2016

Resistance Exercise Elicits Selective Mobilization and Adhesion Characteristics of Granulocytes and Monocyte Subsets

Adam Jajtner
University of Central Florida

RESISTANCE EXERCISE ELICITES SELECTIVE MOBILIZATION AND ADHESION CHARACTERISTICS OF GRANULOCYTES AND MONOCYTE SUBSETS

by

ADAM RAYMOND JAJTNER
BS Valparaiso University, 2009
MS Texas Christian University, 2012

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the College of Education and Human Performance
at the University of Central Florida
Orlando, Florida

Spring Term
2016

Major Professor: Jay R. Hoffman

ABSTRACT

Resistance exercise eliciting muscle damage results in an immune response, leading to increases in circulating cytokines, and immune cell mobilization. Classical monocytes respond to muscle damage, however, little is known about the intermediate or nonclassical monocyte response to resistance exercise. Moreover, the impact of polyphenol supplementation in conjunction with resistance exercise on the innate immune response is unknown. The purpose of this study was to examine the immune response following resistance exercise with (PPB) and without (PL) polyphenol supplementation. Thirty-nine untrained men were randomized into three groups: PPB (n=13, 21.8±2.5yrs, 171.2±5.5cm, 71.2±8.2kg), PL (n=15, 21.6±2.5yrs, 176.5±4.9cm, 84.0±15.7kg) or a control group (CON) (23.3±4.1yrs, 173.6±12.0cm, 77.8±15.6kg). Blood samples were obtained pre- (PRE), immediately- (IP), 1- (1H), 5- (5H), 24- (24H), 48- (48H) and 96- (96H) hours post-exercise (PPB/PL). CON rested for one hour between PRE and IP blood draws. Changes in granulocyte and monocyte subset proportions and adhesion characteristics (CD11b) were assessed via flow cytometry, while plasma cytokine concentrations and markers of muscle damage were analyzed via multiplex and spectrophotometric assays, respectively. Creatine Kinase and myoglobin were elevated at each time point for PPB and PL ($p < 0.050$). Monocyte chemoattractant protein-1 was significantly elevated at IP in PPB ($p = 0.005$) and PL ($p = 0.006$) and significantly greater than CON at 5H (PPB: $p < 0.001$; PL: $p = 0.006$). Granulocyte proportions were elevated at 1H ($p < 0.001$), 5H ($p < 0.001$) and 24H ($p = 0.005$; $p = 0.006$) in PPB and PL, respectively. Classical monocyte proportions were lower in PPB ($p = 0.008$) and PL ($p = 0.003$) than CON at IP, and significantly greater

than CON at 1H (PPB: $p = 0.002$; PL: $p = 0.006$). Nonclassical monocyte proportions were significantly greater in PPB ($p = 0.020$) and PL ($p = 0.028$) than CON at IP. Intermediate monocyte proportions were significantly greater in PPB ($p = 0.034$) and PL ($p = 0.001$) than CON at IP, and significantly lower than CON at 1H (PPB: $p = 0.003$; PL: $p = 0.008$). Intermediate monocyte proportions were also significantly greater in PPB than CON at 24H ($p = 0.016$) and 48H ($p = 0.007$). At PRE, CD11b expression was significantly lower in the PPB group than CON and PL for intermediate ($p = 0.017$; $p = 0.045$) and nonclassical ($p < 0.001$, $p = 0.019$) monocytes, respectively. When groups were combined, CD11b expression was significantly elevated from PRE at IP ($p < 0.001$) and 1H ($p = 0.015$) on granulocytes. CD11b expression on classical monocytes was significantly elevated compared to PRE at 1H ($p < 0.001$), 5H ($p = 0.033$) and 24H ($p = 0.004$) when groups were combined. CD11b expression on intermediate monocytes was significantly elevated compared to PRE at 1H ($p < 0.001$) when groups were combined. Intermediate and nonclassical monocyte proportions also showed significant positive correlations with markers of muscle damage ($r = 0.361$ to 0.775, $p<0.05$). Results indicated that resistance exercise in novice lifters may elicit a selective mobilization of intermediate monocytes at 24h and 48H, and that muscle damage may be related to increases in intermediate and nonclassical monocytes. In addition, polyphenol supplementation appeared to suppress CD11b expression on monocytes to resistance exercise.

To my fiancé Amy, for your love and understanding throughout the past few years, you are truly a blessing in my life!

To my parents Mike and Maureen, for the love and support you have given me throughout my all of my endeavors and the encouragement to chase my dreams!

To my brother and sisters, Eric, Katie and Erin, and all of our foreign exchange brothers and sisters for constantly reminding me why family truly is the most important thing in life; I cannot thank each of you enough!

To my committee members, Dr. Jeffrey Stout, Dr. David Fukuda and Dr. Shlomit Radom-Aizik for your guidance throughout this process!

To my mentor Dr. Jay Hoffman, for providing the opportunities for me to learn and grow as a young professional in the field of Exercise Science. Your guidance will continue impact on my career for years to come!

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

1H	One Hour Post-Exercise
1RM	1-Repetition Maximum
24H	24 Hours Post-Exercise
48H	48 Hours Post-Exercise
5H	Five Hours Post-Exercise
72H	72 Hours Post-Exercise
96H	96 Hours Post-Exercise
ANOVA	Analysis of Variance
APC	Allophycocyanin
AUC	Area Under the Curve
C5aR	Complement C5a Receptor
CCR1	CC Chemokine Receptor 1
CCR2	CC Chemokine Receptor 2
CCR5	CC Chemokine Receptor 5
CD11b	Integrin αM
CD14	LPS Receptor
CD16	FcγRIII
CD16+	Monocytes positive for CD16; Incorporates Intermediate and Nonclassical Monocytes
CD163	Cluster of Differentiation 163; M2 Phenotype marker
CD169	Cluster of Differentiation 169
CD18	Integrin β_2
CD206	Manose Receptor; M2 Phenotype Marker
CD36	Glycoprotein IIIb
CD62L	L-Selectin

CD64	Fc Receptor
CD66b	Cluster of Differentiation 66b; Granulocyte Marker
CD68	Oxidized LDL Receptor; Marker for Macrophages
CK	Creatine Kinase
CLAS	Classical Monocyte
cMoP	Common Monocyte Progenitor Cell
CON	Control Group
CX3CR1	CX_3C Chemokine Receptor 1
CXCR1	CXC Chemokine Receptor 1
CXCR2	CXC Chemokine Receptor 2
DC	Dendritic Cell
ED1	Ectodermal Dysplasia Antibody 1
ED2	Ectodermal Dysplasia Antibody 2
ED3	Ectodermal Dysplasia Antibody 3
ELISA	Enzyme-linked Immunosorbent Assay
F4/80	EGF-like Module-containing Mucin-like Hormone Receptor-like 1
FBS	Fetal Bovine Serum
FITC	Fluorexcein Isothiocyanate
FKN	Fractalkine
sFKN	Soluble Fractalkine
FSC-A	Forward Scatter Area
FSC-H	Forward Scatter Height
G-CSF	Granulocyte Colony Stimulated Factor
GM-CSF	Granulocyte/Macrophage Colony Stimulating Factor
HLA-ABC	Human Leukocyte Antigen - ABC
HLA-DR	Human Leukocyte Antigen – DR; MHC Class II Receptor
HSC	Hematopoietic Stem Cell

ICAM-1	Intracellular Adhesion Molecule 1
IGF-1	Insulin-like Growth Factor 1
IgG	Immunoglobulin G
IL-1ra	Interleukin 1 Receptor Antagonist
IL-1β	Interleukin 1β
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
INF-γ	Interferron γ
iNOS	Inducible Nitric Oxide Synthase
INTER	Intermediate Monocyte
IP	Immediately Post Exercise
K_2EDTA	Ethylenediaminetetraacetic Acid
LDL	Low Density Lipoprotein
LFA-1	Lymphocyte-function Associated Antigen 1
LPS	Lipopolysaccharide
LSD	Least Significant Difference
M1	Early Phase Macrophage
M2	Late Phase Macrophage
MCP-1	Monocyte Chemoattractant Protein-1
MFI	Mean Fluorescence Intensity
MG	Myoglobin
MHC	Major Histocompatibility Complex
MPC	Myogenic Precursor Cell

MPO	Myeloperoxidase
mRNA	Messenger Ribonucleic Acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NETs	Neutrophil Extracellular Traps
NO	Nitric Oxide
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline
PE	Phycoerythrin
PKT	Peak Torque
PL	Placebo Group
PMN	Polymorphoneuclear Cell
PPB	Supplement Group (Proprietary Polyphenol Blend)
PRE	Pre-Exercise
PSGL-1	P-Selectin glycoprotein ligand-1
PVS	Plasma Volume Shifts
ROS	Reactive Oxygen Species
RTD	Rate of Torque Development
SD	Standard Deviation
SE	Standard Error
SSC-A	Side Scatter Area
SSC-H	Side Scatter Height
Th1	T Helper 1 Lymphocyte
Th2	T Helper 2 Lymphocyte
TNF-α	Tumor Necrosis Factor α
VLA-4	Very Late Antigen-4
VO$_2$	Volume of Oxygen Uptake
η^2	Eta Squared

CHAPTER 1: INTRODUCTION

Resistance exercise performed at a sufficient intensity will result in microtrauma to skeletal muscle, which may be reflected by leakage of various biomarkers from the sarcolemma (e.g., creatine kinase or myoglobin), increases in muscle soreness and potential decreases in muscle performance (Clarkson & Hubal, 2002; Jajtner et al., 2015; Paulsen et al., 2005). The mechanical stress associated with the exercise stimulus and the resulting tissue damage signals a profound non-specific immune response (Calle & Fernandez, 2010). This response manifests itself through increases in cytokine and chemokine production from skeletal muscle tissue, endothelial cells, resident macrophages, and other circulating immune cells (Ancuta, Moses, & Gabuzda, 2004; Ancuta et al., 2003; Brigitte et al., 2010; Della Gatta, Cameron-Smith, & Peake, 2014; Nieman et al., 2004). Once released, cytokines and chemokines will elicit a response from the innate immune system, resulting in an accumulation of myeloid cells within a few hours and persist for several days following the muscle damaging exercise protocol (Paulsen et al., 2010).

The infiltration of damaged tissue consists of three phases; preliminary, early and late, with each phase eliciting specific actions within the recovery process (Tidball & Villalta, 2010). The preliminary phase primarily consists of neutrophil infiltration to the site of damage, which promotes an inflammatory environment (Nguyen & Tidball, 2003b; Pizza, Peterson, Baas, & Koh, 2005). Subsequently, during the early phase monocytes become mobilized and differentiate into pro-inflammatory (M1) macrophages replacing neutrophils, further propagating the inflammatory environment (Chazaud, 2014; Nguyen

& Tidball, 2003a; Rigamonti et al., 2013; Song et al., 2000). M1 macrophages then will yield to anti-inflammatory (M2) macrophages, which exhibit regenerative roles during the late phase of recovery (Chazaud, 2014; Song et al., 2000; Tidball & Villalta, 2010). Neutrophils and monocytes are preferentially recruited by specific chemokines, namely interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) and fractalkine (CX3CL1) (Ancuta et al., 2003; Hammond et al., 1995; Yadav, Saini, & Arora, 2010). Although macrophages are derived from circulating monocytes (Yang, Zhang, Yu, Yang, & Wang, 2014), evidence suggests that certain monocyte subsets typically give rise to M1 macrophages, while others differentiate into M2 macrophages (Ancuta et al., 2003; Auffray et al., 2007).

Recently, monocytes have been formally organized into a three subset paradigm (Ziegler-Heitbrock et al., 2010), replacing the two subset model from the late 1980's (Wong et al., 2012). Phenotypically, monocytes are identified based on their expression of the lipopolysaccharide (LPS) receptor, CD14 and the FcγRIIIa receptor, CD16. Briefly, classical (CD14++/CD16-), intermediate (CD14++/CD16+) and nonclassical (CD14+/CD16++) monocytes exhibit different behaviors within the circulation (Van Craenenbroeck et al., 2014). Classical monocytes display phagocytic behavior, while intermediate monocytes exhibit inflammatory actions and function as antigen presenting cells (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). Nonclassical monocytes are patrolling cells residing primarily within the marginal pool monitoring the endothelium (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). With the varied responsibilities of each monocyte subset, healthy individuals typically maintain 80-90% classical monocytes, 5-10% intermediate monocytes and 5-10% nonclassical monocytes (Yang et

al., 2014). During disease and inflammatory states, the proportion of these subsets are altered (Poehlmann, Schefold, Zuckermann-Becker, Volk, & Meisel, 2009; Tallone et al., 2011; Zhu et al., 2015).

Exercise elicits an inflammatory environment, which prompts modulation of the monocyte subsets (LaVoy et al., 2015; Shantsila et al., 2012). Typically, the response to exercise entails a significant decrease in the proportion of classical monocytes with a concomitant increase in the proportion of nonclassical monocytes immediately following exercise (Booth et al., 2010; Radom-Aizik, Zaldivar, Haddad, & Cooper, 2014; Shantsila et al., 2012; Simpson et al., 2009). Within one hour of recovery, the subsets have been demonstrated to return to resting levels (Booth et al., 2010), or flip responses, with an increased proportion of classical monocytes and decreases in the proportion of both intermediate and nonclassical monocytes (Simpson et al., 2009). To the best of our knowledge, no studies have investigated the effect of resistance exercise on monocytes subsets. Ischemic conditions that elicit acute tissue damage, such as stroke and myocardial infarction, have demonstrated relationships with changes in intermediate monocytes and markers of tissue damage (Tapp, Shantsila, Wrigley, Pamukcu, & Lip, 2012; Urra et al., 2009). Although speculative, a mechanical stress such as resistance exercise, which can result in skeletal muscle damage, may induce a similar response.

While changes in leukocyte subsets are observed following exercise (Booth et al., 2010; J. M. Peake, K. Suzuki, G. Wilson, et al., 2005), changes in cellular activation have also been demonstrated (Jajtner et al., 2014; Nielsen & Lyberg, 2004; van Eeden, Granton, Hards, Moore, & Hogg, 1999; Wells et al., 2016). Macrophage-1 Antigen (MAC1), also referred to as Complement receptor 3 (CR3), is a $\beta 2$ integrin involved in the late phases of

transendothelial migration of immune cells following tissue damage (Tan, 2012). Investigations examining the MAC1/CR3 response on both neutrophils and monocytes have utilized various modes of exercise (Jajtner et al., 2014; Nielsen & Lyberg, 2004; van Eeden et al., 1999; Wells et al., 2016). The MAC1/CR3 response to resistance exercise on neutrophils has yielded primarily equivocal results (J. M. Peake, K. Suzuki, G. Wilson, et al., 2005; Pizza et al., 1996; Saxton, Claxton, Winter, & Pockley, 2003). However, changes in the MAC1/CR3 expression on monocytes appear to be more consistent, characterized by an increase in expression for up to one hour post-exercise (Gonzalez et al., 2014; Jajtner et al., 2014; Wells et al., 2016). These latter results, though, did not examine the response of MAC1/CR3 on different monocyte subsets. Limited research has suggested that classical monocytes may have a more robust response to exercise than the other monocyte subsets (Hong & Mills, 2008).

As resistance exercise appears to elicit significant elevations in markers of oxidative stress (Merry & Ristow, 2015; Urso & Clarkson, 2003), antioxidant supplementation has been examined as a potential countermeasure to reduce the oxidative response to resistance exercise (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Jowko et al., 2011; Panza et al., 2008; Paulsen et al., 2014). Specifically, polyphenol supplementation has been demonstrated to reduce force deficits and markers of muscle damage in response to resistance exercise (Bowtell et al., 2011; Jowko et al., 2011; Panza et al., 2008) while others have demonstrated equivocal results (Paulsen et al., 2014). Furthermore, the cytokine/chemokine response following endurance exercise and vitamin A, C and E supplementation has demonstrated a blunted response of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α (Vassilakopoulos et al., 2003). However, the benefits of

polyphenol supplementation in conjunction with eccentric exercise have been ambiguous (Herrlinger, Chirouzes, & Ceddia, 2015; Kerksick, Kreider, & Willoughby, 2010; O'Fallon et al., 2012). Decreases in circulating neutrophil concentrations have been observed 48 hours following eccentric exercise in conjunction with epigallocatechin gallate (EGCG) supplementation (Kerksick et al., 2010), however, the fate of these cells are unknown. As polyphenol incubation results in reduced expression of adhesion molecules on neutrophils and monocytes, along with limited chemotaxis *in vitro* (Graff & Jutila, 2007; Kawai et al., 2004; Takano, Nakaima, Nitta, Shibata, & Nakagawa, 2004) decreased neutrophil concentrations following exercise is not likely explained by increased infiltration. To the best of our knowledge, the specific response of neutrophils and monocyte activation following polyphenol supplementation and resistance exercise is not well understood.

Therefore, the primary purpose of this investigation was to examine the response of neutrophils and monocyte subtype proportion and activation to an acute bout of resistance exercise following 28-days of polyphenol supplementation. Furthermore, a secondary purpose of this study was to investigate the impact of polyphenol supplementation on leukocyte recruitment, behavior, and activation as well as functional recovery in response to resistance exercise.

CHAPTER 2: REVIEW OF LITERATURE

The immune system is composed to two primary arms; the adaptive immune system (or specific immunity) and the innate immune system. Both arms function to maintain the health and integrity of the individual, yet have distinct responsibilities (Parkin & Cohen, 2001). While adaptive immunity can be characterized as a small, but specific response, requiring prior sensitization to the pathogen, innate immunity is characterized by a profound, non-specific response that becomes prominent upon the first encounter with the pathogen (Parkin & Cohen, 2001). While both adaptive and innate immunity are active during and after exercise, the innate immune system plays a primary role in recovery from muscle damaging exercise.

Exercise resulting in muscle damage will initiate an inflammatory cascade that recruits immune cells, primarily neutrophils and monocytes, to the site of damage through the process of chemotaxis (Calle & Fernandez, 2010; Freidenreich & Volek, 2012). When cells arrive at the site of damage they begin the process of diapedesis, and extravasation (Ley, Laudanna, Cybulsky, & Nourshargh, 2007), of which the β2 integrin macrophage-1 antigen (MAC-1), also known as complement receptor-3 (CR3), plays a major role (Ehlers, 2000; Ley et al., 2007; Tan, 2012). Once at the site of tissue injury, immune cells complete a variety of tasks to promote healing of the damaged tissue.

Cytokine Signaling of Immune Cells

For the purposes of this review, a brief overview of cytokine function and classification will be given; however, the function of specific cytokines within the immune response to resistance exercise will be discussed in the later sections. Cytokines are small proteins that are responsible for communication between immune cells (Nicod, 1993). Cytokines are also produced by a variety of cells, including leukocytes and skeletal muscle cells during and following exercise (Calle & Fernandez, 2010). As cytokines are signaling molecules, they are classified based on the type of response they propagate, either pro-inflammatory or anti-inflammatory (Pedersen, 2000). Furthermore, cytokines can be broken into subsets based on their structure, and/or function. Although there is some overlap between the classifications, the most notable cytokine subsets are interleukins (e.g. IL-1β, IL-6) and chemokines (Nicod, 1993). Briefly, interleukin (*"inter"* – between; *"leukin"* – leukocyte) is an umbrella term to classify signaling molecules that mediate communication between leukocytes (Nicod, 1993).

Chemokines serve as chemotactic agents, attracting leukocytes to the site of damage (Nicod, 1993). Furthermore, chemokines are classified into four main subsets based on their protein structure, namely, the position of the first two cysteine molecules near the N-terminus (Rollins, 1997). The three most common chemokine subsets within the immune response to resistance exercise are the CC (β-chemokines), CXC (α-chemokines), and the CX3C chemokines (Nicod, 1993; Rollins, 1997). Briefly, a CC chemokine positions two cysteine molecules adjacent to one another, while 1 or 3 additional amino acids are positioned between the first and second cysteines of CXC or

CX3C chemokines, respectively (Rollins, 1997). Therefore, the nomenclature for chemokines is based on the structure at the N-terminus, followed by the receptor or ligand number. For instance, the chemokine CCL2 (also referred to as MCP-1) is ligand 2 of the CC chemokine family, while CX3CR1 is receptor 1 of the CX3C chemokine family.

<u>Immune cells Involved in the Inflammatory Response</u>

Leukocytes are the cells that make up the immune system, and are classified into three major populations: lymphocytes, monocytes, and granulocytes. Lymphocytes are sub-divided into T-, B- and natural killer (NK) cells, which collectively make up approximately 25% of total leukocytes in healthy individuals (Roussel, Benard, Ly-Sunnaram, & Fest, 2010). Monocytes make up 5-10% of total leukocytes in healthy populations, and give rise to dendritic cells (DC) (Roussel et al., 2010; Ziegler-Heitbrock et al., 2010). Granulocytes are further divided into three cell types: neutrophils, basophils and eosinophils (Roussel et al., 2010). In healthy individuals, neutrophils make up 50-70% of the total leukocyte population, while eosinophils and basophils make up approximately 1-5% and <1%, respectively (Roussel et al., 2010). In general, lymphocytes, namely T- and B- cells make up the adaptive immune system, while monocytes, granulocytes and NK cells make up the innate immune system (Parkin & Cohen, 2001).

Development of leukocytes derive from hematopoietic stem cells (HSC), while lymphocytes develop through a common lymphoid progenitor (CLP), and the lymphoid pathway (Akashi, Traver, Miyamoto, & Weissman, 2000). Monocytes and granulocytes

develop through the myeloid pathway, with a common myeloid progenitor (CMP), and a granulocyte/macrophage precursor (GMP) (Akashi et al., 2000; Summers et al., 2010). Following differentiation to the GMP, granulocytes and monocytes are developed by separate pathways. Granulocytes continue to develop through granulocytic progenitor cells in the mitotic pool before developing into mature immune cells in the post-mitotic pool (Summers et al., 2010). Within the bone marrow, monocytes continue to mature into macrophage/DC precursors followed by the commited monocyte progenitor (cMoP), and then develop into monocytes (Mitchell, Roediger, & Weninger, 2014).

Granulocytes

Granulocytes are broken into three distinct cell types: neutrophils, basophils and eosinophils. Neutrophils are the most numerous leukocyte, and within the context of muscle damage, are the most active granulocyte (Kolaczkowska & Kubes, 2013; Tidball, 2005). Mobilization of neutrophils from the bone marrow is stimulated by granulocyte colony stimulating factor (G-CSF) (Sadik, Kim, & Luster, 2011; Semerad, Liu, Gregory, Stumpf, & Link, 2002), while recruitment to damaged tissue is mediated by interactions with endothelial cells and specific cytokines (Kolaczkowska & Kubes, 2013; Ribeiro, Flores, Cunha, & Ferreira, 1991). The cell surface receptors on neutrophils responsible for the cytokine-mediated recruitment are the CXC chemokine receptors 1 and 2 (CXCR1 and CXCR2) (Futosi, Fodor, & Mocsai, 2013; Hammond et al., 1995), which bind interleukin-8 (IL-8; CXCL8), a potent chemoattractant of neutrophils (Ribeiro et al., 1991; Sadik et al., 2011). Furthermore, recruitment of neutrophils by endothelial interaction is mediated

by adhesion receptors (Kolaczkowska & Kubes, 2013). Neutrophils express several adhesion receptors, including L-selectin (CD62L), P-selectin glycoprotein ligand-1 (PSGL-1), lymphocyte function-associate antigen 1 (LFA-1) and MAC1/CR3 (Futosi et al., 2013). Each of these adhesion molecules perform prominent roles in the leukocyte adhesion cascade, which will be discussed in more detail later (Kolaczkowska & Kubes, 2013; Ley et al., 2007).

Following recruitment, neutrophils are the first cells to arrive at the site of inflammation following a bout of resistance exercise (Tidball & Villalta, 2010). Within damaged tissue, neutrophils exhibit a multitude of functions, including phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs) (Kolaczkowska & Kubes, 2013). The phagocytic response of the neutrophil is represented by the expression of phagocytic receptors, namely the Fc receptors cluster of differentiation- (CD) 64 and CD16, as well as complement receptors on the cell surface (Dale, Boxer, & Liles, 2008; Futosi et al., 2013). Briefly, during phagocytosis, the neutrophil recognizes opsonized debris, engulfs and then sequesters the debris into a phagosome (Dale et al., 2008; Kolaczkowska & Kubes, 2013). Once the cellular debris is isolated, granules within the cytosol of the neutrophil will fuse to the phagosome, releasing enzymes to degrade the debris (Kolaczkowska & Kubes, 2013; Nguyen & Tidball, 2003b; Tidball, 2005).

Myeloperoxidase (MPO), released from the primary granules, and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) are two important enzymes contained within neutrophils (Kolaczkowska & Kubes, 2013; Tidball, 2005). During phagocytosis, as granules fuse to the phagosome, they also fuse to the cell membrane, and

release their contents into the phagosome, or extracellular space (Kolaczkowska & Kubes, 2013; Nguyen & Tidball, 2003b; Tidball, 2005). Once the enzymes are released, a cytotoxic environment is promoted in the phagosome and extracellular space through a specific pathway depicted in Figure 1 (Nguyen & Tidball, 2003b; Tidball, 2005). Briefly, neutrophils consume oxygen that reacts with NADPH oxidase to produce superoxide (Hampton, Kettle, & Winterbourn, 1998). Superoxide can be converted to hydrogen peroxide, by superoxide dismutase, which in turn is converted to hypochlorous acid by MPO, prompting cytolytic effects (Hampton et al., 1998; Kolaczkowska & Kubes, 2013; Tidball, 2005) (Figure 1).
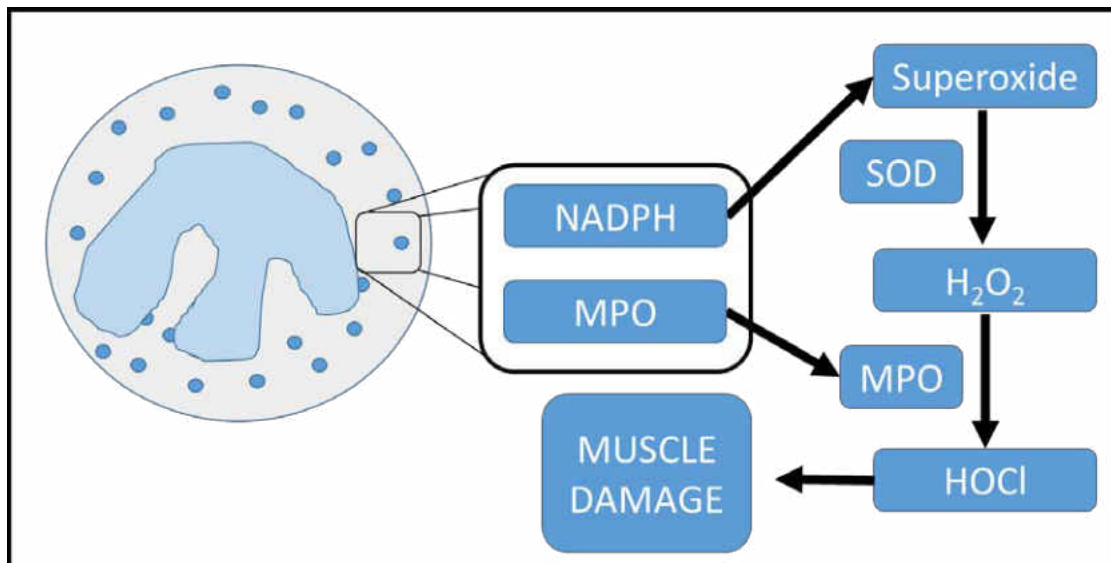
Figure 1: Secondary Damage by Neutrophils.
Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) serves as the precursor to superoxide. Superoxide dismutase (SOD) served as an intermediary, catalyze the conversion to hydrogen peroxide (H_2O_2). Myeloperoxidase catalyzes the reaction to hypochlorous acid (HOCl), which will induce skeletal muscle damage.
Adapted from: Tidball (2005)

The cytotoxic environment produced will prompt additional injury to the skeletal muscle, a process known as secondary damage (Nguyen & Tidball, 2003b; Pizza et al., 2005; Tidball, 2005). This secondary damage caused by neutrophils is enhanced later in recovery, through synergistic action with macrophages (Nguyen & Tidball, 2003a). Nonetheless, researchers have demonstrated that neutrophil mediated secondary damage is not necessary to maintain myofibril recovery, or macrophage populations in the murine model (Nguyen & Tidball, 2003b; Pizza et al., 2005). However, others argue that neutrophils may provide protective effects to skeletal muscle, despite promoting secondary damage (Lockhart & Brooks, 2008; Teixeira et al., 2003; Tidball, 2005). In the human model, neutrophil-mediated secondary damage has been suggested to enhance recruitment of circulating monocytes (Soehnlein, Lindbom, & Weber, 2009). Furthermore, MPO appears to enhance the phagocytic activity of CD68, a significant receptor of macrophages, leading to enhanced macrophage activation (Kolaczkowska & Kubes, 2013; Tidball & Villalta, 2010; Zouaoui Boudjeltia et al., 2004). Therefore, it appears that neutrophils may not be necessary for recovery, however, may aid in the recovery process.

Monocytes

For nearly 25 years, monocytes, which make up approximately 5-10% of the total circulating leukocyte population (Booth et al., 2010; Hong & Mills, 2008), have been sub-divided into two primary categories, classical and nonclassical monocytes (Wong et al., 2012; Ziegler-Heitbrock et al., 2010; Ziegler-Heitbrock & Hofer, 2013). Recent work, however has demonstrated a differential function of the lesser, nonclassical monocyte

12

(Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). This led to further refinement of monocytes, and the newly accepted population of intermediate monocytes (Ziegler-Heitbrock et al., 2010). These monocyte subsets have been widely identified via flow cytometry based on their differential expression of the lipopolysaccharide (LPS) receptor, CD14 and the FcγRIIIa receptor, CD16, as seen in Figure 2 (Wong et al., 2012; Ziegler-Heitbrock et al., 2010). Wong and colleagues (2012), among others (Ziegler-Heitbrock et al., 2010) have previously defined classical monocytes as CD14++/CD16-, intermediate monocytes as CD14++/CD16+ and nonclassical monocytes as CD14+/CD16++. While these subsets have at times been referred to as inflammatory and anti-inflammatory, Ziegler-Heitbrock and colleagues (2010) recommend caution with these labels. They argue that these terms have been interchanged based on the species (murine vs human), and may lead to an oversight of important roles for each population (Ziegler-Heitbrock et al., 2010). Therefore, for the purposes of this review, monocytes will be subdivided into three categories: classical monocytes (CD14++/CD16-), intermediate monocytes (CD14++/CD14+) and nonclassical monocytes (CD14+/CD16+). Due to the recent discrimination between nonclassical and intermediate monocytes, there are limitations on the studies completed prior to this point. In order to examine these studies, discussion of the undifferentiated nonclassical population will be referred to as CD16+ monocytes.
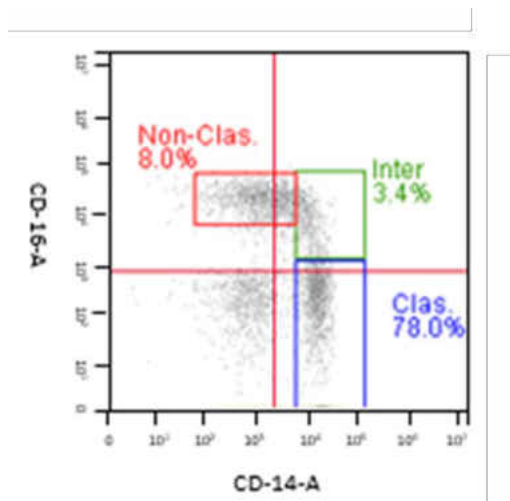
Figure 2: Fluorescent Characteristics of Monocyte Subsets.
Monocytes were analyzed for their differential expression of cluster of differentiation (CD)-14 and CD16. Monocytes were classified as Classical (Clas), Intermediate (Inter) or Nonclassical (Non-Clas).

<u>Monocyte Subsets</u>

Classical Monocytes

Classical monocytes remain in circulation for a few days after release from the bone marrow (Wong et al., 2011; Ziegler-Heitbrock et al., 2010; Ziegler-Heitbrock & Hofer, 2013). It has been suggested that a developmental relationship may exist between classical monocytes with intermediate and nonclassical monocytes (Ancuta et al., 2009; Ziegler-Heitbrock et al., 2010). While there is compelling data demonstrating modulation of key receptors on monocytes *in vitro* (Ramos et al., 2010), formal demonstration of a progressive relationship between monocytes has yet to be provided in humans (Wong et al., 2012; Ziegler-Heitbrock et al., 2010). Classical monocytes make up the majority of the monocyte population, ranging between 80 to 90% of the total monocyte population at rest in healthy individuals (Wong et al., 2011; Ziegler-Heitbrock et al., 2010; Ziegler-

14

Heitbrock & Hofer, 2013). However, this can be greatly altered in several clinical and non-clinical conditions (Booth et al., 2010; Hristov & Weber, 2011; Rossol, Kraus, Pierer, Baerwald, & Wagner, 2012; Simpson et al., 2009; Tapp et al., 2012).

In response to LPS-mediated activation, classical monocytes secrete a variety of chemotactic cytokines, including G-CSF, IL-8, CCL3, CCL5, and MCP-1 in addition to IL-6 and IL-10 at high levels (Cros et al., 2010; Wong et al., 2011; Wong et al., 2012). LPS-stimulation also results in pro-inflammatory cytokine (IL-1β and TNFα) production at moderate levels in classical monocytes (Cros et al., 2010; Wong et al., 2011). Furthermore, classical monocytes demonstrate a high degree of reactive oxygen species (ROS) production in response to LPS stimulation, and the highest phagocytic ability of the three monocyte subsets (Cros et al., 2010; Zawada et al., 2011).

While the secretion of cytokines may explain the potential effects of classical monocytes on the overall milieu of the adjacent area, examination of receptor expression on classical monocytes demonstrates the phagocytic role of this subset. Classical monocytes express greater levels of CD36 and CD64, which serve as a scavenging receptor and receptors that initiate phagocytosis, respectively (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). Classical monocytes also express the greatest proportion of the CCR2 (receptor for MCP-1) and CXCR1 and CXCR2 (receptors for IL-8) when compared to intermediate and nonclassical monocytes (Cros et al., 2010; Wong et al., 2011). Despite the increased expression of CXCR1 and CXCR2 on classical monocytes, the primary chemotactic response of this specific subset is mediated by the MCP-1/CCR2 interaction (Leonard & Yoshimura, 1990; Wong et al., 2012; Yadav et al., 2010; Yang et al., 2014).

Interestingly, expression of CX3CR1 is greatly reduced on classical monocytes; a defining characteristic of classical versus intermediate and nonclassical monocytes (Cros et al., 2010; Wong et al., 2011). Finally, classical monocytes are characterized by high expression of L-selectin (CD62L) and CD11b, which are necessary components of the adhesion cascade, discussed later (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). Taken together, evidence demonstrates the ability of the classical monocyte to migrate to the site of tissue damage, cross the endothelial wall, and scavenge/phagocytose damaged tissue.

Intermediate Monocytes

Intermediate monocytes are the newest subset to be identified, and were formally labeled in 2010 with the consortium statement put forward by the International Union of Immunological Societies and the World Health Organization (Ziegler-Heitbrock et al., 2010). As stated previously, intermediate monocytes appear to have a developmental relationship between classical and nonclassical (Wong et al., 2012; Ziegler-Heitbrock et al., 2010), evidenced by the intermediate expression of several receptors and cytokine production. Typically, intermediate monocytes make up 5-10% of the total circulating monocytes (Wong et al., 2012; Ziegler-Heitbrock et al., 2010; Ziegler-Heitbrock & Hofer, 2013), although this can vary greatly with clinical conditions (Hristov & Weber, 2011; Rossol et al., 2012; Tapp et al., 2012).

Intermediate monocyte display elevated expression of CD11b, a characteristic they share with classical monocytes (Cros et al., 2010; Wong et al., 2012; Zawada et al., 2011).

However, intermediate monocytes also have positive expression of CX3CR1 (receptor for CX3CL1) and lack L-Selectin (CD62L), characteristics they share with nonclassical monocytes (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). While intermediate monocytes have a high capacity for phagocytosis (Cros et al., 2010; Zawada et al., 2011), ROS production from this subset is controversial. Cros and colleagues (2010) reported minimal ROS production, while Zawada, et al. (2011) reported the greatest ROS production from intermediate monocytes. Zawada and colleagues (2011) attribute this difference to the discrepancies between the sampling techniques used. Intermediate monocytes also display an inflammatory function, in addition to functioning as an antigen presenting cell, while also demonstrating an ability to cross the endothelium into damaged tissue (Yang et al., 2014).

The inflammatory nature of the intermediate subset is tied to the pro-inflammatory milieu it promotes. Intermediate monocytes secrete greater amounts of IL-6, and IL-1β, than any other subset, as well as a moderate amount of IL-8 and IL-10 following LPS stimulation (Cros et al., 2010; Rossol et al., 2012; Wong et al., 2011). Additionally, TNF-α likely adds to the pro-inflammatory milieu. In response to LPS, TNF-α production has been shown to both increase (Cros et al., 2010; Rossol et al., 2012) and remain unchanged (Wong et al., 2011), which was suggested to be the result of different clones used to identify CD14 (LPS receptor) (Wong et al., 2011). The use of the M5E2 clone for CD14 (as used by Cros and colleagues (2010)) has demonstrated deleterious effects on the LPS signaling (Power et al., 2004). However, with the elevated expression of CD14 on intermediate monocytes, in conjunction with the positive LPS-mediated response (Cros et al., 2010), it is unlikely the M5E2 clone had an impact on the results. Furthermore, Rossol and

colleagues (2012) demonstrated similar TNF-α production following LPS stimulation, though, the *in vitro* nature of these analyses may impact the findings (Wong et al., 2012). Despite this, in response to LPS signaling it appears intermediate monocytes produce multiple pro-inflammatory cytokines, likely leading to their pro-inflammatory nature.

The antigen presenting capabilities of intermediate monocytes appear to be related to the increased expression of major histocompatibility (MHC) proteins human leukocyte antigen (HLA)-ABC and HLA-DR relative to classical and nonclassical monocytes (Rossol et al., 2012; Wong et al., 2011; Zawada et al., 2011). As these MHC molecules function with T lymphocytes, they are capable of recruiting T cells to the injured area for assistance in the healing process (Grage-Griebenow et al., 2001; Wong et al., 2012). Therefore, modulation of the number of intermediate monocytes in circulation may potentiate or mitigate the T cell response.

Nonclassical monocytes

Nonclassical monocytes are considered the most developed of the three monocyte subsets (Wong et al., 2012; Ziegler-Heitbrock & Hofer, 2013). Nonclassical monocytes make up approximately 5-10% of total monocytes, and are considered the "patrolling" subset, residing primarily in the marginal pool (Cros et al., 2010). Other than the general characteristic of CD14 and CD16, the nonclassical subset is phenotypically characterized by a relatively high expression of CX3CR1, with relatively low expression of CCR2, CCR5, CD11b and CD36 (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). CX3CR1, the primary receptor for CX3CL1 (Ancuta et al., 2003), serves as the primary

receptor involved in the extravasation process for nonclassical monocytes (Cros et al., 2010; Yang et al., 2014). In addition, due to the patrolling nature of nonclassical monocytes, the phagocytic ability and ROS production of these monocytes have been reported to be lower than the other monocyte subsets (Cros et al., 2010; Zawada et al., 2011).

Cytokine production of nonclassical monocytes is rather controversial. In response to LPS, Wong et al. (2011) reported elevated production of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α). However, other investigations have demonstrated production of only IL-1ra, with minimal production of pro-inflammatory cytokines (Cros et al., 2010; Rossol et al., 2012). Additionally, as Cros and colleagues (2010) identified monocytes with the M5E2 clone for CD14, the limited pro-inflammatory cytokine production observed (Cros et al., 2010), may be a function of the deleterious effects of the M5E2 on LPS signaling (Power et al., 2004). Unfortunately, the clone used by Rossol and colleagues (2012), was not reported, and therefore may have the same limitations as reported by Cros et al. (2010). However, Rossol and colleagues (2012) did report minimal pro-inflammatory cytokine production following co-incubation with activated T-cells. Therefore, it is unclear whether pro-inflammatory cytokine production is a prominent feature of nonclassical monocytes.

Overview of the Monocyte Subsets

As stated previously, the relationship between the three monocyte subsets has been suggested to be of a developmental nature, originating from classical monocytes (Ancuta

et al., 2009; Ziegler-Heitbrock & Hofer, 2013). This can be observed through the progression of receptor expression and cytokine production between the subsets depicted in Figure 3. Evidence demonstrating the modulation of key receptors on monocytes, namely CX3CR1 and CD14 in response to specific cytokines has been presented previously (Ramos et al., 2010). Specifically, Ramos and colleagues (2010) demonstrated that IL-10 maintained the already elevated expression of CX3CR1 on cultured monocytes, indicating a maintenance of the moderately matured state (intermediate monocyte). Furthermore, when monocytes were cultured with INF-γ, CX3CR1 expression was also maintained, however, a concomitant decrease in the expression of CD14 was demonstrated (Ramos et al., 2010). This decrease in CD14 results in a phenotype characterized by low CD14, but high CX3CR1 expression, similar to nonclassical monocytes (Wong et al., 2012). Together, the results of Ramos and colleagues (2010) show a potential maturing effect of the monocyte lineage under inflammatory conditions that present INF-γ.

In summary, the phagocytic classical monocytes express high levels of receptors CCR2, CCR5, CD11b and CD62L, in conjunction with low expression of CX3CR1. As classical monocytes mature into the inflammatory intermediate subset, they lose CD62L expression, begin to lose the expression of CCR2, however, gain the expression of CX3CR1 and HLA-DR. As these cells continue to develop into patrolling nonclassical monocytes, the expression of CCR5, CD11b and HLA-DR is reduced, while the expression of CX3CR1 is maintained (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). A depiction of the differential expression of receptors and cytokine production is presented in Figure 3.

	Classical Monocyte			Intermediate Monocyte			Nonclassical Monocyte		
Function	Phagocytosis			Antigen Presenting			Patrolling		
Cytokines Produced	IL-10	IL-6	IL-1β	IL-10	IL-6	IL-1β	IL-10	IL-6	IL-1β
	MCP-1	IL-8	TNF-α	MCP-1	IL-8	TNF-α	MCP-1	IL-8	TNF-α
Receptor Express.	CD11b	CX3CR1	CCR2	CD11b	CX3CR1	CCR2	CD11b	CX3CR1	CCR2
	CD62L	CCR5	HLA-DR	CD62L	CCR5	HLA-DR	CD62L	CCR5	HLA-DR

Figure 3: Phenotypic Differences of Monocyte Subsets.
Receptors and cytokines displayed in red, orange and white are highly, moderately or minimally expressed/produced, respectively. Production of cytokines expressed in grey are contested within literature.
Cytokines displayed: Monocyte chemoattractant protein-1 (MCP-1), Tumor necrosis factor-α (TNF-α), and Interleukins (IL)- 1β, IL-6, IL-8 and IL-10.
Receptors displayed: Cluster of differentiation (CD)-14 (LPS receptor), CD16 (Fc receptor FcγRIIIa), CD11b (α unit of MAC1), CD62L (L-selectin), CC chemokine receptors 2 and 5 (CCR2, CCR5), CX3C chemokine receptor 1 (CX3CR1), and Human Leukocyte Antigen (HLA)-DR

Macrophages

Macrophages are differentiated from monocytes within tissue following transmigration (Yang et al., 2014). Similar to monocytes, macrophages are heterogeneous and their functions are similarly varied (Novak & Koh, 2013; Pilling, Fan, Huang, Kaul, & Gomer, 2009). Macrophages can be primarily separated into two categories, M1 and M2, and the M2 macrophages can be further segregated into three subsets; M2a, M2b and M2c

(Novak & Koh, 2013). These subsets, however, seem to be a function primarily of controlled environments within *in vitro* analysis, and do not always maintain these ridged phenotypes *in vivo* (Mosser & Edwards, 2008; Novak & Koh, 2013). The *in vitro* model, however, may be helpful in understanding the myriad of roles macrophages participate in during skeletal muscle damage and recovery.

Inflammatory (M1) Macrophages

Inflammatory macrophages (M1) are the first cells of the mononuclear phagocyte system observed in skeletal muscle tissue following damage (Novak & Koh, 2013; Tidball & Villalta, 2010). This typically occurs between 1 - 3 days following damage and is preceded by neutrophil invasion, and characterized by a large inflammatory response (Tidball, 2005; Tidball & Villalta, 2010). Furthermore, the macrophage response seems to be required for complete skeletal muscle recovery following exercise induced muscle damage (Cheng, Nguyen, Fantuzzi, & Koh, 2008; Rigamonti et al., 2013; Tidball & Wehling-Henricks, 2007).

M1 macrophages originate from classical monocytes following migration to the tissue, through a process known as classical activation (Yang et al., 2014). Classical activation is primarily mediated by specific inflammatory cytokines, namely INF-γ, although TNF-α has also been implicated in this process (Mosser & Edwards, 2008; Novak & Koh, 2013). M1 macrophages are known to express CD68 (Pilling et al., 2009), a receptor for oxidized low-density lipoprotein (oxLDL) (Tidball & Villalta, 2010; Van Velzen, Da Silva, Gordon, & Van Berkel, 1997). CD68 will become more active with

increased MPO-mediated oxidation of LDL (Zouaoui Boudjeltia et al., 2004), which is released by neutrophils prior to M1 invasion (Kolaczkowska & Kubes, 2013). This leads to the binding of CD68, contributing to the activation of the M1 macrophage (Tidball & Villalta, 2010) and underlining the importance of the neutrophil response to muscle damage. M1 macrophages appear to exclusively express CD80 (Ambarus et al., 2012), however, do not express CD163 or CD206 (markers of M2 macrophages) (Novak & Koh, 2013).

Similar to neutrophils, M1 macrophages are known to contribute to secondary damage, and is amplified in the presence of neutrophils (Nguyen & Tidball, 2003a). This effect is facilitated via a nitric oxide- (NO) mediated reaction through the production of inducible nitric oxide synthase (iNOS), further disrupting the integrity of the tissue (Nguyen & Tidball, 2003a). Moreover, the secondary damage produced by iNOS appears to be required for complete recovery (Rigamonti et al., 2013). M1 macrophages also aid with the proliferation of myogenic precursor cells (MPC), and are the primary source of intramuscular insulin-like growth factor-1 (IGF-1) during the early phase of recovery (Arnold et al., 2007; Song et al., 2000; Tidball & Welc, 2015; Tonkin et al., 2015). Additionally, M1 macrophages produce several pro-inflammatory cytokines, including IL-1β, IL-6, IL-12, and TNF-α (Chazaud, 2014). Furthermore, T-helper 1 (Th1) lymphocytes assist M1 macrophages by also secreting pro-inflammatory cytokines, including INF-γ (Mosser & Edwards, 2008). The secretion of INF-γ from Th1 cells promotes classical activation, and allows for the maintenance of M1 macrophages within the damaged tissue (Mosser & Edwards, 2008).

In conjunction with Th1 cells, M1 macrophages maintain this inflammatory milieu throughout the early phase of recovery (Chazaud, 2014; Mosser & Edwards, 2008). However, as classically activated macrophages begin to phagocytize debris, they begin to polarize into a more anti-inflammatory phenotype (Arnold et al., 2007), a process that is aided by the T-helper-2 lymphocyte (Th2) response (Chazaud, 2014). During this process, the immune cells begin to promote a cytokine milieu consisting primarily of IL-4, and IL-13, prompting "alternative" macrophage activation (Mosser & Edwards, 2008).

Anti-inflammatory (M2) Macrophages

Anti-inflammatory, or M2, macrophages are developed by phagocytosis or alternative activation (Arnold et al., 2007; Mosser & Edwards, 2008). M2 macrophages are the predominant cell of the mononuclear phagocytic system during the late phase of inflammation, between 3 – 7 days following injury (Tidball & Villalta, 2010). The polarization of M1 to M2 macrophages is typically mediated by phagocytosis or alternative activation (Arnold et al., 2007). However, recent evidence from Tonkin and colleagues (2015) suggests that the IGF-1 produced by M1 macrophages may contribute as well. Development of M2 macrophages from nonclassical monocytes is mediated exclusively through the alternative pathway (Brigitte et al., 2010; Nahrendorf et al., 2007). Furthermore, the anti-inflammatory cytokines IL-4, IL-10 and IL-13 govern alternative activation (Arnold et al., 2007). Prior to M2 development, nonclassical monocytes will cross the endothelium via interaction of the CX3CR1 on the immune cell and CX3CL1, which is bound to endothelial cells (Ancuta et al., 2003; Auffray et al., 2007).

Due to the heterogeneity of the M2 subset, and the plasticity of macrophages in general, it is difficult to identify a common receptor on all M2 phenotypes. Moreover, macrophages are able to augment their phenotype and function based on the surrounding environment (Mosser & Edwards, 2008; Novak & Koh, 2013; Stout et al., 2005). With this in mind, macrophages that are cultured in cytokines characteristic of alternative activation (IL-4 and IL-10) result in increased expression of CD163 and CD206 (Ambarus et al., 2012; Buechler et al., 2000). M2 macrophages also promote an anti-inflammatory milieu characterized by increased production of IL-1 receptor antagonist (IL-1ra), IL-10, and transforming growth factor-β (TGF-β) (Ambarus et al., 2012; Chazaud, 2014; Song et al., 2000; Stout et al., 2005). Furthermore, M2 macrophages display reduced expression of pro-inflammatory cytokines TNF-α and IL-1β (Chazaud, 2014; Song et al., 2000; Stout et al., 2005).

During the late phase of recovery, M2 macrophages promote the differentiation of MPC into mature myotubes (Arnold et al., 2007; Martin & Lewis, 2012; Saclier et al., 2013; Song et al., 2000). M2 macrophages also produce fibrous tissue (Song et al., 2000), and continue to secrete IGF-1 within skeletal muscle (Tonkin et al., 2015). Due to the anti-inflammatory environment promoted by M2 macrophages, IGF-1 is suggested to stimulate the p70^{S6K} signaling pathway (Tidball & Welc, 2015; Tonkin et al., 2015). Taken together, M2 macrophages promote an anti-inflammatory environment that is characterized by recovery and regeneration.

Overview of M1/M2 Macrophages

Macrophages that respond to tissue damage are most readily defined by their polarized moieties, M1 and M2. In general, these polarization states are characterized by a pro-inflammatory M1 phenotype, that maintains an environment rich in the pro-inflammatory cytokines INF-γ and TNF-α (Chazaud, 2014). The M2 phenotype is characterized by the maintenance of an anti-inflammatory milieu, consisting of IL-4, IL-10 and IL-13 (Gordon & Martinez, 2010). Furthermore, the M1 polarization contributes to phagocytosis, and secondary damage (Tidball & Villalta, 2010), while also stimulating MPC proliferation (Arnold et al., 2007; Song et al., 2000). The M2 polarization contributes to fiber regeneration, and the promotion of MPC differentiation (Arnold et al., 2007; Mosser & Edwards, 2008; Saclier et al., 2013). Additionally, M1 and M2 macrophages provide the initial source of IGF-1 within skeletal muscle during recovery (Tonkin et al., 2015). Many investigations have also suggested that macrophages exhibit great plasticity, and caution against strict adherence to the M1/M2 characterization (Novak & Koh, 2013; Stout et al., 2005).

Resident Macrophages

Resident macrophages were first identified in skeletal muscle in 1990 using specific labeling with ectodermal dysplasia antibodies- 1, 2 and 3 (ED1, ED2 and ED3) (Honda, Kimura, & Rostami, 1990). These antibodies labeled both monocytes and macrophages (ED1), or were specific to macrophages (ED2 and ED3) (Dijkstra, Dopp, Joling, & Kraal, 1985). Honda and colleagues (1990) identified the presence of ED2 and ED3 macrophages

within the perimysium (Honda et al., 1990), while a follow-up study by McLellan (1993) determined that ED2 and ED3 macrophages did not participate in phagocytic activity (McLennan, 1993). However, upon further examination it was determined that the animals used by McLellan (1993) were likely not sacrificed in a resting state, indicating potential for myeloid cellular invasion prior to analysis, and the results of this study should be interpreted with caution. Subsequent examinations of the ED1, ED2 and ED3 phenotypes have identified close relationships between these antibodies, and receptors currently used to identify macrophages today, namely CD68, and CD163 (Damoiseaux et al., 1994; Pilling et al., 2009; Polfliet, Fabriek, Daniels, Dijkstra, & van den Berg, 2006).

The findings of Honda and colleagues (1990) have subsequently been confirmed with specific phenotyping for myeloid cells (MAC1/CR3), and mononuclear cells (F4/80) (Pimorady-Esfahani, Grounds, & McMenamin, 1997). Recently, Brigitte and colleagues (2010) demonstrated similar results with further discrimination against DC and classical monocytes. Furthermore, they also demonstrated specific recruitment of monocytes and neutrophils from resident macrophages. Briefly, following toxin (notexin) induced injury to skeletal muscle, or stimulation with TNF-α, resident macrophages selectively produce MCP-1 and cytokine-induced neutrophil chemoattractant (CXCL1) (Brigitte et al., 2010). Additionally, patrolling monocytes (nonclassical) have been demonstrated to migrate into tissue, and propagate the inflammatory response (Auffray et al., 2007). Consequently, both resident macrophages and nonclassical monocytes appear to be involved with initiating the immune response to tissue damage.

Exercise itself, may also propagate the inflammatory response. Exercise is known to induce increased mRNA expression of several chemokines in skeletal muscle, including those for neutrophil and monocyte recruitment in humans (Catoire, Mensink, Kalkhoven, Schrauwen, & Kersten, 2014; Della Gatta et al., 2014; Nieman et al., 2004; Stromberg et al., 2016). While these investigations were unable to isolate the source of the mRNA expression, it is possible myeloid cells were responsible. Evidence from a murine model demonstrated myeloid depletion resulting in a significantly reduced mRNA expression of pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) post-exercise (Kawanishi, Mizokami, Niihara, Yada, & Suzuki, 2016). Resident macrophages and monocytes appear to produce these pro-inflammatory cytokines in the recovery period following exercise. Consequently, these cells may be the primary cells responsible for the initiation of the immune response to exercise induced muscle damage.

Endothelial Cells and Leukocyte Recruitment

While endothelial cells are not direct members of the innate immune system, they do have a pivotal role in the development of the immune response (Ley et al., 2007). Endothelial cells express specific adhesion receptors, namely P- and E-selectin (Kolaczkowska & Kubes, 2013; Ley et al., 2007), which when activated will bind PSGL-1, a prominent ligand of neutrophils (Futosi et al., 2013). Furthermore, once activated, endothelial cells will also express CX3CL1, a chemoattractant to nonclassical monocytes and T-cells (Ancuta et al., 2004; Ancuta et al., 2003; Ludwig, Berkhout, Moores, Groot, & Chapman, 2002). As these adhesion receptors and chemoattractants are expressed,

immune cells will bind and initiate transendothelial migration. Activation of endothelial cells is accomplished through various pro-inflammatory cytokines, such as TNF-α, INF-γ and IL-1β (Ludwig et al., 2002; Rollins, Yoshimura, Leonard, & Pober, 1990). Moreover, resistance exercise upregulates the production of IL-1β and TNF-α within skeletal muscle (Buford, Cooke, & Willoughby, 2009; Nieman et al., 2004), likely sourced by resident macrophages (Kawanishi et al., 2016). Therefore, resistance exercise prompts the production of pro-inflammatory cytokines that are responsible for the activation of endothelial cells, indirectly contributing to the migration of leukocytes.

Immune Cell Response to Exercise-Induced Muscle Damage

It is well understood that resistance exercise may result in muscle damage resulting from metabolic, oxidative stress, as well as mechanical stresses (Brancaccio, Lippi, & Maffulli, 2010; Calle & Fernandez, 2010; Clarkson & Hubal, 2002; Clarkson, Kearns, Rouzier, Rubin, & Thompson, 2006; Finaud, Lac, & Filaire, 2006). Myofibrillar damage produced in skeletal muscle following damaging exercise is characterized by leakage of various biomarkers from the sarcolemma (Brancaccio et al., 2010; Clarkson & Hubal, 2002; Clarkson et al., 2006), reduced voluntary contraction (Byrne & Eston, 2002; Paulsen et al., 2005) and muscle soreness (Cheung, Hume, & Maxwell, 2003; Jajtner et al., 2015). These indicators of exercise induced muscle damage are also associated with a pro-inflammatory cytokine response (Buford et al., 2009; Della Gatta et al., 2014; Nieman et al., 2004), resulting in significant leukocyte mobilization (Paulsen et al., 2005; Ramel, Wagner, & Elmadfa, 2003; Saxton et al., 2003; Simonson & Jackson, 2004). Resistance

exercise appears to indirectly contribute to the recruitment of leukocytes by activating resident tissue macrophages (Brigitte et al., 2010; Buford et al., 2009; Kawanishi et al., 2016), which in turn, release potent chemoattractants and cytokines that stimulate endothelial cell-mediated migration (Brigitte et al., 2010; Buford et al., 2009; Imai et al., 1997; Kawanishi et al., 2016; Szmitko et al., 2003).

An overview of the immune cell cascade during recovery from skeletal muscle damage is depicted in Figure 4. Briefly, resident macrophages and nonclassical monocytes are activated, stimulating a response by endothelial cells, neutrophils and monocytes, resulting in their infiltration of the damaged tissue (Brigitte et al., 2010; Kawanishi et al., 2016; Ludwig et al., 2002; Rollins et al., 1990). Neutrophils are the first to arrive at the site of damage, propagating inflammation, while also clearing debris (Kolaczkowska & Kubes, 2013). Subsequently, classical monocytes infiltrate the tissue and develop into M1 macrophages, proliferating inflammation by essentially "priming" the tissue for recovery (Tidball & Villalta, 2010). Simultaneously, Th1 cells are attracted to the site of damage, possibly through antigen presentation performed by intermediate monocytes, and contribute to the inflammatory milieu created during the early phase of recovery (Grage-Griebenow et al., 2001; Wong et al., 2012). As M1 macrophages phagocytize debris within the damaged tissue, polarization to an M2 phenotype occurs, establishing a microenvironment more conducive to repair and healing (Arnold et al., 2007; Tidball & Villalta, 2010). Concurrently, Th1 cells are replaced with Th2 cells, which support the cytokine milieu associated with M2 macrophages during the late phase of skeletal muscle recovery (Mosser & Edwards, 2008). Therefore, during the preliminary (neutrophil) and early (M1 macrophage) phases of recovery, a pro-inflammatory microenvironment is

supported, before yielding to an anti-inflammatory milieu during the late phase of recovery

(M2 macrophages) (Tidball & Villalta, 2010).
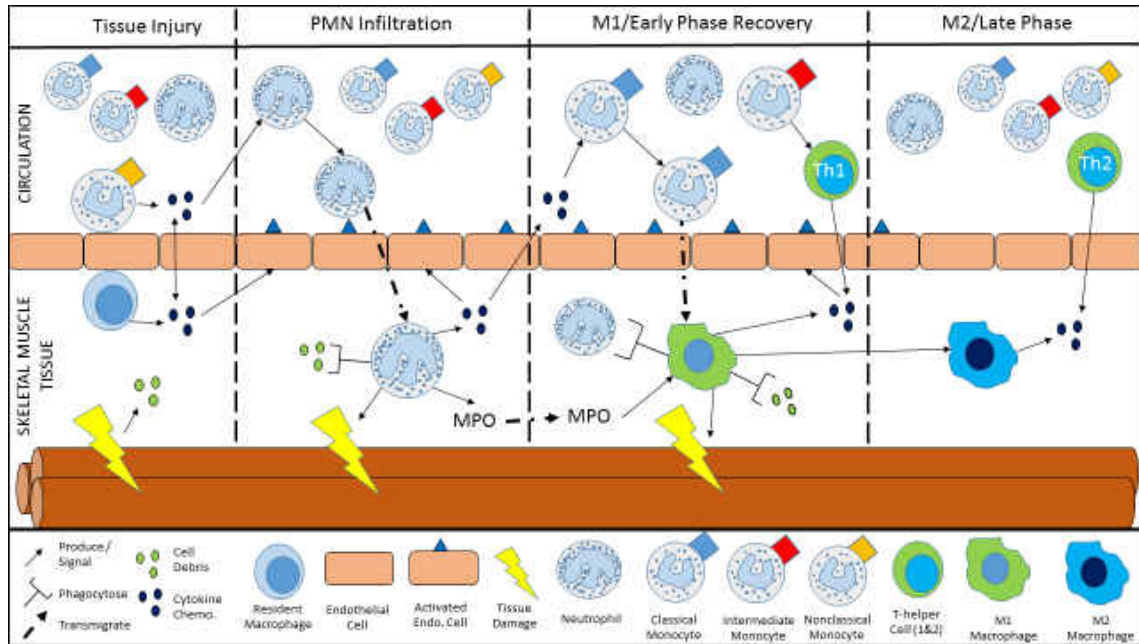
Figure 4: Overview of the Innate Immune Cells involved in Skeletal Muscle Damage.
Resident macrophages and nonclassical monocytes induce a response to skeletal muscle damage. Neutrophils are the first myeloid cell to enter the tissue (PMN Infiltration). Within tissue, neutrophils promote phagocytosis, and release myeloperoxidase (MPO), which contributes to secondary damage. Classical monocytes enter the tissue and classically activate into M1 macrophages. M1 macrophages also contribute to secondary damage, as well as phagocytose apoptotic neutrophils and debris prior to polarizing into M2 macrophages. M2 macrophages support the cessation of inflammation and the recovery of the tissue. T-helper-1 (Th1) and -2 (Th2) cells aid with maintaining the pro- and anti-inflammatory milieu, respectively.

Circulating Neutrophil Response to Exercise

It is well established that the number of neutrophils in circulation increase

following exercise (Miles et al., 1998; Nieman et al., 2004; J. M. Peake, K. Suzuki, G.

31

Wilson, et al., 2005; Radom-Aizik, Zaldivar, Leu, Galassetti, & Cooper, 2008; Ramel, Wagner, & Elmadfa, 2004). Limited studies involving resistance exercise have demonstrated an increase in neutrophil numbers immediately following exercise (Miles et al., 1998; Nieman et al., 2004; Ramel et al., 2004; Simonson & Jackson, 2004). Furthermore, elevations in neutrophil number are maintained for up to two hours post-exercise (Nieman et al., 1995; Ramel et al., 2003). However, data regarding neutrophil population 24 hours or more post-exercise in healthy participants appears to be lacking.

While examination of the neutrophil response to resistance exercise is limited, examination of other modes of exercise may provide additional insight to the overall neutrophil response. As resistance exercise may elicit muscle damage (Brancaccio et al., 2010; Clarkson & Hubal, 2002), examination of the neutrophil response to exercise designed to elicit muscle damage may provide evidence for a time course of neutrophil recovery (Peake, Nosaka, & Suzuki, 2005). Peak neutrophil counts resulting from eccentrically induced muscle damage have been reported at 4- (Saxton et al., 2003), 6- (Malm, Lenkei, & Sjodin, 1999; Malm et al., 2000; Paulsen et al., 2005), and 12-hours (Pizza et al., 1996) post-exercise. Several investigators have further reported neutrophil counts return to resting levels by 24-hrs following exercise induced muscle damage (Malm et al., 1999; Paulsen et al., 2005; Saxton et al., 2003), while others, have reported a rebound effect of total neutrophil count between 48H-96H post-exercise (Pizza et al., 1996). It has also been suggested that the addition of muscle biopsies to an investigation may affect the recruitment of neutrophils, and subsequently influence the rebound effect (Malm et al., 2000). Based upon available evidence it appears that the neutrophil population will expand for up to two hours following resistance exercise. However, if the intensity is sufficient to

elicit muscle damage, this neutrophilia may continue for up to 12 hours before returning to resting levels. While it is unclear whether a typical bout of resistance exercise will respond in similar fashion to exercise designed to elicit muscle damage, there is evidence to support a rebound effect 48 hours following muscle damage.

Circulating Monocyte and Monocyte Subset Response to Exercise

Total monocytes concentrations have been reported to increase in response to both aerobic (Booth et al., 2010; Hong & Mills, 2008; Shantsila et al., 2012) and resistance exercise (Miles et al., 1998; Nieman et al., 2004; Simonson & Jackson, 2004). Typically, total monocyte count is elevated, peaking between immediately post-exercise and two hours post-exercise (Ramel et al., 2003). It appears that both exercise intensity and rest intervals may influence when this peak response occurs, with submaximal resistance exercise prompting a delayed monocytosis (Mayhew, Thyfault, & Koch, 2005; Ramel et al., 2003).

Investigations of monocyte subsets and their response to exercise have been limited. This is likely due to the recent formal redefinition of monocytes to include a third subset six years ago (Ziegler-Heitbrock et al., 2010). To our knowledge, following this redefinition only seven investigations have been published discussing shifts in monocyte subsets following exercise, with one additional investigation conducted in 2009 (Tables 1 and 2). Briefly, these investigations primarily examined cardiorespiratory exercise challenges, and typically assess the monocyte response only once, immediately following

exercise. Additionally, some of these studies examined the changes of monocyte subsets

following exercise in clinical populations (Dimitrov et al., 2013; Dungey, Bishop, Young,

Burton, & Smith, 2015; Hong & Mills, 2008; Van Craenenbroeck et al., 2014) (see Table

2).

Table 1. Monocyte Subset Modulations in response to Exercise in Healthy Populations.

Data reported as Mean (SD)
* Reported significant difference from pre; ~ no SD reported; *Italics:* Absolute Counts (10^9 x L^{-1}).
Classical monocytes (CLAS), Intermediate monocytes (INTER), and nonclassical monocytes (NC)

Author	Participants / Exercise	Pre Exercise			Post Exercise			1H Post Exercise		
		CLAS	INTER	NC	CLAS	INTER	NC	CLAS	INTER	NC
Simpson, et al, 2009	*"Mildly Trained" Males* 45 min Run at 75% VO$_2$ Max	77.8 (7.4)	5.8 (2.5)	9.3 (3.5)	73.8 (9.4)*	7.1 (5.1)	12.4 (6.1)*	86.7 (7.3)*	3.6 (1.3)*	4.0 (1.7)*
Booth, et al, 2010	*Athletes (Men and Women)* 60km cycling TT	86.8 (4.2) *0.38 (0.11)*	5.1 (3.2) *0.02 (0.01)*	11.2 (4.9) *0.05 (0.03)*	77.3 (5.4)* *0.52 (0.14)**	9.7 (4.0) *0.06 (0.03)**	19.7 (4.8)* *0.14 (0.06)**	86.2 (10.1) *0.51 (0.18)*	4.7 (5.5) *0.03 (0.05)*	12.1 (9.7) *0.08 (0.08)*
Shantsila, et al, 2012	*Healthy men and women* Bruce Protocol	~ 85.2 *~ 0.36*	~ 2.1 *~ 0.01*	~ 12.6 *~ 0.05*	~ 85.1 *~ 0.43*	~ 2.9 *~ 0.02*	~ 12.0 *~ 0.06*	~ 85.1 *~ 0.35*	~ 2.9 *~ 0.01*	~ 12 *~ 0.05*
Radom-Aizik, et al, 2014	*Healthy Young Men* 10 x 2min (1min Rest) ~82% of max Cycle Ergometer	Absolute % and Counts Unreported (Depicted in a Figure)			Decreased *Increased*	Unchanged *Unchanged*	Increased *Increased*	N/A	N/A	N/A
LaVoy, et al, 2015	*Healthy Men and Women* Bruce Protocol	65.7 (7.7)	7.6 (2.9)	6.8 (3.9)	53.0 (8.6)*	15.9 (6.7)*	14.1 (6.3)*	N/A	N/A	N/A

Table 2. Monocyte Subset Modulations in response to Exercise in Clinical Populations.

Data reported as Mean (SD)
* Reported significant difference from pre; ~ no SD reported
Classical monocytes (CLAS), Intermediate monocytes (INTER), and nonclassical monocytes (NC)

Author	Participants / Exercise	Pre Exercise			Post Exercise		
		CLAS	INTER	NC	CLAS	INTER	NC
Dimitrov, et al, 2013	*Men and Women with Normal and Pre-hypertension* Bruce Protocol	~ 87.9	~ 4.8	~ 4.2	~ 86.7*	~ 5.0	~ 5.0*
Van Craenenbroeck, et al, 2010	*Chronic Kidney and Heart Disease Patients; Healthy Sedentary* Cycling VO$_2$ Max; 10-20W/min	HS: 88.1 (4.7) CKD: 88.5 (4.3) CHF: 87.4 (3.5)	HS: 4.5 (2.1) CKD: 3.6 (1.7) CHF: 4.7 (2.5)	HS: 7.4 (3.2) CKD: 8.0 (3.6) CHF: 7.9 (2.2)	HS: 83.1 (6.0) CKD: 85.3 (3.7) CHF: 85.9 (4.3)	HS: 5.6 (2.3) CKD: 4.4 (1.6) CHF: 4.8 (2.2)	HS: 11.3 (4.6) CKD: 10.3 (3.0) CHF: 9.4 (3.1)
Dungey, et al, 2015	*Hemodialysis Patients* 30 min Cycle at "somewhat hard" intensity during hemodialysis	82.2 (5.3)	7.5 (2.4)	10.3 (3.9)	83.9 (4.3)	5.5 (1.9)	10.6 (3.9)

Prior to the redefinition, investigations demonstrated selective expansion of CD16+ monocytes following exercise, the magnitude of which was dependent on the intensity of exercise (Steppich et al., 2000). Furthermore, the increase in nonclassical monocytes following exercise may be related to a mobilization from the marginal pool stimulated by the catecholamine response to exercise (Fragala et al., 2011; Kittner et al., 2002). This seems to be consistent even in patients with elevated blood pressure (Hong & Mills, 2008). Despite this, the previous studies demonstrated their results without the incorporation of the intermediate monocyte population. With the new nomenclature, the response of the intermediate subset must be isolated from the nonclassical population, and defined more thoroughly.

While the overall count of all monocyte subsets have been demonstrated to increase with exercise (Booth et al., 2010; Radom-Aizik et al., 2014; Shantsila et al., 2012), the proportion of each subtype in relation to the total population of monocytes may be variable. Immediately following aerobic exercise, a significant decrease in the proportion of classical monocytes is generally observed (Booth et al., 2010; LaVoy et al., 2015; Radom-Aizik et al., 2014; Shantsila et al., 2012; Simpson et al., 2009). This decrease is often associated with increases in the proportion of nonclassical only (Booth et al., 2010; Dimitrov et al., 2013; Radom-Aizik et al., 2014; Simpson et al., 2009), while others report a significant increase in the proportion of both intermediate and nonclassical monocytes (LaVoy et al., 2015). The difference observed between the former and latter investigations may be related to differences in the gender of the participant populations. LaVoy and colleagues (2015), utilized a mixed gender group of participants. The nonclassical monocyte population appears to respond differently to exercise in women than men

(Heimbeck et al., 2010), which suggests that the use of a mixed gender population may result in a different or varied response. Currently, to the best of our knowledge, there does not appear to be any data available regarding the monocyte subset response to resistance exercise.

Only two studies appear to have examined the response of monocyte subsets one hour post-aerobic exercise, and the response was unclear. Initially, Simpson and colleagues (2009) reported a significant increase in the proportion of classical monocytes, and a decrease in the proportion of both intermediate and nonclassical subsets one hour following endurance exercise. Subsequently, Booth and colleagues (2010) reported no differences in the monocyte subset proportions one hour post-exercise. These differences may have been related to differences in either the exercise modality used or the training status of the participants. Briefly, "moderately trained" participants completed 45 minutes of running (Simpson et al., 2009), compared to "athletes" completing a 60km cycling time trial (Booth et al., 2010). Considering that running produces greater muscle damage, especially in moderately trained individuals, than cycling (Millet & Lepers, 2004), it stands to reason that a greater extent of muscle damage may have contributed to the greater response observed with running.

Due to the absence of examinations involving skeletal muscle damage in humans, and the heterogeneity that exists between the murine and human models of monocyte subsets, it is unclear whether the intermediate subset responds to tissue damage. Evidence from the ischemic model of tissue injury indicates a potential link between tissue damage and mobilization of the intermediate subset (Tapp et al., 2012; Zhu et al., 2015). Following acute ischemic injury, patients have demonstrated an elevated proportion of intermediate

from 1-7 days following an acute myocardial infarction (Tapp et al., 2012), and two days following a stroke (Urra et al., 2009). Although speculative, these data suggest a potential relationship between the proportion of intermediate monocytes and tissue damage.

Chemokine Response to Exercise

As stated previously, cytokines function as messengers between cells (Nicod, 1993). Chemokines form a family of cytokines that function primarily as chemoattractants for immune cells (Rollins, 1997). While chemokines typically recruit specific immune cells to the site of tissue damage, several other cytokines elicit specific responses from immune cells (Calle & Fernandez, 2010; Pedersen, Akerstrom, Nielsen, & Fischer, 2007). As the immune cells respond to signals from cytokines and chemokines, a pro- or anti-inflammatory environment is formed, leading to the classification of cytokines as pro- or anti-inflammatory (Calle & Fernandez, 2010). While several cytokines and chemokines are active during recovery from muscle damage, for the purpose of this review, we will focus on the chemokines known to recruit specific immune cells, including interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and fractalkine (CX3CL1).

Interleukin-8 (IL-8)

Interleukin 8 (IL-8) is a CXC chemokine (CXCL8) that has been suggested to perform two primary functions; neutrophil activation and angiogenesis (Pedersen et al., 2007; Rollins, 1997). IL-8 is known to interact with two CXC chemokine receptors

(CXCR1 and CXCR2) (Pedersen et al., 2007; Schraufstatter, Chung, & Burger, 2001), and is produced by a variety of cells/tissues, including monocytes (Cros et al., 2010; Wong et al., 2011), as well as adipose and muscle tissue (Bruun et al., 2004; Chan, Carey, Watt, & Febbraio, 2004; Nieman et al., 2003). Despite the multitude of production locations, it is likely that IL-8 produced by adipose tissue contributes little to the activation of neutrophils (Ribeiro et al., 1991). Therefore, the chemotactic properties of IL-8 on neutrophils are likely due to production from the myeloid cellular lineage or skeletal muscle.

Production of IL-8 within skeletal muscle has been demonstrated following both aerobic (Chan et al., 2004; Nieman et al., 2003) and resistance exercise (Chan et al., 2004; Nieman et al., 2004). Although not directly compared (analyses were completed by the same research group), the change in mRNA expression of IL-8 from pre to post exercise appears to be lower following high volume resistance exercise when compared to high volume running (Nieman et al., 2004; Nieman et al., 2003). Della Gatta and colleagues (2014) also demonstrated an increased mRNA expression of IL-8 following resistance exercise, which corresponded to an increased concentration of IL-8 within skeletal muscle. However, whether this increase in skeletal muscle IL-8 concentration is translatable to IL-8 in circulation is uncertain.

Increases in circulating IL-8 have been reported following exercise consisting of both concentric and eccentric muscle actions (Pedersen et al., 2007). Therefore, it is not surprising to find significant increases in circulating IL-8 following running exercise (Nieman et al., 2003), but not after concentrically based exercises, such as cycling and rowing (Chan et al., 2004; Henson et al., 2000). It stands to reason that with the response of neutrophils to tissue damage (Tidball, 2005), and the chemotactic properties of IL-8 on

neutrophils (Ribeiro et al., 1991; Rollins, 1997) that skeletal muscle damage may be a requisite stimulus for increases in IL-8 concentrations in circulation.

Resistance exercise though has not demonstrated the expected results in regard to circulating IL-8. Most investigations that have examined circulating IL-8 following resistance exercise, or exercise designed to elicit muscle damage, have reported no change (Buford et al., 2009; Paulsen et al., 2005; Ross et al., 2010), while one investigation demonstrated a decrease in response to exercise (Hirose et al., 2004). However, increased circulating IL-8, in conjunction with increased mRNA expression of IL-8 in skeletal muscle, has been reported following high volume resistance exercise (Nieman et al., 2004). A plausible explanation for the different results observed could be related to the total volume of exercise completed.

To our knowledge, only one study has demonstrated a significant increase in circulating IL-8 concentrations following resistance exercise. This study required participants to perform 4 sets of 10 repetitions of 10 different multi-joint, whole body exercises. The first set was performed at 40% of the one-repetition maximum (1RM) and 60% 1RM for sets 2-4 (Nieman et al., 2004). In contrast, no changes in IL-8 concentrations were noted following one investigation requiring participant to perform 300 forced eccentric repetitions of the knee extensor exercise (Paulsen et al., 2005), or 50 eccentric contractions of the leg press exercise (Ross et al., 2010). Furthermore, Buford and colleagues (2009) utilized a dynamic resistance exercise bout requiring 3x10 repetitions of the squat, leg press and leg extension exercises. Although no change in circulation IL-8 concentrations were reported, they did observe a significant increase in the mRNA expression of IL-8 (Buford et al., 2009). Interestingly, Hirose et al (2004) demonstrated a

decrease in IL-8 concentrations following forced eccentric exercise of the elbow flexors (4 x 5 repetitions), which consequently was the lowest volume of all the studies examined(Hirose et al., 2004). Thus, each investigation that reported no change or decreased concentrations of IL-8 incorporated significantly less volume of exercise than Nieman and colleagues (2004). Therefore, IL-8 concentrations may only increase in circulation following exercise of sufficient volume, however, further investigation appears needed.

<div align="center">Monocyte Chemoattractant Protein -1 (MCP-1/CCL2)</div>

Monocyte chemoattractant protein-1 (MCP-1) is a member of the CC subfamily of chemokines, and is also referred to as CCL2 (Yadav et al., 2010). Of the many proposed functions of MCP-1, monocyte chemoattraction appears to be its primary responsibility, principally for classical monocytes (Wong et al., 2012; Yadav et al., 2010; Yang et al., 2014). Furthermore, the primary receptor responsible for ligation of MCP-1 is the CC chemokine receptor CCR2 (Rot & von Andrian, 2004; Yadav et al., 2010). Production of MCP-1 occurs in the endothelial cells, classical monocytes, and within skeletal muscle tissue (Cros et al., 2010; Cushing et al., 1990; Della Gatta et al., 2014; Wong et al., 2011). With the pivotal role of classical monocytes during recovery from resistance exercise, the investigation of MCP-1 in response to resistance exercise is warranted. Despite this, the examination of MCP-1 in circulation following resistance exercise is rather limited.

To our knowledge, only two investigations have examined the effects of dynamic resistance exercise on circulating MCP-1 concentrations. A previous investigation

demonstrated elevated mRNA expression of MCP-1 within skeletal muscle following resistance exercise, which also translated into an increased expression of MCP-1 within the skeletal muscle tissue for up to four hours (Della Gatta et al., 2014). However, this study did not examine circulating changes in MCP-1 concentrations (Della Gatta et al., 2014). In another investigation, Ihalainen and colleagues (2014) demonstrated no change in MCP-1 concentrations following two different exercise bouts (15 x 1-RM or 5 x 10 repetitions at 80% 1-RM) immediately, or 15-min post-exercise. However, a significant decrease in MCP-1 concentrations were observed 30 minutes post-exercise. Conversely, Wells and colleagues (2016) reported a significant, immediate increase of circulating MCP-1 in response to both a high volume and a high intensity bout of resistance exercise for at least 5-hours post-exercise. A reasonable explanation for the discrepancy in these reports is the level of muscle damage that was elicited. Ihalainen et al. (2014) observed 2-fold increase in myoglobin concentrations, versus the 5-8 fold increase observed by Wells and colleagues (2016). Thus, the extent or magnitude of skeletal muscle damage may provide further insight to the cause of increased MCP-1 concentration.

Previous work investigating exercise designed to elicit muscle damage (e.g. downhill running, etc.,) has indicated a significant increase in circulating MCP-1 immediately following exercise (Crystal, Townson, Cook, & LaRoche, 2013; Paulsen et al., 2005; J. M. Peake, K. Suzuki, M. Hordern, et al., 2005). These responses appear to be maintained for at least six hours following damage before returning to resting levels by 24 hours post-exercise (Crystal et al., 2013; Paulsen et al., 2005). Crystal and colleagues (2013) also demonstrated a biphasic response of MCP-1, with initial increases immediately following exercise, and a subsequent increase at 6H that may be attenuated with cold water

immersion (Crystal et al., 2013). Peake and colleagues (2005) also demonstrated significant increases in MCP-1 concentrations at 1-hr post-exercise. Greater increases, however, were observed following high intensity running (85% of VO$_2$max for 60 min) than after downhill running (-10% gradient; 60% VO$_2$max for 45 min) (J. M. Peake, K. Suzuki, M. Hordern, et al., 2005).

It is unclear whether the volume of exercise, or the level of muscle damage regulates circulating MCP-1 concentration. Wells and colleagues (2016) suggested that volume of exercise may explain the different results obtained in the investigations utilizing dynamic resistance exercise. Considering that both of these investigations reported no differences between high and low volume exercise (Ihalainen et al., 2014; Wells et al., 2016), we argue that muscle damage may be a more likely explanation. Nonetheless, further research is warranted to determine the impact of muscle damage or the volume of exercise on MCP-1 concentrations.

Fractalkine (CX$_3$CL1)

Fractalkine is the sole member of the CX3C chemokine family (CX3CL1), and is the primary chemokine responsible for the recruitment and activation of nonclassical monocytes (Bazan et al., 1997), and Th cells (Foussat et al., 2000). Additionally, CX3CL1 will ligate with the CX3C chemokine receptor CX3CR1 (Ancuta et al., 2003), which is prominent on both intermediate and nonclassical monocytes (Cros et al., 2010; Wong et al., 2011). CX3CL1 is produced by endothelial cells under inflammatory conditions, namely through interaction with TNF-α and INFγ (Ludwig et al., 2002) as well as within skeletal muscle tissue following exercise (Catoire et al., 2014; Della Gatta et al., 2014;

Stromberg et al., 2016). CX3CL1 produced in endothelial cells, however, is bound to the endothelial membrane, and serves as an adhesion molecule during transmigration of monocytes and lymphocytes to sites of damage (Ancuta et al., 2003; Fong et al., 1998; Imai et al., 1997). Soluble CX3CL1 (sCX3CL1) is released into circulation, when it is cleaved from the endothelial cell, and will promote chemotaxis of monocytes and Th cells alike (Ancuta et al., 2003; Foussat et al., 2000). The cleaving of CX3CL1 occurs in the presence of pro-inflammatory cues, such as TNF-α and IL-1β (Turner, Mangnall, Bird, Blair-Zajdel, & Bunning, 2010). As a result, elevations of sCX3CL1 can be expected in circulation during inflammatory conditions that increase both TNF-α and IL-1β.

Prior to 2014, no investigations examined the impact of exercise on CX3CL1 or sCX3CL1. Since then, three investigations have been published demonstrating increased skeletal muscle mRNA expression of CX3CL1 (Della Gatta et al., 2014), as well as increased CX3CL1 within skeletal muscle (Stromberg et al., 2016) and plasma (Catoire et al., 2014). Catorie and colleagues (2014) observed a significant increase in mRNA expression of CX3CL1 following one hour of unilateral cycling (at 50% of their unilateral VO$_2$ max). The increase in mRNA expression translated to a significant increase in circulating sCX3CL1 immediately, and 2-hrs post-exercise (Catoire et al., 2014). Following resistance exercise, Della Gatta and colleagues (2014) demonstrated significant increases in mRNA expression of CX3CL1 at 2-hrs, but not 4-hrs, post exercise. Recently, Stromberg et al. (2016) observed significant increases in CX3CL1 mRNA expression 30 minutes following bilateral cycling (Workload: 50% of VO$_2$ max for one hour), and a subsequent return to baseline at 2-hrs post-exercise. The mRNA expression of CX3CL1 paralleled an increase in CX3CL1 protein content observed within skeletal muscle

(Stromberg et al., 2016). However, it is unclear if the CX3CL1 observed within the skeletal muscle was bound to endothelial cells or whether it was soluble.

Evidence is clear that both aerobic and resistance exercise will increase mRNA expression of CX3CL1, however, the time course of sCX3CL1 and CX3CL1 mRNA expression appears to be quite variable. It appears that elevations in mRNA expression of CX3CL1 is delayed following resistance exercise compared to aerobic exercise (Della Gatta et al., 2014; Stromberg et al., 2016). However, Della Gatta and colleagues (2014) did not assess mRNA expression of CX3CL1 prior to 2-hr post-exercise. Consequently, whether the increase in mRNA expression of CX3CL1 observed at 2-hr post-exercise is a sustained or a delayed response remains unclear (Della Gatta et al., 2014). Furthermore, the suggestion that increases in mRNA expression within skeletal muscle prompts sCX3CL1 increases in circulation (Catoire et al., 2014), has yet to be definitively demonstrated following resistance exercise. Therefore, further investigation is required to elucidate the time course of CX3CL1 and sCX3CL1 following resistance exercise.

The αMβ2 Integrin, MAC1/CR3 (CD11b/CD18)

Macrophage-1 antigen (MAC1) or complement receptor 3 (CR3) is a heterodimeric integrin that consists of two subunits, αM (CD11b) and the common β2 subunit (CD18) (Ehlers, 2000). As one of the most studied integrins, the vast array of functions associated with MAC1/CR3 range from a potent phagocytic stimulator (Tan, 2012) to providing a pivotal role in immune cell migration (Ley et al., 2007). Furthermore, MAC1/CR3 can be activated by several methods, including ligation with P-selectin glycoprotein ligand-1

(PSGL1) (Evangelista et al., 1999), or activation through inflammatory cytokines, like TNFα (Montecucco et al., 2008). Despite the vast, and varied roles of MAC1/CR3, the greatest impact on overall immune function appears to be its role in endothelial migration.

As immune cells are attracted to the site of damaged tissue through an intricate signaling network of chemokines (Freidenreich & Volek, 2012; Rollins, 1997), they encounter the endothelial wall, which acts as the final barrier to the damaged tissue (Gerhardt & Ley, 2015). Immune cells will systematically employ specific adhesion molecules that mediate the transendothelial migration to the tissue, a process referred to as the leukocyte adhesion cascade (Ley et al., 2007). Furthermore, molecules within the leucocyte adhesion cascade can be divided into two primary sub-categories, selectins and integrins, while the cascade itself can be divided into five major processes: tethering, rolling, adhesion, crawling and extravasation (Gerhardt & Ley, 2015).

During the leukocyte adhesion cascade, selectins primarily function during the early phases (tethering and rolling), while the integrins function primarily in the later phases (adhesion, crawling and extravasation) (Kolaczkowska & Kubes, 2013). Briefly, the adhesion cascade is initiated by the ligation of PSGL-1 to its receptors on the endothelial wall, P-selectin, and later E-selectin (Evangelista et al., 1999; Kansas, 1996). Following the initial tethering, P- and E-selectin as well as PSGL-1 on the leukocyte continue the rolling process, gradually progressing to a slow rolling state with increasing contribution from L-selectin (CD62L) (Ley et al., 2007). As the rolling phase continues to slow, integrins become more involved, until adhesion can occur. Adhesion is mediated by the cell adhesion molecules intracellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1) located on the endothelial surface, and the integrin

47

lymphocyte function-associated antigen-1 (LFA-1) located on the immune cell (Tan, 2012). Crawling, which is mediated by the interaction of ICAM1 with MAC1/CR3, is initiated once the cells are firmly adhered to the vessel wall. It requires slight movement to position the leukocyte over the endothelial junction to allow for transmigration into the damaged tissue through a paracellular route (Ley et al., 2007). While transcellular migration across the endothelium is possible, evidence suggests immune cells preferentially migrate by paracellular means (Gerhardt & Ley, 2015). Furthermore, MAC1/CR3 aides in the paracellular migration of immune cells by interacting with junctional adhesion molecules near the endothelial junctions (Gerhardt & Ley, 2015). While each process is required for complete migration to the location of damage, the specific modulation of MAC1/CR3 has also been demonstrated to modify immune cell infiltration.

Increased expression of MAC1/CR3 on monocytes has been implicated in increased adherence to the endothelial wall (Weber, Erl, & Weber, 1995). Likewise, decreased expression of MAC1/CR3 leads to decreased adhesion to the endothelial wall (Arakawa et al., 2010). Similarly, mice that are injected with anti-CD11b toxin display limited infiltration into damaged tissue (Rosen & Gordon, 1987). Direct activation of MAC1/CR3 on neutrophils is mediated through the pro-inflammatory cytokine TNF-α (Montecucco et al., 2008). Recent evidence suggests a relationship between MAC1/CR3 expression on monocytes and MCP-1 (Wells et al., 2016). Evidence appears to indicate a significant impact of MAC1/CR3 in the overall recovery of skeletal muscle from exercise stress.

MAC1/CR3 Responses to Exercise Stress

An efficient transmigration of cells into the damaged tissue is paramount to optimal recovery, underlining the importance of MAC1/CR3 in the recovery process. While MAC1/CR3 has been examined frequently, there is still little consensus on the overall response and time course of MAC1/CR3 expression on leukocytes following exercise. This is likely due to the vast array of exercise protocols that have been examined (Gavrieli et al., 2008; Jordan et al., 1999).

Neutrophil Expression of MAC1/CR3 following Exercise

Aerobic exercise has often been used to examine the expression of CD11b in response to exercise. No change in CD11b expression on neutrophils were reported by two investigations in response to moderate duration and intensity exercise (30 min at 75% VO$_2$ max or 60 min at 60% VO$_2$ max) (Gavrieli et al., 2008; Kurokawa, Shinkai, Torii, Hino, & Shek, 1995). Similarly, Jordan and colleagues (1999) demonstrated no significant change in CD11b expression following moderate intensity (50-60%) running for three hours. However, during prolonged endurance events (i.e. a marathon) CD11b expression was demonstrated to both increase (Jordan et al., 1999) and remain at pre-exercise levels (Nielsen & Lyberg, 2004). While these results are conflicting, differences in environmental stresses and hydration status may have influenced these results (Jordan et al., 1999).

As exercise intensity increases, there appears to be greater consistency of the MAC1/CR3 response on neutrophils. Graded exercise tests have been a common mode of

exercise to examine the neutrophil expression of MAC1/CR3 at maximal effort. Studies using this exercise model consistently demonstrate significant increase in CD11b expression immediately following exercise (Jordan et al., 1999; van Eeden et al., 1999). Increases in the expression of CD11b following graded exercise was reported within minutes of the initial exercise stimulus, and remained elevated for at least 30 minutes post-exercise (van Eeden et al., 1999). Furthermore, Gabriel and Kindermann (1998) demonstrated an increase in the expression of CD11b immediately following an intense (110% of anaerobic threshold for approximately 20 minutes) bout of cycling exercise, with a subsequent decrease in expression two hours into recovery.

Exercise designed to elicit muscle damage has also been used to examine MAC1/CR3 expression on granulocytes, producing equivocal results (J. M. Peake, K. Suzuki, G. Wilson, et al., 2005; Pizza et al., 1996; Saxton et al., 2003). Pizza and colleagues (1996) initially examined the effects of forced eccentric elbow flexion on granulocyte CD11b expression. Interestingly, increases were observed only between 24 and 96 hours following exercise (Pizza et al., 1996), long past the expected time course of neutrophil activation (Kolaczkowska & Kubes, 2013). Others observed no changes in neutrophil expression of CD11b following exercise induced muscle damage (J. M. Peake, K. Suzuki, G. Wilson, et al., 2005; Saxton et al., 2003), but they used direct isolation methods to identify granulocytes.

Expression of MAC1/CR3 on granulocytes appears to be modulated primarily by exercise intensity, particularly as a function of VO_2 max (Gabriel & Kindermann, 1998; van Eeden et al., 1999). Of the investigations presented, significant increases were most consistently observed when near maximal effort was used (Gabriel & Kindermann, 1998;

van Eeden et al., 1999). Consequently, it seems that exercise induced muscle damage may not affect neutrophil expression of MAC1/CR3. However, to our knowledge, no investigations have examined neutrophil expression of MAC1/CR3 in response to a dynamic resistance exercise bout, demonstrating the need for further investigation in the area of resistance exercise.

Monocyte Expression of MAC1/CR3 following Exercise

Similar to the MAC1/CR3 expression on neutrophils, the characterization of the MAC1/CR3 response to exercise on monocytes has been ambiguous. Increases in CD11b expression have been previously demonstrated following both a marathon and half-marathon (Nielsen & Lyberg, 2004). Furthermore, the use of dynamic body weight exercises (5 sets of 30 lunge exercises) have also demonstrated significant elevations in CD11b expression on monocytes during recovery (Malm et al., 1999). However, studies using exercise modalities that induce acute muscle damage have yielded inconsistent results. In a study on recreationally active men no change in CD11b expression on monocytes was reported following 50 eccentric contractions of the knee extensors (Saxton et al., 2003). In contrast, Pizza and colleagues (1996) demonstrated significant elevations in CD11b expression 24 hours following 25 eccentric contractions of the elbow flexors in untrained individuals. It is likely that training experience of the participants contributed to these differences. Still, the exercise stimuli employed by both studies were isolated movements, and may not be translatable to dynamic resistance exercise.

Following dynamic resistance exercise the expression of CD11b on monocytes has been reported to increase 30-min following exercise, and return to resting levels at 24- and

51

48-hrs post exercise (Gonzalez et al., 2014; Jajtner et al., 2014). Recently, Wells and colleagues (2016) demonstrated a significant increase in CD11b expression on monocytes immediately, and 1-hr post-exercise. The immediate increase contrasts with the findings of Jajtner et al. (2014) and Gonzalez, et al. (2014), who demonstrated no significant increase immediately following exercise. While each study utilized experienced, resistance-trained participants performing an acute bout of resistance exercise, the differences could be attributable to the identification of monocytes in each study. Gonzalez et al. (2014) and Jajtner et al. (2014) identified monocytes with CD14 exclusively (CD14++). In contrast, Wells and colleagues (2016) utilized CD14 in conjunction with CD16 to identify classical monocytes (CD14++/CD16-). As intermediate and classical monocytes express CD14 at similar levels (Wong et al., 2012), it is likely the CD14++ monocytes analyzed by Gonzalez et al (2014) and Jajtner et al (2014) incorporated both classical and intermediate monocytes. Furthermore, classical and intermediate monocytes are both known to express CD11b (Wong et al., 2012). While speculative, the inclusion of intermediate monocytes may have influenced the expression of CD11b on the CD14++ monocytes reported by Gonzalez et al. (2014) and Jajtner et al. (2014). However, to our knowledge, no investigation has examined the CD11b response on intermediate monocytes. Therefore, future research examining the impact of dynamic resistance exercise on the expression of CD11b on the different monocyte subsets is needed.

Polyphenol Supplementation

Polyphenols are micronutrients that are the most plentiful antioxidant in the diet, and are common in many plant-based foods and beverages, such as fruits, tea and coffee (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Scalbert, Johnson, & Saltmarsh, 2005). Polyphenols are characterized by their structure, which contains multiple hydroxyl groups on aromatic rings (Manach et al., 2004). Based on their structure, polyphenols are broken into four main classifications, phenolic acids, flavonoids, stilbenes, and lignans (Manach et al., 2004). Flavonoids can be further classified into six distinct classifications, and appear to be the most common polyphenol supplement examined with exercise (Manach et al., 2004; Myburgh, 2014).

Human cells continually produced reactive oxygen species (ROS), a process that is intensified during exercise (Merry & Ristow, 2015; Urso & Clarkson, 2003). As polyphenols are known antioxidants, their use during and following exercise has been the subject of recent investigation (Bowtell et al., 2011; Jowko et al., 2011; Panza et al., 2008). Acute polyphenol supplementation has been demonstrated to reduce strength deficits following exercise that elicits muscle damage (Herrlinger et al., 2015; Machin et al., 2014), as well as resistance exercise (Bowtell et al., 2011; Jowko et al., 2011; Panza et al., 2008). Data regarding polyphenol supplementation and the immune response to exercise, however, is limited.

Most investigations that have examined polyphenol supplementation and the exercise response have employed a high volume aerobic exercise model. Nieman and colleagues (2013) assessed changes in cytokine concentration following three days of high

volume running (2.5-hr at 65% VO$_2$max) with or without polyphenol supplementation. While all measured cytokines (IL-6, IL-8, IL-10, MCP-1, G-CSF and TNF-α) increased due to exercise, no differences were observed in response to polyphenol supplementation (Nieman et al., 2013). Similarly, no differences in plasma cytokine concentration (IL-1ra, IL-6, IL-8, IL-10, MCP-1, G-CSF or TNF-α) were reported following a 163km running race between placebo or quercetin supplementation (Nieman, Henson, Davis, et al., 2007). Cycling at ~57% on of VO$_2$max for 3-hr on three consecutive days also demonstrated no differences in immune cell populations between quercetin and placebo groups (Nieman, Henson, Gross, et al., 2007). Additionally, polyphenol supplementation resulted in no differences following downhill running or repeated sprints on a cycle ergometer for TNF-α, IL-1β and IL-6, or IL-6 alone, respectively (Arent, Senso, Golem, & McKeever, 2010; Herrlinger et al., 2015). Similarly, Kerksick and colleagues (2010) showed no differences in TNF-α concentration following 100 eccentric contractions of the leg extensors in conjunction with EGCG supplementation. However, a significant decrease in the number of neutrophils were observed 24-72 hours following exercise with ECGC supplementation for 14 days (Kerksick et al., 2010). Although polyphenol supplementation does not influence the cytokine response to exercise, evidence does suggest that EGCG supplementation can suppress the neutrophil response to eccentric exercise eliciting muscle damage. Nevertheless, no changes in the granulocyte population have been reported following quercetin supplementation and high volume exercise.

Polyphenols, appear to elicit their effects within the context of immune cell adhesion. Evidence from *in vitro* modeling have demonstrated significant downregulation of CD11b on the surface of both monocytes and neutrophils, as well as limited chemotaxis

(Graff & Jutila, 2007; Kawai et al., 2004; Takano et al., 2004). Evidence of reduced CD11b expression on monocytes in response to polyphenol supplementation has also been presented in clinical populations (Chiva-Blanch et al., 2012). However, there does not appear to be any investigations conducted examining the response of CD11b expression to exercise in conjunction with polyphenol supplementation.

Conclusions

Previous investigations of the immune response to exercise and exercise induced muscle damage have elucidated many distinct functions of myeloid and non-myeloid cells during recovery. Following tissue injury, patrolling nonclassical monocytes and resident macrophages initiate the release of specific cytokines and chemokines. These in turn activate endothelial cells, and stimulate the recruitment of myeloid cells. First to arrive at the site of tissue damage are the neutrophils, followed by classical monocytes. Classical activation of these monocytes leads to the development of M1 macrophages, and subsequently, M2 macrophages. During the earliest phases of recovery, neutrophils and M1 macrophages promote an inflammatory environment, while M2 macrophages promote an anti-inflammatory milieu during the late phase of recovery. Despite an inflammatory environment, the early phase of recovery plays a pivotal role in the remodeling of skeletal muscle. MPC are known to proliferate during the M1 phase, while the M2 phase of recovery allows for the differentiation of MPC to mature myotubes, resulting in skeletal muscle recovery.

Within circulation, the myeloid cells also actively contribute to the recovery process. The heterogeneity of monocytes within circulation has gained interest in recent years with the formal definition of an intermediate monocyte population. Furthermore, each monocyte subset has specific responsibilities within the context of skeletal muscle recovery. Classical monocytes infiltrate damaged tissue and give rise to M1 macrophages, while also displaying a strong phagocytic capacity. Intermediate monocytes are inflammatory cells that assist with antigen presentation to T-cells, while nonclassical monocytes patrol the endothelial wall. As each subset has distinct responsibilities, the propensity of each subset to adhere and transmigrate into damaged tissue is of interest. While both classical and intermediate monocytes express the cell adhesion molecule MAC1/CR3, examination of this marker provides an indication of the tendency of these cells to migrate to damaged tissue.

Though many processes of the immune response have been elucidated, many questions remain. The focus of our knowledge on the monocyte subset response to exercise has been primarily achieved using aerobic exercise but our understanding of monocyte subset redistribution in response to resistance exercise is lacking. Furthermore, increases in MAC1/CR3 expression likely lead to extravasation of myeloid cells, however, the response of different monocyte subsets, namely the inflammatory intermediate monocytes, to resistance exercise is unknown. Additionally, polyphenol supplementation is known to suppress the expression of MAC1/CR3 on monocytes and neutrophils. However, the application of polyphenol supplementation to recruitment of myeloid cells following exercise has yet to be examined. Given the necessity of macrophage infiltration for

complete recovery of skeletal muscle, exercise and supplementation programs that maximize myeloid extravasation may be indicative of enhanced recovery.

CHAPTER 3: DESIGN AND METHODOLOGY

Participants

Thirty-six recreationally active males between the ages of 18 and 35 volunteered to participate in this study. Participants were randomly assigned to one of three groups. The first group (PPB; N=13, 21.8 ± 2.5 y, 171.2 ± 5.5 cm, 71.2 ± 8.2 kg, 24.3 ± 2.8 kg/m^2) consumed 2 g per day of the proprietary polyphenol blend supplement; the second group (PL; N = 15, 21.6 ± 2.5 y, 176.5 ± 4.9 cm, 84.0 ± 15.7 kg, 26.9 ± 4.2 kg/m^2) consumed 2 g per day of the placebo and the third group (CON; N = 9, 23.6 ± 4.5 y, 174.0 ± 13.4 cm, 78.3 ± 16.9 kg, 25.7 ± 3.5 kg/m^2) served as control. Following an explanation of all procedures, risks and benefits, each participant provided his informed written consent prior to completing any testing. For inclusion in the study, participants had to complete less than three hours of planned exercise per week, have a body mass index of 18.0-34.9 kg/m^2, be free of physical limitations, and be willing to maintain a habitual diet while abstaining from dietary supplements.

Study Design

All groups reported to the human performance lab for five days of testing (Figure 5). Prior to the first day of testing, PPB and PL completed a 28-day supplementation protocol. Day 1 consisted of 1-repetition maximum (1-RM) testing of the squat, leg press and leg extension exercises, and occurred at least 72 hours prior to the second day of the

study. On day 2, participants arrived in the lab 12 hours postprandial, and provided a resting blood sample (PRE). After blood samples were obtained, participants were provided a small breakfast bar (Cal: 190, CHO: 19g, Protein: 7g, Fat: 13g) and then completed performance testing. After performance testing was complete, CON rested for one hour, while PPB and PL began the acute exercise protocol. Participants provided blood samples immediately post- (IP), 1-hour (1H) and 5-hours (5H) post-exercise, and completed performance testing at 1H following the damaging protocol. Immediately following the performance measures at 1H, participants were provided with a light meal (Cal: 250, CHO: 34g, Protein: 14g, Fat: 6g). Participants returned to the lab in a fasted state 24- (24H), 48- (48H) and 96-hours (96H) later for resting blood samples and performance testing.
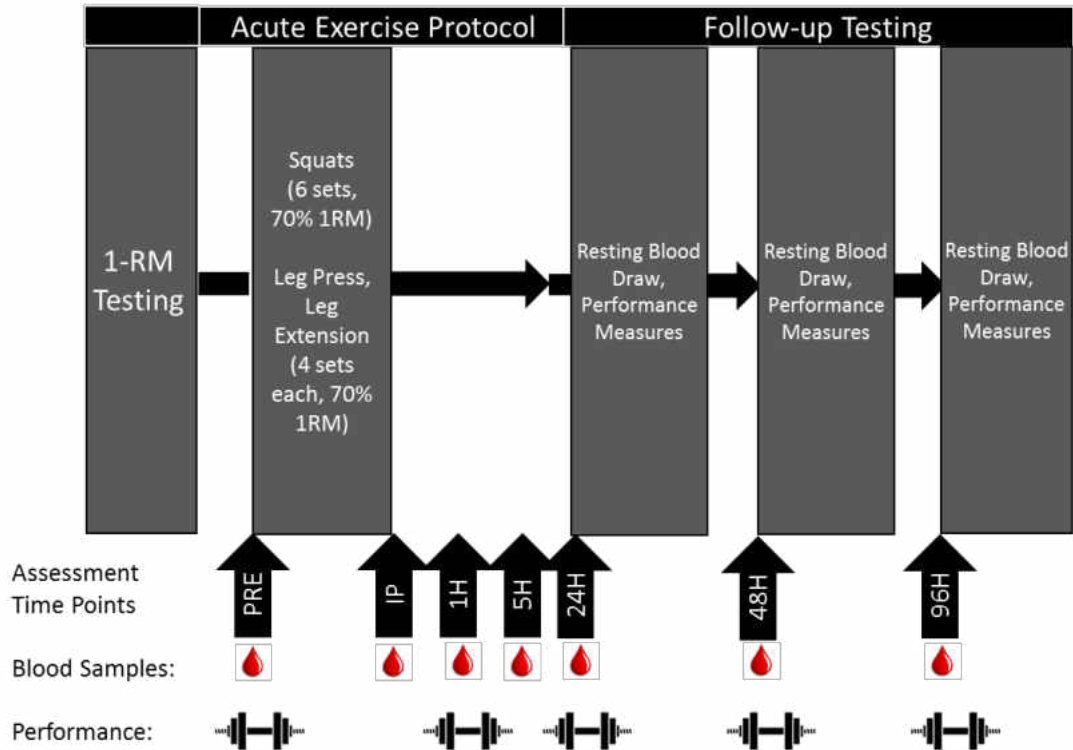
Figure 5: Study Design.
Participants completed 1-RM testing at least 72 hours prior the exercise protocol. During day 2, participants completed a muscle damaging workout, and provided blood samples pre exercise (PRE), immediately (IP), 1 hour (1H) and 5 hours (5H) post exercise. Performance testing was completed at PRE and 1H. Participants provided additional blood samples and completed performance testing 24- (24H), 48-(48H) and 96- (96H) hours following damage. Flow cytometry was completed at every blood sampling except 96H.

Procedures

Supplementing Protocol

Both the PPB and PL groups completed daily supplementation for 28 days with either a proprietary polyphenol blend (PPB) or placebo (PL) (Kemin Foods, L.C., Des Moines, IA, USA). The PPB group consumed a blend of water-extracted green and black tea (Camellia Sinensis) containing at minimum 40% total polyphenols, 1.3% theaflavins,

60

5-8% epigallocatechin-3gallate (EGCG), 7-13% caffeine, 600 ppm manganese. The PL group consumed microcrystalline cellulose in capsules of similar shape and size. All products were tested for toxins including heavy metals, pesticides and excipients by an independent third party.

Briefly, participants reported to the Human Performance Lab three to five days per week to receive the supplement. Participants took one dose (1000 mg PPB or PL) under the supervision of a member of the research team, and were given their prescribed doses in individual containers (1000 mg PPB or PL) for each additional time point. Participants consumed two doses per day for a total of 2000 mg of either PPB or PL daily. Participants were asked to return all empty containers upon their next visit to the lab. Participants that did not maintain 80% compliance in each phase (28 days of supplementation or during the AP) were removed from analysis.

<div align="center">1-Repetition Maximum Testing</div>

Direct measurement of one repetition maximal strength (1-RM) was completed on the squat and leg press exercises, while a predicted 1-RM was performed on the leg extension exercise. All participants completed a standardized warm-up, consisting of 5 minutes on a cycle ergometer against a self-selected resistance, 10 body weight squats, 10 walking lunges, 10 dynamic hamstring stretches and 10 dynamic quadriceps stretches. All 1-RM testing was completed as previously described (Hoffman, 2006). Briefly, each participant completed two warm-up sets consisting of 5-10 repetitions and 3-5 repetitions at approximately 40-60% and 60-80% of his perceived maximum, respectively. Each

participant then performed up to five subsequent trials to determine his 1-RM with 3-5 minutes of rest between each set.

During the squat exercise, participants placed a safety squat bar (Power Lift, Jefferson, IA, USA) across their shoulders and descended to the parallel position, where the greater trochanter of the femur reached the same level as the knee. Participants then ascended to a complete knee extension. Leg press was completed with the participant sitting in a reclined position, with their legs extended. Participants were asked to lower the weight until the lower leg and femur created a 90° angle. Participants were then asked to press the weight up. Participants that were unable to complete the repetition or maintain proper range of motion were given one additional opportunity. If they were still unable to perform the exercise correctly, the last completed weight was recorded as the 1-RM.

For the leg extension exercise, participants were placed in a seated position, and asked to extend their legs straight out in front of them. Participants were asked to perform as many repetitions as possible, and the resulting repetitions and weight used were applied to a prediction equation (Brzycki, 1993). If more than 10 repetitions were performed, the weight was increased and the participant repeated the measure 3-5 minutes later. All testing was observed by a certified strength and conditioning specialist to monitor adherence to form.

Acute Exercise Protocol

Only PPB and PL completed the protocol, while CON rested for an hour. The exercise protocol designed to cause muscle damage in previously untrained individuals was

preceded by a light warm-up as described above. Following the light warm-up, participants

completed a resistance exercise session that consisted of six sets of 10 repetitions of the

squat, as well as four sets of 10 repetitions of the leg press and leg extension exercises. All

exercises were completed at 70% of the subjects previously determined 1-RM with 90 sec

of rest between each set. Participants were provided with assistance if they were unable to

complete 10 repetitions on their own, and weight for the subsequent set was reduced. All

testing sessions were observed by a certified strength and conditioning specialist to monitor

adherence to exercise form.

Blood Measurements

Blood samples were obtained at seven time points throughout the study (PRE, IP,

1H, 5H, 24H, 48H and 96H). The PRE, IP and 1H blood samples were obtained using a

Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male

luer lock adapter and plastic syringe. The cannula was maintained patent using an isotonic

saline solution (Becton Dickinson, Franklin Lakes, NJ, USA). PRE and 1H blood samples

were obtained following a 15-minute equilibration period, while IP blood samples were

taken within 5-min of exercise cessation. The remaining time points (5H, 24H, 48H and

96H) were obtained by a single use disposable needle with the subject in a supine position

for at least 15 minutes prior to sampling. Whole blood (20 ml) was collected in two

Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ), one containing K_2EDTA, and

one containing no anti-clotting agents. Aliquots were removed from the first tube for

hematocrit and hemoglobin measures, as well as flow cytometry analysis, while the second

tube was allowed to clot for 30 minutes prior to being centrifuged at 3,000xg for 15 minutes with the remaining whole blood from the first tube. The resulting plasma and serum was aliquoted and stored at -80°C for later analysis.

Circulating Markers

Hematocrit was analyzed in duplicate from whole blood via microcentrifugation (Statspin®, Critspin, Westwood, MA, USA) and microcapillary technique. Hemoglobin was analyzed in duplicate from whole blood using an automatic analyzer (Hemocue®, Cypress, CA, USA). Coefficient of variation for each assay was 0.20% for hematocrit and 0.46% for hemoglobin. Plasma volume shifts following the workout were calculated via the formula established by Dill and Costill (Dill & Costill, 1974), however, circulating values were not adjusted to account for changes in plasma volume.

Serum concentrations of myoglobin (MG) were obtained via enzyme-linked immunosorbent assay (ELISA) (Calbiotech, Spring Valley, CA, USA), while CK was analyzed using a commercially available kinetic assay (Sekisui Diagnostics, Charlottetown, PE, Canada), per manufacturer's instructions. To limit inter-assay variability, all samples for a particular assay were thawed once, and analyzed by the same technician using a BioTek Eon spectrophotometer (BioTek, Winooski, VT, USA). All samples were analyzed in duplicate with a mean coefficient of variation of 7.57% for MG and 3.66% for CK.

Plasma concentrations of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, monocyte-chemoattractant protein-1 (MCP-1), fractalkine (CX3CL1), interferon-γ (INF-γ)

granulocyte colony stimulating factor (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) were analyzed via multiplex assay, using the human cytokine/chemokine panel one (EMD Millipore, Billerica, MA, USA). All samples were thawed once and analyzed in duplicate by the same technician using the MagPix (EMD Millipore), with average coefficient of variation of 6.17%, 7.74%, 8.04%, 5.33%, 6.84%, 7.18%, 8.21%, 7.82%, and 7.10% for IL-1β, IL-6, IL-8, IL-10, MCP-1, CX3CL1, INF-γ, G-CSF and GM-CSF respectively.

Flow Cytometry

Cell Preparation

Fresh, anti-coagulated (K$_2$EDTA), whole blood (100 µl) was mixed with fluorescent-conjugated monoclonal antibodies specific to CD11b-fluorescein isothiocyanate (FITC; Biolegend, San Diego, CA, USA), CD66b-phycoerythrin (PE), CD14-PerCP Cy5.5 and CD16-allophycocyanin (APC; BD Biosciences, San Jose, CA, USA). Samples were mixed and incubated for 15 minutes in the dark, after which, the samples were lysed with 2 ml of 1 x FACS lysing solution (BD Biosciences), mixed and incubated in the dark for an additional 8 minutes. Following incubation, samples were centrifuged at 300 x g for 8 minutes and washed with 2 ml of 1 x wash buffer containing 1% fetal bovine serum (FBS) in a 1 x phosphate buffered saline (PBS) solution. Samples were centrifuged again at 300 x g for 8 minutes, and the supernatant was removed. Samples were then fixed in 300 µl of 2% paraformaldehyde in PBS.

<u>Data Acquisition and Analysis</u>

Cell preparations were acquired using an Accuri C6 flow cytometer (BD Accuri Cytometers, Ann Arbor, MI, USA) equipped with two lasers providing excitation at 488 and 640 nm, and 4 band pass filters (FL1: 533/30; FL2: 585/40; FL3 670LP; FL4: 675/25). Events were recorded based on size (FSC-A), complexity (SSC-A) and mean fluorescence intensity (MFI). A total of 200 µl were collected for each sample, which ensured at least 8,000 CD14+ events.

Analysis was completed using BD Accuri analysis software (BD Accuri Cytometers). Events were initially gated based on SSC-H and SSC-A as a multiplet cell exclusion criteria (Figure 6 A). Granulocytes were then determined by CD66b positive staining (Figure 6 B, C), while monocytes were discriminated into classical, intermediate and non-classical monocytes initially by FSC/SSC characteristics (Figure 6 D) and secondarily by CD14 and CD16 staining characteristics (Figure 6 E) (Wong et al., 2012), with CD66b exclusion (Figure 6 B, C) (Ziegler-Heitbrock et al., 2010). Mean fluorescence intensity (MFI) for each of these sub-populations was then determined for CD11b (Figure 6 F). Granulocytes are expressed as a percent of leukocytes, and monocyte subsets are presented as a percent of total monocytes.
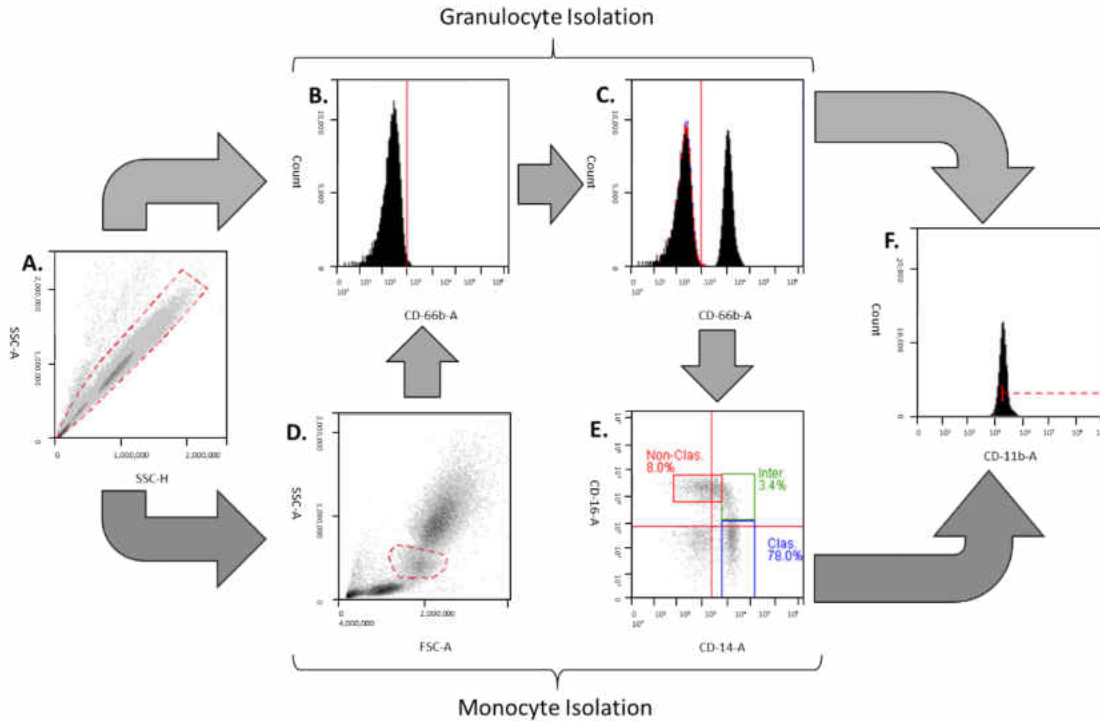
Figure 6: Gating Procedure.
    All samples were initially gated for multiplet exclusion (A). <u>Granulocytes</u> were identified by staining for CD66b in an unstained control sample (B), and compared to samples positively stained for CD66b (C). <u>Monocytes</u> were initially gated in FSC vs SSC characteristic plot with a monocyte gate (D), which were assessed for CD66b expression (B/C). CD66b- monocytes were applied to a CD14 vs CD16 two dimensional histogram to identify classical, intermediate and non-classical monocytes (E). All cell subtypes were analyzed for CD11b expression (F).

<center>Performance Testing</center>

Performance testing was performed at PRE, 1H, 24H, 48H and 96H, and consisted of maximal voluntary isometric contractions, during which peak torque (PKT) and the rate of torque development (RTD) was determined. During each assessment participants were positioned in a BioDex S4 isokinetic dynamometer in a seated position with their hips at a 110° angle. Participants were then secured to the device with straps around the waist and shoulders, after which, the technicians positioned the knee at a 110° angle (with 180°

<center>67</center>

representing full extension). The participants were then prompted to kick as forcefully and as fast as possible, while maintaining their effort for five seconds. Each participant was provided two attempts, and the highest PKT and RTD was recorded.

Statistics

Changes in circulating cytokines, markers of muscle damage, immune cell characteristics and performance measures were analyzed via repeated measures analysis of variance (ANOVA). In the event of a significant F ratio, LSD post-hoc tests were used for pairwise comparisons. Due to non-normality, circulating cytokines and markers of muscle damage were transformed using the natural log (LN). Area under the curve (AUC) was also calculated for changes in the cytokines and myoglobin response using a standard trapezoidal technique. AUC was analyzed via one-way ANOVA. Raw concentrations from PRE, IP, 1H and 5H were used to calculate AUC prior to LN transformation. Additionally, Pearson product moment correlations were calculated to examine selected bivariate relationships between immune cells, and markers of muscle damage measures. Significance was accepted at an alpha level of $p \leq 0.05$ and all data are reported as mean $\pm$ SD of the original, non-transformed data.

CHAPTER 4: RESULTS

Participant Characteristics

Participants were required to maintain 80% compliance during the supplementation period to be included in the final analysis. As a result, 10 participants were removed from the investigation prior to analysis (PPB = 6; PL = 4). Of the 10 participants removed, five participants requested to discontinue testing (PPB = 4; PL = 1). Of the five participants that wished to discontinue, one completed a portion of the resistance exercise protocol prior to discontinuing the study (PPB group), two reported unresolvable scheduling conflicts (both from PPB group), and two discontinued supplementation (PPB = 1, PL = 1). Five additional participants that completed testing were removed from analysis due to lack of compliance (PPB = 2; PL = 3). Four of the five participants were removed for failure to achieve 80% compliance with supplementation (PPB = 2; PL = 2) while one did not adhere to the fasting requirements (PL group). A graphical schematic of this is displayed in Figure 7. Characteristics of the remaining 39 participants are listed in Table 3. No significant differences were observed between groups for participant characteristics or compliance.
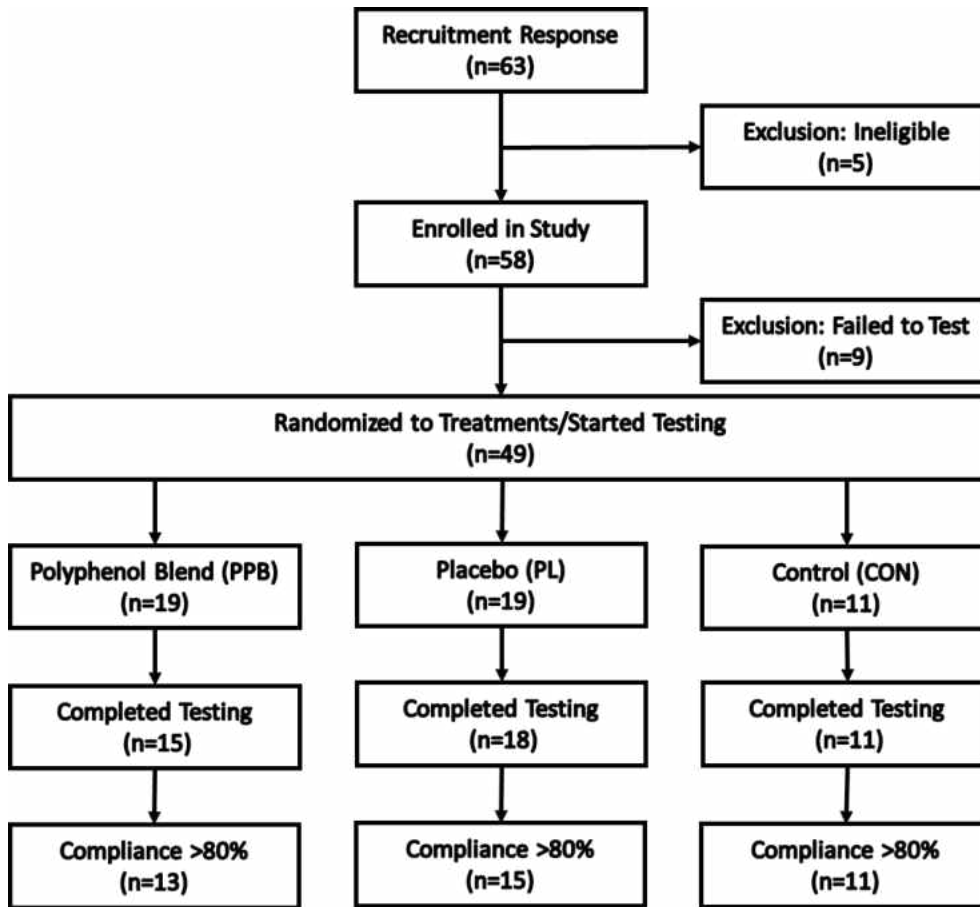
Figure 7: Participant recruitment and randomization.
Participant screening through the duration of the study. Compliance was set *a priori* at >80%.

Table 3: Participant Characteristics.
    Participant characteristics are listed as mean (SD)

Characteristics	Polyphenol Blend (PPB)	Placebo (PL)	Control (CON)	*p*
n	13	15	11	N/A
Age (*Yrs*)	21.8 (2.5)	21.6 (2.5)	23.3 (4.1)	0.344
Height (*cm*)	171.2 (5.5)	176.5 (4.9)	173.6 (12.0)	0.203
Weight (*kg*)	71.2 (8.2)	84.0 (15.7)	77.8 (15.6)	0.060
BMI (*kg*m^{-1}*)	24.3 (2.8)	26.9 (4.2)	25.6 (3.3)	0.181
Squat 1RM (*kg*)	108.2 (14.4)	108.6 (30.5)	121.9 (30.7)	0.400
Leg Press 1RM (kg)	158.0 (41.4)	160.6 (30.5)	197.3 (72.6)	0.199
Compliance (*%*)	95.9 (6.6)	95.4 (6.5)	N/A	0.853

Markers of Muscle Damage

A significant group x time interaction (F = 19.16, *p* < 0.001, η^2 = 0.523) was observed in changes in myoglobin concentrations (see Figure 8). *Post Hoc* analysis indicated a significant elevation at IP in both PPB (*p* = 0.004) and PL (*p* = 0.002) compared to CON. Elevations were maintained at 1H and 5H for both groups (*p's* < 0.001) compared to CON. No significant differences in the myoglobin AUC response were observed between the groups (F = 2.243; *p* = 0.121).

Figure 8: Myoglobin Response to Resistance Exercise.
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for changes in (A) Myoglobin concentration were analyzed PRE, immediately- (IP), one- (1H) and five- (5H) hours post Changes in exercise. (B) Creatine Kinase pre-exercise (PRE) and 24 (24H), 48 (48H) and 96 (96H) hours after exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)

Changes in circulating CK concentrations are depicted in Figure 9. A significant group x time interaction was observed (F = 4.27, p = 0.005, η^2 = 0.201) for CK concentrations. Significant elevations from PRE were noted in both PPB and PL at 24, 48 and 96 hours). However, CK concentrations at 24H, 48H and 96H were significantly greater in PPB compared to PL (p = 0.041, 0.025 and 0.025, respectively) and CON ($p \leq$ 0.001); while PL was significantly greater than CON (p = 0.036) at 24H only.

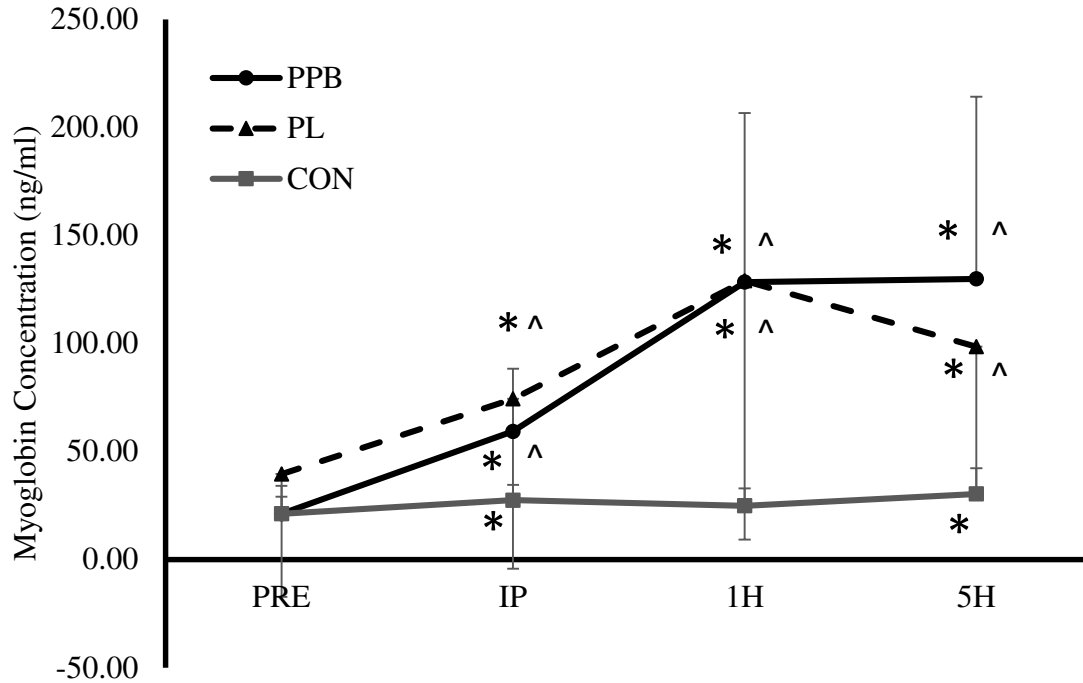**Figure 9: Creatine Kinease Response to Resistance Exercise.**
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for changes in (A) Myoglobin concentration were analyzed PRE, immediately- (IP), one- (1H) and five- (5H) hours post Changes in exercise. (B) Creatine Kinase pre-exercise (PRE) and 24 (24H), 48 (48H) and 96 (96H) hours after exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)
# Significantly different than corresponding value for PL ($p < 0.05$)

<u>Changes in Maximal Voluntary Isometric Contractions</u>

Changes in peak torque in response to resistance exercise are depicted in Figure 10. A significant group x time interaction was observed for percent change in peak torque (F = 4.52, $p = 0.001$, $\eta^2 = 0.201$). Significant reductions from PRE were noted for PPB and PL for every measure (1H – 96H) and for CON from 24H – 48H. The percent reduction from PRE in peak torque at 1H was significantly greater in PBB ($p = 0.001$) and PL ($p = 0.001$) compared to CON. No other between group differences were noted.

73
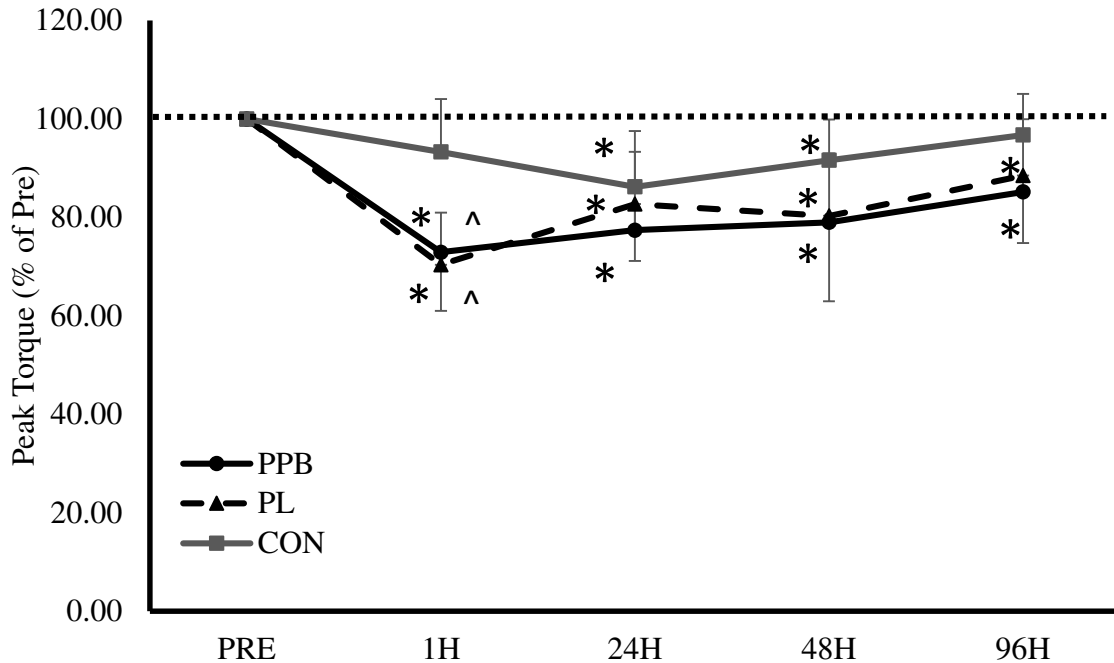
Figure 10: Maximal Voluntary Isometric Torque following Resistance Exercise.
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for
changes in maximal voluntary isometric (A) peak torque and (B) rate of torque
development. Changes are assessed as percent difference from pre exercise to
one- (1H), 24- (24H), 48- (48H), and 96- (96H) hours post exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)

Circulating Cytokines

Changes in circulating MCP-1 in response to resistance exercise are depicted in

Figure 11. A significant group x time interaction was observed between groups (F = 3.17,

$p = 0.003$, $\eta^2 = 0.150$). Significant differences were noted at 5H between CON and both

PPB ($p < 0.001$) and PL ($p = 0.006$). Additionally, a trend ($p = 0.081$) for a difference was

also noted between CON and PPB at 1H. Furthermore, a significant interaction between

groups was observed for AUC of MCP-1 (F = 4.338, $p = 0.021$) (Figure 12). AUC for the

MCP-1 response was significantly greater ($p = 0.006$) in PPB than CON, and trended toward a difference ($p = 0.093$) when compared to PL.
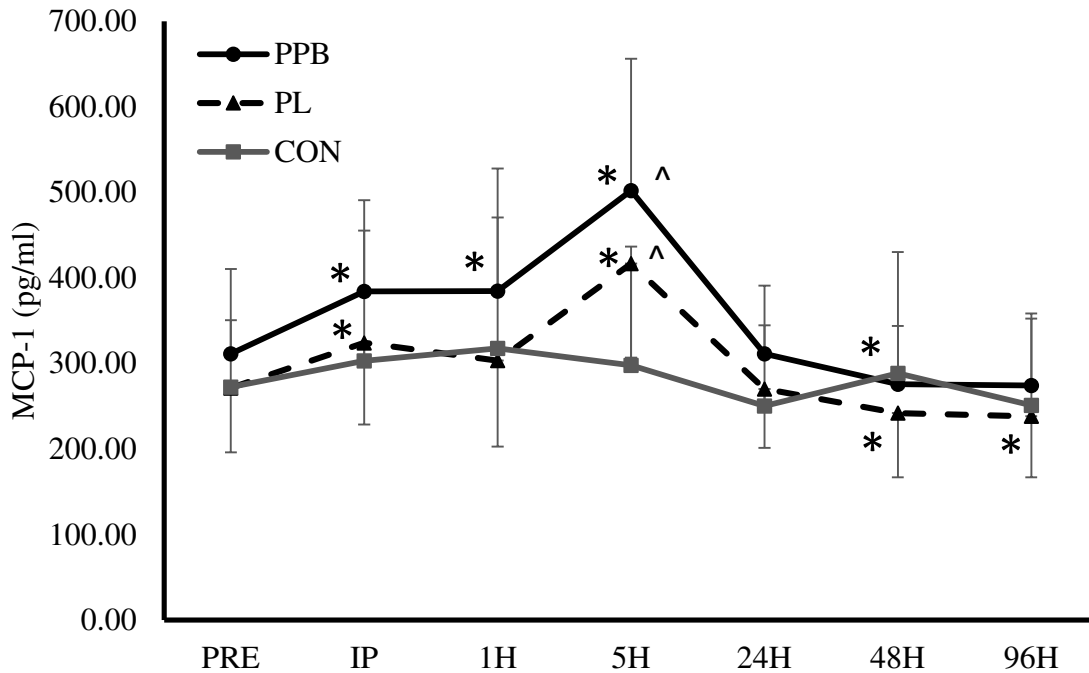
Figure 11: Monocyte Chemoattractant protein-1 (MCP-1) Response to Resistance Exercise.
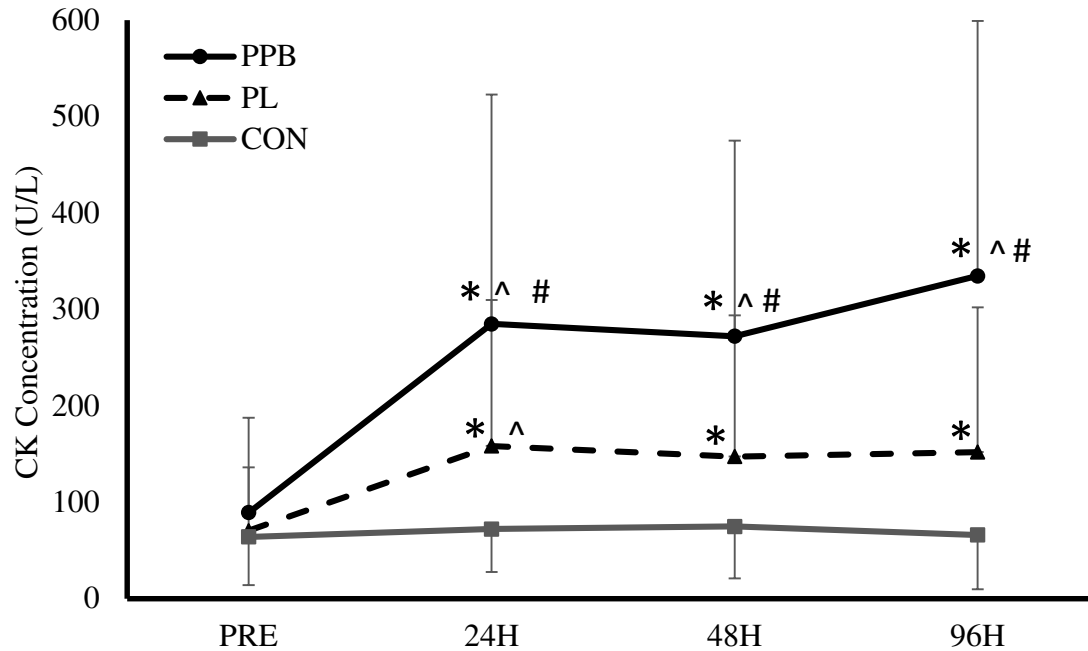Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for changes in MCP-1 pre exercise (PRE), as well as immediately (IP), one- (1H), five- (5H), 24- (24H), 48- (48H) and 96- (96H) hours post exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)
# Significantly different than corresponding value for PL ($p < 0.05$)
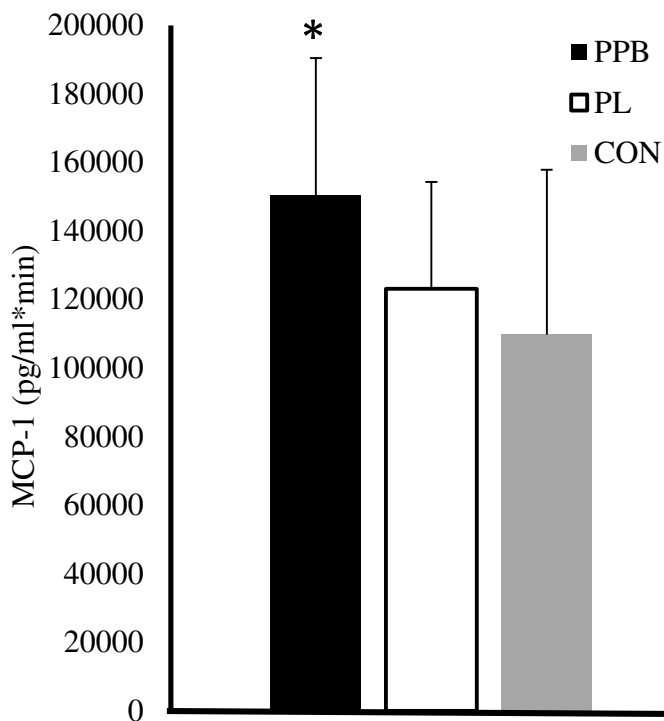
Figure 12: Monocyte Chemoattractant protein-1 (MCP-1) Area under the Curve Response to Resistance Exercise.
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for Area under the curve comparison between PPB, PL and CON.
* Significantly different than CON ($p < 0.05$)

No significant group x time interaction was observed for CX3CL1 (F = 1.081, p = 0.380, η^2 = 0.060), however a significant time effect was observed (F = 16.118, $p < 0.001$, η^2 = 0.322). When collapsed across groups, pairwise comparisons indicated significant elevations from PRE (149.1 ± 84.9 pg/ml) at IP (184.7 ± 70.9 pg/ml; $p < 0.001$) and 1H (171.9 ± 73.8 pg/ml; p = 0.002), and suppression at 48H (130.5 ± 66.4 pg/ml; p = 0.025). In addition, elevations at IP was significantly higher than 5H (165.2 ± 107.8 pg/ml; p = 0.007), 24H (138.9 ± 74.3 pg/ml; $p < 0.001$), 48H ($p < 0.001$) and 96H ($p < 0.001$), while 1H and 5H were significantly elevated over 24H ($p < 0.001$, $p < 0.001$, respectively), 48H

76

($p < 0.001$, $p < 0.001$, respectively) and 96H ($p < 0.001$, $p = 0.011$, respectively). Furthermore, no significant interaction was observed for the CX3CL1 AUC response between groups (F = 0.0859, $p = 0.433$).

No significant group x time interactions were observed for changes in IL-1β (F = 0.47, $p = 0.849$, $\eta^2 = 0.027$), however a significant time effect was observed (F = 17.57, $p < 0.001$, $\eta^2 = 0.341$). Pairwise comparisons indicated that when collapsed across groups, significant elevations (p's < 0.001) from PRE (4.23 ± 2.34 pg/ml) were observed at IP (7.55 ± 5.77 pg/ml) and 1H (6.67 ± 4.89 pg/ml), 5H (4.79 ± 3.52 pg/ml), 24H (4.19 ± 2.91 pg/ml), 48H (3.97 ± 3.34 pg/ml) and 96H (3.71 ± 2.39 pg/ml). When compared to 5H, IL-1β concentrations trended to be lower at 48H ($p = 0.08$) but significantly lower at 96H ($p = 0.015$). No significant differences were observed in the IL-1β AUC response between groups (F = 0.101; $p = 0.904$).

No significant group x time interaction was observed for IL-6 (F = 1.018, $p = 0.422$, $\eta^2 = 0.055$), however a significant time effect was observed (F = 4.801, $p = 0.002$, $\eta^2 = 0.121$). When groups were collapsed, pairwise comparisons indicated a significant increase in IL-6 concentration from PRE (33.2 ± 42.9 pg/ml) at IP (35.2 ± 44.7 pg/ml; $p = 0.044$), 1H (37.7 ± 48.7 pg/ml; $p = 0.004$) and 5H (42.5 ± 51.4 pg/ml; $p = 0.002$). Additionally, 5H was significantly elevated compared to 24H (38.4 ± 49.8 pg/ml; $p = 0.001$), 48H (37.1 ± 50.1 pg/ml; $p = 0.003$) and 96H (28.0 ± 31.6 pg/ml; $p = 0.010$). Furthermore, no significant differences were observed in the IL-6 AUC response between the groups (F = 0.815, $p = 0.451$).

No significant group x time interaction (F = 0.801, $p = 0.532$, $\eta^2 = 0.044$) was observed for IL-10, however, a significant main effect of time was observed (F = 5.608, p

= 0.005, η^2 = 0.138). Pairwise comparisons indicated that when groups were collapsed,

IL-10 concentrations were significantly elevated at 1H (21.09 ± 15.16 pg/ml) compared to

PRE (12.84 ± 14.67 pg/ml, p < 0.001), IP (16.80 ± 14.93 pg/ml; p = 0.009), 5H (14.45 ±

19.41 pg/ml; p = 0.012), 24H (13.15 ± 14.79 pg/ml; p < 0.001), 48H (13.08 ± 15.38 pg/ml;

p < 0.001), and 96H (11.39 ± 11.24; p < 0.001). In addition, IL-10 concentrations at IP

were significantly higher than PRE (p = 0.001), 24H (p = 0.008), 48H (p = 0.018) and 96H

(p = 0.002). The IL-10 AUC response between groups was not different (F = 2.178, p =

0.128).

No significant group x time interaction (F = 0.876, p = 0.534, η^2 = 0.052) was

observed for G-CSF, however a significant main effect for time was observed (F = 7.415,

p < 0.001, η^2 = 0.188). When groups were collapsed, pairwise comparisons indicated

significant elevations from PRE (72.3 ± 37.0 pg/ml) at IP (92.2 ± 55.6; p < 0.001), 1H

(84.9 ± 40.7 pg/ml; p = 0.011) and 5H (80.8 ± 36.3 pg/ml; p = 0.025). Additionally, IP,

1H and 5H were significantly greater than 24H (71.3 ± 40.5 pg/ml; p = 0.001, p = 0.006, p

= 0.001, respectively), 48H (70.6 ± 49.1 pg/ml; p = 0.003, p = 0.004, p = 0.001,

respectively) and 96H (65.8 ± 28.3 pg/ml; p = 0.001, p = 0.001, p < 0.001, respectively).

No significant between group differences were observed for the G-CSF AUC response (F

= 0.146, p = 0.732).

No significant group x time interactions were observed for GM-CSF (F = 0.501, p

= 0.863, η^2 = 0.028), however, a significant main effect for time was observed (F = 8.238,

p < 0.001, η^2 = 0.192). When groups were collapsed, pairwise comparisons indicated a

significant elevation from PRE (54.99 ± 51.38 pg/ml) at IP (75.36 ± 94.35 pg/ml, p < 0.001)

and 1H (73.31 ± 98.20 pg/ml, p = 0.007). GM-CSF was also significantly greater at IP and

1H than 5H (68.40 ± 87.97 pg/ml; $p = 0.013$, $p = 0.049$, respectively), 24H (61.48 ± 78.97 pg/ml; $p < 0.001$, $p = 0.001$, respectively), 48H (59.86 ± 70.52 pg/ml; $p < 0.001$, $p = 0.001$, respectively) and 96H (52.56 ± 62.91 pg/ml; $p < 0.001$, $p < 0.001$, respectively). In addition, GM-CSF concentrations at 5H were significantly greater than 48H ($p = 0.049$) and 96H ($p = 0.040$). No significant differences were noted between the groups in the GM-CSF AUC response (F = 0.315, $p = 0.732$).

No significant group x time interactions (F = 1.169, $p = 0.321$, $\eta^2 = 0.063$ and F = 0.385, $p = 0.855$, $\eta^2 = 0.022$), were observed for changes in IL-8 and INF-γ concentrations, respectively. In addition, no differences were noted in the AUC response (F = 0.122, $p = 0.885$, and F = 0.365, $p = 0.697$, respectively) for these cytokine markers as well.

Plasma Volume Shifts

A significant group x time interaction was observed for changes in plasma volume (F = 10.94, $p < 0.001$, $\eta^2 = 0.378$). Changes in plasma volume at IP were significantly less in CON (-1.4 ± 4.6%) compared to PPB (-17.7 ± 5.8%; $p < 0.001$) and PL (-15.1 ± 4.3%; $p < 0.001$). Furthermore, PL (0.7 ± 3.7%) was significantly greater than PPB (-4.0 ± 7.3%; $p = 0.037$) and CON (-4.5 ± 5.8%; $p = 0.028$). Circulating markers were not corrected for changes in plasma volume.

Leukocyte Population Distributions

Changes in the proportion of granulocytes in circulation in response to resistance exercise are depicted in Figure 13. A significant group x time interaction was observed in the proportion of circulating granulocytes ($F = 5.150$, $p < 0.001$, $\eta^2 = 0.233$). Granulocyte proportions at IP were significantly higher in CON compared to PPB ($p = 0.004$). Furthermore, a trend towards a difference was noted at IP between CON and PL ($p = 0.073$). Additionally, the proportion of granulocytes in CON at 5H was significantly lower compared to PPB ($p = 0.022$) and PL ($p = 0.013$). Granulocyte proportion for PL was significantly greater at 48H than CON ($p = 0.026$) or PPB ($p = 0.022$).

Figure 13: Circulating Granulocyte Proportions following Resistance Exercise.
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for changes in the circulating granulocyte proportions pre exercise (PRE), as well as immediately (IP), one- (1H), five- (5H), 24- (24H), and 48- (48H) hours post exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)
# Significantly different than corresponding value for PL ($p < 0.05$)

Changes in monocyte subset distributions in response to resistance exercise are depicted in Figure 14. A significant group x time interaction was observed for the proportion of classical monocytes of the total monocyte population (F = 0.9552, $p < 0.001$, $\eta^2 = 0.381$). The proportion of classical monocytes were significantly greater in CON at IP compared to PPB ($p = 0.008$) and PL ($p = 0.003$). Furthermore, classical monocytes were significantly reduced in CON at 1H compared to PPB ($p = 0.002$) and PL ($p = 0.006$).

A significant group x time interaction was observed for the proportion of intermediate monocytes of the total monocyte population (F = 7.765, $p < 0.001$, $\eta^2 = 0.334$).

The proportion of intermediate monocytes were significantly lower in CON at IP compared to PPB ($p = 0.034$) and PL ($p = 0.001$). Furthermore, this response was reversed at 1H with significantly greater proportions of intermediate monocytes in CON compared to PPB ($p = 0.003$) and PL ($p = 0.008$). At 24H, the proportion of intermediate monocytes in CON were significantly lower compared to PPB ($p = 0.016$), and trended towards difference in PL ($p = 0.094$). At 48H, the proportion of intermediate monocytes in CON were significantly lower compared to PPB ($p = 0.007$) and PL, and PL trended to be lower than PPB ($p = 0.079$).

A significant group x time interaction was also observed for the proportion of nonclassical monocytes of the total monocyte population (F $= 6.543$, $p < 0.001$, $\eta^2 = 0.297$). The proportion of intermediate monocytes at IP was significantly lower in CON compared to PPB ($p = 0.020$) and PL ($p = 0.028$).

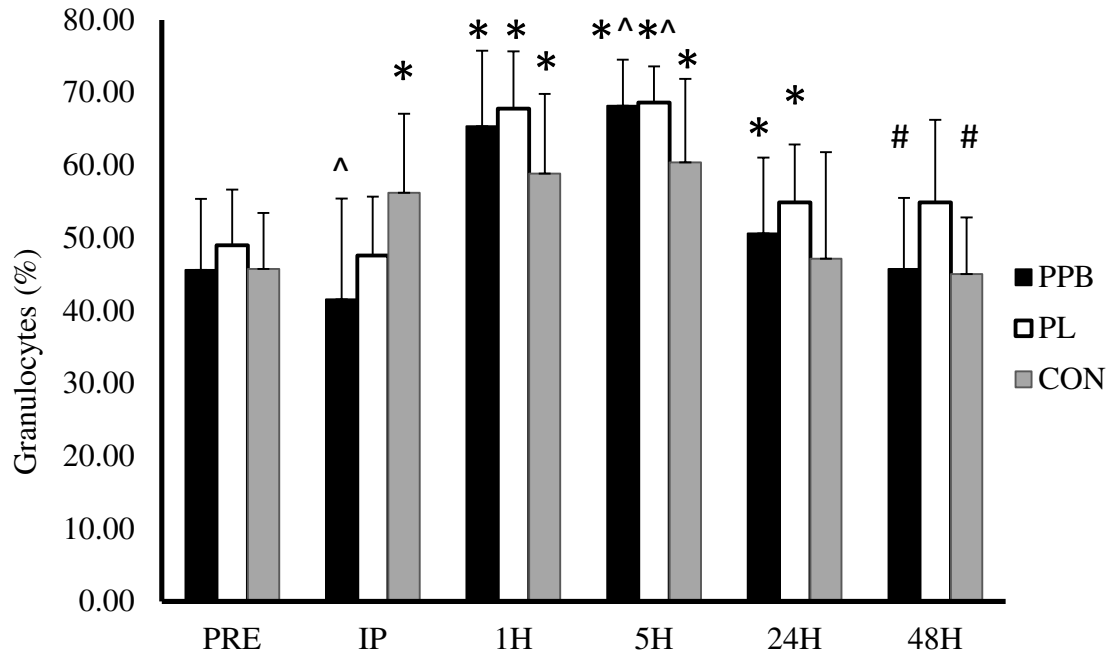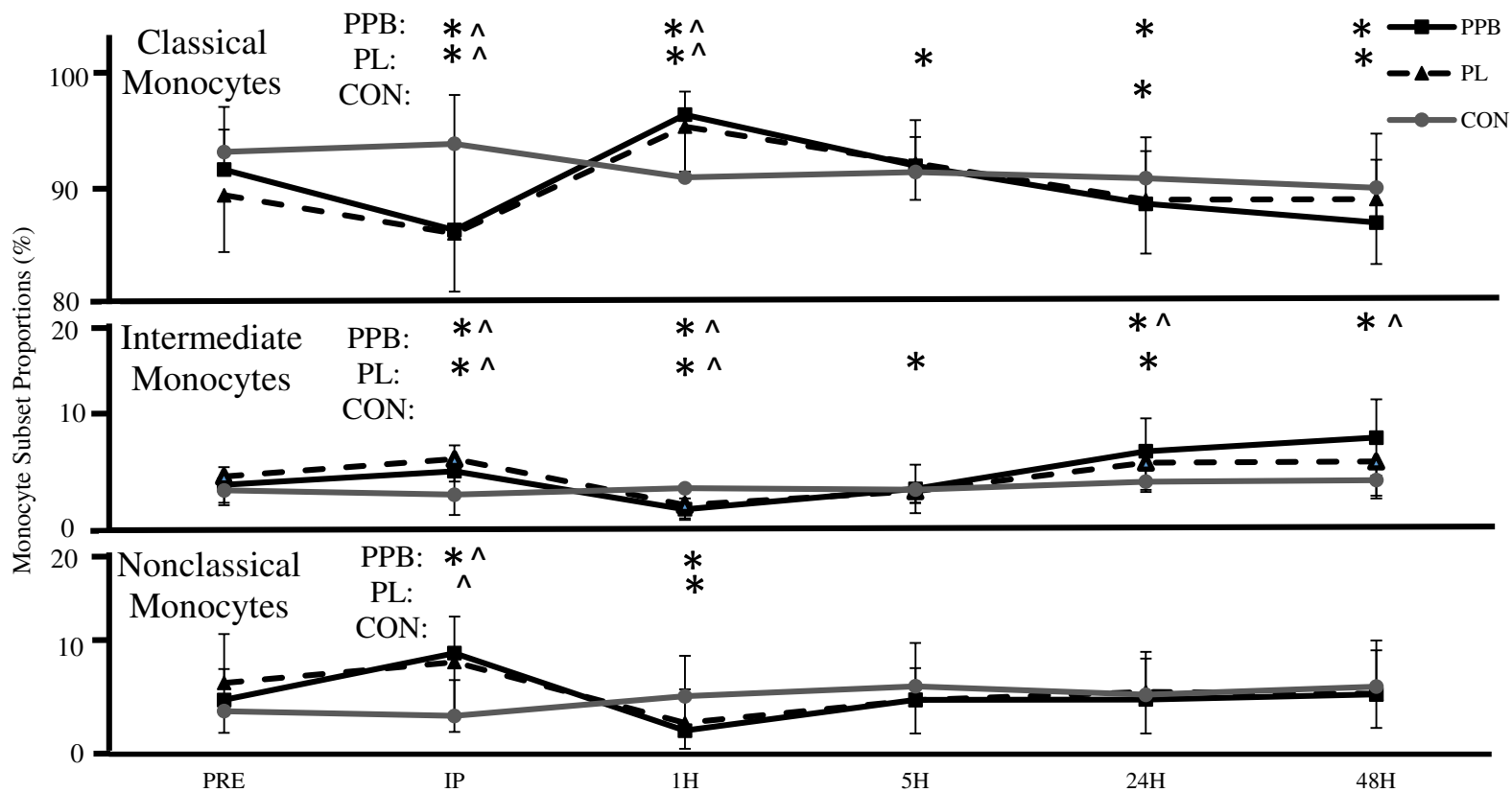**Figure 14: Monocyte Subtype Proportions following Resistance Exercise.**
Supplement (PPB), placebo (PL) and control (CON) groups were analyzed for changes in the monocyte subtype proportions pre exercise (PRE), as well as immediately (IP), one- (1H), five- (5H), 24- (24H), and 48- (48H) hours post exercise.
* Significantly different than corresponding value for PRE ($p < 0.05$)
^ Significantly different than corresponding value for CON ($p < 0.05$)

Leukocyte Subtype Activation

Significant differences were observed between groups at PRE for CD11b expression on granulocytes (F = 5.334, p = 0.010), intermediate monocytes (F = 3.594, p = 0.039) and nonclassical monocytes (F = 7.942, p = 0.002). As such, analyses between groups for expression of CD11b on all leukocyte subsets were analyzed as the percent of resting values (PRE set at 100%).

No significant group x time interaction was observed for the percent change in CD11b expression on granulocytes (F = 1.022, p = 0.421, η^2 = 0.057), however a significant main effect for time was observed (F = 12.059, p < 0.001, η^2 = 0.262). When collapsed across groups , pairwise comparisons indicated that the change in expression of CD11b on granulocytes from PRE to IP (121.6 ± 30.1%; p < 0.001) and 1H (110.5 ± 24.9%; p = 0.015) were significant, while a trend for a significant change in CD11b on granulocytes from PRE to 24H (106.9 ± 23.6%; p = 0.086) was also observed. Additionally, the change from PRE to IP was significantly greater than the change from PRE to 1H (p = 0.001), 5H (99.5 ± 24.3%; p < 0.001), 24H (p = 0.001) and 48H (96.1 ± 19.9%; p < 0.001). Furthermore, the change in CD11b expression on granulocytes from PRE to 1H was significantly greater than the change from PRE to 5H (p = 0.016) and 48H (p = 0.001).

No significant group x time interaction was observed for the percent change in expression of CD11b on classical monocytes (F = 1.788, p = 0.111, η^2 = 0.101), however a significant main effect time was observed (F = 9.663, p < 0.001, η^2 = 0.232). When collapsed across groups, pairwise comparisons indicated that the change from PRE to 1H (130.7 ± 29.8%), 5H (111.2 ± 28.8%), and 24H (113.5 ± 25.9%) were significant (p <

0.001, $p = 0.033$, $p = 0.004$, respectively). Additionally, the change of CD11b expression from PRE to 1H was significantly greater than the change from PRE to IP ($109.4 \pm 33.4\%$; $p < 0.001$), 5H ($p = 0.004$), 24H ($p < 0.001$) and 48H ($102.3 \pm 20.7\%$; $p < 0.001$), while the change from PRE to 24H was significantly greater than the change from PRE to 48H ($p = 0.002$).

No significant group x time interaction was observed for the percent change of CD11b expression on intermediate monocytes (F = 1.859, $p = 0.083$, $\eta^2 = 0.104$), however a significant main effect of time was observed (F = 5.667, $p = 0.001$, $\eta^2 = 0.150$). When collapsed across groups, pairwise comparisons revealed the change from PRE to 1H ($116.6 \pm 20.7\%$) was significant ($p < 0.001$), while the change from PRE to 24H trended toward significance ($105.8 \pm 18.3\%$; $p = 0.070$). Additionally, the change from PRE to 1H was significantly greater than the change from PRE to IP ($104.9 \pm 26.55\%$; $p = 0.003$), 5H ($102.0 \pm 23.0\%$; $p = 0.001$), 24H ($p = 0.001$) and 48H ($100.5 \pm 15.9\%$; $p < 0.001$). Additionally, the change in expression of CD11b at from PRE to 24H was significantly greater than the change from PRE to 48H ($p = 0.029$).

No significant group x time interaction (F = 1.917, $p = 0.084$, $\eta^2 = 0.107$), nor main effect of time (F = 2.099, $p = 0.104$, $\eta^2 = 0.062$) was observed for the percent change of CD11b expression on nonclassical monocytes; however, a significant main effect for group (F = 4.409, $p = 0.020$, $\eta^2 = 0.216$) was observed. Pairwise comparisons indicated that the average percent change from PRE to all time points in CD11b expression on nonclassical monocytes was lower in CON ($89.1 \pm 13.4\%$) compared to PPB (106.0 ± 13.8; $p = 0.006$), while no differences were observed between CON or PPB and PL ($97.6 \pm 12.3\%$; $p = 0.0121$, $p = 0.106$, respectively).

Correlations

Significant correlations were observed between markers of muscle damage and changes in CD11b expression on leukocyte subsets. CK concentrations at 24H were significantly correlated with the change in CD11b expression on intermediate monocytes at IP ($r = 0.439$, $p = 0.001$), and nonclassical monocytes at IP ($r = 0.413$, $p = 0.017$), 1H ($r = 0.392$, $p = 0.024$) and 5H ($r = 0.374$, $p = 0.032$). Furthermore, 48H CK concentrations were significantly correlated with the change in CD11b expression at IP on granulocytes ($r = 0.340$, $p = 0.045$), classical monocytes ($r = 0.365$, $p = 0.037$), and intermediate monocytes ($r = 0.452$, $p = 0.008$). Additionally, the change in CD11b expression on nonclassical monocytes was correlated at IP ($r = 0.450$, $p = 0.009$), 1H ($r = 0.402$, $p = 0.020$), 5H ($r = 0.345$, $p = 0.049$), 24H ($r = 0.446$, $p = 0.009$) and 48H ($r = 0.495$, $p = 0.003$). No correlations were observed between the change of CD11b expression on leukocytes and myoglobin AUC. Correlations with leukocyte subset proportions are displayed in Table 4.

Table 4: Leukocyte Subset Correlations with Markers of Muscle Damage

		Myoglobin AUC				24H CK Concentration				48H CK Concentration			
		GRAN	CLASS	INTER	NC	GRAN	CLASS	INTER	NC	GRAN	CLASS	INTER	NC
IP	r	-0.515	-0.664	-	0.775	-0.635	-0.692	-	0.719	-0.565	-0.583	0.399	0.555
	p	0.001	<0.001		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	0.024	0.001
1H	r	-0.374	-0.359	-	0.535	-	-	-	-	-	-	-	-
	p	0.025	0.040		0.001								
5H	r	-	-0.548	-	0.568	-	-0.361	-	-	-	-	-	-
	p		0.001		0.001		0.042						
24H	r	-	-0.543	-	0.609	-	-0.466	-	0.434	-	-0.434	0.380	-
	p		0.001		<0.001		0.007		0.013		0.013	0.032	
48H	r	-0.408	-0.640	0.412	0.631	-	-0.565	0.452	0.471	-	-0.457	0.387	0.361
	p	0.013	<0.001	0.017	<0.001		0.001	0.009	0.006		0.009	0.029	0.042

CHAPTER 5: DISCUSSION

The results of this study demonstrated significant alterations in the monocyte proportions following acute resistance exercise. Exercise produced significant mobilization of intermediate and nonclassical monocyte subtypes immediately following resistance exercise, followed by a supercompensation of the classical subset. As expected, resistance exercise stimulated an increase of MCP-1 concentrations immediately following exercise, but our results indicated a biphasic response occurring at 5H. Furthermore, CD11b expression was elevated on granulocytes immediately following resistance exercise, and remained elevated for one hour. Classical and intermediate monocytes increased expression of CD11b 1-hr following exercise, while only classical monocytes maintained this elevated expression for 24-hr. Our results also suggest that polyphenol supplementation enhanced the CD11b response to exercise on nonclassical monocytes. Lower resting expressions of CD11b on intermediate and nonclassical monocytes in PBB compared to PL and CON suggest that supplementation may have suppressed this immune marker. Markers of muscle damage, resulting from the exercise protocol, were greater with polyphenol supplementation, and moderately correlated to leukocyte subset distributions, as well as to CD11b expression following exercise ($r = 0.359$ to 0.775).

Skeletal muscle damage produces a potent immune response, characterized by an increased accumulation of phagocytic cells within the damaged tissue (Tidball & Villalta, 2010). MCP-1 plays an integral role in the acute immune response by serving as the primary chemoattractant for classical monocytes (Wong et al., 2012; Yadav et al., 2010). Our results indicated that resistance exercise may mediate a biphasic response of MCP-1

to resistance exercise, characterized by an initial increase immediately following exercise, and a second, larger increase at 5H.

To the best of our knowledge, only two investigations have examined the acute response of circulating MCP-1 following dynamic resistance exercise (Ihalainen et al., 2014; Wells et al., 2016). The immediate increase of MCP-1 observed in this study is in contrast to the decrease in MCP-1 concentration reported 30-min following resistance exercise in untrained men (Ihalainen et al., 2014). However, it is similar to the immediate increase reported following a lower body resistance exercise protocol in resistance trained men (Wells et al., 2016). While Ihalainen et al. (2014) utilized a similar population as this study (e.g., previously untrained men), the exercise stimulus used multiple sets of a single leg press exercise only. In contrast, this study required participants to exercise with multiple lower body exercises, similar to Wells and colleagues (2016). The greater volume of repetitions performed in this study (140 total repetitions compared to 50 in the Ihlalainen et al study) suggest that the immediate increase in MCP-1 following resistance exercise may be driven by volume of exercise, and may be independent of training status. The biphasic response observed though, was absent from any previous study examining the MCP-1 response to resistance exercise (Ihalainen et al., 2014; Wells et al., 2016). However, investigations examining exercise interventions designed to elicit muscle damage have observed the secondary response approximately 5-hr into recovery (Crystal et al., 2013; Paulsen et al., 2005). Furthermore, the participants used in those studies were moderately trained (Crystal et al., 2013; Paulsen et al., 2005), and comparable to the participants used in this study. Therefore, the biphasic response of MCP-1 may also be a function of both volume in conjunction with unaccustomed muscle action.

The recruitment of other monocyte subsets is governed primarily by CX3CL1 (Bazan et al., 1997). Circulating CX3CL1 concentrations increased at both IP and 1H for all groups combined. These results were similar to others that reported increases in circulating CX3CL1 concentrations for two hours following unilateral cycling (Catoire et al., 2014). These investigators also reported a significant increase in mRNA expression of CX3CL1 within skeletal muscle immediately after exercise. Significant increases of CX3CL1 mRNA has also been reported two hours post-resistance exercise (Della Gatta et al., 2014) Although Della Gatta and colleagues (2014) did not examine changes in CX3CL1 concentrations following exercise, they did indicate that mRNA expression returned to baseline levels at 4-hr post-exercise. CX3CL1 is synthesized by endothelial cells (Ludwig et al., 2002), and will remain bound to the endothelial surface unless cleaved in the presence of TNF-α and IL-1β (Turner et al., 2010). Interestingly, the results of this study indicated that IL-1β was elevated concomitantly with CX3CL1 at both IP and 1H. It is likely that elevations in circulating CX3CL1 concentrations were related to the elevations observed in IL-1β.

IL-8 is also a potent chemoattractant for phagocytic cells, namely neutrophils (Ribeiro et al., 1991). However, we saw no change in IL-8 concentrations in response to resistance exercise. This is in contrast with some investigators (Nieman et al., 2004), but is supported by others (Buford et al., 2009). Training status does not appear to have influenced these results, as most investigations utilized trained participants (Buford et al., 2009; Nieman et al., 2004; Ross et al., 2010). However, the volume of exercise used in this study (140 total repetitions) was less than that of Nieman and colleagues (2004) (four

sets of 10 repetitions in 10 different exercises), and may not have been sufficient to elicit a significant increase in IL-8 concentrations.

Expansion of the granulocytic population in this study occurred despite the absence of any change in IL-8 concentrations. This was a bit surprising considering IL-8 is a potent chemoattractant for neutrophils (Ribeiro et al., 1991). However, the expansion of neutrophils, in the absence of increases of IL-8 in circulation has been reported previously (Paulsen et al., 2005), indicating the IL-8 response may not be necessary for granulocytic expansion. In the present study, granulocytes appeared to make up a greater proportion of total leukocytes at 1H, 5H and 24H following exercise. Selective expansion of the granulocyte population is well documented following resistance exercise (Miles et al., 1998; Nieman et al., 2004; Ramel et al., 2003), and exercise designed to elicit muscle damage (Paulsen et al., 2005). Evidence from a muscle damaging study has demonstrated neutrophils to return to baseline concentrations 24-hr following exercise (Paulsen et al., 2005). Despite this, granulocytes are thought to increase immediately following exercise for approximately two hours following resistance exercise (Freidenreich & Volek, 2012). Investigations examining resistance exercise typically do not report the overall granulocyte population following 24 hours of recovery in healthy participants (Miles et al., 1998; Nieman et al., 2004; Ramel et al., 2003). The expansion of the granulocyte population at 24H in this study, however, suggests that granulocytosis may be more prolonged following dynamic resistance exercise than previously reported.

Mobilization of monocytes following resistance exercise has also been demonstrated in conjunction with granulocyte expansion (Mayhew et al., 2005; Nieman et al., 2004; Ramel et al., 2003). The response of specific monocyte subtypes though, has yet

91

to be defined in regards to resistance exercise. In this study, we observed the mobilization of both intermediate and nonclassical monocytes immediately following exercise at the expense of the classical monocyte subset. While selective mobilization of the CD16+ subset may be induced by catecholamines (Kittner et al., 2002), the intensity of exercise also appears to drive this response (Steppich et al., 2000). Previous investigations examining the monocyte response to exercise under the three subset paradigm have demonstrated this mobilization to be primarily from the nonclassical subset (Booth et al., 2010; Radom-Aizik et al., 2014; Shantsila et al., 2012; Simpson et al., 2009). LaVoy and colleagues (2015) demonstrated an increased proportion of both intermediate and nonclassical monocytes following aerobic exercise in a mixed gender group of participants. Women though appear to have a different response of nonclassical monocytes to exercise than men (Heimbeck et al., 2010). Considering that participants in this study were men only, the increase in intermediate monocyte proportions observed immediately following exercise appears to be related to the resistance exercise protocol itself.

Increases of intermediate monocytes at 24H and 48H following exercise occurred at the expense of the classical monocyte subset, with no change in the nonclassical proportion. Furthermore, PPB demonstrated a significantly greater intermediate monocyte population than CON, with a trend compared to PL ($p = 0.079$) at 48H. Although data from ischemic tissue damage has demonstrated significant elevations in intermediate monocytes 24 and 48 hours following injury (Tapp, Shantsila, Wrigley, Pamukcu, & Lip, 2012; Urra et al., 2009), there does not appear to be any previous investigation that has examined monocyte subsets in response to exercise 24 or 48 hours into recovery. Tapp and colleagues (2012) have also reported that changes in intermediate monocytes were

92

associated with the extent of tissue damage. Consequently, the increased intermediate monocyte proportion in PPB at 48H was likely a result of greater muscle damage. Furthermore, the significant correlations observed in this study (r = 0.380 to 0.452) between markers of muscle damage and the intermediate monocyte proportion at 24H and 48H support the potential relationship between tissue damage and intermediate monocyte mobilization.

The coupling of CD11b with CD18 to form MAC1/CR3, and its involvement within the transendothelial migration process (Tan, 2012) makes CD11b a key regulator of phagocyte migration to damaged tissue (Ley et al., 2007). Granulocytes migrate to damaged tissue within the first hours following exercise, monocytes tend to migrate later (Malm et al., 2000; Paulsen et al., 2010). The increased CD11b expression on granulocytes at IP and 1H, followed by classical monocytes at 1H, 5H and 24H observed in this study is consistent with the temporal appearance of these cells within damaged tissue. While the reported time course of CD11b expression on monocytes has been more consistent than on granulocytes, little consensus as to the time course of the granulocyte expression of CD11b has been developed (J. Peake et al., 2005).

Investigations utilizing aerobic exercise (Jordan et al., 1999; van Eeden et al., 1999) and exercise designed to elicit muscle damage (J. M. Peake, K. Suzuki, G. Wilson, et al., 2005; Pizza et al., 1996; Saxton et al., 2003) have previously examined CD11b expression on granulocytes. However, no studies have examined the impact of dynamic resistance exercise on CD11b expression on granulocytes. Our results indicate that resistance exercise can stimulate increases in CD11b expression on granulocytes at IP and 1H. These results are similar to previous investigations examining endurance exercise protocols.

Various studies have reported significant increases immediately following exercise (Gabriel & Kindermann, 1998; Jordan et al., 1999; van Eeden et al., 1999), which may persist for up to 30-min post-exercise (van Eeden et al., 1999). However, our results appear to differ in comparison to studies using eccentric exercise protocols. Following downhill running, Peake and colleagues (2005) reported no changes in CD11b expression during the recovery period in well-trained men. Similarly, studies examining 50 eccentric contractions of the quadriceps (Saxton et al., 2003) or 25 eccentric contractions of the elbow flexors (Pizza et al., 1996) in moderately trained men, reported no change in CD11b expression up to 24 hours post-exercise. However, these latter studies first measured CD11b expression at 4- and 1.5-hr, respectively, post-exercise. We observed an increase in the expression of CD11b on granulocytes at IP and 1H, which was prior to any of the measurements assessed by Saxton et al. (2003) or Pizza et al. (1996). Pizza and colleagues (1996) though, did demonstrate a significant increase in CD11b expression 24 hours following their damaging protocol. The results of this study provides some support as we indicated a trend for an increased expression at 24H ($p = 0.086$).

Expression of CD11b on monocytes has been more extensively examined than granulocytes. Previous reports have demonstrated elevated CD11b expression on classical monocytes immediately and 1-hr post-exercise (Wells, et al. 2014). Other investigations have reported no increase immediately, but significant increases 30-min following a similar exercise stimulus in trained men (Gonzalez et al., 2014; Jajtner et al., 2014). The disparity in the time course reported between the studies may be due to the monocyte population examined. Wells and colleagues (2016) analyzed classical monocytes only (CD14++/CD16-), while Jajtner et al. (2014) and Gonzalez et al. (2014) utilized CD14++

monocytes without differentiating between classical and intermediate monocytes. Consequently, Wells et al. (2016) suggested that the response of CD14++ monocytes may have been influenced by intermediate monocytes, contributing to the lack of any change immediately post-exercise (Gonzalez et al., 2014; Jajtner et al., 2014). The delayed increase of CD11b expression on intermediate monocytes in this study supports this suggestion. However, the delayed expression of CD11b on classical monocytes observed from 1H to 24H contrasts with Wells et al. (2016). Training status of the participants may explain the different results, as this study used untrained participants while Wells et al. (2016) investigated resistance-trained individuals. This study also observed a significant increase in CD11b expression on classical monocytes at 24H. Previous investigations have only examined CD11b expression on CD14++ following resistance exercise in trained men (Gonzalez et al., 2014; Jajtner et al., 2014). This appears to be the first study to examine CD11b expression on monocyte subsets 24 and 48H following exercise.

Polyphenol supplementation (PPB) for 28 days also appeared to reduce CD11b expression on intermediate and nonclassical monocytes at rest compared to CON and PL. CD11b expression on monocytes was not examined prior to the onset of supplementation, therefore we can only speculate in relating this decreased expression of CD11b to polyphenol supplementation. However, there does appear to be support for this from an *in vitro* model, which demonstrated a significant downregulation of CD11b, as well as reduced chemotaxis and adherence in response to incubation with polyphenols (Kawai et al., 2004; Takano et al., 2004). However, others have suggested that decreases in CD11b expression on monocytes may have beneficial health benefits, as polyphenol associated

95

decreases in CD11b expression on monocytes has been previously reported to have potential beneficial effects in cardiovascular disease (Chiva-Blanch et al., 2012).

In this study, no differences in the time course of CD11b expression were observed between groups for classical or intermediate monocytes. However, participants in PPB demonstrated a greater change in CD11b expression on nonclassical monocytes during 48-hr of recovery than CON. While the expression of CD11b on nonclassical monocytes is reduced compared to other subsets (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011), it is unclear why this difference occurred. Nonetheless, the role of CD11b in transendothelial migration (Ley et al., 2007), and the propensity of nonclassical monocytes to polarize to M2 macrophages (Auffray et al., 2007) may indicate no deleterious effects on recovery. Furthermore, it is unclear if reduced CD11b expression on classical and intermediate monocytes throughout the recovery process is detrimental to functional recovery. Therefore, polyphenol supplementation may serve as a potential treatment option to decrease adherence and chemotaxis of monocytes, without affecting the exercise response; however, further research is warranted.

Conclusions

The present study investigated the impact of an acute bout of resistance exercise in untrained participants on the redistribution of monocyte subsets, as well as the expression of adhesion molecules on the surface of phagocytic cells. Circulating MCP-1 concentrations demonstrated a biphasic response to resistance exercise, with an initial increase immediately following exercise, and a second pronounced increase five hours into

recovery. This appears to be the first study to report a sustained increase in the proportion of granulocytes in relation to total leukocyte population up to 24-hr post-resistance exercise. The monocyte subset response to resistance exercise was characterized by a mobilization of intermediate and nonclassical monocytes immediately following exercise, followed by a supercompensation of classical monocytes 1-hr post-exercise. This study also appears to be the first investigation to report a significant increase in the intermediate monocyte subset 24- and 48-hr following exercise. Polyphenol supplementation, possibly due to increased muscle damage, increased the proportion of intermediate monocytes 48H following exercise. The results of this study indicated that an acute bout of resistance exercise in untrained individuals elicits selective mobilization and adherence characteristics of phagocytic cells throughout recovery. Polyphenol supplementation appears to augment the resting expression of CD11b, however, does not affect the exercise response of classical and intermediate monocytes, or granulocytes.

APPENDIX A: UCF IRB APPROVAL LETTER

Notice that UCF will Rely Upon Other IRB for Review and Approval

From :   **UCF Institutional Review Board**
          FWA00000351, IRB00001138

To   :   **Jeffrey Ray Stout**

Date :   **January 21, 2015**

IRB Number: SBE-14-10770

Study Title: The Effect of XSurgeTM Supplementation on Biomarkers of Muscle Damage, Oxidative Stress, Immune Cell Modulation, Tissue Apoptotic Signaling and Recovery of Functional Performance from Acute High Intensity Resistance Exercise

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Designated Reviewer on 1/21/2015. The UCF IRB accepts the New England's Institutional Review Board review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England Institutional Review Board and the consent process will be the process approved by that IRB.**

This project may move forward as described in the protocol. It is understood that the New England's IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

Failure to provide a continuing review report for renewal of the study to the New England IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), an Office for Human Research Protections (OHRP) IRB Authorization form must be signed by the signatory officials of both institutions and a copy of the form must be kept on file at the IRB office of both institutions.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

IRB Coordinator

APPENDIX B: NEIRB APPROVAL LETTER

January 14, 2015

Jeffrey R. Stout, PhD
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Re: (IRB# 14-491): "The Effect of XSurge™ Supplementation on Biomarkers of Muscle Damage, Oxidative Stress,
        Immune Cell Modulation, Tissue Apoptotic Signaling and Recovery of Functional Performance from Acute High
        Intensity Resistance Exercise"

This is to inform you that New England Institutional Review Board (NEIRB)'s Tuesday Board has approved the above-
referenced research protocol and the participation of the above-referenced investigative site in the research. The approval
period is 1/14/2015 to 12/8/2015. **Your study number is 14-491. Please be sure to reference either this number or the
name of the principal investigator in any correspondence with NEIRB.**

Continued approval is conditional upon your compliance with the following requirements:
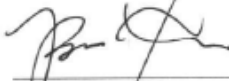
- A copy of the **Informed Consent Document, NEIRB Version 1.0**, approved as of 1/14/2015 is enclosed. Only NEIRB-
  approved informed consent documents should be used. It must be signed by each subject prior to initiation of any protocol
  procedures. In addition, each subject must be given a copy of the signed consent form.

- The following must be promptly reported to NEIRB: changes to the study site, and all unanticipated problems that may
  involve risks or affect the safety or welfare of subjects or others, or that may affect the integrity of the research.

- Approval is valid for enrollment of the number of subjects indicated on your submission form. If you anticipate enrolling
  more than this number of subjects, NEIRB approval must be obtained prior to exceeding the approved enrollment number.

- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until
  approved by the IRB except where necessary to eliminate apparent immediate hazards to the study subjects.

- Compliance with all federal and state laws pertaining to this research, and with NEIRB's SOPs.

- The enclosed subject materials (Medical and Activity History Questionnaire and "Par-Q and You" Questionnaire) have
  been approved. The enclosed subject materials (Flyer and Recruitment Script) have been conditionally approved. Please
  make the indicated revisions and re-submit it to NEIRB for final approval. Advertisements, letters, internet postings and
  any other media for subject recruitment must be submitted to NEIRB and approved prior to use. Please refer to *NEIRB
  Guidelines for Recruitment and Advertising*, available at www.neirb.com.

- All deaths, life-threatening problems or serious or unexpected adverse events, *whether related to the study article or not*,
  must be reported to the IRB. The Serious Adverse Event Form is available at www.neirb.com.

- Any and all necessary FDA approvals must be received prior to your initiation of the trial. If this study is being conducted
  under an IDE, a copy of the FDA IDE approval letter must be submitted to NEIRB.

- The study cannot continue after 12/8/2015 until re-approved by NEIRB. A Study Renewal Report must be completed and
  returned to NEIRB prior to the expiration of the approval period.

NEW ENGLAND INSTITUTIONAL REVIEW BOARD

- When the study is completed, terminated, or if it is not being renewed - complete and submit a Study Completion Report to NEIRB. The Study Completion Report can be accessed via the NEIRB website at www.neirb.com.

Beckett Flynn, BA
Administrator

Copy: NEIRB Chair
        Enclosures

APPENDIX C: RECRUITMENT FLYER

VOLUNTEERS NEEDED FOR RESEARCH STUDY

"THE EFFECT OF XSURGE™ SUPPLEMENTATION ON BIOMARKERS OF MUSCLE DAMAGE, OXIDATIVE STRESS, IMMUNE CELL MODULATION, TISSUE APOPTOTIC SIGNALING AND RECOVERY OF FUNCTIONAL PERFORMANCE FROM ACUTE HIGH INTENSITY RESISTANCE EXERCISE"

DESCRIPTION OF PROJECT:

We are investigating the effects of resistance exercise and training with or without antioxidant (XSurge™) supplementation on muscle damage, oxidative stress, immune function and functional performance

WHO IS ELIGIBLE?

- *Recreationally active, non-specifically trained males*
- *Free of any physical limitations*
- *Between the ages of 18 and 35*

WHAT WILL YOU BE ASKED TO DO?

Complete 2 Resistance Exercise Testing Sessions (5 visits each; separated by 6 weeks) or Complete 1 Resistance Exercise Testing Session with 4 days follow-up testing
Report to the Human Performance Lab for Personalized Resistance Exercise Training
Ingest a placebo or antioxidant supplement (depending on group) for 12 weeks

To learn more, please contact Adam Jajtner or Jeremy Townsend in the Human Performance Laboratory 407-823-2367, or e-mail at humanperformancelab@ucf.edu

104

APPENDIX D: RECRUITMENT SCRIPT

The Institute of Exercise Physiology and Wellness is recruiting participants for a research project investigating how the body responds to antioxidant supplementation and resistance training. Inclusion criteria consist of being a male between 18 and 35 years of age; recreationally active, but not specifically trained; and being free of any physical limitations and other nutritional supplements, medications, or performance enhancing drugs. Following enrollment in the study, you will be enrolled in the control, placebo or active supplementation group. If you are enrolled in the control group you will complete one acute resistance protocol with follow-up testing. If you are enrolled in the placebo or active supplementation group you will complete 30 days of supplementation, followed by a resistance exercise protocol with follow-up testing, three days per week for six weeks of resistance training, and another resistance exercise protocol with follow-up testing. During each resistance protocol, you will perform a lower-body resistance exercise protocol. Our highly qualified research team will collect blood samples and muscle biopsies at designated time points around the workout. If you are interested and would like to learn more about enrolling in this research study, please contact Adam Jajtner or Jeremy Townsend at adam.jajtner@knights.ucf.edu or jeremy.townsend@ucf.edu or 407-823-2367.

APPENDIX E: INFORMED CONSENT

University of Central Florida

The Effect of XSurge™ Supplementation on Biomarkers of Muscle Damage, Oxidative Stress, Immune Cell Modulation, Tissue Apoptotic Signaling and Recovery of Functional Performance from Acute High Intensity Resistance Exercise

Informed Consent

Principal Investigator(s):	Jeffrey R. Stout, Ph.D. (407) 823-2367
Sub-Investigator(s):	Jay R. Hoffman, Ph.D. Leonardo Oliveira, M.D. Adam R. Jajtner, M.S. Jeremy R. Townsend, M.S. Kyle S. Beyer, M.S.
Sponsor:	Kemin Industries Inc.
Study Site(s):	University of Central Florida College of Education Sport and Exercise Science

Introduction: Researchers at the University of Central Florida (UCF) study many topics. To do this we need the help of people who agree to take part in a research study. You are being asked to take part in a research study which is planned to include up to 50 male volunteers and be conducted at UCF. You have been asked to take part in this research study because you are a healthy individual between the ages of 18 and 35 years and complete 3 hours or less of exercise per week.

The investigator conducting the research is Dr. Jeffrey R. Stout.

Who is paying for the study?
A company called Kemin, the study sponsor, is paying for the study. Kemin is also paying the study Investigators to complete the study.

What should you know about this research study?
- Someone will explain this research study to you.
- Your participation is this research study is completely voluntary.
- Whether or not you take part is up to you.
- You should take part in this study only because you want to.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.
- You are not eligible to participate in this study if you are currently enrolled in another clinical investigation.

What is the purpose of the research study?
The purpose of this study is to examine the effect of consuming an antioxidant supplement and performing resistance training on the recovery of your muscles following resistance exercise. In addition, we hope to examine the impact of antioxidant consumption and resistance training on your immune system's response to resistance exercise.

What will you be asked to do in the study?

Screening Visit:

During the Screening Visit, the following will be done in the specified order:

- Informed Consent
- Protected Health Information Authorization Form (HIPAA)
  - Authorization to collect medical history information
- Physical Activity Readiness Questionnaire (PAR-Q)
  - Brief medical history
- Self-reported medical and activity history
  - In-depth questionnaire regarding your medical history
- Your age and race will be collected

During the screening visit, we will review the inclusion/exclusion criteria with you. We will also inform you of the ingredients in the supplement to assess any allergy or intolerance. Allergy or intolerance to any ingredient contained within the supplement or placebo will result in exclusion from the study.

If you are admitted to the study you will complete one of the following groups: 1. Control (CON), 2. Placebo (PL), and 3. XSurge™ (XS), and will be randomized to your group by a random number generator via Microsoft Excel. Participants that are assigned to CON will complete Acute Protocol 1 only, while participants that are assigned to PL or XS groups will complete both phases I and II (these are discussed in further detail below). Participation in this study requires that you are willing to participate in all groups (although you will only be assigned to one). Investigator expectations are the same for all participants, regardless of trial or group. All participants will be asked to complete the exact same protocol.
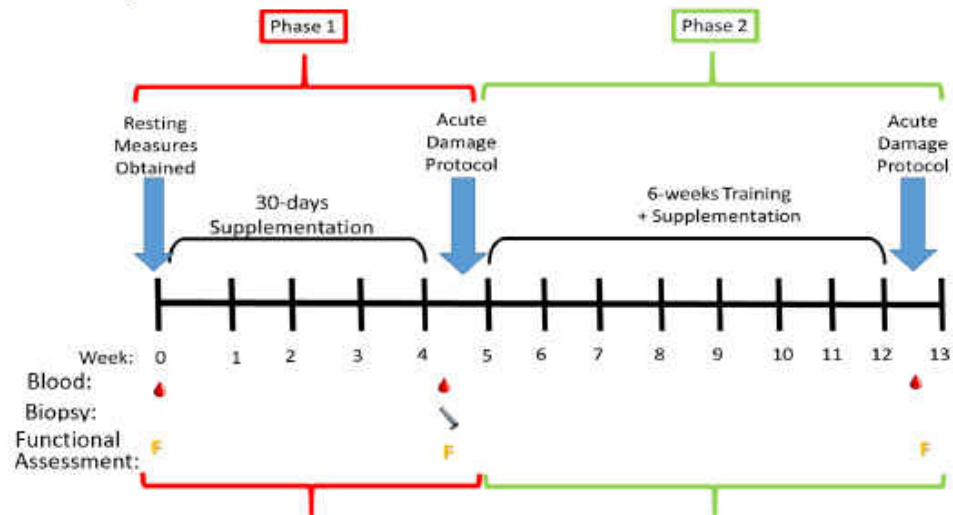
Figure 1.
Visits to the Human Performance Laboratory

- This study will be broken into two different phases: Phase 1 and Phase 2 (Figure 1).
    - Phase 1 will consist of Baseline testing, 30 days of supplementation and acute damage protocol one
    - Phase 2 will consist of six weeks of resistance training and acute damage protocol 2.
    - The CON group will only participate in the Acute Protocol portion of Phase 1 (Approx. 1 week).
- Phase 1
    - Baseline Testing (**XS and PL only**)
        - One Visit (approximately 2 hours)
        - Testing will consist of performance testing as well as supplying a blood sample. The performance testing, and the blood sample is discussed in further detail below.
    - Supplementation (30 days) (**XS and PL only**)
        - You will visit the lab five days per week, for a total of 20 visits (<5 minutes)

3 of 14

- On each visit to the lab, you will consume the first dose of your assigned supplement, and then be provided your second dose of the supplement, as well as every dose of the supplement until you return to the lab in individual containers (if you are not returning to the lab the next day; e.g. weekends). You will be asked to return the empty containers to the lab on your subsequent visit.
  - Acute Protocol 1 (Figure 2) **(ALL GROUPS)**
    - You will report to the Human Performance Lab on 5 occasions (D1 – D5)
    - During D1, you will complete 1-Repetition Maximums (1-RM) for the squat, leg press and leg extension exercises. D1 will be completed approximately 72-96 hours prior to D2. The 1-RM procedure is described in greater detail below.
    - During D2, you will arrive to the human performance lab:
      - In a 12 hr fasted state (without eating; excluding water)
      - Without consuming alcohol for 24 hours
      - Following at least 8 hours of sleep
      - Without exercising within the last 72 hours
    - On D2 you will be asked to complete:
      - Pre-exercise samples including:
        - Blood samples
        - Muscle biopsy
        - Height/weight
        - Performance testing
      - Standardized warm-up which will consist of:
        - Riding a stationary bicycle for 5 minutes against your preferred resistance
        - 10 body weight squats
        - 10 alternating lunges
        - 10 walking knee hugs
        - 10 walking butt kicks
      - Resistance exercise protocol consisting of:
        - 6 sets of the squat
        - 4 sets of leg press
        - 4 sets of leg extension exercises
        - The rest interval between each set will be 90 seconds, and perform 10 repetitions at 70% of your previously measured 1-RM.
      - You will remain in the lab for five hours following the resistance exercise for follow-up testing including
        - Blood draws
        - Muscle biopsies
        - Performance testing
    - During D3, D4 and D5 you will report to the lab in a fasted state (without eating; except water), in the morning. During each of these visits you will supply a blood sample, and complete performance testing. Additionally, on D4, you will supply a muscle sample (muscle biopsy).

- **CON group** will take part in Acute Protocol 1; however, you will not complete the exercise during D2. All other measures (blood and muscle samples, performance and strength testing, and) will be completed at the same corresponding times.
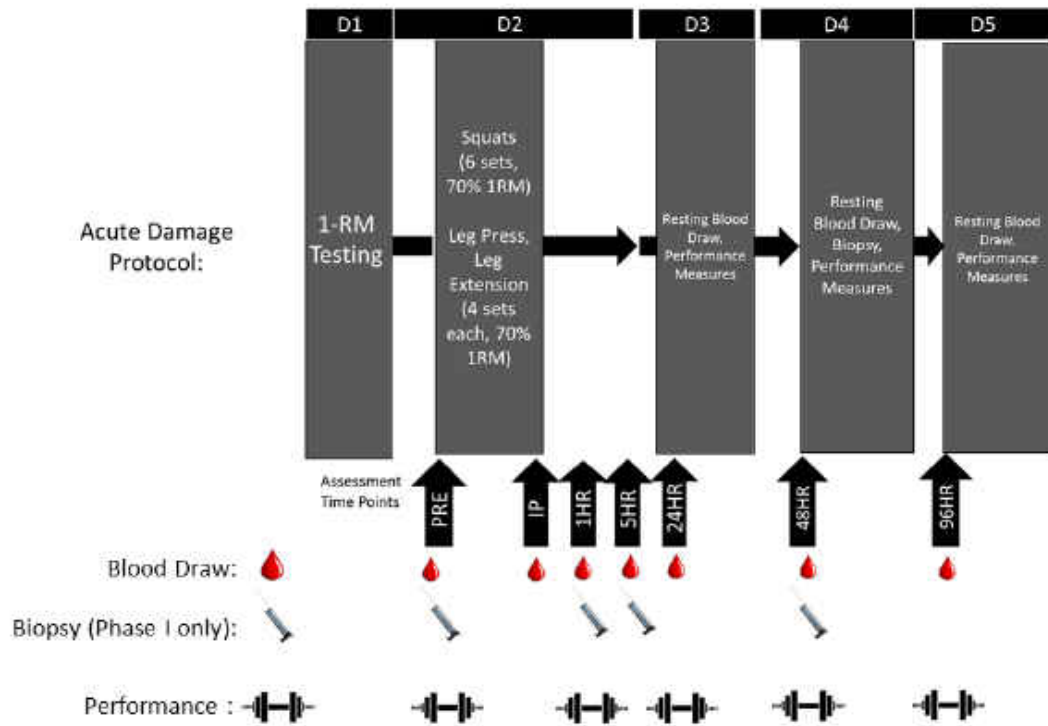
Figure 2.

- Phase 2 (XS and PL only)
  - Resistance Training (6 weeks)
    - You will report to the human performance lab for 17 resistance training sessions (3 sessions per week for 6 weeks). On the sixth week, there will only be two resistance training sessions. During each training session you will complete a standardized warm-up (the same as during the acute protocol). Additionally, you will consume your assigned supplement as described previously throughout the training period. A complete representation of the resistance training protocol is located in Table 1.
  - Acute Protocol 2 (Figure 2)
    - Acute Protocol 2 will be conducted in the same fashion as Acute Protocol 1, however, no muscle samples will be obtained. All other measures will be identical to Acute Protocol 2.

5 of 14

Day 1		Day 2		Day 3	
Smith Machine Squat	4 X 8-10	Leg Press	4 X 8-10	Smith Machine Squat	4 X 8-10
Bench Press	4 X 8-10	Dumbbell Step ups	3 X 8-10	Bench Press	4 X 8-10
Seated Dumbbell press	3 X 8-10	Incline bench press	4 X 8-10	Seated Dumbbell press	3 X 8-10
Low Row	3 X 8-10	Upright Rows	3 X 8-10	Low Row	3 X 8-10
Leg Extension	3 X 8-10	Lat Pulldown	3 X 8-10	Leg Extension	3 X 8-10
Leg Curl	3 x 8-10	Seated Calf Raise	3 x 8-10	Leg Curl	3 x 8-10

Table 1.

Supplementation

You will be asked to consume 2 capsules twice daily (total 4 capsules per day) throughout the study (roughly 12 weeks). You will be asked to report to the human performance lab each weekday for your morning dose of supplement, and then you will be provided with the supplement for your afternoon dose. You will be randomly assigned, to consume either a placebo capsule, the active antioxidant supplement (XSurge™) or no supplement (control). Neither the researchers of this study or you will know which supplement you have been assigned. The two types of supplement (PL or XS) are listed below. The supplement has been formulated under Good Manufacturing Practices. All product lots will be tested for toxins, including heavy metals, pesticides and excipients.

XSurge™ Dosage (per capsule)	XSurge™ (%)[1]	Excipient (%)[2]
500 mg	97	3
0 g (placebo)	0	100

[1] XSurge™ is a 100% water-extracted, granulated, free-flowing, dry powder polyphenolic blend from black tea (*Camellia sinensis*) and green tea (*Camellia sinensis*) containing at least 40% total polyphenols and 1.3% theaflavins.
[2] Avicel PH 105 (microcrystalline cellulose).

Nutrient	Amount per 1 g
Calories	3.65 cal
Calories from Fat	0.02 cal
Total Fat	0 g
Saturated Fat	0 g
Trans Fat	0 g
Cholesterol	<0.01 mg
Sodium	3.72 mg
Total Carbohydrates	0.62 g
Dietary Fiber	0.1 g
Sugars	0.01 g
Protein	0.28 g
Calcium	0.69 mg
Iron	0.07 mg

6 of 14

How long will you be in the study?

We expect that you will be enrolled in this research study for approximately thirteen (13) weeks. If you are enrolled in the control group you will only participate in one week testing. The 13 weeks will be broken into two phases, Phase one (~5 weeks) and Phase two (~8 weeks).

- CON Group
  - Acute Protocol I (~11 hours total; 1 week)
    - D1: ~ 1 hour
    - D2: ~ 7 hours
    - D3: ~ 1 hour
    - D4: ~ 1 hour
    - D5: ~ 1 hour
- Phase I (~13 hours total) (XS and PL Groups)
  - BL Testing (~ 1 hour total)
    - One visit; approximately 1 hour
  - Supplementation Period (~ 1 hour total)
    - 20 visits; less than 5 minutes each
    - This is over the course of 4 weeks
  - Acute Protocol I (~11 hours total; 1 week)
    - D1: ~ 1 hour
    - D2: ~ 7 hours
    - D3: ~ 1 hour
    - D4: ~ 1 hour
    - D5: ~ 1 hour
- Phase II (~45 hours total)
  - Training Protocol (~34 hours)
    - 17 training sessions (~2 hours each)
    - This is over the course of 6 weeks
  - Acute Protocol II (~11 hours; 1 week)
    - D1: ~ 1 hour
    - D2: ~ 7 hours
    - D3: ~ 1 hour
    - D4: ~ 1 hour
    - D5: ~ 1 hour
- Total time commitment: ~ 58 hours over the course of 13 weeks.

Measures:

All testing will be supervised and conducted in the HPL in the College of Education building at the University of Central Florida.

1. Strength Testing
   - You will complete a warm-up (the same warm-up as all exercise sessions), as well as two warm up sets of each exercise.
   - You will make attempts to lift the maximal amount weight in 3 exercises (squats, leg press, and leg extension), with 3-5 minutes rest between each set. All 1RM testing will be supervised by a Certified Strength and Conditioning Specialist.

2. Performance Testing
   - Maximal Voluntary Isometric Contraction (MVIC) and Electromyographic Measurements
     - An apparatus will be applied to your legs, similar to an adhesive bandage, to measure the electrical activity of your muscles.
     - The skin will be shaved cleaned, and abraded prior to placing the apparatus.
     - You will then be seated and secured into an Isokinetic dynamometer (a device similar to leg extension that maintains a constant speed of movement) with belts around your hips, shoulders. Your lower leg will be secured to the device arm in a comfortable location, and will be locked in place.
     - You will then be asked to push against the device arm with your leg for five seconds as hard as possible and repeat this 4 times, with 3 minutes rest in between each.
     - During each of these efforts, the device arm will not move.
   - Isokinetic Contractions
     - After you complete MVICs, you will be asked to complete a series of similar efforts at three different speeds. At each speed you will be asked to complete three maximal efforts.
   - Vertical Jump
     - You will be asked to stand on an elevated platform (force plate) and jump straight up as high as possible three times, with three minutes of rest between each attempt

3. Blood testing - You will report to the HPL following a 10 hour fast.

Blood samples will be obtained from one of your forearm veins using a needle apparatus by an individual trained in phlebotomy. Total number of blood draws and needle sticks are as follows:
   - BL Blood Sample
     - One needle stick
     - 30 milliliters (ml) (~2 tablespoons)
   - Acute Protocol Blood Samples (AP I and II)
     - D2 Blood Samples
       - One venous catheter insertion (same as a needle stick, but a flexible tube will remain in your arm)
         - 3 samples; 30 milliliters (ml) each
         - 90 ml (~6 tablespoons)
       - One needle stick
         - 30 milliliters (ml) (~2 tablespoons)
     - D3 Blood Sample
       - One needle stick
       - 30 milliliters (ml) (~2 tablespoons)
     - D4 Blood Sample
       - One needle stick
       - 30 milliliters (ml) (~2 tablespoons)
     - D5 Blood Sample
       - One needle stick
       - 30 milliliters (ml) (~2 tablespoons)
     - TOTAL for each Acute Protocol
       - 4 needle sticks; 1 venous catheter insertion
       - 210 milliliters (ml) (~14 tablespoons)

A venous catheter is a hollow tube, which can be inserted into the opening of a vein and serve as a channel for the transport of fluid. The venous catheter prevents the need for multiple needle sticks from being performed. The risks associated with the placement of the venous catheter are not any different than that experienced by a normal blood draw using a needle and syringe. The venous catheter will be kept open following each blood draw with an infusion of a saline solution. This solution contains salt that is similar to the osmolarity of the blood and acts to minimize potential blood clotting within the venous catheter that may occur with prolonged use. The venous catheter placement will not interfere with your ability to perform the exercise routine. To put the total volume of blood being drawn in proper perspective, one pint (475 ml) of blood is typically drawn at one time when donating blood. All blood draws will be conducted under sterile conditions. As an additional safeguard in preventing contamination new disposable gloves will be used for all blood draws.

The blood will be divided into smaller tubes and a portion will be frozen and stored for future analysis

4. Muscle Biopsy

During D2, and D4, a small muscle biopsy will be obtained from the upper leg. This will total four biopsies over Acute Protocol 1 (no other biopsies will be completed). This procedure has minimal pain and the feeling has been compared to a "pushing sensation". A small incision (approximately ½ inch), after the area has been numbed with lidocaine. After the incision, a small cannula device will be inserted into the muscle, and a biopsy needle will be inserted through this device to obtain the sample. Approximately 3-6 samples will be taken during each sampling time point, totaling approximately 300 milligrams of muscle. Risks of this procedure include soreness to the area, possible appearance of a scar, and infection. To minimize these risks, only a licensed physician or physician approved technician will perform this procedure using sterile techniques. Additionally, the sampling location will be sterilized prior to insertion.

The samples obtained will be frozen and stored for future analysis.

5. Dietary Recall and Journal

Dietary recall will be collected on each day of testing. You will be asked to remember your food intake the day before acute protocol testing, and throughout the duration of each acute protocol. Additionally, you will be asked to remember you food intake for the two days prior to BL testing. You will also be asked to keep a food journal during the training phase (Phase II); three days per week with two weekdays and one weekend day.

What is Experimental?

All aspects of this study are experimental.

Are there risks?

<u>Risk associated with study product consumption or placebo consumption:</u>

The study product carries a possible risk of allergic reaction to certain ingredients. If you are allergic to green or black tea or related herbs or any ingredients in the snack or meals provided, please inform the study staff. Tea product consumption is very safe and side effects would likely be associated with consumption of the caffeine contained in the tea product including:

- Headache
- Nervousness
- Insomnia
- Vomiting
- Diarrhea
- Irritability
- Irregular heartbeat
- Heartburn
- Confusion
- Convulsions
- Dizziness

<u>Risk associated with the required exercises:</u>

The workout consists of exercises that are common to the training program of individuals with experience in weight lifting. It is expected that you will experience the normal soreness that often accompanies these workouts.

<u>Risk associated with blood testing:</u>

The risks associated with the blood draw may include some momentary pain at the time the needle is inserted into the vein, but other discomfort should be minimal. It is also possible for a bruise to develop at the site that the needle entered the skin or for individuals to report dizziness and possibly faint after the blood is drawn. It is also rare, but possible to develop minor infections and pain after the blood draw. To minimize the risks, the skin area where the needle or venous catheter is inserted will be cleaned and prepared with a disinfectant wipe before the needle or venous catheter is inserted. Needles and venous catheters are sterile, gloves are worn by the person trained in obtaining blood. Following the blood draw the puncture site will be covered with a bandage. Only during D2 will a venous catheter will be used. This is to minimize the number of needle sticks. The total amount of blood drawn during each testing point will not exceed 30 milliliters. This is approximately 2 tablespoons. The total amount of blood that will be obtained during the study will be 450 milliliters (~30 tablespoons). To put the volume of blood being drawn in perspective, one pint (475 ml) of blood is typically drawn when donating blood. To reduce the risk of dizziness and fainting from the blood draws, the cannula will be inserted while you are lying flat on your back, and all blood draws will occur while you lying flat on your back. Risks associated with fasting prior to blood sampling include fainting dizziness, light headedness and fatigue.

<u>Risk associated with muscle biopsies:</u>

The risks associated with muscle biopsies include momentary pain at the site of sampling during the time the needle is inserted, the possible appearance of a scar and potential bruising and/or soreness at the site of sampling. Additional risks include the risk of an allergic reaction to lidocaine, and the risk of infection. To limit these risks, only trained technicians, using sterile techniques will obtain muscle biopsies. Additionally, the sampling site will be sterilized prior to the procedure. There will be a total of 3-6 samples obtained at each time point for a total of 100 milligrams of muscle tissue. This will total approximately 400 milligrams over the course of the study. To put this in perspective, ~25 mg is equal to approximately a grain of rice. To reduce the risk of dizziness and fainting, all procedures will occur while you are lying flat on your back. There are no associated risks regarding participating in multiple research studies involving muscle biopsies over the course of one's lifetime. Participating in multiple studies involving muscle biopsies will not increase your risk for further muscle damage.

All testing and training will be overseen by individuals certified in CPR and AED. An AED is located in the building where testing and training will occur.

If at any time during the study you feel discomfort or that you do not want to continue, please inform the researcher. You may discontinue participation at any time.

You should report any discomforts or adverse effects, an intolerable response perceived to be a result of participation in this study, immediately to the principle investigator Dr. Jay Hoffman (jay.hoffman@ucf.edu), or co-investigators Adam Jajtner (adam.jajtner@knighs.ucf.edu), or Jeremy Townsend (Jeremy.townsend@ucf.edu) or call (407)-823-2367. An adverse effect is defined as an intolerable response, perceived to be a direct consequence of participation in this study.

Will you receive payment?

Upon completion of this study, you will receive a payment of $200. If you only complete certain portions of the study, you will be compensated differently. You will receive $125 for completing phase one (5 weeks), and an additional $75 for completing phase two (7 weeks). No compensation will be provided if you do not complete phase one. Additionally, the $125 compensation for phase one will be paid out upon completion of the study (in the form of the $200 for completion of the study), or upon official disenrollment from the study. If you are assigned to the control, you will be provided $125 compensation following completion of the study (defined as completing Acute Protocol 1). You have the choice to receive this payment as a gift card or check.

How will your information be kept confidential?

The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party preventing the release of your identity. National and state laws are in place requiring the study staff to protect the privacy of your records. All information attained from the medical and activity questionnaires, food logs, laboratory test results, or performance tests results and all other information collected during the study will be held in strict confidence. Individual results will remain confidential. However, there is no guarantee of absolute privacy as the study staff will need your information to complete the study. Individual test results will

be shared with the participant only at their request. Test results will not be shared with participants on a consistent basis. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. Participant names will be kept separately from the study results as a separate electronic file under password protection. This file will be stored on a computer in the Education and Human Performance building. All information will be destroyed 5 years from the end of the study.

Who can you talk to about this study?

If you require emergency care, be sure to tell the emergency care provider about your participation in this study. Contact the study Investigators or staff as soon as possible.

If you have questions, concerns, or complaints, contact Dr. Jay Hoffman, Human Performance Laboratory, Sport and Exercise Science (407) 823-2367 or by email at jay.hoffman@ucf.edu..

Research at the University of Central Florida involving human participants is carried out under the oversight of the University of Central Florida Institutional Review Board (IRB). For information about the rights of people who take part in research, please contact: University of Central Florida IRB. You may also talk to them for any of the following:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You want to get information or provide input about this research.

If you are injured or made sick from taking part in this research study notify the study staff as soon as possible. Study investigators will take all safety precautions to minimize risk to participants and are trained in emergency procedures to ensure proper care is received in the event of an accident. However, financial compensation for such things as lost wages, disability or discomfort due to the injury is not routinely available and is not offered as part of the research project. However, by participating in the research study, individuals do not give up any legal rights. The Study Investigators, the funding agencies, and the University of Central Florida do not provide insurance coverage to compensate for injuries incurred during this research.

If you believe you have been injured during participation in this research project, you may file a claim with UCF Environmental Health & Safety, Risk and Insurance Office, P.O. Box 163500, Orlando, FL 32816-3500 (407) 823-6300. The University of Central Florida is an agency of the State of Florida for purposes of sovereign immunity and the university's and the state's liability for personal injury or property damage is extremely limited under Florida law. Accordingly, the university's and the state's ability to compensate you for any personal injury or property damage suffered during this research project is very limited. You do not give up any of your legal rights by signing this consent form.

Do you have to participate in this study?

Your participation in this study is completely voluntary and you have the right to discontinue your participation at any time without penalty. Your participation in the study may also be terminated at any time by the researchers in charge of the project for the following reasons:

- o Failure to follow the study requirements and instructions
- o The Investigators believe it would be best for you to stop your participation in the study
- o The sponsor or IRB stop the study for any reason

What if you are a UCF employee?

Your participation in this study is voluntary. You are free to withdraw your consent and discontinue participation in this study at any time without prejudice or penalty. Your decision to participate or not participate in this study will in no way affect your continued employment or your relationship with individuals who may have an interest in this study. _____initials.

VOLUNTEER'S STATEMENT

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Adam Jajtner or Jeremy Townsend, student investigators; Jay Hoffman, PhD; or Jeffrey Stout, PhD, if I have any more questions about taking part in the study. The University of Central Florida, employer of Jay Hoffman, PhD., is being funded by the sponsor for my participation in this study.

I understand that my participation in this research project is voluntary. I know that I may quit the study at any time without harming my future medical care or losing any benefits to which I might be entitled. I also understand that the investigator in charge of this study may decide at any time that I should no longer participate in the study.

If I have any questions about my rights as a research subject in this study I may contact:

New England Institutional Review Board
Telephone: 1-800-232-9570
E-Mail: info@neirb.com

By signing this form, I have not waived any of my legal rights.

I have read and understand the above information. I agree to participate in this study. I understand that I will be given a copy of this signed and dated form for my own records.

_____
Printed Name of participant

_____   _____
Signature of participant   Date

_____   _____
Signature of person obtaining consent   Date

_____
Printed name of person obtaining consent

Approved by NEIRB on 1/14/2015
NEIRB Version 1.0

APPENDIX F: MEDICAL HISTORY QUESTIONNAIRE

Confidential Medical and Activity History Questionnaire

Participant #_____

When was your last physical examination? _____

1. **List any medications, herbals or supplements you currently take or have taken the last month:**

Medication	Reason for medication
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

2. Are you allergic to any medications? If yes, please list medications and reaction.

3. Please list any allergies, including food allergies that you may have?

4. Have you ever been hospitalized? If yes, please explain.

Year of hospitalization	Reason
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Approved by NEIRB on 1/14/2015
As Is ✓ As Revised _____ Initials _____

1

Human Performance Laboratory
University of Central Florida

5. Illnesses and other Health Issues

List any chronic (long-term) illnesses that have caused you to seek medical care.

6. Have you undergone major surgery within the previous 16 weeks? If yes, please explain.

7. Have you ever had (or do you have now) active malignant disease or cancer. If yes, please explain.

8. Have you ever had (or do you have scheduled) any procedure with Iodine, Barium, or Nuclear Medicine Isotopes ? (CT and PET scans are examples) If yes, please specify the date of the procedure.

Human Performance Laboratory
University of Central Florida

Have you ever had (or do you have now) any of the following. Please circle
questions that you do not know the answer to.

Cystic fibrosis	yes	no
Water retention problems	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High cholesterol	yes	no
Rheumatic fever	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Dementia	yes	no
Artificial limb	yes	no
Alzheimer's	yes	no

Have you ever had (or do you have now) any of the following. Please circle questions that you do not know the answer to.

Cardiovascular Disease	yes	no
Peripheral vascular disease	yes	no
Cerebrovascular disease	yes	no
Coronary artery disease	yes	no
Aortic stenosis	yes	no
Congestive heart failure	yes	no
Atrial fibrillation	yes	no
"Heart block"	yes	no
Myocardial infarction (Heart Attack)	yes	no
Poorly controlled hypertension	yes	no
Heart pacemaker	yes	no
High blood pressure	yes	no
Heart murmur	yes	no

Pulmonary Disease	yes	no
Chronic obstructive pulmonary disease	yes	no
Asthma	yes	no
Interstitial lung disease	yes	no
Emphysema	yes	no
Chronic respiratory disorder	yes	no

Metabolic Disease	yes	no
Diabetes mellitus (type 1, type 2)	yes	no
Diabetes insipidus	yes	no
Thyroid disorders	yes	no
Renal disease	yes	no
Liver disease	yes	no

Immunodeficiency disorder	yes	no

Any others (specify):_____

_____

_____

126

Do you smoke cigarettes or use any other tobacco products?	yes	no
Do you have a history of drug or alcohol dependency?	yes	no
Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?	yes	no
Do you feel pain in your chest when you do physical activity?	yes	no
In the past month have you had chest pain when you were not doing physical activity?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeats?	yes	no
Do you ever have any arm or jaw discomfort, nausea, or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you ever experience shortness of breath?	yes	no
Do you lose your balance because of dizziness or do you ever lose consciousness?	yes	no
Have you ever had any tingling or numbness in your arms or legs?	yes	no
Has a member of your family or close relative died of heart problems or sudden death before the age of 50?	yes	no
Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?	yes	no
Do you have a bone or joint problem that could be made worse by a change in your physical activity?	yes	no

Approved by NEIRB on 4/14/2015
As Is ____ As Revised ____ Initials _____

5

127

Human Performance Laboratory
University of Central Florida

Has a health care practitioner ever denied or
restricted your participation in sports for any
problem   yes   no

If yes, please explain: _____
_____

Do you know of any other reason why you
should not do physical activity?   yes   no

Are you presently taking any nutritional supplements or ergogenic aids? (if yes, please
detail. _____
_____
_____
_____
_____

I have answered these questions honestly and have provided all past and present health
and exercise information to the best of my knowledge.

_____   _____
Signature   Date

APPENDIX G: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition _and_ that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of _any other reason_ why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to _all_ PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____   DATE _____

SIGNATURE OF PARENT _____   WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE   © Canadian Society for Exercise Physiology www.csep.ca/forms

Approved by NEIRB on _1/14/2015_
As Is ✓   As Revised ____   Initials _AM_

APPENDIX H: NEIRB STUDY CLOSEOUT CONFIRMATION

November 16, 2015

Jeffrey R. Stout, PhD
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Number of Pages { 1 }

Re: NEIRB #14-491: : "The Effect of XSurge™ Supplementation on Biomarkers of Muscle Damage, Oxidative Stress, Immune Cell Modulation, Tissue Apoptotic Signaling and Recovery of Functional Performance from Acute High Intensity Resistance Exercise"

Confirmation of Closure and Conclusion of IRB Oversight

This is to inform you that New England IRB has received and reviewed correspondence regarding the completion of the above referenced study at the above-referenced site (and any applicable additional locations).

At this time, NEIRB considers this study closed at your site(s).

This information has been added to your study file.

REFERENCES

Akashi, K., Traver, D., Miyamoto, T., & Weissman, I. L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature, 404*(6774), 193-197. doi: 10.1038/35004599

Ambarus, C. A., Krausz, S., van Eijk, M., Hamann, J., Radstake, T. R., Reedquist, K. A., . . . Baeten, D. L. (2012). Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. *J Immunol Methods, 375*(1-2), 196-206. doi: 10.1016/j.jim.2011.10.013

Ancuta, P., Liu, K. Y., Misra, V., Wacleche, V. S., Gosselin, A., Zhou, X., & Gabuzda, D. (2009). Transcriptional profiling reveals developmental relationship and distinct biological functions of CD16+ and CD16- monocyte subsets. *BMC Genomics, 10*, 403. doi: 10.1186/1471-2164-10-403

Ancuta, P., Moses, A., & Gabuzda, D. (2004). Transendothelial migration of CD16+ monocytes in response to fractalkine under constitutive and inflammatory conditions. *Immunobiology, 209*(1-2), 11-20. doi: 10.1016/j.imbio.2004.04.001

Ancuta, P., Rao, R., Moses, A., Mehle, A., Shaw, S. K., Luscinskas, F. W., & Gabuzda, D. (2003). Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. *J Exp Med, 197*(12), 1701-1707. doi: 10.1084/jem.20022156

Arakawa, M., Mita, T., Azuma, K., Ebato, C., Goto, H., Nomiyama, T., . . . Watada, H. (2010). Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes, 59*(4), 1030-1037. doi: 10.2337/db09-1694

Arent, S. M., Senso, M., Golem, D. L., & McKeever, K. H. (2010). The effects of theaflavin-enriched black tea extract on muscle soreness, oxidative stress, inflammation, and endocrine responses to acute anaerobic interval training: a randomized, double-blind, crossover study. *J Int Soc Sports Nutr, 7*(1), 11. doi: 10.1186/1550-2783-7-11

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., . . . Chazaud, B. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med, 204*(5), 1057-1069. doi: 10.1084/jem.20070075

Auffray, C., Fogg, D., Garfa, M., Elain, G., Join-Lambert, O., Kayal, S., . . . Geissmann, F. (2007). Monitoring of blood vessels and tissues by a population of monocytes

with patrolling behavior. *Science, 317*(5838), 666-670. doi: 10.1126/science.1142883

Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., . . . Schall, T. J. (1997). A new class of membrane-bound chemokine with a CX3C motif. *Nature, 385*(6617), 640-644. doi: 10.1038/385640a0

Booth, S., Florida-James, G. D., McFarlin, B. K., Spielmann, G., O'Connor, D. P., & Simpson, R. J. (2010). The impact of acute strenuous exercise on TLR2, TLR4 and HLA.DR expression on human blood monocytes induced by autologous serum. *Eur J Appl Physiol, 110*(6), 1259-1268. doi: 10.1007/s00421-010-1616-2

Bowtell, J. L., Sumners, D. P., Dyer, A., Fox, P., & Mileva, K. N. (2011). Montmorency cherry juice reduces muscle damage caused by intensive strength exercise. *Med Sci Sports Exerc, 43*(8), 1544-1551. doi: 10.1249/MSS.0b013e31820e5adc

Brancaccio, P., Lippi, G., & Maffulli, N. (2010). Biochemical markers of muscular damage. *Clin Chem Lab Med, 48*(6), 757-767. doi: 10.1515/CCLM.2010.179

Brigitte, M., Schilte, C., Plonquet, A., Baba-Amer, Y., Henri, A., Charlier, C., . . . Chretien, F. (2010). Muscle resident macrophages control the immune cell reaction in a mouse model of notexin-induced myoinjury. *Arthritis Rheum, 62*(1), 268-279. doi: 10.1002/art.27183

Bruun, J. M., Lihn, A. S., Madan, A. K., Pedersen, S. B., Schiott, K. M., Fain, J. N., & Richelsen, B. (2004). Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. *Am J Physiol Endocrinol Metab, 286*(1), E8-13. doi: 10.1152/ajpendo.00269.2003

Brzycki, M. (1993). Strength testing - predicting a one-rep max from reps-to-fatigue. *J Phys Educ Recr Dance, 64*, 88-90.

Buechler, C., Ritter, M., Orso, E., Langmann, T., Klucken, J., & Schmitz, G. (2000). Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. *J Leukoc Biol, 67*(1), 97-103.

Buford, T. W., Cooke, M. B., & Willoughby, D. S. (2009). Resistance exercise-induced changes of inflammatory gene expression within human skeletal muscle. *Eur J Appl Physiol, 107*(4), 463-471. doi: 10.1007/s00421-009-1145-z

Byrne, C., & Eston, R. (2002). The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. *J Sports Sci, 20*(5), 417-425. doi: 10.1080/026404102317366672

Calle, M. C., & Fernandez, M. L. (2010). Effects of resistance training on the inflammatory response. *Nutr Res Pract, 4*(4), 259-269. doi: 10.4162/nrp.2010.4.4.259

Catoire, M., Mensink, M., Kalkhoven, E., Schrauwen, P., & Kersten, S. (2014). Identification of human exercise-induced myokines using secretome analysis. *Physiol Genomics, 46*(7), 256-267. doi: 10.1152/physiolgenomics.00174.2013

Chan, M. H., Carey, A. L., Watt, M. J., & Febbraio, M. A. (2004). Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. *Am J Physiol Regul Integr Comp Physiol, 287*(2), R322-327. doi: 10.1152/ajpregu.00030.2004

Chazaud, B. (2014). Macrophages: supportive cells for tissue repair and regeneration. *Immunobiology, 219*(3), 172-178. doi: 10.1016/j.imbio.2013.09.001

Cheng, M., Nguyen, M. H., Fantuzzi, G., & Koh, T. J. (2008). Endogenous interferon-gamma is required for efficient skeletal muscle regeneration. *Am J Physiol Cell Physiol, 294*(5), C1183-1191. doi: 10.1152/ajpcell.00568.2007

Cheung, K., Hume, P., & Maxwell, L. (2003). Delayed onset muscle soreness : treatment strategies and performance factors. *Sports Med, 33*(2), 145-164.

Chiva-Blanch, G., Urpi-Sarda, M., Llorach, R., Rotches-Ribalta, M., Guillen, M., Casas, R., . . . Estruch, R. (2012). Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial. *Am J Clin Nutr, 95*(2), 326-334. doi: 10.3945/ajcn.111.022889

Clarkson, P. M., & Hubal, M. J. (2002). Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil, 81*(11 Suppl), S52-69. doi: 10.1097/01.PHM.0000029772.45258.43

Clarkson, P. M., Kearns, A. K., Rouzier, P., Rubin, R., & Thompson, P. D. (2006). Serum creatine kinase levels and renal function measures in exertional muscle damage. *Med Sci Sports Exerc, 38*(4), 623-627. doi: 10.1249/01.mss.0000210192.49210.fc

Cros, J., Cagnard, N., Woollard, K., Patey, N., Zhang, S. Y., Senechal, B., . . . Geissmann, F. (2010). Human CD14dim monocytes patrol and sense nucleic acids and viruses

via TLR7 and TLR8 receptors. *Immunity, 33*(3), 375-386. doi: 10.1016/j.immuni.2010.08.012

Crystal, N. J., Townson, D. H., Cook, S. B., & LaRoche, D. P. (2013). Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *Eur J Appl Physiol, 113*(10), 2577-2586. doi: 10.1007/s00421-013-2693-9

Cushing, S. D., Berliner, J. A., Valente, A. J., Territo, M. C., Navab, M., Parhami, F., . . . Fogelman, A. M. (1990). Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci U S A, 87*(13), 5134-5138.

Dale, D. C., Boxer, L., & Liles, W. C. (2008). The phagocytes: neutrophils and monocytes. *Blood, 112*(4), 935-945. doi: 10.1182/blood-2007-12-077917

Damoiseaux, J. G., Dopp, E. A., Calame, W., Chao, D., MacPherson, G. G., & Dijkstra, C. D. (1994). Rat macrophage lysosomal membrane antigen recognized by monoclonal antibody ED1. *Immunology, 83*(1), 140-147.

Della Gatta, P. A., Cameron-Smith, D., & Peake, J. M. (2014). Acute resistance exercise increases the expression of chemotactic factors within skeletal muscle. *Eur J Appl Physiol, 114*(10), 2157-2167. doi: 10.1007/s00421-014-2936-4

Dijkstra, C. D., Dopp, E. A., Joling, P., & Kraal, G. (1985). The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology, 54*(3), 589-599.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol, 37*(2), 247-248.

Dimitrov, S., Shaikh, F., Pruitt, C., Green, M., Wilson, K., Beg, N., & Hong, S. (2013). Differential TNF production by monocyte subsets under physical stress: blunted mobilization of proinflammatory monocytes in prehypertensive individuals. *Brain Behav Immun, 27*(1), 101-108. doi: 10.1016/j.bbi.2012.10.003

Dungey, M., Bishop, N. C., Young, H. M., Burton, J. O., & Smith, A. C. (2015). The Impact of Exercising During Haemodialysis on Blood Pressure, Markers of Cardiac Injury and Systemic Inflammation - Preliminary Results of a Pilot Study. *Kidney Blood Press Res, 40*(6), 593-604. doi: 10.1159/000368535

Ehlers, M. R. (2000). CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect, 2*(3), 289-294.

Evangelista, V., Manarini, S., Sideri, R., Rotondo, S., Martelli, N., Piccoli, A., . . . Cerletti, C. (1999). Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as a signaling molecule. *Blood, 93*(3), 876-885.

Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative stress : relationship with exercise and training. *Sports Med, 36*(4), 327-358.

Fong, A. M., Robinson, L. A., Steeber, D. A., Tedder, T. F., Yoshie, O., Imai, T., & Patel, D. D. (1998). Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J Exp Med, 188*(8), 1413-1419.

Foussat, A., Coulomb-L'Hermine, A., Gosling, J., Krzysiek, R., Durand-Gasselin, I., Schall, T., . . . Emilie, D. (2000). Fractalkine receptor expression by T lymphocyte subpopulations and in vivo production of fractalkine in human. *Eur J Immunol, 30*(1), 87-97. doi: 10.1002/1521-4141(200001)30:1<87::AID-IMMU87>3.0.CO;2-7

Fragala, M. S., Kraemer, W. J., Mastro, A. M., Denegar, C. R., Volek, J. S., Hakkinen, K., . . . Maresh, C. M. (2011). Leukocyte beta2-adrenergic receptor expression in response to resistance exercise. *Med Sci Sports Exerc, 43*(8), 1422-1432. doi: 10.1249/MSS.0b013e31820b88bc

Freidenreich, D. J., & Volek, J. S. (2012). Immune responses to resistance exercise. *Exerc Immunol Rev, 18*, 8-41.

Futosi, K., Fodor, S., & Mocsai, A. (2013). Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol, 17*(3), 638-650. doi: 10.1016/j.intimp.2013.06.034

Gabriel, H. H., & Kindermann, W. (1998). Adhesion molecules during immune response to exercise. *Can J Physiol Pharmacol, 76*(5), 512-523.

Gavrieli, R., Ashlagi-Amiri, T., Eliakim, A., Nemet, D., Zigel, L., Berger-Achituv, S., . . . Wolach, B. (2008). The effect of aerobic exercise on neutrophil functions. *Med Sci Sports Exerc, 40*(9), 1623-1628. doi: 10.1249/MSS.0b013e318176b963

Gerhardt, T., & Ley, K. (2015). Monocyte trafficking across the vessel wall. *Cardiovasc Res, 107*(3), 321-330. doi: 10.1093/cvr/cvv147

Gonzalez, A. M., Fragala, M. S., Jajtner, A. R., Townsend, J. R., Wells, A. J., Beyer, K. S., . . . Hoffman, J. R. (2014). Effects of beta-hydroxy-beta-methylbutyrate free acid and cold water immersion on expression of CR3 and MIP-1beta following resistance exercise. *Am J Physiol Regul Integr Comp Physiol, 306*(7), R483-489. doi: 10.1152/ajpregu.00542.2013

Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: mechanism and functions. *Immunity, 32*(5), 593-604. doi: 10.1016/j.immuni.2010.05.007

Graff, J. C., & Jutila, M. A. (2007). Differential regulation of CD11b on gammadelta T cells and monocytes in response to unripe apple polyphenols. *J Leukoc Biol, 82*(3), 603-607. doi: 10.1189/jlb.0207125

Grage-Griebenow, E., Zawatzky, R., Kahlert, H., Brade, L., Flad, H., & Ernst, M. (2001). Identification of a novel dendritic cell-like subset of CD64(+) / CD16(+) blood monocytes. *Eur J Immunol, 31*(1), 48-56. doi: 10.1002/1521-4141(200101)31:1<48::AID-IMMU48>3.0.CO;2-5

Hammond, M. E., Lapointe, G. R., Feucht, P. H., Hilt, S., Gallegos, C. A., Gordon, C. A., . . . Tekamp-Olson, P. (1995). IL-8 induces neutrophil chemotaxis predominantly via type I IL-8 receptors. *J Immunol, 155*(3), 1428-1433.

Hampton, M. B., Kettle, A. J., & Winterbourn, C. C. (1998). Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood, 92*(9), 3007-3017.

Heimbeck, I., Hofer, T. P., Eder, C., Wright, A. K., Frankenberger, M., Marei, A., . . . Ziegler-Heitbrock, L. (2010). Standardized single-platform assay for human monocyte subpopulations: Lower CD14+CD16++ monocytes in females. *Cytometry A, 77*(9), 823-830. doi: 10.1002/cyto.a.20942

Henson, D. A., Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Shannon, M., Bolton, M. R., . . . Schilling, B. K. (2000). Influence of carbohydrate on cytokine and phagocytic responses to 2 h of rowing. *Med Sci Sports Exerc, 32*(8), 1384-1389.

Herrlinger, K. A., Chirouzes, D. M., & Ceddia, M. A. (2015). Supplementation with a polyphenolic blend improves post-exercise strength recovery and muscle soreness. *Food Nutr Res, 59*, 30034. doi: 10.3402/fnr.v59.30034

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., & Suzuki, K. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev, 10*, 75-90.

Hoffman, J. R. (2006). *Norms for fitness, performance, and health*. Champaign: Human Kinetics.

Honda, H., Kimura, H., & Rostami, A. (1990). Demonstration and phenotypic characterization of resident macrophages in rat skeletal muscle. *Immunology, 70*(2), 272-277.

Hong, S., & Mills, P. J. (2008). Effects of an exercise challenge on mobilization and surface marker expression of monocyte subsets in individuals with normal vs. elevated blood pressure. *Brain Behav Immun, 22*(4), 590-599. doi: 10.1016/j.bbi.2007.12.003

Hristov, M., & Weber, C. (2011). Differential role of monocyte subsets in atherosclerosis. *Thromb Haemost, 106*(5), 757-762. doi: 10.1160/TH11-07-0500

Ihalainen, J., Walker, S., Paulsen, G., Hakkinen, K., Kraemer, W. J., Hamalainen, M., . . . Mero, A. A. (2014). Acute leukocyte, cytokine and adipocytokine responses to maximal and hypertrophic resistance exercise bouts. *Eur J Appl Physiol, 114*(12), 2607-2616. doi: 10.1007/s00421-014-2979-6

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., . . . Yoshie, O. (1997). Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell, 91*(4), 521-530.

Jajtner, A. R., Fragala, M. S., Townsend, J. R., Gonzalez, A. M., Wells, A. J., Fukuda, D. H., . . . Hoffman, J. R. (2014). Mediators of Monocyte Migration in Response to Recovery Modalities following Resistance Exercise. *Mediators of Inflammation*.

Jajtner, A. R., Hoffman, J. R., Gonzalez, A. M., Worts, P. R., Fragala, M. S., & Stout, J. R. (2015). Comparison of the effects of electrical stimulation and cold-water immersion on muscle soreness after resistance exercise. *J Sport Rehabil, 24*(2), 99-108. doi: 10.1123/jsr.2013-0113

Jordan, J., Beneke, R., Hutler, M., Veith, A., Luft, F. C., & Haller, H. (1999). Regulation of MAC-1 (CD11b/CD18) expression on circulating granulocytes in endurance runners. *Med Sci Sports Exerc, 31*(3), 362-367.

Jowko, E., Sacharuk, J., Balasinska, B., Ostaszewski, P., Charmas, M., & Charmas, R. (2011). Green tea extract supplementation gives protection against exercise-induced oxidative damage in healthy men. *Nutr Res, 31*(11), 813-821. doi: 10.1016/j.nutres.2011.09.020

Kansas, G. S. (1996). Selectins and their ligands: current concepts and controversies. *Blood, 88*(9), 3259-3287.

Kawai, K., Tsuno, N. H., Kitayama, J., Okaji, Y., Yazawa, K., Asakage, M., . . . Nagawa, H. (2004). Epigallocatechin gallate attenuates adhesion and migration of CD8+ T cells by binding to CD11b. *J Allergy Clin Immunol, 113*(6), 1211-1217. doi: 10.1016/j.jaci.2004.02.044

Kawanishi, N., Mizokami, T., Niihara, H., Yada, K., & Suzuki, K. (2016). Macrophage depletion by clodronate liposome attenuates muscle injury and inflammation following exhaustive exercise. *Biochemistry and Biophysics Reports, 5*, 146-151.

Kerksick, C. M., Kreider, R. B., & Willoughby, D. S. (2010). Intramuscular adaptations to eccentric exercise and antioxidant supplementation. *Amino Acids, 39*(1), 219-232. doi: 10.1007/s00726-009-0432-7

Kittner, J. M., Jacobs, R., Pawlak, C. R., Heijnen, C. J., Schedlowski, M., & Schmidt, R. E. (2002). Adrenaline-induced immunological changes are altered in patients with rheumatoid arthritis. *Rheumatology (Oxford), 41*(9), 1031-1039.

Kolaczkowska, E., & Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol, 13*(3), 159-175. doi: 10.1038/nri3399

Kurokawa, Y., Shinkai, S., Torii, J., Hino, S., & Shek, P. N. (1995). Exercise-induced changes in the expression of surface adhesion molecules on circulating granulocytes and lymphocytes subpopulations. *Eur J Appl Physiol Occup Physiol, 71*(2-3), 245-252.

LaVoy, E. C., Bollard, C. M., Hanley, P. J., O'Connor, D. P., Lowder, T. W., Bosch, J. A., & Simpson, R. J. (2015). A single bout of dynamic exercise by healthy adults enhances the generation of monocyte-derived-dendritic cells. *Cell Immunol, 295*(1), 52-59. doi: 10.1016/j.cellimm.2015.02.007

Leonard, E. J., & Yoshimura, T. (1990). Human monocyte chemoattractant protein-1 (MCP-1). *Immunol Today, 11*(3), 97-101.

Ley, K., Laudanna, C., Cybulsky, M. I., & Nourshargh, S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol, 7*(9), 678-689. doi: 10.1038/nri2156

Lockhart, N. C., & Brooks, S. V. (2008). Neutrophil accumulation following passive stretches contributes to adaptations that reduce contraction-induced skeletal muscle injury in mice. *J Appl Physiol (1985), 104*(4), 1109-1115. doi: 10.1152/japplphysiol.00850.2007

Ludwig, A., Berkhout, T., Moores, K., Groot, P., & Chapman, G. (2002). Fractalkine is expressed by smooth muscle cells in response to IFN-gamma and TNF-alpha and is modulated by metalloproteinase activity. *J Immunol, 168*(2), 604-612.

Machin, D. R., Christmas, K. V., Chou, T., Hill, S. C., Van Pelt, D. W., Trombold, J. R., & Coyle, E. F. (2014). Effects of Differing Dosages of Pomegranate Juice Supplementation after Eccentric Exercise. *Physiology Journal, 2014*.

Malm, C., Lenkei, R., & Sjodin, B. (1999). Effects of eccentric exercise on the immune system in men. *J Appl Physiol, 86*(2), 461-468.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B., & Lundberg, I. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol, 529*(1), 243-262.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *Am J Clin Nutr, 79*(5), 727-747.

Martin, N. R. W., & Lewis, M. P. (2012). Satellite cell activation and number following acute and chronic exercise: a mini review. *Cellular and Molecular Exercise Physiology, 1*(1), 1-5.

Mayhew, D. L., Thyfault, J. P., & Koch, A. J. (2005). Rest-interval length affects leukocyte levels during heavy resistance exercise. *J Strength Cond Res, 19*(1), 16-22. doi: 10.1519/R-14113.1

McLennan, I. (1993). Resident macrophages (ED2- and ED3-positive) do not phagocytose degenerating rat skeletal muscle fibers. *Cell Tissue Research, 272*, 193-196.

Merry, T. L., & Ristow, M. (2015). Do antioxidant supplements interfere with skeletal muscle adaptation to exercise training? *J Physiol*. doi: 10.1113/JP270654

Miles, M. P., Leach, S. K., Kraemer, W. J., Dohi, K., Bush, J. A., & Mastro, A. M. (1998). Leukocyte adhesion molecule expression during intense resistance exercise. *J Appl Physiol (1985), 84*(5), 1604-1609.

Millet, G. Y., & Lepers, R. (2004). Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med, 34*(2), 105-116.

Mitchell, A. J., Roediger, B., & Weninger, W. (2014). Monocyte homeostasis and the plasticity of inflammatory monocytes. *Cell Immunol, 291*(1-2), 22-31. doi: 10.1016/j.cellimm.2014.05.010

Montecucco, F., Steffens, S., Burger, F., Da Costa, A., Bianchi, G., Bertolotto, M., . . . Ottonello, L. (2008). Tumor necrosis factor-alpha (TNF-alpha) induces integrin CD11b/CD18 (Mac-1) up-regulation and migration to the CC chemokine CCL3 (MIP-1alpha) on human neutrophils through defined signalling pathways. *Cell Signal, 20*(3), 557-568. doi: 10.1016/j.cellsig.2007.11.008

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat Rev Immunol, 8*(12), 958-969. doi: 10.1038/nri2448

Myburgh, K. H. (2014). Polyphenol supplementation: benefits for exercise performance or oxidative stress? *Sports Med, 44 Suppl 1*, S57-70. doi: 10.1007/s40279-014-0151-4

Nahrendorf, M., Swirski, F. K., Aikawa, E., Stangenberg, L., Wurdinger, T., Figueiredo, J. L., . . . Pittet, M. J. (2007). The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med, 204*(12), 3037-3047. doi: 10.1084/jem.20070885

Nguyen, H. X., & Tidball, J. G. (2003a). Interactions between neutrophils and macrophages promote macrophage killing of rat muscle cells in vitro. *J Physiol, 547*(Pt 1), 125-132. doi: 10.1113/jphysiol.2002.031450

Nguyen, H. X., & Tidball, J. G. (2003b). Null mutation of gp91phox reduces muscle membrane lysis during muscle inflammation in mice. *J Physiol, 553*(Pt 3), 833-841. doi: 10.1113/jphysiol.2003.051912

Nicod, L. P. (1993). Cytokines. 1. Overview. *Thorax, 48*(6), 660-667.

Nielsen, H. G., & Lyberg, T. (2004). Long-distance running modulates the expression of leucocyte and endothelial adhesion molecules. *Scand J Immunol, 60*(4), 356-362. doi: 10.1111/j.0300-9475.2004.01486.x

Nieman, D. C., Davis, J. M., Brown, V. A., Henson, D. A., Dumke, C. L., Utter, A. C., . . . McAnulty, L. S. (2004). Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. *J Appl Physiol (1985), 96*(4), 1292-1298. doi: 10.1152/japplphysiol.01064.2003

Nieman, D. C., Davis, J. M., Henson, D. A., Walberg-Rankin, J., Shute, M., Dumke, C. L., . . . McAnulty, L. S. (2003). Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *J Appl Physiol (1985), 94*(5), 1917-1925. doi: 10.1152/japplphysiol.01130.2002

Nieman, D. C., Gillitt, N. D., Knab, A. M., Shanely, R. A., Pappan, K. L., Jin, F., & Lila, M. A. (2013). Influence of a polyphenol-enriched protein powder on exercise-induced inflammation and oxidative stress in athletes: a randomized trial using a metabolomics approach. *PLoS One, 8*(8), e72215. doi: 10.1371/journal.pone.0072215

Nieman, D. C., Henson, D. A., Davis, J. M., Dumke, C. L., Gross, S. J., Jenkins, D. P., . . . Mayer, E. P. (2007). Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. *J Interferon Cytokine Res, 27*(12), 1003-1011. doi: 10.1089/jir.2007.0050

Nieman, D. C., Henson, D. A., Gross, S. J., Jenkins, D. P., Davis, J. M., Murphy, E. A., . . . Mayer, E. P. (2007). Quercetin reduces illness but not immune perturbations after intensive exercise. *Med Sci Sports Exerc, 39*(9), 1561-1569. doi: 10.1249/mss.0b013e318076b566

Nieman, D. C., Henson, D. A., Sampson, C. S., Herring, J. L., Suttles, J., Conley, M., . . . Davis, J. M. (1995). The acute immune response to exhaustive resistance exercise. *Int J Sports Med, 16*(5), 322-328. doi: 10.1055/s-2007-973013

Novak, M. L., & Koh, T. J. (2013). Macrophage phenotypes during tissue repair. *J Leukoc Biol, 93*(6), 875-881. doi: 10.1189/jlb.1012512

O'Fallon, K. S., Kaushik, D., Michniak-Kohn, B., Dunne, C. P., Zambraski, E. J., & Clarkson, P. M. (2012). Effects of quercetin supplementation on markers of muscle damage and inflammation after eccentric exercise. *Int J Sport Nutr Exerc Metab, 22*(6), 430-437.

Panza, V. S., Wazlawik, E., Ricardo Schutz, G., Comin, L., Hecht, K. C., & da Silva, E. L. (2008). Consumption of green tea favorably affects oxidative stress markers in weight-trained men. *Nutrition, 24*(5), 433-442. doi: 10.1016/j.nut.2008.01.009

143

Parkin, J., & Cohen, B. (2001). An overview of the immune system. *Lancet, 357*(9270), 1777-1789. doi: 10.1016/S0140-6736(00)04904-7

Paulsen, G., Benestad, H. B., Strom-Gundersen, I., Morkrid, L., Lappegard, K. T., & Raastad, T. (2005). Delayed leukocytosis and cytokine response to high-force eccentric exercise. *Med Sci Sports Exerc, 37*(11), 1877-1883.

Paulsen, G., Crameri, R., Benestad, H. B., Fjeld, J. G., Morkrid, L., Hallen, J., & Raastad, T. (2010). Time course of leukocyte accumulation in human muscle after eccentric exercise. *Med Sci Sports Exerc, 42*(1), 75-85. doi: 10.1249/MSS.0b013e3181ac7adb

Paulsen, G., Hamarsland, H., Cumming, K. T., Johansen, R. E., Hulmi, J. J., Borsheim, E., . . . Raastad, T. (2014). Vitamin C and E supplementation alters protein signalling after a strength training session, but not muscle growth during 10 weeks of training. *J Physiol, 592*(24), 5391-5408. doi: 10.1113/jphysiol.2014.279950

Peake, J., Nosaka, K., & Suzuki, K. (2005). Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev, 11*, 64-85.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., & Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur J Appl Physiol, 95*(5-6), 514-521. doi: 10.1007/s00421-005-0035-2

Peake, J. M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., Mackinnon, L., & Coombes, J. S. (2005). Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation. *Med Sci Sports Exerc, 37*(5), 737-745.

Pedersen, B. K. (2000). Special feature for the Olympics: effects of exercise on the immune system: exercise and cytokines. *Immunol Cell Biol, 78*(5), 532-535. doi: 10.1111/j.1440-1711.2000.t01-11-.x

Pedersen, B. K., Akerstrom, T. C., Nielsen, A. R., & Fischer, C. P. (2007). Role of myokines in exercise and metabolism. *J Appl Physiol (1985), 103*(3), 1093-1098. doi: 10.1152/japplphysiol.00080.2007

Pilling, D., Fan, T., Huang, D., Kaul, B., & Gomer, R. H. (2009). Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One, 4*(10), e7475. doi: 10.1371/journal.pone.0007475

Pimorady-Esfahani, A., Grounds, M. D., & McMenamin, P. G. (1997). Macrophages and dendritic cells in normal and regenerating murine skeletal muscle. *Muscle Nerve, 20*(2), 158-166.

Pizza, F. X., Davis, B. H., Henrickson, S. D., Mitchell, J. B., Pace, J. F., Bigelow, N., . . . Naglieri, T. (1996). Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression. *J Appl Physiol, 80*(1), 47-55.

Pizza, F. X., Peterson, J. M., Baas, J. H., & Koh, T. J. (2005). Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *J Physiol, 562*(Pt 3), 899-913. doi: 10.1113/jphysiol.2004.073965

Poehlmann, H., Schefold, J. C., Zuckermann-Becker, H., Volk, H. D., & Meisel, C. (2009). Phenotype changes and impaired function of dendritic cell subsets in patients with sepsis: a prospective observational analysis. *Crit Care, 13*(4), R119. doi: 10.1186/cc7969

Polfliet, M. M., Fabriek, B. O., Daniels, W. P., Dijkstra, C. D., & van den Berg, T. K. (2006). The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production. *Immunobiology, 211*(6-8), 419-425. doi: 10.1016/j.imbio.2006.05.015

Power, C. P., Wang, J. H., Manning, B., Kell, M. R., Aherne, N. J., Wu, Q. D., & Redmond, H. P. (2004). Bacterial lipoprotein delays apoptosis in human neutrophils through inhibition of caspase-3 activity: regulatory roles for CD14 and TLR-2. *J Immunol, 173*(8), 5229-5237.

Radom-Aizik, S., Zaldivar, F., Leu, S. Y., Galassetti, P., & Cooper, D. M. (2008). Effects of 30 min of aerobic exercise on gene expression in human neutrophils. *J Appl Physiol, 104*(1), 236-243. doi: 10.1152/japplphysiol.00872.2007

Radom-Aizik, S., Zaldivar, F. P., Jr., Haddad, F., & Cooper, D. M. (2014). Impact of brief exercise on circulating monocyte gene and microRNA expression: implications for atherosclerotic vascular disease. *Brain Behav Immun, 39*, 121-129. doi: 10.1016/j.bbi.2014.01.003

Ramel, A., Wagner, K. H., & Elmadfa, I. (2003). Acute impact of submaximal resistance exercise on immunological and hormonal parameters in young men. *J Sports Sci, 21*(12), 1001-1008. doi: 10.1080/02640410310001641395

Ramel, A., Wagner, K. H., & Elmadfa, I. (2004). Correlations between plasma noradrenaline concentrations, antioxidants, and neutrophil counts after submaximal

resistance exercise in men. *Br J Sports Med, 38*(5), E22. doi: 10.1136/bjsm.2003.007666

Ramos, M. V., Fernandez, G. C., Brando, R. J., Panek, C. A., Bentancor, L. V., Landoni, V. I., . . . Palermo, M. S. (2010). Interleukin-10 and interferon-gamma modulate surface expression of fractalkine-receptor (CX(3)CR1) via PI3K in monocytes. *Immunology, 129*(4), 600-609. doi: 10.1111/j.1365-2567.2009.03181.x

Ribeiro, R. A., Flores, C. A., Cunha, F. Q., & Ferreira, S. H. (1991). IL-8 causes in vivo neutrophil migration by a cell-dependent mechanism. *Immunology, 73*(4), 472-477.

Rigamonti, E., Touvier, T., Clementi, E., Manfredi, A. A., Brunelli, S., & Rovere-Querini, P. (2013). Requirement of inducible nitric oxide synthase for skeletal muscle regeneration after acute damage. *J Immunol, 190*(4), 1767-1777. doi: 10.4049/jimmunol.1202903

Rollins, B. J. (1997). Chemokines. *Blood, 90*(3), 909-928.

Rollins, B. J., Yoshimura, T., Leonard, E. J., & Pober, J. S. (1990). Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. *Am J Pathol, 136*(6), 1229-1233.

Rosen, H., & Gordon, S. (1987). Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment in vivo. *J Exp Med, 166*(6), 1685-1701.

Ross, M. L., Halson, S. L., Suzuki, K., Garnham, A., Hawley, J. A., Cameron-Smith, D., & Peake, J. M. (2010). Cytokine responses to carbohydrate ingestion during recovery from exercise-induced muscle injury. *J Interferon Cytokine Res, 30*(5), 329-337. doi: 10.1089/jir.2009.0079

Rossol, M., Kraus, S., Pierer, M., Baerwald, C., & Wagner, U. (2012). The CD14(bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. *Arthritis Rheum, 64*(3), 671-677. doi: 10.1002/art.33418

Rot, A., & von Andrian, U. H. (2004). Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu Rev Immunol, 22*, 891-928. doi: 10.1146/annurev.immunol.22.012703.104543

Roussel, M., Benard, C., Ly-Sunnaram, B., & Fest, T. (2010). Refining the white blood cell differential: the first flow cytometry routine application. *Cytometry A, 77*(6), 552-563. doi: 10.1002/cyto.a.20893

Saclier, M., Yacoub-Youssef, H., Mackey, A. L., Arnold, L., Ardjoune, H., Magnan, M., . . . Chazaud, B. (2013). Differentially activated macrophages orchestrate myogenic precursor cell fate during human skeletal muscle regeneration. *Stem Cells, 31*(2), 384-396. doi: 10.1002/stem.1288

Sadik, C. D., Kim, N. D., & Luster, A. D. (2011). Neutrophils cascading their way to inflammation. *Trends Immunol, 32*(10), 452-460. doi: 10.1016/j.it.2011.06.008

Saxton, J. M., Claxton, D., Winter, E., & Pockley, A. G. (2003). Peripheral blood leucocyte functional responses to acute eccentric exercise in humans are influenced by systemic stress, but not by exercise-induced muscle damage. *Clin Sci (Lond), 104*(1), 69-77. doi: 10.1042/

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *Am J Clin Nutr, 81*(1 Suppl), 215S-217S.

Schraufstatter, I. U., Chung, J., & Burger, M. (2001). IL-8 activates endothelial cell CXCR1 and CXCR2 through Rho and Rac signaling pathways. *Am J Physiol Lung Cell Mol Physiol, 280*(6), L1094-1103.

Semerad, C. L., Liu, F., Gregory, A. D., Stumpf, K., & Link, D. C. (2002). G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. *Immunity, 17*(4), 413-423.

Shantsila, E., Tapp, L. D., Wrigley, B. J., Montoro-Garcia, S., Ghattas, A., Jaipersad, A., & Lip, G. Y. (2012). The effects of exercise and diurnal variation on monocyte subsets and monocyte-platelet aggregates. *Eur J Clin Invest, 42*(8), 832-839. doi: 10.1111/j.1365-2362.2012.02656.x

Simonson, S. R., & Jackson, C. G. (2004). Leukocytosis occurs in response to resistance exercise in men. *J Strength Cond Res, 18*(2), 266-271. doi: 10.1519/R-12572.1

Simpson, R. J., McFarlin, B. K., McSporran, C., Spielmann, G., o Hartaigh, B., & Guy, K. (2009). Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. *Brain Behav Immun, 23*(2), 232-239. doi: 10.1016/j.bbi.2008.09.013

Soehnlein, O., Lindbom, L., & Weber, C. (2009). Mechanisms underlying neutrophil-mediated monocyte recruitment. *Blood, 114*(21), 4613-4623. doi: 10.1182/blood-2009-06-221630

Song, E., Ouyang, N., Horbelt, M., Antus, B., Wang, M., & Exton, M. S. (2000). Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. *Cell Immunol, 204*(1), 19-28. doi: 10.1006/cimm.2000.1687

Steppich, B., Dayyani, F., Gruber, R., Lorenz, R., Mack, M., & Ziegler-Heitbrock, H. W. (2000). Selective mobilization of CD14(+)CD16(+) monocytes by exercise. *Am J Physiol Cell Physiol, 279*(3), C578-586.

Stout, R. D., Jiang, C., Matta, B., Tietzel, I., Watkins, S. K., & Suttles, J. (2005). Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol, 175*(1), 342-349.

Stromberg, A., Olsson, K., Dijksterhuis, J. P., Rullman, E., Schulte, G., & Gustafsson, T. (2016). CX3CL1-a macrophage chemoattractant induced by a single bout of exercise in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol, 310*(3), R297-304. doi: 10.1152/ajpregu.00236.2015

Summers, C., Rankin, S. M., Condliffe, A. M., Singh, N., Peters, A. M., & Chilvers, E. R. (2010). Neutrophil kinetics in health and disease. *Trends Immunol, 31*(8), 318-324. doi: 10.1016/j.it.2010.05.006

Szmitko, P. E., Wang, C. H., Weisel, R. D., de Almeida, J. R., Anderson, T. J., & Verma, S. (2003). New markers of inflammation and endothelial cell activation: Part I. *Circulation, 108*(16), 1917-1923. doi: 10.1161/01.CIR.0000089190.95415.9F

Takano, K., Nakaima, K., Nitta, M., Shibata, F., & Nakagawa, H. (2004). Inhibitory effect of (-)-epigallocatechin 3-gallate, a polyphenol of green tea, on neutrophil chemotaxis in vitro and in vivo. *J Agric Food Chem, 52*(14), 4571-4576. doi: 10.1021/jf0355194

Tallone, T., Turconi, G., Soldati, G., Pedrazzini, G., Moccetti, T., & Vassalli, G. (2011). Heterogeneity of human monocytes: an optimized four-color flow cytometry protocol for analysis of monocyte subsets. *Journal of Cardiovascular Translational Research, 4*(2), 211-219. doi: 10.1007/s12265-011-9256-4

Tan, S. M. (2012). The leucocyte beta2 (CD18) integrins: the structure, functional regulation and signalling properties. *Biosci Rep, 32*(3), 241-269. doi: 10.1042/BSR20110101

Tapp, L. D., Shantsila, E., Wrigley, B. J., Pamukcu, B., & Lip, G. Y. (2012). The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. *J Thromb Haemost, 10*(7), 1231-1241. doi: 10.1111/j.1538-7836.2011.04603.x

Teixeira, C. F., Zamuner, S. R., Zuliani, J. P., Fernandes, C. M., Cruz-Hofling, M. A., Fernandes, I., . . . Gutierrez, J. M. (2003). Neutrophils do not contribute to local tissue damage, but play a key role in skeletal muscle regeneration, in mice injected with Bothrops asper snake venom. *Muscle Nerve, 28*(4), 449-459. doi: 10.1002/mus.10453

Tidball, J. G. (2005). Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol, 288*(2), R345-353. doi: 10.1152/ajpregu.00454.2004

Tidball, J. G., & Villalta, S. A. (2010). Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol, 298*(5), R1173-1187. doi: 10.1152/ajpregu.00735.2009

Tidball, J. G., & Wehling-Henricks, M. (2007). Macrophages promote muscle membrane repair and muscle fibre growth and regeneration during modified muscle loading in mice in vivo. *J Physiol, 578*(Pt 1), 327-336. doi: 10.1113/jphysiol.2006.118265

Tidball, J. G., & Welc, S. S. (2015). Macrophage-Derived IGF-1 Is a Potent Coordinator of Myogenesis and Inflammation in Regenerating Muscle. *Mol Ther, 23*(7), 1134-1135. doi: 10.1038/mt.2015.97

Tonkin, J., Temmerman, L., Sampson, R. D., Gallego-Colon, E., Barberi, L., Bilbao, D., . . . Rosenthal, N. (2015). Monocyte/Macrophage-derived IGF-1 Orchestrates Murine Skeletal Muscle Regeneration and Modulates Autocrine Polarization. *Mol Ther, 23*(7), 1189-1200. doi: 10.1038/mt.2015.66

Turner, S. L., Mangnall, D., Bird, N. C., Blair-Zajdel, M. E., & Bunning, R. A. (2010). Effects of pro-inflammatory cytokines on the production of soluble fractalkine and ADAM17 by HepG2 cells. *J Gastrointestin Liver Dis, 19*(3), 265-271.

Urra, X., Villamor, N., Amaro, S., Gomez-Choco, M., Obach, V., Oleaga, L., . . . Chamorro, A. (2009). Monocyte subtypes predict clinical course and prognosis in human stroke. *J Cereb Blood Flow Metab, 29*(5), 994-1002. doi: 10.1038/jcbfm.2009.25

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology, 189*(1-2), 41-54.

Van Craenenbroeck, A. H., Van Ackeren, K., Hoymans, V. Y., Roeykens, J., Verpooten, G. A., Vrints, C. J., . . . Van Craenenbroeck, E. M. (2014). Acute Exercise-Induced Response of Monocyte Subtypes in Chronic Heart and Renal Failure. *Mediators of Inflammation*. doi: Artn 216534

10.1155/2014/216534

van Eeden, S. F., Granton, J., Hards, J. M., Moore, B., & Hogg, J. C. (1999). Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. *J Appl Physiol, 86*(3), 970-976.

Van Velzen, A. G., Da Silva, R. P., Gordon, S., & Van Berkel, T. J. (1997). Characterization of a receptor for oxidized low-density lipoproteins on rat Kupffer cells: similarity to macrosialin. *Biochem J, 322 (Pt 2)*, 411-415.

Vassilakopoulos, T., Karatza, M. H., Katsaounou, P., Kollintza, A., Zakynthinos, S., & Roussos, C. (2003). Antioxidants attenuate the plasma cytokine response to exercise in humans. *J Appl Physiol (1985), 94*(3), 1025-1032. doi: 10.1152/japplphysiol.00735.2002

Weber, C., Erl, W., & Weber, P. C. (1995). Enhancement of monocyte adhesion to endothelial cells by oxidatively modified low-density lipoprotein is mediated by activation of CD11b. *Biochem Biophys Res Commun, 206*(2), 621-628. doi: 10.1006/bbrc.1995.1088

Wells, A. J., Hoffman, J. R., Jajtner, A. R., Varanoske, A. N., Church, D. D., Gonzalez, A. M., . . . Stout, J. R. (2016). Monocyte Recruitment following High-Intensity and High-Volume Resistance Exercise. *Med Sci Sports Exerc*. doi: 10.1249/MSS.0000000000000878

Wong, K. L., Tai, J. J., Wong, W. C., Han, H., Sem, X., Yeap, W. H., . . . Wong, S. C. (2011). Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood, 118*(5), e16-31. doi: 10.1182/blood-2010-12-326355

Wong, K. L., Yeap, W. H., Tai, J. J., Ong, S. M., Dang, T. M., & Wong, S. C. (2012). The three human monocyte subsets: implications for health and disease. *Immunol Res, 53*(1-3), 41-57. doi: 10.1007/s12026-012-8297-3

Yadav, A., Saini, V., & Arora, S. (2010). MCP-1: chemoattractant with a role beyond immunity: a review. *Clin Chim Acta, 411*(21-22), 1570-1579. doi: 10.1016/j.cca.2010.07.006

Yang, J., Zhang, L., Yu, C., Yang, X. F., & Wang, H. (2014). Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark Res, 2*(1), 1. doi: 10.1186/2050-7771-2-1

Zawada, A. M., Rogacev, K. S., Rotter, B., Winter, P., Marell, R. R., Fliser, D., & Heine, G. H. (2011). SuperSAGE evidence for CD14++CD16+ monocytes as a third monocyte subset. *Blood, 118*(12), e50-61. doi: 10.1182/blood-2011-01-326827

Zhu, L., Yin, Y. G., Zhou, R. F., Lin, J., Li, J. M., & Ye, J. (2015). Changes of monocyte subsets in patients with acute coronary syndrome and correlation with myocardial injury markers. *International Journal of Clinical and Experimental Pathology, 8*(6), 7266-7271.

Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D. N., . . . Lutz, M. B. (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood, 116*(16), e74-80.

Ziegler-Heitbrock, L., & Hofer, T. P. (2013). Toward a refined definition of monocyte subsets. *Front Immunol, 4*, 23. doi: 10.3389/fimmu.2013.00023

Zouaoui Boudjeltia, K., Moguilevsky, N., Legssyer, I., Babar, S., Guillaume, M., Delree, P., . . . Remacle, C. (2004). Oxidation of low density lipoproteins by myeloperoxidase at the surface of endothelial cells: an additional mechanism to subendothelium oxidation. *Biochem Biophys Res Commun, 325*(2), 434-438. doi: 10.1016/j.bbrc.2004.10.049