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The Effect of Total Work Performed During Acute Heavy Resistance Exercise on Circulating Lymphocytes in Untrained Men

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The Effect of Total Work Performed During Acute Heavy Resistance Exercise on Circulating Lymphocytes in Untrained Men

Neil Anthony Kelly Jr.

B.S., The College of New Jersey, 2009

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APPROVAL PAGE

Master of Arts Thesis

The Effect of Total Work Performed During Acute Heavy Resistance Exercise on Circulating Lymphocytes in Untrained Men

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Abstract

Lymphocytes are one of many leukocytes which exert a biphasic response to acute intense resistance exercise. Exercise volume (i.e. total work) has been shown to have an immunomodulatory effect. This study evaluates the effect of total work performed during an intense resistance exercise protocol on lymphocyte concentrations in the circulation. Untrained, college-aged (18-35) males who performed high amounts of work (HW) were compared to those who performed low amounts of work (LW). Resistance exercise testing consisted of 6 sets of 10 repetitions of the squat exercise with 2 minutes rest between sets. Both HW and LW performed the same relative intensities (~60% 1RM) throughout the test. At 15 min post-exercise the HW group displayed a significantly higher amount of NK cells in circulation when compared to the LW group. No significant differences were found between groups for any other lymphocytes or physiological responses (i.e. lactate, heart rate), however, both groups suffered lymphocyte suppression 60 min post-exercise. Differing strength levels and thus varied total work performances during a relative acute heavy resistance exercise bout did not result in differences in the time course or pattern of immunomodulation during recovery from exercise. Elevations in NK cell concentrations in the HW group point toward an influence of total work on differential NK cell recruitment patterns in males.

CHAPTER 1

INTRODUCTION

Exercise displays an immunomodulatory effect that could impact the immune system's ability to monitor and protect an individual from disease and to repair damage (40). It is well known that acute exercise is associated with hormonal changes which are known to have an effect on subsequent immune responses (4)(7)(17)(30)(43)(52). In addition to the neuroendocrine influence on immune parameters, exercise-induced muscle damage has been shown to result in transitory increases in leukocyte numbers in circulation (36).

The general leukocytotic response patterns to exercise are well established (9)(19)(13) and although many of the mechanisms of the inflammatory response to exercise are unknown, it is thought that the primary functions of exercise-induced leukocytosis are: attack and breakdown of debris; clearance of cellular debris; and regeneration of muscle cells (2)(20)(35)(42)(47). Lymphocytes are the principal leukocytes responsible for adaptive/specific immunity. In response to an acute bout of intense resistance exercise, lymphocytes have been shown to increase immediately post-exercise, drop below baseline during short-term recovery (i.e. 1-4 hrs), and gradually reach back to baseline during long-term recovery (i.e. 24 hrs) (3)(13)(16)(21)(22)(25)(41).

The current clinical significance of lymphocyte fluctuations focuses on immunosuppression. During the 1-4 hours following exercise lymphocyte counts decline below baseline values. This is termed the 'open window' (50), and is thought to be the time at which individuals are more susceptible to infection. These lymphocyte fluctuations vary depending on specific acute program variables of exercise.

Exercise volume and intensity are two key modulators of hormonal changes, muscle damage, and immune responses to acute exercise (9). Commonly in between-group-designed acute resistance exercise testing protocols, participants endure the same relative intensities. Although this standardizes the variable of intensity, differences in absolute intensities between subjects will cause total exercise volume to vary. This may result in a varied immune response between individuals of differing strength levels. Total resistance exercise volume is often referred to as 'total work' and the role of total work performed during identical resistance exercise sessions is still unclear.

Two studies have investigated the effect of total work on circulating lymphocyte counts. Dohi et al. (3) found no difference between females performing more and less total work during an identical relative acute heavy resistance exercise test. Using similar methods, Miles et al. (22) also showed no difference in females performing high total work and low total work. However, both studies used only female participants. The effect of total work on lymphocyte counts has never been studied in males. The possibility of a gender difference should not be ruled out based on the current knowledge.

Statement of the Problem

It is the purpose of this study to evaluate whether differences in maximal strength, and thus absolute workload, during an acute resistance exercise protocol influence the magnitude of post-exercise lymphocyte concentrations in untrained, college-aged men.

CHAPTER 2

LITERATURE REVIEW

The immune system is greatly affected by the stressor of exercise. The current review provides background on the components of the immune system and exercise variables that are relevant to the current study design. Coverage of lymphocyte biology and the documented effects of exercise on lymphocyte concentrations in the blood will be given priority. Figure 2.1 displays a basic flow chart which this review will follow to accomplish this.

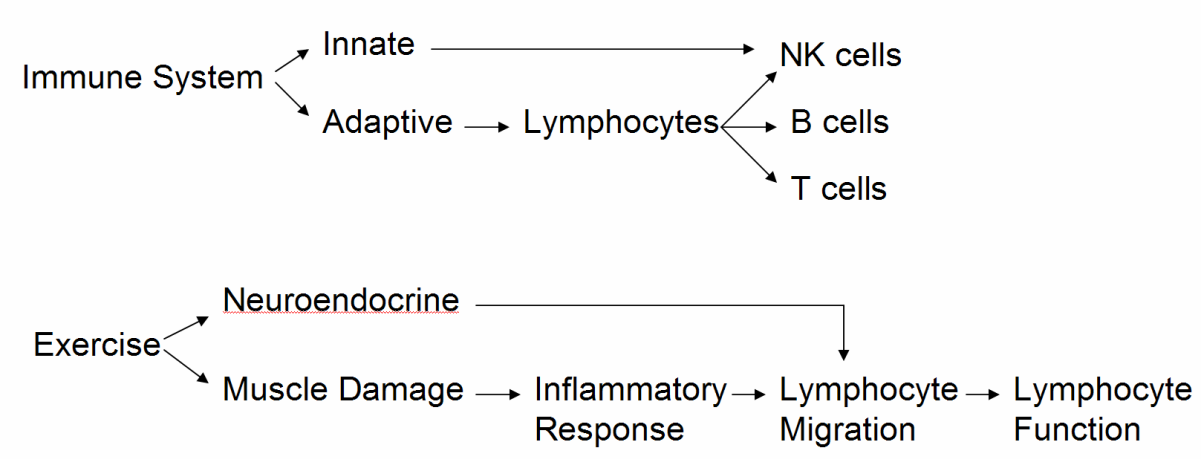

Figure 2.1. Flow chart of review content.

Immune System & Leukocytes

The immune system is a complex, inter-related network of biological structures and processes that serve to protect an organism from pathogens. It can be separated into

two divisions of immunity—innate and adaptive. The innate immune system serves as a first line of defense against pathogens and thus has been named the "non-specific" branch of the immune system. Adaptive immunity is conversely known as the "specific" branch and serves to better protect the organism from re-challenges over time. Both branches of the immune system rely on leukocytes (white blood cells) to protect the body against pathogenic microorganisms, infection, and disease (45). The primary way by which the immune system responds to pathological changes is by increasing leukocyte circulation and proliferation.

The increased concentration of circulating leukocytes is termed leukocytosis. In the adult, leukocytosis is defined as a peripheral white blood cell count of >10,000 mm^{-3} (29). Although total leukocyte count is used to distinguish leukocytosis, there are many differential leukocytes (Table 2.1). All leukocytes have homeostatic mechanisms of production, release, and elimination. These regulatory mechanisms influence which types of leukocytes will be increased and how the leukocytes will be released during leukocytosis (29).

Table 2.1 Leukocyte Subpopulations. Adapted from (29)

Neutrophils	40-75%
Lymphocytes	20-50%
Monocytes	2-10%
Eosinophils	1-6%
Basophils	1%

Leukocytosis can result from several factors including production and immigration from the marrow into the circulation, demargination from blood vessel

walls, and prevention of emigration into tissues (29). Although typically associated with infection, leukocytosis has been shown to arise from many causes, including exercise.

Exercise has an effect on many of the components of the immune system. Since there are many immune cells and many exercise variables, and due to the objectives of the current study, focus will be directed to lymphocytes and acute exercise throughout this review.

Lymphocytes

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition (10). Lymphocytes have been shown to constitute anywhere from 20-50% of the body's total leukocytes (10)(29) and number in the range of, $10^{10} – 10^{12}$ cells, depending on body size and age.

Most lymphocytes undergo development and maturation in the central lymphoid organs–the thymus and bone marrow. After maturation, lymphocytes continually circulate in the blood and lymph, as well as in the lymph nodes and spleen. This provides the means to deliver immunocompetent cells to sites where they are needed, and to allow immunity that is initiated locally to become generalized. Once activated, lymphocytes are able to enter nonlymphoid tissues, where they express effector functions and eliminate local infections (31).

Individual lymphocytes are specialized to respond to a limited set of structurally related antigens. The specificity of each lymphocyte is controlled by receptors (immunoglobulins) on the lymphocyte's surface which detect specific determinants

(epitopes) of an antigen. Lymphocytes differ from each other based on the structure of the combining region of their receptors and therefore the epitopes it can recognize. This means that the ability of an organism to respond to virtually any non-self-antigen is accomplished by the existence of an enormously heterogeneous group of lymphocytes, each bearing receptors specific for a distinct epitope (31).

Along with the difference in specificity of receptors, lymphocytes also differ in function. The vast group of lymphocytes is subdivided into the three broad categories of B cells, T cells, and natural killer (NK) cells based on function and cell-membrane components.

B Lymphocytes

B lymphocytes are derived from lymphoid progenitor cells, which are derived from hematopoeitic stem cells. The molecular mechanisms that ensure B lymphocyte development occur in adult bone marrow. In short, the key events in B cell development involve commitment to the B lineage and repression of the capacity to differentiate to cells of other lineages, and successful completion of the process of immunoglobulin (Ig) gene rearrangement and the expression of the resultant Ig molecule on the cell surface (31).

A mature B cell can be activated directly or indirectly via an encounter with antigen expressing epitopes which are recognized by its specific Ig. Direct activation, known as “cross linkage-dependent B cell activation”, relies on cross linkages formed between Ig molecules and antigens with repetitive epitopes to stimulate intracellular biochemical signals leading to B cell activation, growth, and differentiation. Indirect

activation, known as “cognate help”, utilizes T cells to help activate B cells. If an antigen can not cross-link with an Ig, as long as it is complementary the B cell can bind and endocytose the antigen. This leads to a cascade of events resulting in class II major histocompatability complex (MHC) molecules being brought to the cell surface. These class II MHCs are ligands for the antigen-specific receptors of CD4+ T cells which now interact with the B cell. The CD4+ T cell ultimately activates the B cell and secretes several cytokines which regulate the B cell growth and differentiation (31).

The activation of B cells serves as preparation for division and differentiation into either antibody-secreting cells or memory cells. The antibody-secreting cells migrate to the bone marrow where they produce antibody for up to a year. Memory B cells allow immune responses to a re-challenge to be greater in magnitude and more prompt, along with producing antibody-secreting cells with higher affinity for the antigen (31).

T Lymphocytes

T lymphocytes derive from precursors in hematopoietic tissue, undergo differentiation in the thymus and are then distributed throughout the peripheral lymphoid tissue and added to the recirculating pool of lymphocytes. T cells can be divided into two classes based on which antigen-binding receptors (TCRs) are expressed. Most T cells have TCRs made of α and β chains, while a second group of T cells express TCRs made of γ and δ chains. The α/β T cells can be broken down into two sublineages: those that express the coreceptor molecule CD4 (CD4+ T cells) and those that express CD8 (CD8+ T cells). CD4+ and CD8+ T cells differ in antigen recognition and mediating regulatory and effector functions (31).

CD4+ T cells are known as the helper cells of the immune system, generally inducing a cellular immune response. However, CD8+ T cells most often develop into cytotoxic T lymphocytes capable of lysing specific antigens. Unlike B cells, T cells do not recognize epitopes, they recognize an MHC. The class of MHC is what differentiates CD4+ T cells (class II MHC) from CD8+ T cells (class I MHC) (31).

The activation of T cells depends on several things. The TCR of a T cell is associated with a set of transmembrane proteins, known as the CD3 complex, that play a critical role in signal transduction. The TCR-CD3 complex must interact with its cognate ligand located within a class I or II MHC. Once cross linkage has occurred between TCR-CD3 and its ligand, a signal is transduced within the T cell via the transmembrane properties of CD3 (31).

T cells develop in the thymus, where initially T cell precursors do not express TCR chains, the CD3 complex, or the CD4 or CD8 molecules. Since the T cell precursors are CD4- and CD8- they are referred to as double negative cells. From the double negative pool, thymocytes that are both CD4+ and CD8+ (double positive) and express low levels of TCR and CD3 develop. These double positive cells further differentiate into single positive, mature CD4+ or CD8+ T cells with high expression of the TCR-CD3 complex (31).

The list of functions for T cells is quite large. T cells mediate many immunological functions including: helping B cells develop into antibody-producing cells, increasing the microbicidal action of monocyte/macrophages, the inhibition of certain types of immune responses, direct killing of target cells, and mobilization of the

inflammatory response. Each effect depends on the expression of specific cell-surface molecules and cytokine secretion (31).

Natural Killer Cells

Natural killer (NK) cells are without a precise molecular definition, however they are currently defined by their function—they spontaneously lyse certain tumor cells. NK cells are typically, but not always, large granular lymphocytes. They develop in the bone marrow from an NK cell progenitor to mature NK cells. An important step in NK cell maturation is NK cell receptor acquisition which prompts significant proliferation while the cells are still immature (31).

NK cells are closely related to T cells with the exception that they have activating and MHC receptors instead of TCRs. The activating receptors allow NK cells to recognize features associated with virally infected cells or tumor cells while the MHC receptors shut off their lytic activity. This is important because infected cells that down-regulate expression of MHC may pass by cytotoxic CD8+ T cells, however, they will not pass by NK cells (31).

Mature NK cells are involved in rapid innate defense to an insult. Although they constitute a small population of cells, they respond efficiently and effectively. The immune response of NK cells is accomplished via two mechanisms. One mechanism involves the expression of multiple activation receptors by each NK cell (this contrasts with TCRs limiting T cells to respond to only one antigen), and thus NK cells can respond to multiple activation receptor ligands. The second mechanism relates to NK cells constitutive expression of cytokine receptors which allow many NK cells to be

stimulated by pro-inflammatory cytokines produced early on in an immune response. Therefore, large numbers of NK cells can respond rapidly during an immune response due to multiple activation and/or cytokine receptors (31).

Inflammatory Response

In order to understand the effects of exercise on the immune system, it is integral to know the effects that the inflammatory response has on the immune system. Ultimately, inflammation represents a complex sequence of events that stimulates an immune response. Tissue damage, whether caused by a wound, invading pathogen, or exercise, induces an inflammatory response. The end result of the inflammatory response is to stimulate a specific immune response to the invasion or clearance of debris caused by damage.

There are three major events of the inflammatory response. First, tissue damage causes a release of vasoactive and chemotactic factors that result in an increase in blood flow and capillary permeability. Second, the increase in capillary permeability leads to an influx of fluid and cells causing swelling. And third, the increased capillary permeability results in an influx of leukocytes in a multi-step process which includes rolling, margination and diapedesis (10).

Although various leukocytes can migrate in this multi-step process, this review is focused on lymphocytes and so will discuss only lymphocyte migration. In order for lymphocytes to enter sites of local inflammation, the cells must adhere to and pass between the endothelial cells that line blood vessels. Endothelial cells express leukocyte-specific cell adhesion molecules (CAMs) which are upregulated during an inflammatory

response. As lymphocytes circulate, they interact with endothelial selectins and CAMs causing adhesion to the endothelial cells. These interactions are brief, however, due to the shear force of the circulating blood which detaches the lymphocyte. In what has been termed “rolling”, lymphocytes reattach and detach from endothelial cells becoming activated by various chemoattractants associated with each endothelial cell along the way. Binding of these chemoattractants to receptors on the lymphocyte membrane triggers a conformational change in the lymphocyte resulting in an increased affinity for a specific CAM (Ig superfamily CAM). Subsequent interaction between a lymphocyte and the Ig superfamily CAM stabilizes adhesion (margination). Once firmly secured on an endothelial cell, lymphocytes migrate through the vessel wall into the tissues (diapedesis) (10).

The specificity of the inflammatory response ultimately dictates lymphocyte trafficking and the homeostatic changes in cell counts seen in the exercise immunology literature (44).

Acute Prolonged Exercise and the Immune System

The immune system has been shown to respond to exercise much in the same way that it responds to clinical-physical stressors (i.e. surgery) and infectious disease states (23). These findings display an immunomodulatory effect of exercise that could impact the immune system’s ability to monitor and protect an individual from disease and to repair damage (40).

Exercise can have both positive and negative effects on the immune system. The relationship between exercise and disease susceptibility is well documented as a “J”-

shaped curve (13) (Figure 2.2). This model suggests that engaging in moderate activity enhances immune function above sedentary levels while engaging in prolonged high-intensity exercise may actually impair immune function. This phenomenon has been widely studied with regard to marathoners' seemingly increased susceptibility to upper respiratory tract infections (26).

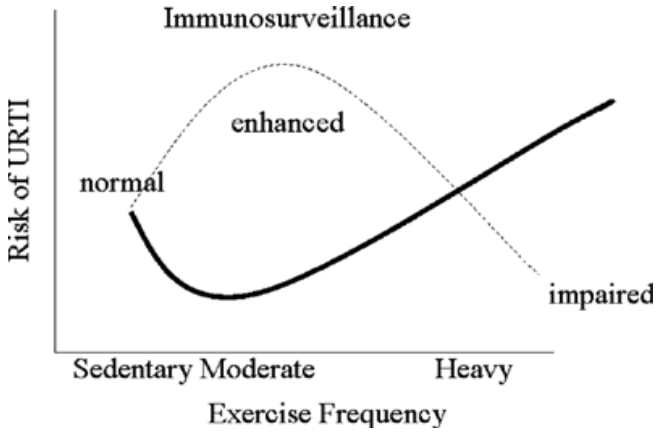

Figure 2.2 Relationship between exercise volume and frequency of contracting URTI. Adapted from Koch 2010 (13).

Many studies have shown that various immune cell functions are transiently impaired following acute bouts of prolonged, high-intensity exercise (9). In general, low concentrations of lymphocytes, suppressed natural immunity, suppressed lymphocyte proliferation, and suppressed levels of secretory IgA in saliva are found simultaneously with high levels of circulating proinflammatory and antiinflammatory cytokines after exercise (34)(13). Although transient, these changes in the immune system are generally more severe with increased exercise volume and intensity (9).

The inflammatory response induced by an acute bout of exercise can be very closely compared to those induced by infection, sepsis, or trauma (33). This response is known as the acute phase response and is characterized by an increase in the number of

circulating leukocytes (primarily lymphocytes and neutrophils) which is typically proportional to both the intensity and duration of exercise (28). There are also increases in the plasma concentrations of various inflammatory markers known to modify leukocyte function (9). Furthermore, it is well known that acute exercise is associated with hormonal changes, including several which are known to have an immunomodulatory effect (i.e. epinephrine, cortisol, growth hormone, and prolactin) (4)(7)(17)(30).

Of these hormonal influences, exercise-induced increases in epinephrine may lead to the demargination of leukocytes (41). Epinephrine has been shown to increase in response to resistance exercise (14) along with an increase in leukocyte sensitivity to epinephrine (25). Epinephrine reduces the affinity of the leukocyte membrane for the vessel wall, resulting in increased leukocyte release into the circulation. Due to the increased blood flow and muscular pumping associated with exercise, the rate at which lymph returns to the blood is greater. This ultimately results in a higher number of lymphocytes in the blood (41). In addition to the neuroendocrine influence on immune parameters, muscle damage has been shown to result in transitory increases in neutrophil numbers in circulation (36). Neutrophils act to infiltrate areas of local muscle damage to eliminate cellular debris and initiate recovery (41). Primary determinants of hormonal response, muscle damage, and immune response are exercise volume and intensity (9).

Lymphocyte changes in response to exercise have been evaluated throughout the literature. In general, lymphocyte concentrations increase during exercise and decrease below baseline values after prolonged exercise (19) (Table 2.2). However, each subpopulation of the lymphocytes does not increase proportionally. For example, the T-

lymphocyte CD4+/CD8+ ratio is decreased (9) reflecting a greater increase in CD8+ T cells than CD4+ T cells. Also, although the absolute number of B cells change, the relative amount of B cells stays constant with exercise (33).

Table 2.2 Effect of strenuous exercise on lymphocytes. ↑, Increase; ↓, Decrease. Adapted from Pederson et al. 2000 (33).

	During Exercise	After Exercise
Lymphocyte count	↑	↓
CD4+ T cell count	↑	↓
CD8+ T cell count	↑	↓
CD19+ B cell count	↑	↓
CD16+56+ NK cell count	↑	↓
Proliferation	↓	↓
NK cell activity	↑	↓

NK cell counts have been studied extensively with regard to exercise and are shown to increase more than any other lymphocyte subpopulation (6). There is a temporary increase in NK cell count in response to acute exercise and following exercise those counts drop as low as half of baseline levels for 2-4 hours; normal resting NK values are usually restored within 24 hours (39). This decrease in NK cell count is accompanied by a loss in NK cell cytolytic activity after exercise, therefore, if the exercise stimulus is both prolonged and of high intensity, both NK cell count and function could decrease during exercise (39).

Lymphocytes have also been shown to exhibit a diminished proliferative response to mitogens (37) and decreased expression of the early activation marker CD69 in response to stimulation with mitogen (24)(37) after exercise. Furthermore, B-lymphocyte Ig production is inhibited (1).

All of the immune responses evaluated in this section have focused on prolonged, high-intensity exercise. This is primarily because until recently other modes of exercise were rarely studied in relation to the immune system. Since exercise of various modes, durations, and intensities induce specific leukocyte recruitment patterns (33) it is a wonder that, until recently, the impact of resistance exercise on immune function had not been studied.

Acute Resistance Exercise and the Immune System

It serves as no surprise that in accordance with prolonged aerobic exercise, resistance exercise also provides an inflammatory stimulus. In both cases there is muscle damage and a subsequent inflammatory response. This muscle damage is currently thought to come primarily from mechanical damage and possibly from metabolic stress as well (12)(46). Although many of the mechanisms of the inflammatory response to exercise are unknown, it is thought that the primary functions of exercise-induced leukocytosis are: attack and breakdown of debris; clearance of cellular debris; and regeneration of muscle cells (2)(20)(35)(42)(47). Leukocytes are attracted to injured muscle cells via various chemotactic factors and infiltrate the tissue in the manner described previously (rolling, margination, diapedesis).

Previous work in the area of resistance exercise and circulating lymphocyte concentrations has shown that resistance exercise causes a similar pattern of lymphocyte response to endurance exercise (3)(13)(16)(21)(22)(25)(41). Although the immune responses to prolonged and resistance exercise serve the same purpose and show similarities in leukocytotic patterns, it seems resistance-exercise-induced leukocytosis is

less pronounced with the exception that lymphocyte response seems to be increased dramatically immediately post-resistance exercise (13) (Figure 2.3).

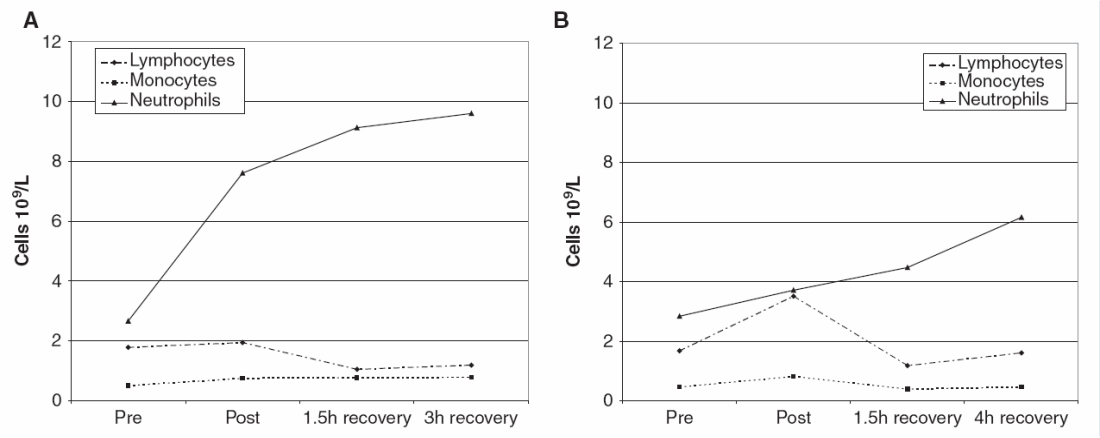

Figure 2.3. Comparison of blood neutrophil, lymphocyte, and monocyte distribution at rest and during recovery from 150 minutes of long-endurance exercise (A) versus 20 minutes of brief, high-intensity resistance exercise (B). Adapted from Koch 2010 (13).

Total Work during Resistance Exercise

Commonly in acute resistance exercise testing protocols, participants from different groups endure the same relative intensities. Although this standardizes the variable of intensity (relatively), differences in absolute intensities between subjects will cause total exercise volume to vary. This may result in a varied immune response between individuals of different strength levels. Total resistance exercise volume is referred to as “total work” and the role of total work performed during identical resistance exercise sessions is still unclear.

Of specific relevance to the current study design, there have been a couple of findings evaluating the lymphocyte response to an acute bout of heavy resistance exercise

with differing workloads. Dohi et al. (3) evaluated lymphocyte responses in eight women with high 1 RM squat strength and eight women with low 1 RM squat strength. Both groups (high strength vs. low strength) of women were matched for age, height, and body mass and completed an acute resistance exercise test of six sets of squat at 10RM with two minutes rest in between sets. By the nature of this testing protocol, the high strength group performed a higher volume of total work. When compared to pre-exercise values, both high strength and low strength groups experienced an increase in T cells, B cells, and NK cells immediately post-exercise. No difference was found in lymphocyte subpopulation response between the high and low strength groups.

Miles et al. (22) expanded on the work done by Dohi et al. (3) by investigating a link between anaerobic intensity, defined as a rise in lactate, and lymphocyte response. Two groups of untrained women were put through a similar protocol of six sets of squats at 75% 1RM for ten repetitions with two minutes rest between sets. It was concluded that workload did not affect lymphocyte response. However, there were significant differences in lymphocyte response when women with the highest rises in lactate (defined as the highest lactate quartile) were compared to women with the lowest rises in lactate (defined as the lowest lactate quartile). The highest lactate quartile experienced a significantly greater rise in T cell and B cell count compared to the lowest lactate quartile.

Although it seems that workload has minimal if any effect on lymphocyte response following acute heavy resistance exercise, both of the previously described studies were characterized by low sample size and female subjects. This effect has never been studied in a larger sample size or males. There may be a possibility of a gender

difference and greater strength disparities among men leading to a more pronounced diversion in total work.

In this study the following hypotheses are tested:

1. Regardless of total work performed, participants will respond with the typical biphasic alterations in circulating lymphocytes. Therefore, both groups will incur immunosuppression during recovery.

2. Greater absolute values of work will provide an increased stimulus to the muscle and thus an increased physiological response. This will lead to a greater inflammatory response and a subsequent increase in the magnitude of lymphocyte count after exercise.

CHAPTER 3

METHODS

Experimental Approach to the Problem

A balanced, between groups design was used to examine the immune response (lymphocyte count) to differing total physical workloads after performing an acute heavy resistance exercise test (AHRET). To reduce experimental variance each subject was familiarized with the AHRET. Familiarizations included a dynamic warm-up and 2 warm-up sets of the squat exercise followed by 6 sets of 10 repetitions of the squat with 2 minutes rest between sets. Load for the squats was set initially at 60% of each subject's pre-determined one repetition maximum (1RM) and modified on subsequent sets to a weight which would only allow 10 repetitions. Lymphocyte count was determined from blood draws taken pre-exercise and 15 and 60 minutes post-exercise. After each set of the AHRET the load and repetitions completed were recorded. Subjects were grouped based on workload performance during the AHRET. Of 84 total subjects, the top ten subjects performing the highest workload were compared to the bottom ten subjects performing the lowest workload for evaluation of the impact of workload on lymphocyte response.

Subjects

College-aged (18-35) men recruited to participate in this investigation were informed of the potential risks associated with their participation and signed an informed

consent document approved by the University of Connecticut Institutional Review Board. All participants were untrained as defined by no regular resistance training within 6 months prior to the study. A total of 84 men participated in the AHRET as part of a larger study. All participants were organized based on total work performed during the AHRET and those performing the highest amount of work (HW; n = 10) and lowest amount of work (LW; n = 10) were grouped. The HW and LW groups demonstrated a significant difference in 1RM squat strength but showed no significant differences in age, height, body mass and body fat percentage (Table 3.1). Body composition was measured using dual-emission X-ray absorptiometry (DXA). None of the subjects were smokers or had any confounding orthopedic, endocrine, or other disorders that would contraindicate their participation. If a subject reported being sick (i.e. cold symptoms) within 72 hours of the AHRET, they were rescheduled.

Table 1.1 Characteristics of the subjects. RM = Repetition maximum

	High Work group (n = 10)		Low Work group (n =10)	
	Mean	SD	Mean	SD
Age (years)	23.0	3.3	22.1	4.9
Height (cm)	169.3	25.3	176.8	7.9
Body Mass (kg)	94.6	31.9	74.3	14.1
% Body Fat	20.6	10.1	25.7	9.1
1 RM Squat (kg)	122.3	17.9	55.9*	5.4
1 RM Squat ($kg \cdot kg\ bodymass^{-1}$)	1.4	0.4	0.8*	0.2
Total Work (kg)	4502.3	506.3	1963.6*	225.8

*$p < 0.05$ from corresponding high strength group value.

Strength Test

Prior to any testing, subjects were familiarized with the proper squatting technique using a Smith machine (Life Fitness: Schiller Park, IL). Subjects reported to the lab at least 48 hours before the AHRET to perform a 1RM squat strength test. Upon

arrival, subjects performed a dynamic warm-up consisting of 5 minutes of cycle ergometer exercise followed by a series of dynamic stretches. Two warm-up sets of squat were then completed, the first at 50% estimated 1RM (8-10 repetitions) and the second at 80% estimated 1RM (2-5 repetitions). Following the warm-up, the 1RM testing protocol consisted of three to five attempts to determine 1RM load in accordance with previous studies (15)(18). The highest mass lifted with proper form was recorded as the 1RM.

AHRET

All subjects performed the AHRET between 6 and 11:30AM after a 12-hour fast. Upon arrival, subject hydration was assessed using refractometry (USG < 1.020). Subsequently subjects were fitted with a heart rate monitor (Polar, Electro, Lake Success, NY) and an indwelling teflon cannula was inserted by a trained phlebotomist into a superficial antecubital forearm vein. After cannula insertion, each subject rested quietly in the seated position for 10 minutes before a pre-exercise blood sample was obtained (i.e. leukocyte and lactate). Subjects then performed the same dynamic warm-up used for the 1RM test followed by one warm-up set of squat at 50% 1RM for 5-6 repetitions.

Next, 6 sets of 10 repetitions of the Smith machine squat were performed with 2 minutes rest between sets. Resistance for the first set was started at 60% 1RM and was subsequently adjusted based on volitional fatigue to attain a 10RM on each set. Heart rate was recorded immediately after each set and those values were used to calculate the average post-set heart rate. Upon completion of the sixth set, subjects immediately consumed a randomized supplement (i.e. carbohydrate, soy, or whey) and were seated in a chair and remained there for 60 minutes post-exercise. A lactate blood sample was

collected immediately post-exercise and leukocyte blood samples were collected at 15 and 60 minutes into recovery from the AHRET. All blood samples were obtained while the subject was in a seated position.

Lactate Concentrations

The liquid lactate assay (Point Scientific #L7596) was performed on human plasma samples as reported by Gutmann et al. (11) and Noll et al. (27), with modifications. 3 µL lactate standards (L7596-STD), controls, and vortexed plasma were added to a 96-well plate. 150 µL Reagent 1 (TRIS Buffer 100mM, 4-aminoantipyrene 1.7mM, Peroxidase (Horseradish) > 10,000 U/L, Surfactant, Stabilizer, Sodium Azide (0.09%) as preservative) was then added to the 96-well plate and incubated at 37˚C for 30 seconds. After incubation, 100 µL Reagent 2 (TRIS Buffer 100mM, Lactate Oxidase (Microbial) > 1,000 U/L, TOOS 1.5mM, Surfactant, Stabilizer, Sodium Azide (0.09%) as Preservative) was loaded onto the plate and incubated at 37˚C for 5 minutes. The assay wavelength was promptly read at 546 nm on a Molecular Devices VERSAmax tunable microplate reader.

Assessment of Blood Cell Populations

Blood cell subpopulations were measured with flow cytometery (FC500, Beckman Coulter) using heparanized whole blood. Aliquots (100 µl) of whole blood were placed in 12mm x 75mm tubes together with 30 µl of a cocktail of fluorescently labeled monoclonal antibodies (Table 3.2). The individual antibodies had been previously titrated. The tubes were incubated at room temperature for 30minutes. The red blood cells were then lysed using 2ml of BD Pharm Lyse buffer (BD Biosciences, San Jose, CA) for

15minutes, vortexed, incubated for another 15minutes and centrifuged at 300 X G for 5 minutes. The supernatants were aspirated, and the cell pellet resuspended in 2ml wash solution (10% PBS, 1% FBS, 1% sodium azide) using sterile filtered research grade water [0.22pm Millipore Steritop disposable, presterilized bottle top filters; Millipore Corporation, Billerica, MA]. The cells were centrifuged (300 X G) for 5 minutes, the wash solution was aspirated, and the cells were resuspended and fixed with 500ul of BD Stabilizing Fixative. 50µl of well mixed SPHERO AccuCount Ultra Rainbow Fluorescent Particles (Spherotech, Inc., Libertyville, Illinois) were added to the stabilizing fixative solution of the tubes dedicated to determining the white blood cell subtype populations and vortexed. The fixed, labeled cell suspensions were kept at 4°C for up to two days before being analyzed. Forward scatter and side scatter were used to estimate lymphocytes, monocytes and granulocyte populations. PE-Texas red (ECD, Beckman Coulter) was used to gate the lymphocyte populations for further analysis based on fluorescent antibody binding. Appropriate isotype controls were used to determine negative staining. CPX Software was used to calculate the percentage of cells in each population. Absolute numbers of cells were calculated using the SPHERO beads added to selected samples. Viability of the blood leukocytes was determined by adding 7-AAD viability dye (Beckman Coulter) to selected tubes of blood in each batch. Viability was generally between 85 and 95%.

Table 3.2. Panels of antibodies used to analyze blood cell population. All lymphocytes were gated with PE-Texas Red CD45 (Beckman coulter PNIM271OU). All other antibodies were purchased from BD Pharmingen ™

Antibody (anti-)	Product	Catalog No.	Cell
Panel A			
CD3	FITC	555339	T lymphocytes
CD8	PA	555367	T cytotoxic lymphocytes
CD4	APC	555349	T helper cells
CD45RA	PE-Cy7	337167	Naïve T Cells
Panel B			
CD 19	FITC	555412	B lymphocytes
CD 56	PE	555516	NK cells
CD 16	FL4	555408	NK Cells
CD 3	APC-Cy7	557832	T lymphocytes

Statistical Analyses

The data sets met the assumptions for linear statistics. Changes in lymphocyte subset concentrations over time (pre-, 15min, 60 min) were compared between groups (HW vs. LW) using a two-way analysis of variance (ANOVA) with repeated measures (2 X 3). Planned comparisons include a repeated contrast for time and pairwise comparisons with Fisher's LSD (all findings were robust, even against the more conservative Bonferroni adjustment). Separate one-way ANOVAs were performed to compare anthropometric, performance test results, lactate, and average post-set heart rate between groups. The variable of supplement was used as a covariate in all analyses which ruled out any effects of acute supplement ingestion. Significance was set at $\alpha = 0.05$.

CHAPTER 4

RESULTS

Lymphocyte Number

There was a significant main effect for time seen in total lymphocytes ($F_{0.05: 2,36}$ = 28.358, p = 0.000), T-CD3 cells ($F_{0.05: 2,36}$ = 11.233, p = 0.000), T-CD4+ cells ($F_{0.05: 2,36}$ = 7.588, p = 0.002), T-CD8+ cells ($F_{0.05: 2,36}$ = 29.198, p = 0.000), B cells ($F_{0.05: 2,36}$ = 13.176, p = 0.000), and NK cells ($F_{0.05: 2,36}$ = 34.417, p = 0.000) (Table 4.1). Only in the HW group were total lymphocytes, T-CD8+, and NK cells increased from pre- to 15 min post-exercise. Both HW and LW groups experienced a significant decrease in all circulating lymphocyte subsets from pre- to 60 min post-exercise. The HW group also decreased significantly in all lymphocyte subsets from 15 min post- to 60 min post-exercise whereas the LW group decreased significantly in all lymphocyte subsets except for T-CD4+ from 15 min post- to 60 min post-exercise.

Table 4.1 Circulating lymphocyte subset concentrations ($x10^9L^{-1}$) for HW and LW groups

	High Work			Low Work		
	Pre	+15	+60	Pre	+15	+60
Lymphocytes	3.24 ± 1.02	4.12 ± 1.44†	2.14 ± 0.69†‡	3.24 ± 1.15	3.07 ± 1.01	1.99 ± 0.53†‡
T-CD3	2.40 ± 0.86	2.39 ± 0.92	1.67 ± 0.53†‡	2.27 ± 0.93	1.98 ± 0.73	1.52 ± 0.46†‡
T-CD4+	1.33 ± 0.50	1.43 ± 0.53	0.89 ± 0.49†‡	1.25 ± 0.64	1.02 ± 0.46	0.93 ± 0.29†
T-CD8+	0.91 ± 0.36	1.19 ± 0.44†	0.54 ± 0.15†‡	1.04 ± 0.41	0.98 ± 0.40	0.60 ± 0.22†‡
B	0.24 ± 0.11	0.26 ± 0.13	0.17 ± 0.07†‡	0.21 ± 0.12	0.21 ± 0.10	0.16 ± 0.08†‡
NK	0.30 ± 0.16	0.77 ± 0.41*†	0.12 ± 0.05†‡	0.39 ± 0.17	0.45 ± 0.11	0.16 ± 0.09†‡

Values are mean ± SD. *$p < 0.05$ between groups; †$p < 0.05$ compared with Pre; ‡$p < 0.05$ compared with +15.

There was also a significant main effect of quartile for NK cells ($F_{0.05:\ 1,18}$ = 5.457, p = 0.031) (Figure 4.1). HW displayed significantly higher circulating NK cells than LW at 15 min post-exercise.

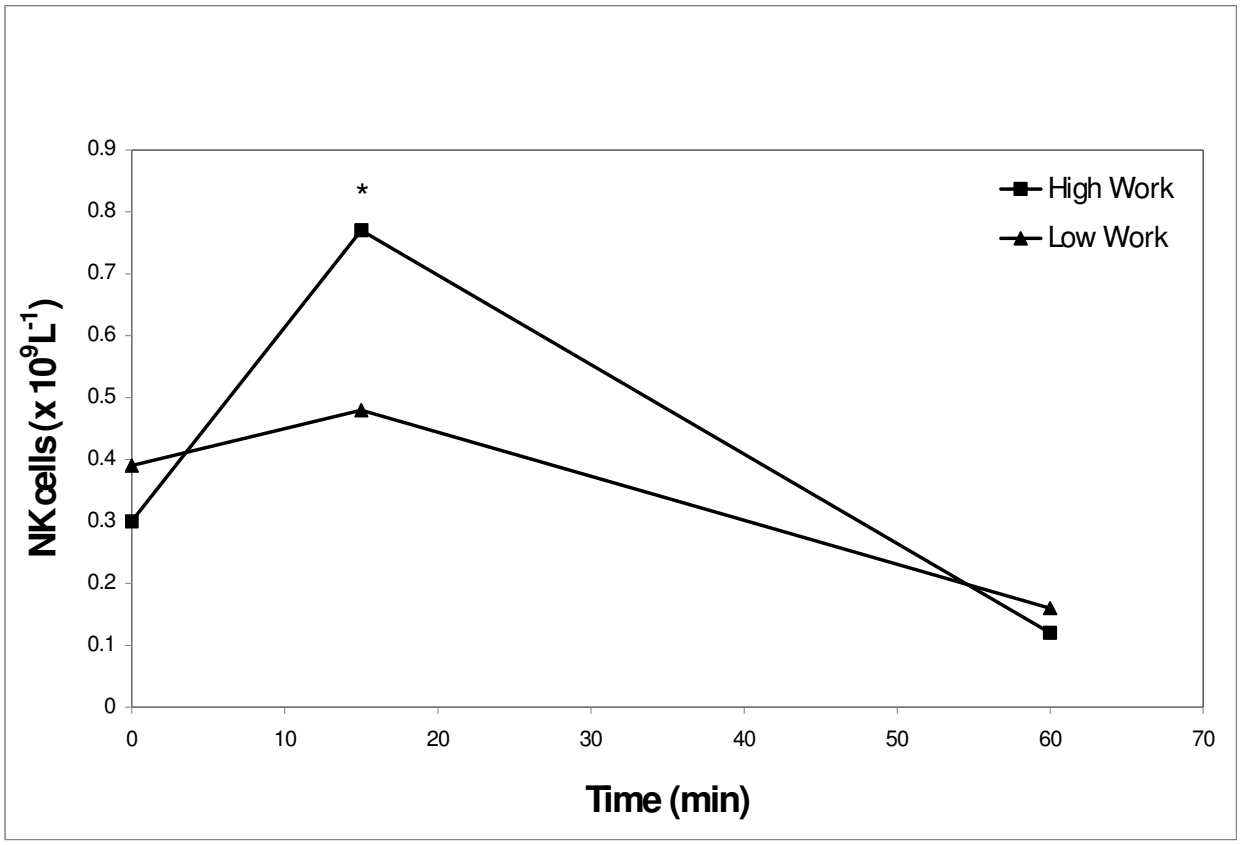

Figure 4.1 NK cell responses to acute resistance exercise in untrained men. Note the difference in NK concentrations between HW and LW groups at 15 min post-exercise. *$p < 0.05$.

Lactate and Heart Rate

The AHRET significantly increased serum lactate concentrations in both HW and LW. There were no significant differences between HW and LW in average post-set heart rate or post-workout lactate (Table 4.2). Pre-exercise heart rate for LW was significantly

higher than HW which is likely due to “white coat syndrome” resulting from high anxiety levels (49).

Table 4.2 Lactate (mmol·l-1) and average post-set heart rate (bpm) values in response to acute heavy resistance exercise.

	High Work		Low Work	
	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise
Lactate	0.98 ± 0.38	11.60 ± 2.57†	1.18 ± 0.42	12.35 ± 2.96†
Heart Rate	68 ± 10	164 ± 17†	78 ± 8*	173 ± 12†

Values are mean ± SD. *$p < 0.05$ between groups; †$p < 0.05$ compared with Pre

CHAPTER 5

DISCUSSION

The current study provides an analysis of lymphocyte circulation in response to an intense resistance exercise stress. The primary finding is that NK cell concentrations were significantly higher in the HW group when compared to the LW group at 15 min post-exercise. Otherwise, all lymphocytes exhibited the same response to exercise regardless of total work performed. The latter results agree with previous investigations which indicate that the total workload during a bout of resistance exercise plays no significant role in resultant lymphocyte responses (3)(22). However, a disparity between NK cell responses in relation to total work performed has never been found.

Many studies have shown there to be a large increase in NK cell concentrations during and immediately after exercise (8)(48)(50). Therefore, it does not serve as a surprise that NK cells increased in the circulation for HW from pre- to 15 min post-exercise. Also it is well accepted that during recovery from exercise NK cell concentrations will decrease steadily even below baseline much like what is found at 60 min post-exercise for both groups in the current study. The fact that LW NK cells are not significantly elevated from pre- to 15 min post exercise and are significantly lower than HW NK cells at the same time point is interesting. Although the recovery rate of both HW and LW resulted in similar NK cell depression at 60 min post-exercise, the disparity at 15 min post-exercise implies that the performance of increased total work leads to an increased NK cell release into circulation.

The HW and LW groups were similar in body composition and anthropometrics which could mean that the musculature of the HW group endured an increased absolute loading and thus suffered more mechanical stress. However, total work most likely influences NK cell release via a differential neuroendocrine response. It is very possible that the perception of lifting an increased load led to an increased anticipatory secretion of catecholamines (i.e. epinephrine) (5). Natural killer cells, like other lymphocytes, express β-adrenergic receptors and thus respond to catecholamines by increasing numbers in circulation (32)(52). Conversely, increases in cortisol concentrations have been shown to decrease NK cell numbers (51)(52)(8) and thus it may also be possible that LW incurred an increased glucocorticoid-producing stress response.

Although all lymphocytes will respond to epinephrine and cortisol, each subpopulation of the lymphocytes does not respond proportionally. NK cells constitute a small population of cells, however, they respond efficiently and effectively. NK cell counts have been studied extensively with regard to exercise and are shown to increase more than any other lymphocyte subpopulation (6). Therefore, even slight differences (which may not be statistically significant) in circulating levels of catecholamines or cortisol would manifest more noticeably in the NK cell response as compared to T or B cells.

With regard to all other lymphocyte subsets and time points there were no differences between groups. Firstly, it can be concluded that total work performed during an acute heavy resistance exercise bout does not impact immunosuppression at 60 min post-exercise. Furthermore, it is likely that, despite strength disparities, the HW and LW groups were a homogeneous sample of physiological and immune responders to

resistance exercise. No differences were observed in lactate and average post-set heart rate between HW and LW. These measures give insight into epinephrine concentrations and shear vascular stress, which are two primary causes of lymphocyte increases in circulation (48)(32). Lactate and epinephrine concentrations in response to acute high intensity exercise have been well correlated (38), while heart rate provides half of the equation for cardiac output, and thus fluctuations in shear stress within the vasculature.

The current study follows work performed by Dohi et al. (3) and Miles et al. (22). Both studies were conducted with similar methods to the current study; however, they only compared lymphocytes at pre-exercise and immediately post-exercise in women. The current study expands this knowledge to show that there were no protective effects of strength, or lack there of, in terms of the lymphocyte immunosuppression suffered at 60 min post-exercise by both groups. Thus, it seems total workload during a relative resistance exercise test does not modulate the 'open window' model of infection susceptibility. Also, based on the current data, it appears there may be a gender effect with respect to total work and NK cell response. This is an interesting finding and one that should be followed up on in future research.

In conclusion, differing strength levels and thus varied total work performances during a relative acute heavy resistance exercise bout did not result in differences in lymphocyte immunosuppression. Individuals who performed more total work had significantly higher concentrations of circulating NK cells at 15 min post-exercise than those who performed less total work. Otherwise, the lymphocyte and physiological responses of both groups were similar.

Practical Applications:

The immunosuppression which results from high intensity exercise is unavoidable even between those who express high levels of strength and those who express low levels of strength when a relative resistance exercise protocol is employed. Untrained men who may be tested for strength or high intensity endurance abilities prior to the beginning of a strength and conditioning program should not be treated differently as long as the testing is relative. An interesting, and as yet unexplained, phenomenon of elevated NK cell concentrations in stronger individuals may exist. The implications of this finding are unknown, however, this does not effect the time course or pattern of immunomodulation during recovery from exercise.

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APPENDIX A

Consent Form for Participation in a Research Project

University of Connecticut

Principal Investigator: Jeff S. Volek and William J. Kraemer

Study Title: The effects of supplementation on responses to resistance exercise

Invitation to Participate

You are invited to participate in this study designed to examine the effects of dietary supplementation with protein versus carbohydrate on responses to resistance training. Resistance training is well known to result in increases in muscle size and strength, but the effects on other health related markers are not as well studied. This project will examine how diet and supplementation with protein and carbohydrate alter responses to 9 months of resistance training in healthy men and women.

Description of Procedures

This research study will take place at the University of Connecticut (UConn) in Storrs and will last approximately 9 months. For this study, you will be required to follow a specific diet and supplementation program and perform resistance training in our facility three times per week for a nine month period. This is specifically what will happen during the research study:

Screening Visit: You will initially be screened, which will include assessment of your medical, nutrition, dietary supplementation, menstrual, and exercise history. We will also determine your height, weight and blood pressure. This visit will take about 30 minutes. We are looking for men and women between 18 and 35 years of age who have not been regularly participating in a high intensity resistance training program. You will be excluded if any of the conditions below are true:

Exclusion Criteria:
1) You have partipated in a resistance training program within the last year.
2) Your body weight is more than 320 pounds.
3) Your blood pressure is more than 150/95.
4) You have diabetes.
5) You regularly use tobacco products.
6) You take cholesterol lowering or blood pressure medications.
7) Your have lost or gained more than 7 pounds in the last 3 months.
8) You are taking anti-inflammatory medication (aspirin, NSAIDs).

9) You consume alcohol more than 3 drinks/day or 18/week.
10) You are pregnant or intend to become pregnant during the 9 mo study period.
11) You have an abnormal menstrual phase.
12) You have an allergy to whey or soy protein.

If you qualify based on the screening visit, we will schedule you for testing. There are a series of tests we will conduct before you start the diet and training portion of the study in order to determine your baseline fitness level. These tests are listed below followed by a brief description of the procedures we will use. We should be able to complete all these tests in three separate visits, but we may need to schedule additional visits depending on your availability.

Testing Measures:
All these tests will be done at baseline and 9 mo of diet and training. In addition, some test will be performed at 3 and 6 months as indicated below. Thus, you will be tested on four separate occasions. We will be asking you to fast for about 12 hours overnight before coming to the laboratory for testing. This means no food or drink that contains calories (including coffee) but you should drink plenty of water. We want you to be well hydrated during all tests. You must also avoid alcohol and strenuous exercise for at least 36 hours prior to coming to the laboratory for testing.

Body weight will be measured on a digital scale.

Body composition (fat, lean, and bone weight) will be determined at four times (baseline, 3, 6, and 9 months) using a machine that will expose you to a small amount of X-ray radiation. You will lie quietly on a table while a scanning arm passes over your body from head to toe. You must remain still for about 5 min during this test. A certified X-ray technician will perform the scan. We will also measure the amount of water in your body by placing two electrodes on your arm and leg while you are comfortably lying down. These tests will take about 1 hour.

Muscle shape will be determined with an ultrasound machine at four times (baseline, 3, 6, and 9 months). We will place a small probe on your upper leg in order to capture various images of the underlying muscle and fat tissues. This test will take about 30 minutes.

Resting Blood pressure will be measured at four times (baseline, 3, 6, and 9 months) by putting a cuff around your arm while you are comfortably seated. Resting blood pressure will take about 15 minutes. We will also attach a monitor that you will wear for an entire day during which time blood pressure and heart rate will be electronically recorded. This will give us an indication of your average blood pressure during the day.

Physical performance will be measured at four times (baseline, 3, 6, and 9 months) by having you lift the most weight in a bench press and squat exercise. Following a standardized warm-up, you will be given multiple attempts to lift as much weight as possible in good form on a specialized machine in our laboratory. Using these same movements, we will assess isometric maximal strength. For this test, you will press up

against an immovable bar as hard as possible while we measure your force output. Muscle power will be assessed in the same movements (squat and bench press). We will load the bar with 30% of your previously determined maximum and ask you to perform the movement in an explosive manner to generate as much power as possible. We will also assess your power by having you jump as high as possible off a force platform while you keep your hands on your waist. These tests will take about 1 hour.

Metabolic rate will be determined twice (baseline and 9 months) early in the morning after you have been lying down on a table for 30 minutes. A ventilated canopy will be placed over your head so we can collect your expired breath for about 20 minutes. The expired breath that is collected will be analyzed for oxygen and carbon dioxide content so that we can calculate the amount of energy (kcal) you are burning. During the test you will be required to rest quietly and breath normally but you will not be allowed to fall asleep. We will also ask you to collect your urine in a container for a 24-hour period starting on the morning of the visit for resting metabolic rate testing. This test allows us to determine how many calories you burn during the day while at rest. This test will take about 1 hour.

Blood will be taken from a vein in your arm to assess resting levels of several health related markers (lipids, hormones, etc.). The amount will be equal to about ½ cup. Thus, over the four visits at baseline, 3, 6, and 9 months we will collect 2 cups of blood total. We will be freezing a portion of your blood that may be used at a later point in time to analyze for specific genes affecting your response to the diet and exercise training. We will not share the results of the genetic analysis with you because they have no direct benefit to you. The blood draw will take about 20 min.

An ***Acute Resistance Exercise Test*** will be performed twice (baseline, 3, 6, and 9 months) to assess how your body responds to an exercise bout. For this test, we will put a flexible catheter into a vein in your arm so that we can draw blood before exercise, immediately after exercise, and 15, 30, and 60 min post-exercise. The total amount of blood during this test will be a little more than ½ cup. The exercise bout will consist of a warm up followed by 6 sets of 10 maximal repetitions of squat. This test will only be done at baseline and after 9 months of diet and training and will take 90 minutes. Thus, the total blood from these tests will be one cup. The total amount of blood collected during the whole study including the resting blood will be a little more than 3 cups.

Supplementation and Diet Assignment:

After baseline testing, you will also be randomly (like pulling a number out of a hat) placed into one of 3 groups. You may also request to be in a control group that only performs the testing described above but does not participate in the supplementation and resistance training.

1. Carbohydrate Supplementation + Resistance Training
2. Whey Protein Supplementation + Resistance Training
3. Soy Protein Supplementation + Resistance Training

Depending on your group assignment, you will be provided with a 2-week supply of the supplements and instructed to consume one serving per day with breakfast on non-training days and immediately after exercise on training days. Each serving contains about 190 kcal. Since it is critical you take the supplement every day, we will ask you to record the time you consumed the beverage each day on log sheets.

In addition to being randomized to a supplementation group, we will counsel you to follow a diet that is designed to meet your caloric needs and that contains a specific amount of protein that should remain constant over the 9 months. The diet will follow general diet guidelines (55-60% carbohydrates, 15-20% protein, and 25-30% fat) emphasizing restriction of saturated fat (<7%) and cholesterol (<300 mg/day). Counseling will focus on making healthy carbohydrate choices, encouraging whole-grain products, fruit and vegetable intake, and lean protein sources.
In order to help you with the diet and monitor compliance, we will ask you to complete a 5-day food record every month. You will be given a small scale to weigh food and specific instructions on how to complete the food logs. We will also ask you to attend regular nutrition meetings one time every two weeks. One of the meetings will be a group meeting and the other a one-on-one meeting with one of our study nutritionists. During the meetings, we will provide you with specific diet advice to help you follow the appropriate guidelines and enhance motivation. We will give you educational materials and counseling regarding the diet including specific lists of appropriate foods, recipes, and example meal plans to help you with the diet. To help with motivation and nutrient assessment, we will be providing you with a Personalized Digital Assistant (PDA) with Palm operating system that has nutrient analysis and graphing software. You will be asked to record the food you eat during a 5-day period each month of the study using the PDA. We will provide you with specific training to make sure you feel comfortable with the software and operation of the device.

Resistance Exercise Training:
All groups will perform resistance training. Training will occur three times per week. We will have designated times you can come to our facility in the Human Performance Laboratory. All sessions will be supervised by a certified personal trainer (CSCS). The program will include a variety of exercises to stimulate major muscle groups and provide variation. The entire workout will take approximately 1 hour.

Risks and Inconveniences

Supplementation Protocol. You should not be in this study if you have any major medical problems. If you are unsure, discuss your health history with the Principal Investigator. There are very few potential risks associated with the procedures used in this study. You should inform us if you have an allergy to soy or whey protein in case you are selected to be in one of these supplementation groups.

Blood Draws. Blood draws with a needle may cause discomfort at the puncture site and the development of a slight bruise. You may also experience lightheadedness or fainting during the blood draw. There is a slight risk of infection from these procedures. All possible precautions to avoid infection will be taken including use of sterile disposable

needles, drapes and gauze and the practice of aseptic techniques during blood sampling. All blood samples will be obtained by trained people. You should refrain from giving blood during the course of the study.

Body Composition. You will be exposed to a very small amount of radiation by the scanner used to measure your body composition. Exposure to any amount of X-ray radiation, no matter how low, may cause abnormal changes in cells. However, the body continuously repairs these changes and the amount of radiation is very low in this study. The total exposure for a whole body scan is approximately 125 times less than the average radiation from a standard chest x-ray. Thus, the radiation levels are extremely low and the health risk minimal. We don't know what effect the radiation could have on an unborn baby so pregnant women should not be in this study. As a precaution we will ask women to take a urine pregnancy test before the scan. For the muscle shape measures, there are no known harmful effects from the use of ultrasound.

Resistance Training and Testing. Even though the resistance exercise program and testing protocols are designed to be safe, there is the risk that you may become injured. The researchers have an extensive experience in conducting short-term and long-term exercise studies, and they will do everything possible to reduce the chance of injury. Every effort will be made to make the study safe by proper supervision of proper technique during testing and exercise sessions. However, if you experience pain, unexpected discomfort, soreness, headache, loss of concentration, dizziness, unusual fatigue or difficulty breathing you should immediately inform one of the supervising members of the research team, who will bring this to the attention of the principal investigators and the medical monitor. The performance of resistance exercise can entail a certain degree of risk from overexertion and/or accident. There are minimal risks for muscle strains or pulls of the exercised muscles. In very rare cases you can experience muscle spasms or tears. Some muscle soreness may be experienced 24 to 48 hours after exercise and this should completely subside with a few days and have no long-lasting effects. The risk of heart attack, although very small, does exist. The chance of any of these events occurring will be minimized by our screening, selection and monitoring procedures, and by the use of properly conducted research procedures. All the research team members are currently certified in CPR.

Urine Collection: There are no risks associated with the 24 hour urine collection, but this may be inconvenient for you. We will provide you a container that you will be asked to collect all your urine for entire day. You should keep the container refrigerated during the collection period.

Genetic Testing. It is not the purpose of this study to look for or provide you with any medical information or diagnoses relating to your present condition or any other disease or illness. Thus, we will not share the results of the genetic analysis with you. The risks associated with this study are mainly psychological and social. You might worry about having a possible genetic disorder. Although unlikely, there is a possibility that incidental findings might be made such as your risk for a certain disease. Your gene results could be used against you if some of these genes are ultimately shown to predict future disease. This could lead to discrimination, potential loss or difficulty in obtaining employment or

insurance. For this reason, your DNA sample will be identified by a code number, and all other identifying information will be removed. The Principal Investigator will keep a code sheet which links the sample code number with your name locked separately and this will be destroyed after two years. This information will not be disclosed to third parties except with your permission.

Benefits

The results of this study will help to determine the role protein supplementation has on responses to weight training and general health, and therefore contribute to a better understanding of dietary recommendations to enhance health. You will be provided with a facility to train under supervised conditions for 9 months during the study. You will also learn your body composition and will most likely improve your fitness and health status.

Economic Considerations

If you complete all training and testing you will receive a stipend of $400 at the end of the study. The stipend will be prorated if you do not complete the study: $50 after completion of baseline testing, $100 after completion of 3 month testing, and $100 after completion of 6 month testing.

If you are selected for the control group that only performs testing (no training) you will receive $200 for completion of all testing sessions. The stipend will be prorated for those who do not complete the study: $25 after completion of baseline testing, $50 after completion of 3 month testing, and $50 after completion of 6 month testing.

Confidentiality

All the data collected will be kept for a minimum of five years and remain confidential and you will never be identified by name in any reporting of results. Further, the results will not be shared with any person outside the investigation without your consent. The results of this study will be kept in locked cabinets under the supervision of Dr. Volek and Dr. Kraemer. You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

Confidentiality of your genetic information will be of high priority to protect the DNA samples from falling into unauthorized possession. All blood samples for gene testing will be identified by a code number, and all other identifying information will be removed. The code number will be linked to the physiological data already obtained from you. The genetic information will be kept at a separate facility where the genetic testing will be done. This information will be kept electronically and/or in locked files. The code sheet which links your sample code number with your name will be kept in a locked

file and office in a different location at the University of Connecticut. This information will be in hard copy form only and not electronic. The code sheet will be destroyed after two years. Your genetic information will not be disclosed to third parties except with your permission.

In Case of Illness or Injury

In the event you become sick or injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If you require medical care for such sickness or injury, your care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed.

If, however, you believe that your illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802.

Voluntary Participation

You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate.

Do You Have Any Questions?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this project or if you have a research-related problem, you may contact the principal investigator, Jeff S. Volek at 860-486-6712. If you have any questions concerning your rights as a research subject, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Authorization:

I have read this form and decided that _____ will
(name of subject)
participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

_____	_____	_____
Participant Signature:	Print Name:	Date:

Relationship (only if not participant):_____

- I agree that my blood sample may be used for gene testing in this study:
  Initials of participant: _____ YES or _____ NO

- I agree that my blood sample and gene data may be used for unspecified future studies:
  Initials of participant: _____ YES or _____ NO

_____	_____	_____
Signature of Person Obtaining Consent	Print Name:	Date: