THE INFLUENCE OF VARIATION IN THE CATECHOL-O-METHYLTRANSFERASE GENE ON PERCEPTUAL RESPONSE TO EXERCISE

By

Nikhil Satchidanand

BS, State University of New York College at Buffalo, 2001

MS, University of Pittsburgh, 2006

Submitted to the Graduate Faculty

of the School of Education in partial fulfillment

of the requirements for the degree

of Doctor of Philosophy

University of Pittsburgh

2010

UNIVERSITY OF PITTSBURGH

SCHOOL OF EDUCATION

This dissertation was presented

by

Nikhil Satchidanand

It was defended on September 8, 2010

and approved by

Robert J. Robertson, PhD, Professor

Robert E. Ferrell, PhD, Professor

Yvette Conley, PhD, Assistant Professor

Dissertation Director: Elizabeth F. Nagle, PhD, Assistant Professor

THE INFLUENCE OF VARIATION IN THE CATECHOL-O-METHYLTRANSFERASE GENE ON PERCEPTUAL RESPONSE TO EXERCISE

Nikhil Satchidanand, PhD

University of Pittsburgh, 2010

INTRODUCTION: Exercise is a psycho-physiological stressor that activates the hypothalamic-pituitary-adrenal axis, eliciting increased catecholamine production. The catechol-O-methyltransferase (COMT) gene codes for the enzyme that catabolizes catecholamines as part of antinonciception. Haplotypes of COMT are implicated in interindividual differences in sensation of pain and possibly exertional perception.

PURPOSE: To examine the influence of haplotypes of COMT on exertional perceptions and lower-body pain (LBP) during a sub-maximal graded exercise test (GXT) among adults.

METHODS: Subjects (n=169, 55% female, mean age: 29.16 $\pm$ 4.10 yrs) completed one sub-maximal GXT to 85% of age-adjusted maximal heart-rate. Oxygen uptake (VO_2), minute ventilation (VE) and heart rate were measured at each stage. Ratings of perceived exertion (RPE; OMNI Scale) were estimated for the overall body (RPE-O), legs (RPE-L), and chest (RPE-C) at 2:45 of each stage. Exercise-induced LBP (Cook Pain Scale) was assessed at 2:55. One sample for DNA extraction was collected. Subject categories were Low Responder (LR), Average Responder (AR), or High Responder (HR). For each subject, linear regression models were generated for RPE or Pain expressed as a function of each physiological criterion variable. A separate slope was calculated for each regression model. Slopes were compared among subgroups via ANCOVA, controlling for age and physical activity.

RESULTS: For males when RPE (Legs, Chest) was expressed as a function of %VO_2max, HR subjects exhibited higher ($p < .001$) slopes than LR subjects. When RPE-L was expressed as a function of VE, HR subjects exhibited higher ($p < .001$) slopes than LR subjects. Finally, among males when RPE (Legs, Chest) was expressed as a function of heart rate, HR subjects exhibited higher ($p < .001$) slopes than LR subjects. No significant differences existed among females for any of the associations. For both males and females, when LBP was expressed as a function of each physiological criterion variable, HR subjects exhibited significantly higher slopes than LR subjects ($p < .001$).

CONCLUSIONS: Haplotypes of the COMT gene appear to influence interindividual differences in exertional perceptions and LBP during a sub-maximal GXT. Subjects with the HR genotype exhibited higher RPEs and LBP than LR subjects at a given workload.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I could not be more grateful to my dissertation director, Dr. Elizabeth F. Nagle for her enduring support and genuine enthusiasm for my dissertation research and development as a professional. Under very challenging personal and professional circumstances, Dr. Nagle dedicated herself to my work and has inspired me to become as nurturing and effective a mentor as she is to me. I am greatly indebted to Dr. Robert J. Robertson, a true force of teaching and research excellence, for his invaluable editorial comments and guidance. I heartily thank Dr. Robert E. Ferrell for his genuine and undying enthusiasm for my work and for his continued feedback throughout this process. I would also like to thank Dr. Yvette Conley for her invaluable editorial comment in the development and implementation of this project. Very special thanks also go to Dr. Vincent C. Arena for all of his statistical consult and career development advice.

I am thoroughly grateful to my fellow students for being a continued source of support and camaraderie. I would like to enthusiastically thank Dr. Ethan E. Hull, Mr. David J. Rice, Ms. Jeanette Garcia, and Ms. L. Denise Edmonds for their continued encouragement and for helping me to laugh when all I wanted to do was cry!

I am forever grateful to my parents, Drs. Sateesh and Yashodhara Satchidanand. They have always inspired me to be my very best and to strive for excellence. I thank them for supporting my educational and career aspirations in every way.

This project simply would not have been possible without the love and support of my wife, Sara. It is proof that with her encouragement I can meet any challenge. I know that together we will always have success and happiness.

It is to each of you that I dedicate this dissertation.

1.0 INTRODUCTION

The objective of this research was to examine the influence that three previously identified haplotypes (variants) of the catechol-O-methyltransferase (COMT) gene have on exertional perceptions and exercise-induced lower body pain during a sub-maximal treadmill graded exercise test, among healthy young adults. This chapter is composed of the following sections: (1.1) Rationale, (1.2) Purpose, (1.3) Specific Aims, (1.4) Research Hypotheses and (1.5) Significance.

1.1 RATIONALE

Physical exercise is a stressor that necessitates initiation of biochemical and physiological processes in an attempt to maintain homeostasis [36, 81,115]. Past research indicates that plasma concentrations of catecholamine hormones increase in response to various modes, intensities, and durations of cardiovascular exercise [1, 6, 18, 23, 25, 31, 36, 42, 43, 60, 81, 90, 118]. The catecholamines epinephrine (E) and norepinephrine (NE) are hormones that are secreted by the adrenal medulla in response to an agent of stress. The release of catecholamines causes excitatory and inhibitory effects in the central and peripheral nervous systems [6, 36, 42, 43, 60]. Heart-rate (HR), ventilation (VE), blood pressure (BP) and myocardial contractility increase. Circulation of blood to the intestinal wall and bronchioles of the lungs decreases [6, 36, 42, 43, 60]. This diversion of energy to the body systems directly involved in self-protection is a critical element in how organisms cope with and adapt to stress. These mechanisms force the organism to physiologically and psychologically experience the stressor [6, 36, 42, 43, 60].

In response to the excitation caused by the increase in catecholamine production, the hypothalamus produces endogenous polypeptides called opioids (endorphins and enkephalins). Opioids are released into circulation by the pituitary gland, and have an affinity for μ-opioid receptors; specialized receptors that are located in various regions of the central nervous system. Opioids will then bind with μ-opioid receptors, producing a sense of well-being and analgesia. This minimizes the excitatory effect of the release of catecholamines, and is an essential adaptation of an organism to survive in the presence of an agent of stress [6, 25, 36, 81, 90, 118].

The catechol-O-methyltransferase (COMT) gene encodes for the enzyme catechol-O-methyltransferase, which is responsible for the catabolism of catecholamines at the synapses of adrenergic neurons. Thus, the COMT enzyme functions to inactivate catecholamines, and is a central component of adrenergic neurotransmission. This process in conjunction with the release of endogenous opioids suppresses the physiological and psychological impact of the stressor on the organism [25, 36, 81]. Without the effect of the COMT enzyme, catecholamines would continue to circulate, prolonging the excitation of the body systems, ultimately having a deleterious effect on the health of the organism [6, 25, 36, 81, 90, 118].

Variation in the COMT gene influences gene transcription and therefore the strength of the COMT enzyme that is produced. This has a profound impact on biochemical processes involving adrenergic neurotransmission, such as response to physiological stress [3, 4, 5, 29, 59, 62, 63].

Recent research indicates that four common single nucleotide polymorphisms (SNPs) of the COMT gene have an association with perceptual response to pain-inducing stimuli [29]. A SNP is a DNA sequence variation occurring when a single nucleotide – Adenine, Thymine, Cytosine, or Guanine – in the genome differs between members of a species. Variations in DNA

sequences are important because they can influence how humans develop disease and how they respond to pathogens, chemicals or drugs [3, 4, 5, 8, 9, 10, 15, 23, 27, 28, 29, 38, 45, 54, 63, 76, 78, 80, 87, 88, 89, 96, 103, 116, 134, 140, 142, 144].

Research indicates that these four SNPs form three haplotypes that are associated with a graded responsiveness to various pain-inducing stimuli. With one haplotype being inherited from each parent, these haplotypes have been previously designated as Low Pain Sensitivity (LPS), Average Pain Sensitivity (APS), and High Pain Sensitivity (HPS). Variability in COMT enzyme function appears to be associated with this graded responsiveness to pain stimuli [29]. The proposed research sought to understand the relation between COMT gene variation and perceptual response to an exercise challenge as an agent of stress. The next section (1.2) provides the purpose of the proposed research.

1.2 PURPOSE

The purpose of this research was to examine variation in the COMT gene that may influence perceptual response to an exercise challenge. Specifically, variation in the COMT gene was examined is association with perception of physical exertion and perception of exercise-induced lower body pain. The variants being examined have previously been designated as Low Pain Sensitivity (LPS), Average Pain Sensitivity (APS), and High Pain Sensitivity (HPS).

1.3 SPECIFIC AIMS

Specific Aims:

- The primary aim of this research was to examine differences in exertional perceptions during a sub-maximal treadmill graded exercise tests among LPS, APS, and HPS haplotypes.

- The secondary aim of this research was to examine differences in exertional pain during a sub-maximal treadmill graded exercise test among LPS, APS, and HPS haplotypes.

1.4 RESEARCH HYPOTHESES

Hypotheses:

- It was hypothesized that with increasing exercise intensity, subjects with one HPS haplotype will report higher exertional perceptions as a function of selected physiological criterion variables than participants homozygous for the LPS or APS haplotypes.

- It was hypothesized that with increasing exercise intensity, subjects with one HPS haplotype will report higher levels of exercise-induced lower body pain as a function of selected physiological criterion variables than subjects homozygous for the LPS or APS haplotypes.

1.5 SIGNIFICANCE

The previously described haplotypes encoding for the COMT enzyme have not been examined in association with exertional perceptions and pain during exercise. The current research focuses to better characterize the influence that these variants have on perceptions of exertion and pain during sub-maximal treadmill graded exercise. Pain and other physical stressors cause organisms to disengage from the agent of the stress. In this research, subjects found to have one HPS haplotype may disengage from physical activity, perceiving it to be a noxious stimulus. Physical activity (PA) is a critical component of a healthy lifestyle. Understanding the biological factors that influence PA behaviors will help interventions efforts to more effectively reverse sedentary tendencies. The more we understand about human behavior and its biological underpinnings, the more prepared we will be to help people alter their behaviors to achieve and maintain health.

2.0 REVIEW OF LITERATURE

The objective of the proposed research was to examine three common haplotypes (variations) of the catechol-O-methyltransferase (COMT) gene, exertional perceptions and exercise-induced lower-body pain in healthy, male and female young adults during sub-maximal treadmill, graded exercise. The proposed research focused on three combinations of four previously identified single nucleotide polymorphisms (SNPs) of the COMT gene that influence catecholamine catabolism in the central nervous system in response to stress-inducing stimuli. Specifically,

these haplotypes have previously been found to influence perceptions of various pain-inducing stimuli. The proposed research sought to extend what is understood about genetic variation that may influence perceptual response to an exercise challenge. The following literature review provides support for the significance of this research.

2.1 CATECHOL-O-METHYLTRANSFERASE

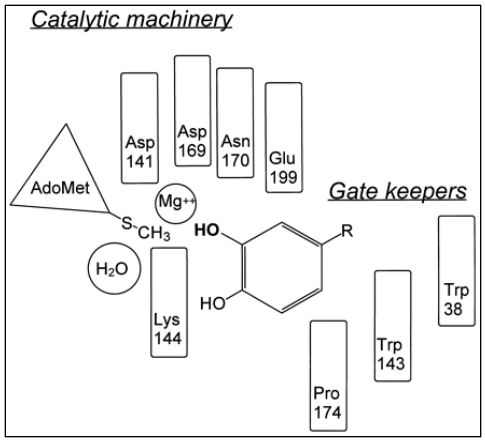
The enzyme-catalyzed O-methylation of catecholamines was first described by Axelrod et al., [3,4]. The enzyme responsible for this O-methylation, catechol-O-methyltransferase (COMT), is essential for the proper catabolism of catecholamines (dopamine, epinephrine and norepinephrine) in the central nervous system. Thus, the COMT enzyme is integral in adrenergic neurotransmission [3, 4, 5, 23, 27, 38, 54, 63, 76, 140, 144]. The vast range of COMT substrates confers significant functional diversity to the COMT enzyme. Therefore, variation in the COMT gene has great potential to influence a number of important health conditions and physiological processes [3, 4, 5, 23, 27, 38, 54, 63, 76, 140, 144]. The following section (2.1.1) describes the structure of the COMT gene and COMT enzyme.

2.1.1 Structural Features of COMT

Based upon early work performed by Salminen et al., [111] and Lundstrom et al., [70, 71], it was found that there is one single gene that encodes for two COMT enzymes; soluble (S-COMT) and membrane-bound (MB-COMT). The human COMT gene contains six exons with the first two exons being non-coding [5, 8, 27, 39]. It is located on chromosome 22, band q11.2 . In addition,

the human S-COMT gene contains 221 amino acids, while the MB-COMT contains 271 amino acids [5, 8, 39, 47, 80, 111, 141].

The COMT enzyme has a single domain ά/β – folded structure in which eight ά-helices are arranged around the central mixed β-sheet. The active site of COMT is made up of the S-adenosyl – L- methionine – (AdoMet) binding domain and the actual catalytic site. The catalytic site is formed by amino acids that are important for the binding of substrate, water and magnesium ions, and for catalysis of O-methylation [5, 8, 39, 47, 80, 111, 141]. The magnesium ion bound to COMT following AdoMet binding converts the hydroxyl groups of the catechol substrate to be more easily ionized. A lysine residue near one hydroxyl group accepts the proton from that hydroxyl group. Then the methyl group from the AdoMet is transferred to the hydroxyl group [5, 8, 39, 47, 80, 111, 141]. Lysine acts as a catalytic base in the nucleophilic reaction. In addition, "gatekeeper" residues (Trp38, Trp143, and Pro174) form hydrophobic "walls" that define the selectivity of COMT toward different side chains of the substrate. This directs the methylation reaction by keeping the planar catechol ring in the correction orientation. These "gatekeepers" participate in the regulation of binding to substrates of COMT [5, 8, 39, 47, 80, 111, 141] (Figure 1.0).



Vidgren et al., (1997) and Vidgren et al., (1999)

Figure 1.0 Schematic of the COMT enzyme

2.1.2 Functional Importance of the COMT Enzyme

The COMT gene encodes for the enzyme catechol-O-methyltransferase that catabolizes a variety of substrates involved in vast physiological processes. This diversity of COMT substrates confers great functional diversity to the COMT enzyme [3, 4, 5, 8, 39, 47, 80, 111, 141]. The physiological substrates of the enzyme include: 1) L-dopa, 2) catecholamines (dopamine, epinephrine, and norepinephrine); 3) hydroxylated metabolites of catecholamines; 4) catecholestrogens [3, 4, 39, 80, 111]; 5) ascorbic acid; and 6) dihydroxylated intermediates of melanin. In addition, a number of dietary and medicinal compounds act as COMT substrates. These include, but are not limited to; dobutamine, and isoprenaline [3, 4, 39, 80, 111].

While the aforementioned substrates are of great functional importance to a variety of physiological processes throughout the human body, the primary role of COMT in catabolizing catecholamines in the central nervous system will receive greater focus in this review. As stated, the catecholamine hormones epinephrine, norepinephrine, and dopamine are inactivated by the enzyme catechol-O-methyltransferase (COMT) [2, 3, 5, 8, 39, 47, 75, 76, 77, 80, 111, 141]. The

enzyme introduces a methyl group (methylation) to the catecholamine which is donated by S-adenosyl methionine (SAM) within the enzyme. The addition of the methyl group to the catecholamine inactivates it, leaving it biologically inert. Thus, the methylation of catecholamines by COMT is critical to the down-regulation of catecholamine hormones in the central nervous system and peripheral tissue [2, 3, 5, 8, 39, 47, 75, 76, 77, 80, 111, 141].

The regulation of catecholamine hormones in the nervous system is of great importance to diverse physiological mechanisms [2, 3, 5, 8, 39, 47, 75, 76, 77, 80, 111, 141]. Of significance to the proposed research was that physiological stressors such as physical exertion associated with exercise, produces an increase in plasma concentrations of catecholamines (epinephrine and norepinephrine) [1, 25, 30, 36, 42, 43, 60, 75, 76, 77, 81, 90]. This mechanism may influence how individuals perceive stress-inducing stimuli such as physical exertion or pain. Past research indicates that variation in COMT enzyme activity influences how individuals perceive a pain-inducing stimulus [1, 29, 63, 87, 88, 89, 134, 144]. The following section (2.2) provides important evidence of cardiovascular exercise as a physiological stressor that causes an increased production of catecholamine hormones as a critical component of the General Adaptation Syndrome (GAS).

2.2 CARDIOVASCULAR EXERCISE AS AN AGENT OF STRESS

2.2.1 Catecholamines

The most abundant catecholamine hormones (dopamine, epinephrine and norepinephrine) are chemical compounds synthesized from tyrosine containing catechol and amine groups. Catecholamines are water-soluble and bound to plasma proteins, allowing them to circulate in the blood. Catecholamine hormones are produced primarily by the chromaffin cells of the adrenal medulla and postganglionic fibers of the sympathetic nervous system [3, 4, 6, 30, 36, 63, 64, 115, 118].

Catecholamines are produced in the central nervous system in response to psychological or physiological stressors such as public speaking and physical exertion. This production aids the body in preparing for the "fight or flight reaction" or acute stress response [3, 4, 6, 30, 36, 63, 64, 115, 118]. The production of catecholamine hormones is a critical step in the General Adaptation Syndrome that will be discussed in the next section (2.2.2).

2.2.2 The General Adaptation Syndrome

The General Adaptation Syndrome (GAS) is a non-specific cascade of biochemical and physiological events initiated in response to the presence of a stressor. A stressor challenges the homeostasis of an organism and endangers life unless it is met with an adequate adaptive response [115]. This adaptability and resistance to the effects of a stressor are essential for survival and every organism has, through evolution, a finite capacity to adapt [115]. A key tenet

of this concept is that the adaptive response of organisms to a given stressor follows very much the same predictable pattern, irrespective of the agent causing the stress [115]. Thus the anxiety, elevated heart-rate, diaphoresis, and nausea elicited by the prospect of public speaking are caused by biochemical processes identical to those associated with being confronted with the threat of physical harm. While the agents of the stress differ significantly, the biochemical and physiological processes that the body experiences in response are very much the same. Psychologist, Hans Selye coined this complex response the General Adaptation Syndrome (GAS) [115]. This section provides a description of the three stages of the GAS and how organisms attempt to cope with stressors.

The GAS is comprised of three stages: alarm reaction, resistance, and exhaustion. If a stressor challenges homeostasis, the body enters the alarm reaction stage. The hypothalamus of the brain controls the autonomic nervous system and secretes two hormones; corticotropin-releasing hormone and thyrotropin-releasing hormone. These neuro-hormones allow the pituitary gland to regulate the biochemical processes performed by other glands in an attempt to adapt to the stress [3, 4, 6, 30, 36, 63, 64, 90, 115, 118]. In response, the pituitary gland stimulates the release of adrenocorticotropin hormone that in turn stimulates the production of the catecholamine hormones epinephrine (E) and norepinephrine (NE) from the adrenal medulla and sympathetic neurons [3, 4, 6, 30, 36, 63, 64, 90, 115, 118]. These hormones elicit an excitatory response causing increased ventilation, heart-rate, and blood pressure. Blood flow to the digestive system, liver and other organs nonessential to self-protection, is diverted in order to supply the skeletal muscle and brain with more oxygen in preparation to escape or fight the agent of the stress [3, 4, 6, 30, 36, 63, 64, 90, 115, 118]. In addition, the brain secretes endogenous opioids; endorphins and enkephalins that act as natural opiates, producing a sense of well being

as well as analgesia. This mechanism makes the effects of the stressor more tolerable, so that the organism can continue to function in the presence of the stressor [3, 4, 6, 30, 36, 63, 64, 90, 115, 118]. The alarm reaction phase lasts only a fraction of a second in most circumstances. Further, the increased production of catecholamines in the central nervous system lasts for just a few milliseconds.

In the resistance stage, the physiological effects that occurred during the alarm reaction stage decline. The pituitary gland decreases the production of hormones that caused the excitation associated with the alarm reaction stage. During resistance, the organism adapts to the stressor. However, full relaxation via balancing the activity of the sympathetic and parasympathetic nervous systems, does not happen immediately [115]. Although the organism begins to adapt, the stressor is still present and the effects of the alarm reaction stage persist long after the stressor has dissipated. The resistance stage represents the organism's attempt to adapt to the stress in order to continue to maintain homeostasis [115]. An example of this mechanism can be seen if one is confronted by a mugger on the street. After the initial panicked feelings elicited by a confrontation, one would begin to adapt to the experience. Feelings of extreme fear that are immediately paralyzing would soon be replaced by the process of reasoning whether to fight or attempt escape. Adaptive reserves will be utilized to process the adversary's actions and intentions in order to identify the best course of action for self-preservation. The resistance stage embodies the organism's flexibility and resilience in the face of adversity.

The final stage of the GAS is exhaustion. The concept of "adaptation energy" is paramount when trying to understand the exhaustion stage of the GAS. This concept posits that every organism has a finite ability to adapt to stressors. When forced to adapt, the organism then loses adaptation energy that, according to Selye, cannot be replaced. Selye theorized that the

depletion of adaptation energy causes diminished ability to adapt effectively to stress each time an organism is confronted [115].

Exhaustion involves the depletion of adaptive resources. The organism may feel overwhelmed and fatigued to the level of inaction. At this time, one of two outcomes will occur. If the stressor is chronic, overwhelmingly strong, and cannot be removed or at the very least diminished, exhaustion can mean death [115]. More likely however, is that the organism will fully recover.

The General Adaptation Syndrome represents a non-specific cascade of biochemical and physiological events initiated in response to the presentation of an agent of stress. It is important to note that although agents of stress vary significantly, activation of the GAS involves identical processes, irrespective of the nature of the stressor [115]. The proposed research sought to demonstrate the importance of this non-specific response to stress-inducing stimuli. The following section (2.2.3) reviews the literature on the increased production of catecholamine hormones in the central nervous system during cardiovascular exercise.

2.2.3 Plasma Concentrations of Catecholamine Hormones Increase with Exercise

In response to a stressor, the Sympathetic-Adrenal-Medullary System causes an increased production of the catecholamines epinephrine (E) and norepinephrine (NE) in the peripheral and central nervous systems [60, 81, 96, 115, 118, 133, 139]. These hormones cause excitatory and inhibitory actions throughout the body. Heart-rate, ventilation, and blood pressure increase to meet an increasing demand for oxygen and nutrients supplied to mobilized skeletal muscle, brain, and heart [60, 81, 96, 115, 118, 133, 139]. This same series of biochemical and

physiological events is observed during cardiovascular exercise of varying intensities and durations [1, 6, 23, 36, 42, 43, 60, 81, 118, 133]. This section provides the scientific basis for cardiovascular exercise as a physiological stressor that elicits an increase in the production of catecholamines in the central nervous system.

Research studies regarding the complex biochemical and physiological responses to exercise have been performed extensively for many years. Studies indicate that catecholamine hormone concentrations within the plasma increase during exercise [1, 6, 23, 36, 42, 43, 60, 81, 118, 133]. As stated in the previous section, the release of catecholamine hormones causes excitatory and inhibitory effects in the central nervous system in order to optimize the response of the skeletal muscle, brain and heart, and adapt to the agent of stress. This indicates that the exertion associated with cardiovascular exercise is a physiological stressor to which adaptation must occur [1, 6, 36, 81, 90].

Various exercise modalities including cycle ergometry and treadmill exercise as physiological stimuli have been used to explore the relation between exercise and plasma concentrations of catecholamine hormones. Two key independent variables explored within this relation are duration and intensity of exercise. Short-duration, intermittent exercise bouts of varying levels of intensity [6, 90] as well as continuous, progressive exercise protocols have been employed [133]. Additional research studies have utilized continuous exercise of extended duration, performed at a comparatively lower relative intensity [23, 33]. In previous protocols, duration has ranged from intermittent 300 meter maximal running (average time: 65.1 sec) [90], to an extended duration treadmill test to exhaustion [42, 133].

Protocols have employed relative intensities of exercise ranging from 60 – 70% of maximal oxygen uptake to supramaximal levels of 105% of individual anaerobic threshold [1, 6,

23, 36, 42, 43, 60, 81, 90, 118, 133]. Plasma catecholamine hormone concentrations have been positively associated with both duration and intensity of exercise [1, 6, 23, 36, 42, 43, 60, 81, 90, 118, 133]. Despite variability in testing paradigms used across studies, plasma concentrations of E and NE increase with increasing duration and/or intensity of exercise. Very-short duration, highly intense exercise bouts elicited a significant increase in NE in a study by Naveri et al., [90]. It was found that 3 x 300m intermittent running was positively associated with an increase in NE of approximately 21.0 nmol/l, equal to approximately 7.7 times above that of resting values. In addition, it was found that progressive exercise of increasing intensity elicited a similar increase in plasma catecholamine hormones. Progressively intense cycle ergometry that increased workload by 50 watts every three minutes until exhaustion elicited a sharp increase in E and NE at the lactate threshold [133].

Since cardiovascular exercise is positively associated with an increase in the concentration of plasma epinephrine (E) and norepinephrine (NE), it is critical to understand the degree of exercise intensity required to elicit such a response. It has been shown that plasma catecholamine concentrations did not increase significantly at a relative intensity below approximately 60 – 70% of maximal oxygen uptake (VO_{2max}) [1, 6, 23, 36, 42, 43, 60, 81, 118, 133]. Furthermore, plasma catecholamine concentrations are significantly associated with intensity of exercise when an exercise bout is at least two-minutes in duration. It has also been shown that exercise intensities above one's individual anaerobic threshold (IAT), will result in increased lactate, E, and NE concentrations until exercise termination [133]. The IAT is defined as the highest metabolic rate where blood lactate (La) concentrations are maintained at steady state during prolonged exercise. Conversely, when lactate concentration does not exceed the IAT, production of E and NE appear to be only modest [133].

Cardiovascular and metabolic responses to exercise are primarily regulated by the neuroendocrine system. Norepinephrine (NE) that is released by sympathetic neurons causes circulatory alterations (Δ HR and cardiac output) according to intensity of exercise. Plasma concentration of NE increases by way of neurotransmitter spillover from neuronal uptake and local metabolism, which increases with increasing exercise intensity [1, 60, 81]. Plasma concentration of Epinephrine (E) increases by way of secretion from the adrenal medulla [60, 81]. During exercise, plasma concentration of both E and NE increases in a curvilinear fashion with increasing exercise workload [1, 60, 81].

Research indicates that the physical exertion associated with various cardiovascular exercise modalities and durations is strongly, positively associated with an increase in the production of plasma epinephrine and norepinephrine [1, 6, 23, 36, 42, 43, 60, 81, 118, 133]. This physiological response as a part of the General Adaptation Syndrome is critical to how humans adapt to a physiological stressor such as exertion. Based upon evidence, it is apparent that cardiovascular exercise at a relative intensity above one's IAT elicits a significant increase in plasma concentration of the catecholamines epinephrine and norepinephrine. This basic physiological mechanism remains of importance to this research. The following section (2.3) is an exploration of the catechol-O-methyltranferase gene.

2.3 VARIATION IN THE CATECHOL-O-METHYLTRANSFERASE GENE

Variation in the catecol-O-methyltransferase gene has been shown to influence diverse biological processes. The proposed investigation will focus on specific single nucleotide polymorphisms of

the COMT gene. A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome (or other shared sequence) differs between members of a species.

2.3.1 Single Nucleotide Polymorphisms of the Catechol-O-methyltransferase gene

The catechol-O-methyltransferase (COMT) gene encodes for the enzyme catechol-O-methyltransferase that catabolizes catecholamine hormones in the central nervous system. Thus, the COMT enzyme is an essential component in adrenergic neurotransmission and the activation of the Sympathetic-Medullary-Adrenal System when responding to various agents of stress [3, 4, 21, 75, 76, 77]. Recent research indicates that polymorphic variability in the COMT gene confers differences in the activity of the COMT enzyme that is synthesized. This variability likely will influence processes that depend upon COMT enzyme activity. Specifically, six single nucleotide polymorphisms (SNPs) have been found to display high polymorphism frequency in the human population (> 40% prevalence) [29, 87, 88, 89].

The first SNP (rs2097903) is located at position – 1217 in the estrogen-sensitive portion of the membrane-bound COMT promoter region [29, 87, 88, 89]. The second SNP (rs6269) is found in the promoter region of soluble COMT [29, 87, 88, 89]. The next three (rs4633, rs4818, and rs4680) are each found within the coding region of both soluble and membrane-bound COMT [29, 87, 88, 89]. It is important to note that SNPs rs4633 and rs4818 are synonymous polymorphisms, meaning that they do not produce a change in amino acid composition. Single nucleotide polymorphism rs4680, however, does produce a substitution of amino acids; valine (val) to methionine (met) at codon 158. The final COMT SNP (rs165599) is located at the end

of the 3'-UTR of the gene [29, 87, 88, 89]. Past investigations indicate that no other COMT SNPs exist with a frequency greater than .15 in the coding region of the gene. The COMT SNP map has been constructed (http://www.ncbi.nlm.nih.gov/SNP>snp_ref.cgi?locusId=1312) .

Previous research indicates that alleles form statistical associations or haploblocks that vary in length. Diatchenko et al., determined that three haploblocks were formed within the COMT locus that encompassed the six previously characterized SNPs. However, statistically significant associations were only found for SNPs within the central haploblock of the COMT locus. Therefore, the proposed research will rely upon this finding, and investigation will focus on the SNPs within this haploblock . In all, four SNPs (rs6269, rs4633, rs4818, and rs4680) were investigated that make up three distinct haplotypes accounting for 95.5% of all haplotypes that were detected. A haplotype is a combination of SNPs that is statistically associated. The following section (2.3.4) will provide a detailed characterization of the three haplotypes that are of interest to the proposed research.

2.3.2 Three Common Haplotypes of the COMT gene and Perceptions of Pain

The human pain experience is complex, influenced by both environmental and genetic factors [66]. It remains a major focus of research to identify candidate genes that influence how individuals perceive various pain-inducing stimuli. As a key element in antinociception, the COMT gene is one likely candidate due to its diverse biological function as well as its critical role in clearing catecholamine hormones in the central nervous system [75, 76, 77].

Past research has found that three distinct haplotypes of the COMT gene have an influence on the level of activity of the COMT enzyme produced, and will likely influences

individual differences in how subjects perceive various pain stimuli. [29, 38, 54, 78, 86, 87, 88, 89, 102, 144]. Diatchenko et al. (2005) found that these haplotypes confer a graded responsiveness to experimental pain. In a sample of pain-free, healthy females aged 18 to 34 years, individual responses to various pain-inducing stimuli including pressure pain, thermal pain, and ischemic pain. It was determined that subjects homozygous for the G_C_G_G haplotype had the lowest pain responsiveness (mean summed z-score = -5.23 ± 1.5). Therefore, the G_C_G_G haplotype was designated the Low Pain Sensitivity (LPS) haplotype. Subjects homozygous for the A_T_C_A haplotype reported intermediate responsiveness to pain (mean summed z-score = 1.75 ± 1.47), this haplotype was designated the Average Pain Sensitivity (APS) haplotype. Finally, the greatest responsiveness to pain was reported by subjects having one A_C_C_G haplotype (mean summed z-score = 8.9 ± 2.9). This was designated the High Pain Sensitivity (HPS) haplotype . Table 3.0 provides a complete list of the possible combinations of the three haplotypes of interest. In all, the three haplotypes accounted for 10.4% of the variance in pain sensitivity in this study ($p < 0.01$).

Table 1.0 Five Combinations of Three Haplotypes of Interest

Haplotype Combinations	Allele Sequence
LPS/LPS	G_C_G_G / G_C_G_G
LPS/APS	G_C_C_G / A_T_C_A
APS/APS	A_T_C_A / A_T_C_A
HPS/LPS	A_C_C_G / G_C_G_G
HPS/APS	A_C_C_G / A_T_C_A

The biochemical basis for this finding involves the fact that composition of the COMT gene influences total enzyme activity. For the functional polymorphism (rs4680), previous research indicates that the valine to methionine substitution produces an enzyme with a lower thermostability resulting in decreased enzyme activity [29]. Thus, the COMT enzyme produced is less effective at clearing catecholamine hormones when there is a valine to methionine substitution at codon 158. In addition, it has been found that, due to this variation, the HPS haplotype produces a deficiency in protein synthesis [29]. Thus, the total amount of protein that is synthesized is reduced when the HPS haplotype is present. The net affect is very much the same, with a decrease in the efficiency of catecholamine catabolism in the central nervous system. In additional work performed by Nackley et al., [87, 88, 89] it was found that differences in the stability of the folded structure of the mRNA are likely contributors to the differences in enzyme activity of COMT. In the HPS haplotype, the structural stability of the mRNA was the highest, conferring the lowest activity of the COMT enzyme. In contrast, the lowest structural stability has been found in the LPS haplotype. Further, when the stable loop in the mRNA of the HPS haplotype was experimentally destroyed without altering amino acid sequence, enzyme activity is similar to that of the LPS haplotype. These results strongly indicate that differences in COMT activity are due not only to amino acid sequence differences, but also to variations in mRNA secondary structure [87, 88, 89].

Polymorphic variability in the COMT gene produces a COMT enzyme that is less efficient at breaking down catecholamine hormones at the synapses of neurons involved in adrenergic neurotransmission. Thus, individuals shown to be less efficient at catabolizing catacholamines may experience a higher level of physiological excitation in response to a stressor, due to the incomplete breakdown of catecholamine hormones. It is then likely that such

individuals will exhibit an exaggerated perceptual response to such stressors as pain and physical exertion associated with exercise. The following section (2.4) will explain the importance of exertional perceptions and scaling methodologies to assess exertional perceptions during an exercise stimulus.

2.4 EXERTIONAL PERCEPTIONS

2.4.1 Exertional Perceptions and the Global Explanatory Model

Perception of exertion is defined as, "the subjective intensity of effort, strain, discomfort, or fatigue that is experienced during physical exercise" [95]. In all human beings, physical exercise elicits feelings of effort which are influenced by the intensity of exercise being performed. Decisions to alter exercise intensity based upon subjective level of comfort can be made.

The global explanatory model of perceived exertion provides a detailed description of the physiological, psychological, and performance-related factors that influence exertional perceptions. It utilizes a "gestalt-like" perspective, synthesizing factors from both internal and external environments. The global model uses perception rather than simply sensation, in expressing exertional perceptions during an exercise challenge [95]. To understand this, an important distinction must be made between sensation and perception. Sensation simply involves the direct stimulation of a sensory end organ, while perception involves "pure sensation and a complex of internal and external stimuli for which there may be no direct link with sensory end organs" [95].

The global explanatory model states that physiological responses to an exercise challenge are the initial mediators that influence the intensity of the associated perceptual signal by altering the tension-producing characteristics of the exercising skeletal muscle. Stimulation from the motor cortex of the brain produces tension in respiratory and/or peripheral muscles during exercise. In turn, the sensory cortex receives this stimulation as a feed-forward command, which is then interpreted as perceptual signals of exertion. Finally, this signal is assessed in comparison to the contents of the perceptual cognitive reference filter. The resultant signal can then be adjusted according to a matrix of past and present events that reflect an individual's psychological characteristics and perceptual style [95]. The perceptual signal to follow can involve the active limbs and/or respiratory system (differentiated) or it can involve the overall body (undifferentiated) [95] (Figure 1.0). Semantic and pictorial scales to assess one's level of perceived exertion have been developed for use in clinical and research settings. The following section (2.4.2) provides an introduction to the OMNI Scale of Perceived Exertion.

2.4.2 Physiological mediators of exertional perceptions

It is clear that complex physiological processes mediate exertional perceptions during an exercise stimulus, the extent to which varies significantly from one process to the other. These physiological mediators act synergistically, influencing level of effort that is perceived [95]. The physiological mediators of exertional perceptions fall into two general categories: respiratory-metabolic mediators and peripheral and non-specific mediators. Respiratory-metabolic mediators include those that influence ventilatory drive during exercise. Peripheral

mediators are those restricted to skeletal muscle within the limbs and trunk. The term non-specific mediator refers to whole-body physiological processes that influence effort sensation associated with an exercise stimulus. An example of this is the production of catecholamine hormones during exercise [95]. The following section provides description of the possible physiological mediators of exertional perceptions (Table 3.0).

Table 2.0 Proposed physiological mediators of exertional perceptions

Respiratory-Metabolic Mediators	- Ventilatory Drive (V_E) - Oxygen Uptake (VO_2) - Carbon dioxide excretion (VCO_2) - Heart-rate (HR) - Blood-pressure (BP)
Peripheral Mediators	- Metabolic acidosis - Contractile properties of muscle tissue - Muscle blood-flow - Blood glucose concentration
Non-specific Mediators	- Temperature Regulation - Pain Reactivity - Hormonal Regulation

2.4.2.1 Respiratory-metabolic Mediators

2.4.2.1.1 Ventilatory Drive and Respiratory Rate Both correlational and experimental research indicates that V_E is an important mediator of exertional perceptions. Simple and multiple regression analyses have demonstrated correlation coefficients between RPE and both V_E and respiratory rate (RR) ranging from 0.61 to 0.94 [19, 26, 31, 33, 35, 40, 44, 46, 56, 66, 67,

72, 79, 94, 95, 100, 105, 106, 108, 110, 112, 113, 122, 130]. Using a multiple regression model, Noble et al. [94], found that V_E and RR accounted for the greatest variance in RPE during treadmill and cycle ergometry in hot and neutral exercise environments. In addition, Robertson et al. [106] experimentally attenuated ventilatory buffering of metabolic acidosis during combined arm and leg exercise using ingestion of $NaHCO_3$. Under the alkalotic condition, VO_2peak, V_E, and RPE-chest were lower when compared to a placebo condition. Furthermore, Robertson et al. [106] demonstrated that RPE increased concurrently with higher rates of ventilation during an exercise stimulus [106]. It also appears that respiratory rate (RR) is a significant mediator of exertional perceptions. Experimentally manipulated cycle ergometer pedaling frequency and blood pH has been shown to produce corresponding alterations in RPE, while tidal volume remains unaltered [105, 106]. Therefore, changes in RR during dynamic exercise appear to be a primary physiological mediator of exertional perceptions [14, 94, 95, 105, 106].

2.4.2.1.2 Respiratory Gases Oxygen uptake (VO_2) and carbon dioxide excretion (VCO_2) during exercise have been demonstrated to mediate exertional perceptions [19, 33, 34, 35, 83, 84, 105, 108, 110]. Perceptual signals associated with VO_2 are mediated by ventilatory drive required to support aerobic metabolism. The ventilatory response to increased aerobic energy demands will result in increased contractility of inspiratory muscles, thereby influencing effort sensation. Thus, oxygen uptake is classified as a signal of respiratory-metabolic exertion [95].

When oxygen supply is sufficient to meet the demand of exercising skeletal muscle, exertional perceptions remain relatively low. However, when oxygen supply is diminished, RPE appears to increase significantly. Robertson et al. [105] showed that RPE remains significantly

higher in a hypoxic exercise condition when compared to a normoxic condition [105]. Similar studies have found correlation coefficients between VO_2 and RPE ranging from r = 0.76 to 0.97 during continuous and intermittent arm and leg cardiovascular exercise [19, 33, 34, 35, 83, 84, 105, 108, 110]. Evidence also exists in support of carbon dioxide excretion as a physiological mediator of exertional perceptions [17, 19, 33]. A higher burden associated with a greater need for CO_2 removal is associated with increased effort sensation. Conversely, when a need for CO_2 removal remains relatively low, effort sensation also remains low [17, 19, 33], suggesting a link between carbon dioxide excretion with exertional perceptions in response to exercise.

2.4.2.1.3 Cardiovascular Responses Correlational evidence exists to support heart-rate (HR) as a physiological mediator of exertional perceptions. Early studies using progressively incremented cycle ergometer power outputs to characterize the relation between HR and RPE, demonstrated correlation coefficients ranging from r = 0.42 to 0.94, for varying exercise modalities [12, 26, 33, 49, 50, 93, 97, 100, 105, 108, 110]. However, stronger evidence suggests a lack of association between HR and RPE when one of the two variables is experimentally manipulated during progressive exercise [95]. The preponderance and strength of experimental evidence indicates that heart-rate is not likely a significant physiological mediator of exertional perceptions. Comparatively little research has been performed to examine the relation between exertional perceptions and blood pressure (BP). The exact relation between RPE and BP is thus far less clearly understood. Additional research to fully characterize this relation is warranted [95].

2.4.2.2 Peripheral Mediators

2.4.2.2.1 Metabolic Acidosis Evidence has shown that peripheral signals of exertion with perturbations in blood pH are associated with high-intensity exercise. As the plasma environment becomes more acidotic, rating of perceived exertion increases during various exercise challenges [106]. In an experimentally induced acidotic exercise condition, RPE-Legs has been shown to be significantly higher compared to a control alkalotic condition. Additionally, it was found that systematically isolating exercising skeletal muscle groups through varying exercise modalities will produce associated shifts in blood pH [106].

Research suggests that in addition to blood pH, blood lactic acid concentration ([HLa]) is a mediator of exertional perceptions during dynamic exercise. It was discovered that during progressive cycle ergometer exercise, blood [HLa] and RPE-legs increase as a positively accelerating function of power output [106]. Additional correlational and experimental evidence also links exertional perceptions with blood [HLa] [18, 44, 66]. At present limited evidence has shown an association between muscle [Hla] and exertional perceptions during dynamic exercise.

2.4.2.2.2 Additional Peripheral Mediators Some evidence has shown that a greater percentage of fast-twitch muscle fibers is associated with higher ratings of perceived exertion (RPE) during cycle ergometry [49, 66, 73, 74, 75]. Blood flow to exercising skeletal muscle has also been shown to mediate exertional perceptions during dynamic exercise [49, 66, 73, 74, 75]. In addition, some research indicates that blood glucose extraction is a significant mediator of exertional perceptions during exercise (15, 33, 62, 66, 82). Carbohydrate metabolism becomes a primary energy source during exercise at intensity above the individual lactate threshold. Availability of glucose as an energy substrate may be associated with exertional perceptions

during an exercise stimulus [22, 90, 107]. At present the exact nature of this relation has not been clearly elucidated and further research is therefore warranted.

2.4.2.3 Non-specific Mediators The non-specific physiological mediators of exertional perceptions that have been examined include: (1) temperature regulation; (2) pain responsiveness; and (3) hormonal regulation.

2.4.2.3.1 Temperature Regulation It has been found that body core temperature and ratings of perceived exertion increase when metabolic heat is produced in response to dynamic exercise. However, experimental evidence does not support a relation between core temperature and exertional perceptions. Correlations coefficients between core temperature and RPE in previous investigations have ranged from 0.14 to 0.20, and were not statistical significance (41, 102). Additionally, a lack of significant associations has been found between core temperature and RPE during exercise in cold (102) and hot (102, 103) environments, involving both arm and leg ergometry (102). This suggests that body core temperature is not a significant mediator of exertional perceptions during exercise. An association between skin temperature and exertional perceptions has not been conclusively identified. Therefore, future research exploring this relation is necessary [95].

2.4.2.3.2 Pain Responsiveness In addition to temperature regulation, another potential mediator of exertional perceptions that warrants discussion is pain responsiveness during exercise. As the proposed research is interested only in non-clinical pain, this review will focus

on pain commonly associated with an exercise stimulus not associated with physical injury or other pathology.

Although limited, evidence linking exertional perceptions with exercise-related pain responsiveness has been shown. It was found that local muscle soreness in swimmers appears to mediate intensity of exertional perceptions associated with an exercise challenge [35, 49, 95]. Recent research indicates moderate to strong correlations between exercise-related pain and rating of perceived exertion during progressive submaximal ergometry [35, 49, 95]. For example, it was found that with increasing workrate in cycle ergomtery exercise, ratings of perceived exertion and sensations of pain both increased as a result. The existing evidence to date indicates that exercise-related pain is an important mediator of exertional perceptions during exercise [95].

2.4.2.3.3 Catecholamines **N**oble and Robertson [95] classify the production of catecholamine hormones as a mediator of exertional perceptions in response to an exercise challenge. Evidence in support of increased plasma catecholamine concentration as a mediator of exertional perceptions suggests a significant association. While most studies have found that production of cateholamine hormones increases with increasing exercise intensity and duration, [1, 22, 23, 31, 36, 81, 104, 109] other studies have found inconsistent results [18, 31]. In supportive research, associations between E and NE and rating of perceived exertion during varying exercise protocols were shown to range from $r = 0.54$ to $r = 0.71$ [1, 22, 23, 31, 36, 81, 104, 109]. These moderate to strong correlations indicate that as plasma concentrations of E and NE increase, a corresponding increase in effort sensation during varying exercise challenges will also occur. Additionally, Docktor et al. [31] demonstrated that both RPE and plasma catecholamines were

attenuated following a five-week training program. This indicates that in response to chronic training, both plasma catecholamines and exertional perceptions decline from pre-training to post-training, suggesting that chronic training diminished exertional perceptions by way of attenuating plasma concentrations of the catecholamines epinephrine and norepinephrine [95].

An examination of the likely biochemical and physiological mechanisms related to catecholamine production may help to characterize this association. Two distinct pathways seem to influence concentration of plasma catecholamines as a mediator of exertional perceptions. These pathways are depicted in Figure 2.0. The first is related to the physiological function of catecholamines in regulating plasma glucose levels during prolonged exercise. Glucose concentrations remain relatively stable at lower exercise intensity and sharply decline as exercise intensity increases beyond 50% – 70% of maximal oxygen uptake [22]. This increase in intensity and declining glucose or glycogen, results in an increased secretion of epinephrine and norepinephrine [6, 81, 23, 49]. Plasma catecholamines have been found to stimulate hepatic and muscle glycogenolysis to compensate for the depletion of carbohydrate substrate as exercise duration increases [6, 49, 81]. It is therefore likely that catecholamines mediate effort sensation through metabolic mechanisms reflecting a depletion of plasma glucose and muscle glycogen as duration of exercise increases [49, 95]. The increase in plasma concentrations of E and NE causes excitatory processes such as elevated heart-rate and myocardial contractility, which influences effort sensation associated with the exercise bout [6, 49, 81].

In addition to the increase in glucose metabolism, psychological stress elicits an increase in plasma concentration of epinephrine. The psychological mechanisms associated with catecholamine production must not be overlooked [31, 49, 81]. Research indicates that concentration of plasma epinephrine (E) increases as a result of the psychological stress

associated with performing an exercise challenge [6, 31]. While the secretion of E and NE during exercise is highly associated with substrate utilization, E also appears closely related to psychological stress associated with exercise [6, 31, 49, 81]. Epinephrine is secreted in response to both physiological and psychological stress inducing stimuli. High intensity exercise represents both a physiological and psychological stressor [95].

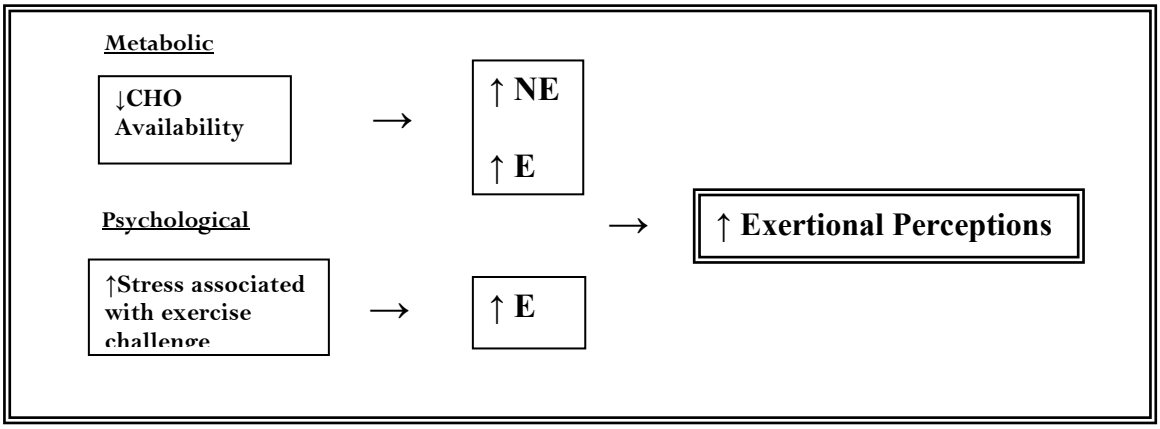


Figure 2.0 Catecholamines as Mediators of Exertional Perceptions

2.5 THE OMNI SCALE OF PERCIEVED EXERTION

Accurate assessment of exertional perceptions in clinical, sports, and pedagogical applications is crucially dependent upon the use of appropriate scaling methodologies [95]. The first numerical, category scale to measure exertional perceptions was developed by psychologist Dr. Gunnar Borg [13]. Recently developed and validated numerical category scales use a finite set of numbers to assess perceptions of exertion, and require that a sensory response continuum be divided into equal intervals. The distance between each category is presumed to correspond to

equal sensory responses [95]. The importance of these scales is that they allow subjects to select a number that represents the intensity of their perception of exertion during exercise. This numerical response is called a Rating of Perceived Exertion or RPE [95].

The proposed research study utilized the Run/Walk format of the OMNI Scale of Perceived Exertion developed by Dr. Robert J. Robertson. The acronym OMNI represents the word omnibus. When applied to the assessment of perceived exertion, OMNI refers to a category scale, having measurement properties that are broadly generalizeable [95]. This instrument utilizes a numerical rating scale from 0 to 10 with both pictorial and verbal descriptors of an individual exercising along a progressive continuum of intensity. The generalizeability of this scale is further enhanced by the interchangeable nature of the pictorial descriptors, which allows use of this scale for varying exercise modalities from cycle ergometry to resistance exercise. While the pictorial descriptors are interchangeable and therefore mode-specific, the verbal descriptors remain the same for each format of the OMNI scale [95]. Of critical importance to the accurate examination of exertional perceptions is the development of reliable and valid instruments. The OMNI scale of perceived exertion has been found to be both reliable and valid. The following section (2.2.4) provides a review of this important evidence.

2.5.1 Reliability of the OMNI Scale of Perceived Exertion

Past research indicates that reliability using the OMNI scale of perceived exertion has been established. Research found reliability coefficients of $r = 0.91$ and $r = 0.95$ for the OMNI scale. This establishes both intra-class and stability reliability for the cycle format of the OMNI scale, respectively [95, 110].

2.5.2 Validity of the OMNI Scale of Perceived Exertion

Previous studies have established concurrent and construct validity of both the adult and child formats of the OMNI scale, using both criterion variables and concurrent variables, in a two-variable research paradigm. The most commonly used criterion variables for aerobic exercise were oxygen uptake (VO_2), percent of maximal oxygen uptake ($\%VO_{2max}$), ventilation (V_E), respiratory rate (RR), respiratory exchange ratio (RER), heart-rate (HR), and glucose oxidation. The concurrent variable used was RPE derived from the various formats of the OMNI scale and/or Borg scale [110]. Robertson et al. [110] used the concurrent variables RPE for the overall body (RPE-Overall), legs (RPE-Legs), and chest (RPE-chest), while submaximal VO_2 and HR served as criterion variables during a cycle ergometry protocol.

To determine concurrent validity, physiological variables such as VO_2, HR, and RER were regressed against RPE-OMNI (overall, legs, chest) and/or RPE-Borg. When appropriate, regression coefficients were calculated separately for males and females.

Results of the linear regression analyses among the various experimental studies indicate that the validity coefficients derived ranged from $r = 0.67$ to $r = 0.95$ [110], establishing concurrent validity for adult and child formats of the OMNI scale of perceived exertion for both cycle ergometry and treadmill exercise modalities.

Construct validity of the OMNI scale was established [110], when RPE-OMNI was regressed against RPE-Borg [13], using both males and females ($r = 0.96$). Additional studies have demonstrated validity coefficients ranging from $r = 0.92$ to $r = 0.97$ [110]. Results of these investigations provide strong evidence of construct validity for adult and child formats of the OMNI scale of perceived exertion.

2.6 SUMMARY OF LITERATURE REVIEW

Exercise represents a stressor that necessitates mobilization of critical physiological processes in an attempt to adapt. As part of the general adaptation syndrome, production of epinephrine and norepinephrine increase. This produces an excitatory response in which heart-rate, blood pressure, and contractility of inspiratory muscles increase to meet the demand of mobilized skeletal muscles, brain, and heart for oxygen, nutrients, and waste removal. To cope with the effects of epinephrine and norepinephrine, the brain releases endogenous opioids that produce a sense of well-being, calm, and analgesia, thus, allowing the organism to function despite the state of excitation.

Strong evidence has shown that increased production of the catecholamines epinephrine and norepinephrine during exercise produces corresponding increases in effort sensation. Catecholamines are catabolized by the enzyme catechol-O-methyltransferase in the nervous system. The break-down of catecholamines reduces the physiological excitation that they caused, and is an essential component of antinociception. Thus, catechol-O-methyltransferase (COMT) is integral to proper balancing of catecholamines in the nervous system.

Polymorphic variation in the COMT gene influences COMT translation and therefore the strength of the COMT enzyme that is produced. During an exercise stimulus, such variability is expected to influence catecholamine clearance in the nervous system, thereby impacting effort sensation as well as perceptions of exertional pain. The proposed research is an examination of COMT gene variation and how it influences perceptual response to exercise as a physiological stressor.

3.0 METHODS

3.1 INTRODUCTION

The primary purpose of this investigation was to examine variation in the Catechol-O-methyltransferase (COMT) gene and exertional perceptions during a maximal treadmill graded exercise test. The secondary purpose was to examine variation in the COMT gene and perceptions of exercise-induced lower-body pain during a sub-maximal treadmill graded exercise test. All dimensions of the proposed research took place at the Center for Health-Fitness Research at the University of Pittsburgh. In addition, the proposed research was been approved by the Institutional Review Board (IRB) at the University of Pittsburgh (Appendix A). Informed consent was obtained prior to participation in the proposed research.

3.2 SUBJECTS

The proposed research was performed as one dimension of a large-scale prospective study. The University of Pittsburgh Physical Activity Study (PittPAS) is a research study designed to examine; (1) psycho-physiological mechanisms to help explain level of participation in physical activity (PA) as well as; (2) spontaneous change in PA over a two-year period in a sample of 848 young adults.

A total of 231 subjects were recruited from this original sample for the proposed research study. In order to participate, subjects had to; 1) be healthy males and females between 27 and

35 years of age; 2) complete one sub-maximal, load-incremented treadmill test and; 3) provide one genetic sample via whole blood or buccal cells. Exclusion criteria were; 1) positive response to one or more questions on the Physical Activity Readiness Questionnaire (PAR-Q) (Appendix B); 2) presence of an unstable medical illness within the last 12 months; 3) positive response to one or more questions on the Final Screening Form (FSF) (Appendix C); 4) knowingly pregnant; 5) having any clinical, musculoskeletal or metabolic contraindications to exercise; 6) having a resting blood pressure greater than either 140 mmHg systolic or 90 mmHg diastolic after two measurements or; 7) being unwilling to voluntarily participate in the testing session. Exclusion was not based on gender, or HIV-status.

3.3 EXPERIMENTAL DESIGN

This investigation employed a multiple observation cross sectional design. Dependent variables were exertional perceptions (differentiated and undifferentiated) and exercise-induced lower body pain as a function of the following physiological criterion variables: oxygen uptake ($l \cdot min^{-1}$), minute ventilation (VE), ventilatory breakpoint and heart-rate (HR), as determined from a sub-maximal treadmill graded exercise test. The independent variable being examined was COMT genotype.

3.4 PROCEDURES

3.4.1 Laboratory Procedures

Subjects were contacted and screened to ensure that they were capable of safely exercising on a treadmill. Upon arrival at the Center for Exercise and Health Fitness Research, all subjects were administered the Physical Activity Readiness Questionnaire (PAR-Q) and Final Screening Form. A flow of the laboratory session is provided (Figure 3.0). Subjects answering "Yes" to any of the screening questions were not eligible to participate in the proposed research without written physician clearance. Subjects reporting health conditions that were not screened for by the PAR-Q or FSF were addressed by the investigators on the parent study on a case-by-case basis. Blood pressure measurement was performed at least twice for each subject prior to completion of the treadmill test. Following assessment of blood-pressure, subjects were oriented to the OMNI Scale of Perceived Exertion and Cook Pain Scale, including a definition of Rating of Perceived Exertion (RPE) and exercise-induced lower body pain with complete instructional anchoring procedures (appendix C and D).

3.4.1.1 Rating of Perceived Exertion Rating of Perceived Exertion (RPE) was assessed using the Walk/Run format of the OMNI Scale of Perceived Exertion (Robertson, 2004) (appendix C) at minute 2:25 of each stage of the sub-maximal treadmill protocol. An undifferentiated rating was obtained for the overall body (RPE-Overall) and two differentiated ratings was obtained for peripheral perceptions of exertion in the legs (RPE-Legs) and respiratory-metabolic perceptions of exertion in the chest and breathing (RPE-Chest). The following orientation script was used to explain the OMNI Scale to subjects.

RPE Scale Orientation:

While you are exercising on the treadmill we will be asking you to give us a rating of how much exertion or effort you feel and how much, if any, pain you are experiencing.

For the exercise you will be doing, exertion is defined as the intensity of effort, strain, discomfort or fatigue that you feel during exercise. Repeatedly during the exercise we will ask you to use the numbers on this scale ***(show the RPE scale)*** *to tell us how different areas of your body feel while you are walking or running on the treadmill.*

Please look at the person at the bottom of the hill who is just starting to walk ***(point to the left-hand picture)****. If you feel like this person when you are walking, the exertion will be extremely easy and your rating should be 0. Now looking at the person who is exhausted at the top of the hill* ***(point to the right-hand picture),*** *if you feel like this person when you are walking or running, the exertion will be extremely hard and your rating should be 10. If you feel that your exertion is somewhere between extremely easy and extremely hard, please give a number between 0 and 10.*

During the exercise we will ask you to use the numbers on the scale to tell us separately how your how your legs feel, how your chest and breathing feel and then how your whole body feels. Please use both the pictures and the words to help you select any number to tell how you feel while you are walking or running. And remember there is no right or wrong answer for this exercise. You will have a mouthpiece in during the treadmill test so we will ask you to use your finger and point to a number; we will say the number and ask you to confirm this with a nod of your head so that we record the correct number.

I'm going to ask a few questions to make sure you understand the exertion scale. Please point to the appropriate number on the scale.

- *How would you rate your level of exertion right now?*
- *If you were running up a hill, how would you rate your exertion?*

- *Think of a time when you exercised as hard as you can ever remember, how would you rate your exertion?*

3.4.1.2 Exercise-induced Lower Body Pain Immediately following the OMNI Scale orientation, subjects were oriented to the Cook Pain Scale, including a definition of lower-body exercise-related pain (appendix D).

Exercise induced pain sensation was measured by the Cook Pain Intensity Scale. This is a 12 category scale having verbal descriptors ranging from "0 = no pain at all" to "10 = extremely intense pain." A point (•) linked to the verbal descriptor "unbearable pain" is positioned at the high intensity end of the response continuum. A pain intensity rating was estimated at minute 2:45 of each stage of the sub-maximal treadmill test. The following orientation script was used to explain the Cook Pain Scale to subjects.

Exercise-related Pain Scale Orientation

(Please emphasize that for our purposes, we are talking about EXERCISE pain).

For the exercise you will be doing, pain is defined as the intensity of hurt that you feel in your lower body, this includes your buttocks and your upper and lower legs. We will refer to these together as your "lower body". This scale ***(show the Pain scale)*** *contains the numbers 0 to 10. We want you to use this scale to describe the feeling of pain in your lower body during the exercise test. Please try to estimate the degree of pain you feel as honestly and objectively as possible. The numbers on the scale represent pain intensity ranging from very faint pain at 0.5* ***(point to scale)*** *to extremely intense pain (almost unbearable) at 10* ***(point to scale).*** *When you feel no pain in your buttocks or legs, please respond with the number 0. You may also respond with a number greater than 10. When the pain you feel is greater than 10, please point to the * on the chart. You should think of a number that represents the pain intensity in comparison with the number 10. In other words, if the pain*

*you feel is twice as great as it felt when it was a 10, respond with the number 20. If you point to the *, I will call out numbers in multiples of 10, for example 20, 30, 40, etc.*

Repeatedly during the test you will be asked to rate the feelings of pain in your lower body. When you rate these pain sensations, be sure to pay attention only to the pain in your lower body and not any other area of your body where you may be feeling pain, for example you may feel discomfort from the mouthpiece. It is very important that your rating of pain intensity reflect only the degree of hurt you feel in your lower body. Also, do not use your rating of pain as an expression of fatigue (the inability of your legs to produce force) or exertion (how much effort you are putting into performing the exercise, this is measured with the RPE scale).

Again, you will have a mouthpiece in during the treadmill test so we will ask you to use your finger and point to a number; we will say the number and ask you to confirm this with a nod of your head so that we record the correct number.

Do you have any questions about the difference between the rating of pain and the rating of exertion?

3.4.1.3 Anthropometric Measures Following the Pain Scale orientation, anthropometic measures were performed. Standing height was measured using a clinic balance beam scale (Health-o-meter KL, Shelton CT). Body weight and an estimation of body composition (% body-fat, % water, % fat-free mass) was assessed via Bioelectrical Impedance Analysis (BIA).

3.4.1.4 Genetic Samples Following anthropometric measures a genetic sample was collected via whole-blood or collection of buccal cells using Scope mouthwash. For the parent study, serial blood lactate samples were collected during the sub-maximal treadmill test via a forearm intravenous catheter. Following collection of the resting lactate sample, an additional 10

ml. (approximately 2 teaspoons) blood sample was collected via the catheter to obtain a DNA sample. Some subjects declined insertion of the intravenous catheter. Alternatively, a blood draw to obtain the DNA sample consisted of a single venipuncture administered by a trained phlebotomist. Risks associated with a single venipuncture include fainting, lightheadedness, temporary local discomfort, bleeding or bruising. The insertion site was monitored for localized discoloration and swelling. There is a rare risk of infection with the insertion of the needle. It is likely that local bruising and/or soreness will occur after blood collection. However, this will be no more than that encountered during blood donation (Institutional Review Board: University of Pittsburgh).

Subjects, for whom collection of a blood sample for DNA extraction is not possible, were asked to provide a sample of buccal cells. For buccal cell collection, subjects were first instructed not to eat or brush their teeth within one hour of the mouthwash cell collection. After anthropometric measures were completed, subjects were asked to vigorously swish 10 ml. of Scope mouthwash around in their mouths for 45 seconds. They then spit the mouthwash into a centrifuge tube and secured the top. Subjects were instructed not to gargle or clear their throats while swishing the mouthwash.

The DNA samples are being stored without identifiers in the Human Genetics Laboratory in the Department of Human Genetics at the University of Pittsburgh. After collection of the genetic sample, laboratory tests were conducted that included physiological assessments, and a sub-maximal treadmill graded exercise test. Following collection of the blood or buccal cells, the sub-maximal oxygen graded exercise test was completed.

3.4.1.5 Sub-maximal Treadmill Graded Exercise Test A load-incremented, sub-maximal treadmill, graded exercise test will be used consisting of progressive intensity 3-minute stages. Metabolic data during the treadmill test will be collected using a ParvoMedics TrueOne Respiratory-Metabolic Analyzer (Sandy Lake, UT) with a Hans-Rudolph respiratory valve (Shawnee, KS). The treadmill protocol will be performed on a Trackmaster TMX425C treadmill (Newton, KS).

The multistage Modified Balke protocol consists of 3-minute stages as follows; Stage 1 – 3.0 mph at a 0% grade, Stage 2 – 3.0 mph at a 2.5% grade, Stage 3 – 3.0 mph at a 5.0% grade, Stage 4 – 3.0 mph at a 7.5% grade, Stage 5 – 3.0 mph at 10% grade, Stage 6 – 3.0 mph at 12.5% grade. Each stage thereafter will increase in graded at a rate of 2.5%. Measures of Heart-rate (HR), Rating of Perceived Exertion (RPE), oxygen uptake (VO_2), Carbon dioxide excretion (VCO_2), and minute ventilation (VE) will be assessed at each stage. Termination will correspond to 85% of age adjusted maximal heart-rate or volitional exhaustion. Upon completion of the sub-maximal, subjects will be given a 2 minute cool-down at 2.0 mph at a 0% grade until HR decreases to < 110 $B \cdot min^{-1}$.

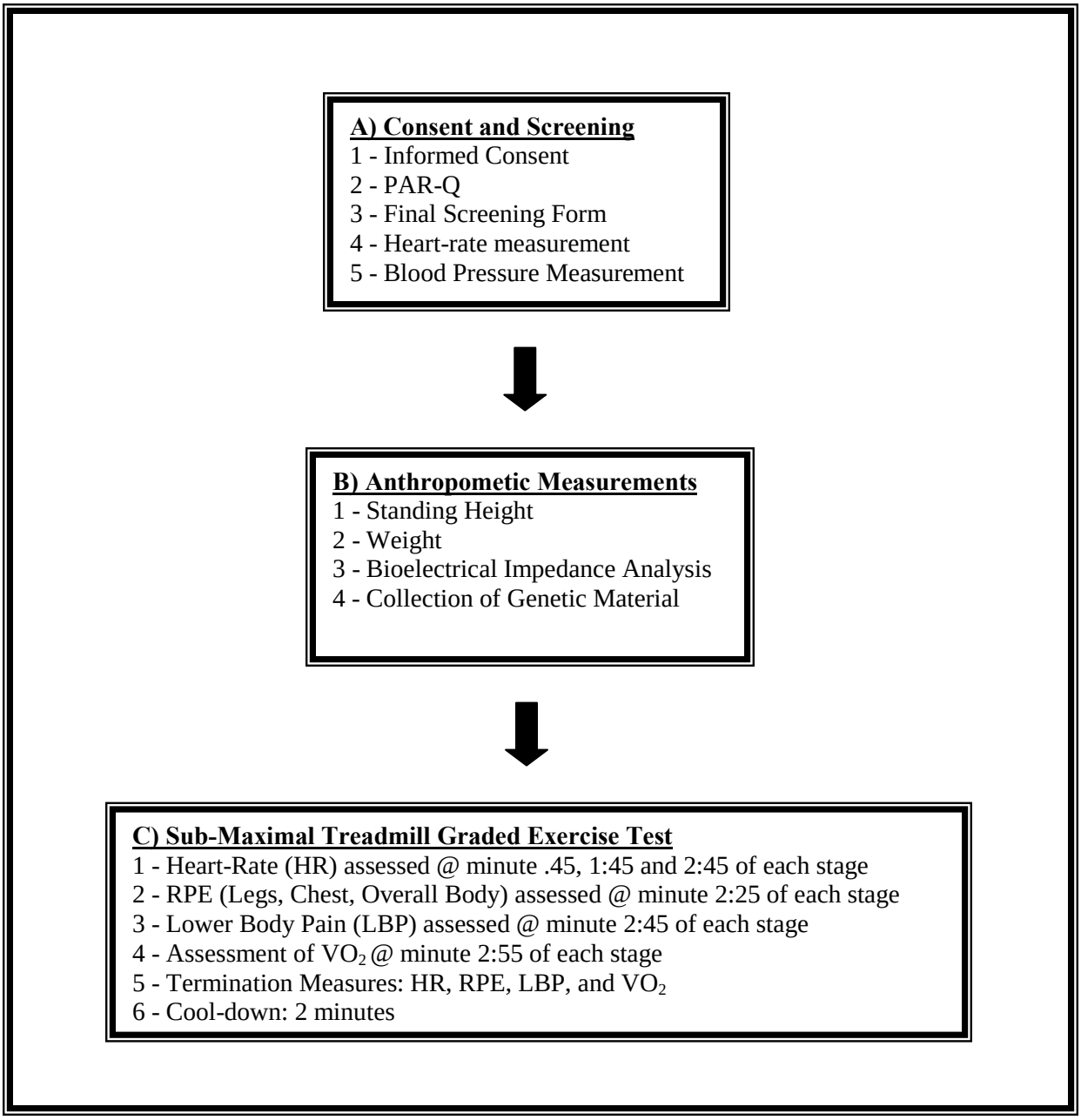


Figure 3: Flow of Laboratory Session

3.5 GENETIC PROCEDURES

Genetic samples were transported from The Center for Health-Fitness Research to the Human Genetics Laboratory within the Department of Human Genetics, at the University of Pittsburgh by research personnel on the parent study. Samples were transported in collection tubes (blood) and centrifuge tubes (buccal cells) isolated in biohazard bags (one sample per bag to prevent cross-contamination).

Trained laboratory technicians performed all genetic analyses. Single Nucleotide Polymorphism genotypes were determined by amplification of each site using sequence specific primers designed using dbSNP (www.ncbi.nlm.nih.gov/Database). Amplification by polymerase chain reaction followed by fluorescence polarization followed. Alleles were assigned by comparison to samples of known genotype run on the same gel. Fit to the expectations of Hardy-Weinberg equilibrium was tested by chi square analysis. COMT haplotypes were estimated using the program PHASE. Table 3.0 provides the three previously designated LPS, APS, and HPS haplotypes. All genetic laboratory procedures were supervised by Dr. Robert Ferrell at the University of Pittsburgh's School of Public Health, Department of Human Genetics.

Table 3.0: Three Haplotypes of the COMT Gene

Haplotype	DNA Sequence
Low Pain Sensitivity (LPS)	G_C_G_G
Average Pain Sensitivity (APS)	A_T_C_A
High Pain Sensitivity (HPS)	A-C_C_G

3.6 STATISTICAL CONSIDERATIONS

Statistical analyses were performed using SAS 9.2. The original physiological database and genotype data exist in Microsoft Access format and were exported to SAS 9.2 for all statistical procedures. Frequency distributions were performed on each variable to check for outliers and entry errors. Additionally, 10% of all the records were randomly selected and checked for errors by comparing laboratory sheet data with electronically input data. Descriptive statistics were calculated for all variables. Measures of central tendency (mean, median, and percentiles) and dispersion (standard deviation and ranges) were computed for continuous variables such as RPE and Pain. Additionally, distributions of the data were examined using histograms and box and whisker plots.

The three previously designated haplotypes (LPS, APS, and HPS) served as the basis for grouping subjects. Recalling that one haplotype is inherited from each parent, six diplotypes (combination of two haplotypes) were theoretically possible. Please refer to Table 4.0 for the formation of these six diplotypes. SAS 9.2 will be used to estimate haplotype frequencies. Given that presence of even one LPS haplotype confers a diminished responsiveness to pain, and

presence of one HPS haplotype confers an exaggerated responsiveness to pain, only three distinct categorizations were required. Therefore, subjects were categorized as Low Responders (LR), Average Responders (AR), or High Responders (HR). Subjects with one LPS haplotype belonged in the LR group. Subjects with at least one APS haplotype will belong in the AR group, and subjects with one HPS haplotype were placed in the HR group.

Perceptual response to exercise (RPE and Pain) within each sub-group was examined using linear regression models, with RPE and Pain expressed as a function of each of the physiological criterion variables (oxygen uptake, ventilation, HR). From this linear regression equation, a slope for each subgroup was calculated. Differences in slopes among the three genotype sub-groups were examined using analysis of covariance (ANCOVA). Covariates examined were gender, age, BMI, total PA, and maximal oxygen uptake. Appropriate post hoc procedures were be used to identify differences among the sub-groups. Statistical significance was accepted at the $p < .05$ level.

Table 4.0: Six Diplotypes of the COMT Gene

Diplotypes	
LPS/LPS	G_C_G_G / G_C_G_G
APS/APS	A_T_C_A / A_T_C_A
HPS/HPS	A_C_C_G / A_C_C_G
LPS/APS	G_C_G_G / A_T_C_A
LPS/HPS	G_C_G_G / A_C_C_G
APS/HPS	A_T_C_A / A_C_C_G

4.0 RESULTS

4.1 INTRODUCTION

The objective of this research was to examine the influence that three previously identified haplotypes of the Catechol-O-methyltransferase (COMT) Gene have on perceptual response to treadmill graded exercise, among healthy young adults. The primary aim was to examine differences in exertional perception during a sub-maximal treadmill graded exercise test, among LR, AR, and HR haplotypes of the COMT gene. The secondary aim was to examine differences in exercise-induced lower-body pain during a sub-maximal treadmill graded exercise test, among LR, AR, and HR haplotypes of the COMT gene. This chapter is composed of the following sections: (1) Sample; (2) Haplotypes of the COMT Gene; (3) Identification of Covariates; (4) Results of Specific Aims; and (5) Discussion.

4.2 SAMPLE

A total of 231 subjects were recruited for this study. Of these, 169 (73%) subjects met inclusion criteria for this research. Sixty-two (27%) subjects in this study were excluded due to an inability to attain physiological criteria identified for achieving maximal oxygen uptake (VO_2max). The criteria for attainment of VO_2max utilized for this study was having attained a: (1) heart-rate (HR) $\pm$ 10 b $\cdot$ min^{-1} of age adjusted maximal heart-rate (AAMHR); or (2)

Respiratory Exchange Ratio (RER) $\geq$ 1.10; or (3) plateau in oxygen uptake (VO_2) despite increasing workload. Descriptive characteristics of the 169 included subjects and 62 excluded subjects are provided in Table 5. Results indicated that a significantly higher ($p<.001$) proportion of females were ineligible than males (76.5% vs. 23.5%). Additionally, ineligible subjects demonstrated a significantly ($p<.001$) higher body mass index (BMI) than eligible subjects (28.40 $kg \cdot m^2$ vs. 25.21 $kg \cdot m^2$). Finally, the 62 ineligible subjects reported significantly lower ($p=.013$) median leisure-time physical activity (PA) than the 169 eligible subjects (299.78 $min \cdot wk^{-1}$ vs. 474.41 $min \cdot wk^{-1}$). Due to an inability to accurately assess VO_2max among ineligible subjects, a comparison of aerobic fitness between groups was not possible. In addition, due to racial differences in haplotype frequency of the COMT gene, and the extremely low (n = 7) proportion of non-white, eligible subjects, only white subjects were included in the present analyses. No significant differences were found between eligibility groups with respect to age and genotype.

Table 5: Demographic and Anthropometric Characteristics of Subjects by Eligibility Group

Covariate	Eligible (n=169)	Ineligible (n=62)	p-value
Gender (%)			
Male	45.0	23.5	**<.001***
Female	55.0	76.5	
Age	29.16 ± 4.10	29.24 ± 4.60	.832
Body Mass Index (BMI) ($kg \cdot m^2$)	25.21 ± 2.14	28.40 ± 1.17	**<.001***
Physical Activity (PA) ($min \cdot wk^{-1}$)	474.41 ± 1154.98	299.77 ± 1064.32	**.013***

Values are mean ± standard deviation
* $p < .05$

Descriptive statistics by gender for the eligible subjects are provided in Table 6. A significant (p=.023) difference in mean age was found between males and females, with males slightly older than females (29.30 ± 1.14 vs. 29.08 ± 1.01). Males in this study also reported significantly higher (p=.013) PA than females (1208.01 minutes ± 1224.00 vs. 314.00 minutes ± 945.01), and also exhibited significantly (p=.043) higher mean VO_2max levels than females (42.30 $ml \cdot kg \cdot min^{-1}$ ± 7.78 vs. 33.29 $ml \cdot kg \cdot min^{-1}$ ± 6.63). No significant differences in BMI categories were found between males and females.

Table 6: Demographic and Anthropometric Characteristics by Gender

	Males	Females	p-value
Age (years)	29.30 ± 1.14	29.08 ± 1.01	**.023***
BMI ($kg \cdot m^2$)	26.69 ± 5.10	26.47 ± 4.82	.748
PA ($min \cdot wk^{-1}$)	1208.01 ± 1224.00	314.00 ± 945.01	**.013***
VO_2max ($ml \cdot kg \cdot min^{-1}$)	42.30 ± 7.78	33.29 ± 6.63	**.043***

Values are mean ± standard deviation
*$p < .05$

4.3 HAPLOTYPES OF THE COMT GENE

Fit to the expectation of Hardy-Weinberg Equilibrium was tested using Chi Square Analysis. The resulting p-values were not significant, suggesting that these loci were in equilibrium in the population. Table 7 provides the frequency and Chi Square statistic for each genotype.

Table 7: Genotype Frequencies for COMT Single Nucleotide Polymorphisms

SNP	Genotype	Observed	Expected	x^2	p
4633	CC	53	49.6	1.344	.281
	CT	76	82.6		
	TT	38	34.6		
4680	AA	39	47.9	.410	.523
	AG	80	84.1		
	GG	50	36.9		
4818	CC	69	67.7	.173	.681
	CG	76	78.5		
	GG	24	22.7		
6269	AA	59	61.3	.602	.442
	AG	82	77.3		
	GG	22	24.3		

Haplotype estimation was performed using the program PHASE on a Macintosh platform. The three distinct haplotypes being investigated composed 92.2% of all haplotypes observed for the COMT gene in this sample. Subjects with haplotypes other than those of interest in addition to subjects for whom haplotype estimation yielded uninterpretable results, were excluded from the current study. Table 7 provides the proportion of the three haplotypes estimated for this sample. As shown, the LPS haplotype was observed 58 times. Similarly, the APS haplotype was observed 77 times. Finally, the HPS haplotype was observed 21 times.

Table 8: LPS, APS, and HPS COMT gene haplotypes

Haplotype	Number
LPS	58
APS	77
HPS	21

The distribution of genotypes and corresponding perceptual response categories was then determined. Of the 169 subjects, 51 (28 males, 23 females) subjects were categorized as Low Responders (LR) based upon genotype. There were 64 (28 males, 36 females) Average Responders (AR), and 54 (26 males, 28 females) High Responders based upon genotype. A statistically significant difference ($p = .032$) in gender composition was found within only the AR genotype sub-group.

Table 9: COMT Genotype Distributions for Combined Sample, Males and Females

Genotype	Perceptual Response Category	Total	Males	Females
LPS / LPS	LR	51	28 (55%)	23 (45%)
APS / APS APS / LPS	AR	64	28 (44%)	36 (56%)*
HPS / LPS HPS / APS	HR	54	26 (48%)	28 (52%)

LR (Low Responder); AR (Average Responder); HR (High Responder)
*$p < .05$

4.4 IDENTIFICATION OF COVARIATES

Covariates examined were: (1) gender; (2) age; (3) BMI; (4) Total leisure-time physical activity (PA); and (5) maximal oxygen uptake (VO_2max). Descriptive data by genotype subgroup are provided in Table 9. Significant differences in age were found among Low Responders (LR), Average Responders (AR), and High Responders (HR) genotype subgroups. Results of the analysis of variance (ANOVA) with a Bonferroni post hoc adjustment indicate that the LR subgroup was significantly ($p=.012$) younger than the HR subgroup (28.38 years vs. 29.36 years). The LR subgroup also reported significantly ($p=.046$) higher median minutes of leisure-time physical activity per week, than the HR sub-group (315.67 min$\cdot$ wk^{-1} vs. 216.90 min $\cdot$ wk^{-1}). No significant differences in BMI or VO_2max were found among the three genotype subgroups. Therefore, for the following analyses, only age and PA were entered into the Analysis of

Covariance (ANCOVA) model, in addition to the slope of the regression line for each association of interest. The following section provides a thorough examination of the primary and secondary specific aims of this research.

Table 10: Demographic and Anthropometric Characteristics by Genotype Sub-group

		LR (n=51)	AR (n=64)	HR (n=54)	p-value
Gender	**Male (n=82)**	28	28	26	
	Female (n=97)	23	36	28	
Age (Yrs)		28.38 ± .991	28.78 ± .924	29.36 ± 1.399	**.012***
BMI ($kg \cdot m^2$)		27.05 ± 4.64	26.29 ± 5.51	26.10 ± 5.00	.640*
PA ($min \cdot wk^{-1}$)		315.67 ± 124.25	237.15 ± 138.79	216.90 ± 167.54	**.046***
VO_2max ($ml \cdot kg \cdot min^{-1}$)		36.32 ± 7.03	37.83 ± 9.30	37.75 ± 8.90	.577

Values are Mean ± standard deviation LR (Low Responder); AR (Average Responder); HR (High Responder)
*$p < .05$

4.5 RESULTS OF SPECIFIC AIMS

The two specific aims of this research intended to explore the influence that variation of the Catechol-O-Methyltransferase (COMT) Gene has on perceptual response to sub-maximal treadmill graded exercise. The primary aim was to examine differences in exertional perception during a sub-maximal treadmill graded exercise test among LR, AR, and HR subgroups. The secondary specific aim was to examine differences in exercise-induced lower-body pain during a sub-maximal treadmill graded exercise test among LR, AR, and HR subgroups.

4.5.1 Primary Aim

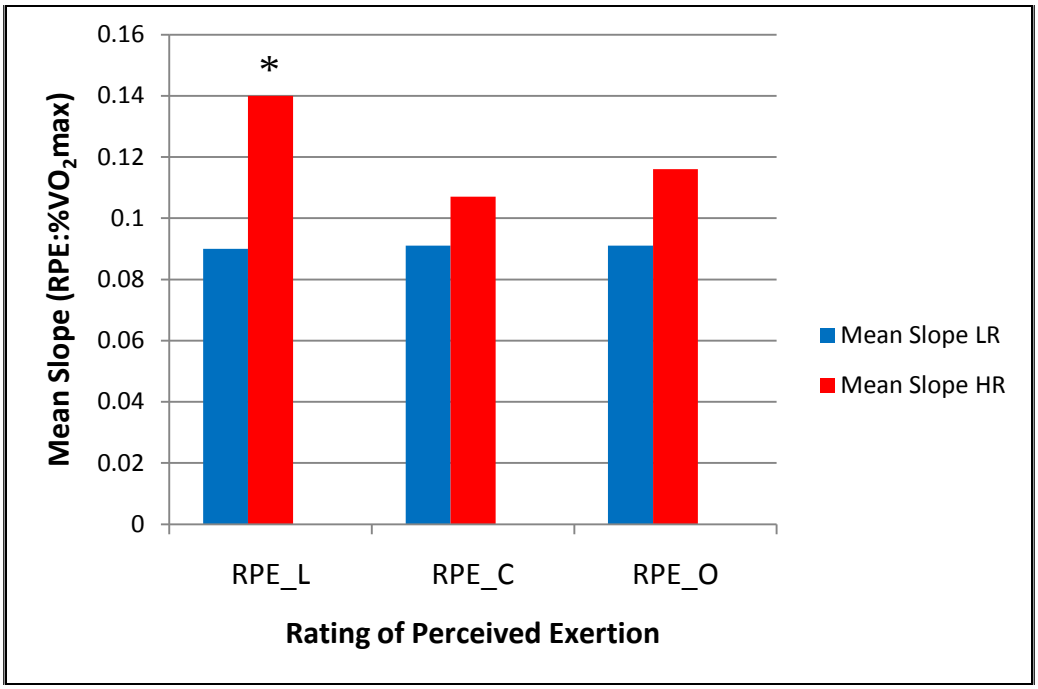
This analysis examined the influence that genotypic variation in the COMT gene had on differentiated and undifferentiated ratings of perceived exertion (RPE) during a sub-maximal treadmill graded exercise test. For each genotype subgroup, a regression equation was calculated with RPE expressed as a function of a physiological criterion variable (%VO_2max, VE, or heart-rate). Differences in the slope of each line among genotype subgroups were examined using Analysis of Covariance (ANCOVA) with age and PA entering the ANCOVA model as covariates.

Results indicated that no significant differences in slope were found between the AR subgroup and either of the remaining subgroups (LR, HR) with respect to any of the associations being examined. However, significant differences in slope did occur between the LR and HR subgroups with respect to select associations between RPE and a given physiological criterion variable. Therefore, all proceeding results will compare the LR and HR sub-groups. In addition,

due to gender differences in age and PA, all proceeding results are presented as both gender-specific and for the combined sample.

4.5.1.1 Rating of Perceived Exertion as a Function of % VO_2max

Figure 4 presents the results of the ANCOVA for RPE expressed as a function of %VO_2max. When RPE_Legs (RPE_L) was expressed as a function of %VO_2max, the ANCOVA revealed that genotype was a significant predictor of the slope of the regression lines ($F_{(1, 103)} = 12.183$, $p = .001$), when controlling for both PA and age. The mean slope of RPE_L as a function of %VO_2max (RPE_L:%VO_2max) for the LR sub-group was .090, while the mean slope for the HR sub-group was .140. However, when RPE_Chest (RPE_C) was expressed as a function of %VO_2max (RPE_C:%VO_2max), genotype was not found to be a significant predictor of the slope of the regression lines ($p = .240$). Similarly, for the slope RPE_Overall: %VO_2max, genotype was not a significant predictor ($p = .055$).



* p < .001

Figure 4: Analysis of Covariance for Rating of Perceived Exertion Expressed as a Function of %VO_2max for Combined Sample

4.5.1.2 Rating of Perceived Exertion as a Function of Minute Ventilation

Figure 5 presents the results of the ANCOVA for RPE expressed as a function of minute ventilation (VE) with PA and age as covariates. Results indicate that for the slope of RPE_L: VE, genotype was not found to be a significant predictor of the slope of the regression lines ($F_{(1, 102)} = 3.358$, $p = .069$). Very similar results were discovered for both RPE_C and RPE_O ($p = .989$ and $p = .598$, respectively). In all, RPE as a function of VE was not found to be significantly different between the two genotype sub-groups (LR, HR).

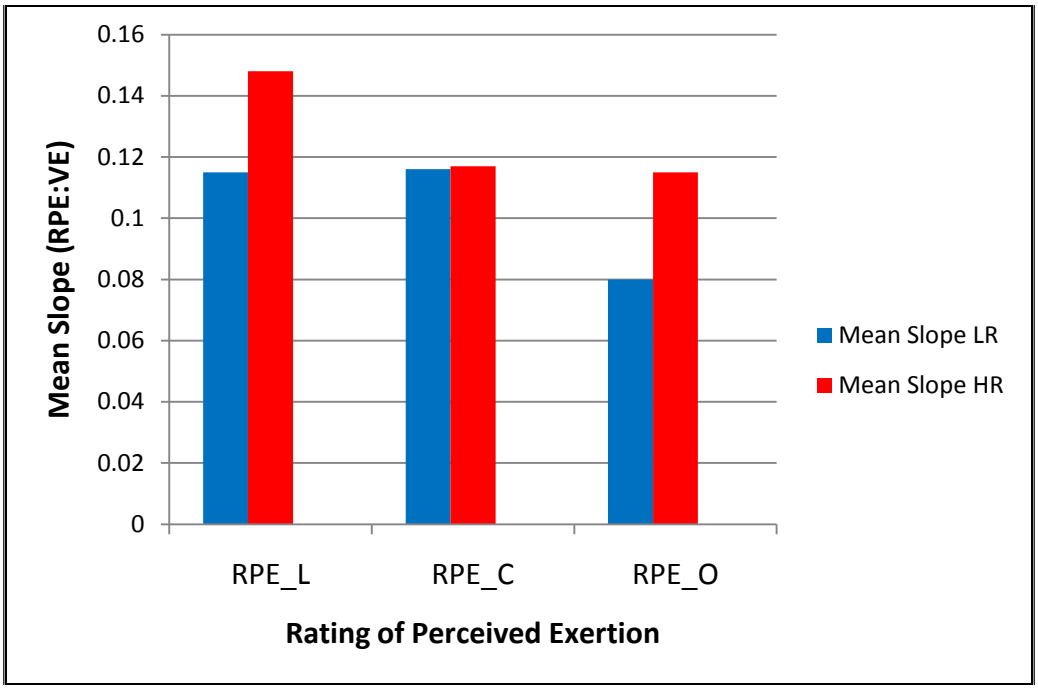
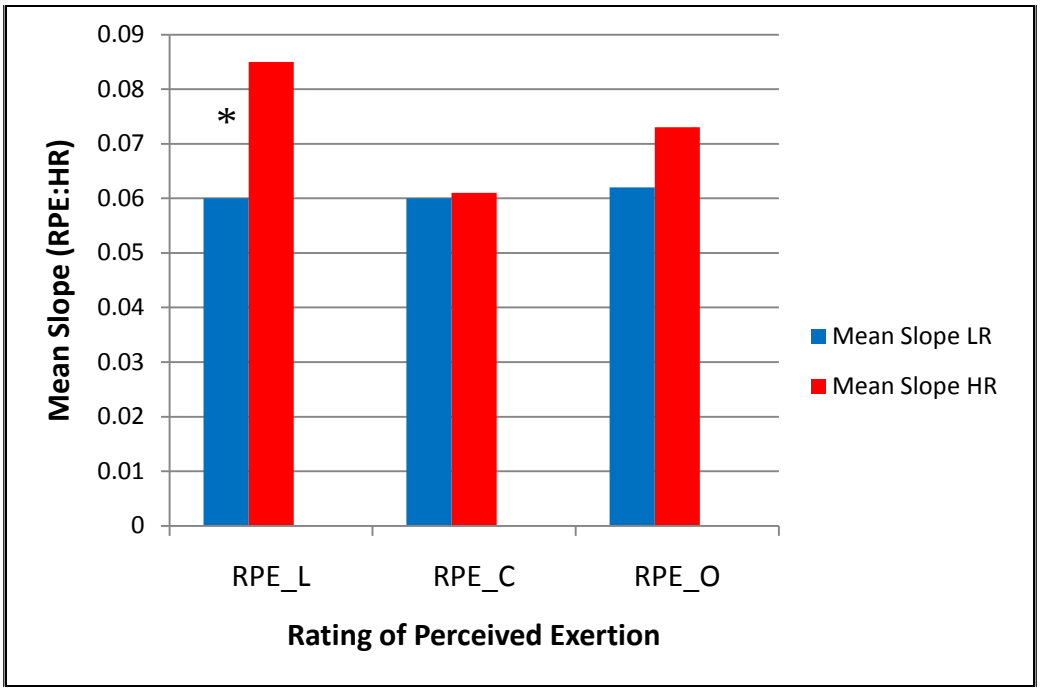


Figure 5: Analysis of Covariance for Rating of Perceived Exertion Expressed as a Function of Minute Ventilation for the Combined Sample

4.5.1.3 Rating of Perceived Exertion as a Function of Heart-rate Figure 6 presents the results of the ANCOVA for RPE expressed as a function of heart-rate. Interestingly, similar to the results of RPE expressed as a function of %VO_2max, genotype was found to be a significant predictor of slope, when controlling for total PA and age. It was found that the slope of RPE_L: HR, was significantly different between the two genotype sub-groups ($F_{(1, 102)} = 9.223$, $p = .003$). The mean slope for the LR sub-group equaled .060, while the mean slope for the HR sub-group equaled .085.



* p < .001

Figure 6: Analysis of Covariance for Rating of Perceived Exertion Expressed as a Function of Heart-rate for the Combined Sample

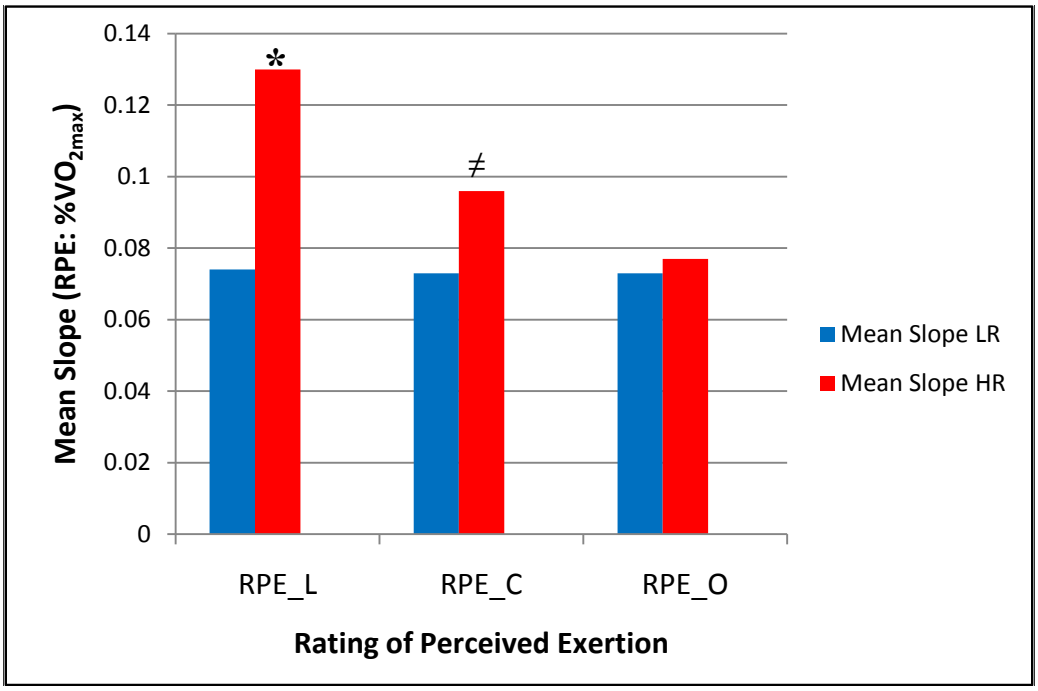
4.5.2 Results of Gender-specific Analyses for Rating of Perceived Exertion

Due to significant differences in PA and age between males and females, gender specific analysis was performed in order to fully examine the psycho-physiological relations of interest as well as any differences that might exist between genders.

4.5.2.1 Rating of Perceived Exertion expressed as a Function of %VO_2max by Gender

Figures 7 and 8 provide the gender-specific results of the ANCOVA for RPE expressed as a function of %VO_2max with total PA and age entered as covariates. Significant differences in slope were discovered between the genotype sub-groups for males and females separately, controlling for total PA and age. Among males, genotype was a significant predictor of the slope of RPE_L: %VO_2max ($F_{(1, 52)} = 21.369$, $p = .000$). The mean slope for the LR sub-group

equaled .074, while the mean slope for the HR sub-group equaled .130. Similarly, it was discovered that for the slope of RPE_C: $\%VO_2max$, genotype was a significant predictor, ($F_{(1, 52)} = 4.389$, $p = .039$). However, there were no significant differences in RPE_O: $\%VO_2max$ between genotype sub-groups, controlling for total PA and age, among males. Among females, associations of RPE (Legs, Chest, Overall) expressed as a function of $\%VO_2max$ were not different between genotype sub-groups ($p = .083, .634, .388$, respectively).



* $p < .001$;
≠ $p < .05$

Figure 7: Analysis of Covariance for Rating of Perceived Exertion Expressed as a Function of $\%VO_2max$ for Males

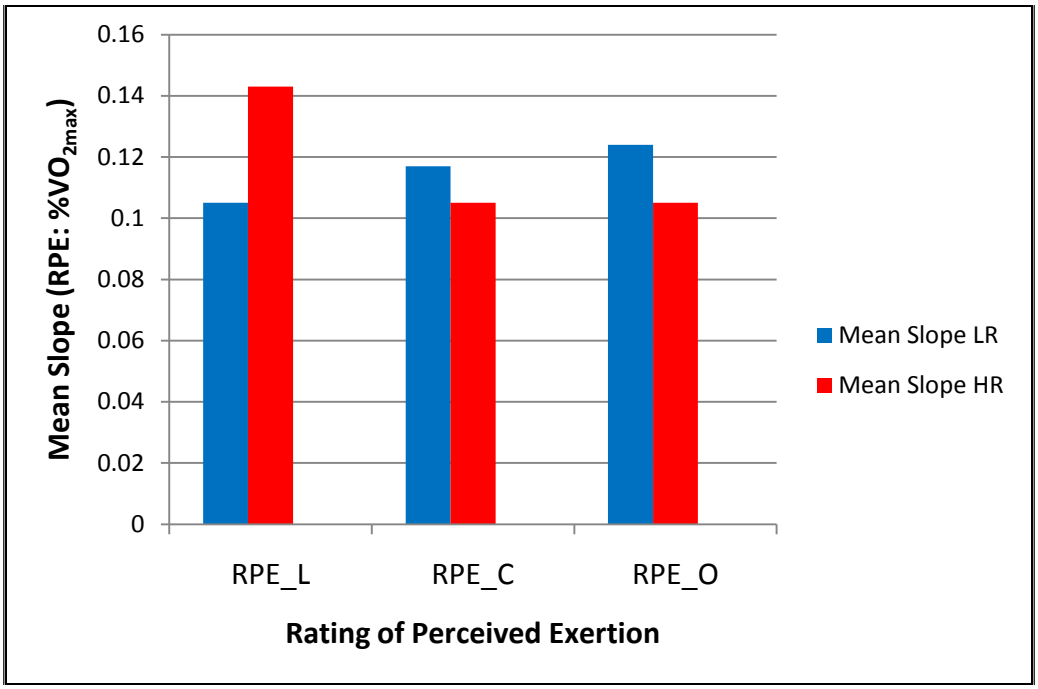
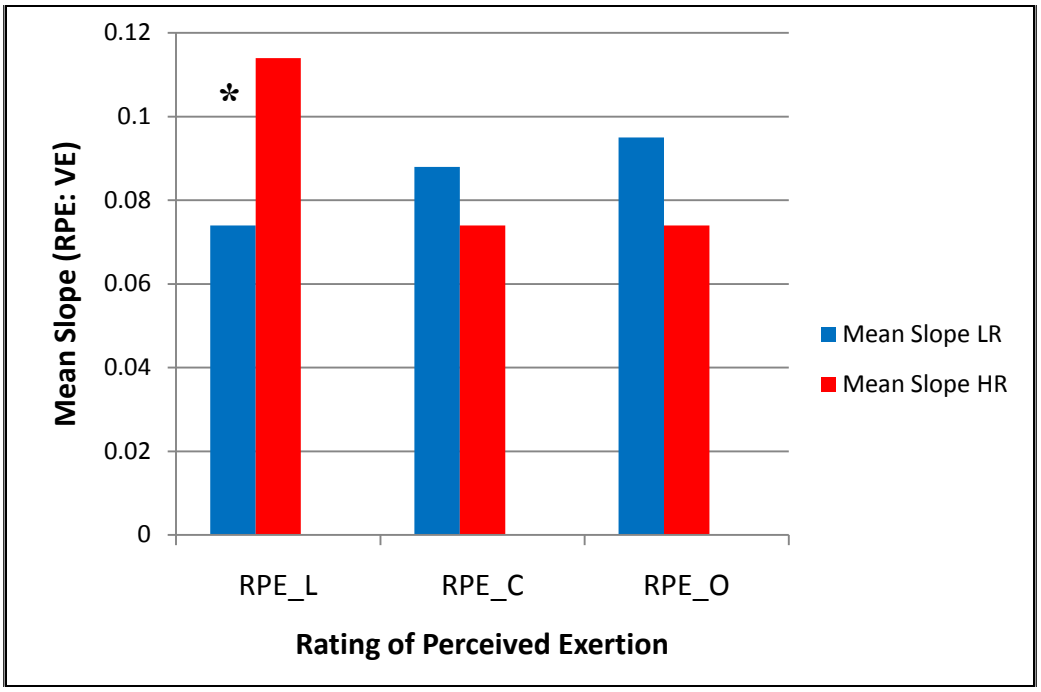


Figure 8: Analysis of Covariance for Rating of Perceived Exertion Expressed as a Function of %VO_2max for Females

4.5.2.2 Rating of Perceived Exertion as a Function of Minute Ventilation by Gender

Figures 9 and 10 present the results of the ANCOVA for RPE expressed as a function of VE for males and females separately, controlling for total PA and age. Among males, it was found that for the slope of RPE_L: VE, genotype remained a significant predictor ($F_{(1, 51)} = 11.690$, $p = .001$). The mean slope among the LR subjects equaled .074, while the HR subjects reported a mean slope of .114. However, significant differences in slope were not discovered for RPE_C and RPE_O ($p = .167$ and $p = .058$, respectively), when expressed as a function of VE. Among females none of the associations of RPE (Legs, Chest, Overall) with VE were found to be statistically significantly between genotypes.



* p < .001

Figure 9: Analysis of Covariance for Rating of Perceived Exertion as a Function of VE for Males

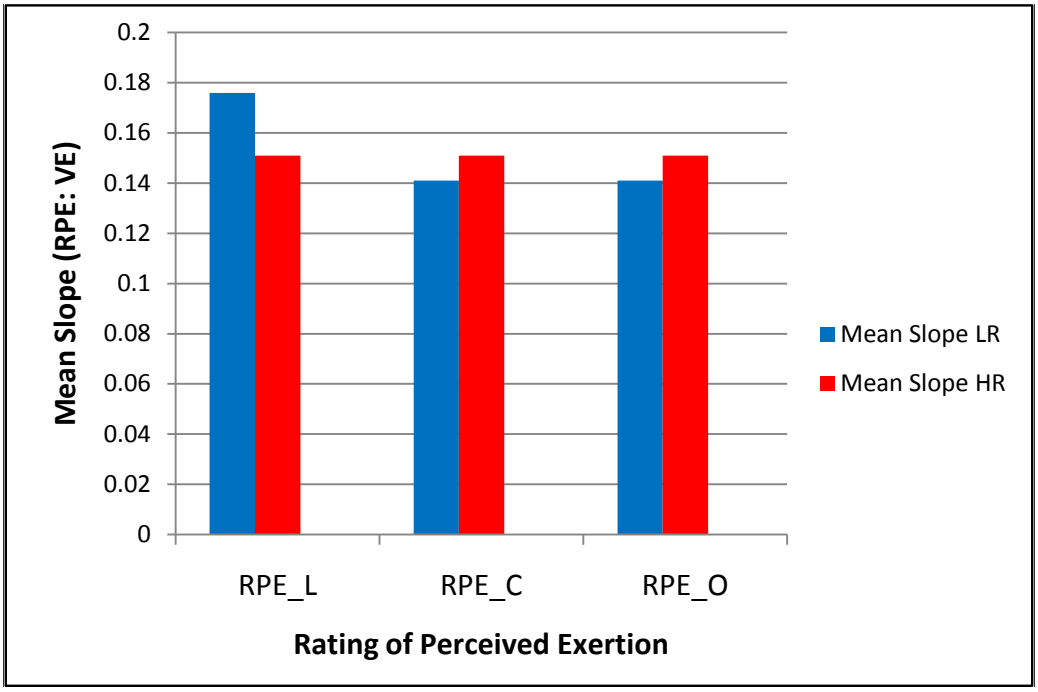
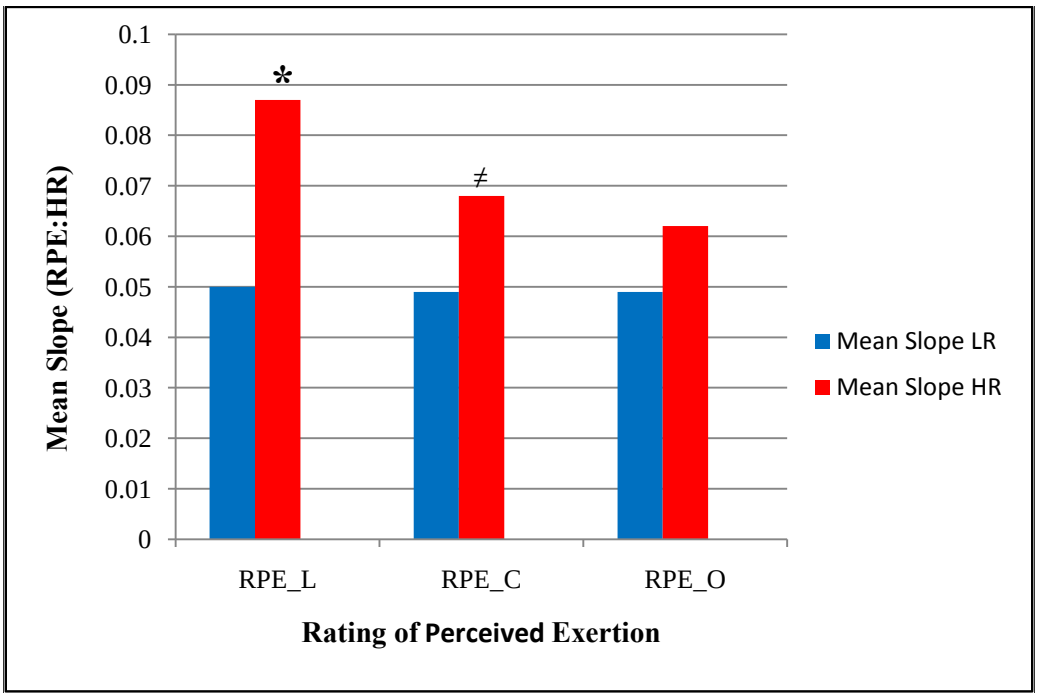


Figure 10: Analysis of Covariance for Rating of Perceived Exertion as a Function of VE for Females

4.5.2.3 Rating of Perceived Exertion as a Function of Heart-rate by Gender Figures 11 and 12 present the results of the ANCOVA for RPE expressed as a function of heart-rate, for males and females, with total PA and age being covariates. Results indicate that among males only, a significant difference in the slope of RPE_L: HR exists by genotype ($F_{(1, 52)} = 22.178$, $p = .000$). The mean slope for LR subjects is .050, while the HR subjects reported a mean slope equal to .087. In addition among males, for RPE_C: HR, genotype was a significant predictor of the slope of the regression lines ($F_{(1, 52)} = 5.833$, $p = .018$). The mean slope among LR subjects equaled .049, while the mean slope for heart-rate subjects equaled .068. Finally, genotype was not a significant predictor of slope for RPE_O: HR ($p = .074$). Among females, no statistically

significant differences in the slope of the regression lines for RPE as a function of heart-rate existed between genotypes (p = .295, p = .665, .p = 797, respectively).



* p < .001
≠ p < .05

Figure 11: Analysis of Covariance for Rating of Perceived Exertion as a Function of HR for Males

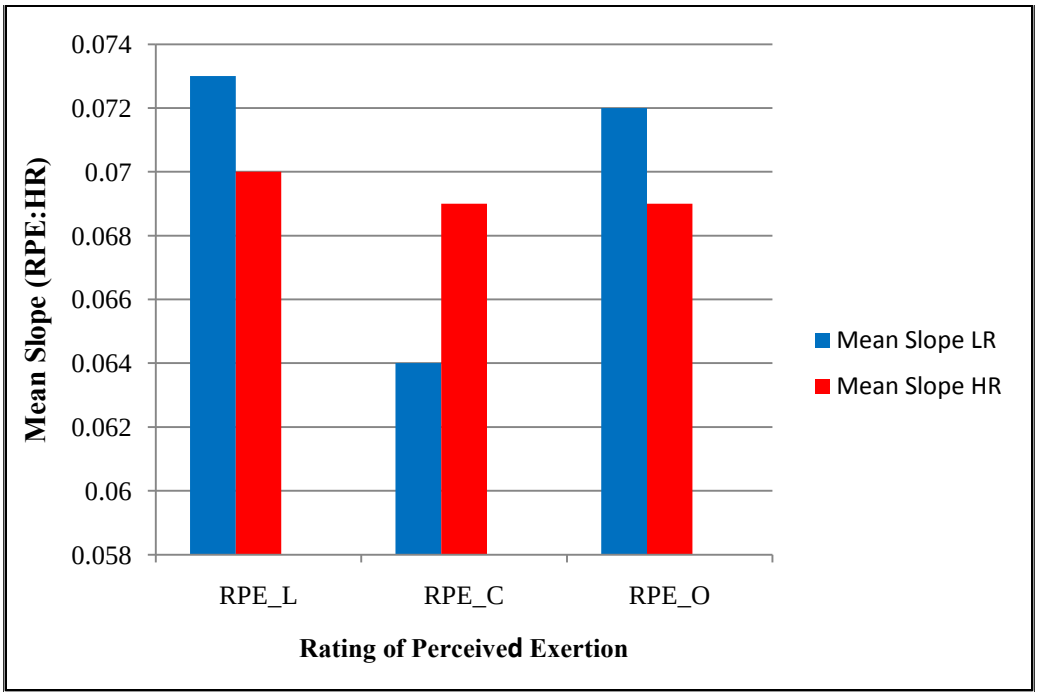


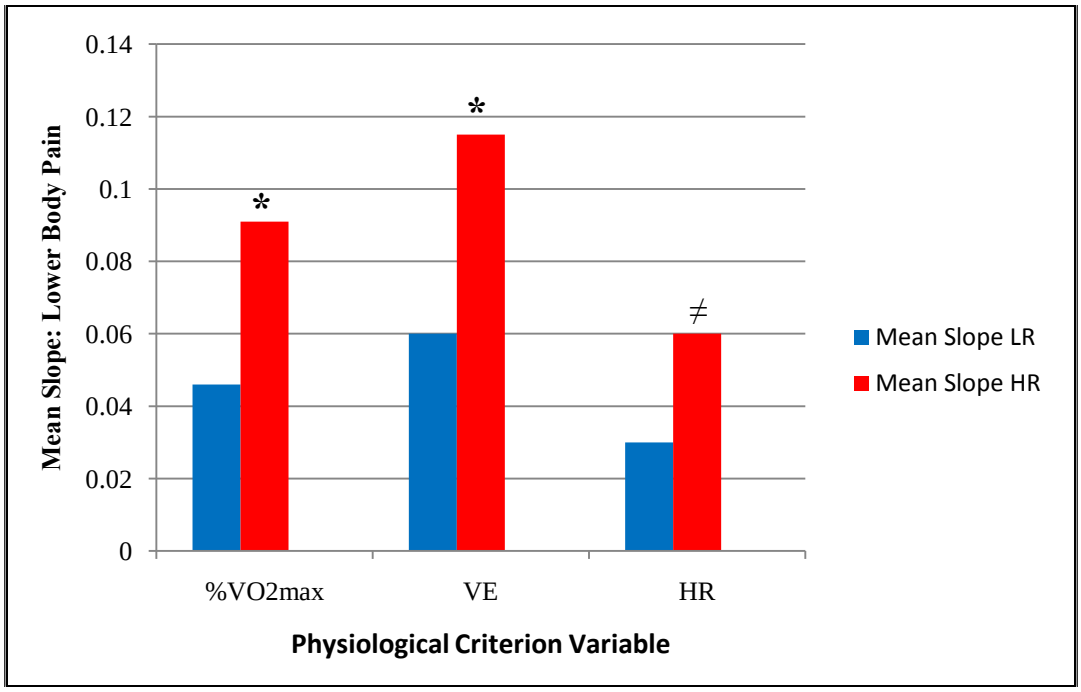
Figure 12: Analysis of Covariance for Rating of Perceived Exertion as a Function of HR for Females

4.5.3 Secondary Aim

This analysis examined the influence that variation in the COMT gene has on rating of exercise-induced lower-body pain during a sub-maximal treadmill graded exercise test. For each genotype subgroup, a regression equation was calculated with Pain expressed as a function of a physiological criterion variable (percent of maximal oxygen uptake (%VO_2max), minute ventilation (VE) or heart-rate (HR)). Differences in the slope of each line among genotype subgroups were examined using Analysis of Covariance (ANCOVA) with age and total PA entering the ANCOVA model as covariates. Figures 10 and 11 provide the results of the ANCOVA for rating of exercise-induced pain and each physiological criterion variable (%VO_{2max}, VE, HR) for combined and gender-specific samples.

Results indicated that no significant differences in slope were found between the Average Responder (AR) subgroup and either of the remaining subgroups (LR, HR) with respect to any of the associations being examined. However, significant differences in slope were discovered between the LR and HR subgroups with respect to select associations of interest. Therefore, all proceeding results will only compare the LR and HR sub-groups. In addition, due to gender differences in age and PA, all proceeding results are presented for the combined and gender-specific samples.

4.5.3.1 Exercise-induced Pain Expressed as a Function of %VO_2max Results of the ANCOVA indicate that when exercise-induced LBP was expressed as a function of %VO_2max, significant differences exist in the slope of the regression lines, between genotype sub-groups ($F_{(1, 102)} = 15.307$, $p = .000$), for the combined sample. Figure 13 illustrates LBP expressed as a function of %VO_2max, VE, and heart-rate. It was found that among LR subjects the mean slope equaled .046, while HR subjects exhibited a mean slope equal to .091. Similar results were found when LBP was expressed as a function of VE. For the combined sample, the mean slope among LR subject equaled .060, while among HR subjects the mean slope equaled .115. In addition, when LBP was expressed as a function of heart-rate, significant differences in the slope of the regression lines were discovered between genotype sub-groups (Figure 10).



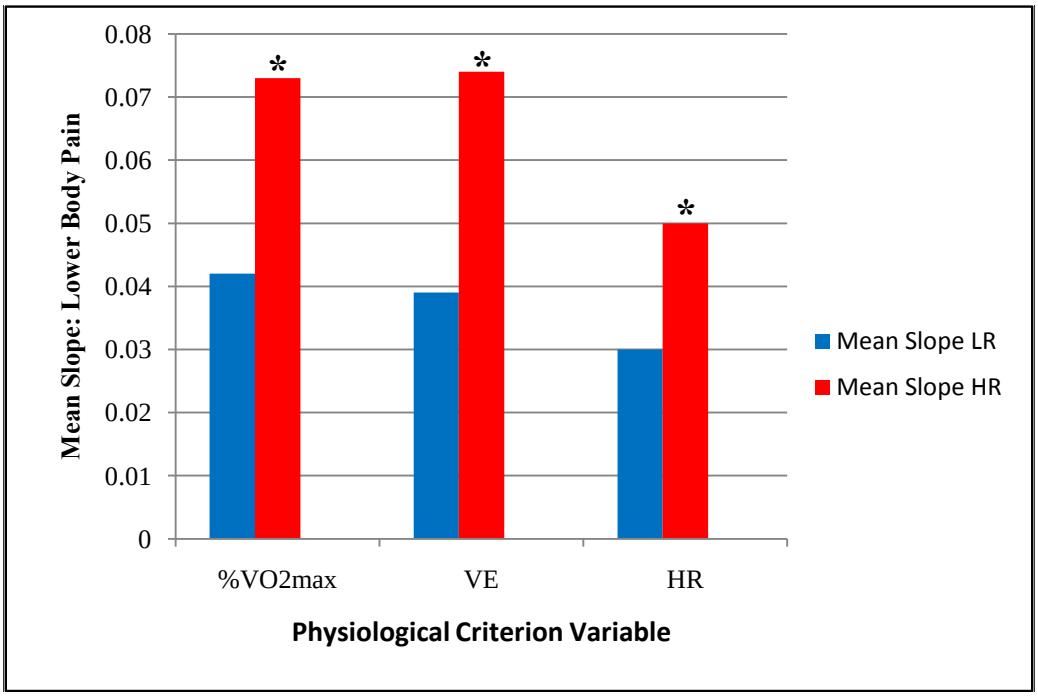
* $p < .001$;
≠ $p < .05$

Figure 13: Analysis of Covariance for Lower-body Pain as a Function of $\%VO_{2max}$, VE, and HR for the Combined Sample

4.5.4 Results of Gender Specific Analyses for Lower Body Pain

Among males and females, it was found that when LBP was expressed as a function of $\%VO_{2max}$, VE and HR significant differences in slope between the genotypes were discovered for each criterion variable. Among males, when LBP was expressed as a function $\%VO_2max$, genotype was found to be a significant predictor of the slope of the regression lines ($F_{(1, 52)} = 6.775$, $p = .011$), when controlling for age and PA. Similar results were discovered for LBP expressed as a function of VE and HR ($p = .006$, $p = .008$, respectively). In all, genotype was found to be a significant predictor of the slope of the regression lines for each association of

interest, with the HR sub-group reporting significantly higher mean slopes than the LR sub-group (Figure 14).

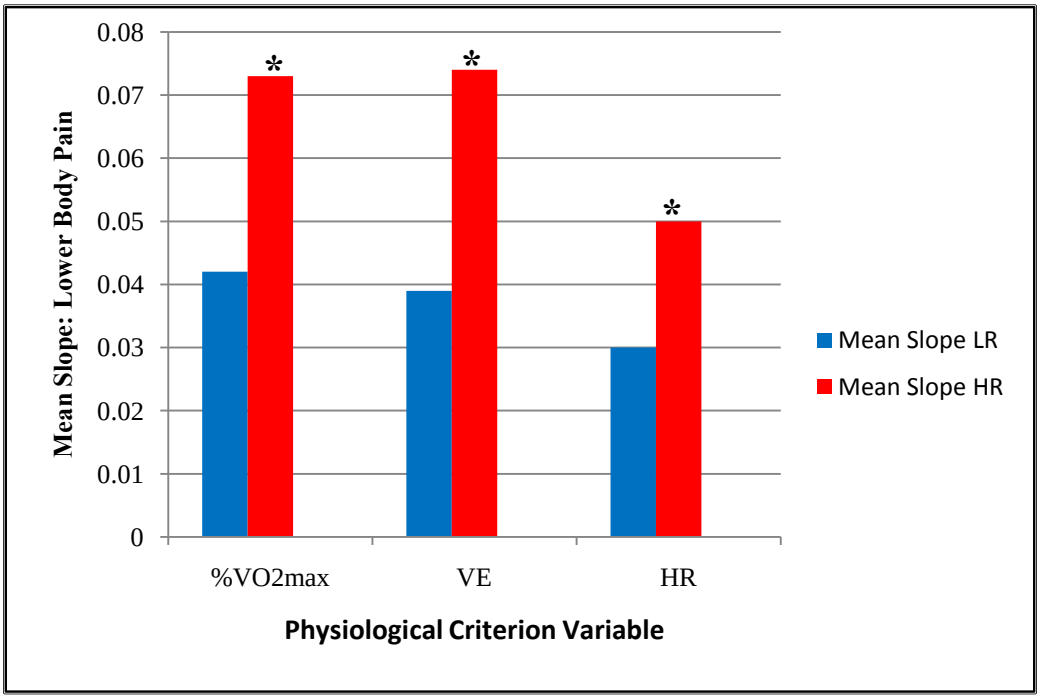


* p < .05

Figure 14: Analysis of Covariance for Lower-body pain as a Function of %VO_2max, VE and HR for Males

It was also found that among females, genotype was a significant predictor of each association of interest (%VO_2max, VE, and HR). When LBP was expressed as a function of %VO_2max, genotype was a significant predictor of the slope of the regression lines ($F_{(1, 51)}$ = 9.269, p = .003). It was also found that when LBP was expressed as a function of VE and HR, genotype was a significant predictor of the slope of the regression lines (p = .002, .012, respectively), when controlling for age and total PA. In all, genotype in females was found to be

a significant predictor of each of the associations of interest, with HR subjects reporting significantly higher mean slopes than LR subjects (Figure 12).



* p < .05

Figure 15: Analysis of Covariance for Lower-body pain as a Function of %VO_2max, VE and HR for Females

4.6 SUMMARY OF RESULTS

The specific aims of this study attempted to elucidate the influence that variation in the COMT gene may have on perceptual response to exercise. The results of these specific aims are summarized in the following section and presented in Tables 10 and 11.

4.6.1 Summary of Primary Aim

For the combined sample, genotype was a significant predictor of the slope of the regression lines for Rating of Perceived Exertion in the legs (RPE_L) expressed as a function of percent of maximal oxygen uptake (%VO_2max), controlling for both age and total leisure-time physical activity (PA). In addition, for the combined sample, genotype was a significant predictor of the slope of the regression lines for RPE_L expressed as a function of minute ventilation (VE), controlling for both age and PA. In both of these associations the High Responder (HR) subjects reported significantly higher mean slopes than the Low Responder (LR) subjects.

When examined separately, for males, genotype was a significant predictor of the slope of the regression lines when RPE_L and RPE_C were expressed as functions of %VO_2max. Furthermore, when RPE_L was expressed as a function of both VE and HR, significant differences by genotype were identified between the HR and LR subgroups. Finally, for males it was found that when RPE_C was expressed as a function of heart-rate, significant differences in the slopes between LR and HR genotypes were discovered. In each significant association, the HR sub-group experienced higher mean slopes than the LR sub-group.

Analysis of female subjects indicated that no significant differences in the slopes of the regression lines existed between LR and HR genotypes, when RPE was expressed as a function of a given physiological criterion variable.

Table 11: Summary of Results of Primary Specific Aim

Association	Combined	Male	Female	Result
RPE_L / %VO_2max	♦	♦		HR slope > LR slope
RPE_C / %VO_2max		♦		HR slope > LR slope
RPE_O / %VO_2max				
RPE_L / VE		♦		HR slope > LR slope
RPE_C / VE				
RPE_O / VE				
RPE_L / HR	♦	♦		HR slope > LR slope
RPE_C / HR		♦		HR slope > LR slope
RPE_O / HR				

♦ Significant association (ANCOVA with age and PA as covariates)

4.6.2 Summary of Secondary Aim

For the combined sample, genotype was a significant predictor of the slope of the regression lines for LBP expressed as a function of %VO_2max, VE, and heart-rate. Overall, each LBP association when expressed as one of the three physiological criterion variables was significantly different between LR and HR groups. In each instance, the HR subjects reported significantly higher mean slopes than the LR subjects, when controlling for age and PA. Interestingly, the same results were discovered when the males and females were analyzed separately using ANCOVA with age and PA as covariates. Each association of interest was significantly

different between HR and LR subjects, with HR subjects having higher mean slopes than LR subjects (Table 12).

Table 12: Summary of Results of Secondary Specific Aim

Association	Combined	Male	Female	Result
Pain / $\%VO_{2max}$	♦	♦	♦	HR slope > LR slope
Pain / VE	♦	♦	♦	HR slope > LR slope
Pain / HR	♦	♦	♦	HR slope > LR slope

♦ Significant association (ANCOVA with age and PA as covariates)

5.0 DISCUSSION, CONCLUSIONS, AND FUTURE DIRECTIONS

5.1 INTRODUCTION

This genetic association study was performed to develop an understanding of possible biological influences on perceptual response to exercise stimuli, and builds upon an underlying rationale that exercise represents a psycho-physiological stressor, from which some individuals may disengage [1, 3, 29, 36, 42, 43, 60, 81, 118, 144]. Complex factors may influence how individuals react to an exercise stimulus, thus potentially impacting willingness to adopt and maintain a physical activity and exercise program. In the present study, overall findings suggest that interindividual differences in psycho-physiological reactivity to an exercise stimulus may be influenced by genetic composition. To our knowledge, this research is among the first to identify genetic influences associated with interindividual differences in perception of exertion and exercise-induced lower-body pain (LBP) during an exercise challenge. This chapter is divided into the following sections: (1) Discussion; (2) Strengths; (3) Limitations; and (4) Future Directions.

5.2 DISCUSSION

5.2.1 Subjects

Included subjects were 169 males and females between 27 and 32 years of age. It was found that a greater proportion of females were ineligible than males. A total of 47 females and 15 males

were ineligible due to their inability to attain physiological criteria for maximal oxygen uptake (VO_{2max}). While 100% eligibility cannot be expected, 27% ineligible is enough of a proportion that requires discussion. It is reasonable to believe that if subject experience with VO_{2max} testing were greater, the comfort associated with one's ability to attain a level of intensity would be appropriate for a maximal treadmill graded exercise test. If provided with sufficient opportunity for orientation to the treadmill test, a higher proportion of subjects may have been capable of attaining the appropriate level of exercise intensity.

Subjects' relative lack of experience with high intensity cardiovascular exercise may have hindered their ability to reach a maximal exercise intensity necessary for an accurate assessment of maximal aerobic power. For the parent study, a supervised treadmill test orientation was administered to each subject. Subjects walked on the treadmill for 2 – 3 minutes at increasing grades in order to become more comfortable with the testing procedures. Feedback provided by testing staff noted that subject experience with treadmill exercise varied considerably. Subjects who demonstrated discomfort with the process were provided with ample time to increase their proficiency with treadmill walking. This standardized orientation process was adequate to maximize subject proficiency with treadmill walking, given the constraints of time. However, the orientation process did not include running on the treadmill which was required of subjects during the maximal treadmill graded exercise test. Therefore, subjects for whom treadmill running was a new experience or who elicited discomfort may not have received sufficient practice with higher intensity treadmill exercise. Perhaps additional time to practice on the treadmill would have enhanced subject comfort with the treadmill exercise tests.

It is possible that when challenged with maximal physical effort, some individuals were psychologically intimidated and experienced high levels of state anxiety in response. Therefore

their performance was negatively impacted, and did not satisfy criteria for having attained true physiological VO_{2max}. Furthermore, the protocol for the parent study required that subjects perform the maximal treadmill graded exercise test approximately 10 – 12 minutes after completion of the sub-maximal walking treadmill graded exercise test. Physical and psychological fatigue potentially influenced willingness to perform a maximal test having just completed a relatively long duration, sub-maximal graded exercise test (approximately 12 – 18 minutes). Therefore subjects may have felt overwhelmed by the idea of maximal performance, particularly when faced with both lack of experience and fatigue.

Interestingly, this subset is worthy of more in depth study, given that they responded negatively when presented with maximal intensity exercise. It may be that genotype in combination with environmental characteristics drove a disengagement from the exercise stimulus. Additional examination should explore psychosocial factors such as self-efficacy for physical activity (PA), social support, enjoyment, and barriers to PA in these subjects. Additionally, past experience with PA or exercise (i.e. during childhood and adolescence), may have significantly influenced one's willingness to engage in high intensity exercise.

Preliminary evidence suggests that significant differences in selected psycho-social variables occurred between subjects who attained physiological criteria for VO_{2max} and those who do not. Within the present cohort, analyses showed that perceived benefits of PA and enjoyment of PA were significantly lower in subjects unable to attain physiological criteria for VO_{2max}. Perceived stress and depression were found to be significantly higher in this subset [113]. This suggests that psycho-social factors may impact the ability of some healthy young adults to reach a physical effort appropriate for measurement of VO_{2max}.

It also appears that a gender differential exists, with a higher proportion of females who did not attain criteria for VO_{2max}. It may be that the influence of specific environmental factors on ability to attain VO_{2max} differs between males and females. Factors responsible may include past experience with sport and exercise, parental influence, self-efficacy for PA as well as a gender difference in how directions were interpreted. The exact nature of the influence of gender on ability to attain physiological criteria for VO_{2max} should be further explored.

Social norms and perceptions of gender roles also may significantly influence choice of physical activities as well as the intensity at which they are practiced. This influence may be in the form of differences in availability of specific sports or activities in the school setting, how parents encourage their children to adopt specific activities based on gender, or in how peers influence one another to adopt specific activities [7, 24, 65, 121]. Some might argue that males are more likely to be encouraged to engage in high-intensity exercise or PA than females [7, 24, 65, 121]. Each of the aforementioned factors may have contributed to the disproportionate number of subjects who did not meet criteria for VO_{2max}.

For this study, it is likely that complex differences exist between eligible and ineligible subjects. These differences are not exclusive to performance on a treadmill test rather they may encompass many dimensions of the PA or exercise experience. It would therefore prove highly beneficial to apply the same paradigm to excluded subjects, in order to better understand how genetic composition might contribute to sedentary tendencies. The excluded subjects consisted of a higher proportion of females than males, were less physically active, and had a higher mean BMI. Factors associated with the overall PA experience that warrant a more detailed exploration include gender-specific psycho-social factors such as self-efficacy, social support, and socio-cultural differences. It is expected that subjects, who were excluded from the current analysis

due to their inability to attain VO_2max, would have a higher proportion of the highly reactive genotype. Consequently they would exhibit lower levels of PA. Additionally, subjects with the HR genotype might demonstrate exaggerated state anxiety when challenged with an exercise stimulus. Overall, their perceptual and consequently emotional reaction to the exercise stimulus might be exaggerated.

Complex factors influence attainment of physiological criteria for VO_{2max}, which at some level, may reflect one's willingness to engage in high-intensity exercise. The application across many dimensions of exercise-based research could include human performance and training and clinically developed methods to improve fitness assessment. In many ways, it is this group of largely sedentary subjects that should be targeted to better understand how their genetics might influence their psycho-physiological reactivity to an exercise challenge, and consequently how this association might impact PA participation. As we discover new candidate genes that are likely associated with perception of physiological and psychological stressors, the potential to include other genes in a similar analysis continues to grow each day. The following section provides an exploration of the association between genotype and rating of perceived exertion.

5.2.2 The Association between Genotype and Rating of Perceived Exertion

A wealth of research indicates that diverse physiological and psychological factors mediate perception of exertion during various forms of arm and leg exercise [1, 12, 13, 14, 17, 18, 19, 20, 31, 32, 33, 34, 35, 40, 58, 72, 73, 92, 93, 94, 95, 97, 98, 99, 100, 104, 105, 122, 124, 129]. It is therefore unlikely that differences in exertional perception during an exercise stimulus will be attributed exclusively to genetic variability. Rather, diverse factors occurring in a complex

psycho-physiological milieu converge to formulate an established subjective rating of effort sensation [107]. The objective of this research was to examine the extent to which genetic variation may contribute to interindividual differences in exertional perception and lower-body pain during exercise.

For this study, ANCOVA was utilized to test the hypothesis that variation in the Catechol-O-Methyltransferase gene would differentially influence exertional perception during a sub-maximal treadmill graded exercise test, among healthy young adults. Overall results indicate that, at a given workload, subjects in the HR sub-group reported significantly higher exertional perception than subjects in the LR sub-group. The following sections provide a thorough examination of each association that was investigated.

5.2.3 Undifferentiated Rating of Perceived Exertion

Results of the ANCOVA clearly indicate that genotype was not a significant predictor of undifferentiated rating of perceived exertion (RPE_Overall) expressed as a function any of the physiological criterion variables (%VO_{2max}, VE, and HR). This was found for the combined sample and with gender-specific analysis. Possible explanations for this result are provided in the following section.

Previous research indicates that during walking exercise, the dominant perceptual signal is provided by the exercising skeletal muscles (RPE_L) [105]. Robertson et al. [105] found that during treadmill exercise at higher velocities with load carriage, young women experienced higher RPEs differentiated to the legs [105]. Additionally, Robertson et al. [110] found that in a cohort of white and African American male and female children, RPE_legs was significantly

higher than RPE-Chest and RPE_Overall at 25, 50, 75, and 100 watts, during cycle ergometry [110]. Furthermore, studies by Mahon et al. found similar results using the Borg 6 – 20 Scale of Perceived Exertion, in which RPE differentiated to the legs provided the dominant signal shaping perception of exertion [73, 74].

Though it remains unclear how the brain interprets afferent feedback to induce exertional perception during exercise, several mechanisms (mediators) have been proposed in previous research. Gamberale et al. (1972) found that during cycle ergometry, peripheral RPE (exertion in the active muscles and limbs), was higher than respiratory-metabolic RPE (ventilation and heart-rate) [44]. These results suggest that localized changes in the exercising skeletal muscle were associated with increased lactate concentration and recruitment of type II muscle fibers and provide the dominant signal that influences rating of perceived exertion [49].

It has also been hypothesized that localized sensations of fatigue in exercising skeletal muscles, arise from proprioceptor feedback and Golgi tendon activity which mediate an overall sensation of effort [49]. It was also demonstrated that as subjects cycled at equivalent power outputs at cadences of 40, 60, and 80 rpm, the 40 rpm cadence elicited higher RPEs than the 80 rpm cadence despite similar values for HR and VO_2 [98, 99]. This result supports the concept that increased biomechanical efficiency significantly influences muscular fatigue. As efficiency increased, muscular fatigue decreased. Pandolf et al. [99] suggested that local sensations of muscular strain are a primary mediator of exertional perception during lower-body exercise because a higher proportion of Type II muscle fibers are recruited [99]. The aforementioned studies strengthen the hypothesis that RPE_Legs provides the dominant signal that informed effort sensation.

5.2.4 Differentiated Rating of Perceived Exertion

For this study, differentiated RPE was estimated during the sub-maximal treadmill graded exercise test. The differentiated ratings encompassed the exercising lower-body (RPE_L) as well as chest and breathing (RPE_C). For the combined sample, genotype was a significant predictor of RPE_L when expressed as a function of $\%VO_{2max}$, and when expressed as a function of heart-rate. However, among males and females combined, RPE_C was never found to be significantly different when expressed as a function of any of the physiological criterion variables.

It appears that RPE_Legs provided the dominant signal that informed RPE among males and females combined. As described in the previous section, it is possible that localized mechanisms (i.e. lactate concentration, recruitment of type II muscle fibers, and mechanical strain) in the exercising skeletal muscle had a significant impact on mediating overall effort sensation [48]. This manifested in higher RPE differentiated to the legs.

Gender-specific analysis revealed that among males only, when RPE_L and RPE_C were expressed as a function of $\%VO_{2max}$ and heart-rate, RPE differentiated to the legs and chest were significantly different between the LR and HR sub-groups. In each instance the HR sub-group reported higher RPE than the LR sub-group. Possible explanations for these findings are offered in the following section.

When males and females were examined separately, males demonstrated significant differences in RPE-Legs and RPE-Chest. As previously described, effort sensation is driven by a series of peripheral and respiratory-metabolic mediators [1, 12, 13, 14, 17, 18, 19, 20, 31, 32, 33, 34, 35, 40, 58, 72, 73, 92, 93, 94, 95, 97, 98, 99, 100, 104, 105, 122, 124, 129]. While the role of RPE_Legs has been hypothesized, additional attention must be provided to RPE differentiated to

the chest. Past research suggests that the respiratory-metabolic mediators associated with aerobic fitness are heart-rate and VO_2. These respiratory-metabolic mediators are strongly believed to influence sensations of effort felt in the chest and muscles of respiration [104, 105, 106]. Research by Borg et al. [13] found a high correlation between perceived exertion and heart-rate [13]. Additional research has also discovered similar correlations across gender and mode of exercise [12, 112, 113, 119, 120]. These studies provide evidence that effort sensation differentiated to the chest appears to be driven by central signals closely linked to aerobic fitness.

5.2.5 The Association between COMT Gene variability and RPE between genders

When gender-specific analysis was conducted, a significant gender main effect was revealed. It was discovered that among males, differences in perception of exertion between LR and HR subjects were statistically significant. However, no significant differences in RPE were detected between LR and HR sub-groups among females. A number of factors that may explain this sexual dimorphism will be discussed.

Interestingly, emerging research in neuropsychopharmacology identifies sex differences in the genetic epidemiology and clinical manifestations of some psychiatric disorders [10, 132, 140, 142]. The COMT gene is a leading candidate in the identification of genes that contribute to this dimorphism. Genetic associations between COMT and various psychiatric phenotypes frequently differ between males and females. For example the functional $Val^{158}Met$ polymorphism is associated with anxiety phenotypes in women, obsessive-compulsive disorder among males, and has a greater impact on cognitive function in male children than in female children [10, 132, 140, 142]. In addition, recent research indicates that the MET allele of the

COMT gene preferentially transmits Attention Deficit with Hyperactivity Disorder to males, [10, 132, 140, 142].

Sex-specific effects of COMT are frequently attributed to transcriptional regulation by estrogens. Women have been shown to have lower COMT activity than men [15, 39, 45, 55], due to the downregulation of COMT by estradiol [55]. Additionally, Gogos and colleagues (1998) demonstrated sexually dimorphic effects on dopamine levels and associated behaviors in COMT-deficient mice [45]. Although not yet fully explored, there exists compelling evidence demonstrating that COMT differentially impacts central nervous system function and dysfunction in males and females [15]. Evidence clearly exists in support of a sexual dimorphism associated with COMT enzyme activity which can differentially influence psycho-physiological processes that depend upon dopaminergic and adrenergic neurontransmission [10, 132, 140, 142]. Results of such studies help to explain differences between males and females, with regard to the associations being examined in the current study. In the current study, RPE was only significantly different among males based upon genotype sub-group. It may be that COMT gene function differentially influences this phenotype between males and females. Clearly, there exist differences in COMT gene activity between males and females. The extent to which these differences influence behavior is not currently known. However, COMT gene variability between males and females may influence sensitivity to physiological stressors, imparting a higher level of sensitivity to males. It is important to note that no evidence exists to directly support this rationale.

The current study represents only a preliminary effort to explain the relation between genetics and exertional perception and pain during exercise, and thus corroborative literature is not available that directly supports the validity of a gender main effect. The exact nature of this

finding can only be hypothesized based upon similar results related to the COMT gene as a strong candidate that produces sexual dimorphism in susceptibility to, and genetic epidemiology of disease.

While a genetic basis for sexual dimorphism may help explain the gender main effect discovered in the current study, it is also important to examine additional possible contributing factors. Two additional methodological and perceptual factors may have influenced the gender main effect. Male subjects on average completed a greater number of exercise stages during the sub-maximal treadmill exercise test than females. It is possible that the regression models developed for females lacked the necessary number of valid data points to reveal a significant association between genotype and exertional perception, due to comparatively lower variability in exertional perception among females. Perceptually, it may be that male subjects were better able to interpret physiological cues to effort sensation. We know that males were more physically active overall. Therefore their comparatively greater experience with PA may have aided them in accurately interpreting effort sensation during the graded exercise test. Thus they may have been better able to accurately assess their level of exertion. However, no direct evidence exists to support this hypothesis.

The exact nature of the gender main effect discovered in this research can only be hypothesized, given the innovative nature of this research and lack of directly supportive literature. Likely it is the result of a combination of genetic, perceptual and methodological factors. Clearly more research is required to further investigate factors that influence exertional perception during exercise, between males and females.

5.2.6 COMT Gene Variability Influences RPE during Exercise

As previously stated the exertion associated with exercise represents a psycho-physiological stressor that activates the hypothalamic-pituitary-adrenal (HPA) axis. This results in the increased production of the catecholamine hormones epinephrine and norepinephrine. In response to increasing metabolic and cardiorespiratory demands as well as the psychological stress of performing the exercise challenge, physiological excitation occurs forcing an individual to physically and emotionally experience an effect of the stressor [81, 115].

Polymorphic variability in the COMT gene appears to differentially influence perception of exertion during an exercise stimulus. Haplotypes of the COMT gene impact the amount and efficiency of the COMT enzyme that is produced, thus creating interindividual differences in the interpretation of the physiological excitation that is experienced by the individual [1, 29, 87, 88, 89, 144]. Subjects with the highly responsive haplotype produce a COMT enzyme that is less effective at catabolizing catecholamine hormones [1, 29, 87, 88, 89, 144]. Based upon this rationale, in the current study, HR subjects are more reactive when provided with the same exercise experience than subjects with the LR haplotype. Subjects with the highly reactive haplotype are believed to more readily disengage from the exercise experience, perceiving it to be a noxious stimulus. Further, it is hypothesized that subjects with the HR haplotype will demonstrate higher levels of circulating catecholamines due to a comparatively less effective COMT enzyme. Therefore, the physiological and psychological excitation experienced by HR subjects will be relatively higher than that experienced by LR subjects. The result is an exaggerated perceptual response to the given exercise challenge. Figure 13 provides an illustration of the hypothesized associations among COMT gene variability, catecholamine catabolism and exertional perceptions during exercise.

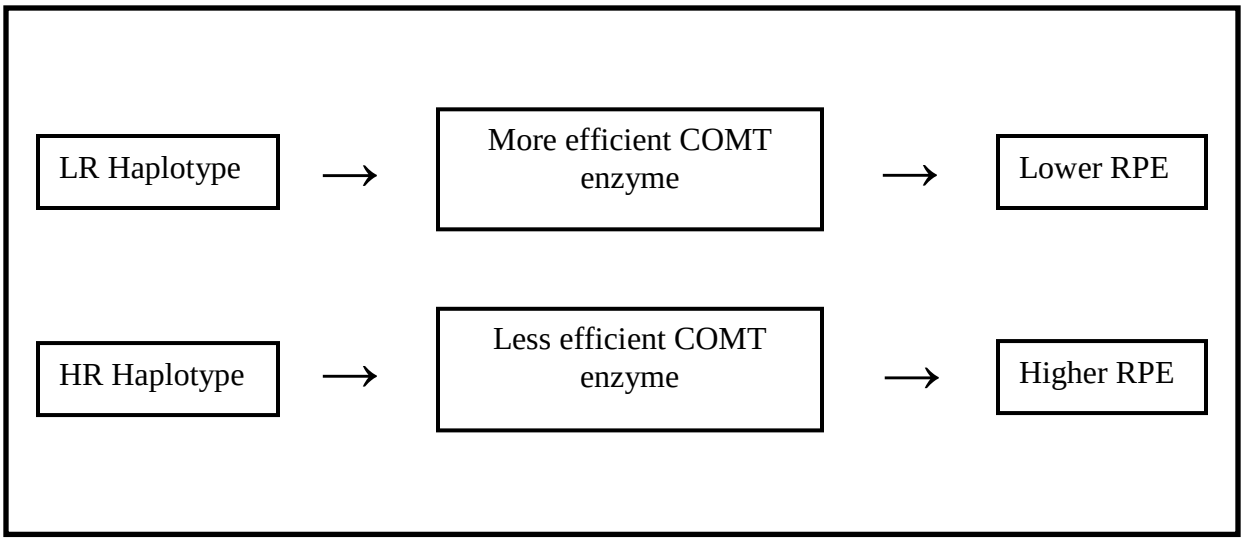


Figure 16: Proposed Mechanism by which COMT gene Haplotype Effects Rating of Perceived Exertion

5.2.7 Association between Genotype and Exercise-induced Lower Body Pain

When lower-body pain was expressed as a function of each physiological criterion variable (%VO_{2max}, VE, and HR), significant differences were discovered between genotypes, for the combined sample, as well as for males and females separately. In other words, at a given workload, highly responsive subjects experienced significantly higher sensations of lower-body pain than LR subjects. As far as we know, this appears to be the only evidence demonstrating genotypic differences in exercise induced pain in healthy adults. The following section provides a brief review of the past research that may help explain these findings.

Past research indicates that COMT gene variability is associated with interindividual differences in various pain-inducing stimuli including cancer pain, thermal and pressure pain, as well as susceptibility to chronic pain conditions [1, 29, 87, 88, 89, 144]. Diatchenko at al. [29]

demonstrated that COMT haplotypes were strongly associated with a graded responsiveness to experimental pain stimuli [29]. It is the Diatchenko haplotypes that served as the basis for this research. While a 3-grade (low, average, high) responsiveness to exercise-induced lower body pain was not demonstrated in the current study, significant differences were discovered between LR and HR subjects. It is not fully understood why the current study did not reveal a similar pattern of pain responsiveness. However a number of factors may have been influential.

One possibility is that the stimulus of the submaximal protocol, across genotype sub-groups, was not sufficient to identify more subtle differences in perceptual response among LR, AR (Average Responder), and HR sub-groups. While the sub-maximal test was chosen to maximize the number of data points entering each regression model, the relatively low intensity of the Modified Balke protocol may have elicited only relatively low levels of exercise-induced pain, thus decreasing the total variability in the pain scores. Exercise-intensity may not have reached a high enough level to elicit a sufficient range of pain scores, within each of the three genotypes.

Another possible explanation for this difference is related to sample size. The Diatchenko et al. study included 202 subjects in all [29]. This study was only able to test 169 eligible subjects. It is possible that a lower sample size may have further diminished the variability of the pain scores that entered the analysis.

Additionally, the study populations differed significantly between the two studies. The Diatchenko study utilized a sample of 202 apparently healthy females, while our study utilized a sample of both males and females. Previously, it was described in detail why gender may have played a critical role in the results of this study. Again, it must be cautioned that the exact nature

of these results can only be speculated. Due to the unique nature of this research, abundant supportive evidence for these results is not available.

5.2.8 The Influence of COMT Gene Variation on the Association of Pain with $\%VO_{2max}$, Heart-rate, and Ventilation

Results indicate that for both males and females, the slope of Lower Body Pain (LBP) expressed as a function of $\%VO_{2max}$, heart-rate, and ventilation were significantly different between the LR and HR sub-groups, with the HR sub-group evidencing higher slopes than the LR sub-group. The following section provides a possible explanation for the observed differences.

As previously stated, the COMT gene encodes the COMT enzyme that breaks down catecholamines at the synapses of andrenergic neurons in the central nervous system, rendering them biologically inert [1, 29, 87, 88, 89, 144]. COMT gene variability has been found to modulate perception of various experimental and disease-related pain stimuli as well as susceptibility to some chronic pain conditions [29, 38, 48, 54, 59, 86, 87, 88, 89, 134, 144]. Studies indicate that the low activity variants of the COMT gene negatively impact many aspects of physiology and behavior [29, 38, 48, 54, 59, 86, 87, 88, 89, 134, 144]. The important role of COMT in regulating response to pain stimuli makes it an ideal candidate gene when investigating biological influences on perception of exercise-induced pain.

Exercise-induced LBP is a psycho-physiological stressor that activates the hypothalamic-pituitary-adrenal (HPA) axis. This results in the increased production of the catecholamine hormones epinephrine and norepinephrine, producing physiological excitation [81, 118]. Polymorphic variability in the COMT gene appears to influence perception of LBP during treadmill exercise. Haplotypes of the COMT gene influence efficiency of the COMT enzyme

that is produced, thus causing interindividual differences in how a pain stimulus is perceived [29, 38, 48, 54, 59, 86, 87, 88, 89, 134, 144]. Results of the current study support the primary hypothesis that subjects with the HR haplotype are more reactive to the pain stimulus when provided with the same exercise experience than subjects with the LR haplotype. Subjects with the highly responsive haplotype are therefore more likely to disengage from the exercise experience, perceiving it to be a noxious stimulus. Specifically, subjects with the HR haplotype are proposed to have higher levels of circulating catecholamines in response to the pain stimulus compared to subjects with the LR haplotype, due to a less efficient COMT enzyme. Consequently, HR subjects reported relatively higher perceptions of lower-body pain than LR subjects. Figure 14 describes the proposed mechanism by which COMT gene variability influences rating of lower-body pain during exercise.

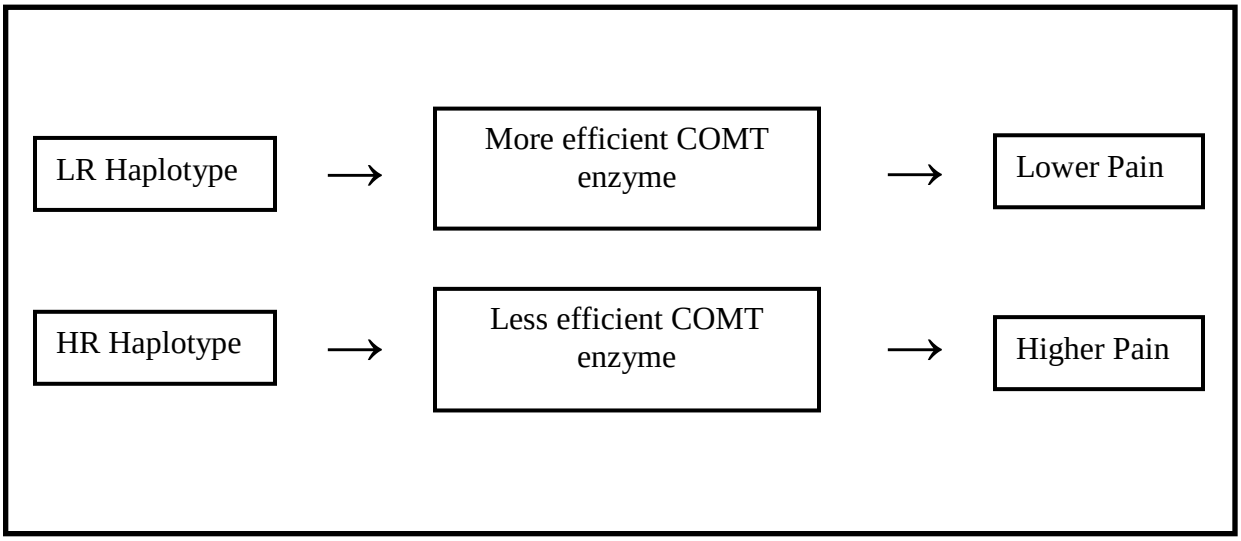


Figure 17: Proposed Mechanism by which COMT Gene Haplotype Effects Rating of Lower-Body Pain

5.2.9 Strengths

Very few studies examining physiological or psychological responses to an exercise stimulus have successfully collected over 100 treadmill graded exercise tests. An extremely important benefit of utilizing the cohort from the University of Pittsburgh Physical Activity Study (PittPAS) was the ability to collect data on almost 200 subjects. This robust sample size is of critical importance in the implementation of genetic association studies. Additionally, the relatively similar proportion of males and females in the combined sample as well as within each genotype sub-group is an important strength to recognize. Only the AR sub-group which was not included in any advanced analyses, showed significant differences in the ratio of males to females.

An additional strength of this study was the availability of an established foundation built by Diatchenko et al. [29]. Catechol-O-Methyltransferase gene haplotypes did not have to be identified prior to conducting this research. The association between response to various pain stimuli and genotype has already been thoroughly investigated and established. This study served more simply as an extension of Diatchenko's work into the area of response to exercise as an additional physiological stressor that is biochemically similar to perception of pain.

Additionally, indirect calorimetry (IC), the established gold standard for estimating energy expenditure and measurement of VO_{2max} was utilized for both sub-maximal and maximal GXTs. Since VO_{2max} was measured and not estimated, it provided a far more accurate assessment of a physiological variable that was critical to this study. Although more costly, the use of IC is of critical importance to the validity and scientific merit of this study.

An additional strength of this study was its innovative nature. An examination of biological, physiological, and psychosocial relations using a large sample in a controlled testing

environment was feasibly conducted. To our knowledge this is one of the first studies demonstrating genotypic variations that seem to partially explain interindividual differences in perceptual response to an exercise challenge. This research contributes an important and highly under-investigated dimension to the study of exertional perception as well as perception of pain during exercise.

5.2.10 Limitations

While significant associations between exertional perception and genotype were discovered among males, the total sample size of 169 subjects may have been a limiting factor. Although this did not seem to negatively impact the overall results, it is possible that a larger sample size would have yielded significant differences in RPE among females.

One major challenge to obtaining a sufficient number of subjects was associated with interindividual variability in the capacity of subjects to attain physiological criteria for VO_{2max}. Sixty-two subjects had to be excluded because they did not achieve any of the physiological criteria, and therefore an accurate estimation of their aerobic fitness could not be determined. In addition, the testing paradigm of the parent study may have further influenced inclusion of subjects. Both lack of experience with VO_{2max} testing or high intensity cardiovascular exercise and fatigue may have hindered some subjects' ability to attain VO_{2max}, after completing a sub-maximal treadmill walking test of relatively long duration. As previously detailed in the beginning of the discussion section, complex factors may have contributed to subject difficulty in attaining physiological criteria for VO_{2max}.

Perhaps a larger study population would have yielded different results. A significant association between exertional perception and genotype was not found among females.

Therefore is appears that gender differences in the proportion of subjects able to attain maximal oxygen uptake may have played a critical role in shaping the results of this study.

Another significant limitation related to sample size is the lack of non-white subjects in this research. It has been shown that there are racial/ethnic differences in the frequency of COMT gene haplotypes (). Therefore, haplotype estimation must be performed separately for each distinct racial or ethnic group. This study was performed in Allegheny County, Pennsylvania, and used a cohort that was predominantly white. Given the extremely small number of eligible non-white subjects (n = 7), only white subjects were included in the analysis. Therefore, generalizeability is diminished and must be reserved to only those subjects who met the demographic characteristics of the study population.

A few interesting methodological limitations warrant discussion. Collection of the biological sample for DNA extraction was performed using two different methods. For subjects agreeing to the I.V. catheter insertion or single venipuncture, collection of DNA was performed via whole-blood. As an alternative, collection was also performed using *Scope* mouthwash for subjects refusing to provide a blood sample. Although proven to be both valid and reliable [69]. the mouthwash collection was far more economically costly and time-intensive due to the need to amplify the relatively small amount of DNA extracted from the saliva.

An estimate of body composition was performed using bio-electrical impedance analysis (BIA). BIA has been shown to be less accurate than Dual X-Ray Absorpiometry, with an average correlation of 0.92 with this gold-standard [16]. It is possible that an inaccurate assessment of body composition may have negatively impacted measurement of relative VO_2max, producing a less accurate assessment of aerobic fitness relative to body composition. If percent of fat-free mass was over or underestimated, this may have produced a less accurate

estimate of caloric expenditure and consequently aerobic fitness. Another methodological limitation that is of importance is the assessment of physical activity. Physical activity was assessed via an interviewer administered past-year physical activity questionnaire. This provides room for bias if a subject wants to appear more physically active than they truly are, possibly being influenced by social desirability. Additionally, given the nature of past one-year recall, respondents may not have been able to accurately assess their level of activity over a one-year period. Overall these factors may diminish the validity of the recall physical activity questionnaire. Yet, given the large-scale nature of the parent study, an objective measure of PA was not a feasible option for the entire study population. Additionally, in this study total PA appeared to be over-estimated. However, it also appears that total PA was over-estimated across the study population and was not reserved to only males or only females or another distinct sub-group.

Finally, it must be mentioned that only one gene was examined in relation to the phenotype being studied. It is clear that complex factors influence perception of exertion and lower-body pain during an exercise challenge. It is therefore highly likely that multiple genes will be associated with this phenotype. While the results of this analysis are encouraging, they must represent only the beginning of investigation of this complex phenotype. Many genes are associated with physiological and psychological response to stress and therefore should be included in this investigation as part of the long-term objective.

5.2.11 Future Directions

Given its complex nature, it is likely that multiple genes are involved in influencing this exercise-based phenotype. The results of this research are extremely encouraging and interesting, and therefore must be expanded upon in a systematic manner. Many genes are associated with perception of physiological and psychological stressors through modulation of dopaminergic and adrenergic neurotransmission and may serve as an expansion point. For example, variation in the mu-opioid receptor gene ($OPRM_1$) has been shown to influence the experience of human p]in [38, 54, 59]. Therefore investigation of the $OPRM_1$ gene within the context of the same phenotype may be a worthwhile undertaking. Another viable candidate that may be associated with this phenotype of interest is the Cannabinoid Receptor Gene (CNR_1). Interestingly, research indicates that variability in the CNR_1 gene has been associated with differences in perception of various pain stimuli [51, 52] and has recently been implicated in the phenomenon known as "Runner's High" [30, 123]. Although the biochemical mechanisms are not identical to those demonstrated by the COMT enzyme, the endocannabinoid system is integral in the processes of nociception and antinociception. Therefore variability in the CNR_1 gene may significantly influence the phenotype being examined. The complexity of this phenotype dictates that as more evidence in support of candidate genes emerges, the direction of this research will continue to evolve appropriately. Future research questions might include:

- What is the association of the 118A $\rightarrow$G single nucleotide polymorphism of $OPRM_1$ gene with perception of exercise-induced pain during high intensity treadmill running?
- What is the association of the 118A $\rightarrow$G single nucleotide polymorphism of $OPRM_1$ gene with perception of exertion during high intensity treadmill running?

- What is the association of variation in the CNR_1 gene with perception of exercise-induced lower-body pain during moderate to high intensity treadmill running?

- What is the association of variation in the CNR_1 gene with perception of exertion during moderate to high intensity treadmill running?

- Does variation in the COMT, CNR_1, and $OPRM_1$ genes influence exertional perception during moderate to high intensity treadmill running?

While the primary objective of this research was to explore genetic variation that may influence perceptual response to exercise as a physiological and psychological stressor, an important extension of this work may be to investigate how perceptual response might influence participation in physical activity. Subjects in the LR sub-group reported significantly more leisure-time physical activity than HR subjects. This provides some evidence that variability in effort sensation and/or perception of exercise-induced lower-body pain may influence level of physical activity. Conversely, it may prove very interesting to examine how physical activity participation may influence rating of perceived exertion and exercise-induced LNP A more thorough investigation of this association appears warranted based upon our preliminary results.

In addition, it might prove very interesting to not only examine total physical activity but also participation in specific types of activity based upon level of exercise intensity. Individuals having the highly reactive genotype may select physical activities that are of a lower intensity, and therefore less likely to elicit this exaggerated perceptual response. In addition, individuals having the highly reactive genotype might disengage from physical activity entirely, viewing it to be a noxious stimulus. It would be very interesting to examine how differences in exertional perception might influence the inclination to engage in physical activity of varying levels of intensity. Possible future research questions are:

- Do subjects with the HR haplotype of the COMT gene exhibit comparatively lower levels of total physical activity than subjects with the LR haplotype?
- Does having the HR haplotype predispose subjects to select physical activities that are of a comparatively lower relative intensity compared to subjects with the LR haplotype?

5.2.12 Implications

While the notion of altering a population's genetic composition to promulgate healthier behaviors is one wrought with social, legal and ethical complications, the results of this research offer an important glimpse into the underpinnings of human behavior. If by their very nature, some members of our community are predisposed to disengaging from physical activity or exercise, concerted efforts must be made to facilitate their adoption and maintenance of health behaviors. This research has the potential to inform future intervention efforts targeting individuals who appear to be predisposed to sedentary lifestyles.

Progressive, step-up interventions should be developed to facilitate adoption of physical activity and exercise programs. Patients can be taught how to more effectively self-regulate exercise intensity to limit feelings of anxiety or extreme physical discomfort that may be a common and overwhelming feeling. Additionally, patients can be taught through various intervention efforts how to recognize that some level of discomfort can be expected when performing various modes and intensities of exercise and that this discomfort is not associated with dysfunction or injury. In addition, patients must be taught that this discomfort is not something to be afraid of. Rather, it can through chronic training be turned into a source of positive feedback eliciting feelings of accomplishment as an individual increases their level of

fitness. The importance of this research lies in the development of a better understanding of the complex factors that influence individuals to adopt and maintain healthy behaviors, and in a better understanding of how to optimally intervene to increase participation in physical activity and exercise as well as other important health behaviors.

It must also be understood that if genetic composition influences us to adopt sedentary lifestyles and other deleterious behaviors, additional resources must be made available and created to meet this important need. Young children must be provided every opportunity to excel at physical activity or exercise to discover for themselves the activities that drive them to participate despite some discomfort or feelings of anxiety. A child's experience with physical activity and sport may influences adoption and maintenance long-term. If many positive experiences are provided to our children, we can help them overcome feelings of physical discomfort and anxiety that they may associate with participating in physical activity or exercise. Those feelings may lead to withdrawal from activity that is so critical to health maintenance.

In addition the environment may hinder physical activity participation long term. Family members may or may not share COMT gene haplotypes. However, they do often share health-related attitudes and resultant behaviors such as cigarette smoking and poor eating habits. It is critically important that we facilitate, through education and more engaging programming, the adoption of healthy behaviors such as sustained physical activity and exercise.

5.2.13 Conclusions

This study demonstrated that variation in the Catechol-O-Methyltransferase gene has an influence on exertional perception and perception of exercise-induced lower-body pain, during a sub-maximal treadmill graded exercise test. While these results only offer a glimpse into these

important associations, they are of great interest and warrant further investigation. It appears that significant differences in psycho-physiological reactivity to an exercise stimulus exist between the two genotypes. Additionally, a gender main effect was discovered in which only male subjects demonstrated these significant associations based on genotype. The explanations discussed also warrant a more in-depth examination.

We cannot alter our genetic composition to combat sedentary behaviors. However, studying genetic influences on psycho-physiological response to physical activity and exercise provides us with a new perspective on the dynamics of human behavior. If we can understand the biological forces that drive the most complex behaviors, we can develop targeted intervention efforts to optimally assist individuals achieve lasting behavior change, not only in the realm of physical activity participation and adherence to exercise, but in all dimensions of a healthy lifestyle. The future of exercise-based research must incorporate genetic research to not only inform intervention efforts but to develop a more refined understanding of the genetic susceptibility to some lifestyle oriented diseases such as obesity, cardiovascular disease, and type 2 diabetes.

Few studies exist to directly support the results of this investigation. Many conceptual bridges were explored to strengthen the need for this research. More importantly, this study represents only the beginning of the examination of genetic influences on psycho-physiological reactivity to exercise. If within our very architecture there are forces hindering us from adopting and maintaining physically active lifestyles, efforts must mobilize resources in every dimension of our lives, to combat the pandemic of physical inactivity, in order to achieve and maintain healthy lifestyles and prevent chronic illness.

APPENDIX A

CONSENT FORMS

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Psycho-physiological Influences on Physical Activity

PRINCIPAL INVESTIGATOR: **Robert J. Robertson, Ph.D.**
Professor
Department of HPA
University of Pittsburgh
107 Trees Hall
Telephone: 412-648-8258

CO-INVESTIGATORS:

Elizabeth Nagle, Ph.D.
Assistant Professor
Department of HPA
University of Pittsburgh
109 Trees Hall
Telephone: 412-648-8268

Nikhil Satchidanand M.S.
Research Coordinator
Department of HPA
University of Pittsburgh
134A Trees Hall
Telephone: 412-648-8759

Vincent Arena, Ph.D.
Associate Professor
Department of Biostatistics
University of Pittsburgh
318 Parran Hall
Telephone: 412-624-3023

Jeanette Garcia
Graduate Student Researcher
Department of HPA
University of Pittsburgh
134 Trees Hall
Telephone: 412-648-6743

SOURCE OF SUPPORT: National Institutes of Health (NIH), National Cancer Institute (NCI)

Why is this research being done?

Being physically active is an important component of health and has been found to decrease the risk of certain diseases such as heart disease and diabetes. However, many adults do not get the recommended amount of physical activity. In order to help develop better programs to help people increase their activity it is important to understand how and why people's physical activity changes. The purpose of this study is to identify factors that contribute to increases, decreases, or maintenance of physical activity. A total of 848 subjects will be enrolled in this study.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you have been participating in a study to examine changes in physical activity from adolescence to young adulthood. This research study is a continuation of the research projects you have been participating in since 1990.

What procedures will be performed for research purposes?

If you decide to take part in this research project, you will complete two testing sessions separated by approximately two years and the information collected for this study will be compared with prior data collected in the "Epidemiology of Physical Activity: Teenage to Adult Years" study. All procedures will take place at the Human Energy Research Laboratories located in Trees Hall at the University of Pittsburgh. Each testing session will last approximately 2 ½ hours and include the following procedures.

1. Measurements of your height and weight. The amount of fat in your body will be estimated by the method of bioelectric impedance analysis (BIA). BIA is a common method of assessing body composition. It involves passing a small electric current through your body and measuring the impedance or opposition to the current flow. You will not feel the current.

2. You aerobic fitness level and physical work capacity will be measured by having you walk on a motorized treadmill. Prior to beginning the treadmill test you will complete a medical history and Physical Activity Readiness Questionnaire. If you have an orthopedic, cardiovascular, and/or metabolic disease (e.g., coronary artery disease, prior myocardial infarction, peripheral vascular disease, chronic obstructive pulmonary disease, and diabetes mellitus) you will be excluded from participation in the treadmill testing component of the protocol.

   If you do not have any conditions that would limit your ability to exercise on a treadmill, you will complete two exercise sessions separated by a period of approximately 5 minutes. During the exercise sessions we will place a heart rate monitor around your chest and secure it with an elastic strap. A rubber mouthpiece, connected to a headset, will be placed in your mouth to determine the amount of oxygen that you use during exercise. A clip will be attached to your nose to insure that all of the air that you breathe comes in and out through your mouth. Some individuals become anxious when fitted with the nose clip and mouthpiece. If this occurs to you, please inform the individual performing the test and the test will be stopped. The test will begin with a 2 minute warm-up. Then every 3 minutes the workload will be increased. The first exercise session will continue until your heart rate has reached a predetermined level. For the second exercise session you will be encouraged to continue until fatigued. You may stop the tests at any time. Following completion of the second test, you will cool-down by walking slowly for 2 minutes. Your heart rate and the amount of oxygen your body uses will be measured during each minute of the test. You will also be asked to give a rating (1-10) of your exertion and any pain/discomfort associated with the exercise.

3. During the first exercise session we will be obtaining blood samples to measure the amount of lactic acid in your blood. Lactic acid is an indication of muscle fatigue. Ten minutes before the first exercise session a trained phlebotomist will insert a catheter in a vein in your forearm. A small sample of blood (approximately 1 teaspoon) will be taken at rest, every three minutes during the exercise session, and 5 minutes following the completion of the test. A total of 15 to 25 ml (approximately 2-3 tablespoons) of blood will be sampled throughout the entire test.

4. We will also obtain an additional 10 ml blood sample (2 teaspoons) from the catheter inserted into the forearm vein. This sample will be used to examine genetic factors that are related to physical performance and/or perceptual response. If we are unable to obtain a blood sample, we will ask you to swish a small amount of Scope mouthwash around in your mouth, or to spit into an Oragene saliva self-

collection container to obtain some loose cells from your mouth. It is important that you refrain from eating, drinking, or rinsing your mouth for one hour before collection of the saliva sample. The genetic material, along with other study data, will be de-identified. All genetic specimens (DNA) will be stored without identifying information in the Human Genetics Laboratory, University of Pittsburgh, Graduate School of Public Health. All genetic specimens will be under the control of Dr. Robert Farrell.

5. You will complete a one-on-one interview with a trained research assistant. The interview will include an assessment of physical activity during the past year. Your current physical activity patterns will be compared to your physical activity patterns reported 2-3 years ago. The interviewer will ask you to identify factors that may have contributed to any change (either increase or decrease) in your usual level of physical activity. We are audio taping these interviews so that we can capture every word that you say. It would not be possible for the research assistant to remember everything that was said or to write everything down. Your name will not be disclosed on any printed or published reports that are produced; ID numbers will be used for all participants in the study. All audio tapes will be confidential and stored in my locked office on the university campus. Tapes will only be reviewed by the investigators and research assistants who will transcribe the tapes. The tape will be destroyed when the project is complete.

6. You will complete several questionnaires about your medical history, lifestyle characteristics, psychological characteristics, and other health behaviors such as diet and tobacco and alcohol use. The questionnaires should take less than an hour to complete. You may choose not to answer any question that you are not comfortable with.

7. You will be asked to provide information that will be used to contact you in approximately 2 years. At that time you will receive a letter indicating that a research assistant will be contacting you to schedule your second visit.

Also, you may be able to participate in future studies related to this one that might focus on other factors related to physical activity and health. For this reason, Dr. Aaron and/or her co-workers may try to contact you during the next 10 years to tell you about these other projects. They may ask if you are willing to give a telephone interview, fill out a mailed questionnaire, or attend another clinic visit(s) during which you would undergo physical measures or give another blood specimen. They would explain what any additional project activities were before you would agree to volunteer.

☐ I do agree to be contacted for future studies.

☐ I do *not* agree to be contacted for future studies.

What are the possible risks, side effects, and discomforts of this research study?

The possible risks of this research study may be due to the treadmill testing, the blood test and/or the BIA test. You may chose not to participate in any tests that you are not comfortable with.

Risks of the Treadmill Tests

Abnormal responses, such as excessive rises in blood pressure, mental confusion, shortness of breath, chest pain, heart attack, and death, to maximal exercise tests in young healthy adults are rare, occurring in less than 1% of people (less than 1 out of 100 people tested). However, some common risks, occurring in 1% to 25% of people (1 to 25 out of 100 people tested), of maximal exercise testing include; heavy breathing, dizziness, muscle fatigue, headache, and overall fatigue. To minimize risks associated with maximal exercise testing, you will be asked to complete a Physical Activity Readiness Questionnaire which asks questions about your current health status and a medical history questionnaire before you begin to exercise on the treadmill. If you have any orthopedic, cardiovascular, and/or metabolic problems that may be worsened by maximal exercise (e.g., coronary artery disease, prior myocardial infarction, peripheral vascular disease, chronic obstructive pulmonary disease, and diabetes mellitus) you will be excluded from participation in the treadmill testing component of the study. However, you will complete the other components of the study. If you are a female, you will be asked if you are currently pregnant. If you are pregnant at the time of your clinic visit you will be rescheduled for a visit at least 3 months after the termination of your pregnancy. Risks associated with study monitors (e.g. heart rate monitor, mouth piece, etc.) include redness, irritation, and chafing. If an abnormal response occurs during exercise, the test will be immediately stopped and you will be given proper medical attention. Emergency equipment will be on site for all testing procedures and staff personnel are certified in CPR and First Aid by the American Red Cross. If you have an abnormal response to the treadmill test, you will be told of the findings and will be encouraged to contact your primary care clinician.

Risks of the BIA Test

According to the U.S. Department of Health and Human Services, there have been no reported adverse events induced by BIA. However, if you have an implanted device, such as a cardiac defibrillator, you will be excluded from the BIA testing due to possible electrical interference.

Risks of the Blood Test:

Trained phlebotomists will perform the blood draw. The risks associated with the blood draw include fainting, light headedness, temporary local discomfort, bleeding or bruising. There is a rare risk of infection with the insertion of the needle. It is possible that you may have some minor bruising and/or soreness after blood collection. However, this will be no more than you would encounter during blood donation.

Risks of the Collection of Genetic Material

There is a possibility that if the results of the research studies involving your genetic material were to become generally known, this information could impact future insurability, employability, or reproductive plans, or have a negative impact on family relationships, and/or result in stigmatization.

What are possible benefits from taking part in this study?

You will likely receive no direct benefit form taking part in this research study. However, you will receive important information regarding your fitness level. In addition, a benefit of the study is obtaining generalizable knowledge on factors that contribute to adoption, maintenance and resumption of physical activity.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any procedures performed for the purpose of this research study.

Will I be paid if I take part in this research study?

You will be paid a total of $200 for completing all parts of this research study. Specifically, you will be paid $100 for completing each testing session approximately two years apart. In addition, any parking fees related to your participation in this study will be paid for by the study. If you are found to be ineligible for the treadmill testing during a study visit and complete the other components of the protocol you will receive the full payment of $100 for completing the visit. If you are a female and indicate that you are pregnant at the time you come for a study visit, you will receive $20 compensation for your time. In addition, you will receive the full payment of $100 for completing the study visit following the termination of your pregnancy.

Who will pay if I am injured as a result of taking part in this study?

University of Pittsburgh researchers and their associates who provide services at UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of the UPMC. It is possible that the UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment,

you will be responsible for the cost of this follow-up unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confident (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identify on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medial information?

This research study will not involve the use or disclosure of any identifiable medical information.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

- Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.
- In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.
- Authorized people sponsoring this research study, NIH, because they need to make sure that the information collected is correct, accurate and complete, and to determine the results of this research study.
- Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of five years after final reporting or publication of a project.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers to protect your safety or you are unable or unwilling to complete the research protocol.

****

VOLUNTARY CONSENT:

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained to the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions that the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX B

SCREENING INSTRUMENTS

ID# _____
Round: _____

University of Pittsburgh Physical Activity Study (PittPAS)

Physical Activity Readiness Questionnaire (PAR-Q)

Now I'm going to ask you a few questions to determine if you are eligible to complete the treadmill exercise...

5. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

   No ___ Yes ___ If yes, specify:
   _____

6. Do you feel pain in your chest when you do physical activity?

   No ___ Yes ___ If yes, specify:
   _____

7. In the past month, have you had chest pain when you were not doing physical activity?

   No ___ Yes ___ If yes, specify:
   _____

8. Do you lose your balance because of dizziness or do you ever lose consciousness?

   No ___ Yes ___ If yes, specify:
   _____

9. Do you have a bone or joint problem that could be made worse by a change in your physical activity?

   No ___ Yes ___ If yes, specify:
   _____

10. Is your doctor currently prescribing drugs (for example, water pills) for a blood pressure or heart condition?

    No ___ Yes ___ If yes, specify:
    _____

11. Do you know of any other reason why you should not do physical activity?

    No ___ Yes ___ If yes, specify:
    _____

6. What is your age? ___

Women Only

7. Are you currently pregnant?

No ___ Yes ___ If yes, approximate due date: _____

ID#

_____

Round: _____

University of Pittsburgh Physical Activity Study (PittPAS)

Final Screening Form

I'm going to ask you a few questions to determine if you eligible to complete all of the tasks today...

1. Have you ever been told by a doctor or other medical person that you have had any of the following? *(If yes to any of the following, participant is eliminated from the treadmill exercise)*

		If yes, specify:
Angina	No ___ Yes	_____
Heart Attack	No ___ Yes	_____
Peripheral Vascular Disease	No ___ Yes	_____
COPD	No ___ Yes	_____
Diabetes	No ___ Yes	_____

2. Do you have a temporary or chronic physical injury or disability that would prevent you from exercising on a treadmill?

   No ___ Yes ___ If yes, specify:

   _____

   If temporary, what is estimated length of recovery?

   _____

3. Do you have an implanted device such as a pace maker or cardiac defibrillator? *(If yes, participant is eliminated from taking the BIA test)*

   No ___ Yes ___ If yes, specify:

   _____

4. Are you presently being treated by a physician or health care provider for any type of health problem? *(If yes, determine if participant can participate in all parts of the study)*

   No ___ Yes ___ If yes, specify:

   _____

5. Are you currently taking any prescribed medications?

   No ___ Yes ___ If yes, what medications are you currently taking?

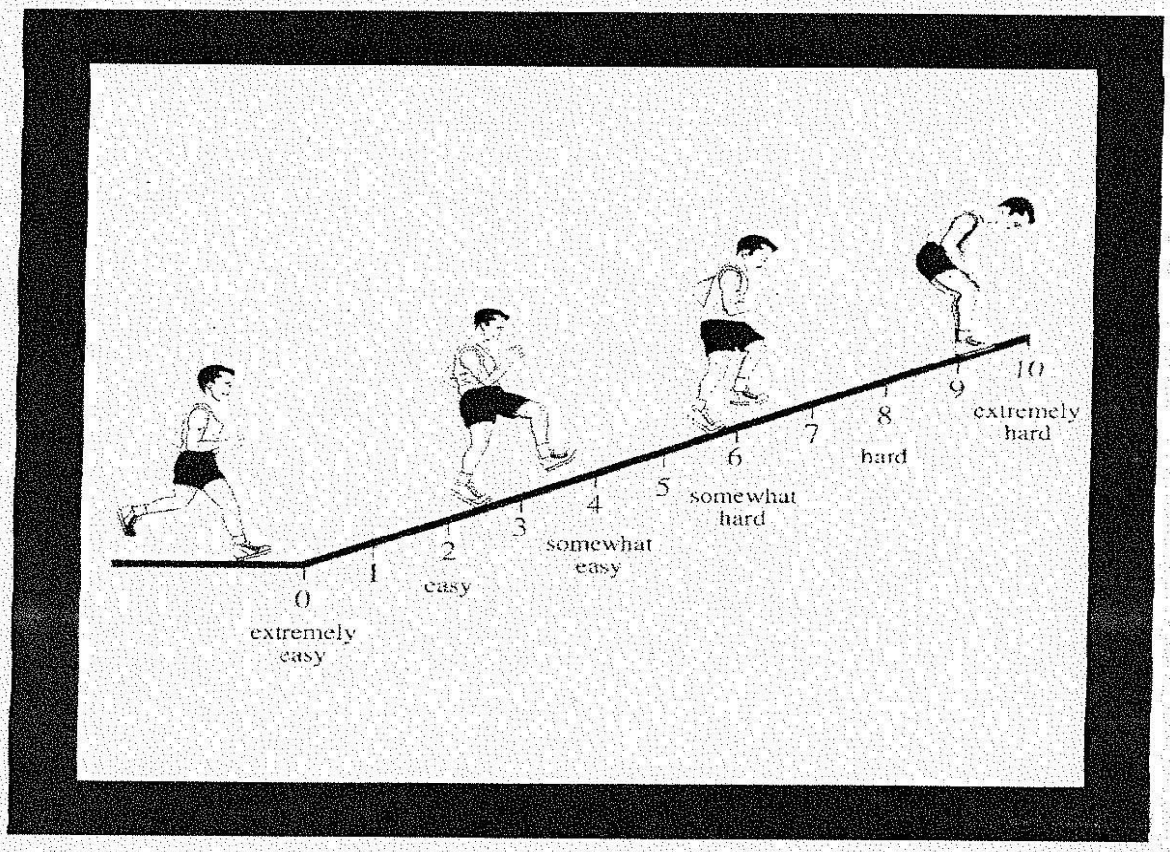
   _____

   _____

   _____

APPENDIX C

OMNI SCALE OF PERCEIVED EXERTION


0
extremely
easy
1
2
easy
3
4
somewhat
easy
5
6
somewhat
hard
7
8
hard
9
10
extremely
hard

APPENDIX D

COOK PAIN SCALE

0 No pain at all

0.5 Very faint pain (just noticeable)

1 Weak pain

2 Mild pain

3 Moderate pain

4 Somewhat strong pain

5 Strong pain

6

7 Very strong pain

8

9

10 Extremely intense pain (almost unbearable)

- Unbearable pain

APPENDIX E

DATA COLLECTION FORM

ID #_____

Round_____

Date of Visit _____ / _____ / _____

LABORATORY DATA COLLECTION FORM

Lab Technicians/Interviewer:

A: _____ **B:** _____ **C:** _____ **D:** _____

RADIAL PULSE

☐ **Not Completed** **Reason:** _____

Measurement 1 ___ ___ ___ bpm in 30 sec Measurement 2 ___ ___ ___ bpm in 30 sec

Enter the number of beats counted in 30 seconds. Do not calculate beats per minute.

BLOOD PRESSURE

☐ **Not Completed** **Reason:** _____

Sitting Blood Pressure	Measure 1	Measure 2	Measure 3
Systolic	___ ___ ___ mmHg	___ ___ ___ mmHg	___ ___ ___ mmHg
Diastolic	___ ___ ___ mmHg	___ ___ ___ mmHg	___ ___ ___ mmHg

If M1 and M2 (systolic or diastolic) differ > 10 mm, take M3.

	Measure 4
Systolic	___ ___ ___ mmHg
Diastolic	___ ___ ___ mmHg

BP > 140/90 ⇨ Have participant rest 5 minutes, take M3.

M3 > 140/90 ⇨ Do not complete treadmill testing. Have participant complete all questionnaires and give physician's clearance form to take home.

Only take M4 when there was a difference > 10mm between M1 & M2 and the participant's BP is > 140/90

ID #_____

Round_____

STANDING HEIGHT

☐ Not Completed Reason: _____

Measurement 1 ___ ___ ___ . ___ in.

Measurement 2 ___ ___ ___ . ___ in.

BIA Height: _____ ft _____ in

If M1 and M2 differ by more than 0.5 inches, do measurement 3.

Measurement 3 ___ ___ ___ . ___ in.

BIA

☐ Not Completed Reason: _____

Standard Mode		Athletic Mode	
Body Mass Index	___ ___ . ___ ___		
Body Weight (lb)	___ ___ ___ . ___		
Fat (%)	___ ___ ___ . ___	Fat (%)	___ ___ ___ . ___
Fat Mass (lb)	___ ___ ___ . ___	Fat Mass (lb)	___ ___ ___ . ___
Fat Free Mass (lb)	___ ___ ___ . ___	Fat Free Mass (lb)	___ ___ ___ . ___

Note to examiner: Please attach BIA printouts below.

ID #_____

Round_____

Submaximal Exercise Test: ☐ **Not Completed** **Reason:** _____

AGE: _____ AAMHR: _____ 85% AAMHR: _____

Note: Pre Exercise RPE is the predicted value for the entire test.

Treadmill Protocol				"B" Person	"A" Person	"B" Person				"C" Person
						RPE @ 2:30			Pain @ 2:45	Blood Draw @ 3:00
Stage	Speed	Grade	Minute	HR	VO_2 @ 3:00	L	C	O		Y or N If no, reason*.
Pre Exercise			0							1. No Catheter Insertion 2. No Blood Flow 3. Equip Malfunction
1	3.0	0%	1							
			2							
			3							
			4							
2	3.0	2.5%	1							
			2							
			3							
			4							
3	3.0	5.0%	1							
			2							
			3							
			4							
4	3.0	7.5%	1							
			2							
			3							
			4							
5	3.0	10%	1							
			2							
			3							
			4							
6	3.0	12.5%	1							
			2							
			3							
			4							
7	3.0	15%	1							
			2							
			3							
			4							
8	3.0	17.5%	1							
			2							
			3							
			4							

ID #_____

Round_____

Submaximal Exercise Test: AGE: _____ AAMHR: _____ 85% AAMHR: _____

Protocol				HR	VO_2	L	C	O	Pain	Blood Draw
9	3.0	20%	1							
			2							
			3							
			4							
10	3.0	22.5%	1							
			2							
			3							
			4							
11	3.0	25%	1							
			2							
			3							
			4							
5 Minutes Post Exercise										

Note: Post Exercise RPE is the session rating for the entire submaximal test.

Total steps during submaximal test _____

ID #_____

Round_____

Maximal Exercise Test: ☐ **Not Completed** **Reason:** _____

AGE: _____ AAMHR: _____ 85% AAMHR: _____

Note: Pre Exercise RPE is the predicted value for the entire test.

Treadmill Protocol				"B" Person HR	"A" Person VO_2 @ 3:00	"B" Person			
						RPE @ 2:30			Pain @ 2:45
Stage	Speed	Grade	Minute			L	C	O	
Pre Exercise			0						
1	1.7	10%	1						
			2						
			3						
2	2.5	12%	1						
			2						
			3						
3	3.4	14%	1						
			2						
			3						
4	4.2	16%	1						
			2						
			3						
5	5.0	18%	1						
			2						
			3						
6	5.5	20%	1						
			2						
			3						
Maximal Effort Total Treadmill Time: _____									
5 Minutes Post Exercise									

Note: Post Exercise RPE is the session rating for the entire maximal test.

Did the subject start running? Yes ____ No ____
(Stage: _____ Minute: _____)

Total steps during maximal test _____

Post RPE time delay_____

HR After 2-minute cool down _____

APPENDIX F

PAST YEAR PHYSICAL ACTIVITY QUESTIONNAIRE

ID#

University of Pittsburgh Physical Activity Study

Past Year Physical Activity Questionnaire

Date Completed: __ __ / __ __ / __ __ __ __

Round: _____

Reviewed by: _____

ID#

PAST YEAR LEISURE-TIME PHYSICAL ACTIVITY

For in personal interview:

Think back to _____ (month/year). Please look at the list and tell me which activities you have participated in **at least 10 times in the past year**. Make sure you include all individual and team recreational activities or sports that you participated in during the last year from _____ (month/year) through _____ (month/year).

Are there any other activities that you did at least 10 times in the past year that are not included in this list? If yes, list activities on the next page in the open blocks.

For each activity that you participated in I am going to ask you (a) which months of the past year you participated in that activity, (b) approximately how many days per week you participated in that activity and (c) on average, how many minutes per day you participated in that activity.

As a reminder, the past year is referring to _____ (month/year) through _____ (month/year).

For telephone interview:

Think back to _____ (month/year). I am going to read you a list of activities that you may have participated in during the past year. As I read through the list please tell which activities you have participated in **at least 10 times in the past year**. Make sure you include all individual and team recreational activities or sports that you participated in during the last year from _____ (month/year) through _____ (month/year).

Are there any other activities that you did at least 10 times in the past year that are not included in this list? Please tell me what other activities you have done in the past year. *[List activities on the next page in the open blocks.]*

For each activity that you participated in I am going to ask you (a) which months of the past year you participated in that activity, (b) approximately how many days per week you participated in that activity and (c) on average, how many minutes per day you participated in that activity.

As a reminder, the past year is referring to _____ (month/year) through _____ (month/year).

Date Completed: Round: ID#

Activity	Activity done in past year at least 10 times?		Which months in the past year did you do this activity?											On average, how often did you do this activity?		
	No	Yes	JAN	FEB	MAR	APR	MAY	JUN	JLY	AUG	SEP	OCT	NOV	DEC	Months	Days/Wk	Mins/Day
Aerobics																	
Baseball																	
Basketball																	
Bicycling (Mountain)																	
Bicycling (Stationary)																	
Bicycling (Street)*																	
Bowling																	
Dance Class																	
Football																	
Garden/Yard Work																	
Gymnastics																	
Hiking																	
Ice Skating																	
Roller Skating																	
Running for Exercise																	
Skateboarding																	
Snow Skiing																	
Soccer																	
Softball																	
Street Hockey																	
Swimming Laps																	
Tennis																	
Volleyball																	
Water Skiing																	
Weight Training																	
Walking for Exercise*																	

Are there other activities that you did at least 10 times in the past year? If yes, list activities below.

*** Note: Walking and/or biking to and from work should not be included in this section.**

APPENDIX G

MEAN RATING OF PERCEIVED EXERTION AND LOWER-BODY PAIN PER STAGE OF EXERCISE

Mean Rating of Perceived Exertion and Lower-body Pain per Stage of Exercise for Low Responders and High Responders

Exercise Stage	Low Responder Genotype				High Responder Genotype			
	RPE-L	RPE-C	RPE-O	LBP	RPE-L	RPE-C	RPE-O	LBP
1	1.56	1.74	1.53	0.31	1.62	1.86	1.57	0.50
2	2.14	2.16	2.23	0.47	2.23	2.26	2.01	0.61
3	2.94	2.54	2.50	0.51	3.08	3.36	3.07	0.83
4	3.32	2.90	3.00	0.60	4.32	3.94	4.00	1.17
5	4.15	3.75	3.85	1.11	5.37	4.77	4.51	1.67
6	4.71	3.88	4.11	1.32	6.03	5.76	4.71	1.78
7	5.43	4.89	4.86	1.54	7.30	6.70	6.85	2.37
8	6.00	5.60	5.00	2.00	7.59	7.65	6.90	2.49

Bibliography

1. Acevedo EO, Kraemer RR, Kamimori GH, Durand JG et al. Stress hormones, effort sense, and perception of stress during intremental exercise: an exploratory investigation. *J Strength Cond* 2007; 21(1): 283 – 288.

2. Agarwal N, Pacher P, Tegeder I, Amava F et al. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci* 2007; 10(7): 870 – 809.

3. Axelrod J, Senoh S, Witkop B. O-methylation of catechol amines in vivo. *J Biol Chem* 1958; 233: 697 – 701.

4. Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* 1958a; 233: 702 – 705.

5. Ball P, Knuppen R. Catechoestrogens (2-and-4-hydroxoestrogens): chemistry, biogenesis, metabolism, occurrence and physiological significance. *Acta Endocrin* 1980; 93(232); 1 – 127.

6. Banister EW, Griffith J. Blood levels of adrenergic amines during exercise. *J Appl Physiol* 1972; 33(5): 674 – 676.

7. Beets MW, Vogel R, Forlaw L, Pitetti et al. Social support and youth physical activity: the role of provider and type. *Am J Health Behav* 2006; 30(3): 278 – 289.

8. Bertocci B, Miaggiano V, Da Prada M et al. Human catechol-methyltransferase: cloning and expression of the membrane-associated form. *Proc Natl Acad Sci* 1991; 88: 1416 – 1420.

9. Beuten J, Payne TJ, Ma JZ, Li MD, Significant association of catechol-O-methyltransferase (COMT) haplotypes with nicotine dependence in male and female smokers of two ethnic populations. *Neuropsychopharmacology* 2006; 31(3): 675 – 684.

10. Biederman J, Woo KJ, Doyle AE, Fagerness J et al. Sexually dimorphic effects of four genes (COMT, SLC6A2, MAOA, SLCA4) in genetic associations of ADHD: a preliminary study. *Am J Med Genet: Neuropsychiatric Genet* 2008; 147B(8): 1511 – 1518.

11. Boecker H, Sprenger T, Spilker ME, Henriksen G et al. The runner's high: opioid mechanisms in the human brain. *Cerebral Cortex* 2008; 18(11): 2523 – 2531.

12. Borg GA, Dahlstrom H. A pilot study of perceived exertion and physical work capacity. *Acta Soc Medic Upsal.* 1962; 76: 91 – 93.

13. Borg GA. A note on a category scale with “ratio properties” for estimating perceived exertion. *Rep Inst Appl Psychol* 1973; 366. Univ. of Stockholm.

14. Borg GA. Perceived Exertion. *Exercise Sport Sci Rev* 1974; 2: 131 – 153.

15. Boudikova B, Szumlanski C, Maidak B, Wienshilboum R. Human liver catechol-O-methyltransferase pharmacogenetics. *Clin Pharmacol Ther* 1990; 48: 381 – 389.

16. Bracco D, Thiebaud D, Chiolero R, Landry M et al. Segmental body composition assessed by biolelectrical impedance analysis and DEXA in humans. *J Appl Physiol* 1996; 81: 2580 – 2587.

17. Cain WS, Stevens JC. Constant-effort contractions related to the electromyogram. *Med Sci Sports Exercise* 1973; 5(2): 121 – 127.

18. Cafarelli E. Peripheral contributions to the perception of effort. *Med Sci Sports Exercise* 1982; 14(5): 382 – 389.

19. Cafarelli E, Noble BJ. The effect of inspired carbon dioxide on subjective estimates of exertion during exercise. *Ergonomics* 1976; 19(5): 581 - 589.

20. Carton RL, Rhodes EC. A critical review of the literature on rating scales for perceived exertion. *Sports Med* 1985; 2(3): 198 – 222.

21. Casal DC, Leon AS. Failure of caffeine to affect substrate utilization. *Med Sci Sports Exercise* 1985; 17: 174 – 179.

22. Coyle EF, Cogan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise. *J Appl Physiol* 1986; 61: 165 – 172.

23. Chwalbinska-Moneta J, Krysztofiak H, Ziemba A et al. Threshold increases in plasma growth hormone in relation to plasma catecholamineand blood lactate concentrations during progressive exercise in endurance-trained athletes. Eur J *Appl Physiol O* 1996.

24. Davison KK, Cutting TM, Birch LL. Parents’ activity-related parenting practices predict girls’ physical activity. *Med Sci Sports Exercise* 2003; 35(9): 1589 – 1595.

25. De Kloet R. Hormones and the stressed brain. *Ann NY Acad Sci* 2004; 1018: 1 – 15.

26. DeMello JJ, Cureton JK, Boineau RE, Singh MM. Ratings of perceived exertion at the lactate threshold in trained and untrained men and women. *Med Sci Sports Exercise* 1986; 19: 354 – 362.

27. Demille MM, Kidd JR, Ruggeri V et al. Population linkage disequilibrium across the COMT gene considering promoter region and coding region variation. *Human Genet* 2002; 111: 521 – 537.

28. DeYoung CG, Gretchell M, Koposov RA, Trigollen CM et al. Variation in the catechol-O-methyltransferase Val158Met polymorphism associated with conduct disorder and ADHD symptoms, among adolescent male delinquents. *Psych Genet 2010;* 20(1): 20 - 24.

29. Diatchenko L, Slade GD, Nackley AG et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Mol Genet* 2005; 14: 135 – 143.

30. Dietrich A, McDaniel WF. Endocannabinoids and exercise. *Br J Sports Med* 2004; 38:536–541.

31. Docktor R, Sharkey BJ. Note on some physiological and subjective reactions to exercise and training. *Percept Mot Skills* 1971; 32(1): 233 – 234.

32. Dunbar CC, Robertson RJ, Baun R, Blandin MF et al. The validity of regulating exercise intensity by rating of perceived exertion. *Med Sci Sports Exercise* 1992; 24: 94 – 99.

33. Edwards RH, Melcher A, Hesser CH, Wigertz O et al. Physiological correlates of perceived exertion in continuous and intermittent exercise with the same average power output. *Eur J Clin Invest* 1972; 2(2): 108 – 114.

34. Ekblom B, Goldbarg AN. The influence of physical training and other factors on the subjective rating of perceived exertion. *Acta Physiol Scand* 1971; 83: 399 – 406.

35. Eston RG, Williams JG. Exercise intensity and perceived exertion in adolescent boys. *Br J Sports Med* 1986; 20: 27 – 30.

36. Farrell PA, Gustafson AB, Morgan WP et al. Enkephalins, catecholamines, and psychological mood alterations; effects of prolonged exercise. *Med Sci Sports Exercise* 1986; 19(4): 347 – 353.

37. Felig P, Cherif A, Minagawa A, Wahren J. Hypoglycemia during prolonged exercise in normal men. *New Engl J Med* 1982; 306: 895 – 900.

38. Fillingim RB, Kaplan l, Staud R et al. The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. *J Pain* 2005; 6: 159 – 67.

39. Floderus Y, Wetterberg L. The inheritance of human erythrocyte catechol-O-methyltransferase activity. *Clin Genet* 1981; 19: 392 – 393.

40. Frankenhaeuser M, Post B, Nordheden B et al. Physiological and subjective reactions to different physical workloads. *Percept Mot Skills* 1969; 28(2): 343 – 349.

41. Franklin BA, Vander AL, Wrisley D, Rubenfire M. Aerobic requirements of arm ergometry: implications for exercise training and testing. *Physiol Sports Med* 1983; 11: 81 – 90.

42. Galbo H. 1983. Hormonal and metabolic adaptations to exercise. New York: George Thieme Verlag: 46 – 51.

43. Galbo H, Holst JJ, Christensen NJ. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. *J Appl Physiol* 1975; 38(1): 70 – 76.

44. Gamberale F. Perceived exertion, heart rate, oxygen uptake and blood lactate in different work operations. *Ergonomics* 1972; 15(5): 545 – 550.

45. Gogos JA, Morgan M, Luine V, Santha M et al. Catechol-Omethyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Nat Acad Sci* 1998; 95: 9991 – 9996.

46. Goslin BR, Rorke SC. The perception of exertion during load carriage. *Ergonomics* 1986; 29: 677 – 686.

47. Grossman MH, Emanuel BS, Budarf ML. Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q-11.1 – q11.2. *Genomics* 1992; 12: 822 – 825.

48. Gursoy S, Erdal E, Herken H, Madenci E et al. Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. *Rheumatology Int* 2003; 23: 104 – 107.

49. Hampson DB, Gibson ASC, Lambert MI, Noakes TD. The influence of sensory cues on perceived exertion during exercise and central regulation of exercise. *Sports Med* 2001; 31(13): 935 – 952(18).

50. Horstman DH, Weiskopf R, Robsinson S. The nature of perception of effort at sea level and high altitude. *Med Sci Sports Exercise* 1979; 11(1): 6 – 11.

51. Ibrahim MM, Porreca F, Lai J, Albrecht P et al. CB_2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci* USA 2005; 102(8): 3093 – 3098.

52. Ibrahim MM, et al. CB2 cannabinoid receptor mediation of antinociception. *Pain* 2006; 122:36 - 42.

53. Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. *Med. Sci. Sports* 1979: 11; 1:6 – 11.

54. Janicki P, Schuler G, Francis D, Bohr A et al. A genetic association study of the function A118G polymorphism of the human μ-opioid receptor gene in patients with acute and chronic pain. *Anesthesia Analgesia 2006*; 103(4): 1011 – 1017.

55. Jiang H, Xie T, Ramsden DB, Ho SL. Human catechol-O-methyltransferase down-regulation by estradiol. *Neuropharmacology* 2003; 45: 1011 – 1018.

56. Jones NL, Dyspnea in exercise. *Med Sci Sports Exercise* 1984; 16: 14 – 19.

57. Juhani I, Pekka S, Timo A. Strain while skiing and hauling a sledge or carry a backpack. *Eur. J. Appl. Physiol*. 1986; 55: 597 – 603.

58. Kamon E, Pandolf K, Cafarelli E. The relationship between perceptual information and physiological responses to exercise in heat. *J Human Ergol*. 1974; 3: 45-54.

59. Klepstad P, Rakvag TT, Kaasa S, et al. The 118 A_G polymorphism in the human micro-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease. *Acta Anaesthesiol Scand* 2004; 48:1232–9.

60. Kjaer M. Adrenal medulla and exercise training. *Eur J Physiol* 1998; 77: 195 – 199.

61. Kloet R, Joels EM, Holsboer F. 2005. Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience* 6 (6): 463-475.

62. Kopin I. Catecholamine metabolism: basic aspects and clinical significance. *Pharma Rev* 1985; 37: 333-364.

63. Lachman HM, Papolos DF, Saito T et al. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996; 6: 243 – 250.

64. Li T, Ball D, Zhao J et al. Family-based linkage disequilibrium mapping using SNP marker haplotypes: application to a potential locus for schizophrenia at chromosome 22q11. *Mol Psychiatry*; 5: 452.

65. Li W, Lee AM, Solomon MA. Gender differences in beliefs about the influence of ability and effort in sport and physical activity. *Sex Roles 2006*; 54(1) 147 – 156.

66. Ljunggren G, Ceci R, Karlsson J. Prolonged exercise at a constant load on a bicycle ergometer: ratings of perceived exertion and leg aches and pain as well as measurements of blood lactate accumulation and heart rate. *Int J Sports Med* 1987; 8: 109– 16.

67. Lollgren H, Ulmer HV, and Nieding G von. Heart rate and perceptual responses to exercise with different pedaling speed in normal subjects and patients. *Eur. J. Appl. Physiol.* 37: 297 – 304. 1977.

68. Lotta T, Vidgren J. Tilgmann C et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 1995; 34: 4202-4210.

69. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol. Biomark. Prev* 1998; 7: 719–724.

70. Lundstrom K, Salminen M, Jalanko A et al. Cloning and characterization of human placental catechol-O-methyltransferase cDNA. *DNA Cell Biol* 1991 10: 181-189.

71. Lundstrom K, Tenhunen J, Tilgmann C et al. Cloning, expression, and structure of catechol-O-methyltransferase. *Biochem Biophys Acta Prot Struct Mol Enzymol 1995;* 1251: 1-10.

72. Mahon AD, Marsh ML. Reliability of the rating of perceived exertion at ventilatory threshold in children. *Int. J. Sport Med.* 13 (8): 567 – 571. 1992.
73. Mahon AD, Gay JA, Stolen KQ. Differentiated ratings of perceived exertion at ventilatory threshold in children and adults. *Eur J Appl Physiol O* 1998; 78(2): 115 – 120.

74. Mahon AD, Stolen KQ, Gay JA. Differentiated perceived exertion during submaximal exercise in children and adults. *Ped Exercise Sci* 2001; 13: 145 – 153.

75. Mannisto PT, Tuomainen P, Tuomainen RK. Different in vivo propoerties of three new inhibitors of catechol-O-methyltransferase in the rat. *Brit Journ Pharma* 1992; 105: 569-574.

76. Mannisto PT, Ulmanen I, Lundstrom K et al. Characteristics of catechol-O-methyltransferase (COMT) and properties of selective COMT inhibitors. *Prog Drug Res* 1992; 39: 291-350.

77. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharma Rev.* 1999; 51 (4) 593-628.

78. Marbach JJ, Levitt M. Erythrocyte catechol-O-methyltransferase activity in facial pain patients. *J Dent Res* 1976; 55: 711.

79. Maresh CM, Deschenes MR, Seip RL, Armstrong LE et al. Perceived exertion during hypobaric hypoxia in low- and moderate-altitude natives. *Med. Sci. Sports Exercise.* 25: 945 – 951. 1993.

80. Maruyama W, Naoi M, Narabayashi H. The metabolism of L-dopa and L-threo-3,4 dihydroxyphenylserine and their effects on monoamines in the human brain: analysis of the intraventricular fluid from parkinsonian patients. *Journ Neurol Sci* 1996; 139: 141-148.

81. Mastorakos G, Pavaltou M.. Exercise as a stress model and the interplay between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes. *Metab Res* 2005; 37: 577 – 584.

82. Methods for discovering and scoring single nucleotide polymorphisms. National Institutes of Health Guide; 1998

83. Mihevic PM. Sensory cues for perceived exertion: a review. *Med Sci Sports Exercise* 1981; 13 (3): 150-63.

84. Mihevic PM, Gliner JA, Horvath SM. Perception of effort and respiratory sensitivity during exposure to ozone. *Ergonomics* 1981; 24:365-374.

85. Miyashita MK, Onodera, TI. How Borg's scale has been applied to Japanese. In Perceptual exertion in physical work, ed. G Borg and D. Ottoson., 27 – 34. London: Macmillan, 1986.
86. Mogil JS, The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc. Natl. Acad. Sci* 1999; 96: 7744 – 7751.

87. Nackley AG, Tan KS, Fecho K, Flood P, et al. COMT modulates pain sensitivity and cytokine production through both β2 and β3 adrenergic receptors. Society for Neuroscience; Washington DC: 2005.

88. Nackley AG, Shabalina SA, Tchivileva IE et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 2006; 314 (5807): 1930 – 1933.

89. Nackley AG, Soo Tan K, Fecho K, Flood P et al. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both B_2 and B_3 adrenergic receptors. *Pain* 2007; 128(3): 199 – 208.

90. Naveri H, Kuoppasalmi, Harkonen M. Plasma glucagon and catecholamines during exhaustive short term exercise. *Eur. J. Appl. Phys.* 2004; 53 (4): 308 – 311.

91. Nieman DC, Carlson KA, Brandstater ME et al. Running endurance in 27-h-fasted humans. *J. Appl. Physiol.* 63: 2502 – 2509. 1987.

92. Noble BJ, Borg GA, Jacobs I, et al. A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Med Sci Sports Exercise* 1983; 15 (6): 523-8.

93. Noble BJ, Borg G, Jacobs I et al. A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Med. Sci. Sports Exercise.* 14: 406 – 411. 1982.

94. Noble BJ, Metz KF, Pandolf KB, Cafarelli E. Perceptual responses to exercise: a multiple regression study. *Med Sci Sports* 1983; 5:104–109.

95. Noble BJ, Robertson RJ. Perceived Exertion. Champaign, IL: Human Kinetics; 1996.

96. Oswald LM, McCaul M, Choi L et al. Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary-adrenal axis responses to naloxone: a preliminary report. *Biol Psychol* 2004; 55: 102 – 105.

97. Pandolf KB, Cafarelli E, Noble BJ, et al. Perceptual responses during prolonged work. *Percept Mot Skills* 1972; 35 (3): 975-85.

98. Pandolf KB, Noble BJ. The Effect of pedaling speed and resistance changes on perceived exertion for equivalent power outputs on the bicycle ergometer. *Med Sci Sports Exerc* 1973; 5(2): vi – xi.

99. Pandolf KB. Differentiated ratings of perceived exertion. *Med Sci Sports Exerc* 1982; 14(5):397 – 405.

100. Pederson PK, Welch HG. Oxygen breathing, selected physiological variables and perception of effort during submaximal exercise. G Borg (ed.) *Physical Work and Effort.* New York: Pergamon Press, 1977, pp. 385 – 400.

101. Perkins KA, Sexton JE, Solberg-Kassel RD, Epstein LH. Effects of nicotine on perceived exertion during low-intensity activity. *Med Sci Sports Exercise* 1991; 23:1283–1288.

102. Reyes-Gibby CC, Shete S, Rakvak R et al. Exploring joint effects of gene and the clinical efficacy of morphine for cancer pain: *OPRM1* and *COMT* gene. *Pain* 2007; (130) 25 – 30.

103. Qian Q, Wang Y, Zhou R, Li J et al. Family-based and case-control association studies of catechol-O-methyltranferase in attention deficit hyperactivity disorder suggest genetic sexual dimorphism. Am J Med Genet B *Neuropsychiatr Genet* 2003; 118(1): 103 – 109.

104. Robertson RJ, Gillespie RL, McCarthy J et al. Differentiated perceptions of exertion: part II: relationship to local and central physiological responses. *Percept. Mot. Skills* 49: 691 – 697. 1979.

105. Robertson RJ. Central signals of perceived exertion during dynamic exercise. *Med Sci Sports Exercise* 1982; 14 (5): 390-6.

106. Robertson RJ, Falkel JE, Drash AL, Swank AM, Metz KF, Spungen SA, LeBoeuf JR. Effect of blood pH on peripheral and central signals of perceived exertion. *Med. Sci. Sports Exercise* 1986; 18: 114 – 22.

107. Robertson RJ, Stanko RT, Goss FL et al. Blood glucose extraction as a mediator of perceived exertion during prolonged exercise. *Eur. J. Appl. Physiol.* 61: 100 – 105. 1990.

108. Robertson RJ, and Metz KF. Ventilatory precursors for central signals of perceived exertion. G Borg and D Ottoson (eds). *The Perception of Exertion in Physical Work.* London: Macmillan, 1996, pp. 111 – 121.

109. Robertson RJ, Gillespie RL, McCarthy J et al. Differentiated perceptions of exertion: part I: Mode of integration of regional signals. *Percept. Mot. Skills* 1997; 49: 683 – 689.

110. Robertson RJ, Moyna NM, Sward KL, Millich NB et al. Gender comparisons of RPE at absolute and relative physiological criteria. *Med Sci Sports Exerc*; 32(12): 2120 – 2129.

111. Salminen M, Lundstrom K, Tilgmann C et al. Molecular cloning and characterization of rat liver catechol-O-methyltransferase. *Gene* 1990; 93: 241-247; 1990.

112. Sargeant AJ, Davies CT. Perceived exertion during rhythmic exercise involving different muscle masses. *J Hum Ergol Tokyo* 1973; 2 (1): 3-11.

113. Sargeant AJ and Davies CT. Perceived exertion of dynamic exercise in normal subjects and patients following leg injury. G Borg (ed). *Physical Work and Effort.* New York: Pergamon Press, 1977, pp. 345 – 356.

114. Satchidanand N. Psychosocial differences in attainment of physiological criteria for maximal oxygen uptake. *Med Sci Sports Exercise* 2009; 41(5): 84.

115. Selye H. The general adaptation syndrome and the diseases of adaptation. South Med Surg. 1951; 113: 315 – 323.

116. Shifman S, Bronstein M, Sternfeld et al. A highly significant association between a COMT haplotype and schizophrenia. *Am J. Hum Genet* 2002; 71: 1296-1302.

117. Simoneau JA, Bouchard C. Human variation in muscle fiber type and enzymatic activities. *Endo and Metab* 1989; 257 (4): E567 – E572.

118. Singh A, Petrides JS, Gold PW et al. 1995. Differential hypothalamic-pituitary-adrenal axis reactivity to psychological and physical stress. *J. Clin Endo Metab* 84: 1944 – 1948.

119. Skinner JS, Borg G, Buskirk ER. Physiological and perceptual reactions to exertion of young men differing in activity and body size. *Exercise and fitness*. Ed. BD Franks, 53 - 66. Chicago: Athletic Institute. 1969.

120. Skinner JS, Hutsler R, Bergsteinova V, et al. Perception of effort during different types of exercise and under different environmental conditions. *Med Sci Sports* 1973; 5 (2): 110-5.

121. Smith A. Peer relationships in physical activity contexts: a road less travelled in youth sport and exercise psychology research. *Psychol Sport and Exercise* 2003; 4(1): 25 – 39.

122. Smutok MA, Skrinar GS, Pandolf KB. Exercise intensity: subjective regulation by perceived exertion. *Arch Phys Med Rehabil* 1980; 61 (12): 569-74.

123. Sparling PB, Giuffrida A, Piomelli D, Rosskopf L et al. Exercise activates the endocannabinoid system. *NeuroRep* 2003; 14(17): 2209 – 2211.

124. Squires RW, Rod JL, Pollock ML, Foster, C. Effect of propranolol on perceived exertion soon after myocardial revascularization surgery. *Med. Sci. Sports Exercise* 1982; 14(4): 276 – 280.

125. Sullivan P. Spurious genetic associations. *Biol Psychol* 2006; 61 (10): 1121 – 1126.

126. Stevens JC, Krimsley A. Build-up of fatigue in static work: role of blood flow. *In Physical work and effort*. Ed. G. Borg, 145 – 56. New York: Pergamon Press. 1977.

127. Tilgmann C, Kalkkinen N. Purification and partial characterization of rat liver soluble catechol-O-methyltransferase. *FEBS Lett* 1990; 264: 95-99.

128. Tilgmann C, Kalkkinen N. Purification and partial sequence analysis of the soluble catechol-O- methyltransferase from human placenta; comparison to the rat liver enzyme. *Biochem Biophys Res Commun* 1991; 174: 995-1002.

129. Toner MM, Drolet LL, Pandolf KB. Perceptual and physiological responses during exercise in cool and cold water. *Percept Mot Skills* 1986; 62:211–220.

130. Toner MM, Drolet LL and Pandolf KB. Perceptual and physiological responses during exercise in cool and cold water. *Percept. Mot. Skills* 70: 591 – 594. 1990.

131. Tong HJ and Hwang O. Dopamine β-hydroxylase: biochemistry and molecular biology. *Ann. New York Acad. Sci* 2006; 493: 342 – 350.

132. Turnbridge EM, Harrison PJ, Weigberger DR. Catecholo-O-methyltransferase, cognition, and psychosis Val158Met and beyond. *Bio Psychiatry* 2006; 60: 141 – 151.

133. Urhausen A, Weiler B, Coen B et al. Plasma catecholamines during endurance exercise of different intensities as related to the individual anaerobic threshold. *Eur Journ Appl Physiol* 1994; 69: 16-20.

134. Vargas-Alarcon G, Fragoso J, Cruz-Robles D et al. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. *Arthritis Res and Thera.* 9 (110) 1 -7.

135. Vidgren J. Tilgmann C, Lundstrom K et al. Crystallization and preliminary x-ray investigation of a recombinant form of rat catechol-O-methyltransferase. *Prot Struct Funct Gen* 1991; 11: 233- 236.

136. Vidgren J, Svennsen LA, Lijas A. Crystal structure of catechol-O-methyltransferase. *Nature (Lond)* 1994; 368: 354-358.

137. Vidgren J, Ovaska M. Structural aspects in the inhibitor design of catechol-O-methyltransferase, in *Structure-based Drug Design (*Veerapandian P ed.) pp 343-363, Marcel Dekker, Inc. NY. 1997.

138. Vidgren J. Ovaska M, Tenhunen et al. Catechol-O-methyltransferase, *in Structure and Function of AdoMet-dependent Methyltransferases* (Cheng X and Blumenthal RM eds.) pp 55-91, World Scientific, Singapore. 1999.

139. Viru A. 1985. Hormones and muscular activity. Boca Raton: CRC Press. vol. 1: 195.

140. Williams-Gray C, Hamshire A, Robbins T, Owens A et al. Catechol-O-methyltransferase val158met genotype influences frontoparietal activity during panning in patients with Parkinson's disease. *J Neurosci* 2007; 27: 4832 – 4838.

141. Winqvist R, Lundstrom K, Salminen M et al. The human catechol-O-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows frequent RFLP with *Bg/*I. *Cytogen Cell Genet 1992;* 59: 253-257.

142. Woo J-M, Yoon K-S, Choi Y-H, Oh K-S et al. The association between panic disorder and the L/L genotype of catechol-O-methyltransferase. J Psychiatr Res 2004; 38: 365 – 370.

143. Xie T, Ho SL, Ramsden D. Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. *Mol Pharma* 1999; 56: 31-38.

144. Zubieta JK, Heitzeg MM, Smith YR et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 2003; 299: 1240-1243.