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Brent C. Creighton
Unniversity of Connecticut, BrentCreighton@gmail.com

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The Influence of Resistance Training on Primary Hemostatic Responses

Brent Clark Creighton

B.S., University of Connecticut, 2009

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Masters of Arts

at the

University of Connecticut

2011

APPROVAL PAGE

The Influence of Resistance Training on Primary Hemostatic Responses

Master of Arts Thesis

Presented by

Brent Clark Creighton, B.S.

Major Advisor _____

William J. Kraemer, Ph.D.

Associate Advisor _____

Carl M. Maresh, Ph.D.

Associate Advisor _____

Jeff S. Volek, Ph.D., R.D.

Associate Advisor _____

Brian R. Kupchak, Ph.D.

University of Connecticut

2010

Acknowledgements

The completion of this thesis was only possible with the help of my professors, lab mates, friends and family.

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Abstract

Objective: The rise in casualties of acute cardio vascular disease has increased the investigation of potential ways to combat these problems. Long term resistance training has been viewed as one possible approach in helping to reduce the hyperaggragability of platelets following acute strenuous exercise. The present investigation was designed to explore the effects of an acute resistance exercise test (AERET) and recovery on the primary hemostatic system in both resistance trained and untrained individuals.

Methods: Ten resistance trained (RT) (Age, 26.0 ± 1.42 yr; Height, 175.12 ± 2.7 cm; Weight, 79.56 ± 4.29 kg) and ten untrained individuals UT (Age, 26.4 ± 1.97 yr; Height, 170.31 ± 2.36 cm; Weight, 67.88 ± 5.34 kg) performed an AERET (6 sets of 10 repetitions of heavy squats). Blood samples were obtained before exercise, immediately post and at 15, 60 and 120 minutes following the exercise test. Blood samples were analyzed for platelet count, von Willebrand Factor (vWF), Beta Thromboglobulin (β-TG) and Platelet Factor 4 (PF4).

Results: Results found significant differences between the RT group and the UT group for measurements of plasma β-TG. Platelet count, vWF and β-TG all increased significantly following the resistance exercise test. PF4 had no significant change. All measured variables returned to baseline 120 minutes following exercise.

Conclusion: RT individuals demonstrated reduced platelet activation *in vivo* in response to an acute bout of heavy resistance exercise compared to UT individuals. Reduced platelet activation could be attributed to training status as shown by reduction in plasma levels of β-TG measured in the RT group.

Chapter 1: Introduction

Cardio vascular disease (CVD) is the leading cause of morbidity and mortality within the Western world (15). Acute cardiovascular disease, normally onset by strenuous physical exercise, can result in sudden cardiac death, acute myocardial infarctions or stroke. Such acute syndromes result from an increased hyperaggregability of platelets following strenuous physical exercise (25). Increased platelet activation after exercise is thought to be the primary cause of acute CVD aside from preexisting atherosclerosis (54). Such acute events occur most commonly in people with manifestations of chronic plaque deposition (76) as unanticipated disruptions in atherosclerotic plaque further lead to platelet activation and thrombosis. Rupture of atherosclerotic plaque followed by platelet adhesion is clinically termed atherothrombosis, when a thrombus forms over a pre-existing atheroma. Sedentary individuals with increased physical inactivity are at much higher risks of developing CVD and atherosclerosis, predisposing such individuals to subsequent acute syndromes (76).

Inactivity combined with diets high in fat are the two primary causes of unhealthy lifestyles among sedentary individuals (35, 43, 68). These factors coupled with normal aging can lead to reduced muscle mass, dyslipidemia, diabetes mellitus and hypertension, all of which strongly contribute to an increase in atherosclerosis and plaque formation among this population (10, 77). CVD is a largely preventable condition (88) when appropriate measures are taken to modify sedentary lifestyle. Resistance exercise training has been shown to be an effective means for reducing various CVD risk factors by preventing muscle atrophy associated with aging and inactivity (10). Skeletal muscle is

important for glucose and triglyceride metabolism, increasing basal metabolic rate and maintaining a normal healthy lifestyle (10).

Muscular strength is a vital factor in an individual's ability to perform daily activities, such as lifting boxes, moving furniture, and carrying groceries. Hence, improvements in muscular strength permit individuals to live a more independent lifestyle. In addition, regular resistance training improves bone density, (10) strengthens connective tissue, and increases lean body mass (89). Furthermore, reports have demonstrated that an increase in muscular strength allows an individual to work at a lower level of maximal contraction for a given load, (56, 58) thereby placing less stress on the heart and cardiovascular system. Consequently, CVD complications following acute bouts of strenuous exercise have been shown to be significantly less prominent in resistance trained individuals (76). Resistance training clearly improves muscular function; it remains unclear whether this type of exercise can truly reduce the likelihood of cardiovascular and thrombotic events (20, 37).

Platelets, small anucleaet cells that circulate within the blood, are necessary in the process of primary hemostasis. Platelet adhesion, activation and aggregation are the processes comprising primary hemostasis which allow platelets to bind to injured endothelium and form a platelet plug arresting bleeding. Endothelial denudation is the main signal by which platelet activation occurs; however, inflammation or strenuous exercise appear to be other modes which may activate platelets (29). Exercise intensity seems to play a significant role in platelet hyperactivity as increasing intensity leads to a greater hypercoagulability state. The release of catecholamines with exercise also appears to be intensity dependent and may be the primary initiator of platelet activation observed

with exercise, though this has not been fully proven (55). Various studies have shown increases in platelet activation following acute strenuous exercise (2, 3), however these physiological mechanisms are not fully understood.

Exercise plays a beneficial role (28, 36, 57, 65) in helping combat the progression and advent of cardiovascular disease, yet how this occurs is not fully understood. Previous studies (2, 3, 62, 64) investigating the effect of exercise training on primary hemostasis *in vivo* have assessed platelet activation by quantifying different surface receptors and platelet biomolecules such as βeta-Thromboglobulin and Platelet Factor 4. These molecules are released from the α-granules of platelets and can be measured in the plasma to assess platelet activation *in vivo* (40)**.** Additionally, von Willebrand Factor, a glycoprotein released from platelets and stressed endothelium, can also be measured in plasma as a marker of platelet activation and endothelial dysfunction. Ahmadizad et al and El-Sayed et al have shown increases in all three variables following acute resistance exercise (2, 3).

Chronic physical training has shown favorable adaptations such as reductions in platelet activation and aggregation in response to strenuous physical exercise (8, 44, 83). Additional physiological alterations seen with resistance training exercise that may have a beneficial impact on primary hemostasis include an increase in plasma volume (18), an increase in vessel diameter and stability (66), as well as increases in antithrombtic chemicals by the endothelium (9, 30, 33). Evidence suggests exercise training, independent of sex, has a positive effect on platelet activation in both men and women (47). Endurance exercise and its effects on hemostasis has been previously studied (21-24) however there has been a lack of research focused on the influence of habitual

resistance training on the primary hemostatic system and its' possible beneficial effects during periods of heavy physical exertion. For this reason, the purpose of the present investigation was to explore the effects of an acute resistance exercise test on βeta-Thromboglobulin, Platelet Factor 4 and von Willebrand Factor in both resistance trained and untrained individuals.

Chapter 2: Review of Literature

This review will examine the physiology of normal endothelial function as well as the structure and function of platelets. The process of primary hemostasis will be described in moderate detail followed by the implications of platelets in the formation of thrombosis and atherosclerosis. Lastly, this review will conclude with the effects exercise has on primary hemostasis. Both acute and chronic exercise will be evaluated.

What is Hemostasis?

Hemostasis is the process by which the body elicits cessation of the blood at a site of injury while simultaneously keeping blood in a fluid state. The term hemostasis is derived from Latin, *hemo* (blood) and *stasis* (to halt). This process initiates when damage occurs to blood vessels such as from a cut or puncture. However, any damage to the endothelium can elicit the process of hemostasis. Endothelial denudation occurs frequently throughout life, from normal endothelial cell death to infectious agents, acidosis, hypoxia, inflammation, and hypotension (61). Hemostasis can be divided into two distinct pathways: primary hemostasis which comprises the formation of a primary platelet plug and secondary hemostasis which consists of the intrinsic and extrinsic pathways. Primary hemostasis can be further broken down into three dynamic processes that are defined separately yet strongly intertwined and dependent on one another. These processes are platelet adhesion, platelet activation and platelet aggregation.

Normal Endothelial function:

Endothelial cells are not only important in the maintenance of hemostasis but are also essential in the regulation of other physiological processes such as response to

infection and sepsis, alteration of blood flow and wound healing (34, 61). To understand the mechanisms involved in primary hemostasis and conceptualize the interplay between endothelial cells and platelets, it is necessary to first comprehend how the endothelium responds under normal physiological conditions. Endothelial cells line the blood vessels of the body and contain a rod shaped organelle called the Weibel-Palade body (34). These organelles contain von Willebrand factor, a glycoprotein released by damaged endothelial cells which readily binds platelets (34). Endothelial cells continuously secret anti-platelet and anti-coagulant molecules to maintain vascular homeostasis and prevent thrombotic episodes (61). However, when affected by infection, a stressor, hypertension, or dyslipidemia, endothelial cell function can become compromised to the point where it no longer is able to adequately produce these molecules and quickly becomes procoagulant (34, 61).

By inhibiting platelet adhesion, activation and the subsequent recruitment of additional platelets, endothelial cells prevent primary hemostasis from initiating. The only location within the vessel where blood needs to be arrested is at the site of vascular injury. This inhibitive nature of endothelial cells is essential considering blood needs to be maintained in liquid form. To maintain rheological homeostasis, endothelial cells release many different molecules, three of which directly affect primary hemostasis. They include nitric oxide (NO), a naturally occurring gas, prostacyclin (PGI_2) an eicosanoid, and ADP diphosphatase (CD39) (34, 41, 61). Both NO and PGI_2 are synthesized and secreted by endothelial cells and work to inhibit platelet adhesion, activation and aggregation. They also function as vasodilators by relaxing vascular smooth muscle (41). The surface expression of endothelial membrane-associated CD39 helps return platelets

to their resting state and prevents platelet recruitment by metabolizing ADP and ATP, two platelet agonists, to AMP which is non stimulatory (34). Endothelial cells also express heparin sulfate on their surfaces which act as a potent anitcogaluant by participating in the inactivation of thrombin (69). Thus, by secreting various anti-platelet substances, when platelets enter in close proximity to endothelial cells, they become unresponsive.

One other physiological phenomenon that can greatly affect primary hemostasis is shear rate or shear stress. Shear rate reflects the differences in flow velocity, greatest at the center of a vessel, as a function of distance from the blood vessel wall, where it is slowest (69). As blood vessels decrease in diameter, shear rate increases; the reverse is true as well, an increase in vessel diameter leads to a decrease in shear rate. Being highest in small arterioles and lowest in large arteries and veins, shear rates can vary considerably throughout the vasculature (74). These factors determine the number of platelets passing by a single point in a given time interval as well as the amount of time a platelet has to interact with the blood vessel wall or other platelets. The rate of dilution of platelet activating agents and the forces tending to pull a platelet from the vessel wall or another platelet will also be affected by shear rate (74). High shear rates can specifically affect platelet adhesion via a mechanism that involves vWF binding to GPIb/IX followed by intracellular signaling, discussed below.

Platelet Formation:

Platelets or thrombocytes are small, 2-3µm in diameter, anucleated, discoid shaped cell fragments that are fundamental in the process of hemostasis (39, 42). Platelets are one of the primary cell types along with red and white blood cells that circulate within

the blood and average a life span of roughly 10 days in humans with normal platelet counts (42). The average adult human produces roughly 1×10^{11} platelets, a level of production that can increase 10 to 20 fold in times of increased demand, usually from additional platelet stores within the spleen (42). Not only do they carry out their principal role of aggregation and formation of a primary platelet plug at sites of vascular injury, platelets also play a key role in cell cross talk, specifically cells involved with the inflammation process.

Like many other cells within the human body, platelets are derived from hematopoietic stem cells (HSC) within the bone marrow (38). Their production is dependent on the differentiation of HSC to the megakaryocyte lineage (42).This process of platelet generation, known as thrombopoiesis, is regulated by the glycoprotein hormone thrombopoietin (TPO) and in most physiologic and pathologic states the platelet count is inversely related to plasma thrombopoietin levels (42). TPO, a cytokine produced primarily in the liver, increases platelet production by increasing megakaryopoiesis: both the size and number of megakaryocytes are affected (38). TPO bears a very close homology to erythropoietin, a cytokine necessary in red blood cell production (42). HSC exposure to TPO induces proliferation and maturation of megakaryocyte progenitors and induces an amplification of megakaryocyte DNA which leads to the generation of platelet specific proteins and granules (38).

An atypical feature of megakaryocyte development is endomitosis, a unique form of mitosis in which the DNA is repeatedly replicated in the absence of nuclear or cytoplasmic division. This process yields cells that are highly polyploidy, containing more than two paired sets of chromosomes, and prepares the megakaryocyte for platelet

differentiation (42). Following endomitosis, platelet production begins when one pole of the megakaryocyte spontaneously elaborates pseudopodia and microtubules aggregate in the cell cortex (38, 42). These primary large blunt pseudopodia later thin and branch into proplatelets. The branching off of proplatelets from the megakaryocyte seems to be dependent on a localized assembly of actin within the cell, and subsequently can be inhibited by drugs that disrupt actin filaments (38, 42) (42). Intracellular organelles and granules are transported to the platelet buds along microtubule tracks via long pseudopodia-like shafts. Platelets are assembled mostly at the ends of the proplatelets where they are consequently released (38).

Platelet Function:

Platelets are cells adapted to seal damaged blood vessels, aggregate to one another and facilitate the generation of thrombin (74). Under normal conditions platelets circulate and do not interact with other cells or platelets. The initial signal for platelet deposition and activation is stimulation by an agonist; exposure of the subendothelium caused by endothelial cell denudation is almost always the primary agonist (1). Important parameters that control the platelet response include the depth of injury, the type of vascular bed that has incurred damage, age of the individual, hematocrit, and shear rate. Deeper tissue damage exposes a greater amount of platelet-reactive materials and tissue factors. Mucocutaneous tissues rely more highly on platelets for hemostasis than do the vessels within muscles and joints. Hematocrit measures the proportion of erythrocytes, red blood cells, which occupy the blood. An increased number of erythrocytes tend to enhance platelet interactions with the endothelium because erythrocytes occupy the axial

region of the vessel which forces platelets to the periphery of the bloodstream (74). The final product of platelet adhesion, activation and aggregation is the formation of a primary platelet plug which is reinforced when thrombin, a serine protease, converts soluble fibrinogen to insoluble strands of fibrin (74) Fibrinogen is a plasma glycoprotein produced by the liver and its conversion to fibrin is characteristic of secondary hemostasis (74).

Platelets achieve adhesion primarily via their surface receptors which can bind adhesive glycoproteins (74). There is a redundancy to the type and amount of surface receptors found on the platelet membrane with the purpose of enabling the cell to establish multiple contacts (1). Important surface receptors directly involved in binding to collagen, vWF, or fibrinogen are expressed in the greatest quantities (11). These include the GPIb/IX/V complex with roughly 15,000 to 25,000 copies, supporting platelet adhesion by binding von Willebrand Factor, and the integrin $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) receptor with nearly 80,000 copies, which is platelet specific and mediates platelet aggregation by binding fibrinogen and/or von Willebrand factor (74). Platelets also promote activation and aggregation when they become activated and release alpha granule, dense body and lysosomal contents. The molecules released from activated platelets not only affect platelet function, but also impact innate immunity, cell proliferation, vascular tone, fibrinolysis and wound healing (74). Additionally, activated platelets initiate an inflammatory response by expression of surface P-selectin, which mediates interactions with leukocytes, and CD40 ligand, which activates a number of proinflammatory cells and releases chemokines (74). Overall, platelets are not just simply involved with

hemostasis, they are capable of communicating with, and are affected by, other blood cells and endothelial cells.

Platelet Structure:

Structurally, platelets are discoid in shape with a flat undistinguished membrane apart from receptors covering the surface. The platelet plasma membrane is composed of a bilayer of phospholipids in which cholesterol, glycolipids, and glycoproteins are embedded (74). The other feature noticeable within the plasma membrane is the open cannicular system (OCS). This widespread system of internal membrane conduits tunnels through the interior of the platelet and serves as passageways to the outside of the cell into which granular contents are released (38, 74). This system also functions as a reservoir of plasma membrane, membrane receptors, and proteins that can be tapped into during platelet activation (38) Both the OCS and the plasma membrane are supported by a highly structured and well-defined cytoskeletal system (74).

The purpose of platelets is to initiate a primary platelet plug and pave the way for secondary hemostasis. As previously stated, platelets operate by secreting various contents stored within granules inside the cell body. The two primary storage granules, the alpha and dense granules, rarely fuse with the plasma membrane, and instead exocytose into the OCS (38).

Platelet Granule Contents

Dense Granules (Delta):

Platelets contain roughly three to eight electron dense organelles called dense granules or delta granules that range in size from 20 to 30 nm in diameter (74). High

concentrations of positively charged calcium ions are stored within these granules and are what give the organelles their "electron-dense" name (67, 74). Dense granules, the second and smaller of the two platelet granules, secrete mediators that function only for a brief period of time to recruit additional platelets to sites of vascular injury by modulating platelet behavior and endothelial vascular tone (1, 74). Discharge of dense granule content into the OCS is initiated upon platelet activation.

Of the contents stored within the dense granules, serotonin, ADP and calcium are by far the most significant. High concentrations of serotonin within the dense granules are taken up from plasma by plasma membrane carriers and then trapped inside the delta granules (74). Serotonin functions as a potent vasoconstrictor at the point of injury and most likely plays a role in decreasing hemorrhage and facilitating platelet and fibrin deposition via its effect on blood flow (74). Serotonin is a weak platelet agonist where as released ADP acts as a more potent positive feedback agonist (74). In areas where endothelial damage has occurred, ADP levels increase with platelet activation and promote further increases in platelet aggregation. ADP is the primary agonist inducing platelet shape change at the beginning of platelet activation (1). Platelets roll through an area with increased ADP concentrations and bind ADP on $P2Y_1$ and $P2Y_{12}$, purinergic chemoreceptors for ADP, where they become activated through a G-protein-coupled receptor initiated signal transduction pathway (1, 74). As a positively charged ion, calcium plays various roles in platelet hemostasis. It reacts with negatively charged carboxylated glutamic acid residues on coagulation factors and helps attach them to the phospholipid surface of the platelet cell membrane by tightly binding the coagulation

factors down (74). Calcium is also necessary to help fibrinogen and vWF bind to activated $\alpha_{IIb}\beta_3$ receptors.

Alpha Granules (α):

In comparison to dense granules, alpha granules are both larger (200 to 500 nm in diameter) and more abundant, numbering approximately 50 to 80 per platelet (67, 74). Alpha granules contain upwards of 300 identified proteins involved in cell adhesion and coagulation, most of which are stored within the granule itself (1, 74). These identified proteins include adhesive proteins, coagulation factors, protease inhibitors, chemokines, and angiogenesis regulatory proteins; all function to enhance the adhesion of platelets, promote cell–cell interactions, regulate angiogenesis, and stimulate vascular repair (74). Alpha granules also contain glycoprotein receptors in their membranes which fuse with the plasma membrane of the platelet once activated (74). The important receptors expressed in alpha granules include P-selectin, a cell adhesion molecule responsible for leukocyte recruitment, and a portion of $\alpha_{IIb}\beta_3$ and the glycoprotein Ib/IX/V complex (GPIb-IX-V; a receptor for vWF) (74). Alpha granule proteins derive from different origins; some by biosynthesis (primarily at the megakaryocyte level) and others by endocytosis (74).

Of the diverse proteins stored within the alpha granules, platelet factor 4 (PF4) and beta-thromboglobulin are the highest in concentration, roughly 20,000 times higher in the platelet than in the plasma (67, 74). Their high concentrations within the granules enable them to attain a high local concentration when released at sites of vascular injury (74). Their release from platelets has been widely identified as a marker of in vivo

platelet activation. Both molecules have similar amino acid sequence homology, belong to the chemokine family and bind to heparin, but with varying affinities (74).

Platelet Factor 4 (PF4):

The primary role of PF4 when released from platelets is to bind with high affinity to heparin and neutralize heparin's anticoagulant activity (74). When PF4 binds to heparin it forms a "PF4-heparin complex", either free floating in the blood or on the surface of endothelial cells (74) These complexes are important clinically, as they represent the target antigen in heparin-induced thrombocytopenia (74). Other than actively binding heparin, PF4 has also been attributed to numerous other roles including: histamine release from basophils, inhibition of tumor growth and megakaryocyte maturation, reversal of immunosuppression, enhancement of fibroblast attachment to substrata, potentiation of platelet aggregation, inhibition of contact activation and monocyte responsiveness to lipopolysaccharide (74). PF4's clearance from the circulation is dependent on multiple factors. Hepatocytes bind and catabolize PF4 in the liver and so clear PF4 more rapidly than β-TG, which is excreted by the kidneys. In addition, due to its high affinity to heparin, PF4 may be cleared from the plasma as it binds to heparin sulfate on the surface of endothelial cells (74).

βeta-Thromboglobulin (β-TG):

Compared to PF4, β-TG has a lower affinity for binding heparin and thus is less effective at neutralizing heparin. β-TG belongs to the β-Thromboglobulin family which includes: platelet basic protein, low-affinity PF4 (connective tissue-activating peptide III [CTAP-III]), β-thromboglobulin, and β-thromboglobulin-F NAP2 (74). As noted earlier,

PF4 is cleared by the liver while β-TG is cleared from the circulation by the kidney (74). Other than binding to heparin, β-TG is a chemoattractant for fibroblasts, cells which synthesize new collagen and extra cellular matrix tissue (74).

von Willebrand Factor (vWF):

von Willebrand Factor (vWF) is a multimeric adhesive glycoprotein essential in initiating the process of platelet adhesion to the subendothelium and aiding in platelet aggregation (74). vWF is synthesized in megakaryocytes and endothelial cells and is stored respectively within the alpha granules of platelets and the Weibel-Palade body organelles of the endothelial cells (34). Additionally, vWF circulates within the plasma acting as a carrier protein for Factor VIII, stabilizing it within the circulation and protecting it from inactivation or degradation (34). Factor VIII is a procofactor important for clotting. Indivuduals with deficient or defective vWF are diagnosed with von Willebrand Disease (vWD) which is associated with a decreased or inhibited ability to form clots.

vWF is the initial bridge between circulating platelets and damaged blood vessels and is therefore essential to platelet adhesion. When the subendothelium and collagen become exposed, vWF attaches to the surface where it becomes exposed to high shear rates. This exposure to high shear stresses uncovers the vWF A1 domain, the site for platelet- binding (34). vWF factor, by way of deposition from the plasma and release from damaged endothelial cells or activated platelets, binds to the newly exposed collagen (34). Platelets bind to vWF via the GPIb/IX/V receptor complex, which is one of many receptors on the platelet surface. The interaction between vWF and the GPIb/IX/V

receptor complex seems to be most important for platelet adhesion at moderate to high shear force (34).

The bonds formed between vWF and GPIb/IX/V are not strong enough to maintain adhesion of the platelets, and instead, break and reform quickly along the site of vascular injury. This detachment and reattachment of the GPIb/IX/V receptor complex with vWF on the exposed collagen slow the platelet down and cause it to roll along the site of vascular injury. This rolling effect allows the platelet to bind GPVI, a low affinity receptor potent for initiating signal generation, to the exposed collagen which it would have been unable to do at normal flow speeds. These initial interactions between vWF and GPIb/IX/V, as well as GPVI with collagen, result in activation of the integrin receptor $\alpha_{IIb}\beta_3$ (GPIIb/IIIa, which more strongly binds vWF and stabilizes the platelet (34). Attachment of vWF to the luminal side of already adhered platelets promotes additional binding of more platelets. This "stacking effect" is the process of platelet aggregation.

Lysosomes:

The third category of granule organelles found within platelets are lysosomes. Measuring roughly 175-250 nm, lysosomes contain various acid hydrolases and enzymes required for particle digestion and elimination of the platelet aggregate (67). Lysosomal contents require strong agonists in order to be released from the cell, and are thus more slowly and less completely excreted. A specific enzyme, platelet-associated heparatinase, is thought to cleave heparin-like molecules from the surface of endothelial cells producing an antiproliferative particle that may inhibit growth of smooth muscle cells (1, 74).

Primary Hemostasis: A Three Step Process

Platelet Adhesion:

Platelet adhesion is the first step of primary hemostasis and is defined as the process by which platelets adhere to a non-platelet surface other than a platelet. As previously noted, adhesion is initiated when normal endothelial cells sustain damage and the underlying sub-endothelial collagen becomes exposed. vWF factor adheres to the newly exposed collagen either by deposition from the plasma or from released stores in the damaged endothelial cells and activated platelets (34). Exposure of vWF to high shear stresses uncovers the active site on the glycoprotein and allows for platelet-binding (34). The GPIb/IX/V receptor complex on the platelet membrane is the initial receptor that binds to this active site of vWF. The subsequent rolling of these cells over an area of vascular injury exposes them not only to vWF but also to two specific collagen receptors, GPVI and $\alpha_2\beta_1$, which help in adhering the platelet to the vessel wall (11, 29). The interaction between vWF and GPIb/IX/V is not strong enough to hold platelets in place, and therfore is where receptors GPVI and $\alpha_2\beta_1$ play a large role. The initial interactions between vWF and GPIb/IX/V are stabilized by vWF and the integrin receptor $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) soon after platelet activation, deemed firm adhesion (34, 54). Binding of the platelet to these four particular receptors, vWF, GPVI, $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ not only functions to arrest the platelet from circulation but also activates a G-protein coupled receptor in the cell that initiates platelet activation (11, 29).

Platelet Activation:

The initial event of platelet activation is reorganization of the platelets' actin cytoskeleton which alters the cells' discoid shape to an irregular spherical shape with

multiple filopodial projections, also known as platelet spreading (1). Reorganization of the cells' matrix concentrates the cells' contents towards the center, more easily facilitating the release of stored cellular contents. Spreading of the platelet along the injured area also allows the platelet to more strongly adhere to the exposed collagen. Secretion of α and dense granule contents occurs through a complex signaling pathway dependent on small GTPases, protein kinases and members of the SNARE/SNAP family (1). Once agonists bind and induce signaling, granules fuse with the plasma membrane and the OCS.

As previously mentioned, adhesion of platelets to the subendothelium or collagen, particularly the binding of VWF to GPIb/IX/V, as well as thrombin, is suggested to be the primary signal of platelet activation (74). Activation of platelets triggers many intracellular signals other than granule secretion. Activation of phospholipase enzymes breaks down intracellular phospholipids into arachidonic acid, which gets converted to Thromboxane A2 (TXA_2), a potent vasoconstrictor and platelet aggregator. Once formed, TXA_2 can diffuse across the plasma membrane and activate other platelets through signaling pathways (1). Like TXA_2, the release of ADP and serotonin amplify signals to further enhance platelet activation and aggregation. Hormonal regulation of platelets most likely occurs via epinephrine (1).

Agonist binding also results in the activation of $\alpha_{IIb}\beta_3$ receptors on the luminal side of the platelet, leading to the high-affinity ligand-binding conformation (74). Activated $\alpha_{IIb}\beta_3$ receptors bind not only VWF but also fibrinogen and other ligands (29). The binding of these agonists signal $\alpha_{IIb}\beta_3$ receptors via an "inside out" signaling mechanism which can take place within seconds of platelet activation. A mixture of

agonists may play a significant role in platelet activation as agonists are likely to change as the process of primary hemostasis ensues. Many of these platelet agonists initiate platelet activation by binding to seven-transmembrane heterotrimeric, G-protein-coupled receptors. The effect of multiple agonists' is probably both additive and synergistic, depending on the mechanism involved. These positive feedback mechanisms insure an adequate hemostatic response (74).

Secreted platelet contents not only lead to further platelet activation but also have a potent effect on initiating the inflammatory response. This response is driven by the release of vasoactive and mitogenic agents, as well as chemokines and the appearance of P-selectin on the cell surface, a cell adhesion molecule important for the recruitment of leukocytes (74). An α-granule membrane protein, P-selectin is absent from the surface of resting platelets and fuses with the membrane upon cell activation (1).

Platelet Aggregation:

Platelet aggregation is the final step of primary hemostasis and is defined as the process whereby platelets stick or aggregate to one another, forming the primary platelet plug. After the initial platelets adhere to the exposed collagen on the subendothelium, they release the contents of their granules and promote the activation of additional platelets exposing surface receptors and propagating aggregation. The activation of $\alpha_{IIb}\beta_3$ receptors on the luminal side of the platelet, and the subsequent adoption of their high-affinity ligand-binding conformation, promotes further binding of vWF and fibrinogen helping to bridge platelets together (1, 74).

Aggregated platelets have an important role in facilitating thrombin generation by various mechanisms: recruitment of bloodborne tissue factor, synthesis or activation

of tissue factor, formation of procoagulant microvesicles, exposure of activated factor V, exposure of negatively charged phospholipids, and possibly, activation of the contact system (74). Generated thrombin further activates platelets and coagulation and most notably initiates the deposition of fibrin strands. Fibrin helps to reinforce the primary platelet plug as well as serve as an additional site for vWF deposition (74). Furthermore, thrombin appears to help in the transition from platelet adhesion to aggregation by down regulating GPIb/IX/V and up regulating $\alpha_{IIb}\beta_3$ receptors.

The $\alpha_{IIb}\beta_3$ receptor plays a more significant role in platelet aggregation than any other part of primary hemostasis. Due to the extraordinarily high density of receptors on the platelet surface, $\alpha_{IIb}\beta_3$ is critical in determining the extent of platelet aggregation. As previously mentioned, the receptor is not actively in its high-affinity ligand-binding state on resting platelets and instead needs to be activated by such agonists as ADP, serotonin, thrombin, collagen and TXA_2 (74). A number of different signal transduction mechanisms have been identified that convert these agonist signals into a change in conformation of the $\alpha_{IIb}\beta_3$ receptor.

Left unchecked, platelet aggregation can create thrombi which can break off and occlude blood vessels. The endothelial cells' generation of prostacyclin and NO at sites of vascular injury or inflammation may provide a physiological mechanism to help limit platelet accumulation (74).

Implications of platelets in thrombosis and atherosclerosis (atherothrombosis):

Hemostasis is the normal physiological formation and breakdown of a thrombus within the vasculature. The pathological condition, thrombosis, occurs when a blood clot obstructs the flow of blood through the lumen of a blood vessel. Highly recognized as an

inflammatory associated disease, atherosclerosis can be a primary cause of thrombosis especially upon rupture of atherosclerotic plaque (50) The formation of a thrombus due to rupture, tearing or disruption of atherosclerotic plaque is referred to as atherothrombosis, both the acute and chronic manifestation of arterial disease (69). This plaque destabilization exposes the lipid core and collagen, one of the most thrombogenic substances in atherosclerotic plaques upon which platelets adhere rapidly (15, 50, 54).

Thrombosis can, however, occur independently of plaque formation within the vasculature, typically within the venous system where blood flow is decreased resulting in venous thrombosis (15). The buildup of atherosclerotic plaque within the vasculature is of major concern among the Western adult population, especially in the United States, and is the leading cause of cardiovascular disease (15). Consequently, acute coronary syndromes (ACS) resulting in atherosclerotic alterations of the coronary arteries and thrombotic occlusion are the leading cause of morbidity and mortality in the Western world (54). A thrombus formed over an atherosclerotic lesion can easily break off the vascular wall and travel through the circulation leading to occlusion of vessels elsewhere in the body. This mobile thrombus is clinically known as an embolus, and leads to commonly known clinical manifestations such as myocardial infarctions or strokes.

The normal formation and breakdown of clots within the vasculature occurs in response to endothelial perturbations. However, current research suggests that endothelial denudation may not be a prerequisite for platelet activation, and platelet adhesion may persist in response to vascular dysfunction (29). Inflamed endothelial cells develop properties in which they down regulate production of antithrombotic mediators such as NO or PGI_2, rendering them adhesive to platelets. This adherence of platelets to an intact

but inactivated endothelial lining has been demonstrated by in vitro studies both with human and animal models (29, 50). As previously noted, activated platelets secrete numerous thromboinflammatory mediators accelerating inflammatory processes and cell recruitment, namely leukocytes (50, 54) (50). It is well established that individuals with coronary syndromes have an increased interaction between platelets and leukocytes (50, 79, 86).

The release of PF4 from platelets acts as a chemoattractant for monocytes and stimulates their differentiation into macrophages. This recruitment of macrophages is necessary for ingestion of cellular debris following platelet aggregation, but current research suggests PF4 may enhance both the esterification and uptake of oxidized LDL by macrophages (60). New investigative research indicates that PF4 may promote the retention of lipoproteins on cell surfaces by inhibiting their degradation by the LDL receptor (70). Furthermore, PF4 has been found in atherosclerotic lesions in connection to macrophages and foam cells, supporting the idea that PF4 may have a larger association with promoting vascular inflammation and atherogenesis than previously thought (29, 79). New research is beginning to reveal an increased association of platelets to atherosclerosis, due to the cells' link between initiating inflammation and atherogenesis, the developmental process of atheromatous plaques. Plaque formation within the vascular system can be attributed to a number of factors including hypertension, smoking, diabetes and hyperlipidaemia (15). Hyperlipidaemia or hypercholesterolemia, an increasing result of poor nutrition and diet within Western cultures, may present a high risk in increasing atherosclerotic plaque, a consequence of increased LDL levels within the circulation (35, 43, 68). These LDL particles pass through the endothelial wall entering the intimal layer

of the vessel. Here they are metabolized abnormally and become oxidized by reactive oxygen species, resulting in inflammation (69). Inflammation draws in macrophages which extravasate through the endothelial wall and ingest the LDL particles in an attempt to combat inflammation, in turn becoming foam cells (6, 79). Inflammation can activate platelets, further recruiting additional macrophages to the inflamed area. Foam cells may not directly be harmful, but with considerable accumulation or lysing of their lipid contents within the epithelial lining, they can form soft plaque. Smooth muscle cell proliferation and collagen synthesis solidify the soft plaque establishing the onset of atherosclerosis. Over time as lipid deposition increases and the plaque hardens, the vessel narrows and impedes blow flow. A resultant symptom, hypertension, can increase shear stress within the arteries affected by plaque. High shear stress itself is known to activate platelets within the circulation through the VWF-GPIb/IX/V complex pathway (69). This concept may offer another possible link between the blood vessel narrowing due to plaque formation and platelet activation. Likewise, recurrent infections are linked to an increased risk of coronary syndromes as the proinflammatory state associated with pathogenic infections may promote activation of platelets in the local area (69).

Comprehending the interplay between platelets, leukocyte recruitment, inflammation and the endothelial wall is an essential strategy in developing new therapeutic strategies to aid with atherosclerosis and thrombosis. Antiplatelet therapy drugs such as aspirin, clopidogrel or platelet inhibitors are frequently used to help treat patients with coronary artery disease, yet further research is essential to develop more efficient drugs (80). Expanding the knowledge of platelet adhesion, activation and aggregation is a necessary strategy in improving the prevention of thrombosis and

atherosclerosis; it is recognized that exercise can play a positive role (28)in reducing the development of both. However, this effect of exercise is not fully understood and even more unclear is the effect resistance exercise may have on thrombogenesis, atherosclerosis or platelet activation.

The Effects of Exercise on Primary Hemostasis

The significance of acute coronary artery disease among the Western population has lead to an increase in research investigating the continued rise and steps for the prevention of these diseases. The role platelets may play in atherosclerotic manifestation (6, 50, 69, 86) as well as the potential health benefits of exercise are well recognized (28). For this reason, the potential positive effect of exercise on primary hemostasis and platelet activation is an area of much needed research. The effects of exercise on hemostasis are both unclear and poorly understood. Even less well known is the role exercise plays on the primary hemostatic system. As is such, the literature describing these effects is both variable and contradictory. The analysis of exercise effects on primary hemostasis can be strongly controversial as the analytical methods used to study platelets are plagued with various methodological problems (25).

Research investigating endurance exercise and its effects on hemostais has been previously studied (21-24). Resistance training and its effects on hemostatic measurements have only recently gained interest; most likely due to endurance exercise having always been preferentially chosen over resistance training by the average exercising individual. Studies investigating the effects of exercise on hemostatic parameters have included healthy individuals as well as patients with coronary artery disease. The purpose of utilizing patients with coronary syndromes was to elucidate the

positive or the negative effects exercise may have on the hemostatic system of individuals already established with ACS. Few studies have investigated the role of training status on platelet parameters. Sex differences in primary hemostatic measurements do not appear to be significant, although many studies have focused predominantly on men. The current effects of exercise training on platelet activation and function is not yet well understood and is a primary reason for the importance of future studies (25).

Acute Effects of Exercise:

Factors such as exercise intensity, exercise duration and the physical fitness status of the individual can all affect exercise induced changes in platelet response. Increases in platelet aggregation and activation are associated with strenuous exercise while moderate-intensity exercise suppresses platelet function (25, 51). In general, acute exercise generates a considerable increase in platelet count. This short lived increase in platelet count has been associated with a release of platelets from the spleen, bone marrow and intravascular pool of the pulmonary circulation and lungs (9).

The exact role of epinephrine on platelets in response to exercise is vaguely understood, however exercise induced increases in epinephrine may be the reason for a release of platelets from the spleen, wherein a third of the body's platelets are stored (72). Wang et al recently hypothesized that during exercise, epinephrine has been shown to bind to α_2-adrenoreceptors on the platelet membrane resulting in platelet activation (84). Results from their investigation suggest strenuous exercise increases the density of α2-adrenoreceptors on the cell surface, producing platelets more sensitive to increases in epinephrine such as occur during strenuous exercise. Still, other studies have revealed by

β-blockage that exercise induced platelet hyperaggregation cannot be entirely attributed to the α_2-adrenoreceptor pathway (5).

Exercise associated factors possibly contributing to exercise induced increases in platelet aggregation/activation include lactic acidosis, an increase in temperature and exercise induced hemoconcentration. The increase in hydrogen ion concentration linked to lactic acidosis following exercise has been strongly associated with a rise in platelet aggregates (25). Furthermore, it is speculated mechanical stress factors and endothelial microlesions exposing collagen are possible stimuli for platelet activation during exercise, depending on exercise intensity (25).

The platelet markers of β-TG and PF4, as previously noted, are released from the α-granules of the platelet and are used as markers of *in vivo* platelet activation (53). The plasma level of β-TG is determined by the rate of its release from the platelet and the rate of its clearance from the circulation. As such, measurements of β-TG demonstrating exercise related changes in platelet reactivity and increases in β-TG following physical exercise have been shown (2). However these increases cannot assess whether exercise directly activates platelets (25). Subsequently, PF4 levels in the plasma are more difficult to determine as PF4 binds with strong affinity to heparin sulfate on endothelial cells and is metabolized rapidly by the liver. The clearance of PF4 from the circulation raises questions about its effectiveness as a marker for *in vivo* activation. Measurements of β-TG and PF4 within the plasma can indirectly determine platelet activation induced by exercise but cannot quantify changes in platelet reactivity stimulated by exercise (25, 44). Whether or not β-TG and PF4 are effective in measuring *in vivo* activation of platelets in response to exercise still remains unclear. vWF, upon release from activated platelets and

damaged endothelium, has been reported to increase post exercise, although the mechanism to support this is not fully understood (9).

Chronic Effects of Exercise:

The chronic effects of exercise training on primary hemostasis have been far less studied than the acute effects. It has been demonstrated that chronic physical training has favorable adaptations on the primary hemostatic system, especially with regard to platelet count, function, and reduction in platelet adhesion and aggregation, particularly in response to acute exercise (8, 44). Wang et al showed that physical training attenuated a previously seen increase in platelet activity prior to training in response to an acute strenuous exercise bout. In addition detraining of the individuals resulted in a reverse effect on the resting and post-exercise effects (83). It is important to note that the physical training status of individuals varied within observed studies.

Following exercise, exercise-induced thrombocytosis, an increase in platelet number, is less pronounced in physically trained individuals compared with untrained individuals. Trained individuals tend to show a slightly lower platelet count than untrained individuals, a possible result of an expansion of plasma volume (19). Plasma volume expansion is an adaptation that results from chronic exercise, and it has been suggested that this resulting expansion of plasma volume may shorten the contact time of platelets with the vessel wall (12, 14, 81). Resistance training has also been associated with an increase in vessel diameter and an increase in vessel stability (66). A decrease of platelet interaction with the vessel wall would thus make platelet less likely to be activated by slight endothelial damage. An increase in plasma volume may not only reduce contact time between the endothelial wall and platelets, but potential exercise

induced increases of prostacyclin(9) would also decrease platelet interaction with the vessel wall. Furthermore, exercise increases the release of nitric oxide from the endothelium for vasodilation; it also acts as a potent anti-platelet agent suppressing platelet activity (30, 33).

Overall, the role of exercise on the primary hemostatic system is still unclear. However, progress has recently been made in an attempt to understand the precise mechanisms underlying exercise-induced effects on platelets. Studies have shown (8, 44, 83) favorable adaptations of the hemostatic system to exercise training, although the methodology and analytic measures used were highly varied. The potential benefits of exercise on platelet activation and aggregation require further investigation to be carried out in order to substantiate current findings. With evidence linking platelets to atherothrombosis increasing, the potential positive effects of exercise in preventing or ameliorating cardiovascular symptoms are significant. Unquestionably, further research is needed to ascertain the true significance of exercise on the primary hemostatic system.

Hypothesis:

1. We hypothesized that differences in primary hemostatic markers will be seen between resistance trained and non-resistance trained individuals following an acute exhaustive resistance exercise test.
2. Resistance trained individuals will have reduced platelet activation as measured by markers of B-TG, PF4 and vWF in the plasma when compared against non-resistance trained individuals.

Chapter 3: Methods

Experimental Approach to the Problem

To examine the effects of strenuous exercise on the Primary Hemostatic system, we utilized a research design that incorporated ten resistance trained and ten untrained men and women. Our goal was to examine the effects of a single session of an acute exhaustive resistance exercise test (AERET) on the primary hemostatic system. Each subject completed three laboratory visits to the Human Performance Laboratory at the University of Conneciticut. Individuals were familiarized with the protocol, tested for their one repetition-maximum (1-RM: a weight that can only be lifted once, no more or no less) and approximately one week later carried out the AERET which consisted of six sets of ten repetitions at 80% of each individual's previously determined 1-RM. Blood was drawn at five separate times points for later analysis to determine exercise effects on platelets: Before exercise (Pre), immediately-post exercise (IP), 15, 60 and 120 min after the exercise test.

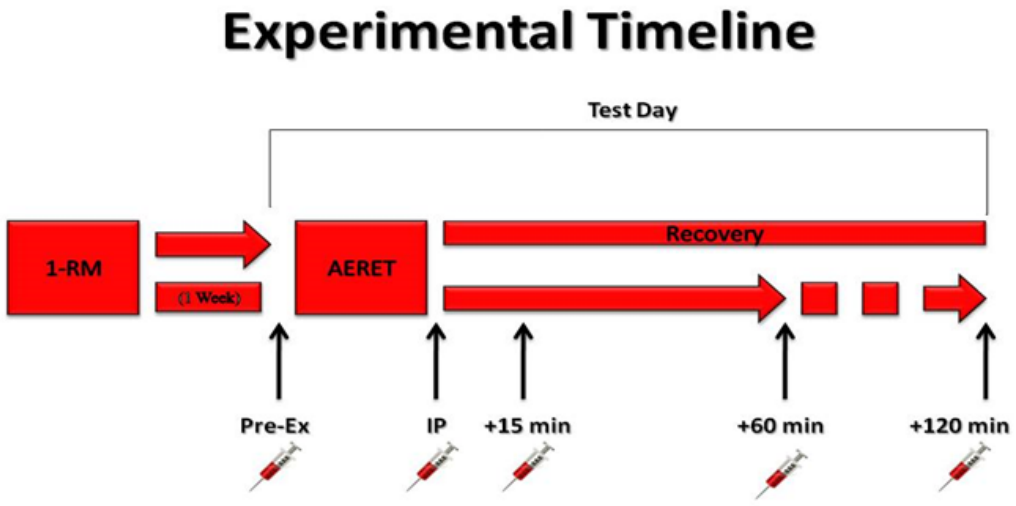

Figure 1: Experimental Timeline. RM=Repetition Maximum. AERET=Acute Exhaustive Resistance Exercise Test [6 sets of 10 repetitions of squats at 80% of tested 1RM]

Individuals

This investigation was approved by the University of Connecticut's Institutional Review Board for the use of human individuals in research. In an information session, each subject was verbally instructed on the study procedures, as well as the risks, inconveniences, and benefits of participation. At the end of this information session, each subject volunteered his or her participation via a signed written consent form (See Appendix A). All individuals completed a medical history questionnaire, which was reviewed by our Medical Monitor, and a self-reported physical activity level questionnaire, which was reviewed by study personnel. Each subject, considered healthy, was screened by the medical monitor as having no medical conditions that would confound any of the study variables.

Upon medical clearance and confirmation of the inclusion/exclusion criteria, participants were allowed to proceed with the study protocol. Inclusion criteria included men and women between 18-39 years of age at the start of the study. Of the 20 recruited individuals, 10 constituted the resistance trained group (5 males & 5 females, Age: 26.0yr ± 1.42, Height: 175.12cm ± 2.7, Weight: 79.56kg ± 4.29) and met the criteria of consistently performing a structured resistance training program (2-3x/week) for at least 2 years prior to the start of the study. The other 10 individuals constituted the untrained group (5 males & 5 females, Age: 26.4yr ± 1.97, Height: 170.31cm ± 2.36 Weight: 67.88kg ± 5.34) and were defined as not having participated in a resistance exercise training program within the last six months, defined as training 3x a week for 6 months, and not participating in cardiovascular activity more than 2x a week. Exclusion criteria included: participation in an endurance exercise training program (consistently for 3

months, 3x week) within the last year, contraindications based on health history by the study physician, dyslipidemia (TG>400 mg/dl, cholesterol>240 mg/dL), hypertension (SBP>150 or DBP>95 mmHg), diabetes (fasting glucose >126 mg/dL), use of tobacco products, cholesterol lowering and blood pressure medications, change in body weight >3 kg during past 3 months, use of anti-inflammatory medication (aspirin, NSAIDs), alcohol consumption >3 drinks/day or 18/wk, musculoskeletal injuries or physical limitations affecting ability to exercise, anticoagulant medication (coumarin), use flax or fish oils, use of hormonal substances including, testosterone, anabolic steroids, or growth hormones.

Table 1: Descriptive Characteristics of Subjects

Variable	Resistance Trained N=10	Non-Resistance Trained N=10
Age (yr)	26.0 ± 4.5	26.4 ± 6.2
Ht (cm)	175.12 ± 8.54	170.31 ± 7.45
Wt (kg)	79.56 ± 13.56	67.88 ± 16.90
BMI	25.7 ± 2.1	23.2 ± 4.3
% fat	11.71 ± 3.59	15.59 ± 4.60
LBM (kg)	70.28 ± 11.47	57.23 ± 10.08
Waist (cm)	77.0 ± 8.1	73.6 ± 12.1
Hip (cm)	102.0 ± 7.8	96.3 ± 7.4
Umbilicus	82.6 ± 7.8	79.7 ± 10.6
WHR	0.76 ± 0.08	0.76 ± 0.08

Values are displayed as mean ± SE. LBM=Lean Body Mass; WHR=Waist to Hip Ratio; BMI=Body Mass Index. *Significantly different from Resistance Trained Group ($p < 0.05$)

Procedures

Individuals reported to the Human Performance Laboratory (HPL) at the University of Connecticut on three separate occasions.

Visit 1:

The first visit to the HPL was used to gather Anthropometric data (height, weight and skin-fold thickness via calipers) in order to assess each individual's body mass index. All anthropometrics measurements were obtained by the same two individuals throughout the study (i.e. male measured males, female measured females) on the right side of the subject's body. Skinfold measurements were obtained using skinfold calipers (Bodycare Harpenden caliper, England) at the chest, abdomen, and thigh for the males, and the tricep, suprailiac, and thigh for the females following procedures previously described (52)**.** Three measurements were obtained with the means being utilized. A three-site skinfold equation was used to estimate percent body fat. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany) and total body mass to the nearest 0.1 kg on a digital scale (OHAUS Corp., Florham Park, NJ). Participants wore only light athletic clothing and no shoes for measurements of height and body mass. BMI was calculated as weight (kg)/height (m^2). Waist, hip and umbilicus circumferences were assessed using a standard flexible tape measurer with a spring-loaded handle (Perform Better, Cranston, RI, USA) extended to the same marking with each trial. Measurements were taken in triplicate of the natural waist, between the navel and iliac crest, and at the widest part of the hips. Rotation through the measurement sites allowed skin to regain normal texture and was retested if a measurement was not within 5mm at any one site. Tests were performed by the same staff member each time. Blood pressure was recorded with a sphingometer after individuals were sitting for 5 minutes.

Each subject was then familiarized with a standard warm-up and the testing protocol in order to reduce learning effects. The standardized warm-up routine consisted

of five minutes of cycling on a stationary bicycle pedaling at 60 RPM followed by various dynamic stretching exercises (forward lunges, lateral lunges, body weight squats, butt kicks, toy soldiers and knee hugs). Following the warm-up individuals were verbally familiarized with the numerical "Rating of Perceived Exertion" (RPE) scale to assess how hard each subject felt he or she was working during his or her exercise bout. Individuals were then familiarized with the proper technique for performing the squat exercise using the Plyometric Power System. The Plyometric Power System utilizes a modified Life Fitness® (Franklin Park, IL, USA) Smith's Machine that allows only vertical translation of the bar. Linear bearings attached to either side of the bar allow it to slide up and down two steel shafts with minimal friction. Once individuals demonstrated proper technique in the squat exercise, they were scheduled for their second visit anytime within the following week.

Visit 2:

Upon the second visit to the HPL individuals arrived in the morning having been asked to fast for 12 hours, abstain from caffeine for 24 hours and exercise for 48 hours. Once the individuals arrived they were seated for fifteen minutes and a blood sample was taken to assess metabolic markers (glucose, cholesterol, triglycerides, etc.). Individuals were then allowed to consume a light snack (cereal, oatmeal, protein bar) before they proceeded with their 1-RM in order to ensure they had energy for the exercise test after arriving fasted. In testing each individual 1-RM, strength was measured using the methods described by Fleck and Kraemer (27). They performed the same standard warm-up which they had been familiarized with (five minutes of cycling on a stationary bicycle pedaling at 60 RPM followed by various dynamic stretching exercises). Individuals then

performed 8–10 squat repetitions on the Smith Machine at ~50% of their estimated 1-RM which was then followed by another set of 2–5 squat repetitions at ~85% of estimated 1-RM. Subsequently, 4–5 one-repetition trials were used to determine the 1-RM. All sets were separated by a three minute rest period. All exercise protocols were supervised by a National Strength and Conditioning Association Certified Strength and Conditioning Specialist

Visit 3: Acute Exhaustive Resistance Exercise Test (AERET)

Individuals reported to the HPL after being asked to fast for 12 hours as well as abstain from alcohol, drugs and high doses of caffeine (more than 2 cups on the prior day, or any on the day of) for 24 hours. Participants were also asked to abstain from exercise for 48 hours prior to the test day. All AERET trials were performed in the early morning (0700–0900) and time of day was standardized (±1 hr) to avoid confounding influences from diurnal hormonal variations.

To ensure adequate hydration status before performing the exercise trial, the night before the AERET, individuals were instructed to drink approximately 0.5 liters (about 2 cups) of water. Individuals were also instructed to drink another 0.5 liter of water the following morning before arriving at the HPL. Upon arrival to the lab on the AERET day, individuals were asked to provide a urine sample for a measurement of hydration status which was determined via urine refractometry (Model A300CL, Spartan, Japan); individuals were considered euhydrated if they had urine specific gravity <1.025 gml−1. Each subject received a standardized breakfast consisting of a bagel with cream cheese or butter and a banana in order to ensure individuals had energy for the exercise test after arriving fasted. No current literature shows that food intake affects platelet functions.

Exercise commencement did not begin until at least 45 minutes after individuals consumed their standardized breakfast.

Individuals were then seated, and an indwelling Teflon catheter was inserted into an antecubital forearm vein. A baseline blood sample was taken after the subject was seated for approximately 15 minutes; blood pressure was taken, a heart rate monitor was placed around the subject's sternum and all individuals were again familiarized with the RPE scale. Individuals then performed the same standardized warm-up as they were previously shown in the familiarization visit followed by one warm-up set of squats at 50% of their 1-RM. Individuals performed six sets of ten repetitions of the squat with two minutes of rest in between sets at an initial load of 80% of their 1-RM. If individuals were unable to complete at least 8 repetitions per set, the weight was adjusted accordingly so as to allow the individuals to perform 8-10 repetitions in the following set. After each set was performed during the AERET, each subject gave a numerical number based on the RPE scale and his or her heart rate was taken and recorded. Immediately after the 6th set of squats, individuals were seated in a wheel chair and the immediate post blood draw was taken. Three additional blood samples were obtained at 15, 60 and 120 min into recovery from exercise.

Blood Draws

As mentioned earlier, prior to the AERET, individuals were asked to remain seated for five minutes, after which time a trained phlebotomist inserted an indwelling Teflon cannula into a superficial forearm antecubital vein. The cannula was kept patent with a saline solution. Prior to each blood draw, 3ml of blood was extracted and discarded to avoid inadvertent saline dilution of the blood sample.

Blood Processing

Approximately 20 ml of blood was drawn off and collected in serum, EDTA, Sodium Citrate, and CTAD vacutainers. Serum tubes were allowed to clot at room temperature while all other blood samples were kept in ice blocks before being spun. EDTA and serum tubes were centrifuged at 3,000 rpm at 4 ◦C for 15 min. Sodium Citrate and CTAD tubes were centrifuged at 2,000 rcf at 4 ◦C for 30 minutes to obtain platelet poor plasma. Serum, plasma and platelet poor plasma aliquoted and then stored at −80 ◦C until later analysis.

Biochemical Assays

Hematocrit was measured in EDTA-treated whole blood immediately following blood collection. Hematocrit was measured in triplicate by centrifugation of heparinized micro-hematocrit capillary tubes (Fisherbrand®, Pittsburgh, PA). EDTA-treated whole blood was used for platelet count assessment and was performed by Quest Diagnostics INC. (Willimantic, CT). CTAD samples were analyzed in duplicate for plasma values of B-TG and PF4 via enzyme linked immunoassays (ELISA) (Asserachrom B-TG, Diagnostica Stago, INC, USA), & (Asserachrom PF4, Diagnostica Stago, INC, USA), using an automatic microplate reader (VERSAmax tunable, Molecular Devices, CA, USA). Assay sensitivity for β-TG was 5 IU/ml, intra-assay coefficients of variation were below 5.5% and inter-assay coefficients of variation were below 9.0%. Assay sensitivity for PF4 was 1 IU/ml, intra-assay coefficients of variation were below 4.8% and inter-assay coefficients of variation were below 8.7%. Sodium Citrate samples were analyzed in duplicate for plasma values of vWF via enzyme linked immunoassays (ELISA) (Asserachrom vWF, Diagnostica Stago, INC, USA), using an automatic microplate

reader (VERSAmax tunable, Molecular Devices, CA, USA). Assay sensitivity for vWF was 1%, intra-assay coefficients of variation were below 7.6% and inter-assay coefficients of variation were below 10.1%. All frozen samples were analyzed at the same time in the same microplate to minimize differential analysis effects.

The liquid lactate assay (Point Scientific #L7596) was performed on human plasma samples as reported by Gutmann et al. and Noll et al., 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 using an automatic microplate reader (VERSAmax tunable, Molecular Devices, CA, USA).

Statistical Analyses

Data are presented as mean ± the standard deviation unless otherwise stated; figures are presented with means +- the standard error. Data was analyzed using a 2(training status) x 5(time) repeated measures anova (two-way ANOVA) to identify variance within the whole group, as well as between each group. In the event of a significant F-score, a Fishers LSD post-hoc analysis was performed. Data for each variable were evaluated to determine if the assumptions (e.g. normalcy, sphericity) for linear statistics were met. For each analysis means, SD, F ratio, 95% confidence intervals

and effect sizes were computed. Significance for this study was set at $p \leq 0.05$. No significant gender differences were shown for the measured variables except β-TG; a covariate was run as gender was not an interest of the current investigation. Gender was collapsed across training groups. Analysis was run for area under the curve, however no unique findings were found beyond the repeated measures anova. The level of significance for this study was set at $p \leq 0.05$.

Chapter 4: RESULTS

Directly following the AERET a significant main effect for time was found in both the resistance trained and untrained groups for platelet count ($F_{0.05\ (4,\ 72)} = 167.085$, P=0.000), β-TG ($F_{0.05\ (4,\ 64)} = 29.346$, P=0.000), PF4 ($F_{0.05\ (4,\ 72)} = 3.26$, P=0.016) and vWF ($F_{0.05\ (4,\ 72)} = 11.064$, P=0.000). No significant differences were found among individuals for any of the characteristics measured.

Platelets:

Although a significant main effect for time was shown in both groups for platelet count, there were no significant differences between groups at any time point, and thus, no significant main effect for training status was shown. Repeated contrasts indicated a significant interaction between time and training status between groups. There was a significant difference in the delta from IP to +15 $F_{0.05\ (4,\ 72)} = 5.018$, P=0.001, between the two groups; ΔRT= -38, ΔUT= -19.3. Significant differences were shown in the resistance trained group at time points IP and +15 which were different from all other time points. The untrained group showed that all time points were significantly different from each other except, Pre and +120.

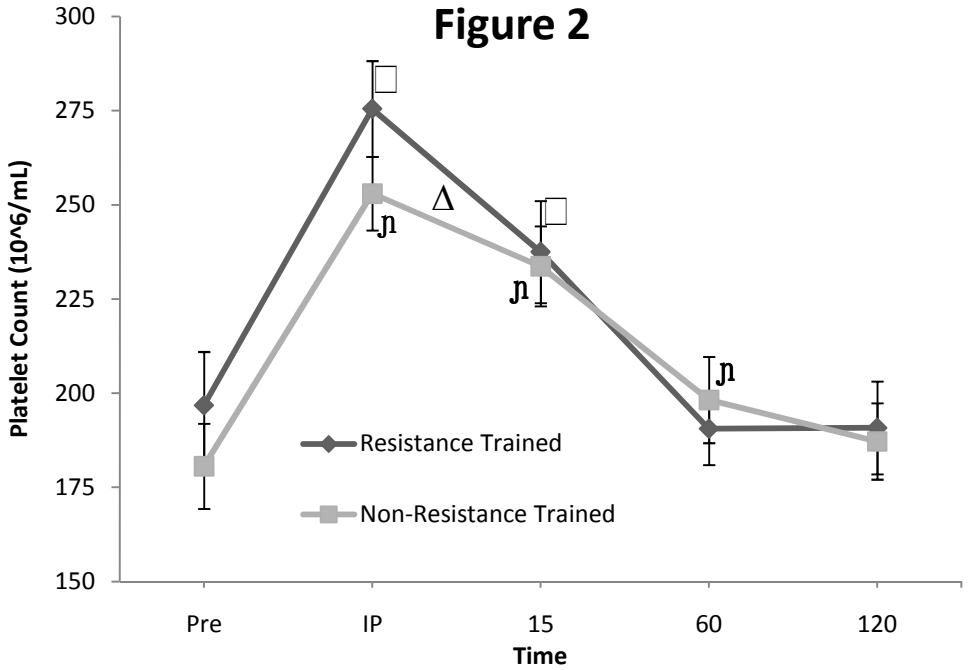

Figure 2: Platelet count values: pre, immediately-post, and +15, +60, +120 post AERET (10^6/mL). Values are displayed as mean ± SE. Significant difference in RT from all other time points within group denoted by □ ($p<.05$). □ indicates a significant difference in UT from all other time points within group ($p<.05$). Δ denotes a significant difference in the rate of change between RT and UT from IP to +15 (ΔRT= -38, ΔUT= -19.3), ($p<.05$).

vWF:

Although a significant main effect for time was shown in both groups for vWF, no significant differences were shown between groups at any time point, and thus, no significant main effect for training status was shown. No significant interaction between time and training status was shown. In the resistance trained group, IP was significantly different from Pre +60 and +120; +15 was also significantly different from Pre. The untrained group demonstrated that Pre was significantly different at every time point and in addition +15 was significantly different from +60 and +120.

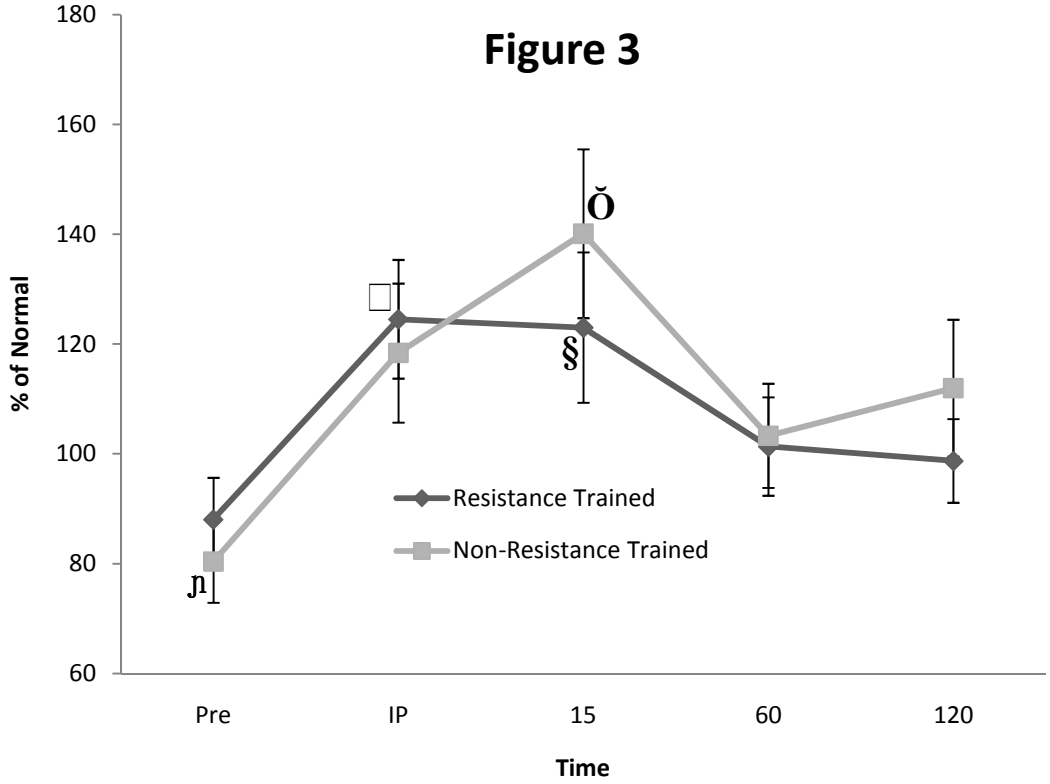

Figure 3: Plasma vWF values: pre, immediately-post, and +15, +60, +120 post AERET (% of Normal). Values are displayed as mean ± SE. Significant difference from Pre, +60 and +120 within group RT denoted by □ ($p<.05$). **§** denotes a significant difference from Pre in the RT group ($p<.05$). □ indicates a significant difference in UT from all other time points within group ($p<.05$). **Ŏ** denotes a significant difference from +60 and +120 in UT group ($p<.05$).

PF4:

Although a significant main effect for time was shown in both groups for PF4, no significant differences were shown between groups at any time point, and thus, no significant main effect for training status was shown. No significant interaction between time and training status was shown. The resistance trained group showed no significant differences at any time point. However, in the untrained group, time point +120 was significantly different from IP and +15.

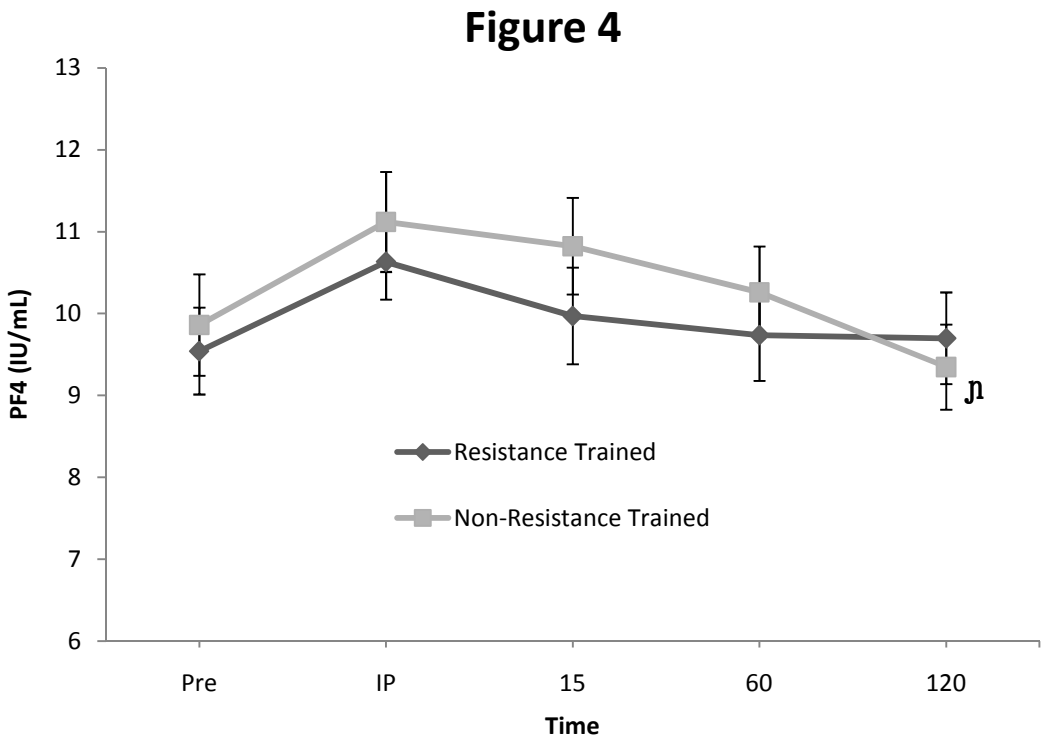

Figure 4: Plasma PF4 values: pre, immediately-post, and +15, +60, +120 post AERET (IU/mL). Values are displayed as mean ± SE. □ indicates a significant difference in UT from IP and +15 ($p<.05$).

β-TG:

A significant difference between groups was shown for β-TG at time points +15 and +60. In the resistance trained group, both IP and +15 were significantly different from Pre and +120. Significant differences were shown between every time point except IP and +60 in the untrained group.

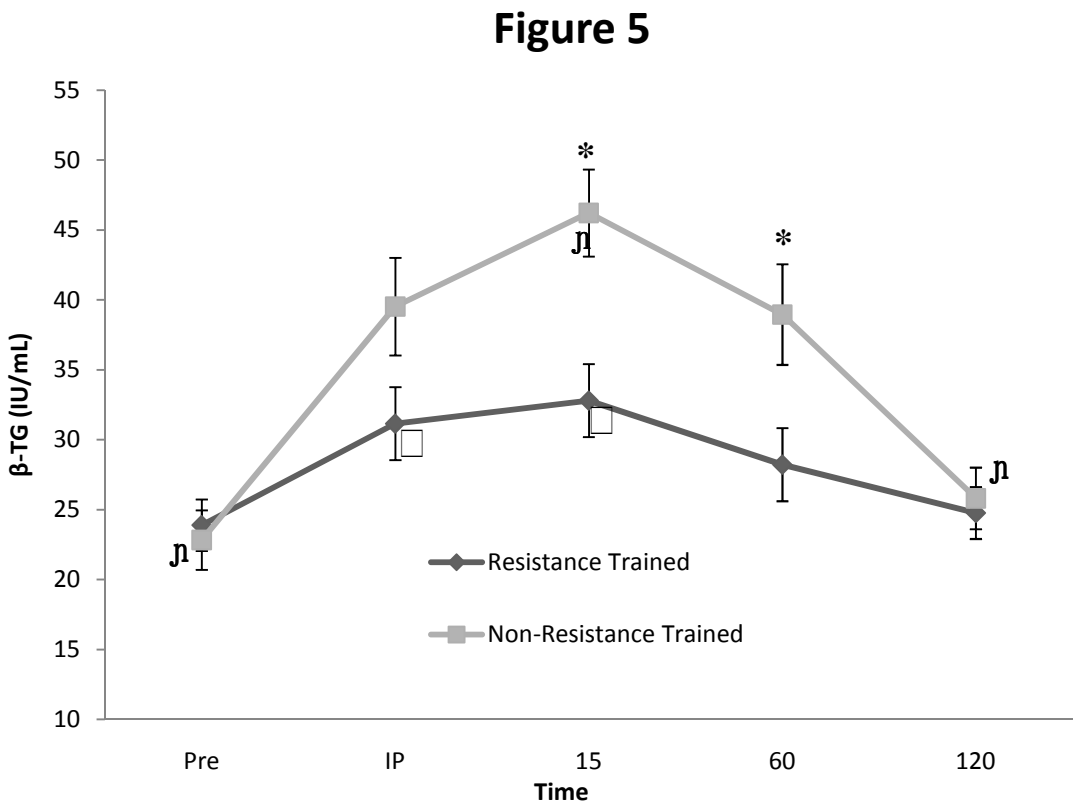

Figure 5: Plasma β-TG values: pre, immediately-post, and +15, +60, +120 post AERET (IU/mL). Values are displayed as mean ± SE. * Significant difference between groups (p<.05). Significant difference from Pre and +120 within group in RT denoted by □ (p<.05). □ indicates a significant difference in UT from all other time points within group (p<.05).

Lactate, HR, RPE:

Lactate values, heart rate and RPE were used together to assess physical exertion during exercise. No differences between groups were shown for any variables. Lactate values of both groups showed significant differences between all time points except between Pre and +120. Heart rate for both groups showed a significant difference between Pre and all sets performed. Similarly, +120 post exercise was significantly different from all sets performed. Pre and +120 post exercise were not significantly different from each other. RPE for RT showed significant differences between all sets, except 4 and 5. Similarly, RPE for UT showed significant differences between all sets, except 2 and 3.

Table 2.1: Lactate & Average Heart Rate

	Resistance Trained N=10			**Non-Resistance Trained N=10**		
	Pre Exercise	Immediately Post Exercise	120Min Post Exercise	Pre Exercise	Immediately Post Exercise	120Min Post Exercise
Lactate (mmol/L)	**1.07 ± 0.2**	**11.55 ± 2.9†**	**1.04 ± 0.3**	**1.04 ± 0.3**	**9.66 ± 2.6†**	**1.26 ± 0.6**
Heart Rate (BPM)	**73.5 ± 8.4**	**179.2 ± 11.5†**	**67.8 ± 9.1**	**76.0 ± 5.6**	**188.0 ± 12.7†**	**79.0 ± 9.0**

Values are displayed as mean ± SE. † significantly different from Pre ($p < 0.05$).
BPM=Beats Per Minute

Table 2.2: Rating of Perceived Exertion (RPE)

	Resistance Trained N=10	**Non-Resistance Trained N=10**
1st Set	**6.5 ± 2.1**	**6.4 ± 1.4**
2nd Set	**7.3 ± 1.6**	**8.0 ± 1.3**
3rd Set	**8 ± 1.4**	**8.4 ± 1.7**
4th Set	**8.8 ± 1.2**	**9.3 ± 1.4**
5th Set	**9.3 ± 0.9**	**10.1 ± 1.4**
6th Set	**10.1 ± 1.0**	**10.8 ± 1.9**

Values are displayed as mean ± SE. significantly different from all other sets ($p < 0.05$).

Chapter 5: DISCUSSION

The present study is the first to examine the effects of an acute exhaustive resistance exercise test (AERET), utilizing a six sets by ten repetition format, on the primary hemostatic system in both resistance trained and untrained individuals. It is important to distinguish that although previous studies have implemented a resistance training protocol at 80% 1-RM (2, 3)no study to date has utilized an AERET. In addition, no previous studies investigating the interaction between resistance exercise and primary hemostasis have extended recovery to 120 minutes post exercise, nor have they measured PF4. In accordance with our hypothesis, resistance trained individuals' demonstrated significantly lower platelet activation as measured by plasma β-TG compared to the untrained group. However, β-TG was the only variable to show significant differences between groups. Platelet count revealed a significant interaction between groups in the change from IP to +15; the change was greater in the RT group -38 (10^6/mL) compared to the UT group -19.3 (10^6/mL).

The analysis of primary hemostasis can be particularly difficult because sample collection, handling and processing are all factors capable of effecting can affect platelets *in vitro*. Methodological differences among the few studies that have examined resistance exercise and platelet activation allow for variation among the results, and thus, differences among findings.

Platelet Count

Platelet count significantly increased in both the resistance trained and untrained groups by 40 percent from pre to immediately post exercise. Resistance exercise induced increases and subsequent post exercise decreases in platelet count following acute

exercise have been disclosed in previous studies by Ahmadizad et al. (2, 3). Studies implementing endurance exercise have also shown similar increases in platelet count (16, 63). A rise in platelets following acute strenuous exercise may be related to a subsequent rise in epinephrine levels along with an increase in shear stress which cause demargination of platelets into the circulation (59, 91). This transient release of fresh platelets stems from vascular endothelium of the spleen, bone marrow and the pulmonary circulation in the lungs (9). Exercise-induced hemoconcentration is also another mechanism by which platelet count rises after acute exercise (13).

A return to near resting levels 60 to 120 minutes post exercise probably occurs as a result of a reduction in epinephrine concentration and shear stress resulting in a subsequent margination of platelets back into the spleen, bone marrow and lungs (59, 91). The larger rate of change shown in the resistance trained group from immediately post exercise to fifteen minutes post exercise compared to the untrained group is most likely associated with an adaptive response seen in trained individuals. Sensitization to hormones, namely epinephrine, as well as repeated exposure to exercise induced stress enables trained individuals to more efficiently respond and recover post exercise compared to untrained individuals (26, 46). Although the effects epinephrine has on primary hemostatic factors are poorly understood, epinephrine has been shown to stimulate the release of platelets by the spleen (72) . Consequently, trained individuals more adept at responding to exercise-induced stressors may more rapidly cease signaling the need for additional platelets. Also, redistribution of plasma volume and subsequent regression of hemodilution is probably more rapid in trained individuals.

von Willebrand Factor

Plasma levels of circulating von Willebrand Factor (vWF) are typically measured to assess endothelial dysfunction *in vitro*, as well as platelet activation (7). vWF plasma levels increased significantly in both groups from pre to immediate post exercise, hence corresponding to other studies with similar increases (82, 85, 90). However, many previous studies utilized only endurance exercise or clinical individuals in their investigations. This transient rise in plasma vWF immediately following exercise probably occurs due to a rapid release of stored vWF from both the endothelial vasculature and platelets in response to various agonists such as thrombin, collagen, epinephrine or vasopressin.(32, 78) Vasopressin has a compensatory effect in response to exercise as it functions to increase blood pressure and vascular resistance. These mechanisms consequently increase shear stress; a resultant release of vWF from the endothelium may help ensure platelets are still capable of adhering with an increase in shear rate. Likewise, catecholamines such as epinephrine induce the release of vWF from endothelial cells and stimulate platelet activation releasing stored vWF (32).

The resistance trained group peaked at immediate post exercise while the untrained group continued to increase up to fifteen minutes post exercise before showing a decrease. Although the intensity of the exercise for every individual was relative to their own pre determined 1-RM, it can be concluded that the untrained group peaked at a higher plasma concentration since the resistance trained group had been previously exposed to resistance training stress and was thus favorably adapted. In response to training, individuals may decrease resting and short term plasma catecholamine levels reducing the subsequent release of vWF from both the endothelium and platelets (55).

Reduction of plasma vWF occurred fifteen minutes post exercise in both groups due to proteolysis and clearance by macrophages in the liver and spleen, as well as reductions in epinephrine levels (71). A reduction in vWF has previously been shown in response to exercise (17), although subject population and mode of stimulus differed. In contrast, other studies have shown elevated levels of vWF persisting up to an hour post exercise (62). Differences in results may be attributed to a number of factors including the subject population used, the mode of exercise stimulus and the methods used to measure vWF *in vitro*.

Platelet Factor 4 & βeta-Thromboglobulin

Platelet factor 4 (PF4) and βeta-Thromboglobulin (β-TG) are measured within the plasma as markers of *in vivo* platelet activation. However, they cannot quantify changes in platelet reactivity stimulated by exercise. Although released in similar amounts from platelets, plasma concentrations of β-TG exceed those of PF4 within the plasma. PF4 and β-TG can be measured independently. When measured together, they can be used to rule out *in vitro* activation if β-TG levels exceed those of PF4 by a high enough ratio (40). The most probable reason for exercise induced release of PF4 and β-TG is platelet activation in response to epinephrine and increased shear stress within the vasculature (84).

No significant differences between groups were shown for PF4 although the resistance trained group did maintain an overall lower PF4 level. This difference, though not significant, may suggest an underlying reduction in platelet activation. As mentioned similarly with vWF, trained individuals may decrease their resting and short term plasma catecholamine levels (55) resulting in reduced sensitivity of platelets to epinephrine via

the a-2-adrenorecptor on platelets (48), ultimately reducing platelet activation. Still, adaptive sensitivity to epinephrine is probably not the only mechanism by which trained individuals reduce platelet activation during exercise.

Plasma levels increased from pre exercise and peaked immediately post exercise in both the resistance trained and untrained groups. The clearance of PF4 from the circulation and its high affinity for binding to heparin sulfate on the endothelium are the most likely reasons an increase in PF4 was not evident (40). Since no previous studies have examined the effects of an acute bout of resistance training on plasma PF4 levels, it is hard to speculate why a significant increase was not observed. One study, by Strauss et al (75) utilizing both healthy and cardio vascular diseased males, showed no significant increases in PF4 in response to a treadmill test. However, other studies measuring PF4 in response to aerobic exercise (49, 64) did measure noticeable increases in plasma PF4 levels when older individuals, individuals with CVD or both were utilized. Higher amounts of plasma PF4 in CVD individuals compared to healthy individuals is most likely due to an inability of diseased individuals to effectively bind PF4 to the endothelium, a result of endothelial dysfunction from plaque formation.

The primary findings of the current study showed significant differences between the resistance trained and untrained groups of β-TG in the plasma at 15 minutes and 60 minutes post exercise. These findings are similar to studies carried out by Ahmadizad et al who also showed increases in β-TG in response to an acute resistance exercise bout (2, 3). As mentioned previously, the significant difference between the two groups may be partially attributed to a decrease in resting and short term plasma catecholamine levels seen in trained individuals (55). A reduction in catecholamine levels might result in a

reduced sensitivity of platelets to epinephrine via the a-2-adrenorecptor on platelets (48), reducing platelet activation. Still, adaptive sensitivity to epinephrine is probably not the only mechanism by which trained individuals reduce platelet activation during exercise.

β-TG levels peaked in both groups at 15 minutes post exercise before decreasing to near baseline levels. A less rapid clearance of β-TG from the plasma compared to PF4 may explain the extended temporal decrease seen after acute resistance exercise. The exact reasons for exercise induced increases in β-TG are difficult to determine from the present study and the current literature has no definitive answers.

Lactate, Heart Rate & Rating of Perceived Exertion

Lactate values, heart rate and RPE values were used together to assess physical exertion during exercise. Significant increases in lactate and heart rate from pre to immediately post AERET and a subsequent significant decrease at 120 minutes post exercise testing within both groups follow previous studies that utilized high volume, high intensity, short rest period resistance training workouts (4, 45, 73, 87). RPE values in both groups also saw significant increases from the first set to the sixth set demonstrating a perceptual increase in fatigue over the course of the AERET. Similar increases in RPE have been shown in previous studies in relation to resistance exercise (31).

Conclusion

The limitations of the current study as well as those of numerous other investigations lie with the use of the subject population. The individuals used within this study tended to be young and healthy, making it difficult to extrapolate the results to older populations or individuals with cardiovascular complications. Additionally, the

effects of acute resistance exercise were examined on two different groups of individuals, resistance trained and untrained. Thus, only the response to an acute bout of exercise can be ascertained from the individuals used and not the long term potential benefits on primary hemostasis.

In conclusion, the results of the current investigation revealed that resistance training does invoke a varying response to acute exercise when compared against individuals who are untrained. A significant difference in β-TG levels measured between resistance trained and untrained individuals suggests reduced *in vivo* platelet activation in response to an acute bout of resistance exercise. The exact mechanism underlying this adaptation seen with resistance training is not entirely understood. Further experiments are necessary to determine this interaction between resistance training and primary hemostasis as the knowledge would be beneficial for both CVD patients as well as sedentary individuals.

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Appendix A

Consent Form for Participation in a Research Project

University of Connecticut

Principal Investigators: Brian R. Kupchak, Ph.D. and Jeffery S. Volek, Ph.D.,R.D.
Study Title: The Effects of Resistance Training on Coagulation, Fibrinolytic, and Endothelium Parameters

Invitation to Participate
You are invited to participate in this study which will investigate the effects of a single acute exhaustive resistance exercise on coagulation and fibrinolytic systems in non –resistance and resistance trained individuals.

2. Purpose
The purpose of this study is to examine the effects of a single session of resistance exercise on the coagulation and fibrinolytic systems. The findings may provide data to further elucidate possible mechanism underlying the relationship between exercise and clot formation.

3. Description of Procedures
Before you can be approved for participation you must sign the consent form, complete a medical history and training questionnaire to ensure that you meet all the inclusion and none of the exclusion criteria at this on-site screening session.

Inclusion:
To be considered eligible for this study you must be between the ages of 18-39 years. In addition, resistance trained individuals must be working out (at least 3 times per week) during the 6 months preceding the study. Untrained individuals must have not participated in any resistance exercise training regimen in the past 6 months will be recruited.

Exclusion:
Upon review of your medical history form by our medical monitor (Jeffrey Anderson, M.D.), you will not be allowed to participate in this study if you have a pre-existing medical condition which may put you at risk while performing the strength-testing exercises or that might influence the outcomes of this study. Individuals will be medically screened for the following exclusion criteria:

1) non resistance trained individuals must not have participated in a resistance exercise training program within the last six months, which is defined as training 3x a week for 3 months
2) contraindications based on health history by the study physcian
3) body weight >145 kg (weight capacity of DXA scanner)
4) dyslipidemia (TG>400 mg/dL, cholesterol>240 mg/dL)

5) hypertension (SBP>150 or DBP>95 mmHg)
6) diabetes (fasting glucose >126 mg/dL)
7) use of tobacco products
8) cholesterol lowering and blood pressure medications
9) change in body weight >3 kg during past 3 mo.
10) use of anti-inflammatory medication (aspirin, NSAIDs)
11) alcohol consumption >3 drinks/day or 18/wk
12) musculoskeletal injuries or physical limitations affecting ability to exercise.
13) anticoagulant medication (coumarin)
14) use flax or fish oils.
15) use of Vitamin E
16) use of hormonal substances including, testosterone, anabolic steroids, growth hormones, or spironalactone (Aldactone®), a common drug with opposing actions to testosterone. .

In addition, we reserve the right to dismiss you from this study at any time, if we believe that you do not follow the instructions provided for this study.

Visits:

This study will require that you come to the University of Connecticut Human Performance Laboratory (HPL) located in the basement of the Gampel Pavilion for three experimental visits (not including this on-site screening session). During Visit 1, 2 and 3, you will be required to come to HPL.

Blood Screening (~15 minutes)

Once you are approved for participation, you will arrive in the morning to the HPL, and abstained from food for 12 hours and caffeine for 24 hours. You will be seated for 15 minutes and a blood sample will be taken to access metabolic markers (glucose, cholesterol, triglycerides, etc.).

Visit 1 (~1 hour)

If your blood tests (glucose, lipids) are normal you will be able to proceed in the study, if not you will be informed of your results and will be suggested to contact your primary care physician. Once approved for participation, you will arrive to HPL in the morning or afternoon, we will then demonstrate and teach you the standardized warm-up routine. The standard warm-up consists of 5 minutes on a stationary bike followed by a variety of dynamic stretches, which include 10 reps of body weight squats, side lunges, heel kicks, and high kicks. All of this will be explained and briefly demonstrated. You will be familiarized with the verbal numerical "Rating of Perceived Exertion" (RPE), which accesses how hard the subject feels that they are working during exercise exertion bouts. We will also demonstrate and familiarize individuals with the use of the Smith machine and allow them to try the movement. We will teach you how to properly perform the squat exercise on the smith machine. Additionally, we will measure each participant's height, weight, and skin-fold thickness via calipers.

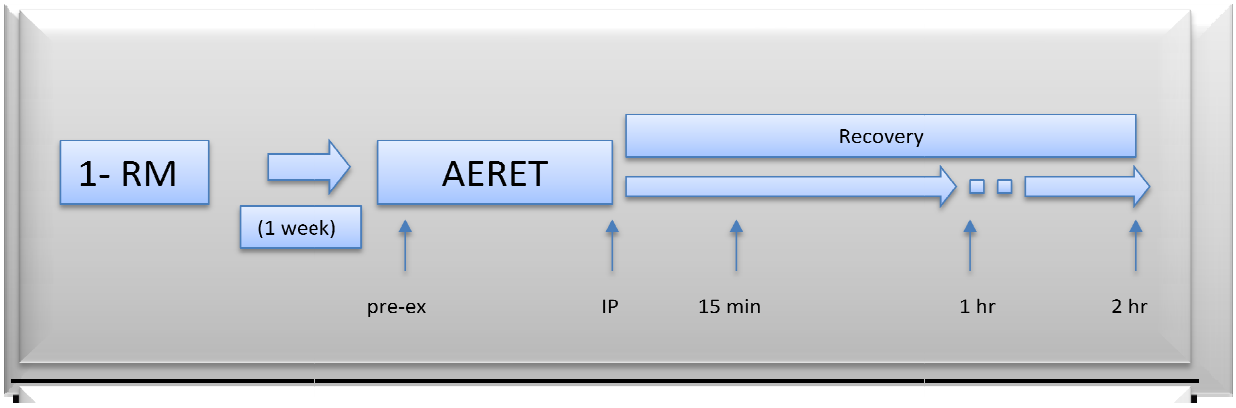
Visit 2 (~1 hour and 15 minutes)

You will arrive to HPL in the morning or afternoon to test your 1 Repetition Maximum lift (1-RM), you will first perform a light warm-up that includes riding a stationary bicycle and dynamic stretching. Then, you will perform 6-8 repetitions of the squat exercise using a light resistance. Next, you will perform 2-5 repetitions of the squat exercise at a moderate intensity. Finally, you will perform progressively heavier lifts, one at a time until they have reached the maximal weight that they can lift using correct and safe technique. We will give you plenty of rest throughout to ensure that they are not fatigued. Visit 2 and visit 3 will be separated by one week.

Visit 3 (~2 hours and 30 minutes)

The night before the acute exhaustive resistance exercise trial (AERET), you will be instructed to drink approximately 0.5 liter (about 2 cups) of water. Before you arrive at the HPL the next morning, you will be instructed to drink another 0.5 liter of water. This will ensure that they are adequately hydrated before performing the exercise trial. We will confirm adequate hydration by obtaining a small urine sample in a small cup. Hydration assessment will occur at the beginning of every subsequent experimental visit. You will arrive at the laboratory fasted, and a 24-hour abstinence from alcohol, drugs, and high doses of caffeine (more than 2 cups on the prior day, or any on the day of). You should abstain from exercise for 48 hours prior to baseline. You will receive a standardized breakfast consisting of a bagel with cream cheese and a banana. Once you have eaten your breakfast, you will wait one half hour to commence the exercise protocol. First you will be seated and an indwelling catheter will be inserted into an antecubital vein. A baseline blood sample will be taken once the indwelling catheter has been inserted for 15 minutes. During this time we will again familiarize the subject with the RPE scale. After the baseline blood sample is taken, we will measure your blood pressure and heart rate. You will then perform a light warm-up that includes riding a stationary bicycle and dynamic stretching. Then, you will warm-up by performing 50% of their 1 RM for 8-10 repetitions. The AERET (diagram of exercise shown in Figure 1) will then commence where you will perform 80% of their 1 RM for 6 sets for 10 repetitions with 2 minutes rests in between sets. If you cannot do at least 8 repetitions in a set the weight will be adjusted so you will be able to perform 8-10 repetitions in the following set. After each set is performed you will give a numerical number based on the RPE scale and their heart rate will be taken. As soon as the AERET is finished, you will be seated and the immediate post blood draw will occur. This will be followed by blood samples taken and 15 minute, 1 hour and 2 hour post exercise from the indwelling catheter.

Figure 1.



1-RM = 1-repitition maximal; AERET = acute exhaustive resistance exercise test; IP = immediate post.

4. Risks and Inconveniences

Resistance Exercise Workout. The squat exercise protocol used in this project is extremely physically demanding, especially for those individuals who have not participated in regular resistance exercise. Performing 6 sets of 10 repetitions with two minutes of rest between each set is exhausting. While this is a protocol that has been used safely on numerous occasions occasions in the past, extreme physical reactions to the intense exercise are not unusual. Physical symptoms such as dizziness, lightheadedness, nausea, and vomiting occur with regularity. Some individuals in the past have even passed out briefly. You will be closely attended to by laboratory personnel during the exercise in order to protect your safety should you experience any of these symptoms.

All of the above symptoms, while unpleasant, are temporary in nature. It is possible, but much less likely, that more serious and lasting injury can occur during this exercise protocol. The most likely site of injury with the squat exercise is in the low back. Acute muscle strains and muscle spasms can occur. Injuries to the discs in the low back can also occur, but the likelihood of this is small with one isolated squat exercise protocol. Knee injuries can occur, but it is very unlikely that anything worse than temporary knee pain will result from a one-time squat exercise protocol. It is also conceivable that other musculoskeletal injuries can occur, but the likelihood of these is very small. Proper instruction and supervision will be provided to you, and they can diminish the likelihood of any of these injuries occurring.

Finally, it is important to note that more serious, and potentially catastrophic medical problems can occur with exercise. The risk of sudden death associated with exercise is currently estimated at 1 in 250,000 young, healthy individuals in the United States. This is obviously an extremely small risk, and those who are at risk are nearly as likely to experience sudden death while working out on their own as they are in an exercise protocol such as this one. All laboratory personnel are CPR and AED certified, there is an AED immediately accessible in the laboratory, and the laboratory has emergency action protocols should an emergency arise.

Blood Draws. Blood samples will be obtained from a small, flexible tube (catheter) which will be inserted in a forearm vein. There is a slight risk of infection and/or fainting 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 a trained person in the Human Performance Laboratory. We will obtain blood draws in a fasted state with only water allowed for 8-12 hrs prior to the blood sampling. Blood draws by venapuncture 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 a trained person in the Human Performance Laboratory. You will be informed to refrain from giving blood during the course of the study. During the blood collection aspect of this investigation, experienced individuals will be responsible for inserting all catheters as well as collecting blood samples. Blood collection may be performed by any of the faculty in the Human Performance Laboratory (HPL) or graduate research assistants who have received training. All personnel in the HPL who are designated as potential phlebotomists are trained carefully by a person with extensive experience in phlebotomy including universal precautions and the responsible procedures to follow. All blood drawing materials will be sterile and aseptic technique will be used. You may experience a small initial pain attributed to inserting the catheter and bruising may occasionally occur after the procedures are completed. In addition, you may experience lightheadedness or faint which is common when individuals provide blood samples.

Various Inconveniences. The time requirement of this study is ~4.5 hours (0.5 hour for blood screening, 1 hours for the familiarization visits, ~3.0 hour for each resistance exercise session and blood draws.

5. Benefits

By participating in this study you will learn how to perform squat exercise in the Smith's Machine with correct technique and how strong you are in this exercise. We will explain all of your individual data collected from this study to you and interpret them along with answering any questions you might have in relation to these so that you can gain a maximum amount of educational understanding and use of your collected data. In general, this study will provide you a better understanding of how your coagulation and fibrinolytic levels change in response to a bout of resistance exercise. You may also gain knowledge on the possible benefits of resistance training and its potential to prevent stroke or deep vein thrombosis.

6. Economic Considerations

There are no costs to you, but there will be compensation given. Completion of Visits 1 (Familiarization) and 2 (Pre Blood Draw and 1-RM) will be given $5 each, while Visit 3 (Testing Protocol) will be compensated with $20.

7. Confidentiality

Electronic databases will be kept on a password protected computer in a locked office in the Human Performance Labaratory and backed up. Data files will be kept by unidentifiable numbers assigned to participants, so that identity can not be determined. All data will be coded with a unique identifier consisting of the first letter of Resistance Exercise and Coagulation, first letter of Resistance or Non-Resistance in parenthesizes depending on trained state, and two random numbers (e.g. REC(R)-01) in order to maintain confidentiality of the data. Subject codes will be used when statistical analyses are performed or when experimental feedback sheets for participants are produced. Data will be reviewed by research personnel as it becomes available. Any unusual observations will be brought to the attention of the principal investigator. All records will be kept for five years past the end of the study. All investigators are aware of the confidentiality involved with this study. All individuals will be made aware that the data will not be available or divulged to anyone outside of the experimental research team.

All participants will be informed prior to enrollment that they have the option to withdraw at anytime throughout the course of the study for any reason without any consequence. In the rare case of an adverse event, the participant will be given the opportunity to withdraw from the study without consequence.

It is the belief of the research team that this study does not involve more than minimal risk. Never-the-less, procedures have been carefully outlined to ensure minimization of any perceived risk to study participants.

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 that reviews research studies to protect the rights and welfare of participants

8. In Case of Illness or Injury

In the event you become sick or injured during the course of the research study, immediately notify the primary investigator, Dr. Brian R. Kupchak (office 860-486-2649 or cell phone 860-967-6609) or Dr. Jeffery S. Volek (office 860-486-6712 or cell phone 860-655-4235).

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.

9. Voluntary Participation

You do not have to be in this study if you do not want to participate. 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.

10. 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 investigators (Brian R. Kupchak, Ph.D., 860-486-2649 or Jeffery S. Volek, Ph.D. 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.

Signature: _____

Relationship: _____

Date: _____

_____ _____

Signature of Primary Investigator Phone

or

_____ _____

Signature of Person Obtaining Consent Phone