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Exercise-induced alterations in melanocortin receptor expression and inflammation

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EXERCISE-INDUCED ALTERATIONS IN MELANOCORTIN RECEPTOR EXPRESSION AND INFLAMMATION

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LIST OF ABBREVIATIONS

ACC - acclimation day

ACTH – adrenocorticotrophic hormone

αMSH – alpha melanocyte stimulating hormone

AgRP - agouti-related peptide

ARC - arcuate nucleus

β-AR - beta adrenergic receptor

BMI – body mass index

BW – body weight

Ca - intracellular calcium

CD – cluster of differentiation

C/EBPβ - CCAAT/enhancer binding protein beta

CHD – coronary heart disease

CNS – central nervous system

CRF - corticotrophin-releasing factor

CRH - corticotrophin-releasing hormone

CREB - cAMP response element binding factor

CRP – C-reactive protein

CVD- cardiovascular disease

DXA - Dual X-ray Absorptiometry

Epi – epinephrine

EWAT - epididymal white adipose tissue

FcγR - Fc receptors for immunoglobulin G

FFA - free fatty acid

FM – fat mass

GDP - guanine diphosphate

GPCR – G protein coupled receptor

Gs – stimulatory G protein

GTP - guanine triphosphate

HDL – high density lipoprotein

HPA - hypothalamus-pituitary-adrenocortical

IFNγ **-** interferon gamma

IgG – immunoglobulin

IL-1β – interleukin 1 beta

IL-4 - interleukin 4

IL-6 – interleukin 6

IL-10 – interleukin 10

IL-13 - interleukin 13

IL-12 – interleukin 12

IP3 - inositol phosphate

IPAQ - International Physical Activity Questionnaire

Jak2 - janus kinase 2

LBM – lean body mass

LBP - LPS binding protein

LDL – low density lipoprotein

LPS – lipopolysaccharide

LRR - leucine-rich repeat

LSU – Louisiana State University

MAPK - mitogen activated protein kinase

MCR – melanocortin receptor

MD2 - lymphocyte antigen 96

MFI – mean fluorescence intensity

NE – norepinephrine

NF-kB - nuclear factor-kappa B

NOS - nitric oxide synthase

NPY - neuropeptide Y

PDE - prostaglandin E

PKA – protein kinase A

POMC – proopiomelanocortin

POST – after training

PRE – before training

PVN - paraventricular nucleus

RER – respiratory exchange ratio

RMR- resting metabolic rate

SH2 - Scr homology 2

SNS – sympathetic nervous system

SOCS - suppressors of cytokine signaling

STAT - signal transducer and activator of transcription

T2D – type 2 diabetes

TLR – toll like receptor

TNFα – tumor necrosis factor alpha

Tyk2 - tyrosine kinase 2

ABSTRACT

Inflammatory cytokines play a significant role in the pathogenesis of obesity-related diseases and have been implicated as integral factors in both early and late phases of atherosclerosis. Lifestyle modifications such as increasing physical activity and making dietary changes to induce weight loss are part of the primary prescription for the treatment of metabolic syndrome. Additionally, physical activity has been implicated as a potentially effective regimen for the control of inflammation, yet little is known about the anti-inflammatory mechanistic alterations induced by physical activity.

Exercise training causes acute changes in inflammation immediately post exercise, evidenced by upregulation of inflammatory cytokines and increased activity of leukocytes. Production of inflammatory cytokines leads to increased circulating levels of monocytes and macrophages as well as other immune cells. Additionally, chronic changes in inflammation occur after recurring bouts of exercise, evidenced by upregulation and production of anti-inflammatory cytokines and increased sensitivity of macrophages to stimuli. Previous publications from our laboratory have found that whole blood samples from exercise trained individuals possess significantly increased sensitivity to lipopolysaccharide (LPS) stimuli, resulting in lower levels of interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis alpha (TNFα). Others have also noted the decrease in circulating levels of inflammatory cytokines chronically with exercise training.

The research presented in this dissertation is novel in that it explores the expression of the melanocortin receptors (MCR) on the plasma membranes of systemic monocytes, indicating a novel role for the MCRs as anti-inflammatory receptors that are regulated by chronic exercise training. The melanocortin 1 receptor (MC1R), melanocortin 3 receptor (MC3R), and

melanocrotin 5 receptors (MC5R) are expressed on immune cell populations. Several lines of *in vitro* research have shown that activation of the MC3R on cultured macrophages leads to upregulation of the anti-inflammatory pathways, suggesting a novel role for MCRs in mediating exercise-induced inflammation. Here, I show that 12 weeks of resistance training in a young, healthy population decreases both MC1R and MC3R receptor density on systemic monocytes in conjunction with decreases in C-reactive protein (CRP). Furthermore, the percentage of circulating monocytes expressing MC1R increases whiles those expressing MC3R decreases in response to resistance training.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Inflammation and Macrophages

Inflammation has emerged as a focal point in the development of many diseases, including obesity, type 2 diabetes (T2D), metabolic syndrome, and cardiovascular disease (CVD), and is recognized as a potential causative agent and side effect of atherosclerosis and insulin resistance [1-5]. Inflammation refers to the recruitment and activation of leukocytes, such as macrophages to injured areas of the body as well as upregulation and increased secretion of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor alpha (TNFα) [1-2]. Recent investigations examining the etiology of inflammatory associated diseases show that chronic inflammation, evidenced by activation of immune cells such as monocytes and macrophages, can augment the onset of such diseases [1-5]. Additionally, pro-inflammatory cytokine serum concentrations, as well as concentrations within various tissue microenvironments, are significantly elevated in several disease states [3-4]. Studying leukocytes, specifically monocyte and macrophage populations, is essential for understanding immune abnormalities associated with chronic inflammatory disease states.

Monocyte and macrophage populations are key leukocytes responsible for the local production of a variety of pro-inflammatory and anti-inflammatory cytokines that play an important immunomodulatory role in the body [6-9]. Systemic, circulating monocytes are some of the first responders to tissue damage within the body and are large contributors to pro-inflammatory cytokine production, including IL-6, TNFα, and IL-1β, during times of inflammation. Conversely, monocytes and macrophages help alleviate inflammation by secreting anti-inflammatory cytokines, such as IL-10 [1-2]. Anti-inflammatory cytokines provide negative feedback in both autocrine and paracrine manners by inhibiting further production of pro-inflammatory cytokines from monocytes as well as other leukocytes. Shifts in macrophage

activation from a pro-inflammatory to an anti-inflammatory state may ameliorate the effects of chronic inflammation and slow the progression of related diseases.

1.2 Effects of Exercise on Inflammation

Exercise training has been used as a successful intervention in changing inflammatory profiles in several populations, including populations afflicted with obesity, T2D, and CVD [10-13]. A large body of evidence supports the role of exercise training in decreasing chronic inflammation [13-22]; yet, the mechanisms involved in creating these favorable changes remain elusive. The lack of a consensus on the underlying mechanisms responsible for exercise-induced decreases in chronic inflammation may be partially due to the high variation in exercise training regimens utilized during intervention studies, as studies indicate that modality, intensity, and duration may impact changes in inflammatory profiles. The degree of metabolic demand and the level of fatigue are major contributors to changes in cytokine production that may be linked to complex interactions between hormonal changes, metabolic by-products, motor unit recruitment, myofibrillar disruption, and increases in body temperature [23-25]. Several lines of research support the theory that metabolic changes in conjunction with muscle damage are responsible for exercise-induced inflammation [23-29]. For example, high levels of IL-6 are found in conjunction with glycogen depletion [26]. Evidence also shows a positive correlation between lactate levels and IL-6 in young male wrestlers [27] as well as between muscle soreness and IL-6 [24, 28]. Furthermore, recruitment of larger muscle groups during an exercise bout leads to larger numbers of circulating leukocytes afterwards [29-30]; and high intensity exercise (≥ 75% VO2max) leads to secretion of IL-1 and IL-6 more so than moderate or low intensity exercise [29-30]. Other causes of change observed in acute systemic cytokine levels following an exercise bout may also be explained by blood redistribution following the exercise bout [31]. While strenuous exercise leads to a redistribution of blood flow away from the splanchnic organs and to

contracting muscle, less strenuous exercise allows for normal blood flow to the splanchnic, including renal blood flow [32]. Additionally, cytokine production with strenuous exercise may show a larger systemic increase due to less clearance through urinary output, as previous studies show that cytokines are removed in the urine following an exercise bout [33]. Thus, the increased circulating time for inflammatory cytokine clearance during strenuous exercise may cause the larger downstream adaptation to anti-inflammatory cytokine production.

In addition to the regulatory processes involved in immune response adaptations to acute and chronic exercise, the physiological changes that occur in response to chronic exercise that also contribute to changes in the inflammatory profile remain unknown. Exploration of the regulatory pathways that may be modified by exercise training is convoluted by differences in the length, intensity, duration, and modality of the exercise regimens prescribed. Despite these differences, the strength of varying protocols is that an overall consensus still develops in the realm of exercise science, as several investigations utilizing differing interventions have noted changes in the inflammatory profile in response to exercise training [14-15, 21-23, 25, 28, 34-39]. Interactions of metabolic demand, fatigue, and muscle damage may also contribute to physiological adaptations present after chronic, repeated bouts of resistance training and may be the most crucial factor in contributing to changes in inflammatory cytokine production, as dramatic physiological changes in muscle have been noted after the first few resistance training bouts [40]. It is possible that the effects of chronic exercise training may cause adaptations of the immune response to occur due to repeated acute upregulation of pro-inflammatory cytokines that may desensitize leukocytes to these cytokines [38]. Conversely, it is my hypothesis that there may be no desensitization but rather leukocyte sensitization to pro-inflammatory cytokines in response to chronic exercise, leading to a faster or more efficient response of the anti-inflammatory pathways to stimuli. This action may eventually result in a blunted response,

where TNFα and other pro-inflammatory cytokines are acutely depressed after an exercise bout in trained individuals compared to untrained individuals. Despite immense variation in exercise training protocols, significant advances have been made in recent years towards identifying possible regulatory processes involved in chronic inflammation through leukocyte stimulation [13, 16, 18-19, 41-42]. Specifically, advances in the study of exercise training's effects on systemic monocytes have largely been made by identifying extracellular receptors that regulate pro-inflammatory pathways and secretion of pro-inflammatory cytokines from monocytes [18-19].

Interestingly, there are no studies exploring the possible identification of plasma membrane receptors that regulate anti-inflammatory pathways and secretion of anti-inflammatory cytokines from monocytes as a consequence of resistance exercise training. This section will focus on the effects of exercise training on circulating markers of inflammation that are known to regulate inflammation.

1.2.1. Acute Exercise and Inflammation

Exercise training causes acute changes in inflammation immediately post exercise, evidenced by upregulation of inflammatory cytokines and increased activity of monocytes and macrophages [1-2]. Specifically, heavy resistance training has been shown to acutely upregulate systemic inflammatory cytokines and increase circulating levels of monocytes and other immune cells [15, 17, 20-22, 25, 27, 30, 43-46].

IL-6: IL-6 is a major pro-inflammatory cytokine studied in the area of exercise physiology. IL-6 is involved in the signaling cascade activated acutely after exercise training that leads to upregulation of other pro-inflammatory cytokines and acute phase proteins, including CRP. In addition to recruiting other immune cells to the area of damage, IL-6 and IL-1β are also known to regulate the synthesis of CRP [47-49]. Specifically, IL-6 can regulate transcription of

CRP by increasing CCAAT/enhancer binding protein beta (C/EBPβ) binding to the C/EBP binding site centered at position -53 on the CRP promoter [47, 50].

CRP: Due to its high correlation with the development of atherosclerosis and CVD, measurement of CRP levels have become increasingly popular in establishing risk of myocardial infarction and may serve as a more reliable and accurate marker of chronic inflammation than pro-inflammatory cytokines [51-58]. Thus, CRP is rising in importance in medical communities as a global marker of inflammation that may be as reliable as measuring blood cholesterol high density lipoproteins and low density lipoproteins in an effort to determine health status [57].

1.2.2 Chronic Exercise and Inflammation

Contrary to the acute manifestations of exercise, chronic, recurring bouts of exercise lead to an overall decrease in inflammation, evidenced by decreased recruitment of leukocytes into the circulation as well as increased sensitization of macrophages to inflammatory stimuli. Studies suggest that after repeated bouts of exercise, neutrophil recruitment to damaged muscle is attenuated by 10-45% and inflammatory response receptor presentation is altered [23]. Recruitment of leukocytes may be largely dependent on beta adrenergic receptor (β-AR) stimulation on circulating leukocytes by catecholamines, as blockade of the β2-AR by propanolol ameliorates recruitment of monocytes and lymphocytes acutely post exercise [59-60]. Furthermore, catecholamine stimulation decreases whole blood levels of TNFα and IL-1β and increases secretion of IL-10 from macrophages [42, 61-62]. Thus, a viable hypothesis of exercise-mediated decreases in chronic inflammation may be that the transient increases in catecholamine and pro-inflammatory cytokine release seen acutely, leads to activation of monocyte plasma receptors and anti-inflammatory pathways effectively dowregulating pro-inflammatory cytokine production. Indeed, chronic changes in inflammation occur after

recurring bouts of exercise, evidenced by increased anti-inflammatory cytokines, such as IL-10, in circulation and increased sensitivity of macrophages to stimuli [15].

IL-10: IL-10 is considered an anti-inflammatory cytokine due to its ability to inhibit the release of the inflammatory cytokines from monocytes and macrophages, including IL-6 [8]. IL-10 works to decrease inflammation through activation of the JAK-STAT signaling pathway. Briefly, IL-10 binds its receptor on the plasma membrane of the monocyte, activating the tyrosine kinases, janus kinase 2 (Jak2) and tyrosine kinase 2 (Tyk2). Jak2 and Tyk2 then phosphorylate the signal transducers and activators of transcription (STAT1, STAT2, and STAT5) and help regulate gene expression. IL-10 acts in an anti-inflammatory manner by inhibiting pro-inflammatory responses through inhibition of nuclear factor-kappa B (NF-kB) [63].

1.3 Melanocortin 1 Receptor and Melanocortin 3 Receptor

Physical activity is known to create favorable changes in the cytokine profile of individuals and is a viable treatment for slowing the progression and preventing the onset of associated low-grade inflammatory diseases [10, 13, 15-16, 18, 41]. Although physical activity is known to decrease circulating inflammatory cytokine levels, the mechanism behind these changes remains unknown. With the recent finding that stimulation of melanocortin 3 receptors (MC3R) on immune cells leads to upregulation of anti-inflammatory cytokine production, MC3R activation provides a potential mechanism for exercise-induced changes in cytokine profiles [41, 64-65]. MCRs are G-protein coupled transmembrane receptors that act through Gs (stimulatory G protein) to increase cAMP [66]. There are 5 known melanocortin receptors, MC1R through MC5R, whose roles are diverse and global in the body. Thus, while the finding that MC3R activation on macrophages upregulates IL-10, two other melanocortin receptors, melanocortin 1 receptor (MC1R) and melanocortin 5 receptors (MC5R), are also found on

immune cells and the MC1R may support the role of MC3R in its anti-inflammatory effects, although MC5R does not appear to play a role in immunomodulation on monocytes [6-7, 66-68]. This section will focus on specific MCRs and their regulation of the immune response within monocyte and macrophage populations.

1.3.1 Melanocortin 1 Receptor

MC1R and MC3R have been shown to have redundant immunomodulatory roles within macrophage cell lines. MC1R activation by agonist, including pro-opiomelanocortin peptide (POMC) cleavage products alpha melanocortin stimulating hormone (α-MSH) or adrenocorticotrophic hormone (ACTH), leads to upregulation and secretion of IL-10 [69-70]. MC1R is thought to increase anti-inflammatory actions through downregulating cluster of differentiation 86 (CD86) expression on monocytes [69], a co-stimulatory protein that leads to secretion of cytokines and activation of immune cells.

1.3.2 Melanocortin 3 Receptor

Like MC1R, activation of the MC3R by agonist, alpha melanocortin stimulating hormone (α-MSH) or adrenocorticotrophic hormone (ACTH), causes secretion of IL-10 [71]. Studies have shown that exercise increases levels of ACTH acutely which then acts on the adrenal cortex to cause secretion of cortisol, a stress response hormone, but the ACTH response is blunted with exercise training [72]. Acute increases in ACTH may have significant implications for the present project since it may activate MC1R or MC3R on systemic monocytes, leading to secretion of anti-inflammatory cytokines, such as IL-10, that then inhibit IL-6, TNFα, and IL-1β secretion [6, 8, 41, 71, 73]. Indeed, activation of the MC3R on macrophages is known to inhibit IL-6 and TNFα secretion [8, 71, 73-75].

1.3.3 Research Focus

Overall the goal of this research project is to analyze changes in protein expression and to determine how modifications to monocyte function can contribute to chronic inflammation and associated disease states, such as CVD, obesity, and T2D. Specifically, I will examine the role of resistance training in modulating anti-inflammatory receptors on the plasma membrane of systemic monocytes. The work included in this dissertation explores a novel role for the MC1R and MC3R in regulating anti-inflammatory processes associated with chronic exercise training. The specific aims for the project are as follows.

Specific Aim 1: Hypothesis: MC1R and MC3R expression and density on plasma membranes of circulating monocytes changes in response to chronic, repeated resistance training bouts.

Objectives:

A. Determine if MC1R cell surface expression on systemic monocytes changes in response to resistance training.
B. Determine if MC3R cell surface expression on systemic monocytes changes in response to resistance training.

Specific Aim 2: Hypothesis: Resistance training will alter CRP levels in conjunction with changes in pro and anti-inflammatory cytokine profiles in college aged individuals.

Objectives:

A. Determine whether resistance training induces a change in CRP levels.
B. Determine whether resistance training induces changes in representative systemic pro-inflammatory cytokines.
C. Determine whether resistance training induces changes in a representative systemic anti-inflammatory cytokine.

Specific Aim 3: Hypothesis: MC1R or MC3R expression correlates to changes in the inflammatory profile in college-aged individuals in response to resistance training.

Objectives:

A. Correlate resistance-training induced changes in MC1R on immune cells and specific monocyte populations in whole blood with changes in markers of inflammation.
B. Correlate resistance-training induced changes in MC3R on immune cells and specific monocyte populations in whole blood with changes in markers of inflammation.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Impaired energy homeostasis leads to obesity, a low grade inflammatory state that precedes metabolic syndrome and its associated diseases, such as T2D, CVD, and atherosclerosis [76]. Currently, more than half of all U.S. adults are overweight or obese. Metabolic syndrome, itself, is generally characterized by dyslipidemia, hypertension, insulin resistance, and obesity and was estimated to affect 76 million people in the United States alone in 2007 [77]. Recent investigations examining the etiology of obesity-related diseases show that chronic inflammation, evidenced by activation of immune cells such as monocytes and macrophages, can be a determinant in the onset of obesity [1-5]. In addition, macrophages are responsible for the local production of a variety of cytokines, including the inflammatory cytokines IL-1β, IL-6, and TNFα as well as anti-inflammatory cytokines, such as IL-10 [6-9]. These cytokines play an immunomodulatory role in the development of obesity, and serum levels of IL-6, IL-1β, and TNFα, as well as levels within various tissue microenvironments, are significantly elevated in metabolic syndrome [3-4]. Physical activity is known to create favorable changes in the inflammatory profile of individuals and is a viable treatment for slowing the progression and preventing the onset of metabolic syndrome, T2D, CVD, and atherosclerosis [10, 13, 15-16, 18, 41]. Consequently, lifestyle modifications such as increasing physical activity and making dietary changes to induce weight loss constitute the primary prescription for the treatment of metabolic syndrome [78]. Even though physical activity has been implicated as a potentially effective regimen for the control of inflammation, little is known about the anti-inflammatory mechanistic alterations that occur with exercise.

Physical activity may affect inflammation through changes in cytokine expression in the

central nervous system (CNS) as well as in peripheral tissues. Extensive research in the 1990s has focused on the role of the CNS in the immune response, yet little attention has been paid to how these effects manifest in the peripheral and tissue-specific immune response. Melanocortin 3 and 4 receptors (MC3R and MC4R, respectively) are G-protein coupled receptors that are found in the CNS [79-81], peripheral tissues, and leukocytes [67, 82] and may act in mediating the inflammatory response [7-8, 83-84]. Thus, the MCRs may play a crucial role in advancing our knowledge of how the CNS communicates with the periphery in affecting inflammation. Current research on the MC3R and MC4R focuses on their role in obesity, a disease state associated with chronic inflammation [85-87]. More novel research on the MC3R has begun to focus on understanding its role in the immune response. The most established model for MCR activation is best understood in its regulation in a signaling cascade that controls adiposity and satiation within the hypothalamus [85-88]. This model states that MCRs are activated in the hypothalamus when leptin, secreted from adipocytes, binds leptin receptors on the pro-opiomelanocortin (POMC) neurons, causing the activation of POMC neurons and release of the POMC polypeptide. Once translated, POMC is then proteolytically cleaved into various peptides, including α-, β-, and γ- melanocyte-stimulating hormone (MSH), and adrenocorticotropic hormone (ACTH). In the hypothalamus, ACTH, γ-MSH, or α-MSH (cleaved from ACTH) can bind to the MCR, acting as an activating agonist [83, 89-90].

In other independent studies involving the culture of macrophages, studies show that activation of the MC3R on macrophages can occur by subjecting the cells to media containing either cyclic α-MSH (MT-II) or ACTH1-39. Once activated, there is an increase in IL-10 found in the cell-free supernatant from the macrophages [91]. IL-10 is known for its effects as an anti-inflammatory cytokine, inhibiting pro-inflammatory cytokine (IL-6, TNFα, and IL-1β)

secretion from macrophages [15, 62, 74, 84]. ACTH production, itself, can be induced by IL-1β. IL-1β is one of several pro-inflammatory cytokines secreted by macrophages during the acute phase inflammatory response. IL-1β is also known to activate corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) located in the hypothalamus [89]. PVN neurons project to the hypophyseal portal system and noradrenergic neurons in the brainstem and POMC neurons in the hypothalamus. Induction of CRH neurons leads to activation of the POMC neurons that secrete α-MSH and ACTH which may lead to increased activation of MC3R in the hypothalamus as well as in peripheral systemic macrophages [92]. Thus, with the recent finding that immune cells possess MC3Rs and stimulation of the MC3R with agonist leads to upregulation of anti-inflammatory cytokine production from macrophages, the MC3R provides a potential mechanistic link for exercise-induced changes in cytokine profiles [41, 64-65].

2.2 Pro-inflammatory and Anti-inflammatory Response

2.2.1 Inflammation and Macrophages

Inflammation involves several physiological responses of the body to tissue/cellular damage or infection, may be specific or nonspecific, and may be beneficial or harmful in specific situations. The nonspecific inflammatory response is induced by infection or injury, resulting in vasodilation and increased blood flow to the injured/infected area, increased infiltration of bodily fluids into intracellular spaces, and increased number of leukocytes, such as neutrophils, lymphocytes, and monocytes, in the area [93]. Responses are dependent on the presence of adhesion molecules and inflammatory mediators, such as prostaglandins, histamine, and serotonin [94]. Additionally, pro- and anti-inflammatory cytokines are chemical signals that play a role in recruiting leukocytes to the area of injury. Leukocytes are defined as white blood cells or derivations of hematopoietic stem cells from the bone marrow and are found in several lymphoid tissues as well as the blood and lymph circulation. Leukocytes are broadly classified

into two groups; granulocytes, containing granules within the cytoplasm, and agranulocytes, without granules in the cytoplasm. Within these classifications, leukocyte populations can be subdivided into five different types. Granulocytes include neutrophils, basophils, and eosinophils. Agranulocytes include lymphocytes and monocytes. As part of the acute cellular response to injury, neutrophils are the first line of defense, followed by lymphocytes and monocytes as part of the chronic cellular response when sufficient damage or infection occurs [95].

As the immune system is extensive in its responses to infection/injury and involves the communication and function of various cell types, it is impractical to review all immune responses. Thus, the focus of this review will be on monocyte/macrophage responses and populations, because they large producers of inflammatory cytokines that are widely studied in response to exercise training within the peripheral circulation. Monocytes are the immature form of macrophages that exist in the vascular endothelium and, in small fractions, in circulating blood. Monocytes make up 10-15% of the circulating leukocyte population and primarily function in phagocytosis, antigen presentation, cytokine production, and cytotoxicity [96-98]. There are several different factors involving various signaling cascades that induce maturation of bone marrow derived monocytes into macrophages upon stimulation, including induction by autocrine, paracrine, and endocrine cytokines. Maturation requires several steps; hematopoietic stem cell to myeloid stem cell to monoblast to promonocyte to monocyte in the bone marrow and then monocyte in the blood vessel [99]. During the various stages of differentiation, monocytes express different cell surface proteins with specific receptor or adhesion molecule functions [100]. Chemoattractants lead to upregulation of adhesion molecules on the cell surface of circulating monocytes and signal through the various cell surface receptors to recruit derived and differentiated monocytes to an injured tissue [101]. Adhesion molecules allow the binding of

monocytes to endothelial cells for movement of the monocytes into the tissue. In addition, the immune response leads to vasodilation, effectively slowing blood flow and monocyte movement through the injured/infected area to allow for better recruitment [100]. Thus, monocytes are allowed to move through diapedesis into the affected tissue, where they will then differentiate into macrophages. Different stages of differentiation and combinations of cell surface protein expression help determine the function of the monocyte/macrophage within the immune response [100]. For example, monocytes express membrane-bound co-stimulatory molecule recetpors, such as CD14 that acts as a receptor in the recognition of gram negative bacteria [102-103]. Additionally, CD11a, CD25, CD35, CD45RO, and CD45RA are found on the cell surface of circulating monocytes [96, 98, 100]. Once monocytes travel to the site of injury, they differentiate into activated macrophages that remain viable for varying periods of time, lasting up to several months. Macrophages also express several cell surface proteins that are used in their identification and classification, separate from those of monocytes. Macrophages are known to express CD11a, CD16, and CD45RO [96, 98, 100]. CD14 and CD16, a mediator of phagocytosis, are the most widely used markers in the exercise literature for the recognition and differentiation between monocyte and macrophage populations [102-103]. Heterogeneous populations of macrophages exist within injured/infected tissues and are maintained by continual influx from the circulation as well as local proliferation [102]. Fully differentiated macrophages create a signaling cascade that induces recruitment of other leukocytes to the site of injury and continue to regulate the inflammatory response as well as play a role in angiogenesis [104-105]. Chemical signals produced from macrophages include: bioactive lipids (prostaglandins, prostacyclins, thromboxans, leukotrienes), glutathione, complement components, clotting factors, proteinases (elastase, collagenase, angiotensin convertase, stromelysin), protease inhibitors, peptidases, lipases, ribonucleases, heat shock proteins, apolipoprotein E, and

cytokines (IL-1, IL-6, IL-8, TNFα, interferon γ (IFNγ), macrophage inflammatory proteins, regulatory growth factors) [99]. Due to the secretion of various cytokines and other recruiting factors, macrophages are part of the nonspecific cell-mediated immune response. Although they may use antibody receptors such as CD receptors to recognize pathogens, macrophages are not considered part of the humoral immune response, as they do not secrete antibodies in response to antigens in order to neutralize the antigen [106].

2.2.2 Cytokines: IL-6 and IL-10

Pro- and anti-inflammatory cytokines help maintain inflammatory homeostasis [15, 62, 74, 84]. Inflammation is tightly regulated, as injury / infection requires a quick response in the form of physiological changes at the site of injury / infection as well as a quick reversion of the response to its former physiological state. An inability to revert leads to chronic inflammation generally associated with several disease states [1-5]. Thus, cytokines are soluble intercellular regulatory proteins that play a critical role in mediating the inflammatory response by acting as paracrine, endocrine, or autocrine hormones. Cytokines are secreted by leukocytes, as well as tissues such as muscle, and bind extracellular receptors on target cells to induce activation of second messenger systems, leading to specific gene transcription [107]. Pro-inflammatory cytokines are generally acute phase stimulants that signal to the hypothalamus to increase body temperature through the production of prostaglandins of group E (PGE2) as well as acting to alter metabolism and gene regulation within tissues such as the liver [99]. Pyrogenic pro-inflammatory cytokines include: IL-1β, IL-6, and TNFα.

IL-6: IL-6 is both a cytokine and myokine, meaning it's released from immune cells and myocytes [41, 108]. Its role in the inflammatory response is, at best, controversial. In myocytes, it is known to correlate with energy mobilization and body temperature [109]. IL-6 acts by binding cytokine receptors that activate the Janus kinase-signal transducers and activators of

transcription (JAK-STAT) pathway [110]. The IL-6 receptor is a homodimer found within the plasma membrane in which each protein possesses a single-pass transmembrane [111]. Activation of the receptor activates associated JAK molecules that in turn phoshorylate the cytokine receptor [111], Phosphorylation of the cytokine receptor recruits proteins containing phosphotyrosine-binding Scr homology 2 (SH2) domains, such as STATs. Recruited STATs are also phosphorylated by JAK molecules at the site of the receptor, forming a scaffold for the binding and phosphorylation of additional STATs [110]. STATs form homodimers that translocate to the nucleus and act as transcription factors. Negative regulation of cytokine receptors occurs by inhibiting STAT phosphorylation, involving suppressors of cytokine signaling (SOCS), mechanically blocking activated STAT at the promoter region by protein inhibitors of activated STATs (PIAS), or by dephosphorylation of cytokine receptors and STATs by protein tyrosine phosphates [110].

IL-10: Upregulation of the pro-inflammatory cytokines leads to significantly increased circulating numbers of monocytes, lymphocytes, and granulocytes [41-42] and a subsequent induction and upregulation of anti-inflammatory cytokines. IL-10 is an anti-inflammatory cytokine that acts to inhibit the release of the inflammatory cytokines, TNFα, IL-6, and IL-1β, from monocytes and macrophages [8] as well as block neutrophil activation [99], thus alleviating inflammation at the site of injury / infection. Once IL-10 binds its receptor, the JAK-STAT signaling pathway is activated. Jak2 and Tyk2, both tyrosine kinases, are activated and phoshporylate the transcription factors STAT1, STAT2, and STAT5, ultimately controlling gene expression. IL-10 is thought to inhibit pro-inflammatory responses of the cell by inhibiting NF-kB activity [63].

2.2.3 Acute Phase Protein: CRP

CRP is an acute phase protein secreted mainly by the liver in response to inflammatory stimuli as part of the acute phase reaction within the immune system [112]. CRP is known to bind to low density lipoprotein (LDL), at type of cholesterol that is increased in atherosclerosis, and thus CRP plays a role in atherogenesis [113]. Large chronic increases in plasma CRP levels are now commonly used as a marker of inflammation and as a possible risk factor for CVD [113]. Upregulation of CRP production and secretion from hepatocytes is dependent on the presence of the pro-inflammatory cytokine, IL-6 [114]. IL-6 may be released from systemic immune cells, including monocytes and macrophages, and upregulates transcription factors, such as C/EBPβ, that are needed for CRP transcription [115]. Regulation of IL-6 levels may also lead to regulation of CRP levels at the transcriptional level. Additionally, measure of CRP plasma levels may be a better indicator of inflammation in resting plasma samples due to its persistent upregulation during chronic inflammation, unlike more transient cytokines such as IL-6 or IL-10 measurements in plasma, whose levels appear to be more transient in nature.

2.2.4 Pro-inflammatory Cytokines and the CNS Connection in the Periphery

Intercommunication between the nervous system and the lymphatic tissues exists, where the CNS, particularly the hypothalamus-pituitary-adrenocortical axis (HPA), controls the immune and inflammatory responses through the use of complex pathways involving electrochemical signals [83, 89, 116]. This interconnecting web of pathways has been demonstrated in many publications using lipopolysaccharide (LPS), a protein found on the outer membrane of gram-negative bacteria, to mimic bacterial infection and immunostimulation [83, 89, 117]. Whole body responses to LPS include release of IL-1β and TNFα from macrophages and, consequently, an increase in T-lymphocyte cell activation [118-119]. T-lymphocytes then secrete IL-6, increasing the number of activated macrophages, leading to increased secretion of

pro-inflammatory cytokines [120]. Furthermore, studies show that IL-1β, TNFα, and IL-6 are all potent stimulators of the HPA axis. When rats are injected intracranially or peripherally (subcutaneous) with LPS, there is an acute upregulation of plasma ACTH and corticosterone levels and pro-inflammatory TNFα, IL-6, and IL-1β protein levels [83, 89]. This same response can be seen in human whole blood samples exposed to LPS [62]. The existing data suggest that the first pro-inflammatory responder to LPS is TNFα followed by IL-1β and IL-6. Chronic stimulation with LPS leads to attenuation of ACTH and corticosterone circulating plasma levels with chronically upregulated circulating IL-1β levels but not IL-6 or TNFα [83, 89]. The chronic hypersecretion of IL-1β seen with LPS injection creates a blunted and short-lived acute response of rats to LPS injection [83]. It has also been shown that a reciprocal pathway between the HPA and periphery occurs, where stimulation of neurons with IL-1β, TNFα, or IL-6 leads to secretion of corticotrophin-releasing hormone (CRH) and, subsequently, ACTH [84]. Similar inflammatory responses occur within the systemic circulation and CNS in response to LPS stimulation.

2.2.5 Anti-inflammatory Cytokine IL-10 in the Periphery and CNS

Interleukin 10 is an anti-inflammatory cytokine produced by macrophages in response to LPS stimulation. IL-10 is referred to as an anti-inflammatory cytokine because it acts to inhibit the release of the pro-inflammatory cytokines, TNFα, IL-6, and IL-1β, from monocytes and macrophages [8]. Peripheral administration of IL-10 in rats causes inhibition of IL-1β secretion in response to LPS stimulation in the brain stem and periphery but not the hypothalamus [9]. Conversely, central administration of IL-10 causes inhibition of IL-1β secretion in response to LPS stimulation in the hypothalamus and brain stem but not in the periphery [9]. This suggests a major role of IL-10 in subduing activation of the HPA axis in response to pro-inflammatory

cytokines, where pro-inflammatory cytokines, such as IL-1β, in the periphery cause activation of the HPA and secretion of corticotrophin-releasing factor (CRF), ACTH, and POMC.

2.3: Melanocortin Receptors in the Central Nervous System

2.3.1 Melanocortin Receptors are G Protein Coupled Receptors

Melanocortin receptors belong to a family of intramembrane receptors known as G protein coupled receptors (GPCRs). GPCRs possess 7 transmembrane domains, with an extracellular ligand binding site and an intracellular carboxy terminus linked to a G protein [121]. Activation of the receptor occurs when ligand binds and the associated G protein is activated. G proteins exist as heterodimers consisting of three separate subunits; Gα, Gβ, and Gγ [122]. Over twenty different isoforms of Gα subunit can be found in mammalians, all of which bind to guanine nucleotides, both guanine diphosphate (GDP) and triphosphate (GTP) [122]. In the case of MCRs, Gαs (s meaning stimulatory) is the isoform activated when ligand binds to the receptor. Gαs, formerly associated with GDP, undergoes an allosteric change that allows GTP to replace GDP in the guanine nucleotide binding site and Gαs dissociates from Gβ and Gγ. Gαs plus GTP then activate the enzyme, adenylyl cyclase [123]. GTP is hydrolyzed to GDP by the GTPase activity of Gαs and adenylyl cyclase converts ATP to cyclic AMP (cAMP) [124]. The now inactive Gαs associates with the Gβ and Gγ subunits again, the receptor is phosphorylated to reduce its response to ligand [125]. cAMP is a second messenger that causes amplification of the ligand's effect on the cell by activating cAMP response element binding factor (CREB) and protein kinase A (PKA) [126]. CREB is a transcription factor that binds to the nucleic acid sequence 5'-TGACGTCA-3' within the promoter region of various genes causing their subsequent upregulation [65].

In addition to the upregulation of the cAMP second messenger system, evidence exists to suggest that activation of several MCRs (MC2R and MC3R) by ligand activates other second

messenger systems involving inositol phosphate (IP3) and intracellular calcium (Ca) [127]. Upregulation of the IP3 / Ca second messenger is indirectly related to upregulation of cAMP production in agonist-stimulated MC3R transfected cells. PKA is a negative regulator of IP3 / Ca, thus induction of MCR and subsequent increases in intracellular cAMP, leading to concominant increases in PKA, may be the regulatory pathway controlling inhibition of IP3 / Ca upregulation after MCR stimulation [127]. The third transmembrane domain of MCR is thought to be the regulatory loop of the receptor that couples to the necessary G protein, conferring upregulation of specific, separate second messenger systems [127]. One explanation for the dependence of the specific systems is a post translation modification, such as phosphorylation of the third transmembrane loop. Increases in PKA may lead to phosphorylation of the regulatory domain of MCR, changing the conformation of the protein and leading to the binding of different G protein subunits with subsequent upregulation of separate second messenger systems.

2.3.2 Melanocortin Receptors and Metabolism: MC3R and MC4R

The brain is the most complex organ of the body and is involved in many functions, including intercepting and interpreting endocrine signals from other organs. Specifically, the hypothalamus is considered the central unit of satiety comprehension and metabolic signaling [80, 85, 128-130]. The brain is a major player in the battle against obesity and T2D. It is known from extensive research on the arcuate nucleus (ARC) and lateral hypothalamus that there are specific neurons, POMC neurons, which receive endocrine signals from peripheral adipose tissue in the form of hormones [80, 85, 128, 131]. Leptin is one such hormone which binds to receptors located on the POMC neurons [80, 128, 131]. Leptin is an endocrine hormone secreted from adipocytes, the circulating amount of which is directly dependent on the amount of adipose tissue present. Leptin travels through the blood stream, crosses the blood brain barrier, and binds to leptin receptors located on the plasma membrane of POMC neurons. Once bound, the protein

POMC is released from the neurons and later proteolytically cleaved into various differing peptides, including: ACTH, α, β, and γ-MSH [132]. ACTH, β-MSH, and α-MSH are all agonistic peptides able to bind the melanocortin 3 (MCR3) and 4 (MCR4) receptors, located in the ARC, lateral hypothalamus, dorsomedial hypothalamic nucleus (DMH), and the PVN of the hypothalamus [79, 132]. The MC3R and MC4R are also under the control of agonistic competition through the effects of agouti-related peptide (AgRP) and neuropeptide Y (NPY), peptides also released by neurons in the hypothalamus and controlled by leptin expression [80, 85, 128-130]. When leptin is present in the brain and the MC3R and MC4R are bound by their agonistic peptides, the sympathetic nervous system (SNS) is stimulated to release norepinephrine (NE) leading to increased thermogenesis and energy expenditure as well as inhibition of food intake [86, 133], positively correlating with an increase in lipolysis and fatty acid oxidation [134-135]. When leptin is absent, AgRP binds to MC3R and MC4R, preventing agonists from binding [136], whereas NPY binds to separate receptors located on the POMC neurons, antagonizing the release of agonistic peptides [137]. Under antagonistic conditions, hyperphagia ensues, energy expenditure decreases, and fatty acids are converted to triglycerides for storage in adipose tissue.

Although the pathway of leptin signaling in the brain seems complete, our understanding of the mechanisms underlying the roles of MC3R and MC4R are not fully understood and the distinct actions of each receptor with regards to nutrient sensing, satiety, and metabolism remain to be elucidated. Thus, it becomes necessary to speak of both receptors when the role of one receptor is being examined. Extensive studies have found that mice lacking the MC4R are hyperphagic, eating as much as three times that as WT mice, hypometabolic, and exhibit increased body weight as well as increased fat mass [87-88, 138-140]. MC3R knock out (KO) mice also develop increased fat mass, although this occurs through a different mechanism than that of MC4R KO mice. MC3R KO mice are not hyperphagic, hypometabolic, or overweight.

Instead they exhibit differences in nutrient partitioning, where fat free mass (FFM) is sacrificed in order to conserve and even increase fat mass (FM) [87-88, 139]. Furthermore, when injected with leptin, MC4R KO mice exhibit ablation of hyperphagia with compromised SNS activation, whereas MC3R KO mice show full activation of the SNS with failure of leptin to reduce food intake [86]. When MC4R KO mice are fed a diet with moderate (7%) increases in fat, metabolic efficiency is further exacerbated, leading to larger increases in adiposity [87, 141-143]. When MC3R KO mice are fed a diet with moderate (7%) increases in fat they still do not exhibit increases in body weight or become hyperphagic, although they do exhibit increases in adiposity and metabolic efficiency [87, 141-143].

Thus, it is obvious that MC4R and MC3R are mediators not only in food intake and SNS activation, but also in nutrient partitioning, and metabolic efficiency. The separate roles of each remain to be completely elucidated, but it can be concluded that, generally, MC4R is used in the comprehension of satiation and food intake, whereas the MC3R is mainly used in the comprehension of nutrient composition of food and subsequently nutrient partitioning.

2.4. Melanocortin Receptors and the Immune Response

The immune response is greatly interconnected with various parameters of metabolism, where metabolic pathways are highly regulated, independent of metabolic changes, during inflammation. Disturbance in metabolic homeostasis can lead to hypoxia, decrease in intracellular pH, release of lysosomal enzymes, disruption of cell membranes, and decreased mitochondrial function. Dysfunctional mitochondria are the cause of impaired oxidative phosphorylation and insufficient ATP production [144-146]. Cells must utilize anaerobic respiratory pathways, glycogen, and creatine phosphate stores to create ATP for survival. Depletion of glycogen and creatine phosphate rapidly occurs, leading to accumulation of intracellular sodium and water in addition to potassium leakage [146]. Protein synthesis halts as

a result of damaged endoplasmic reticulum and ribosomes. Calcium accumulates in the mitochondria and necrosis of the cell occurs. This process is recognized by the body as cellular damage and induces the inflammatory response [99]. The inability to maintain metabolic homeostasis has been associated with infection and inflammation and is generally believed to be the product of changes in cytokine expression rather than the initiators of changes in cytokine expression. Therefore, researchers hypothesize that low level chronic inflammation may actually precede the manifestation of several diseases, such as T2D, metabolic syndrome, and cancer.

2.4.1 Melanocortin Receptors and the Immune Response in the CNS: MC3R

The melanocortin system, meaning the POMC neurons and MCRs and their agonists and antagonists, is one pathway that is highly regulated in the CNS to control metabolic homeostasis as well as inflammation. It has been shown that LPS injections increase production of leptin from peripheral adipose tissue and decreases food intake in hamsters. Futhermore, injections of TNFα or IL-1 also increase secretion of leptin from epididymal white adipose tissue (EWAT) despite decreases in food intake [147-148]. As stated previously, leptin is an endocrine hormone that travels through the blood from peripheral adipose tissue to the brain, where is can bind leptin receptors on POMC neurons, leading ultimately to activation of MC3R. In addition to increased secretion of leptin from adipose tissue, intraperitoneal injections of human IL-1β in rats increase plasma ACTH levels with a concomitant decrease in ACTH anterior pituitary levels, increases in POMC mRNA in the anterior pituitary, and no difference in CRF in the hypothalamus [149]. It has also been shown that CRF directly upregulates POMC expression in the anterior pituitary [149]. In studies using the AtT-20 corticotroph tumor cell line, it has been shown that IL-6 and Il-1β, but not TNFα, significantly upregulate ACTH secretion [150]. In the same study, by luciferase assay, CRH increases POMC promoter activity. Promotor activity is also minimally upregulated with TNFα, IL-6, or IL-1β, but when CRH is added along with either cytokine, the

effects of CRH alone on POMC promoter activity are potentiated [150]. Thus, modulation of the HPA axis during the immune response can be initiated by cytokines in the hypothalamus or pituitary, causing activation of POMC neurons by leptin, transcription and secretion of POMC by IL-6, IL-1β, TNFα and CRH, leading to increased ACTH secretion by protease activity of the POMC peptide. Speculation leads to the conclusion that MC3R activation by ACTH or its derivative peptide, α-MSH, in the HPA axis is important in mediating pro- and anti-inflammatory responses as well as associated diseases, including obesity.

2.4.2 Melanocortin Receptors and the Immune Response in the Periphery: MC1R, MC3R, and MC5R

The role of MCRs in the inflammatory response extends from the CNS to peripheral tissues where MC3Rs are known to exist on granulocytes, lymphocyres, macrophages, and monocytes [67, 82]. The inflammatory and metabolic pathways involving the MC3R in the CNS is mimicked in these peripheral immune cells, especially macrophages. POMC is expressed in lymphocytes subpopulations and monocytes [67]. POMC-derived ACTH, α-MSH, β-MSH, and γ-MSH are also expressed within immune cells, including lymphocytes. Studies show that activation of MC3R on macrophages by α-MSH or ACTH inhibits IL-6 and TNFα production in macrophages [7, 75]. In human monocytes, there is an increase in α-MSH production in response to IL-6 or TNFα stimulation [151]. Though macrophages are known to possess both MC1R and MC3R, and both are activated by ACTH and α-MSH, recent studies indicate that MC1R or the MC3R on macrophages are sufficient in mediating the peripheral anti-inflammatory response in monocytes and macrophages while the function of MC5R on monocytes / macrophages remains unknown [69-70].

Other recent studies have explored the role of MCRs in inflammation by using the MC4R KO and MC3R KO mouse models. MC4R KO mice exhibit increased macrophage infiltration into adipose tissue, a phenomenon normally associated with inflammation. In addition, when

placed on a high fat diet where MC4R KO mice become extremely obese, macrophage infiltration in adipose tissue is exacerbated and chronic inflammation ensues, leading to insulin resistance [152-153]. On the other hand, MC3R KO mice only become obese when placed on a high fat diet, yet an increase in macrophage number within the adipose tissue of obese MC3R KO mice is not seen nor is an upregulation of TNFα mRNA [152-153]. One explanation is that the MC3R presence on macrophages and within the CNS is crucial for the inflammatory response to take place. Thus, it is clear that the role of MC3R in mediating the immune response is necessary in the CNS as well as in peripheral tissues, and these pathways are intertwined.

In order to fully understand the biological mechanisms underlying the immune response and how we can manipulate the response in order to help diseased individuals, it is necessary to study the connections between the immune response in the CNS and periphery. Studies have extensively shown that activation of the POMC neurons occurs during inflammation in response to pro-inflammatory cytokines; IL-6, IL-1β, and TNFα [154-155]. This translates to increased ACTH secretion in the hypothalamus [154]. Studies also show that circulating POMC and ACTH levels are increased during inflammation [154-155]. In other independent studies, it is known that macrophages, responders to stress and initiators of the immune response, possess MC3Rs. MC3R are important in regulation of metabolic responses in the CNS. They are activated by various derivatives of POMC, including ACTH, α-MSH, β-MSH, and γ-MSH, in the hypothalamus. Furthermore, MC3Rs on cultured peritoneal macrophages show activation in response to stress, mimicking the response of MC3R in the CNS. In response to activation at the MC3R, macrophages secrete IL-10, an anti-inflammatory cytokine, which in turn inhibits secretion of IL-6, IL-1β, and TNFα from macrophages. Additionally, activation of the MC1R on macrophages shows a redundant role of these MCRs in mediating the anti-inflammatory processes of macrophages. It remains unclear if the MC1R and MC3R may be activated in

response to different external stimuli or cause differential cellular processes to occur, as both receptors are able to bind the same agonists and seem to elicit the same cellular responses. Hence, research that focuses on the changes of MC1R and MC3R during acute and chronic inflammation will help to elucidate the mechanisms behind MCR activation and their subsequent effects on infection and disease, providing the necessary connection between peripheral and central inflammatory pathways and disease prevention.

2.5 Effects of Exercise on the Immune System

2.5.1 Effects of Exercise on Inflammation Related Disease Development

Exercise is an easily controlled and utilized form of therapy for many diseases, such as T2D, atherosclerosis, metabolic syndrome, and CVD. A major therapeutic effect of regular exercise is its potential to decrease inflammation, as some of the most prominent diseases in today's society coincide or are preceded by the presence of chronic inflammation. For instance, CVD is currently the leading cause of death in Western countries, accounting for approximately 40% of annual deaths in the United States. There are several forms of CVD; coronary heart disease (CHD), cerebrovascular disease, hypertension, heart failure, and rheumatic heart disease. The leader in deaths amongst the various forms of CVD is CHD, with CHD killing approximately 445,687 deaths in 2005 alone [77]. Development of CHD involves atherosclerosis, a buildup of plaque in the arteries that leads to luminal narrowing and decreased compliance. Initiation of atherosclerosis begins with injury and endothelial dysfunction, causing recruitment of leukocytes, specifically monocytes, to the area. Monocytes then migrate into the intima of the vessel wall, where they mature into macrophages and begin endocytosis of low density lipoprotein (LDL) cholesterol [156-157]. Once LDL cholesterol molecules are engulfed by the macrophage, the cell is referred to as a foam cell. Foam cells remain present in the intima chronically and increase in number over time. Eventually, the foam cells form plaque within the

vessel walls. Atherosclerosis is initiated as an inflammatory response to injury of the vessel and proceeds into chronic inflammation of the area [158]. Build up of plaque causes decreased responsiveness of the vessel to stimuli that normally control dilation of the vessel and compliance to changes in blood pressure [159]. Additionally, dislodging of the plaque from the vessel wall into the circulation may cause vascular blockage and tissue ischemia, stroke, or death. Because initiation of atherosclerosis is believed to be due to injury and subsequent recruitment of monocytes as well as additional leukocytes to the area, CHD is largely controlled by inflammatory cells and cytokines [57].

Whereas CHD is the number one killer of Americans, T2D was listed in 2005 as the seventh leading cause of death in the United States. T2D may be correlated with CHD, as 68% and 16% of the T2D deaths in 2005 in people aged 65 or older also exhibited heart disease and stroke, respectively [160]. Additionally, 75% of those diagnosed with T2D also possess hypertension. Given that CVD and T2D appear to afflict the same populations, it is no surprise that T2D is also linked to chronic low-grade inflammation. Diabetics exhibit increased levels of blood glycoproteins, acute phase proteins, and elevated leukocyte counts [161-163]. Elevated cytokine levels themselves are thought to be a predictor of T2D [163]. Furthermore, elevated levels of inflammatory cytokines are positively correlated with insulin resistance, the distinguishing factor in type 2 diabetics. Also correlated with T2D is obesity, a major cause of metabolic syndrome [164]. Obesity promotes inflammation in adipose tissue as well as the circulation. It is thought that obesity-related inflammation can lead to insulin resistance and further tissue damage in the pancreas [165]. Thus, obesity may be an underlying factor in the etiology of T2D that creates a vicious cycle of chronic inflammation and insulin resistance.

Closely related to T2D, metabolic syndrome is a newly recognized disease that encompasses several of the risk factors seen not only in T2D but also in CHD. Metabolic

syndrome, also referred to as syndrome X or dysmetabolic syndrome, is characterized by abdominal obesity, insulin resistance or glucose intolerance, hypertension, dyslipidemia, and, most notably, inflammation. It was estimated that in 2007 approximately 76 million people in the United States can be classified as having metabolic syndrome [77]. Coincidently, 44% of obese adolescents develop metabolic syndrome [77]. One of the most highly associated factors of metabolic syndrome is chronic inflammation. Sufferers of metabolic syndrome exhibit high levels of circulating pro-inflammatory cytokines, including IL-6 and TNFα [166]. Because obesity is a major contributing factor to metabolic syndrome, it is plausible that chronic inflammation associated with metabolic syndrome is a direct result of increased adiposity. Several researchers note an association between obesity and chronic inflammation, with some hypothesizing that the pro-inflammatory state of adipose tissue is a precursor to obesity [166].

As some of the most prevalent diseases in today's society all involve a state of low level chronic inflammation, reduction in risk factors for these disease states all include increasing physical activity. Indeed, a large body of evidence that shows an inverse relationship between increased physical activity levels and decreased inflammation supports the anti-inflammatory effects of exercise. Studies show that fitness level is a stronger predictor for death than smoking, high cholesterol, and diabetes [167]. Notably, exercise alone can increase high density lipoprotein (HDL) cholestrol and insulin sensitivity, improve body composition, and decrease plasma triglycerides, cholesterol, blood pressure and inflammatory cytokines [13, 159, 168-169]. Studies show that those who take part in a regular exercise program have much lower incidence of CHD than sedentary counterparts [167]. Additionally, development of CHD in physically active populations, if it does occur, has been shown to be less severe and develop at a later age [167]. Exercise promotes insulin independent uptake of glucose into working muscles, helping to control plasma glucose levels [170]. As a result, tissues become more insulin sensitive over time

[167]. Physical activity helps to alleviate insulin resistance in T2D and metabolic syndrome, even allowing some diabetics to lower or stop taking medications related to these diseases. Exercise decreases circulating levels of pro-inflammatory cytokines, such as those associated with T2D, obesity, and metabolic syndrome [171-172]. Not only is exercise an excellent therapeutic strategy to alleviate the development of a multitude of diseases, but it is also an excellent model to use in the study of the immune response due to its ease of implementation and few detrimental side effects.

2.5.2 Effects of Exercise on Pro and Anti-Inflammatory Cytokines in Peripheral Tissues

During exercise, there is tissue injury leading to inflammation. Thus, the effects of exercise mimic the initiation of the immune response. During muscle contraction, IL-6 is secreted from myocytes as well as systemic leukocytes. IL-6 is known to activate macrophages, leading to secretion of IL-1β and TNFα. Secretion of IL-1β and TNFα recruit other immune cells to the site of injury. The inflammation associated with exercise is generally referred to in two separate phases, the acute and chronic phases. Acute changes in inflammation occur immediately post exercise and can continue for over four hours after the completion of exercise [15]. Chronic changes in inflammation can last for weeks or months after chronic, recurring exercise. The two phases are distinctive from each other due mainly to the differences in cytokine expression. During acute phase, local tissue injury causes upregulation of circulating and muscle specific pro-inflammatory cytokines, including IL-6, IL-1β, and TNFα [15, 108]. Upregulation of the pro-inflammatory cytokines in turn leads to significantly increased circulating levels of macrophages, NK cells, monocytes, lymphocytes, granulocytes, T-helper, and T-cytotoxic cells [41-42]. Acute exercise also induces increased activity of macrophages [42]. Mechanisms underlying the acute increase in pro-inflammatory secretion observed following exercise can be contributed to activation of the p38 mitogen activated protein kinase (MAPK) and nuclear factor-

Kβ (NF-Kβ) pathways [147]. MAPKs are serine / threonine kinases that are responsible for controlling gene expression through phosphorylation of various proteins, including nitric oxide synthase (NOS). NOS is responsible for producing NO, which has antimicrobial and tumoricidal activity, in macrophages. NF-Kβ is a transcription factor responsible for enhancing NO production in macrophages by enhancing transcription of NOS [173].

Generally, after chronic exercise, there is an increase in circulating levels of IL-10, leading to decreased secretion of pro-inflammatory cytokines, IL-1α and TNFα [11]. There is also increased sensitivity of macrophages to stimuli and increased levels of NO production [10]. Although there may also be an increase in IL-6 during chronic exercise, this may be due to the fact that IL-6 is both a myokine (secreted by myocytes) and a cytokine. In fact, it has been postulated that the production of IL-6 from muscle during exercise induces the secretion of IL-10. IL-10 may then act to decrease circulating levels of IL-1β and TNFα [34].

2.5.3 Effects of Exercise on CRP

Several studies support the role of exercise in decreasing circulating CRP levels [38, 174-177]. While studies utilizing aerobic exercise interventions show beneficial effects of exercise in lowering body weight, adiposity, and increasing fitness, aerobic exercise has not been shown to create the same level of favorable changes in CRP levels as resistance training [38, 174-177]. For example, our lab, in conjunction with Dr. Tim Church's lab at Pennington Biomedical Research Center, has shown that 6 months of regular aerobic exercise at incremental doses of (50%, 100%, and 150%) of the NIH Consensus Panel physical activity recommendation on cardiorespiratory fitness failed to reduce CRP in healthy, overweight/obese postmenopausal women [37, 178].

Conversely, several independent studies have shown that resistance training significantly decreases CRP levels, decreases IL-6 and IL-1β concentrations, increases the anti-inflammatory cytokine IL-10, and induces overall alterations in chronic inflammation [39, 174-175]. Given

that CRP is transcriptionally regulated by the presence of IL-6 [15, 47, 50], resistance training induced alterations in CRP transcription and translation may be aided by alterations in the pro-inflammatory cytokine, IL-6 as well as IL-1β. IL-6 and IL-1β production from monocyte / macrophages is further negatively impacted by the synthesis and production of the anti-inflammatory cytokine, IL-10 [15]. Thus, even in younger, healthy populations, it appears that resistance training exerts the most beneficial effects on chronic inflammation by changing circulating inflammatory cytokine profiles and inducing changes in global markers of inflammation, such as CRP.

2.5.4 Effects of Exercise on Inflammation in the CNS

Given the relationship between the CNS and periphery in the inflammatory response, it's no surprise that exercise exerts its effects not only in peripheral tissues but also through signaling pathways in the CNS. Exercise, like IL-1β, activates CRH neurons in the hypothalamic PVN [89]. PVN neurons in turn project to the hypophyseal portal system and noradrenergic neurons in the brainstem and POMC neurons in the hypothalamus. Thus, induction of CRH neurons as a result of exercise leads to the release of the neurotransmitters, NE and epinephrine (Epi), as well as activation of the POMC neurons that secrete α-MSH and ACTH. Exercise also causes an increase in MC3R mRNA in the hypothalamus [179]. In the periphery, NE inhibits TNFα and IL-6 production through activation of beta adrenergic receptors (β-AR) in human whole blood extracts stimulated with LPS [62]. Studies also show that isoproteronol, a β-AR agonist, inhibits TNFα but not IL-6 production after exercise [41-42]. Furthermore, it is known that muscle secretes IL-6 in response to contraction. Thus, it appears that the peripheral changes seen during exercise may stimulate the pathway associated with the immune response in the CNS, which in turn may regulate peripheral inflammation during the acute and chronic phases of exercise.

2.6 Immune Cell Response to Exercise

2.6.1 Population Response to Exercise

Acute bouts of exercise not only lead to an increase in inflammatory cytokine secretion from leukocytes in the periphery, but also lead to acute leukocytosis, or increased leukocyte recruitment into the circulation [14, 20]. The increases seen are proportionally related to the intensity of the exercise bout [23]. It is believed that increases in circulating leukocytes are due to increases in SNS stimulation, and leukocytosis is largely due to increases in NK cells and neutrophils [14, 20-21, 25]. Muscle damage that occurs during an exercise bout, causing NK cell and neutrophil recruitment, induces release of pro-inflammatory cytokines into the circulation and recruitment of other leukocytes to the area. After the exercise bout, macrophages become the predominant leukocyte type in the damaged muscle [180]. Furthermore, pro-inflammatory cytokines are replaced by anti-inflammatory cytokines being released from the newly activated macrophages. Macrophages may remain within the tissue, releasing cytokines, for up to fourteen days after the exercise bout, whereas neutrophils are transiently recruited for up to twenty-four hours after the exercise bout [29]. Studies report that activated neutrophils and macrophages release the pro-inflammatory cytokines IL-1β and TNFα up to 5 days after an exercise bout [29]. These transient systemic increases in pro-inflammatory cytokines acutely after exercise may be attributed to secretion from contracting myocytes instead of secretion from recruited leukocytes [181]. Support for this hypothesis has been documented in several studies that reveal that acute spontaneous cytokine production within circulating monocytes is not the source of increased TNFa or IL-6, although these cytokines are significantly elevated in serum after exercise [21]. Transient increases in pro-inflammatory cytokines, whether initially produced from circulating monocytes or contracting myocytes, lead to recruitment and activation of leukocyte subpopulations, resulting in possible production and secretion of pro-inflammatory cytokines

from monocytes. Furthermore, activation of monocytes by pro-inflammatory cytokines, independent of their source of production, will result in changes in anti-inflammatory cytokine production from activated monocytes. Evidence shows that, acutely, exercise simultaneously upregulates circulating levels of pro-inflammatory cytokines as well as anti-inflammatory cytokines and pro-inflammatory cytokine "inhibitors" [23, 25, 27, 29, 33]. The hypothesis of simultaneous secretion is that muscle contraction leads to the production of the pro-inflammatory cytokines, recruiting and activating leukocytes, which in turn produce and secrete anti-inflammatory cytokines [29].

Contrary to the acute manifestations of exercise, chronic, recurring bouts of exercise lead to an overall decrease in inflammation, evidenced by decreased recruitment of leukocytes into the circulation as well as increased sensitization of macrophages to inflammatory stimuli [17]. One of the first endocrine responses to an exercise bout is an increase in the secretion of Epi and NE from the adrenal glands. Increases in SNS output help to increase heart rate, cardiac output, oxygen uptake and metabolism and acts to maintain homeostasis of oxygen uptake to carbon dioxide production, waste production to waste removal, and heat production to heat loss. Chronic exercise training may lead to a desensitization of leukocytes by downregulation of receptors, specifically β-ARs, the receptors responsible for responsiveness of leukocytes to SNS activation [182]. Thus, chronic recurring bouts of exercise leads to attenuated recruitment of certain leukocyte subpopulations into the circulation after an acute bout of exercise. It is plausible that exercise exerts its anti-inflammatory effects, alleviating chronic inflammation, by effectively decreasing leukocyte recruitment to inflamed areas. Indeed, studies suggest that after repeated bouts of exercise, neutrophil recruitment to damaged muscle is attenuated by 10-45% and inflammatory response receptor presentation is altered [29]. Recruitment of leukocytes may be largely dependent on ß-AR stimulation by catecholamines, as blockade of the ß2-AR by

antagonist ameliorates recruitment of monocytes and lymphocytes acutely post exercise [59-60]. Furthermore, catecholamine stimulation decreases whole blood levels of TNFa and IL-1ß and increases secretion of IL-10 from macrophages [61-62]. Another viable hypothesis of exercise-mediated decreases in chronic inflammation may be that the transient increases in catecholamine and pro-inflammatory cytokine release seen acutely leads to activation of macrophage plasma receptors and anti-inflammatory pathways affectively dowregulating pro-inflammatory cytokine production.

2.6.2 Classically Activated vs. Alternatively Activated Macrophages vs. Type II Activated Macrophages

Macrophages exert differential effects through activation of divergent pathways, pro- or anti-inflammatory. Differential effects are a result of a complex hierarchy of macrophage phenotypes, activation, and pathways. Currently, three macrophage phenotypes are recognized; classically activated, alternatively activated, and type II activated macrophages. Classically activated macrophages are characterized by Th1 type response to inflammation, meaning they work to recruit other immune cells to the site of injury, release pro-inflammatory cytokines such as interferon gamma (IFNγ), degrade extracellular matrices, and promote apoptosis [183-184]. Conversely, alternatively activated macrophages are characterized by a Th2 type response, release anti-inflammatory cytokines such as IL-10, construct extracellular matrices, and promote angiogenesis [184]. A critical balance between pro- and anti-inflammatory statuses is maintained by the recruitment of differential macrophage phenotypes to an injured area [183].

Differentiation of macrophages into classically versus alternatively activated is dependent on activation of priming pathways through binding of circulating proteins to plasma receptors on the macrophages. IFNγ, once bound to IFNγ membrane receptors, is an adequate priming factor for classically activated macrophages. After being primed macrophages then commit to the classically activated pathways when challenged by inflammatory stimuli such as

LPS. Classically activated macrophages are responsible for eliciting significant tissue damage when unchecked by promoting degradation of tissue as well as promoting chronic inflammation in the area. Alternatively activated macrophages are used by the body to balance the inflammatory response, counteracting the effects of classically activated macrophages. Alternatively activated macrophages release anti-inflammatory cytokines leading to prevention of chronic inflammation and tissue destruction [184]. Alternatively activated macrophages do not require priming before committing to the alternative pathway; instead circulating cytokines interleukin 4 (IL-4), interleukin 13 (IL-13), or glucocorticoids seem to be sufficient in activating alternative macrophages [184]. When activated, these macrophages also undergo morphological changes, recruit leukocytes to the injured area, and secrete several factors involved in promoting extracellular matrix construction as well as cell proliferation [183].

In addition to classically and alternatively activated macrophages, scientists now recognize a third macrophage phenotype, the type II activated macrophage [184]. Type II activated macrophages are characterized by altered cytokine release upon stimulation. Stimulation causes downregulation of interleukin 12 (IL-12) and increased secretion of IL-10, which in turn causes secretion of IL-4 from T cells. Activation of type II macrophages requires ligation of the Fc receptors for immunoglobulin G (IgG) (FcγR) by antigen IgG complexes plus inflammatory stimuli. Both unprimed and IFNγ primed macrophages may be stimulated to express the type II activated phenotype. In response to stimulation, these macrophages mimic a Th2 response, much like the alternatively activated macrophages [6, 184].

Response to Exercise

Changes seen in cytokine production from circulating macrophages in response an acute versus chronic exercise may be due to differential macrophage phenotype. Studies have shown that chronic resistance and aerobic exercise decrease IFNγ levels in whole blood samples [11,

185]. Given that classically activated macrophages require priming by IFNγ for activation, exercise may attenuate recruitment and stimulation of classically activated macrophages in response to injury. This agrees with the literature that promotes exercise as a therapy for decreasing chronic inflammation, as classically activated macrophages promote inflammation in a Th1 manner [6]. Furthermore, decreases seen in circulating pro-inflammatory cytokines with chronic exercise correlate with the hypothesis that long-term adherence to an exercise training program may preferentially stimulate differential macrophage phenotypes within the circulation [13]. Conversely, exercise may promote recruitment of the alternatively activated macrophage phenotype into the circulation. Exercise has been shown to upregulate IL-4 and increase endocrine glucocorticoids, notably cortisol [116, 186]. Both factors are sufficient to stimulate the alternatively activated macrophage phenotype, possibly increasing levels of circulating anti-inflammatory cytokines [187].

2.6.3 Macrophage Response to External Inflammatory Stimuli

Endotoxin, specifically LPS, is the most prevalent stimuli used to induce inflammation through activation of the innate immune response in experimental research. LPS is a component of gram negative bacteria and acts as an endotoxin to elicit an immune response. LPS is composed of three main components; O polysaccharide, core antigen, and lipid A [117]. Lipid A exists as a phosphorylated dimer of N-acetylglucosamine plus six or seven fatty acid chains that functions as the anchoring region of LPS, conferring the toxicity of LPS to the host [188]. The O polysaccharide confers immunogenicity by binding to tissues. It varies largely in structure amongst bacterial strains, with polysaccharide chains ranging in length and in structure [189]. Once LPS is in the circulation, it promotes inflammation by binding to soluble LPS binding protein (LBP) and soluble or CD14 [190]. CD14 delivers the LPS to the recognition site of the LPS recognition complex on the plasma membrane of the leukocyte, composed of a toll

like receptor (TLR) and lymphocyte antigen 96 (MD2) [191]. When inflammatory stimulus, such as LPS, is recognized by macrophages, it causes phagocytosis and degradation of the pathogen / pathogen component within the macrophage [192]. Morphological changes as well as secretory changes occur, leading to recruitment of neutrophils, immature dendritic cells, NK cells, and activated T cells to the site. LPS stimulated macrophages further recruit leukocytes by secreting pro-inflammatory cytokines, including IL-1β, IL-6, and TNFα .

Whereas LPS creates a pro-inflammatory environment through activation of TLRs on macrophages, β-ARs are known to be involved in creating an anti-inflammatory response when activated on macrophages. Much like MCRs, β-ARs are GPCRs that activate G proteins, activating the enzyme adenylate cyclase, resulting in formation of the transcriptional factor cAMP. Three subtypes of β-AR have been identified; β1-AR, β2-AR, and β3-AR. All three receptor subtypes can lead to activate of the G protein subtype, Gs (stimulatory) and increase cAMP. Additionally, the β2-AR can also activate the Gi subtype (inhibitory), inhibiting increases in cAMP. β-ARs can be stimulated by several agonists, including the catecholamines Epi and NE. Varying β-AR subtypes can be localized to specific tissues throughout the body. Most notably, the predominanat β-AR subtype on macrophages is the β2-AR. Catecholamines have been shown to play a role in inflammation by inhibiting IL-6, IL-1, and TNFα production from mononuclear cells, most likely through β-AR activation [193-194]. In addition, stimulation of β2-ARs on whole blood leukocytes have been shown to attenuate LPS induction of TNFα production and secretion [42, 195]. Catecholamines also decrease IL-1β secretion in whole blood in response to LPS [62]. It is suggested that decreases in pro-inflammatory cytokine production in whole blood by LPS stimulation preceded by catecholamine or β-AR agonist is mediated by increases in IL-10, as IL-10 increases are seen after catecholamine stimulation [62]. Additionally, LPS stimulation of macrophages causes decreases in β2-AR in macrophages [196].

Given the attenuation of inflammation normally associated with LPS treatment of macrophages in cultures first stimulated with β-AR agonist, it appears that there may be cross talk between β-ARs on macrophages and TLRs. It is plausible that the anti-inflammatory effects of exercise are partially mediated by elevated catecholamines during exercise, leading to increases in β-AR activation on circulating macrophages, that in turn decreases the pro-inflammatory response to muscle damage that normally induces activation of the TLRs and production and secretion of pro-inflammatory cytokines.

Response to Exercise

Exercise creates direct and indirect adaptations of the immune system to inflammatory stimuli. Several studies remark on the ability of chronic exercise to alter immunological response. In addition to exogenous LPS derived from gram negative bacteria being used as an initiator of inflammation in biological studies, endogenous LPS has also been shown to be elevated within the circulation of endurance athletes after long races (89.4km) by 81% and these changes seemed to correlate with fitness level of the athlete [197]. Additionally, three weeks after the race, these same athletes experienced no change in LPS concentration following a 21.1km run, perhaps due to exercise-induced adaptation of increased IgG binding to circulating LPS [197]. Changes in LPS concentration found acutely after exercise coincide with acute changes in pro-inflammatory cytokines. Increases in circulating TNFα have been found following 2.5h of running [198] and IL-6 immediately after a 10km [197] or 20km run [199].

Other studies using exogenous LPS stimulation of cultured macrophages from whole blood or cultured peripheral whole blood access the effects of exercise conditioning on immune response; whether exercise enhances or attenuates the immune response. LPS stimulation has been shown to increase TNFα production in peripheral blood cultures following 30 minutes of rowing when compared to controls that did not complete a rowing bout [200]. Chronic, moderate

intensity endurance exercise training in mice also leads to increased sensitivity to LPS stimulation, acutely, with increases in TNFα and IFNγ and concominant decreases in IL-10 (24h after exercise and 24h after LPS stimulation) [196]. Conflicting data on the effects of exercise-induced inflammatory modulations on LPS response in culture do exist. For example, male cyclists showed attenuated TNFα increases in response to LPS stimulation after 3 hours of cycling [36]. Differences in changes of TNFα seen with LPS stimulation may be due to the intensity, duration, or modality of exercise used in the protocol. Also, sampling and assay procedure may affect the outcome of the experiments and account for different results. Another explanation for differences in results may be due to diet in the experimental groups. For example, cyclists were allowed to consume water, tea, or coffee during their experimental trial. Because both coffee and tea contain caffeine, and caffeine increases prostaglandin E (PDE), a protein responsible for inhibiting the breakdown of the transcriptional factor cAMP, variation due to differences in transcriptional regulation of cytokines and differences in catecholamine secretion may account for the differences seen between the aforementioned studies.

Other macrophage plasma receptors also change in response to exercise, independent of inflammatory stimuli. For examples, studies show that exercise changes presentation of several CD receptors on the plasma membranes of circulating macrophages. Increases in CD64L have been found 24h after exercise as well as decreases in CD4 expression at 6 h after exercise, indicating a decrease in monocyte activation [201]. CD64L is a cell surface antigen that allows monocytes to attach to endothelium. Thus, increases in CD64L may be induced during an exercise bout in order infiltrate damaged muscle and help recruit leukocytes to the area to initiate repair. CD4 is a cell surface antigen found on T helper cells and monocytes that is used to "activate" monocytes and induce inflammation. Thus, downregulation of this receptor may lead to changes in inflammatory cytokine production from circulating macrophages / monocytes after

exercise. Additionally, CD64 expression, along with CD18 expression, is shown to decrease after repeated bouts of exercise [29]. Transient increases in activated macrophages may be an indication of enhanced immunity within exercise populations dependent on exercise intensity or duration, corresponding to muscular damage initiated during the bout. Thus, concomitant decreases in damage during chronic exercise may correlate with decreases in CD receptor expression on activated macrophages.

Although CD receptors are good indices of changes in immunity, recent focus on indirect adaptations that may confer alterations with physical activity explore the possibility of TLR2 and TLR4 as the mediating key plasma receptors in the model of exercise-induced inflammation. TLRs are a six member family of transmembrane receptors possessing an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic domain [202-204]. TLRs found on several cell types, including macrophages, which may explain exercise-induced alterations in immune response. TLRs function to recognize pathogens and link the innate immune response to the adaptive immune response. Stimulation of the inflammatory response through TLR2 and TLR4 causes a signaling cascade that promotes inflammation through activation of NF-kB [205-207]. Activation of TLRs on macrophages ultimately leads to production of TNFα, IL-6, and IL-1β [208]. Other adaptations to TLR activation on macrophages include presentation of CD80/86 and major histocompatibilty complex II (MHC II) on the macrophage plasma membrane, both of which allow recognition of the macrophages by other recruited leukocytes in the area [209]. Furthermore, production of pro-inflammatory cytokines is directly correlated with TLR4 expression on macrophages [210]. TLRs may retain the ability to control inflammation in response to exercise, whereby TLR activation on macrophages due to exercise leads to the production and secretion of pro-inflammatory cytokines into the circulation. A viable

explanation whereby repeated bouts of exercise decreases chronic inflammation may be due to changes in cell surface expression of TLRs on systemic macrophages.

Several studies support this hypothesis of exercise-induced changes in the pro-inflammatory receptor, TLR, and inflammation. Prolonged aerobic exercise in the form of cycling (two and half hours at 60% Vo2 max) has been shown to decrease cell surface expression of TLRs on macrophages [209, 211]. Furthermore, decreases in TLR2 and TLR4 with exercise training coincide with decreases in IL-6 production and MHC II and CD80/86 presentation [211]. Other studies utilizing resistance training have confounding conclusions. In a cross sectional study, resistance trained elderly women showed significantly decreased in TLR4 mRNA in conjunction with decreased production of IL-6, IL-1β, and TNFα after LPS stimulation of whole blood cultures when compared to sedentary controls [212]. Yet, in a separate training study, when blood is taken immediately post exercise, TLR4 is significantly increased compared to the control group resting sample but is not different from the exercise trained resting sample [212]. In the same study, however, resting blood levels of TLR4 decreased in exercise-trained individuals compared to untrained control individuals. Furthermore, no changes were seen in TLR4 following a twelve week resistance and endurance training intervention when compared to baseline TLR4 values as well as resting control TLR4 values [22]. Conversely, TLR4 has been shown to decrease in response to a combined endurance and resistance exercise training regimen [38]. Limitations to the present body of data on exercise-induced changes in TLR expression include the training regimen and blood sampling time, which may explain the differences seen in TLR expression in response to training.

2.6.4 Influence of Exercise Mode, Intensity, and Duration on Macrophage Function

In all exercise based studies, it is important to note the differences and similarities in exercise mode, intensity, and duration used as treatment. Mode, intensity, and duration all play a

role in determining activation of differential metabolic systems during an exercise bout; oxidative phosphorylation or glycolytic respiration [213-214]. Oxidative phosphorylation involves the metabolism of glucose, either plasma glucose / glycogen derived glucose or beta oxidation of free fatty acids (FFA), by type I oxidative muscle fibers or type IIa intermediate muscle fibers. Utilization of either glucose of FFA is further dependent on the duration of the exercise bout [213]. Glucose is first utilized during low intensity bouts, followed by oxidation of FFA during longer duration low intensity exercise bouts. Both fuel sources require the presence of oxygen within the mycocyte mitochondria as the final electron acceptor.

Several factors influence the rate at which aerobic versus anaerobic respiration take place. Perhaps foremost oxygen delivery and utilization by myocyte mitochondria determines the type of respiration [215]. Upon inspiration oxygen crosses from the aveoli within the lungs to surrounding capillaries where it binds hemoglobin within the red blood cells (RBC) [216] Oxygenated blood then flows from the lungs to the left atrium then the left ventricle and out to the systemic circulation. During exercise, blood flow is redistributed from the splanchnic organs to working muscles where formation of ATP is critical for continued muscle contraction [217]. Increased amounts of oxygenated blood are delivered to the myocyte. Once at the myocyte, oxygen dissociates from hemoglobin, crosses the sarcolemma, and binds to high affinity myoglobin within the muscle cells. Myoglobin works to deliver the oxygen from the outside of the myocyte to the mitochondria [218]. At the mitochondria, oxygen can be used as the final electron acceptor for hydrogen ions formed from oxidation of NADH and FADH in the electron transport chain, ultimately forming water. The ability of aerobic respiration to take place is dependent on fuel availability, mitochondrial content, mitochondrial enzyme function, hemoglobin content, RBC count, myoglobin content, and fuel source. Conversely, anaerobic respiration utilizes plasma glucose or glycogen derived glucose or phosphocreatine, without the

presence of oxygen, as possible fuel sources for ATP formation and, thus, is not dependent on mitochondrial number or function, hemoglobin, myoglobin, or RBC count. Instead, anaerobic respiration is dependent on fuel availability and enzyme activity.

Additionally, oxidative versus glycolytic forms of respiration are dependent on differential recruitment of muscle fiber type. There are two general phenotypes of recognized muscle fibers; type I and type II fibers. Type I fibers are distinctive under a light microscope by their dark coloration, due to high amounts of myoglobin within the fibers. Type I fibers possess large amounts of mitochondria and are innervated by small diameter alpha motor neurons, causing a slower transmission of electrical signals from the spinal cord needed to depolarize the myocyte. Thus, type I fibers are considered slow twitch fiber types that utilize forms of oxidative phosphorylation [219]. Muscle groups compromised of mostly red fibers are mostly type I fibers and generate less power and velocity of shortening yet can sustain cycling contraction / relaxation for long durations [32]. Type II fibers are lighter in color than type I fibers, indicating less mygoblin content. These fibers also contain less mitochondria than type I fibers and are innervated by larger diameter alpha motor neurons, allowing faster transmission of electrical signals from the spinal cord [219]. Type II fibers can be further subcategorized based on the type of myosin ATPase present in the fiber. Type IIa fibers are generally referred to as intermediate fibers, as they have characteristics found in type I fibers. Type IIa fibers can use either aerobic or anaerobic pathways for ATP formation. Type IIx fibers are generally referred to as fast glycolytic fibers, as they are used during high intensity physical activity of short duration [219]. Muscle groups composed of white fibers are mostly type II fibers that can generate large power output and a faster velocity of shortening but can only sustain cycling contraction / relaxation for short periods of time. In addition some muscles are mixed muscles, meaning they contain both

red and white fibers and can perform either low power long duration or high power short duration exercises [32].

Due to differential recruitment of fiber phenotype with exercise modality, intensity, and duration, there are differential by-products and end products. With both aerobic and anaerobic exercise, by-products include hydrogen ions from the intermediate reactions of glycolysis, lactate from pyruvate if there is little oxygen present, and conversion of lactate into lactic acid [220-222]. Consequently, anaerobic respiration will cause formation of elevated levels of lactate and lactic acid in the circulation in comparison to aerobic respiration. It is important to take these by-products into consideration when evaluating the immune response to exercise, as several researchers have noted that they contribute to decreases in blood and muscle pH, with larger decreases seen in muscle pH. When pH is decreased below optimal levels, respiration will be unable to continue due to dysfunctional enzymes, and exercise performance decreases [222]. It is also plausible that formation of varying amounts of by-products may cause differences in the circulating inflammatory profile and cytokine production. In fact, a positive direct correlation exists between lactate levels and the anti-inflammatory cytokine, IL6, in young male wrestlers [27].

Because various muscle groups are innately different from each other, it follows that recruitment of differing fiber types as well as the amount of fibers or motor units recruited during an exercise bout may play a role in differences seen in exercise-induced inflammation. Evidence suggests that changes in systemic leukocyte number may be due to muscle recruitment, where recruitment of larger muscle groups during an exercise bout leads to larger numbers of circulating leukocytes afterwards and vice versa [29]. Researchers believe that the initial increase in circulating pro-inflammatory cytokines is due to damage of the muscle during an exercise bout. It seems plausible that contraction of large muscle groups and recruitment of more motor

units during an exercise bout may potentially lead to increased damage and inflammation. A positive correlation exists between leukocyte number after acute exercise and degree of soreness, a possible measurement of muscle damage [201].

Another possible explanation for inflammatory differences associated with muscle size may be due to the ability of larger muscle groups to increase core body temperature more effectively than small muscle groups. Increases in body temperature increase activation of heat shock proteins. Heat shock proteins in turn have been implicated in promoting a pro-inflammatory state, in conjunction with pro-inflammatory cytokines [13]. For example, heat shock protein 70 (HSP70) has been shown to increase acutely after an intense endurance exercise Ironman triathlon) bout involving whole body muscular contraction, especially large muscle groups such as the quadriceps and hamstrings [24].

As previously stated, modality, intensity, and duration may all affect the peripheral changes seen in circulating cytokines as well as leukocytes. Studies show high intensity exercise (≥ 75% VO2max) leads to secretion of IL-6 more so than moderate or low intensity exercise [14]. A positive correlation exists between leukocyte number after acute exercise and exercise intensity and soreness [201, 223-224]. Researchers have found that short duration, low to moderate intensity exercise is not without inflammatory benefits. Five or fifteen minutes of low or moderate aerobic exercise in mice both lead to increases in the number of total leukocytes and peritoneal macrophages as well as an increase in the phagocytic activity of the macrophages [225]. Other increases in macrophage phagocytic activity have been noted after an acute bout of endurance exercise (one hour of swimming) in rats and mice (thirty, sixty, and one hundred twenty minutes of treadmill running) [35, 226]. In addition, peritoneal macrophage phagocytosis has been shown to significantly increase in response to anaerobic exercise (plyometrics, 48h after

exercise) in rats that have been immunocompromised by injection with tumor inducing cells [227].

In addition to leukocyte recruitment and differential activation of macrophages as well as increased phagocytic activity of macrophages associated with exercise, mode, intensity, duration, and muscle group contraction also affect cytokine production. Studies show that several modalities of exercise (cycling, running, eccentric, and knee extensor exercises) all significantly increase levels of IL-6 acutely [181]. Other studies report that eccentric elbow flexion in young (20-22 years), untrained male individuals leads to no changes in IL-6; although, the protocol for the eccentric elbow flexion did not mention how much weight was lifted by participants and how this related to the intensity of the eccentric bout. Furthermore, the exercise session only included eccentric elbow flexion, 5 sets of 6 reps with 2 minutes of rest in between sets [17]. It appears that perhaps intensity may play a larger role in exercise-induced inflammation than modality. For instance, changes seen in IL-6 and IL-1 acutely after eccentric exercise directly vary with intensity of the eccentric exercise, where changes are seen with moderate intensity eccentric exercise but larger increases are seen with high intensity (≥ 75% VO2max) eccentric exercise [29]. Changes in other pro-inflammatory cytokines also appear to be dependent on exercise intensity. TNFα has been shown to significantly increase acutely after a moderate shuttle walk test, and this increase was magnified using a higher intensity strenuous shuttle walk test in the same group [228]. After one month of daily training in these same participants, TNFα decreased after an acute shuttle walk test. Exercise intensity plays a large role in determining inflammatory response to exercise, and exercise training leads to adaptations in response to repeated acute upregulation of pro-inflammatory cytokines that may desensitize leukocytes to these cytokines. Conversely, there may be no desensitization, but rather sensitization of leukocytes to pro-inflammatory cytokines with chronic exercise, leading to a sensitization of anti-inflammatory

pathways and a faster or more efficient response of the anti-inflammatory pathways to stimulus, eventually causing the decreases in TNFα and other pro-inflammatory cytokines seen acutely after an exercise bout in trained individuals.

Other evidence exists to support the role of exercise intensity and duration in regulating the immune response. After an intense Ironman triathlon, IL-6 and IL-10 were elevated acutely (thirty minutes post exercise) and returned to baseline levels within one day [24]. Marathon racers also experience increases in IL-6, IL-10, TNFα, and Il-1β acutely after a race [29]. Furthermore, IL-6, TNFα, and IL-1β are acutely elevated when a combination of intense aerobic and anaerobic wrestling bout is performed in young male wrestlers [27].

2.6.5 Age May Influence Inflammatory Responses to Exercise

In addition to intensity, duration, and modality, evidence is suggestive of a role of muscle fiber type and size in inducing inflammation acutely after an exercise bout. It follows that age of participants may also affect the inflammatory response to exercise. With age, atrophy of muscle occurs, decreasing strength and mass of the muscle. Additionally, changes in fiber type occur with aging. Over time the predominant muscle fiber type becomes type I, slow twitch oxidative fibers in elderly individuals. The change in muscle fiber type and increases in atrophy lead to reduced velocity of shortening of the muscle during contraction and decreases in force development and, consequently, power output [29]. Studies show that changes in leukocytosis also occur with aging. Elderly males have exhibited attenuated response of neutrophils in the systemic circulation, with 20% less recruitment when compared to young males [29]. Changes in systemic cytokines in response to exercise have also been reported to be dependent on age. IL-6 production and secretion after downhill running in elderly males is only equal to 90% of the IL-6 produced and secreted during the same exercise bout in young males [29]. In addition, IL-6, TNFα, IL-1β, and IL-10 exhibit attenuated elevation in response to LPS stimulation of

macrophages in aged mice when compared to younger mice [229-230]. Although changes in circulating cytokine production have been seen in response to exercise and LPS stimulation in humans and mice, respectively, the mechanism for these changes in unclear and little research has explored the mechanism behind the differences associated with aging. It does appear, however, that the changes seen with age may not involve changes in receptor expression on macrophages. Studies show no significant changes in TLR expression in young versus older humans, although aged mice do exhibit decreased amounts of TLR4 when compared to younger mice [18, 230-232].

2.7 Literature Review Summary

Extensive research has been conducted on changes in pro- and anti-inflammatory cytokines in response to exercise bouts and training. Several representative studies have been reported here in order to demonstrate the changes seen acutely and chronically after exercise. All agree that exercise induces secretion of acute phase reactants into the systemic circulation, promoting inflammation, and that chronic phase reactants are found in the systemic circulation in response to long term exercise training. Where these studies differ is in the explanation of where and how these circulating cytokines are secreted. While several studies propose that pro-inflammatory cytokines are secreted from circulating leukocytes acutely after exercise, others express the conclusion that pro-inflammatory cytokines are produced from myocytes in response to muscle damage caused by the bout itself. Whether pro-inflammatory cytokines are secreted by circulating leukocytes or myocytes remains an area of debate, while a consensus exists amongst researchers regard to the source of production and secretion of anti-inflammatory cytokines in response to chronic exercise. Researchers agree that the presence of pro-inflammatory cytokines seen acutely after exercise lead to concomitant increases in anti-inflammatory cytokines from

circulating leukocytes. These adaptations to chronic exercise help to explain the beneficial effects of exercise training in diseased populations that exhibit low grade chronic inflammation.

While it is agreed that exercise training leads to beneficial adaptations within the inflammatory response, the mechanism behind these exercise-induced changes in systemic cytokine profile remains unclear. It is most likely, given the redundancy of the biological system, that several adaptations to exercise training culminate to create a reduction in chronic inflammation. Changes in immune cell response are affected by several different mechanisms. It is possible for one researcher to illuminate these changes due to the magnitude of the task. First, there are several subpopulations of leukocytes to take into account in affecting inflammation. Second, subpopulations are derived from different tissues in response to different signals. Third, different types of stimuli on the immune system cause differential expression of populations of immune cells. Furthermore, within subpopulations, there is differential phenotype expression of immune cells. Fourth, populations exhibit various expression patterns of recognition receptors in response to varying stimuli. Activation of different receptors leads to differing downstream gene and protein expression patterns. Additionally, various receptors, either on the same cell or other immune cells, may communicate through cross-talk, complicating pathway activation or repression in order to regulation gene and protein expression. Although a consensus is seen in the end product of exercise training on inflammation, elucidating the mechanisms behind these changes remains a daunting task.

I have concentrated this review and my studies on the immune response of monocytes and macrophages to exercise, acutely and chronically. Several mechanisms for changes in inflammatory cytokine secretion from macrophages have been proposed and studied. Most notably, differential expression of the β-ARs and TLRs have been researched as effectors of exercise-induced changes in inflammation. Each class of receptor seems to play a crucial and

opposite role in mediating cytokine production in macrophages. TLR2 and TLR4 induce inflammation by causing upregulation of pro-inflammatory cytokine production; whereas, β-ARs are noted to cause upregulation of anti-inflammatory cytokines. β-ARs and TLRs respond to different stimuli. β-ARs respond to catecholamines, Epi and NE, while TLRs respond to LPS and other endotoxin pathogens. It appears that induction of the pro-inflammatory response is due to damage of myocytes during an exercise bout, and induction of the anti-inflammatory response is due to changes in endocrine hormone circulation during and after an exercise bout. Although, the β-ARs and TLRs appear to regulate exercise-induced changes in inflammation, studies of changes in receptor expression in response to exercise remain controversial.

Few other macrophage plasma receptors have been extensively studied in response to exercise. It is known that macrophages, integral in the response to stress and initiators of the immune response, possess MCRs. MC3Rs are important in regulation of metabolic responses, such as nutrient partitioning, in the hypothalamus within the CNS, where they are activated by various derivatives of the propeptide POMC, including ACTH, α-MSH, β-MSH, and γ-MSH.

In addition to the potential role of the MC3R in the CNS in regulating nutrient partitioning, recent investigations have concentrated on MC3R expression in peripheral tissues. One additional role of MC3R may be its ability to regulate circadian rhythms through integration with the food entrainable oscillator. Another function of MC3R in the periphery focuses on its expression on macrophages. Studies have shown that activation of the POMC neurons occurs during inflammation in response to pro-inflammatory cytokines; IL-6, IL-1β, and TNFα. This translates to increased ACTH secretion in the hypothalamus. Studies also show that circulating POMC and ACTH levels are increased during inflammation in the periphery. MC3Rs have been shown to play a role in cytokine production from macrophages in response to stress. Activation of the MC1R and MC3R causes macrophages to secrete IL-10, an anti-inflammatory cytokine,

which in turn inhibits secretion of IL-6, IL-1β, and TNFα from leukocytes. It appears that the MC3R may function in a comparable manner within the periphery as within the CNS, where the melanocortin system is expressed in both locations, but different outcomes are present in response to MC3R activation. In peripheral tissues, MC3R may function to regulate inflammation through its expression on systemic macrophages. The immunomodulatory role of the MC3R on monocytes may further be amplified by activation of the MC1R. Furthermore, MC1R and MC3R expression may be affected by exercise and activation seems a plausible regulatory adaptation of the anti-inflammatory response to chronic exercise.

CHAPTER 3. RESISTANCE EXERCISE ALTERS MONOCYTE MELANOCORTIN RECEPTOR EXPRESSION

3.1 Introduction

Exercise training has been shown to create favorable changes in inflammatory profiles. Specifically, resistance training has been shown to dowregulate systemic pro-inflammatory cytokines associated with chronic inflammation and upregulate anti-inflammatory cytokines [39, 174-175]. It has been established that exercise training results in chronic downregulation of inflammatory cytokine production and secretion within circulating monocyte populations via downregulation of the toll-like receptors [205-208]. Whereas regulatory pro-inflammatory receptors have been identified on monocytes, few studies have examined possible anti-inflammatory receptors that may regulate changes in anti-inflammatory pathways associated with chronic exercise training. The MC3R is a member of five GPCRs that make up the melanocortin receptor family, MC1R through MC5R. MC3R is known to increase secretion of the anti-inflammatory cytokine, IL-10, from cultured macrophages [71, 74]. Additionally, activation of MC3R with agonist leads to downregulation of the pro-inflammatory cytokines, IL-6 and TNFα, from cultured macrophages in response to inflammatory stimuli, likely due to upregulation of IL-10 [74-75]. In addition to MC3R, monocytes as well as several other immune cell populations have been shown to express MC1R and MC5R [67, 82]. Whereas MC5R has not been shown to be critical in regulating cytokine expression and secretion from monocytes and macrophages, MC1R and MC3R have both been shown to have redundant modulatory roles within monocyte and macrophages cell lines [69-70].

Although multiple lines of evidence suggest that MC3R plays an immunomodulatory role through regulation of gene expression in macrophage cell lines, to our knowledge, no studies have explored the effects of exercise training on monocyte MC3R expression. I recently found that MC3R mRNA expression increased 13-fold in whole blood samples taken after a 12 week

resistance training intervention. Thus, my current efforts have been focused on determining whether changes in MC3R mRNA translate to changes in MC3R protein expression on the plasma membrane of systemic monocytes.

In the present study, I examined the modulation of MC1R and MC3R expression in response to resistance training in young, healthy adults. My results clearly demonstrate that resistance training increases the number of monocytes expressing MC1R but decreases the number expressing MC3R. In addition, I show that MC1R and MC3R receptor density on monocytes decreases in response to resistance training. I also show that changes in MC3R but not MC1R expression negatively correlates to changes in body composition. Resistance training also leads to changes in the inflammatory profile, evidenced by decreases in CRP and transient upregulation of IL-6 and chronic decreases in IL-10 protein expression.

3.2 Materials and Methods

3.2.1 Participants and Screening

Thirty-nine healthy, physically active college-aged students were recruited for this study. The resistance training group (n = 23) consisted of interested males and females from a senior level Kinesiology course (KIN 4501-The Physiology of Strength Training) at Louisiana State University. Students learned advanced techniques in strength training and completed the below measurements as part of the class. Students interested in the training group portion of this study were asked if they were interested in allowing us to use data collected in the class for research purposes. Students were not penalized for not participating in the study. An “active control” group (n = 19) consisted of active males and females who participated in an endurance or resistance training program on a regular basis. The active control group participants were recruited by word of mouth. This project was approved by the LSU Intuitional Review Board.

3.2.2 Preliminary Testing

Medical Screening, Health History and Physical Activity Questionnaires

Subjects in the training group received permission to participate from a State licensed M.D. All study participants (training and active control) completed a medical history form. Additionally, all participants filled out the International Physical Activity Questionnaire (IPAQ)-Short Format [233]. The IPAQ-short format is an instrument that can be used to obtain internationally comparable estimates of physical activity with adults aged 18-65 yr. It is designed to assess health-related aspects of physical activity and sedentary behaviors. The short version contains 4 items (7 questions) targeting time spent in vigorous- and moderate-intensity activity, walking, and in sedentary activity. The screening and questionnaires were designed specifically with the participant's health in mind by: 1) preventing those with pre-existing conditions from participating in a running program/study and 2) allowing the researchers to become aware of any potential health issues.

3.2.3 Baseline Resting Measurements

Baseline measurements in both training and control groups were taken in the Exercise Physiology Laboratory at Louisiana State University (LSU). Subjects were instructed to refrain from exercising on the day of testing. Upon arrival, the subjects were given the Informed Consent form and given time to review the document. Then, the investigator explained the experimental protocol and answered any questions.

Body Composition

Body density was assessed using Dual X-ray Absorptiometry (DXA) in the Nutrition and Health Assement Laboratory in the School of Human Ecology at LSU. Subject age, gender, ethnicity, and height and weight were entered into the DXA apparatus (Prodigy Pro, GE Lunar Medical Systems, Madison, WI). Participants were then asked to remove all of their jewelry and

metal objects and lie on their back on the DXA table. They remained on their back for the duration of the scan, about 5 minutes or less. All measurements were taken by a trained DXA operator (Tara M. Henagan).

Resting Metabolic Rate (RMR)

Subjects underwent a test for resting metabolic rate (RMR) at the beginning of the training intervention (or normal activity in active control) (PRE) and end (POST) of the 12-week experimental period. RMR was obtained while subjects rested quietly for 30 minutes and breathed into a mouthpiece so that expired air was collected and analyzed for oxygen consumption, carbon dioxide production and total ventilation as previously described [234].

Blood Sampling

Participants were asked to report to the lab after an overnight fast and refraining from exercise from the previous 72 hours between 0800-1100 h and rest quietly for at least 15 minutes before the baseline, pre-training blood sample were obtained PRE. Approximately 8 mL of blood was obtained by a registered nurse from an antecubital vein. Aliquots were treated with EDTA (BD-Pharmingen, USA). At the end of the twelve week training period, blood was collected again POST from both the active control and trained group 72 hours after their last resistance training bout.

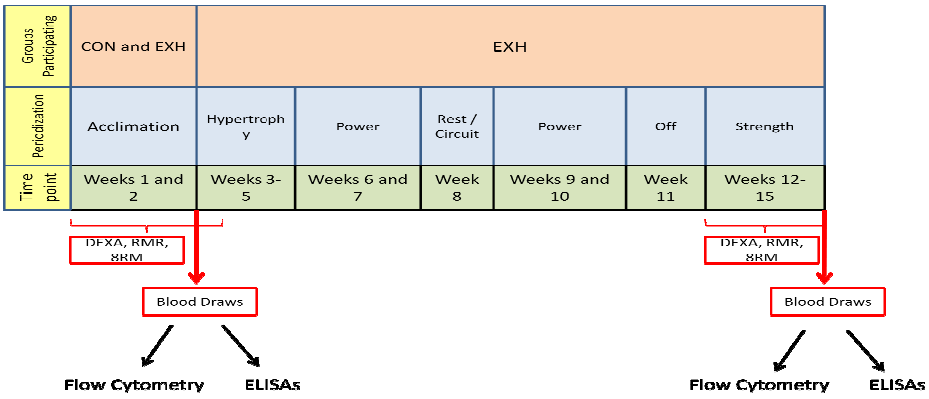

FIGURE 1. Study Timeline

3.2.4 Acclimation and Training

Acclimation

Both the active control and training groups completed a 2 week-long acclimation period to the following resistance exercises: bench press, bent over rows, shoulder press, barbell curls, dumbell shrugs, tricep pressdowns, crunches, squat, deadlift, walking lunges, lying leg curl, leg extension, and standing and seated calve raises. They performed four acclimation exercise sessions on non-consecutive days (Monday and Wednesday or Wednesday and Friday). On acclimation day 1 (ACC 1), participants were taught proper lifting techniques for each of the seven upper body exercies to minimize the risk of injury. Each subject in the control and trained groups performed one set of 10-15 repetitions of bench press, bent over rows, barbell curls, dumbell shrugs, tricep pressdowns, and crunches. On the second acclimation day (ACC2), participants were taught proper lifting techniques for each of the seven lower body exercies to minimize the risk of injury. Participants then performed one set of 10-15 repetitions of each exercise; squat, deadlift, walking lunges, lying leg curl, leg extension, and standing and seated calve raises. On the third acclimation day (ACC3), participants performed the same exercises as ACC1 but performed two sets of each at 10-15 repetitions. One the fourth acclimation day (ACC4), participants performed the same exercises as ACC2 but performed two sets of each at 10-15 repetitions. 8-RM for bench press, squat, and deadlift exercises was assessed on ACC3 and ACC4. All acclimation and exercise sessions were preceded by 5-10 min of cycle ergometry or walking. All acclimation sessions and resistance exercise sessions were completed in the Stadium Weight Room or University Recreational Center at LSU under the supervision of trained technicians. (See Figure 1.)

8 RM testing

Following the acclimation period but before the intervention period and following the intervention period, CON and RT individuals underwent 8RM testing for bench press and squat. Subjects were asked to perform one set of 10 – 12 repetitions of lift weight as a warm up set. Subjects then rested for 2-3 mins. Based on the individuals assessment, a suitable weight was determined in the use of the 8RM testing. The subject was asked to perform 8 repetitions, coming to complete muscle failure by the eighth repetition. If the subject could not complete 8 repetitions or completed more than 8 repetitions at the current weight, the weight was increased or decreased by 5-10% accordingly. The subject was then asked to perform the lift again with the goal being complete muscle failure by the eighth repetition. The subject rested 2-3 mins between tries if the 8RM was not reached on the first try. The subject was allowed 3 tries to reach the 8RM for a specific lift. Upon competion of the 8RM, the weight was recorded for each subject. If the subject did not reach the 8RM by the third try, the weight lifted and the number of repetitions was recorded. The 8RM was then determined using the CSCS calculation for RM testing.

Training

Following the acclimation and testing period, the training group completed 12 weeks of progressive resistance training on non-consecutive days (3x / week) and were asked to maintain their normal diet. Subjects in the training group were provided with a training routine to follow. The training was periodized and progressive (Appendix 1). Progression was implemented by asking participants to complete a given repetition range for a specified number of sets for each lift until failure. If the participant was able to complete and repetitions for all sets of a given lift without reaching failure, the load was increased during the following training period until failure was reached. Participants were supervised to ensure proper lifting techniques for each of the exercise to minimize the risk of injury and proper progression through the training plan. The

active control group did not undergo supervised exercise sessions, but were asked to maintain their normal activity and eating habits.

3.2.5 Post Intervention Data Collection

Post intervention measures were performed on all participants following the 12 week intervention. Procedures for post-training measurements were identical to the protocol followed for the baseline resting and exercise measures outlined above.

3.2.6 Blood Analyses

Flow Cytometry

EDTA-treated blood was incubated with either MC1R (Santa Cruz) or MC3R (Amolone) or CD14 (eBioscience) antibody as well as a three-color isotope control. The percent variation in MC3R over a 3 day time period was 7.35% ($P = 0.89$) for the percentage of monocytes expressing MC3R and 0.50% ($P = 0.94$) for MC3R MFI. Primary gates were established on leukocyte populations using forward and side scatter light; secondary gating was established using CD14 to identify and analyze monocyte populations. All experiments were performed using the FACS Laboratory at the LSU School of Veterinary Medicine.

Serum Cytokine Profiles

Plasma was collected from EDTA-treated whole blood samples by centrifugation (14000 rpm, 4°C, 10 minutes) immediately following blood collection and was used in commercially available ELISA kits to measure amounts of CRP (ALPCO) and circulating cytokines IL-6 (eBioscience) and IL-10 (eBioscience). Intra-assay coefficients of variation were 6%, 4.9%, and 6.8% Inter-assay coefficients were 13.8%, 6.0%, and 7.5% for CRP, IL-6 and IL-10 respectively.

3.2.7 Statistical Procedures

The two-tailed Student t-test, linear regression, and a 2 (PRE vs. POST) X 2 (CON vs. RT) repeated measures analysis of variance (ANOVA) were used for determining the

significance of the experimental differences when appropriate. Significance was set at $P < 0.05$. Bonferroni post hoc tests were used when appropriate. All statistical analyses were performed using GraphPad Prism or JMP statistical software. Correlations were determined using linear regression analysis in GraphPad Prism software. All values reported are means ± SEM.

3.3 Results

Nineteen subjects were enrolled in the CON group and twenty-three subjects were in the RT group, two CON subjects dropped out over the course of the 12 week intervention period and all RT participants completed the study. Even though this study was not designed to determine a gender effect, relatively equal numbers of men and women were used in this study. The CON and EX group consisted of 7, 12 and 11, 12 male and female participants, respectively.

Age, height, and body weight (BW) variables were not different at baseline in CON and RT and remained unchanged and not significantly different POST. Age ranged from 20 to 27 yr in the CON group and from 18 to 27 yr in the RT group. Height ranged from 152.2 to 190.5 cm in the CON group and from 156.2 to 184.7 cm in the RT group (TABLE 1). BW ranged from 47.17 to 136.08 kg in the CON group and from 53.43 to 158.3 kg in the RT group before the intervention and did not change in both groups after the intervention period (TABLE 1). BMI ranged from 19.85 to 37.5 kg/m^2 in CON and from 19.78 to 45.42 kg/m^2 in RT prior to the intervention period and did not change significantly in either CON or RT after the intervention period (TABLE 1).

There were no differences in lean body mass (LBM) percentage or LBM or body fat percentage between CON and RT groups at baseline (FIGURES 2 and 3 and 6), however participants in the RT group significantly increased LBM by 1.15 ± 0.27 kg (POST – PRE), a 1.84 ± 0.46% increase (FIGURES 4 and 5). This increase was significantly larger than the change in LBM seen in CON (0.29 ± 0.28 kg, POST – PRE), a 0.63 ± 0.34% change (FIGURES

4 and 5). Furthermore, the change in body fat percentage was significantly different in RT, who had a 1.55 ± 0.45% decrease in body fat percentage (POST – PRE), and CON, who had a 0.54 ± 0.35% decrease in body fat percentage (POST – PRE, FIGURE 7). There were no significant changes in bone mineral content (BMC) PRE or POST in CON (2.74 ± 0.17 kg PRE vs. 2.48 ± 0.17 kg POST) and RT (2.98 ± 0.13 kg PRE vs. 2.97 ± 0.12 kg POST). There were no significant differences in RMR or RER in either CON or RT (TABLE 2).

TABLE 1. Descriptive data for subjects.
Descriptive data for the subjects before (Pre) and after (Post) the 12-week resistance training intervention. CON, active control group; RT, resistance training group.
* denotes significant differences between CON and RT groups.

Variable	Time	CON (n=18)	RT (n=22)
Age		21.4 ± 0.42	22.2 ± 0.38
Height (cm)		166.32 ± 2.14	171.85 ± 1.94
Body Weight (kg)	Pre	68.12 ± 4.98	75.49 ± 4.61
	Post	67.76 ± 4.88	74.84 ± 4.41
BMI (kg/m^2)	Pre	24.18 ± 1.19	24.23 ± 1.04
	Post	24.07 ± 1.10	24.06 ± 0.99
8RM Test (% change)	Bench Press	-11.44 ± 4.04 (n=7)	5.58 ± 3.01* (n=15)
	Squat	-5.18 ± 4.28 (n=6)	11.82 ± 3.02* (n=14)

3.3.1 Training Program Results

Compliance was recorded weekly through self recorded workout logs All exercise participants completed 92% of the prescribed exercise sessions. The RT group experienced significant increases in 8RM in both bench press and squat and the percent change in strength was significantly greater in RT compared to CON after the intervention period. The RT experienced 5.58% ± 3.01 (143.05 ± 17.92 PRE vs. 156.75 ± 18.58 POST) and 11.82% ± 3.02 (170.83 ± 15.93 PRE vs. 193.11 ± 19.29 POST) increases in the bench press and squat respectively (TABLE 1). The amount of work performed each week by the RT group varied, as an undulating periodized routine was utilized over the twelve week period. The lowest work completed was during weeks 3 and 9 and the highest at weeks 7 and 8. The number of repetitions recommended ranged throughout the 12 week intervention from 442 repetitions per week to 1275 repetitions per week (Appendix A).

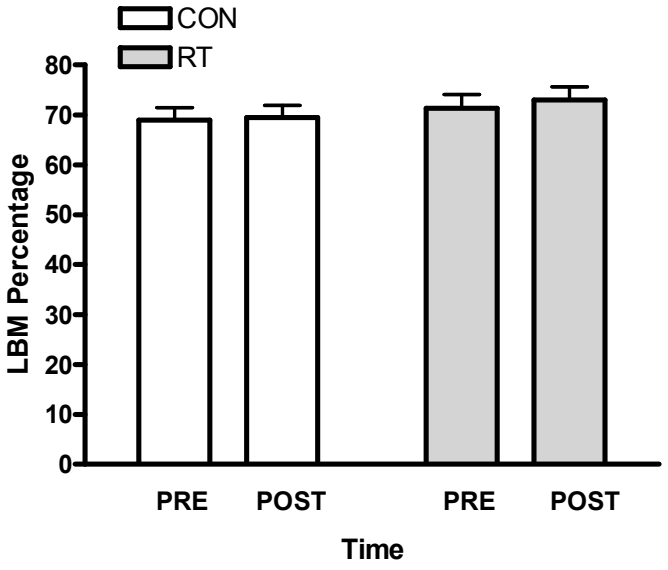

FIGURE 2. LBM percentage.
Lean body mass (LBM) percentage before (PRE) and after (POST) the intervention period. CON, active control group; RT, resistance training group. There were no significant differences in LBM percentage in RT or CON PRE to POST.

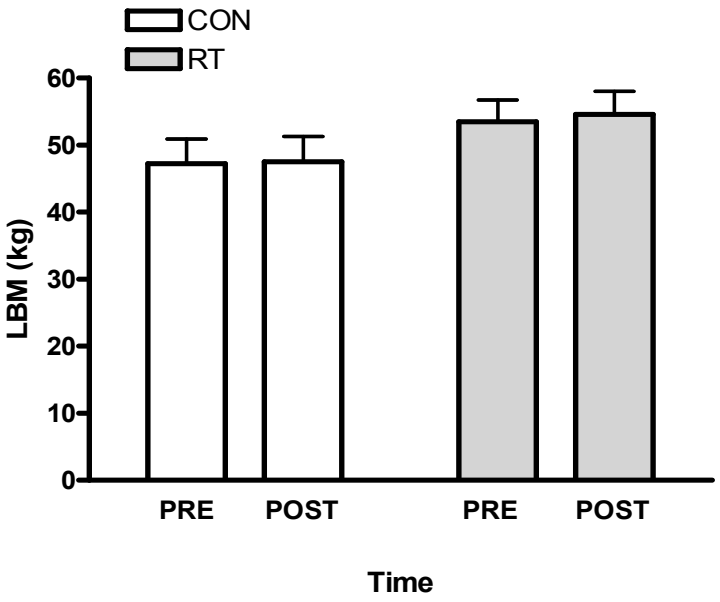

FIGURE 3. LBM.
Lean body mass (LBM) before (PRE) and after (POST) the intervention period. CON, active control group; RT, resistance training group. There was a significant interaction between time and treatment effect on LBM ($P < 0.05$ by two way ANOVA).

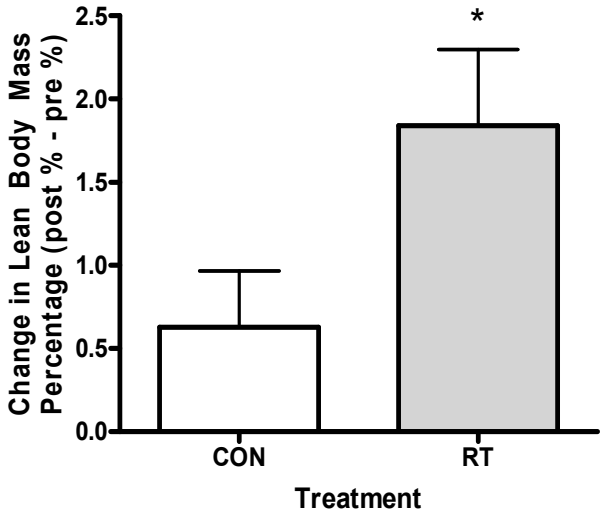

FIGURE 4. Change in LBM percentage (POST – PRE).
CON, active control group; RT, resistance training group. The change in lean body mass (LBM) percentage (POST – PRE) was significantly larger in the RT vs. the CON group ($P < 0.05$ by t test). *denotes significant difference between CON and RT groups

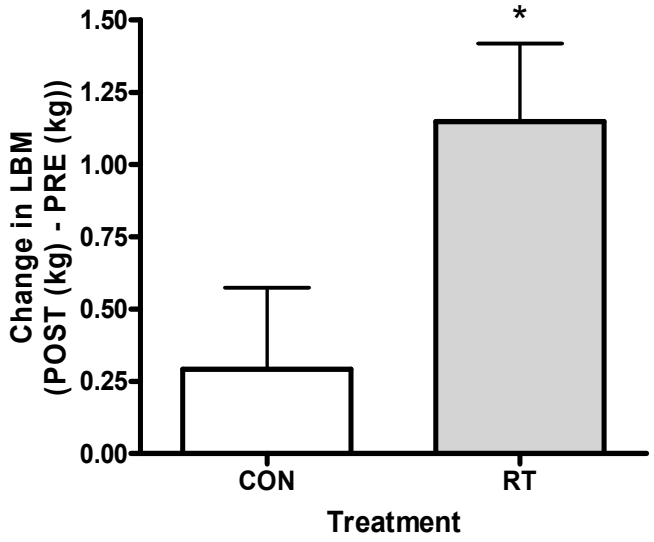

FIGURE 5. Change in LBM (POST – PRE).
CON, active control group; RT, resistance training group. The change in lean body mass (LBM; POST – P5E) was significantly larger in the RT vs. the CON group ($P < 0.05$ by t test). *denotes significant difference between CON and RT groups

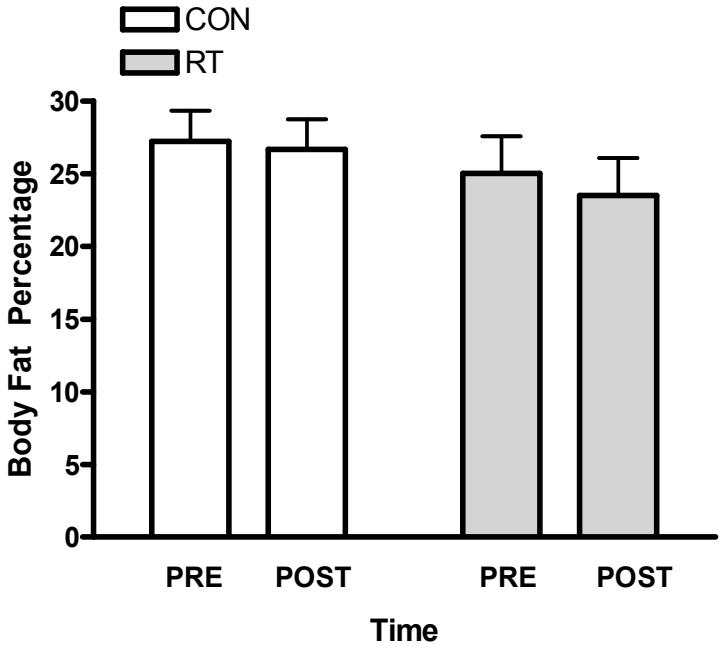

FIGURE 6. Body fat percentage.
Body fat percentage before (PRE) and after (POST) the intervention period. CON, active control group; RT, resistance training group. There were no significant differences in body fat percentage in RT or CON.

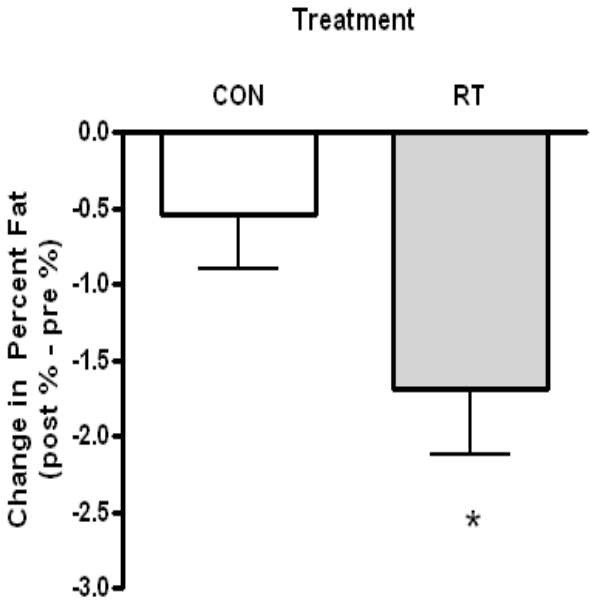

FIGURE 7. Change in body fat percentage (POST – PRE).
Change in body fat percentage (POST – PRE). CON, active control group; RT, resistance training group. Decreases in body fat percentage were significantly large in RT than CON. ($P < 0.05$ by Students t test). *denotes significant difference between CON and RT groups.

TABLE 2. RMR and RER.
Resting metabolic rate (RMR) and respiratory exchange ratio (RER) before (Pre) and after (Post) the intervention period. Values are means ± SEM. CON, active control group; RT, resistance training group

Variable	**Time**	**CON (n=19)**	**RT (n=22)**
RMR (kcal / day)	Pre	1915.64 ± 117.91	2045.36 ± 106.88
	Post	1839.15 ± 76.94	2106.19 ± 110.29
RER	Pre	0.80 ± 0.02	0.81 ± 0.01
	Post	0.79 ± 0.01	0.83 ± 0.01

3.3.2 Monocyte Cell Surface Expression of MC1R and MC3R

Prior to the intervention, there were no significant differences between CON and RT in the percentage of monocytes expressing MC1R (82.16 ± 1.89% in CON vs. 84.11 ± 1.16% in RT) or MC3R (7.75 ± 1.52% in CON vs. 12.33 ± 2.25% in RT; FIGURES 8 and 9). The percentage of systemic monocytes expressing MC1R significantly increased from 84.11 ± 1.16% to 91.41 ± 0.79 % in response to RT whereas the percentage of monocytes expressing MC1R in the CON group did not change (82.16 ± 1.89% PRE vs. 83.36 ± 1.93% POST; FIGURE 8). The percentage of monocytes expressing MC3R significantly decreased in RT (12.33 ± 2.25% PRE vs. 6.47 ± 0.84% POST) with no change in CON (7.75 ± 1.52% PRE vs. 6.58 ± 1.3% POST), although there was a significant effect of time (FIGURE 9). No differences in the amount of MC1R and MC3R receptor density, determined by the mean fluorescence intensity (MFI), were detected in baseline samples.

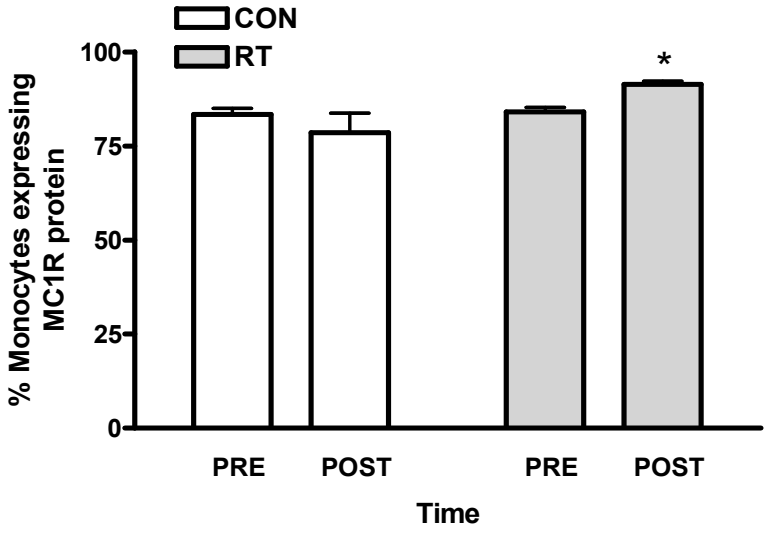

FIGURE 8. Percentage (%) of systemic monocytes expressing melanocortin 1 receptor (MC1R) protein on the cell surface.
Pre, before intervention; Post, after intervention. CON, active control group; RT, resistance training group. The percentage of monocytes expressing MC1R protein increased in RT but not CON after the intervention period ($P < 0.001$ by two way ANOVA). *denotes significant difference between Pre and Post values within CON or RT groups

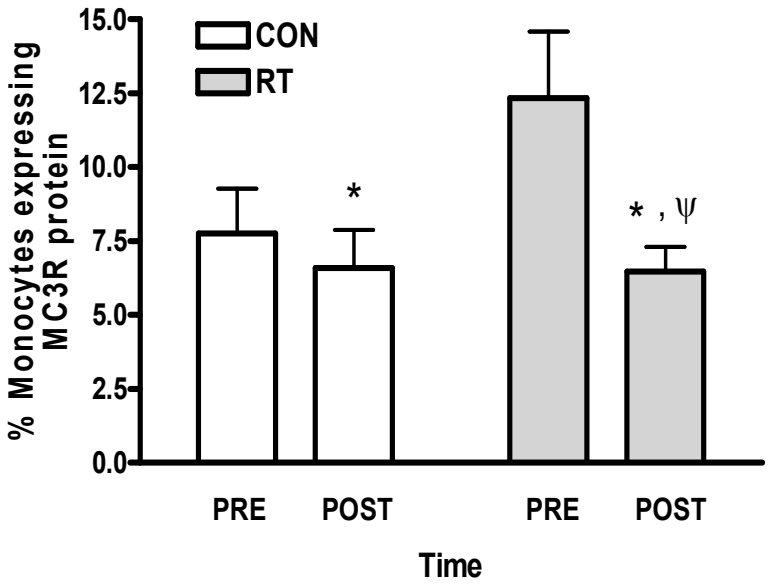

FIGURE 9. Percentage (%) of systemic monocytes expressing melanocortin 3 receptor (MC3R) on the cell surface.
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group. The percentage of monocytes expressing MC3R protein decreased in response to RT, and there was a significant effect of time in the model ($P < 0.05$ by two way ANOVA). *denotes significant effect of time; Ψ denotes significant effect of treatment

At the POST time point, the amount of MC1R expressed on individual monocytes or the MC1R MFI significantly decreased from 33.82 ± 2.31 PRE to 15.05 ± 0.92 POST in RT with no change in MC1R MFI in CON (32.75 ± 2.33 PRE vs. 27.85 ± 2.94 POST; FIGURE 10). MC3R MFI also significantly decreased in RT (20.3 ± 2.66 PRE vs. 11.85 ± 1.37 POST) and there was a significant effect of time on MC3R MFI (FIGURE 11). MC1R MFI was not significantly altered in CON 16.11 ± 1.62 PRE vs. 10.68 ± 1.35 POST; FIGURE 10).

Changes in the percentage of monocytes expressing MC1R did not significantly correlate with MC1R MFI, body fat percentage, body fat (g), or RMR (kcal / d) (FIGURE 12, TABLE 3). Changes in the percentage of monocytes expressing MC3R (POST – PRE) significantly correlated with changes in MC3R MFI, body fat percentage, body fat (g), and RMR (kcal / d).

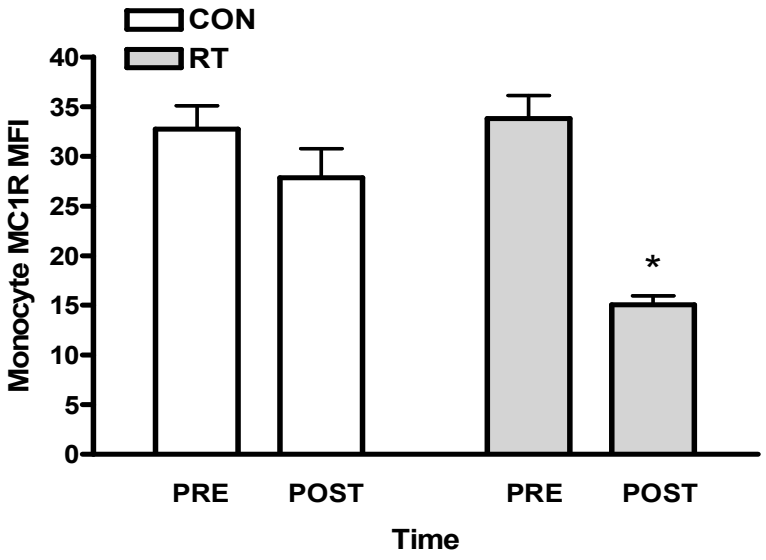

FIGURE 10. Plasma membrane expression of melancortin 1 receptor (MC1R) per monocyte.
CON, active control group; RT, resistance training group. Mean fluorescent intensity (MFI) of MC1R expression in the RT group after the intervention ($P < 0.001$ by two way ANOVA). *denotes significant difference in Pre and Post values within CON or RT groups.

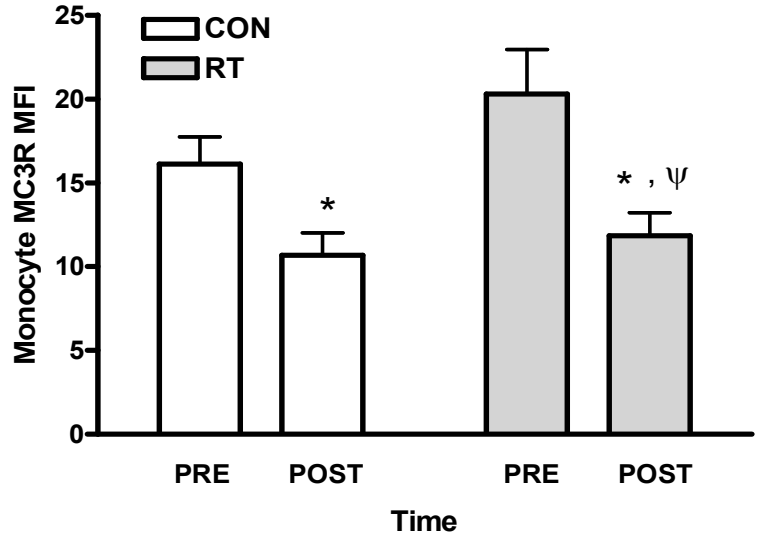

FIGURE 11. Melanocortin 3 receptor (MC3R) density on systemic monocytes.
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group. The mean fluorescent density (MFI) of MC3R decreased in RT after the intervention and there was a significant effect of time in the model ($P < 0.01$ for treatment by two way ANOVA). *denotes significant effect of time; Ψ denotes significant effect of treatment

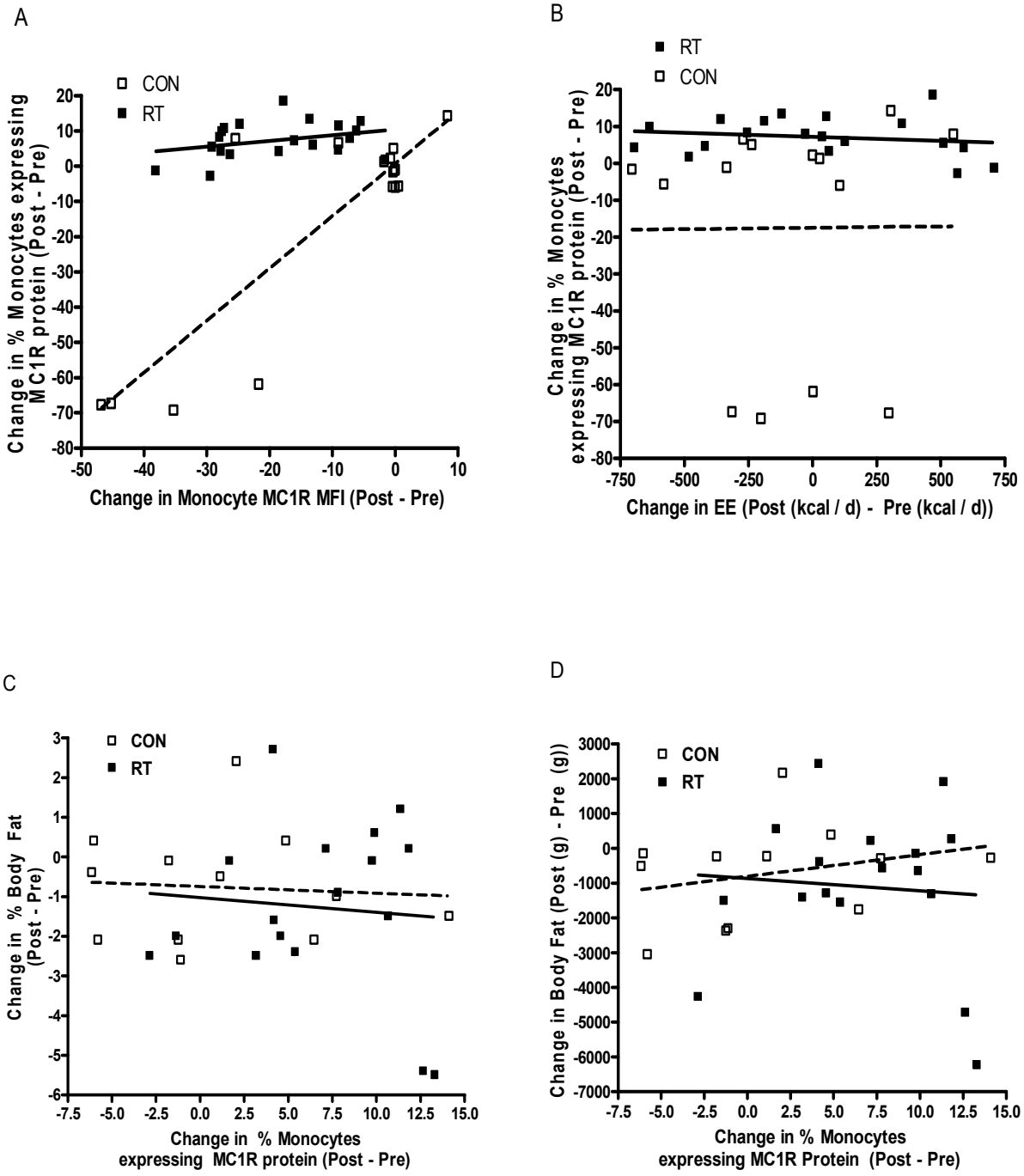

FIGURE 12. Correlations between the change in the percentage of monocytes expressing MC1R and (A) the change in MC1R receptor density, (B) change in body fat percentage, (C) change in body fat, and (D) change in energy expenditure.
CON, active control group; RT, resistance trained group.

TABLE 3. Summary of correlations between changes in the percentage of monocytes expressing MCRs and changes in MC3R or MC1R plasma receptor density, changes in body fat percentage, changes in body fat, or changes in energy expenditure.
CON, active control group; RT, resistance training group. MFI, mean fluorescence intensity. EE, energy expenditure. Values are reported as the correlation coefficient (top) and P value (bottom). * indicates significance.

	Treatment Group	**Correlation Variables**				
Correlation Variables		Change in MC3R MFI	Change in MC1R MFI	Change in % Body Fat	Change in Body Fat	Change in RMR (kcal / day)
Change in percent monocytes expressing MC3R protein	CON	0.6528, 0.0002*		0.01213, 0.6847	0.06851, 0.3274	0.06629, 0.3742
	RT	0.7121, < 0.001*		0.295, 0.0198*	0.2946, 0.0199*	0.2553, 0.0273*
Change in percent monocytes expressing MC1R protein	CON		0.734, < 0.0001*	0.07297, 0.3958	0.005561, 0.8178	0.00074, 0.9787
	RT		0.1039, 0.1657	0.005873, 0.77	0.00747, 0.7487	0.0313, 0.4688

The largest decreases in the percentage of monocytes expressing MC3R significantly correlated with the smallest decreases in MC3R MFI ($r^2 = 0.7121$; $P < 0.0001$) (FIGURE 13A). The largest decreases in percent body fat ($r^2 = 0.295$; $P < 0.05$) and fat ($r^2 = 0.295$; $P < 0.05$) significantly correlated with the smallest decreases in the percentage of monocytes expressing MC3R (FIGURES 13B and 13C). Changes in RMR (kcal / day) significantly correlated with changes in the percentage of monocytes expressing MC3R protein ($r^2 = 0.2553$, $P < 0.05$; FIGURE 13D; TABLE 3).

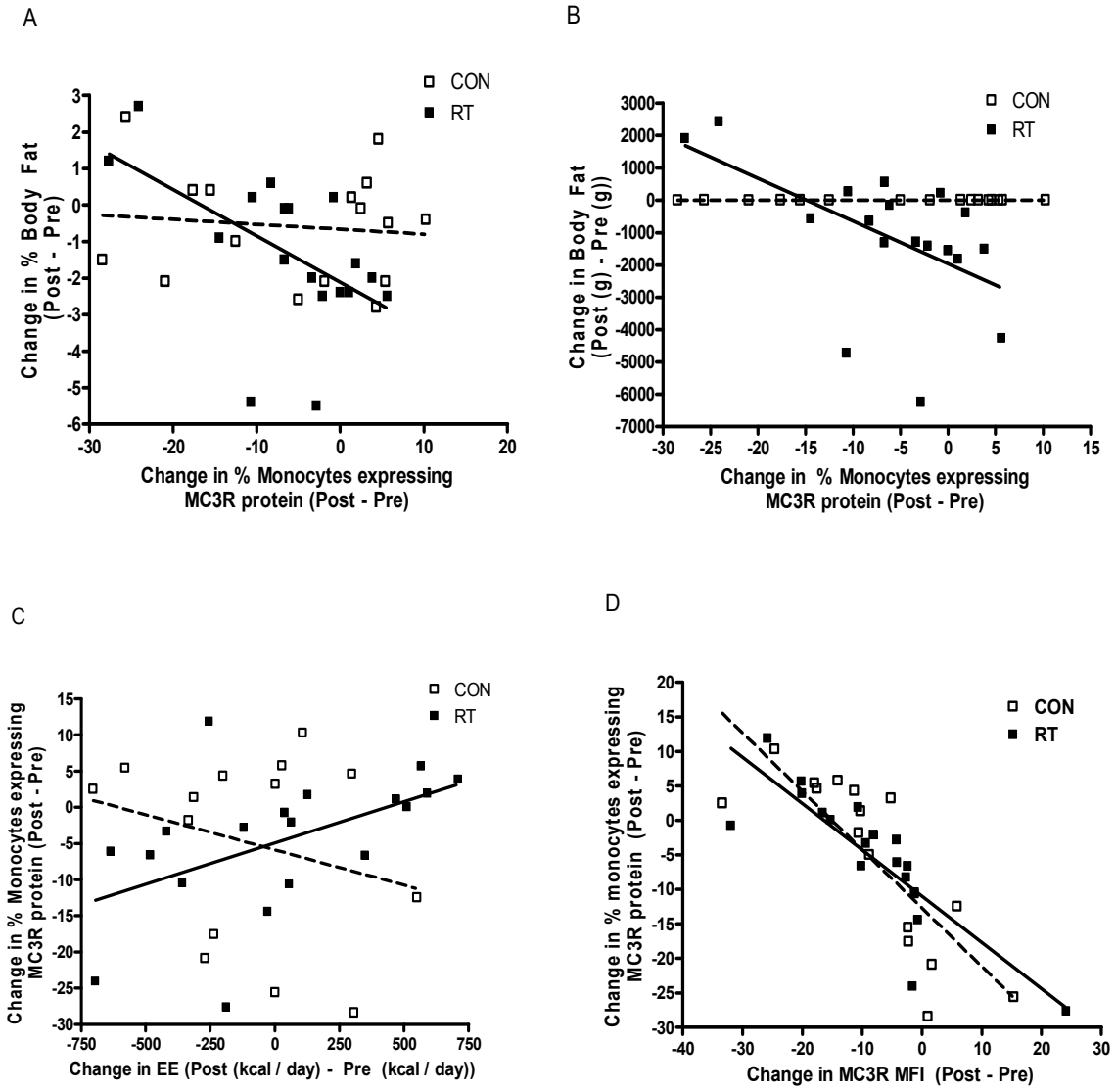

FIGURE 13. Correlations between the change in the percentage of monocytes expressing melanocortin 3 receptor (MC3R) and (A) the change in MC3R receptor density, (B) change in body fat percentage, (C) change in body fat, and (D) change in energy expenditure.
CON, active control group; RT, resistance trained group

3.3.3 Plasma CRP and Cytokine Concentrations

There were no differences in CRP between CON and EX groups at baseline. CRP plasma levels did not change significantly in RT or CON individuals (FIGURE 14). However, CRP levels in RT decreased from a moderate risk for CVD category at the beginning of the study

(1.71 ± 0.59) to a low risk category Post (0.9 ± 0.26 mg/mL). Additionally, when CRP levels were placed in tertiles, the highest baseline tertile in the RT group experienced a significant decrease in CRP at the end of the 12 week intervention (2.08 ± 0.34 PRE to 1.09 ± 0.34 POST), with no changes seen in CON in any tertile (TABLE 4).

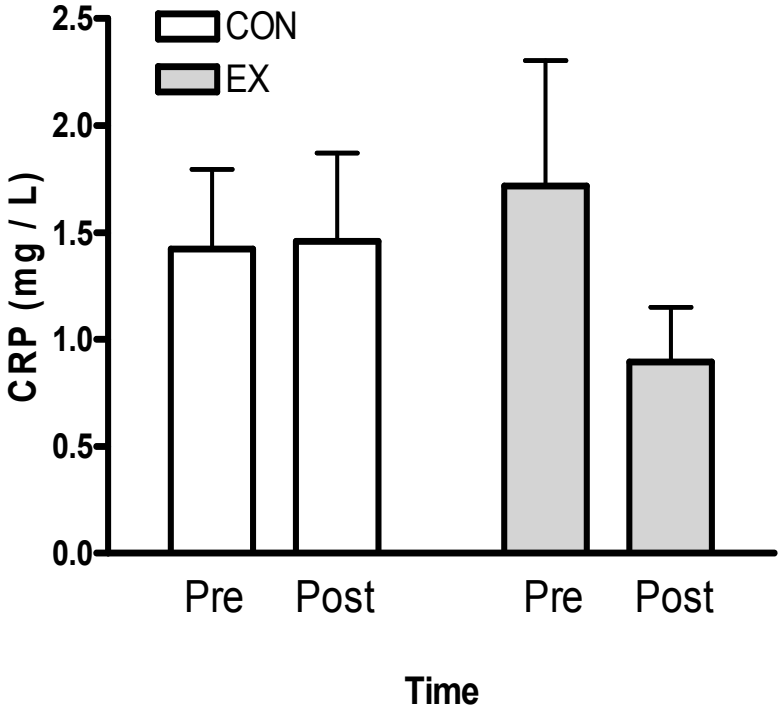

FIGURE 14. C-Reactive Protein (CRP) plasma protein levels.
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group. There were no significant differences in CRP in either CON or RT groups Pre to Post.

There were no differences in IL-6 or IL-10 between CON and RT groups at baseline. After the intervention there were no changes in IL-6 protein in RT or CON (FIGURE 15, TABLE 4). IL-10 protein plasma levels significantly decreased in RT, but almost the same degree of decrease was seen in CON individuals after the 12 week intervention (FIGURE 16, TABLE 4). There was also a significant effect of time on IL-10 in both CON and RT (FIGURE 16, TABLE 4).

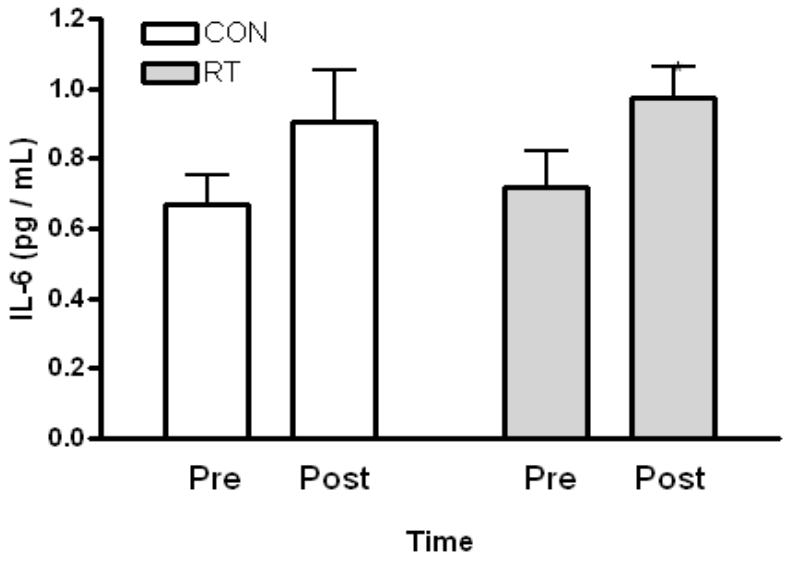

FIGURE 15. Plasma interleukin-6 (IL-6) protein levels.
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group. There were no significant differences in IL-6 in either CON or RT groups.

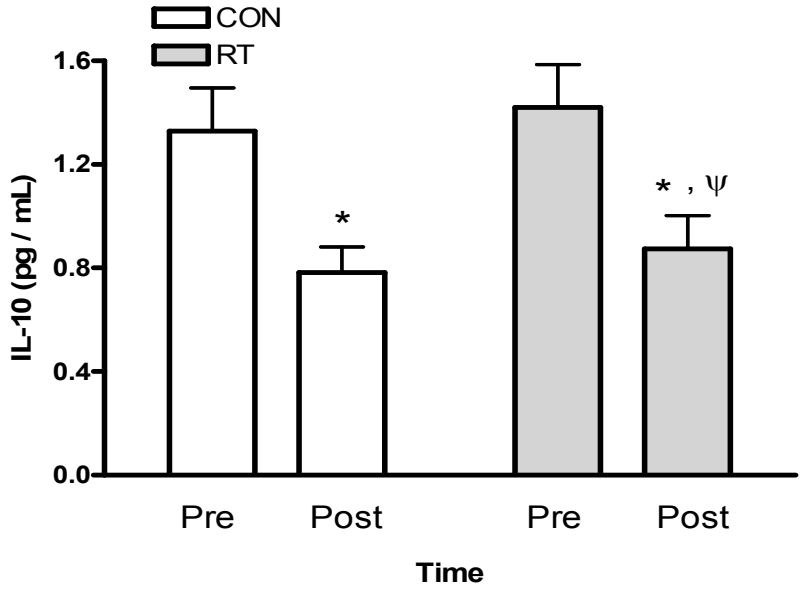

FIGURE 16. Interleukin-10 (IL-10) plasma protein levels.
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group. IL-10 decreased in RT Pre vs. Post values. There was a significant effect of time ($P < 0.0001$ by two way ANOVA). *denotes significant effect of time

TABLE 4. Summary of plasma protein levels of C-reactive protein (CRP), interleukin 6 (IL-6), and interleukin 10 (IL-10).
Pre, before the intervention period; Post, after the intervention period. CON, active control group; RT, resistance training group *denotes significant difference between Pre and Post values within CON or RT groups

Variable	Time	CON	RT
CRP (total)	Pre	1.42 ± 0.38 (n = 14)	1.71 ± 0.59 (n = 16)
	Post	1.46 ± 0.41	0.9 ± 0.26
CRP (highest tertile)	Pre	3.32 ± 0.38 (n = 6)	2.08 ± 0.34 (n =9)
	Post	2.56 ± 0.60	1.09 ± 0.34*
CRP (middle Tertile)	Pre	1.04 ± 0.05 (n = 5)	0.41 ± 0.03 (n = 5)
	Post	0.98 ± 0.34	0.35 ± 0.08
CRP (lowest tertile)	Pre	0.38 ± 0.08 (n = 5)	0.24 ± 0.02 (n = 5)
	Post	1.23 ± 0.67	0.39 ± 0.09
IL-6	Pre	0.88 ± 0.23 (n = 19)	0.72 ± 0.10 (n = 21)
	Post	0.97 ± 0.14	0.98 ± 0.09
IL-10	Pre	1.32 ± 0.17 (n = 19)	1.42 ± 0.17 (n = 21)
	Post	0.78 ± 0.10*	0.87 ± 0.13*

Changes in CRP correlated with changes in body composition. Larger decreases in body fat percentage and body fat and larger gains in LBM directly correlated with larger decreases in CRP (FIGURE 17, TABLE 5). Additionally, changes in IL-6 but not IL-10 also correlated with changes in body composition. Larger increases in IL-6 correlated with larger decreases in body fat percentage and body fat and larger increases in LBM percentage (FIGURE 17, TABLE 5).

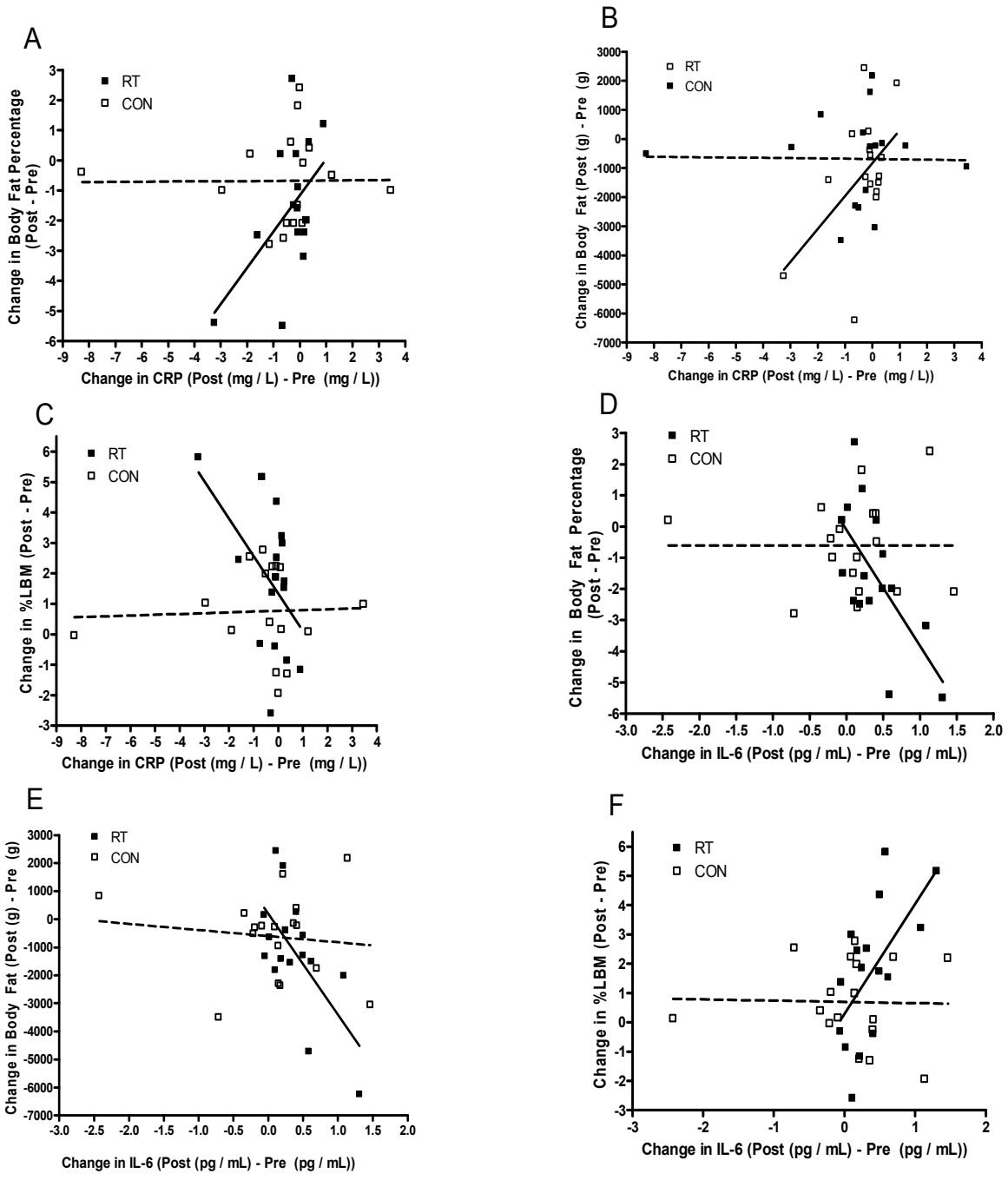

FIGURE 17. Correlation graphs between changes in CRP plasma protein levels and changes in body composition.

Correlation graphs between changes in CRP plasma protein levels (Post – Pre) and (a) changes in body fat percentage (Post – Pre), (b) changes in body fat (Post – Pre), and (c) changes in lean body mass (LBM) percentage (Post – Pre). Also shown are correlations between changes in IL-6 plasma levels (Post – Pre) and (d) changes in body fat percentage (Post – Pre), (e) changes in body fat (Post – Pre), and (f) changes in lean body mass (LBM) percentage (Post – Pre)

TABLE 5. Correlations between changes in plasma protein levels of C-reactive protein (CRP) or interleukin-6 (IL-6) and changes in body composition.
Summary of correlations between changes in plasma protein levels of C-reactive protein (CRP) or interleukin-6 (IL-6) and changes in body fat percentage, changes in body fat, or changes in lean body mass (LBM) percentage. CON, active control group; RT, resistance training group. Values reported are the correlation coefficient (top), P value (bottom). * denotes significance.

	Treatment Group	**Correlation Variables**		
Correlation Variables		Change in Body Fat %	Change in Body Fat	Change in LBM %
Change in plasma CRP levels	CON	0.00011, 0.9693	0.00023, 0.9559	0.001763, 0.8773
	RT	0.2749, 0.0371*	0.2625, 0.0425*	0.2496, 0.0488*
Change in plasma IL-6 levels	CON	0,	0.01379, 0.6535	0.0006046, 0.9254
	RT	0.4269, 0.0061*	0.4298, 0.0058*	0.3758, 0.0116*

3.4 Discussion

Exercise is an easily manipulated and well studied form of therapy for many chornic diseases, such as T2D, atherosclerosis, CVD, osteoporosis and cancer. While several studies focus on the inflammatory pathways induced acutely after an exercise bout, here we explore the response of MC1R and MC3R on monocytes to repeated, chronic bouts of RT in an effort to propose a novel role for the melanocortin receptors in affecting anti-inflammatory exercise-induced responses. My results are the first to show that chronic RT does affect the protein expression levels of MC1R and MC3R on systemic monocytes. While RT increases the number of cells expressing MC1R, indicating an increase in the anti-inflammatory capacity of the cells to stimuli, it decreases the number of cells expressing MC3R. One possible explanation for these conflicting results in MCR protein expression may be that the increase in MC1R may be a

mediating factor in the dowregulation of MC3R expression. Alternatively, exercise-induced decreases in MC3R cell surface expression may be compensated for by MC1R upregulation, allowing for the possible increase in anti-inflammatory processes within the monocyte. Interestingly, my data show that smaller decreases in MC3R expression per monocyte are significantly correlated with larger decreases in the percentage of monocytes expressing MC3R. Thus, the increases in the percentage of cells expressing MC1R may manifest anti-inflammatory effects in conjunction with the smaller decreases in MC3R expression per cell, where cells expressing MC1R may express less MC3R and vice versa. Although MC1R and MC3R have redundant immunomodulatory roles in monocytes, they may have alternate mechanisms for activation and translocation to the plasma membrane. For example, MC1R and MC3R bind to differing agonists and antagonists with differing affinities and upon stimulation with agonist, MC1R and MC3R may then translocate from the plasma membrane to vesicle by endoytosis, ultimately leading to changes in gene regulation [235-237].

As the current study measures only cell surface expression of MC1R and MC3R, there may be intracellular changes in protein expression not noted here that may also help to explain decreases in cell surface MC3R expression. Exercise training may increase the reservoirs of MC3R within vesicles in the monocyte when immune cells are stimulated with pro-inflammatory cytokines, and are released acutely after an exercise bout, allowing for a greater induction of gene transcription. In this scenario, MCR agonists such as POMC or ACTH are secreted acutely during and after a RT bout and act either in an autocrine or paracrine manner on the monocytes. MC3R may then translocate to the membrane of the monocyte in an acute manner where activation can occur. Hence, the monocyte can respond acutely to increase MC3R cell surface expression and sensitize the cell to produce a faster and larger anti-inflammatory response. Because the change in MC3R expression may occur acutely and may be transient in nature,

chronic, resting plasma samples obtained at 72 hours after the last RT bout may not have captured the complete nature of MC3R cell surface expression. This hypothesis seems plausible as my previous study examining MC3R mRNA expression 24 hours after a resistance training bout show significantly increased MC3R in participants undergoing a 12 week RT intervention (unpublished data). Additional support for this hypothesis lies in the expression of MC1R and MC3R on individual monocytes. My data show that both MC1R and MC3R cell surface expression, when explored on individual monocytes, actually decreases in response to RT. Thus, although a greater or lesser number of monocytes express MC1R and MC3R, respectively, all monocytes that express each receptor possess a lower abundance of each receptor. There is a time-dependent decreased in MC3R plasma protein density in both RT and CON but the decrease in MC3R in CON showed no effect from treatment, while the decrease in MC3R receptor density was dependent on the treatment (RT). Thus, the time dependent decreases seen in MC3R may be due to seasonal variation in temperature and photoperiod, leading to differences in receptor expression. Indeed, several studies show that MC3R exhibits circadian rhythms and helps maintain circadian rhythms of several other genes in hepatocytes [238-239]. In addition to possible seasonal variation in MC3R, studies also show seasonal variation in IL-10, which may also explain the decreases in IL-10 in both CON and RT as a function of time, although only the decreases in IL-10 were also significantly impacted by treatment (RT) [240]. It is also possible that seasonal changes in MC3R expression in monocytes are responsible for the seasonal variation seen in IL-10, as stimulation of MC3R leads upregulation and secretion of IL-10 from monocytes [7] and MC3R expression in hepatocytes is needed to maintain circadian rhythms of several proteins involved in metabolism [228-229].

I also note that changes in MC1R and MC3R expression coincide with changes in systemic cytokine expression. Here, IL-6 increases, although not significantly, in response to RT

while IL-10 decreases. The present data is in agreement with previous studies that have noted no change in IL-6 at the 72 hour time point but is in disagreement with previous findings for IL-10 which show increases or no change in IL-10 chronically in response to exercise [17, 46, 241-242]. As my main goal in the present study was to determine chronic changes in MCR expression in resting plasma samples, I chose the 72 hour time point to ensure that acute changes in receptor expression would not confound the results. For example, Langberg et al 2002 show that 180 mins of running at steady pace acutely upregulates IL-6, and IL-6 levels return to baseline levels at 48 hours after the running bout [241]. Croisier shows that IL-6 increases acutely after 30 maximal eccentric contractions of the knee flexor and extensor, and these levels return to baseline 48 hours after the resistance training bout [46]. Hirose et al 2004 also report the return of IL-6 and IL-10 levels to baseline levels by 24 hours post, following 6 sets of 5 eccentric contractions of the elbow flexors [17]. Margeli et al 2005 also show IL-6 returning to baseline by 48 hours after an ultradistance endurance race [243]. Conversely, others, such as Markovitch et al 2008 show no change in IL-6 or IL-10 during or after a bout of treadmill walking at an intensity of 50% VO2max [242]. It is interesting to note that MC3R and MC1R, both known anti-inflammatory receptors, decrease in response to RT in agreement with the changes in IL-10 expression, where IL-10, an anti-inflammatory cytokine, decreases. Thus, it is possible that the 72 hour time point may still contain the manifestations of the acute response to the last bout of RT training.

Although my samples exhibit changes in MCR and cytokine expression that favor a pro-inflammatory rather than anti-inflammatory response at the 72 hour time point, CRP tends to decrease ($P = 0.2641$) in response to RT. CRP is a much more stable marker of chronic inflammation than more transient cytokine levels and has recently gained acceptance in the medical community as a marker of CVD. Exercise has been shown to decrease CRP [38, 174-

177]. Thus, I believe that the overall decrease in CRP in the resistance training group may be a consequence of the acute changes seen here. For example, changes in MCR expression may elicit sufficient changes in cytokines or other factors that impact CRP levels, leading to its chronic downregulation. Acute upregulation of pro-inflammatory cytokine, IL-6 has been shown to directly upregulate transcription factors, such as C/EBP, that then upregulate CRP expression. Others have shown that, with exercise training, downregulation of IL-6 occurs chronically possibly due to upregulation of IL-10 [7, 75]. In conjunction with previous reports, the lack of change seen in IL-6 in the current project coincides with the lack of a significant change in CRP levels. However, those individuals in RT expressing higher baseline levels of CRP did significantly decrease their CRP while no changes were seen in CON individuals expressing higher baseline CRP levels. Furthermore, CRP levels in RT but not CON decreased from a moderate risk category for CVD (1-3 mg/mL) to a low risk category for CVD (<1 mg/mL), indicative of decreases in chronic inflammation possibly due to acute changes in cytokines not represented in the present data.

Studies have noted that changes in inflammation may be dependent on changes in adiposity, as increased percent fat correlates with increased inflammation [244]. Indeed, obesity is a disease state that is considered to be a type of low-grade chronic inflammation [245]. Here, I show that RT significantly decreases body fat percentage. Interestingly, changes in MC3R negatively correlate with changes in adiposity, where individuals losing the highest percentage of body fat also show the smallest decreases in the percentage of monocytes expressing MC3R. The role of the MC3R in the development of obesity has been studied extensively in rodent models, mainly in MC3R KO mice. Studies focus on the role of MC3R in the CNS, but little has been published on the effects of MC3R in peripheral tissues on adiposity. MC3R KO mice have been shown to exhibit increased adiposity despite hypophagia in comparison to wild type mice [87-88,

139]. Additionally, the adipose tissue of obese MC3R KO show decreased macrophage infiltration [87]. These previous studies indicate that peripheral MC3R expression on macrophages mediates macrophage activation and recruitment to sites of inflammation. Based on our current knowledge of the MC3R, one would expect that a decrease in adiposity and inflammation would correlate with increased MC3R expression on monocytes, and my data here supports this hypothesis. Again, I propose that while MC3R expression is decreased on the plasma membranes of systemic monocytes obtained from resting plasma samples, MC3R protein may be increased inside the monocyte where it resides in vesicles until stimulation. MC1R expression, on the other hand, did not correlate with changes in adiposity.

In support of the idea that changes in inflammation are partially related to changes in body composition, the current study also shows that changes in body composition correlate to changes in circulating IL-6 and CRP protein levels but not IL-10 protein levels. Larger decreases in body fat percentage and body fat are directly correlated with larger decreases in CRP and increases in IL-6. Conversely, larger gains in LBM percentage also correlated with larger decreases in CRP and increases in IL-6. Because IL-6 is both a cytokine and a myokine, it is not surprising that IL-6 is somewhat elevated in response to increases in lean body percentage in the present study [181]. It is possible that the increases in acute levels of IL-6 in response to muscle contraction may then lead to the larger decreases in circulating levels of CRP.

In addition to changes in MC3R correlating with changes in adiposity, MC3R was also negatively correlated with changes in resting metabolic rate, with participants experiencing the largest increases in resting metabolic rate also showing the smallest decreases in the percentage of monocytes expressing MC3R. These results are not surprising, as it has been discussed in several earlier publications that the intensity and duration of an exercise bout affects changes in metabolism and these changes in metabolism may lead to alterations in the inflammatory profile

[246]. Based on the body composition changes in the present study, the high metabolic demand placed on the participants as a result of our progressive, intense resistance training plan may be a leading cause of alterations in MC3R expression and, ultimately, monocyte activation and changes in cytokine production and secretion. Indeed, I did observe significant correlations between the changes in the number of monocytes expressing MC3R but not MC1R and changes in body composition. This data may further support the hypothesis that while both MC1R and MC3R possess similar properties such as the same agonists and downstream anti-inflammatory effects upon stimulation, there may be alternate mechanisms of activation and expression for each receptor on systemic monocytes.

In summary, my data shows that 12 weeks of RT leads to changes in the number of monocytes expressing MC1R and MC3R, as well as the amount of each receptor being expressed on individual monocytes. To my knowledge this is the first investigation to study the expression of MC1R and MC3R on systemic monocytes as a novel exercise-induced anti-inflammatory receptor. Future studies should focus on measurement of the MCRs at various time points after a resistance training bout in trained and untrained individuals and correlations in MCR expression with cytokine expression. Exploration into the percent of intracellular versus plasma membrane expression MC1R and MC3R protein also bears merit.

CHAPTER 4. GENERAL SUMMARY

4.1 Summary

The findings in the present study for RMR and strength gains in response to the RT plan are consistent with those found in other studies utilizing a young, healthy population as well as some diseased populations [247-249], thus I believe that the training plan was not a limitation in the present study. While some laboratories have reported larger increases in strength gains in response to resistance training than the present study, these publications did not utilize an acclimation period and measurement of strength was not completed using free weights and as in the present study; furthermore, strength assessment may have also been performed previously for small muscle groups. For example, Flynn et al. show that 10 wks of resistance training leads to a 34% strength increase in leg extension and a 50% in plantar flexion in 1RM assessments [250]. Frontera et al show that 12 wks of resistance training in men leads to a 116.7% increase in knee extensor strength and 226.7% increase in knee flexor strength using 1RM assessments on a Cybex, a pneumatic machine [251]. Both studies, as well as most resistance training intervention studies, utilize a guided machine for each lift and assess strength in a more isoloated manner than the testing utilized in the present study. Here we used unassisted barbell bench press and squat for the 8RM testing which requires not only the strength to move the load but also stabilization of the load during the lift and muscle synergy, making it more difficult than machine assisted lifts. Furthermore, both the CON and RT groups in the present study underwent a 4 day acclimation period including the lifts used for the 8RM assessment before the intervention period. No acclimation period was utilized in the other aforementioned studies [250-251]. The acclimation period may explain the smaller changes seen in strength gains in the RT group, as it provided familiarization with the weight equipment as well as increased confidence in the CON and RT groups in the particular lifts used during the 8RM testing. Indeed, it has been shown that

at least 3-4 familiarization sessions with weight lifting are needed to accurately assess 1RM measurements in young subjects [252]. Furthermore, the present study utilized an 8RM protocol as a more accurate assessment of strength changes than a 1RM protocol, as the repetition range for a single set in the RT regimen was usually between 8-10 repetitions. The comparison of 8RM measurements to 1RM measurements is not a fair comparison, as derivation of an individual's 1RM from 8RM testing is only an estimate of the 1RM due a nonlinear association between the two. Although the changes in strength reported here are small to moderate, we do see large increases in LBM, a 1.45kg average increase, and decreases in percent fat, 1.56% decrease, in response to the undulating periodization utilized in the present study compared to other studies utilizing a progressive resistance training routine in a young, healthy population. For example, Poehlman et al. show that 6 months of progressive resistance training leads to no change in FM and a 2kg increase in LBM [253], and, in a separate study, 6 months of RT leads to no change in FM and a 1.3kg increase in LBM [247]. Rakobowchuk et al report a significant increase of 2.3lb in FM and a 3.5% increase in LBM [254]. The changes in body composition seen herein are indicative of an intense resistance training regimen that decreased the chance of habituation to the exercise stressor. In addition to determining the effects of RT in young, healthy individuals on RMR and strength, I set out to determine the following:

Specific Aim 1: Determine MC1R and MC3R expression and density on plasma membranes of circulating monocytes in response to chronic, repeated resistance training bouts.

Conclusion: MC1R and MC3R cell surface expression change in response to resistance training. The percentage of systemic monocytes expressing MC1R significantly increased in response to RT whereas the percentage of monocytes expressing MC1R in the CON group did not change. Conversely, the percentage of monocytes expressing MC3R significantly decreased in RT with

no change in CON. Additionally, MC1R and MC3R plasma density decreased in RT with no change in MC1R MFI in CON.

Specific Aim 2: Determine if resistance training alters CRP levels in conjunction with changes in pro and anti-inflammatory cytokine profiles in college aged individuals.

Conclusions: CRP levels decreased from the moderate risk category (1-3 mg/mL) to the low risk category (<1 mg/mL) in RT but not CON individuals, although the decrease in CRP was not significantly different in POST RT compared to PRE RT. However in RT, CRP levels significantly decreased POST in those individuals expressing higher baseline levels of CRP. RT participants also experienced no chane in IL-6 and significant decreases in IL-10 in conjunction with the changes in CRP. Concominant decreases in IL-10 were also noted in CON. Thus, changes in IL-10 may be due to seasonal variation rather than treatment.

Specific Aim 3: Determine if MC1R and MC3R expression correlates to changes in the inflammatory profile in college-aged individuals in response to resistance training.

Conclusions: There were no correlations between changes in MCR expression or the percentage of monocytes expressing MCR with changes in representative cytokine or CRP expression. There were, however, significant correlations between increases in the percentage of monocytes expressing MC3R but not MC1R and decreases in body fat percentage and increases in lean body mass percentage. Changes in the percentage of monocytes expressing MC3R but not MC1R may be dependent on changes in body composition.

4.2 Conclusions

MC1R did not correlate to changes in body composition or energy expenditure in RT, whereas changes in MC3R correlated with changes in body fat percentage, changes in body fat, and changes in energy expenditure. Additionally, changes in MCRs did not correlate to changes in cytokines. However, changes in both CRP and IL-6 correlated with changes in body fat

percentage, changes in body fat, and changes in lean body mass but not changes in energy expenditure. Changes in IL-10 did not correlate with changes in body composition or energy expenditure. Changes in CRP did not correlate with changes in either IL-6 or IL-10.

Limitations

There are several limitations to the current study worth mentioning, including the time point at which samples were obtained, cell surface vs. intracellular expression of MCR, cytokines measured, and the sample population used. For example, my previous findings show that resistance training increases MC3R mRNA expression 13-fold in whole blood samples in older overweight women. In the current study, in an effort to avoid the effects of anti-inflammatory drug use in the older population, I proposed to examine the expression of MC3R in healthy, younger individuals, both men and women, with exercise training. Thus, I may not have observed the same changes in protein expression due to the difference in study population. However, I did observe a significant change in all principle outcome variables indicating that resistance training can influence the inflammatory profile of this population. Additionally, the inclusion of measurements of MC1R and MC3R mRNA or intracellular protein levels may have added significance to the current study, and allowed a better comparison to the findings of my previous study in the older postmenopausal women. I also chose the most widely measured cytokines in exercise science, Il-6 and IL-10, as representative cytokines for changes in pro- or anti-inflammatory pathways, respectively. The inclusion of additional cytokines may have added more significance to our finds. Also, the measurement of MCR expression at different time points would have allowed us to determine if the effects of the resistance training plan in the current study shifted the time at which systemic cytokine and MCR monocyte expression return to baseline levels.

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APPENDIX 1. PROGRESSIVE RESISTANCE TRAINING ROUTINE

ACC1

Exercise	Reps	Rest
Bench press	12	1.5
Bent over rows	12	1.5
Shoulder Press	10	1.5
Straight bar curls	10	1.5
Dumbell shrug	10	1.5
Pressdowns	10	1.5
Crunches	15	1
Crunches	12	1
Crunches	10	

ACC2

Exercise	Reps	Rest
Squats	15	1.5
Deadlift	15	1.5
Walking lunges	15	2
Extensions	12	1.5
Lying leg curls	12	1.5
Standing calves	10	1.5
Seated calves	10	

ACC3

Exercise	Reps	Rest
Bench press	12	1.5
Bench press	10	2
Bent over rows	12	1.5
Bent over rows	10	2
Shoulder Press	10	1.5
Shoulder Press	8	2
Straight bar curls	10	1.5
Straight bar curls	8	2
Dumbell shrug	10	1.5
Dumbell shrug	8	2
Pressdowns	10	1.5
Pressdowns	8	2
Crunches	15	1
Crunches	12	1
Crunches	10	

ACC4

Exercise	Reps	Rest
Squats	15	1.5
Squats	12	2
Deadlift	15	1.5
Deadlift	12	2
Walking lunges	15	1.5
Walking lunges	12	2
Extensions	15	1.5
Extensions	12	2
Lying leg curls	12	1.5
Lying leg curls	10	2
Standing calves	12	1.5
Standing calves	10	2
Seated calves	12	1.5
Seated calves	10	

Week 3

Exercise	**Reps**	**Rest**
Bench press	12	1.5
Bench press	10	1.5
Bench press	8	2
Bent over rows	12	1.5
Bent over rows	10	1.5
Bent over rows	8	2
Shoulder Press	10	1.5
Shoulder Press	8	1.5
Shoulder Press	6	2
Straight bar curls	10	1.5
Straight bar curls	8	1.5
Straight bar curls	6	2
Dumbell shrug	10	1.5
Dumbell shrug	8	1.5
Dumbell shrug	6	2
Pressdowns	10	1.5
Pressdowns	8	1.5

Exercise	**Reps**	**Rest**
Squats	15	1.5
Squats	12	1.5
Squats	10	2
Deadlift	10	1.5
Deadlift	8	1.5
Deadlift	6	2
Walking lunges	16	1.5
Walking lunges	12	1.5
Walking lunges	10	2
Leg extensions	18	1.5
Leg extensions	15	1.5
Leg extensions	12	2
Lying leg curls	12	1.5
Lying leg curls	10	1.5
Lying leg curls	8	2
Standing calves	12	1.5
Standing calves	10	1.5
Standing calves	8	2
Seated calves	12	1.5
Seated calves	10	1.5
Seated calves	8	

Pressdowns	6	2
Crunches	20	1
Crunches	15	1
Crunches	12	

Week 4

Exercise	Reps	Rest
Bench press	10	1.5
Bench press	8	1.5
Bench press	6	2
Incline press	10	1.5
Incline press	8	1.5
Incline press	6	2
inlcine push up	15	1.5
incline push up	15	2
Shoulder press	10	1.5
Shoulder press	8	1.5
Shoulder press	6	2
Lateral raises	8	1.5
Lateral raises	8	1.5
Lateral raises	8	2
Rear delt rows	10	1.5
Rear delt rows	8	1.5
Rear delt rows	6	2
Overhead DB extension	10	1.5
Overhead DB extension	8	1.5
Overhead DB extension	8	2
Pressdowns	10	1.5
Pressdowns	8	

Exercise	Reps	Rest
Seated rows	10	1.5
Seated rows	8	1.5
Seated rows	6	2
Back extensions	20	1.5
Back extensions	15	1.5
close grip pulldowns	12	1.5
close grip pulldowns	10	2
wide grip pulldowns	10	1.5
wide grip pulldowns	8	2
Straight bar curls	10	1.5
Straight bar curls	8	1.5
Straight bar curls	6	2
Hammer curls	10	1.5
Hammer curls	8	1.5
Hammer curls	ø	2
Crunches	25	1
Crunches	25	1
Crunches	25	2
Elbow to knee	20	1
Elbow to knee	20	1
Elbow to knee	20	

Exercise	Reps	Rest
Squats	12	1.5
Squats	10	1.5
Squats	8	1.5
Squats	6	2
Deadlifts	8	1.5
Deadlifts	6	1.5
Deadlifts	4	2
Walking lunges	18	1.5
Walking lunges	15	1.5
Walking lunges	12	2
Extensions	10	1.5
Extensions	8	1.5
Extensions	6	2
Lying leg curls	10	1.5
Lying leg curls	8	1.5
Lying leg curls	6	2
Standing calves	10	1.5
Standing calves	8	1.5
Standing calves	6	2
Seated calves	30	1.5
Seated calves	25	1.5
Seated calves	20	1.5

Week 5

Exercise	Reps	Rest
Bench press	10	1.5
Bench press	8	1.5
Bench press	6	2
Incline press	10	1.5
Incline press	8	1.5
Incline press	6	2
incline push up	15	1.5
incline push up	15	2
swimmer press	10	1.5
swimmer press	8	1.5
swimmer press	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1
Rear delt rows	10	1.5
Rear delt rows	8	1.5
Rear delt rows	6	2
isometric DB T hold-	30sec	0

Exercise	Reps	Rest
Seated rows	10	1.5
Seated rows	8	1.5
Seated rows	6	2
Back extensions	20	1.5
Back extensions	15	1.5
Back extensions	12	2
close grip pulldowns	12	1.5
close grip pulldowns	10	2
wide grip pulldowns	10	1.5
wide grip pulldowns	8	2
Straight bar curls	10	1.5
Straight bar curls	8	1.5
Straight bar curls	6	2
Hammer curls	10	1.5
Hammer curls	8	1.5
Hammer curls	6	2
Crunches	25	1

Exercise	Reps	Rest
Squats	12	1.5
Squats	10	1.5
Squats	8	1.5
Squats	6	2
Deadlifts	8	1.5
Deadlifts	6	1.5
Deadlifts	4	2
Walking lunges	18	1.5
Walking lunges	15	1.5
Walking lunges	12	2
Extensions	10	1.5
Extensions	8	1.5
Extensions	6	2
Lying leg curls	10	1.5
Lying leg curls	8	1.5
Lying leg curls	6	2
Standing calves	10	1.5
Standing calves	8	1.5

135		
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1
Overhead DB extension	10	1.5
Overhead DB extension	8	1.5
Overhead DB extension	8	2
Pressdowns	10	1.5
Pressdowns	8	

Crunches	25	1
Crunches	25	2
Elbow to knee	20	1
Elbow to knee	20	1
Elbow to knee	20	

Standing calves	6	2
Seated calves	30	1.5
Seated calves	25	1.5
Seated calves	20	

Week 6

Exercise	Reps	Rest
medicine ball throws	30	0.5
Dumbell press	10	1
Dumbell press	8	1
Dumbell press	6	2
medicine ball throws	30	0.5
decline push ups	15	1
decline push ups	12	2
Cable crossovers	12	1
Cable crossovers	10	2
Close grip bench press	10	1
Close grip bench press	8	1
Close grip bench press	6	2
overhead medicine ball tosses	25	0.5
Lying tricep extension	10	1
Lying tricep extension	10	1
overhead medicine ball tosses	25	0.5
swimmer press	10	1.5
swimmer press	8	1.5
swimmer press	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1
front raises	10	1.5
front raises	8	1.5
front raises	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1

Exercise	Reps	Rest
T bar row	12	1
T bar row	10	1
T bar row	8	2
Stiff legged deadlifts	12	1
Back extensions	30	1
Close grip pulldowns	12	1
Close grip pulldowns	10	2
Close grip pulldowns	8	2
Stiff legged deadlifts	10	1
Back extensions	25	1
Dumbell curls	10	1
Dumbell curls	8	1
underhand medicine ball toss	25	0.5
incline dumbell curl	10	1
incline dumbell curl	8	2
underhand medicine ball toss	25	0.5
Concentration curls	12	1.5
Concentration curls	12	2
Butterfly crunches	40	1
Butterfly crunches	30	1
Butterfly crunches	30	1.5
medicine ball twist	30	1
medicine ball twist	25	1
medicine ball twist	20	
scissors	40	1
scissors	30	1
scissors	30	1.5

Exercise	Reps	Rest
jump squats	20	0.5
side leg jump holds	15	0.5
squats on plank	10	1.5
squats on plank	8	1.5
squats on plank	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
side step squats	10	1.5
side step squats	8	1.5
side step squats	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
step ups	12	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	10	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	8	1.5
one legged standing calves	30	1
one legged standing calves	30	1
one legged standing calves	25	1
one legged standing calves	50	2
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	1
seated calve raises	50	2

Week 7

Exercise	Reps	Rest

medicine ball throws	30	0.5
Dumbell press	10	1
Dumbell press	8	1
Dumbell press	6	2
medicine ball throws	30	0.5
decline push ups	15	1
decline push ups	12	2
Cable crossovers	12	1
Cable crossovers	10	2
Close grip bench press	10	1
Close grip bench press	8	1
Close grip bench press	6	2
overhead medicine ball tosses	25	0.5
Lying tricep extension	10	1
Lying tricep extension	10	1
Lying tricep extension	8	2
Front raises	12	1
Front raises	10	1
Front raises	8	2
Upright rows	10	1
Upright rows	8	2
rear delt cable pulls	12	1
rear delt cable pulls	10	1
rear delt cable pulls	8	2

T bar row	12	1
T bar row	10	1
T bar row	8	2
Stiff legged deadlifts	12	1
Back extensions	30	1
Close grip pulldowns	12	1
Close grip pulldowns	10	2
Close grip pulldowns	8	2
Stiff legged deadlifts	10	1
Back extensions	25	1
Dumbell curls	10	1
Dumbell curls	8	1
underhand medicine ball toss	25	0.5
incline dumbell curl	10	1
incline dumbell curl	8	2
underhand medicine ball toss	25	0.5
Concentration curls	12	1.5
Concentration curls	12	2
Butterfly crunches	40	1
Butterfly crunches	30	1
Butterfly crunches	30	1.5
medicine ball twist	30	1
medicine ball twist	25	1
medicine ball twist	20	
scissors	40	1
scissors	30	1
scissors	30	1.5

jump squats	20	0.5
side leg jump holds	15	0.5
squats on plank	10	1.5
squats on plank	8	1.5
squats on plank	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
side step squats	10	1.5
side step squats	8	1.5
side step squats	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
step ups	12	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	10	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	8	1.5
one legged standing calves	30	1
one legged standing calves	30	1
one legged standing calves	25	1
one legged standing calves	50	2
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	1
seated calve raises	50	2

Week 8

Exercise	**Reps**	**Rest**
push ups	20	0
lawnmowers	12	0
military push ups	15	0
wide grip pulldowns	12	0
decline push ups	20	0
underhand pulldowns	12	0
side step pushups	18	0
close grip pulldowns	10	0
triangle pushups	16	0
seated rows	8	0
push ups	20	0
lawnmowers	10	0.5
military push ups	15	0
wide grip pulldowns	15	0.5

Exercise	**Reps**	**Rest**
Curl and Press	12	0
pushdowns	12	0
Straight bar curls	12	0
switch grip kickbacks	12	0
lateral raises	10	0
hold and curl	12	0
skull crushers	10	0
upright rows	12	0
pronating curls	10	0
skull crushers	10	0
upright rows	12	0.5
hold and curl	10	0
standing kickbacks	10	0
seated straight and leaning shoulder flyes	12	0
Curl and Press	12	0

Exercise	**Reps**	**Rest**
Squats	30	0
walking toe lunges	20	0
run stance squats	30	0
step ups	25	0.5
Squats	30	0
walking toe lunges	20	0
run stance squats	30	0
step ups	25	0.5
wall squat	1min	0.1
one legged floor touches	25	0
one legged wall squat	1min	1.5
lunge and kick	15	0
wall squats	1min	0.1
one legged floor	25	0.5

decline push ups	20	0
underhand pulldowns	12	0.5
side step pushups	18	0
close grip pulldowns	10	0.5
triangle pushups	16	0
seated rows	8	0.5
holds	1min	0
holds	1min	0
holds	30sec	0
frog	30	0
one leg holds	1min	0
one leg holds	1min	0
scissors	30	0
reach and cross toe touch	50	0
fist twists	50	0

pushdowns	12	0
Straight bar curls	12	0
switch grip kickbacks	12	0
lateral raises	10	0
pronating curls	10	0
standing kickbacks	10	0
seated straight and leaning shoulder flyes	12	0

touches		
one legged wall squat	1min	1.5
lunge and kick	15	0
wall squat	1min	0.1
one legged floor touches	25	0.5
holds	1min	0
holds	1min	0
holds	30sec	0
frog	30	0
one leg holds	1min	0
one leg holds	1min	0
scissors	30	0
reach and cross toe touch	50	0
fist twists	50	0

Week 9

Exercise	**Reps**	**Rest**
medicine ball throws	30	0.5
Dumbell press	10	1
Dumbell press	8	1
Dumbell press	6	2
medicine ball throws	30	0.5
decline push ups	15	1
decline push ups	12	2
Cable crossovers	12	1
Cable crossovers	10	2
Close grip bench press	10	1
Close grip bench press	8	1
Close grip bench press	6	2
overhead medicine ball tosses	25	0.5
Lying tricep extension	10	1
Lying tricep extension	10	1
overhead medicine ball tosses	25	0.5
swimmer press	10	1.5
swimmer press	8	1.5
swimmer press	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1
front raises	10	1.5
front raises	8	1.5
front raises	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	

Exercise	**Reps**	**Rest**
T bar row	12	1
T bar row	10	1
T bar row	8	2
Stiff legged deadlifts	12	1
Back extensions	30	1
Close grip pulldowns	12	1
Close grip pulldowns	10	2
Close grip pulldowns	8	2
Stiff legged deadlifts	10	1
Back extensions	25	1
Dumbell curls	10	1
Dumbell curls	8	1
underhand medicine ball toss	25	0.5
incline dumbell curl	10	1
incline dumbell curl	8	2
underhand medicine ball toss	25	0.5
Concentration curls	12	1.5
Concentration curls	12	2
Butterfly crunches	40	1
Butterfly crunches	30	1
Butterfly crunches	30	1.5
medicine ball twist	30	1
medicine ball twist	25	1
medicine ball twist	20	
scissors	40	1
scissors	30	1
scissors	30	

Exercise	**Reps**	**Rest**
jump squats	20	0.5
side leg jump holds	15	0.5
squats on plank	10	1.5
squats on plank	8	1.5
squats on plank	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
side step squats	10	1.5
side step squats	8	1.5
side step squats	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
step ups	12	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	10	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	8	1.5
one legged standing calves	30	1
one legged standing calves	30	1
one legged standing calves	25	1
one legged standing calves	50	2
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	1
seated calve raises	50	

Week 10

Exercise	Reps	Rest
medicine ball throws	30	0.5
Dumbell press	10	1
Dumbell press	8	1
Dumbell press	6	2
medicine ball throws	30	0.5
decline push ups	15	1
decline push ups	12	2
Cable crossovers	12	1
Cable crossovers	10	2
Close grip bench press	10	1
Close grip bench press	8	1
Close grip bench press	6	2
overhead medicine ball tosses	25	0.5
Lying tricep extension	10	1
Lying tricep extension	10	1
overhead medicine ball tosses	25	0.5
swimmer press	10	1.5
swimmer press	8	1.5
swimmer press	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	1
front raises	10	1.5
front raises	8	1.5
front raises	6	2
isometric DB T hold-135	30sec	0
isometric DB T hold-90	30sec	0
isometric DB T hold-45	30sec	

Exercise	Reps	Rest
T bar row	12	1
T bar row	10	1
T bar row	8	2
Stiff legged deadlifts	12	1
Back extensions	30	1
Close grip pulldowns	12	1
Close grip pulldowns	10	2
Close grip pulldowns	8	2
Stiff legged deadlifts	10	1
Back extensions	25	1
Dumbell curls	10	1
Dumbell curls	8	1
underhand medicine ball toss	25	0.5
incline dumbell curl	10	1
incline dumbell curl	8	2
underhand medicine ball toss	25	0.5
Concentration curls	12	1.5
Concentration curls	12	2
Butterfly crunches	40	1
Butterfly crunches	30	1
Butterfly crunches	30	1.5
medicine ball twist	30	1
medicine ball twist	25	1
medicine ball twist	20	
scissors	40	1
scissors	30	1
scissors	30	

Exercise	Reps	Rest
jump squats	20	0.5
side leg jump holds	15	0.5
squats on plank	10	1.5
squats on plank	8	1.5
squats on plank	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
side step squats	10	1.5
side step squats	8	1.5
side step squats	6	2
jump squats	20	0.5
side leg jump holds	15	0.5
step ups	12	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	10	1.5
2 box jump	15	0.5
cross the river	10	0.5
step ups	8	1.5
one legged standing calves	30	1
one legged standing calves	30	1
one legged standing calves	25	1
one legged standing calves	50	2
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	1
seated calve raises	50	

Week 12

Exercise	Reps	Rest
Close grip bench press	10	1
Close grip bench press	10	1
Close grip bench press	10	2
standing switch grip kickbacks	14	1
standing switch grip kickbacks	14	2
wide rope pushdowns	8	1
wide rope pushdowns	8	2
reverse cable dumbell curls	8	1
reverse cable dumbell curls	8	2
incline dumbell curl	8	1
incline dumbell curl	8	2
pronating curls	8	1
pronating curls	8	2
pullovers	8	1
pullovers	8	1

Exercise	Reps	Rest
Squats	15	1.5
Squats	8	2
Squats	6	2
Squats	4	2
Squats	2	2
Extensions	8	1.5
Extensions	8	2
Lying leg curls	8	1.5
Lying leg curls	8	2
Standing calves	8	1.5
Standing calves	6	2
Seated calves	30	1.5
Seated calves	25	1.5
Seated calves	20	

Exercise	Reps	Rest
bench press	8	1
bench press	8	2
bench press	6	2
bench press	6	2
incline press	6	1
incline press	6	2
flyes	8	1
flyes	8	2
seated rows	12	1
seated rows	12	1
seated rows	10	1
Stiff legged deadlifts	15	1
Stiff legged deadlifts	12	1
switch grip pulldowns	10	1
switch grip pulldowns	10	1
switch grip pulldowns	10	

military press	10	1
military press	10	2
reverse pullovers	8	1
reverse pullovers	8	2
plank	40	0.5
plank	30	0.5
plank	30	1
medicine ball twist	30	0.5
medicine ball twist	25	0.5
medicine ball twist	20	1
leg press ups	40	0.5
leg press ups	30	0.5
leg press ups	30	

Week 13

Exercise	**Reps**	**Rest**
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	0.5
seated calve raises	50	
Lying leg curls	10	1.5
Lying leg curls	10	1.5
Lying leg curls	10	1.5
mary catherine lunges	15	2
Extensions	10	1.5
Extensions	10	1.5
Extensions	10	1.5
mary catherine lunges	15	2
Squats	8	1.5
Squats	8	1.5
Squats	8	1.5
mary catherine lunges	15	2
Deadlifts	8	1.5
Deadlifts	8	1.5
mary catherine lunges	15	

Exercise	**Reps**	**Rest**
bench press	15	1
bench press	8	2
bench press	6	2
bench press	4	2
bench press	2	2
incline press	6	1
incline press	6	2
flyes	8	1
flyes	8	2
seated rows	12	1
seated rows	10	1
Stiff legged deadlifts	15	1
Stiff legged deadlifts	12	1
switch grip pulldowns	10	1
switch grip pulldowns	10	

Exercise	**Reps**	**Rest**
Close grip bench press	10	1
Close grip bench press	10	1
Close grip bench press	10	2
standing switch grip kickbacks	14	1
standing switch grip kickbacks	14	2
wide rope pushdowns	8	1
wide rope pushdowns	8	2
reverse cable dumbell curls	8	1
reverse cable dumbell curls	8	2
incline dumbell curl	8	1
incline dumbell curl	8	2
pronating curls	8	1
pronating curls	8	2
pullovers	8	1
pullovers	8	1
military press	10	1
military press	10	2
reverse pullovers	8	1
reverse pullovers	8	2
plank	40	0.5
plank	30	0.5
plank	30	1
medicine ball twist	30	0.5
medicine ball twist	25	0.5
medicine ball twist	20	1
leg press ups	40	0.5
leg press ups	30	0.5
leg press ups	30	

Week 14

Exercise	**Reps**	**Rest**
deadlifts	15	1
deadlifts	8	2
deadlifts	6	2
deadlifts	4	2
deadlifts	2	2
deadlifts	2	2
switch grip pulldowns	10	1

Exercise	**Reps**	**Rest**
holds	1min	0
holds	1min	0
holds	30sec	0
frog	30	0
one leg holds	1min	0
one leg holds	1min	0
scissors	30	0

Exercise	**Reps**	**Rest**
seated calve raises	30	1
seated calve raises	30	1
seated calve raises	25	0.5
seated calve raises	50	
Lying leg curls	10	1
Lying leg curls	10	2

switch grip pulldowns	10	2
bench press	8	1
bench press	8	2
bench press	6	2
bench press	6	2
decline push ups	6	1
decline push ups	6	2
flyes	8	1
flyes	8	2

reach and cross toe touch	50	0
fist twists	50	0
standing switch grip kickbacks	14	1
standing switch grip kickbacks	14	2
wide rope pushdowns	8	1
wide rope pushdowns	8	2
reverse cable dumbell curls	8	1
reverse cable dumbell curls	8	2
pronating curls	8	1
pronating curls	8	2
pullovers	8	1
pullovers	8	1
military press	10	1
military press	10	2
reverse pullovers	8	1
reverse pullovers	8	2

3 angle lunge and kick	30	0
mary catherine lunges	15	0
Extensions	10	1
Extensions	10	2
3 angle lunge and kick	30	0
mary catherine lunges	15	0
Squats	8	1.5
Squats	8	1.5
Squats	8	2
3 angle lunge and kick	30	0
mary catherine lunges	15	0
Deadlifts	8	1.5
Deadlifts	8	

APPENDIX 2. PARTICIPANT CONSENT FORM

1. Study Title: **Strength Training Induced Alterations in Markers of Immune Function**

2. Performance Site: **Louisiana State University**

   Baton Rouge, LA 70803

3. Investigators: The following investigators are available for questions about this study,

   M-F, 8:00 a.m.- 10:40 a.m., 1:40 p.m. - 4:30 p.m.

   Principal Investigator: Laura K. Stewart, Ph.D.
   Co-investigator: Tara Henagan,

4. Purpose of the Study:

   The purpose of this research project is to examine the effect of a 12 week intensive strength training intervention on markers of immunity.
   Subject Inclusion:
   Participants in both the control and training groups must be at least 18 years of age and currently enrolled as students at LSU and may not be pregnant. Students in the training group must have medical clearance following a complete comprehensive physical from a State licensed M.D. or D.O. The cleared student must provide documentation of permission by returning a signed "Physician Approval Form" after the physical and before participating in the study. Students must complete a physical activity readiness questionnaire (PAR-Q) before participating.

5. Number of subjects = 60 (n = 30 in the training group and n = 30 in the control group).

6. Study Procedures:

   Baseline: Before the 12 week training program begins, you will have baseline measurements taken. This will include height, weight, blood pressure, heart rate, body composition (body fat percentage, bone mineral density) taken with a DXA and skinfold measurements taken with a Lange skinfold caliper. At this time, you will also fill out questionnaires. We will take a resting/fasting venous blood sample from your antecubital vein between 6 and 9am. An assessment of your physical fitness will be estimated by having you perform a 8RM test with blood lactate measurements taken by finger prick every 5 minutes up to 30 minutes after your resistance training bout.

   12 Week Intervention Program: At the start of the program, you will be provided with a suggested training program (training groups only). If you choose not to follow this training program, you will not be penalized. Throughout the 12 week training period, all groups will fill out a training log and a 3-day diet recall log, as well as a sickness and injury inventory questionnaire. This should take about 20 minutes weekly to complete. Participants in the control group will be instructed to continue ususal day to day activities. Participants in both groups will continue to complete weekly training and diet logs, and sickness inventory questionnaires.

Post-Training: During the last week of class, you will undergo post-training measures. Procedures for post-training measurements will be identical to the protocol followed for preliminary measures.

7. Benefits:

   While no guarantee of benefits can be made, you will be given training instruction and a body composition analysis at no cost to you. You may also learn more about your physical capacity for strength and should significantly improve your anaerobic fitness. Subjects will be provided a comparison of his/her pre-training and post-training results at the end of the semester.

8. Risks/Discomforts:

   Exercise Training & Testing: As with any exercise program, there is a chance that you will experience muscle soreness, fatigue, or even injuries such as sprains or strains. There is also a remote risk of a heart attack or stroke and in very rare cases, death. Precautions to minimize this risk have been taken by requiring medical clearance to participate as well as completion of a health history questionnaire. Your cooperation in obtaining approval from your physician as well as providing honest answers in completing the health history form will decrease this risk.

   DXA: The Dual Energy X-Ray Absorptiometry test uses an x-ray technique to assess the density of your body and can then provide an accurate estimate of body fat percentage and lean body mass. The whole body DXA scan exposes subjects to low levels of ionizing radiation. No discomfort will be felt during the DXA scan. There is a risk of radiation exposure from this test, but it is well below the level that would provide any adverse effects. The total body DXA Scan will produce less than 0.37 μSV (0.037 mrem) of ionizing radiation skin entrance dosage. The skin entrance dose of 0.37 μSV is well below the yearly public limit dose for incidental exposure of 1,000 μSV (100 mrems).

   Skinfold measurements: Since a slight pinching of the skin is required to measure subcutaneous fat through calipers, you may experience mild bruising in the measurement sites (chest, midaxillary, triceps, subscapular, abdomen, suprailiac, and thigh).

   Blood Sampling: There is a risk of bruising and a remote risk of infection with the blood sampling techniques. You may also become lightheaded and faint during these procedures. These risks will be minimized by having trained technicians using sterile, single-use supplies for blood sampling. You will be seated during blood sampling, but you should tell us if you feel dizzy or faint.

In addition to the risks listed above, you may experience a previously unknown risk or side effect.

9. Injury/Illness:

   In the unlikely event of injury or medical illness resulting from the above procedures, contact **Laura Stewart, Ph.D., 225-578-3549**. You will be referred for treatment, but the expense of medical treatment will be your responsibility. No compensation is available in case of study-related illness or injury.

10. Right to Refuse:

You may choose not to participate or to withdraw from the study at any time with**out penalty or loss of any benefit** to which you might otherwise be entitled.

11. Privacy:

Your identity will remain confidential unless disclosure is required by law. In other words, data will be kept confidential unless release is legally compelled. All data collected will be handled only by the investigators and kept in a secure location. Results of the study may be published using group means only and names or identifying information will not be included in the publication.

12. Financial Information:

There is no cost to you, nor is there any compensation for participating in the study.

13. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have any questions about subjects' rights or other concerns, I can contact Robert C. Matthews, Institutional Review Board at (225) 578-8692. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of this consent form.

_____ _____

Participant's Signature Date

The study subject has indicated that s/he is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

_____ _____

Reader's Signature Date

APPENDIX 3. IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

APPENDIX 4. PHYSICIAN'S APPROVAL FORM

Physician Approval Form
Kinesiology 4501
The Physiology of Endurance Training
This physical must be completed by a State licensed M.D. or D.O. only

I have completed a full comprehensive physical examination of _____ *(print student's full name)* as required for him/her to participate in Kinesiology 4501 (The Physiology of Weight Training). I understand that the student will be engaging in an intensive resistance training and conditioning program which will involve optional participation in a course-designated weight lifting routine for the Spring 2010 semester. Based on my physical findings, I am making the following recommendation related to the demands of the course:

____ Patient is cleared to participate **without restriction**.
____ Patient is cleared to participate with the following restrictions or guidelines.
_____
_____ Patient is **not** cleared to participate.

Physician Signature: _____
Physician Name (Print):_____ _
Date: _____
Address: _____
Phone: _____
Fax: _____
Email: _____

VITA

Tara M. Henagan was born to Margaret Edana Henagan and Charles Rory Henagan in Baton Rouge, Louisiana. After several moves as a child, Tara became a permanent resident of Louisiana at the age of ten and attended St. Louis King of France School until the eighth grade. Tara graduated from St. Michael the Archangel High School in 2000. She attended Louisiana State University with a Honors TOPS scholarship and graduated with a Bachelor of Science degree in biological sciences with a minor in anthropology in 2004.

Upon completion of her degree program in 2004, Tara worked as a Research Assistant in Dr. Thomas W. Gettys' laboratory at the Pennington Biomedical Research Center, where her appreciation of science and scientific research blossomed. While working with Dr. Gettys, Tara met a fellow researcher and postdoctoral fellow, Dr. Laura K. Stewart. Due to their mutual regard for scientific research, Tara followed in Dr. Stewart's footsteps and entered the graduate program with Dr. Stewart as her mentor in 2007. While in graduate school, Tara received the Corbett Graduate Student Summer Fellowship and a recurring teaching assistantship in the School of Education through the Department of Kinesiology. In 2010, Tara passed her Certified Strength and Conditioning examination. She plans to utilize her knowledge in weight lifting to help others achieve a healthier lifestyle. Tara plans to graduate from the graduate program at LSU in December 2010, only three short years after joining the program, with a Doctor of Philosophy in kinesiology and a minor in pathobiological sciences.