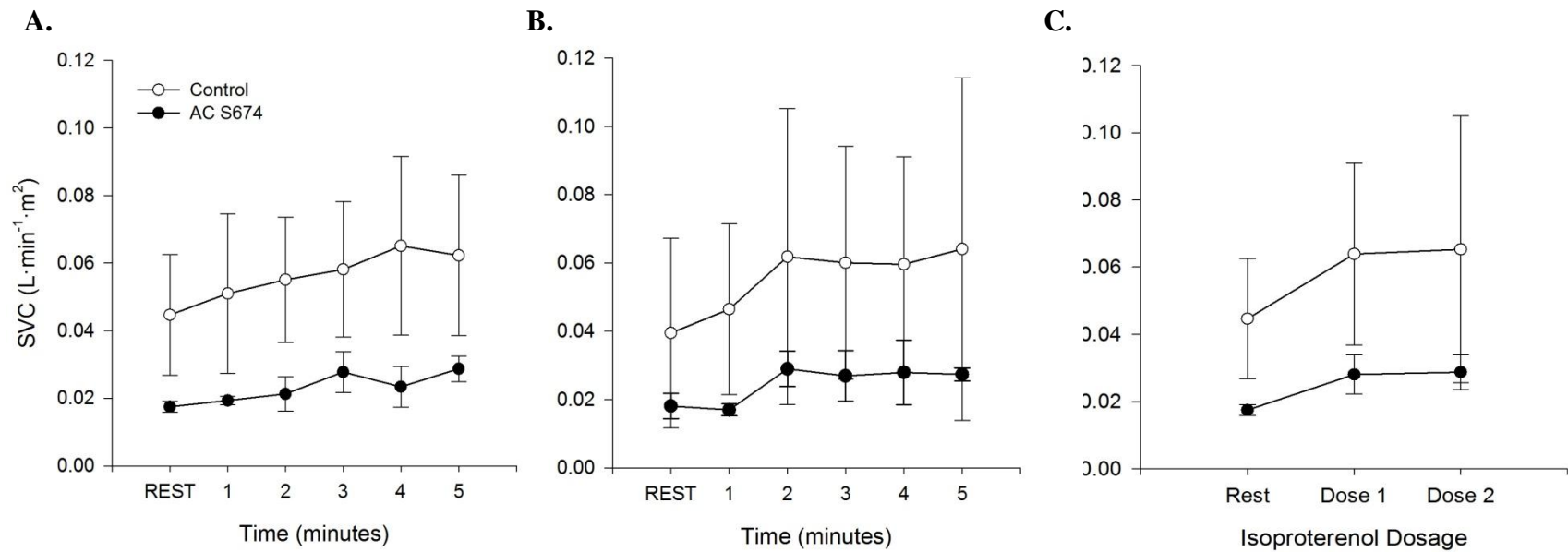
Figure 4.11 Systemic vascular conductance (SVC) responses to isoproterenol. **A.** SVC response to first isoproterenol dose (0.01μg·kg^{-1}); **B.** SVC response to second isoproterenol dose (0.02μg·kg^{-1}); **C.** Maximum SVC values obtained during dose one and two. There was no effect of isoproterenol dose or group on SVC (**C**; p = NS; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
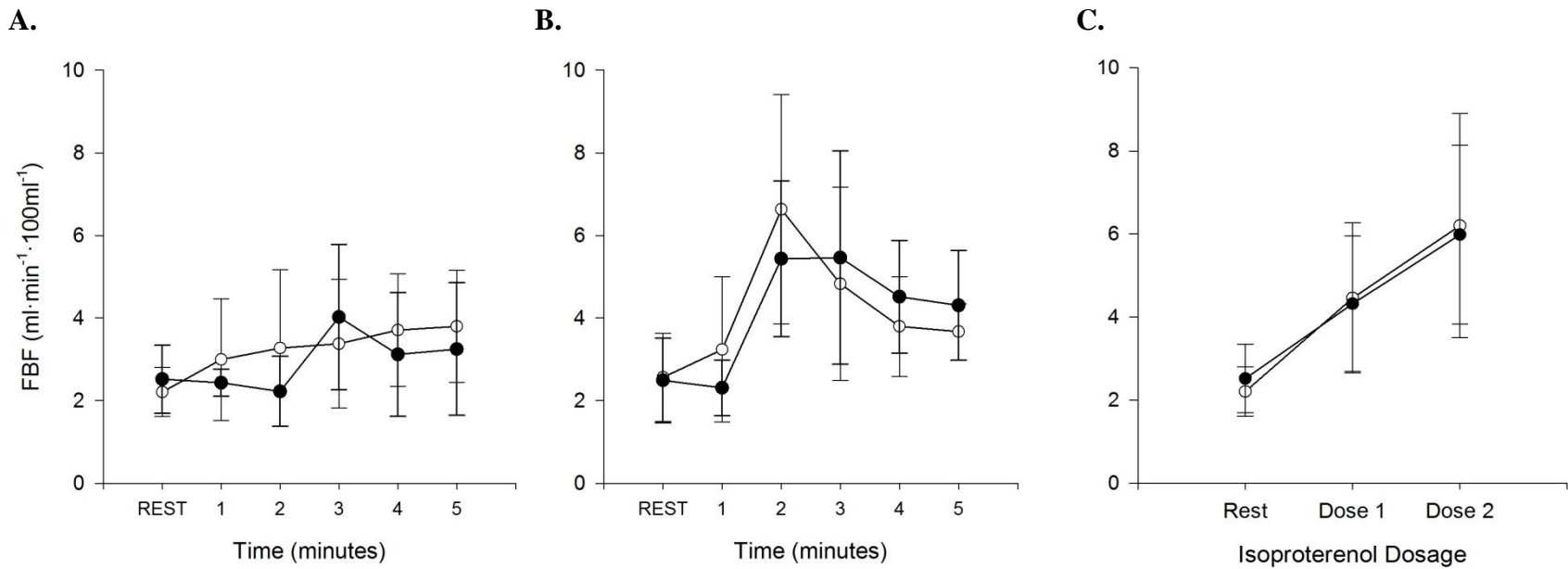
Figure 4.12 Forearm blood flow (FBF) responses to isoproterenol. **A.** FBF response to first isoproterenol dose (0.01μg·kg^{-1}); **B.** FBF response to second isoproterenol dose (0.02μg·kg^{-1}); **C.** Maximum FBF values obtained during dose one and two. A main effect of isoproterenol dose on FBF occurred (**C**; $p < 0.05$; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
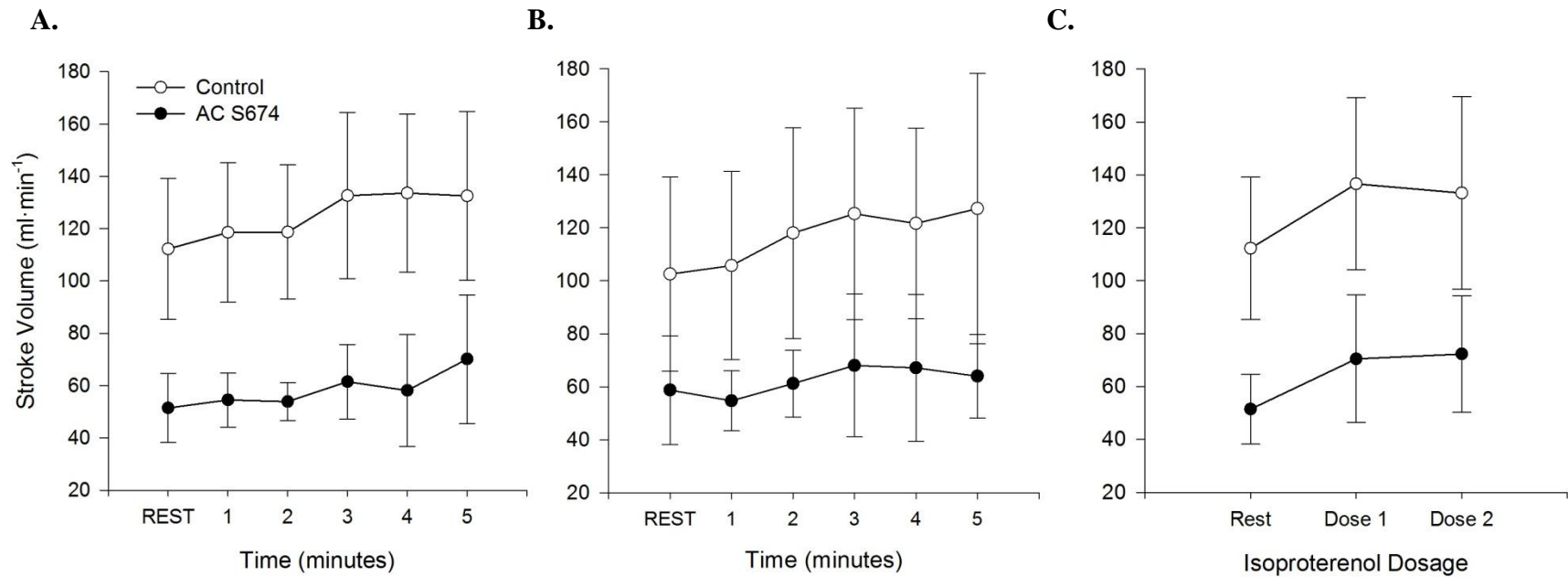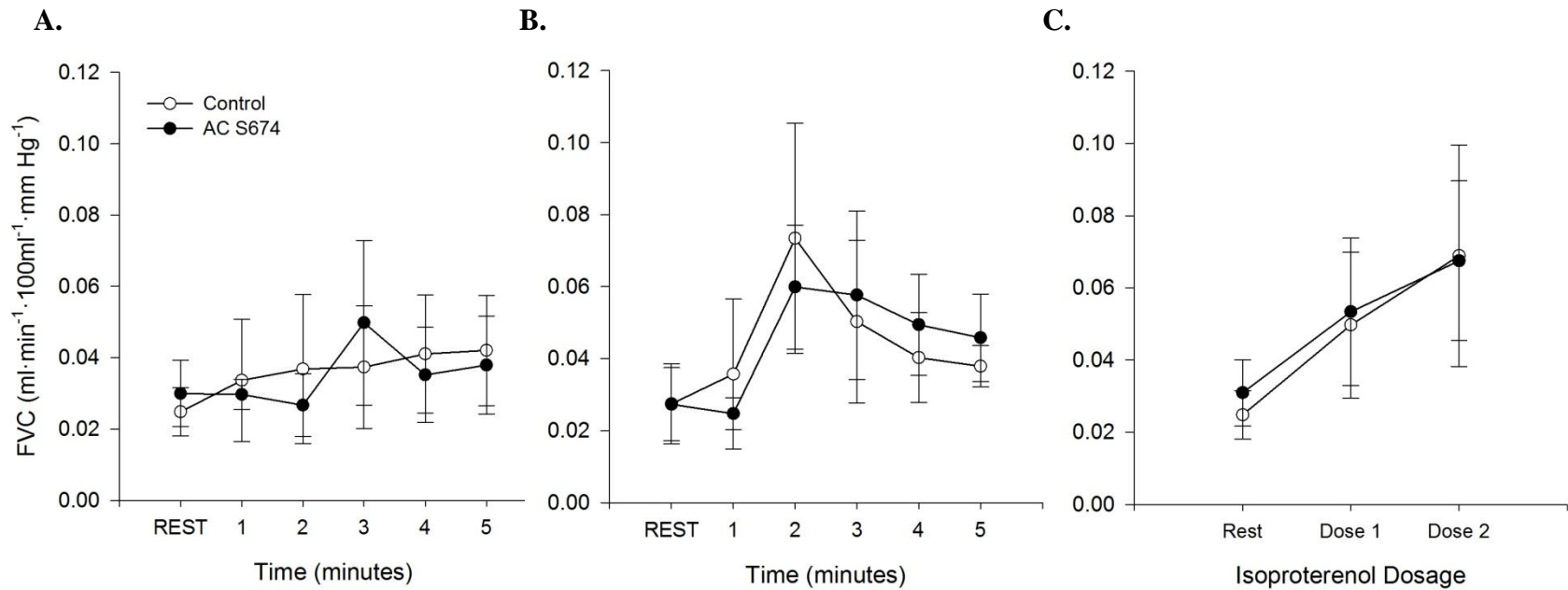
Figure 4.13 Forearm vascular conductance (FVC) responses to isoproterenol. **A.** FVC response to first isoproterenol dose (0.01μg·kg^{-1}); **B.** FVC response to second isoproterenol dose (0.02μg·kg^{-1}); **C.** Maximum values obtained during dose one and two. A main effect of isoproterenol dose on FVC occurred (**C**; $p < 0.05$; controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.

Figure 4.14 Cardiac responses to propranolol infusion. Left: time course response, Right: change (Δ) from rest to 15 minutes. **A.** No significant differences between groups on ΔHR; **B.** No significant differences between groups on ΔSV; and **C.** No significant differences between groups on ΔCO (controls: n = 6; AC S674: n = 3). Values are means $\pm$ standard deviation.

Figure 4.15 Systemic responses to propranolol infusion. Left: time course response, Right: change (Δ) from rest to 15 minutes. **A.** No significant difference between groups on ΔMAP; **B.** No significant difference between groups on ΔSVC (controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
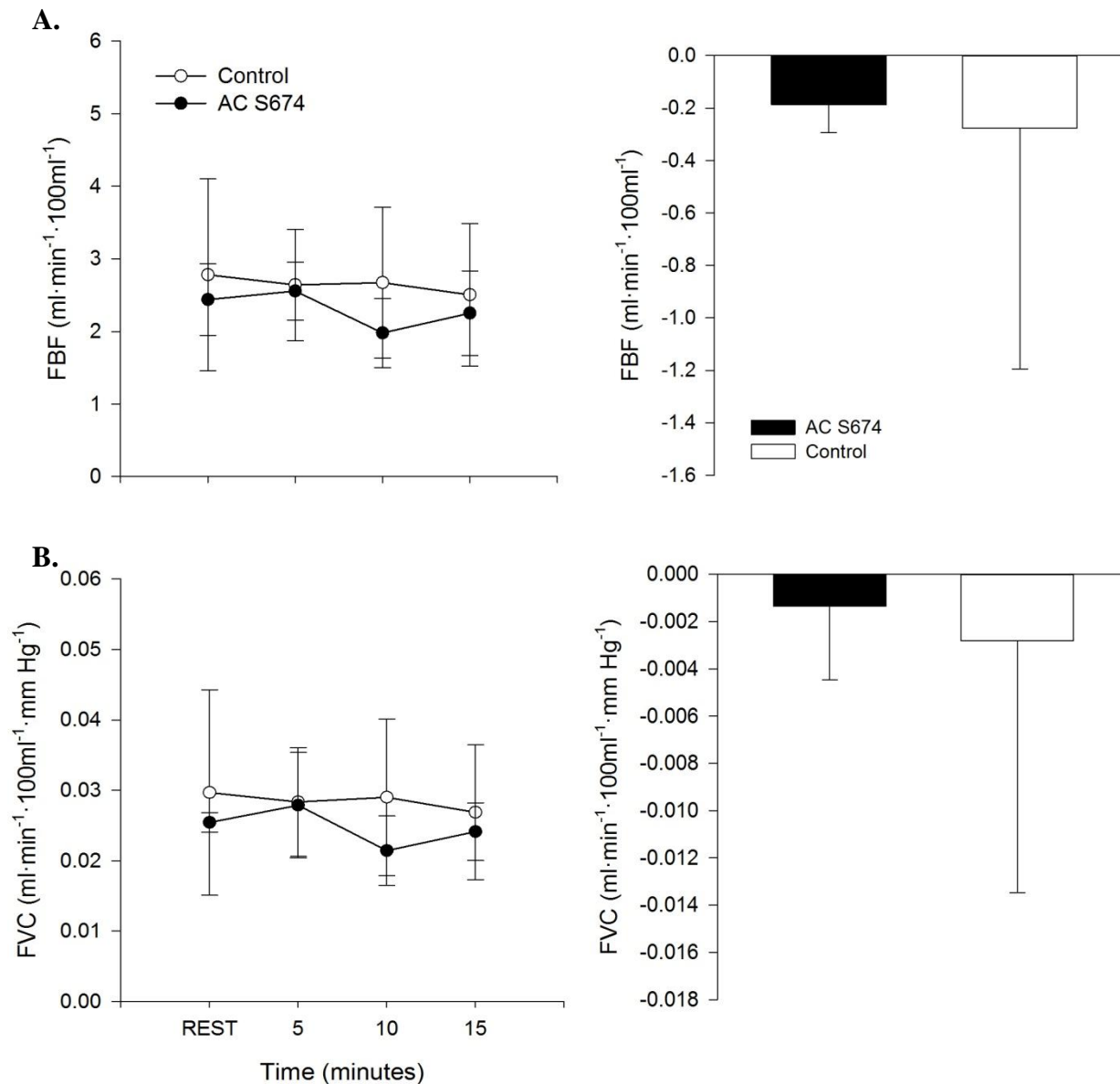
Figure 4.16 Forearm vascular responses to propranolol infusion. Left: time course response, Right: change (Δ) from rest to 15 minutes. **A.** No significant difference between groups on ΔFBF; **B.** No significant difference between groups on ΔFVC (controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
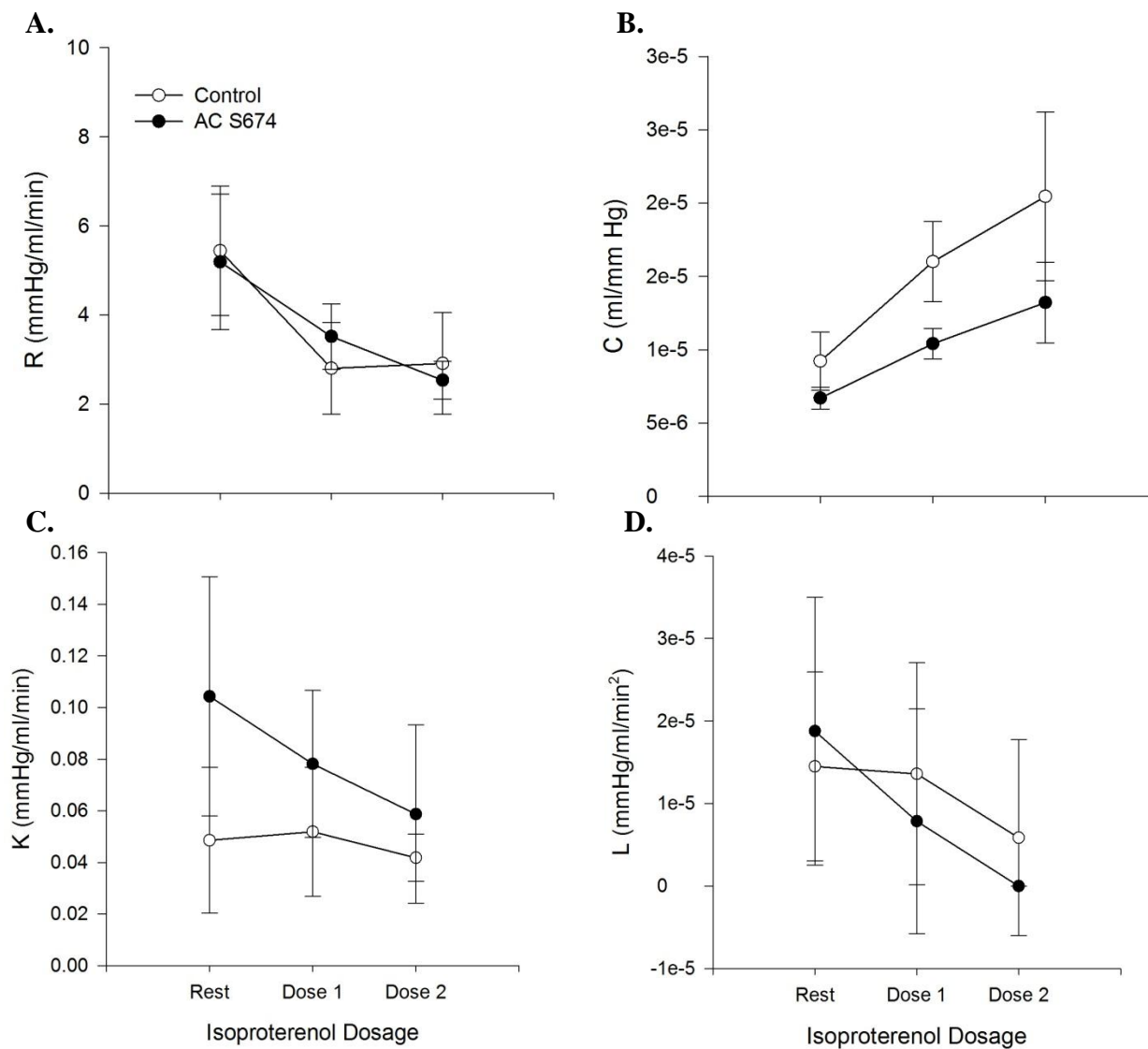
Figure 4.17 Maximal forearm vascular bed mechanic responses to isoproterenol. **A.** Main effect of isoproterenol on resistance. **B.** Main effect of isoproterenol on compliance. **C.** No significant effect of isoproterenol or group on viscoelasticity. **D.** Main effect of isoproterenol on inertia (controls: n = 5; AC S674: n = 3). Values are means ± standard deviation.

A.

B.
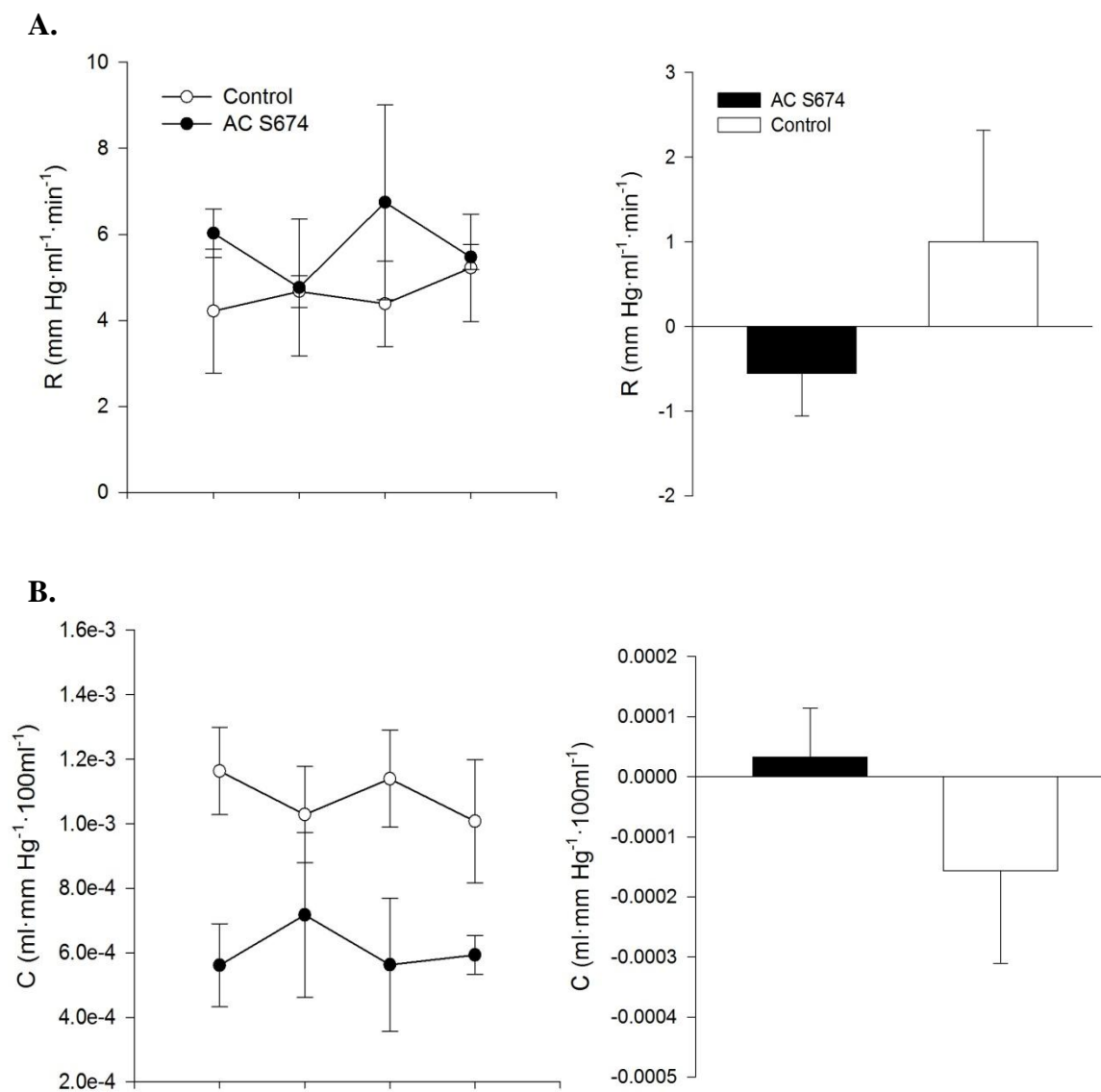
Figure 4.18 Forearm vascular bed mechanic responses to propranolol. Left: time course response, Right: change (Δ) from rest to 15 minutes. **A.** No significant difference between groups on Δ resistance; **B.** No significant difference between groups on Δ compliance (controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.
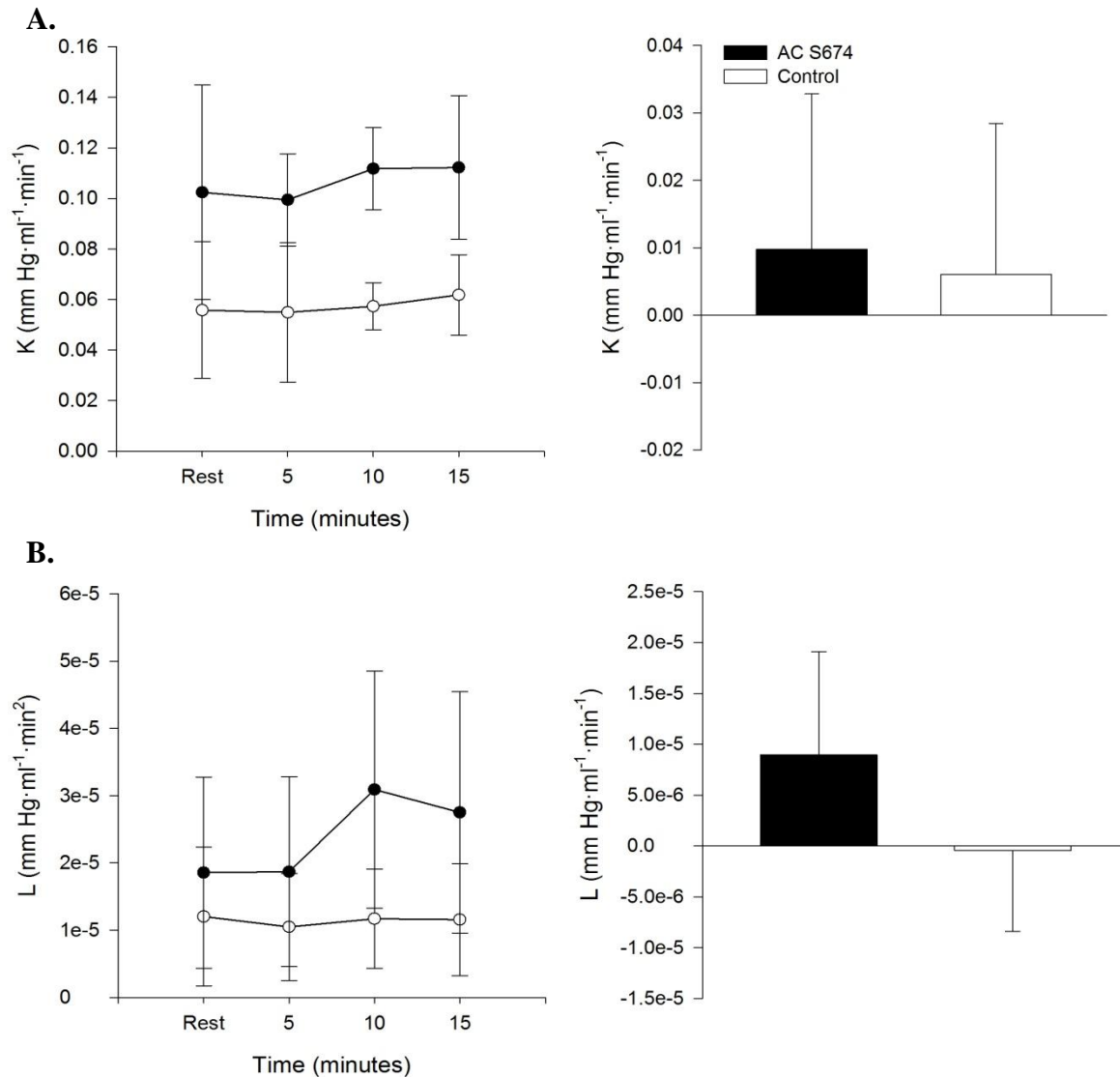
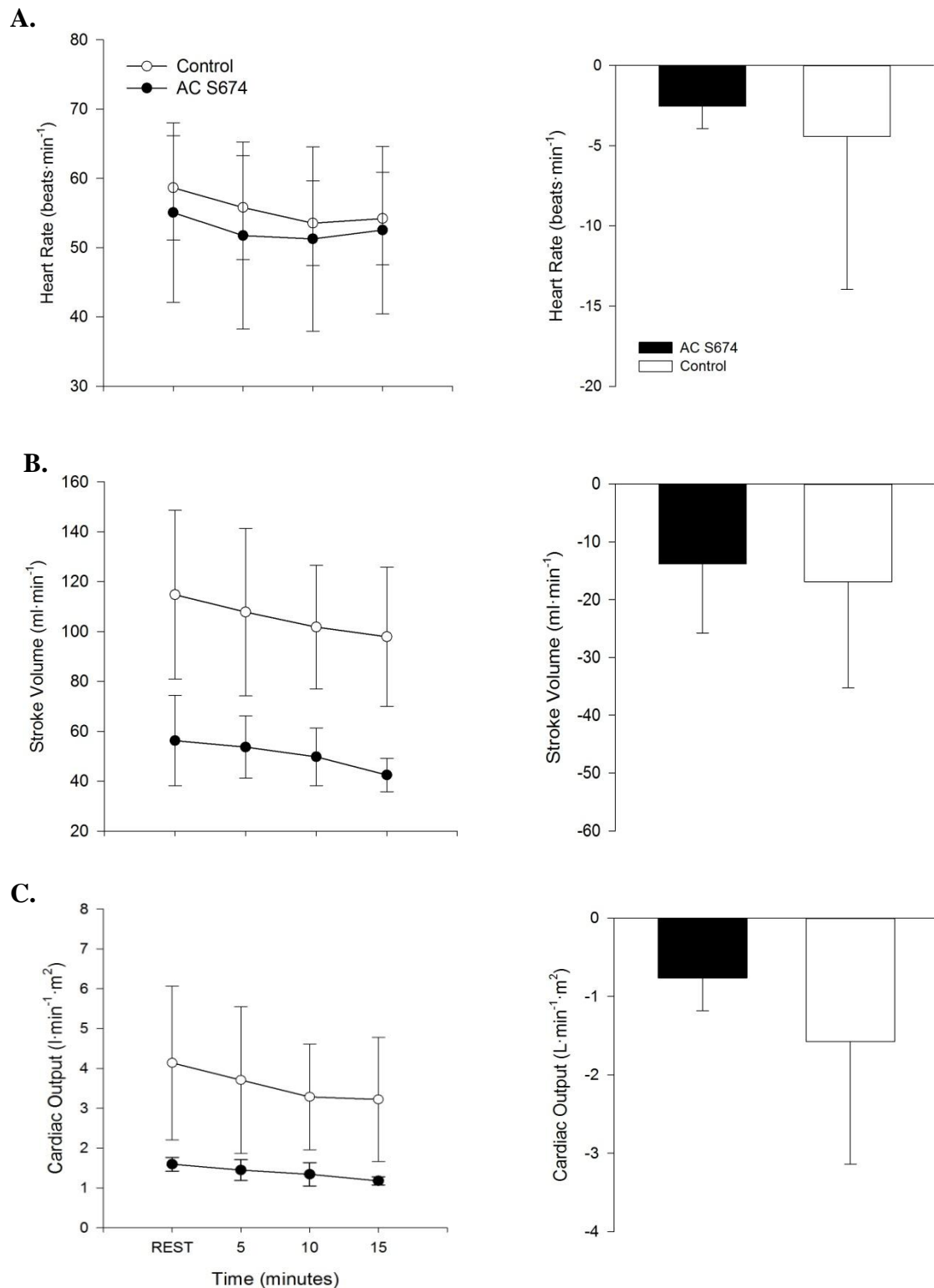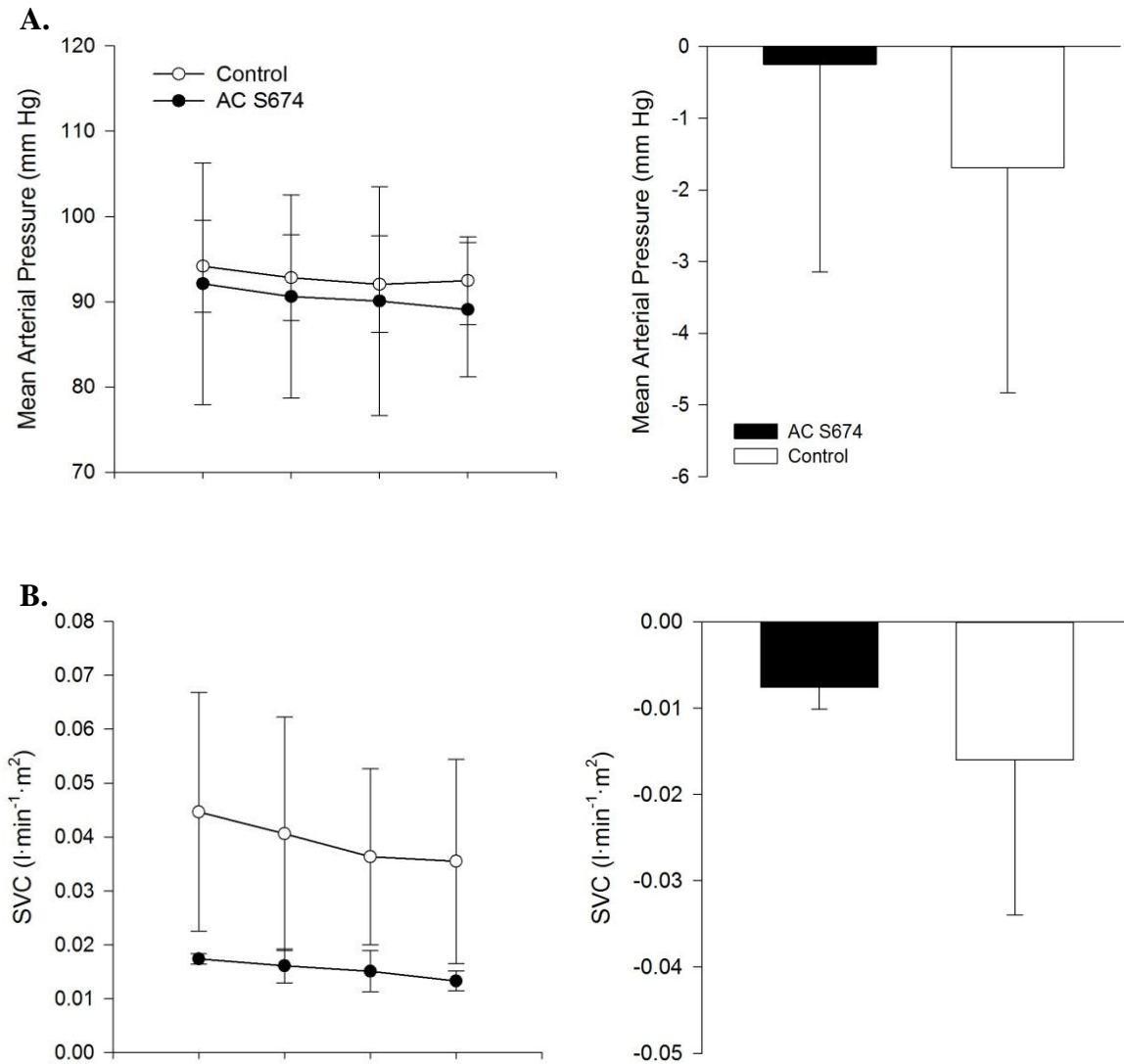**Figure 4.19** Forearm vascular bed mechanic responses to propranolol. Left: time course response, Right: change (Δ) from rest to 15 minutes. **A.** No significant difference between groups on Δ viscoelasticity; **B.** No significant difference between groups on Δ inertia (controls: n = 6; AC S674: n = 3). Values are means ± standard deviation.

Chapter 5

5. Discussion

The purpose of the present study was to identify the role of AC S674 in cardiac and vasomotor control. First, compared with non-variant, individuals with AC S674 expressed decreased baseline cardiac contractility (SV and CO), altered blood pressure characteristics (decreased DBP, MAP; increased PP), decreased forearm vascular bed compliance, and increased vasomotor contractile state (decreased SVC). However, neither the systemic nor forearm responses to beta-adrenergic activation or blockade, nor the exercise-induced dilation during handgrip, were affected by AC S674. Therefore, the major impact of this adenylyl cyclase variant appears to affect baseline hemodynamics and not responsiveness to vasoactive stimuli.

5.1 Baseline

The AC S674 genetic variant has been referred to as a hyperfunctional enzyme compared to the more common AC A674 genotype (Gros, 2007; Hodges, 2010). As such, it is possible that there is increased activity of the AC S674 enzyme and cAMP production at rest. Additionally, this increase in AC S674 activity would be expected to promote greater cardiac contractility, smooth muscle relaxation, enhanced vasodilation of arteries, decreased vasomotor tone and, therefore, increased vascular conductance. However, the results of the current study indicate the opposite, as evidenced by decreased cardiac output and vascular bed compliance.

Blood flow to vascular beds within the human body is determined by perfusion pressure (mean arterial pressure) and vascular contractile state (conductance). However, MAP was significantly decreased in AC S674 individuals and relied on a reduced SVC to maintain the same level of blood flow as controls. The three-fold smaller CO at rest in AC S674 individuals was the determining factor of reduced SVC. In turn, the smaller CO was related first to a smaller aortic diameter. This is the first report of altered CO and aortic diameter in this variant group and suggests that a major impact of AC S674 affects cardiac structure. As this finding was unexpected, additional studies are needed to address this important possibility. Further, the enhanced PP in this group is consistent with reduced aortic size and indicates an increased vascular stiffness of the central arteries. This observation is consistent with the diminished compliance of the forearm vascular bed. For reasons that are not known, the intervening brachial

artery conductance was not different between groups. Overall, however, the altered cardiac and vascular structure appears to be a major outcome in this AC variant.

The mechanisms mediating this altered structure remain to be determined, but could include elevated adrenergic or angiotensin II stimuli. Under baseline conditions, sympathetic vasomotor nerves regulate arterial pressure and control the distribution of CO at rest (Dampney, 2002). It is possible that AC S674 individuals express greater sympathetic tone or increased levels of vasoconstrictor metabolites. However, previously observed baseline levels of norepinephrine were similar in AC S674 variant individuals and non-variants (Hodges, 2010). Additionally, elevated angiotensin II could also affect cardiac and vascular structure and mechanics. Importantly, AC S674 carriers were found to have increased levels of circulating plasma renin (Hodges, 2010). Renin is a crucial enzyme that regulates the production of angiotensin II, a potent vasoconstrictor. In the current study, reduced SVC in AC S674 could be a reflection of the chronic effects of increased renin activity, leading to enhanced aldosterone-mediated effects on blood volume in addition to possible increased ambient angiotensin II-mediated effects (Hodges, 2010).

In the same way that vascular conductance (or resistance) influences steady state flow, a change in compliance affects the impedance of the vascular bed in pulsatile flow. Additionally, arterial compliance is influenced by vascular smooth muscle contractility and is an indicator of vasomotor control (Zamir, 2007). In the present study, baseline forearm vascular bed compliance was lower in AC S674 individuals. This reduction in compliance may be related to increased vasomotor tone (Frances, 2011). However, compliance represents the elastic ability of the arterial wall and, is therefore associated with elastin fibres (Zamir, 2009). Whether this diminished compliance is due to increased neurogenic vasoconstrictor effects or altered vascular stiffness remains to be determined.

The AC S674 group consisted of young, physically active individuals free of cardiovascular abnormalities. It is possible that the cardiac data may vary from general population due to above-average fitness levels of participants in both groups. However, the long-term effect of chronically reduced SVC and diminished vascular bed compliance is a point of interest for continued study for this group. Furthermore, AC S674 individuals expressed lower DBP and greater PP compared to controls. These two variables are the preeminent markers for

vascular stiffening. Thus, further research is required to determine the cause of increased vascular resistance in AC S674 individuals.

5.1.1 Methodological Considerations

The hemodynamic profile of the AC S674 group was significantly different compared to controls. The AC S674 group expressed a significantly lower cardiac output and reduced SVC. Specifically, the decreased stroke volume in AC S674 was the primary cause for reduced cardiac output as heart rate was similar between groups. The difference in stroke volume between groups may be due to a decreased aortic diameter in AC S674 compared to controls. It is not known if this difference in aortic diameter is a characteristic of the AC S674 population as this is the first study to measure stroke volume velocity in this cohort. Previously, cardiac output collected via Finometer has been shown to be increased in AC S674 compared to controls (Hodges, 2010). In the present study, cardiac output values were based on Doppler ultrasound measures. However, to contrast the current data with those measured previously, the cardiac output feature of the Finometer was also measured. This value of cardiac output was similar between groups, contrasting with the Doppler data. However, calculation of cardiac output from the Finometer uses the patented ModelFlow algorithm that predicts cardiac stroke volume centred on a transfer function-based calculation of the aortic pulse pressure assuming a level of aortic compliance based on age and sex (*Pulse Pressure = Stroke Volume/Compliance*). An aorta with greater than expected stiffness will increase the calculated PP and lead to a SV that is erroneously high. This may be the case in the current study.

5.2 Handgrip Exercise

To our knowledge, this study was the first to examine the effect of adenylyl cyclase on different levels of submaximal dynamic exercise in humans. In the present study, the hyperemic response to 20, 40 and 60% dynamic handgrip exercise were not significantly different in AC S674 and control groups. Specifically, there was no significant difference in either FBF or FVC kinetics during handgrip exercise at the various intensities. The total response of ΔFBF and ΔFVC were similar between groups as well. Thus, the genetic variant AC S674 does not produce enhanced vasodilatory effects in response to dynamic exercise. These observations stand in contrast to those observed earlier by Hodges . (2010) who also used handgrip exercise. However,

in that earlier study, the flow was measured during a five second recovery period after a 5 second handgrip. The regulation of post-exercise blood flow differs from that during more rhythmic contractions(Shoemaker, 1997; Brock, 1998). Thus, it appears that the AC S674 variant may exert its vascular effects on the recovery phase of exercise.

Adenosine and prostaglandins stimulate adenylyl cyclase to induce vascular smooth muscle relaxation and arterial vasodilation during exercise (Joyner, 2007; Clifford, 2011). Adenosine has been a leading candidate for metabolic vasodilation because it is a potent vasodilator released from contracting skeletal muscle (Radegran, 2001). As well, arterial infusion of prostacyclin (a powerful vasodilating prostaglandin) increases blood flow in the human forearm (Stiegler, 1988). However, recent evidence in humans indicates that not all people respond to adenosine and that the overall contribution of prostaglandins to exercise blood flow control remains small (Shoemaker, 1996; Martin, 2007; Joyner, 2007). In the present study, blood flow responses were similar in AC S674 and control groups. As such, it is hypothesized that these metabolites did not increase contributions of AC S674 on vasodilation. Further research must be conducted to assess the functionality of these metabolites on the AC S674 enzyme during exercise.

The requirements of skeletal muscle blood flow directly correlate to metabolic demand. In addition to adenosine and prostaglandins, nitric oxide, potassium and acetylcholine spillover contribute to vasodilation in response to active skeletal muscle contraction (Clifford, 2004; Joyner 2007). This overlap in metabolic pathways makes it difficult to tease out the vasodilator effects of any individual mechanism, such as adenylyl cyclase. Specifically, neither nitric oxide nor acetylcholine individually affects the magnitude or rate of increase in FBF exercise (Shoemaker, 1997). Thus, AC S674 is not the sole determinant of increased skeletal muscle blood flow during dynamic exercise, as other pathways may up or down regulate their activity to meet the metabolic requirements of the exercising muscle.

Overall, the current data suggest that the AC S674 enzyme does not exhibit increased vasodilator effects in response to submaximal dynamic exercise. The similar FBF and FVC responses in both AC S674 individuals and controls can be explained by the classical "metabolic milieu" response to exercise. Additional research must be conducted to parse out the overlapping effects of vasodilator metabolites.

5.3 Beta-adrenergic Control

Isoproterenol is a non-selective beta-adrenergic agonist that exerts potent stimulation of $\beta 1$ and $\beta 2$ receptors. In the current study, isoproterenol infusion produced significant increases in HR and CO in both AC S674 and control groups. However, the presence of AC S674 did not provide an increased cardiac response to isoproterenol infusion. The positive chronotropic effects of isoproterenol on the heart are consistent with previously reported results in supine humans (Gortex, 1961). To our knowledge, this was the first study to examine the beta-adrenergic stimulation of AC S674 in the heart.

Intravenous infusion of isoproterenol primarily elevates FVC in skeletal muscle (Harman & Limbird, 1996). Forearm vascular bed conductance and compliance increased in both groups during isoproterenol infusion. These alterations to forearm vascular bed mechanics are indicative of vascular smooth muscle relaxation. However, FBF and FVC were similar in both groups. Thus, the AC S674 enzyme did not appear to present increased levels of vasodilation in response to beta-adrenergic activation.

The above results do not support the hypothesis that this adenylyl cyclase variant would exert differential beta-adrenergic control of cardiac or vascular function in response to excitatory or inhibitory stimuli. The determinants of impact for this enzyme over baseline hemodynamics, but not stimulation-induced changes remain to be determined. Previously, others have observed differences in venous vasomotor control in this AC variant group (Gros, 2007). However, arterial and venous smooth muscle responses to beta-adrenergic stimulation are not identical (Stein, 1997). Additional studies examining local artery blood flow and vascular conductance responses to beta-adrenergic control are warranted.

5.4 Limitations

The focal limitation of the present study is the small sample size of the AC S674 group. A total of 80 individuals were recruited and analyzed for DNA sequencing. Two participants were known carriers of the AC S674 variant from previous studies. Therefore, the AC S674 variant prevalence was 2.5% in the current study population compared to a previously reported 7% frequency (Gros, 2005). Due to time constraints and limited resources it was not possible to identify more carriers for study participation. However, using a two-tailed t-test, $p < 0.05$, Power

of 0.80, and effect size of 2.47 describing the difference in SVC between groups, a minimum of four individuals per group is necessary to replicate this outcome. Therefore, the small sample size used in this study is sufficient to achieve statistical significance.

Furthermore, it appears that a more comprehensive analysis of the endocrinological and visceral hemodynamic control is needed to fully characterize the impact of the AC S674 variant in humans. It would seem important that future studies obtain resting muscle sympathetic nerve activity (MSNA) in addition to epinephrine, norepinephrine, and plasma renin activity, angiotensin II, and vasopressin levels to obtain true values of vasomotor tone in AC S674 individuals.

5.5 Conclusion and Perspective

The results of the current study indicate that expression of the dysfunctional genetic variant AC S674 has profound effects on systemic hemodynamics. At rest, AC S674 individuals have decreased SVC and vascular bed compliance indicative of elevated vasomotor tone and/or remodeling. Chronic elevation in vascular contractile state may result in vascular stiffening and enhanced pulse pressures with detrimental long-term consequences for cardiovascular health. Specifically, genetic variants of beta-adrenergic receptors and G-proteins are associated with the development of hypertension(Felder, 2002; Feldman, 2006). Coupled with the results of the current study, adenylyl cyclase and cAMP production appear to be important factors in the regulation of cardiovascular health.

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Appendices

Appendix 1A: 20% handgrip exercise

Variable	F Value	η^2	Significance
FBF (ME HG)	$F(3, 21) = 27$	0.37	$p < 0.001$
FVC (ME HG)	$F(3, 23) = 30$	0.47	$p < 0.001$
MAP (ME group)	$F(1, 7) = 6$	0.43	$p = 0.042$

ME HG, main effect of handgrip; ME group, main effect of group

Appendix 1B: 40% handgrip exercise

Variable	F Value	η^2	Significance
FBF (ME HG)	$F(3, 20) = 101$	0.67	$p < 0.001$
FVC (ME HG)	$F(3, 21) = 121$	0.75	$p < 0.001$
MAP (ME group)	$F(1, 7) = 6$	0.41	$p = 0.044$

ME HG, main effect of handgrip; ME group, main effect of group

Appendix 1C: 60% handgrip exercise

Variable	F Value	η^2	Significance
FBF (ME HG)	$F(3, 18) = 89$	0.71	$p < 0.001$
FVC (ME HG)	$F(3, 19) = 86$	0.75	$p < 0.001$

ME HG, main effect of handgrip

Appendix 2: Isoproterenol dose response

Variable	F Value	η^2	Significance
HR (ME dose)	$F(2,14) = 8$	0.32	$p = 0.003$
SV (ME group)	$F(1, 7) = 48$	0.85	$p < 0.001$
CO (ME dose)	$F(2, 14) = 5$	0.08	$p = 0.021$
CO (ME group)	$F(1, 7) = 6$	0.38	$p = 0.040$
FBF (ME dose)	$F(2,11) = 18$	0.44	$p < 0.001$
FVC (ME dose)	$F(2,11) = 15$	0.44	$p < 0.001$

Appendix 3: Manual Blood Pressure Data

	Control (n = 6)	AC S674 (n = 4)
SBP (mm Hg)	114 ± 10	116 ± 2
DBP (mm Hg)	76 ± 6	71 ± 10

Values are mean ± standard deviation.

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Approved Local Adult Participants:200
Approved Local Minor Participants:0
Protocol Title: The Role of a Single Nucleotide Variant of Adenylyl Cyclase 6 in Aerobically Trained Athletes (REB#18813)
Department & Institution: Health Sciences\Kinesiology,Western University
Sponsor: Natural Sciences and Engineering Research Council

Ethics Approval Date: May 16, 2012
Ethics Expiry Date: February 28, 2013

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Letter of Information & Consent	Part 1 and 2	2012/03/20
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This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

This is an official document. Please retain the original in your files.

Research Development & Services • *The University of* Western Ontario
Room 5150, Support Services Building • London, Ontario • CANADA – N6A 3K7
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Curriculum Vitae

Name	James C. Corkal
Position	Master of Science (MSc.)
	Neurovascular Research Laboratory
	School of Kinesiology, Rm. 2310 Thames Hall
	The University of Western Ontario
	London, Ontario, N6A 3K7

EDUCATION

MSc.	The University of Western Ontario, Faculty of Health Sciences, Department of Kinesiology, Neurovascular Research Laboratory Specialty in Integrative and Exercise Physiology Advisor: Dr. J. Kevin Shoemaker Thesis: *The role of a single nucleotide variant of adenylate cyclase 6 on blood flow control in healthy adults*	2013
BSc.	The University of Western Ontario, Faculty of Health Sciences, Department of Kinesiology Honours Specialization Undergraduate Thesis: *Central and peripheral vascular mechanics: a comprehensive analysis of cardiovascular disease risk in older adults*	2009

TEACHING ASSISTANTSHIPS

1.	**Kinesiology 4433: Physiology of Exercise Training:** This course investigated the knowledge, prescription and physiological effects of training with an emphasis on aerobic, anaerobic, and strength training. Primary responsibilities involved grading exams and meeting students to review lecture materials.	2013
2.	**Kinesiology 4432: Physiology of Exercise:** This course provided an in depth analysis of cardiovascular function and limitations during exercise. In addition to grading exams, I conducted group learning sessions that included reviewing lecture material and demonstrating lab techniques such as ultrasound. I was also available to meet with students weekly.	2012
3.	**Anatomy and Cell Biology 2221: Functional Human Anatomy:** This course investigated musculoskeletal, cardiovascular, respiratory, nervous, digestive, excretory, and reproductive systems with an emphasis on locomotion and function. Primary responsibilities included leading and instructing cadaver laboratories, grading exams, and meeting with students weekly.	2011 - 2012

TECHNICAL SKILLS

1.	**Doppler Ultrasonography** Measures arterial diameters and blood flow velocity. These measures are used to conduct clinical tests to assess cardiovascular health.	2010 - present
2.	**Indirect Calorimetry** Technique used to estimate oxygen consumption and carbon dioxide exhalation. These measures are used to assess metabolic rate at rest and during exercise, as well as, estimating the contribution of carbohydrate and fat to total energy expenditure.	2007 - present
3.	**Finometer and Electrocardiogram** These can be used to measure beat-by-beat blood pressure and heart rate. In combination, they can be used to assess stroke volume, cardiac output, pulse pressure and other clinical assessments such as arterial stiffness.	2010 - present
4.	**Clinical Testing** Includes cardiovascular and metabolic assessments such as: flow mediated dilation, pulse wave velocity (arterial stiffness) and incremental exercise tests to exhaustion (e.g. VO_2 max tests). Additional experience performing physical readiness assessments and functional activity measures.	2009 - present
5.	**Computer Software** Expertise with Powerlab/Labchart, Microsoft Office, MATlab and statistical software programs such as SPSS and SigmaPlot.	2009 - present

ACADEMIC PRESENTATIONS

1.	Metabolic syndrome does not affect forearm vascular mechanics Canadian Society for Exercise Physiology, Quebec, Que. AGM.	2011

PUBLICATIONS

1.	**Published Abstract:** **J. Corkal**, H. Edgell, M. Frances, M. Zamir, and J.K. Shoemaker. Metabolic syndrome does not affect forearm vascular mechanics. *Applied Physiology Nutrition and Metabolism*. Proceedings: S311.	2011
2.	**Manuscript near submission:** **J. Corkal**, Edgell, H., Frances, M., and Shoemaker, JK. Central and peripheral vascular mechanics: a comprehensive analysis of cardiovascular disease risk in older adults.	Present

PROFESSIONAL EXPERIENCE

1.	**Owner/Operator**, FSK Health & Fitness London, Ontario • Personal strength and conditioning training and consulting business that is focused on promoting functional strength and movement patterns • Specialize in training competitive athletes, injury rehabilitation, and fitness gains	2010 - present
2.	**Research Assistant,** Neurovascular Research Laboratory The University of Western Ontario, London, Ontario • Developed and conducted research projects focusing on vascular health in metabolic syndrome and coronary artery disease patients • Performed assessments and instructed participants through exercise intervention protocols	2010 - present
3.	**Project Coordinator**, Canadian Centre for Activity and Aging The University of Western Ontario, London, Ontario • Responsible for the organization and dissemination of the Get Fit for Active Living – Diabetes exercise program across Canada • Certified course instructor: Functional Activity Measures • Seminar and conference lecturer (United States and Canada)	2010
4.	**Personal Trainer (Level 2)**, GoodLife Fitness London, Ontario • Specialized in exercise prescription and development, functional assessments, weight loss, strength and conditioning, and rehabilitation • Secondary responsibilities included developing sales techniques and selling personal training packages	2009-2010

VOLUNTEER & LEADERSHIP ROLES

1.	**Physiotherapy Volunteer**, PT Health Physiotherapy Clinics London, Ontario • Assist with treatment of patients through rehabilitative exercises, administer laser therapy, transcutaneous electrical nerve stimulation, interferential current treatment and ultrasound	2012-2013
2.	**Vice President of Student Affairs**, Kinesiology Graduate Board The University of Western Ontario • Represent kinesiology graduate students' academic interests	2012-present
3.	**Varsity Football Player,** Western Mustangs The University of Western Ontario • Receiver for the Western Mustangs varsity football team (2005-2007) • Receiver coach for various high school and varsity teams in Sudbury and London	2005-present

HONOURS, AWARDS, & GRANTS

1.	**Western Graduate Research Scholarship** The University of Western Ontario	2011-2013
2.	**Health Sciences Travel Award** Faculty of Health Sciences	2011
2.	**Dean's Honor List** The University of Western Ontario	2010-2011
3.	**Canadian Millennium Scholarship of Excellence** Government of Canada	2005-2009
4.	**Western Scholarship of Excellence** The University of Western Ontario	2005