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The Ohio State University

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ON IN VIVO PLATELET RELEASE
IN MODERATELY TRAINED FEMALES:
RADIOIMMUNOASSAY OF PLATELET FACTOR 4
AND BETA-THROMBOGLOBULIN

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy
in the Graduate School of
The Ohio State University

By
Sally V. Rudmann, B.A., M.S.

*****

The Ohio State University
1986

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1. Sigma Delta Epsilon, Graduate Women in Science
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CHAPTER I

INTRODUCTION

Introductory Statement

In the past decade there has been a resurgence of interest in physical exercise as an essential component of a healthy lifestyle. In the 1979 Surgeon General's report on health promotion and disease prevention, regular physical fitness activities were associated with numerous health benefits including: more energy, need for less sleep, weight loss, increased strength and flexibility, greater self-reliance, decreased anxiety, improved cardiac efficiency, and a lower risk of cardiovascular disease. The Surgeon General's report emphasized the responsibility of the individual to initiate behavioral changes that would lead to the improvement of his/her own health status. Increased exercise constitutes a behavioral change that, along with other lifestyle factors, is effective in reducing cardiovascular risk. Exercise has been reported to result in an improvement in the overall efficiency of the cardiovascular system as well as a reduction in serum lipid levels. Interest in exercise as a mechanism for improvement of health status and the resultant decrease in health care cost, has resulted in the emergence of numerous workplace health promotion efforts.
In addition to the increased use of exercise to reduce risk among healthy individuals, exercise is becoming a more frequently prescribed treatment and rehabilitation modality in the chronically ill patient population. Exercise has been identified as a potential mechanism for improvement of the health status and functional capacity of patients with such conditions as pulmonary disease, diabetes mellitus, obesity, hypertension, peripheral vascular disease and arthritis.4

Along with the increased use of exercise prescription comes the responsibility of the health professional to further investigate the physiological consequences of exercise. Many researchers have concentrated their investigative efforts in the area of exercise physiology. This has led to the development of a rapidly expanding data base on the physiological effects of exercise.

A review of the literature supports the hypothesis that exercise has an effect on the hemostatic mechanism. Hemostasis has two major functions: maintaining blood in a liquid state while confined within the vascular system; and arresting bleeding in the event of vascular trauma.5 Disruptions in this mechanism may result in excessive bleeding or development of intravascular clotting.6 Hemostasis is dependent upon a set of complex interactions between blood vessels, plasma coagulation factors, circulating inhibitors, platelets, and the fibrinolytic system. Data from the literature provide experimental evidence of hemostatic changes during and following exercise. Data suggest that changes occur in the soluble plasma coagulation system, the fibrinolytic system and in platelet number and function.
Information regarding the nature and extent of the hemostatic responses requires further clarification. Such research data will provide the health professional with information from which to make appropriate decisions with regard to the consequences of exercise. These data are most essential when exercise prescription is aimed toward populations that may be hemostatically compromised consequent to an ongoing disease process or treatment regimen.

Platelets play an active role in primary hemostasis, blood coagulation and vascular integrity. Data suggest that platelets undergo physiological changes (quantitative and qualitative) during exercise. The effect of exercise on platelet function remains controversial and difficult to interpret due to lack of standardization of exercise protocols, procedural variations in in vitro platelet testing and the lack of suitable controls.

Statement of Purpose

The purpose of this study was to investigate the effect of a single episode of aerobic exercise on in vivo platelet functional parameters: more specifically this study was designed to determine whether platelets undergo activation and subsequent release of their alpha granule contents during aerobic exercise. Alpha granule release was determined by measuring the immediate post exercise levels of two platelet specific proteins: platelet factor 4 (PF4) and beta-thromboglobulin (BTG).
This study focused on the following research hypotheses:

1. The mean plasma level of BTG of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.

2. The mean plasma level of PF4 of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.

In order to determine the effects of aerobic levels of exercise on platelet alpha granule release it was necessary to establish for each subject an exercise workload that would insure that each exercising subject would be working at approximately the same level of their functional capacity or VO2 maximum. To accomplish this each female volunteer was maximally tested on a bicycle ergometer. Functional capacities were calculated from these maximal test data. Subjects were screened prior to testing in order to assure that each had no medical contraindications to exercise, and no history of medication or disease that is known to effect platelet function. An exercise history and demographic data were taken in order to assure that each subject met the criteria established for this protocol. All subjects were informed by the principle investigator with regard to the nature of the investigation and inherent risks. Each subject signed a written consent form.

Subjects were randomly assigned to exercise and control groups. Exercising subjects worked on a bicycle at a workload of 65 to 75 percent of their maximal functional capacity for twenty minutes.
Controls rested for twenty minutes. Both exercise test subjects and control subjects had refrained from exercise for 24 hours and from all medications for seven days prior to the test protocol. Blood specimens for measurement of the two dependent variables (BTG and PF4) were drawn within five minutes of the twenty minute exercise or resting period.

BTG and PF4 were measured by a competitive binding radioimmunoassay procedure at The Ohio State University Hospital Laboratory. All results were determined using a standard curve prepared during the test procedures. PF4 and BTG values were reported as the mean of duplicate values.

Limitations of the Study

The proposed research is limited by the following:

1. The number of subjects in the study was limited to twenty due to constraints of budget, time and accessibility of equipment and trained exercise technician support. Generalizability of data is limited with such small sample numbers. Small samples also result in decreased precision. In general, the standard error of the mean is inversely proportional to the square root of n.

2. Sampling was also limited to an accessible population of females willing to volunteer for the exercise protocol. Generalizability of the data to the population is limited since the sample was not representative of the population.

3. The research was limited to a study of the effect of one particular mode of aerobic exercise (bicycle ergometer) on the measured dependent variables. The study would have to be repeated using other forms of aerobic exercise in order to generalize the results for all aerobic exercise modalities.
4. Subjects were all active moderately trained females, therefore, results can be generalized only in the population of moderately trained females.

5. Maximal testing was performed using a bicycle ergometer. In subjects who are not trained bicyclers this test may provide an underestimate of maximal uptake by as much as ten percent. Ergometer maximal testing is also somewhat influenced by the ability, experience and motivational levels of the subjects. Variations in these factors limit the precision of the test.

6. Medication histories and health data were self-reported. No mechanism was available to validate the accuracy of these histories.

Definition of Terms

Activated partial thromboplastin time (APTT) and Partial thromboplastin time (PTT):

Procedural modifications of a laboratory screening test which assays for all coagulation factors except for factor VII and platelet factor 3.

Aerobic exercise:

An exercise level that relies heavily upon oxygen for energy production. For this protocol aerobic exercise was further defined as rhythmic exercise using large muscle groups that continues for 15 to 30 minutes at a workload of 65 to 75 percent of the functional capacity.

Aggregation:

The process by which platelets attach to one another during platelet plug formation.

Beta-thromboglobulin:

A platelet-specific protein that is contained within platelet alpha granules.

Bovine fibrin plates:

A laboratory assay used to measure plasma fibrinolytic activity.
Bruce protocol:
A standard treadmill protocol used in maximal exercise testing.

Euglobulin clot lysis time:
A laboratory assay used to measure plasma fibrinolytic activity.

Factor VIII, Fibrinogen, Prothrombin, Factor XII:
Coagulation proteins that participate in the physiological process of secondary hemostasis.

Fibrin Split Products:
Breakdown products resulting from the action of plasmin on fibrin(ogen).

Fibrinolytic activity:
The activity of the plasma fibrinolytic system, i.e., the ability to lyse or dissolve a clot.

Functional Capacity:
Maximal ability to utilize oxygen, as an energy source expressed as maximal oxygen consumption or VO\textsubscript{2} max (ml/kg/min) (See VO\textsubscript{2} max).

Hemostasis:
A physiologic process which effectively maintains a fluid circulation of blood within the vasculature, and the cessation of bleeding at the site of vascular damage.

Kilipond-meter (kpm):
The work done when a mass of one kilogram is lifted one meter against the force of gravity.

Moderately trained:
Individuals undertaking a consistent program of aerobic exercise which would meet the ACSM guidelines for cardiorespiratory exercise prescription. The ACSM recommends the following:

1. Exercise involving large muscle masses.
2. A frequency of at least three times per week.
3. An average conditioning intensity between 60 and 70 percent of functional capacity.

4. An exercise duration of at least 15 minutes per session.

**Plasminogen:**

A plasma substance that can be activated to plasmin. Plasmin is the enzyme responsible for the fibrinolytic activity of plasma.

**Platelet aggregometry:**

An in vitro test measuring platelet aggregation responses to a variety of agonists such as ADP, epinephrine and collagen.

**Platelet factor 4:**

A platelet-specific protein that is contained within the platelet alpha granules.

**Platelet release:**

After activation in vivo platelets undergo a secretory reaction releasing the contents of platelet granules into the circulation.

**Primary hemostasis:**

That process which involves the interaction between blood vessels and platelets resulting in the formation of the platelet plug.

**Prostacyclin (PGI$_2$):**

A potent inhibitor of platelet function synthesized by endothelial cells.

**Secondary hemostasis:**

Interactions between a series of plasma proteins resulting in the formation of a fibrin network which stabilizes the platelet plug.

**Thromboxane A$_2$ (TXA$_2$):**

A substance produced by platelets that functions as a potent platelet agonist and vasoconstrictor.

**$\text{VO}_2\text{max}$:**

Maximal oxygen consumption expressed in ml/kg/min (See Functional capacity).
Pulmonary Gas Abbreviations:

- $V$ - volume (flow) per unit time
- $F$ - fractional concentration of gas
- $E$ - expired
- $I$ - inspired
- STPD - standard temperature, pressure and dry

Moist gas volumes are corrected to a standard temperature (0°C), barometric pressure (760 mmHg) and dry (free of water vapor) using the formula:

$$\text{STPD factor} = \frac{273}{273 + °C \text{ insp}} \times \frac{P_B - P_{H_2O}}{760}$$

where:

- $°C \text{ insp} = \text{observed temperature of inspired air}$
- $P_B = \text{barometric pressure}$
- $P_{H_2O} = \text{water vapor pressure of inspired air}$
CHAPTER II

REVIEW OF THE LITERATURE

Introduction
Hemostasis involves the following physiological functions: maintaining a fluid circulation of blood within the vasculature, and the cessation of bleeding at the site of vascular damage. Hemostasis can be divided into two steps: primary and secondary. Primary hemostasis involves the interaction between the blood vessel and platelets, resulting in the formation of the primary platelet plug. Secondary hemostasis involves interactions between a series of plasma proteins, and results in the formation of a fibrin network which stabilizes the platelet plug. In addition, the body has a mechanism by which the fibrin clot is dissolved. This process is known as fibrinolysis. Data from the literature of applied physiology and exercise science provide evidence of numerous changes in primary and secondary hemostasis as a consequence of exercise.

Effects of Exercise on Secondary Hemostasis and Fibrinolysis
Many researchers have gathered data regarding the effects of exercise on secondary hemostatic function and fibrinolysis. Although
data are conflicting, many studies have documented both an increase in coagulability and in fibrinolytic activity. Collen et al. measured hemostatic changes in sixteen untrained healthy subjects after strenuous physical exercise on a bicycle ergometer. Exercise consisted of eight sessions of exhaustive physical exercise at a workload of 1200 Kilopond-meters (kpm). Most subjects were exhausted within five minutes. These researchers reported a decrease in the partial thromboplastin time (PTT) and an increase in factor VIIIc levels after exercise. The authors reported an apparent increase in fibrinolytic activity evidenced by an increase in the euglobulin clot lysis value (expressed in units), and an increase in the lysis zone on bovine fibrin films. Levels of fibrinogen, plasminogen and prothrombin did not change, nor was there a detectable increase in fibrin degradation products. These researchers also reported a significant increase in the turnover of fibrinogen and plasminogen suggestive of consumption. After exercise there was a significant increase in degraded alpha chains of fibrinogen.

Other research data support the hypothesis that secondary hemostatic changes are induced by exercise. Iatridis and Ferguson exercised 59 normal male subjects to exhaustion on a treadmill. The period of exercise varied from six to thirty-two minutes. Blood was drawn immediately after exercise. These researchers reported a significant shortening of the partial thromboplastin time (PTT); an increase in Factor VIIIc and Factor XII levels, and an increase in
fibrinolytic activity as measured by the euglobulin clot lysis and plasma plate methods.

Knudsen et al., measured several hemostatic parameters immediately following long distance running (12 and 28 kilometers). Euglobulin clot lysis time was shortened indicating an increase in fibrinolytic activity for both groups (12K and 28K). Mandalaki et al., assessed blood coagulation and fibrinolytic changes in a group of 38 runners, 20 minutes after completion of a marathon run. Runners were tested three consecutive years following a marathon run over the same course. In all three years, these authors reported a decrease in the activated partial thromboplastin time (APTT), a decrease in euglobulin lysis time and an increase in fibrin degradation products. Hyers and coauthors reported similar decreases in the APTT, decreased euglobulin clot lysis time and increased fibrin plate lysis. In each case change was progressive throughout the exercise protocol, and peaked either at maximal exercise levels or during a ten minute recovery phase. Davis et al., measured hemostatic variables during and following a progressive exercise protocol on a bicycle ergometer. These authors noted similar changes in coagulation and fibrinolysis. Major changes in fibrinolytic activity were achieved after 70-80% maximum heart rate (MHR) was achieved and peaked at maximal exercise. Increases in factor VIIIc were noted between 95 and 100% MHR and peaked at 5-10 minutes post-exercise. Data for this experiment demonstrated the importance of carefully standardized exercise
protocols and exerting subjects and controls at the same relative level. Vogt et al.,13 exercised ten healthy males to exhaustion on a bicycle ergometer. Blood was drawn before and immediately after exercise. Data from this study support other research findings. These researchers reported a decreased APTT and a shortening of the euglobulin clot lysis time. Britton et al.,15 demonstrated that activation of fibrinolysis during exercise was not mediated by a beta adrenergic receptor.

While the majority of investigators have reported coagulation and fibrinolytic changes during and/or following exercise, conflicting data have been published. For example, Britton et al.,15 reported no change in Factor VIIIc levels after five minutes of strenuous exercise on a bicycle ergometer. Collen et al.,8 reported no change in Factor XII levels after a similar exercise session. Huisveld et al.,16 reported decreases in post-exercise levels of Factor XII and other coagulation factors after these figures were corrected for changes in plasma volume.22

These and other conflicting reports are due in some part to the following:

1. Lack of exercise standardization within and between studies.
2. Lack of standardized testing procedures and uniform blood sampling techniques.
3. Lack of correction for blood volume changes in pre- and post-test measurements.
4. Lack of suitable controls in many studies.
5. Lack of detailed medical and medication histories on subjects and/or controls.

The Effect of Exercise on Platelet Number and Function

Exercise has been shown to effect platelet size, number and function. Burghuber et al.,18 demonstrated a decreased sensitivity to prostacyclin (PGI₂) immediately following 20 minutes of either jogging or squash. Dix and co-workers19 demonstrated an increased sensitivity of platelets to PGI₂ when measurements were taken 24 hours after a marathon race. Many researchers have reported either an increase in platelet count20,21,22,23,24 or an increase both in platelet count and platelet size21,23 following exercise. Bennett25 was unable to demonstrate an increase in platelet count after two exercise sessions. The conflicting data generated by this study are most probably due to the low level of exercise used in the research protocol (walking 3.2 km or 9.6 km). Although results are mixed, some researchers have reported changes in platelet aggregation with ADP, epinephrine and/or collagen following exercise sessions. Mandalaki et al.,11 reported increased aggregability with ADP and collagen after a marathon run in the extreme heat. No changes in aggregation were noted following two other marathons run at lower temperatures. Warlow and Ogston20 reported increased aggregation of platelets with ADP, epinephrine and collagen on 24 healthy untrained subjects following fifteen minutes of running. Siberba et al.,26 exercised twenty highly trained subjects to exhaustion on a bicycle ergometer. These researchers reported an increase in both the rate and extent of
aggregation with ADP and collagen, as well as an increase in the number of reversible platelet microaggregates. Siess et al.,\textsuperscript{27} reported no increase in platelet aggregation with arachidonic acid, collagen, epinephrine and ADP after eight minutes of treadmill exercise. Disparity between these two reports may be due to differences in relative exercise intensity. Data regarding the effects of exercise on platelet aggregation must be interpreted with caution. Platelet aggregometry is variable and difficult to standardize procedurally. A correlation between platelet function in vivo, and platelet aggregation in vitro has not been well established.\textsuperscript{28}

Recently two specific platelet proteins, platelet factor 4 (PF4) and beta-thromboglobulin (BTG) have been isolated and characterized.\textsuperscript{29,30,31,32,33,34,35} Platelets contain three secretory storage granules. Platelet factor 4 and BTG are stored within platelet alpha granules. Platelets may become activated in response to a number of stimuli or agonists. Activation results in subsequent shape change, aggregation and release of granular contents.\textsuperscript{36,37,38,38} PF4 and BTG have been demonstrated to be essentially platelet specific.\textsuperscript{30} Both proteins can be utilized as specific markers of in vivo platelet alpha granule release.\textsuperscript{29,30,31,40}

A sensitive radioimmunoassay has been developed for both proteins.\textsuperscript{41,42,43,44} When suitable procedural controls are in place to insure that in vitro platelet release is inhibited, the presence of elevated levels of these substances in the plasma is a specific indicator of platelet release in vivo.\textsuperscript{29,32,39,43}
Some data have been published regarding the effect of exercise protocols on plasma levels of BTG and/or PF4. Because of the hypothesized association of platelet activation and coronary atherosclerosis much of the data has been generated from protocols testing the hypothetical relationship between platelet activation and exercise-induced myocardial ischemia. Green et al. exercised forty coronary artery disease (CAD) patients on a treadmill using a symptom limited Bruce Protocol. In this study the authors reported significant elevations in PF4 levels in those CAD patients with exercise-induced cardiac ischemia. Rolmensch et al. reported similar increases in postexercise PF4 concentrations in six of seven patients with positive exercise tolerance tests. These authors demonstrated an increase in PF4 levels over time when specimens were drawn through an indwelling catheter as opposed to venipuncture. Such increases in PF4 were concluded to be artifactual. The authors suggest that such elevations were most probably due to the disruption of the vascular lining by the catheter with subsequent platelet activation and release. Levine et al. reported a significant increase in plasma PF4 concentrations after standardized treadmill tests. These researchers, however, could not confirm the observation of Green that increases in plasma PF4 were related to the development of myocardial ischemia. Stratton et al. reported an increase in exercise induced PF4 levels in CAD patients, and an increase in both PF4 and BTG after exercise in normal subjects and CAD patients. These researchers used a
symptom-limited standard Bruce treadmill exercise protocol. Mehta and Mehta\textsuperscript{49} exercised CAD patients and normal subjects on a treadmill using a symptom limited Bruce protocol. These authors report increases in post-exercise BTG both in normal controls and CAD patients. These authors reported that the mean increase in BTG was greater in those with positive exercise tests than in those with negative tests. Strauss et al.,\textsuperscript{50} reported experimental data that did not support the hypothesized association between myocardial ischemia and the release of PF4 and BTG during exercise. In this study mean values for PF4 and BTG were the same for normal subjects, for CAD patients with positive exercise tests and for CAD patients with negative exercise tests. Other researchers reported no exercise induced increases in PF4 and/or BTG. Ek et al.,\textsuperscript{51} exercised CAD patients on a bicycle ergometer using a graded, symptom-limited exercise protocol. These researchers reported no increase in PF4 levels following exercise. Mant et al.,\textsuperscript{52} reported no changes in plasma concentrations of PF4 and BTG after normal subjects underwent maximal exertion on a bicycle ergometer. Hughes et al.,\textsuperscript{53} reported no increase in PF4 and BTG levels after maximal treadmill exercise.

Only one study has looked at the effects of prolonged exercise on PF4 levels. Knudsen et al.,\textsuperscript{14} reported an increase in plasma levels of PF4 after distance running (12 kilometers and 28 kilometers). No reports are available regarding the effect of prolonged exercise on BTG levels.
Significance of Platelet Release

Studies which accurately measure the in vivo release of platelets would be of significance both in disease and in health. Acquired platelet functional defects have been found to be associated with a number of clinical conditions. These abnormalities are similar to those occurring in patients with platelet storage granule deficiencies. Such disorders are believed to be due to the presence of "exhausted" platelets: i.e., platelets that have undergone activation and release following exposure to in vivo activators such as damaged endothelium and immune complexes. A similar platelet dysfunctional status has been noted following cardiopulmonary bypass. These transient platelet disorders are associated with selective alpha granule release. Doyle et al. studied the relationship between the plasma levels of PF4 and BTG and platelet survival in vivo. These researchers reported a significant correlation between elevated plasma levels of these proteins and decreased platelet survival time. These data would suggest that after release of their alpha granule contents (as measured by increased plasma PF4 and BTG), platelets have a shortened life span and altered functional capacity in vivo. Information regarding the hypothesized in vivo activation of platelets during exercise would provide evidence relating to the "functional status" of platelets post-exercise.

In addition, platelets have been implicated as having a role in the development of atherosclerotic lesions. Atherosclerotic
plaque has been reported to consist of proliferative lesions of smooth muscle cells, connective tissue secretory products and accumulated lipid. The genesis of these lesions is believed by many to begin with endothelial tissue damage. Platelets adhere to exposed subendothelial surfaces with subsequent aggregation, and local release of platelet constituents. Smooth muscle cells then migrate into the site and undergo platelet-mediated proliferation. Smooth muscle cells form a connective tissue matrix resulting from secretion of collagen and elastic fiber proteins. Lipid then accumulates at the lesion site. The formation of atherosclerotic lesions appears to be dependent upon the activation of platelets, and the subsequent release of platelet granule constituents.59

In addition to the role of platelets in atherosclerogenesis, platelet activation has been experimentally associated with the pathophysiology of sudden cardiac death.60,61,62 Hammon and coworkers,60 studied the effects of platelet inhibition on the occurrence of ventricular fibrillation in dogs. Occlusion of the circumflex coronary artery in dogs results in a high incidence of ventricular fibrillation. This intervention was described as resulting in maximal platelet activation. Ventricular fibrillation after occlusion was largely limited to the first ten minutes after the intervention which is analogous to the rapid occurrence of many instances of sudden cardiac death in humans. These researchers reported a reduction in ventricular fibrillation from 53% to 6% after infusion of a potent platelet
inhibitor, PGI$_2$. These researchers further suggested that protection from ventricular fibrillation was due to the production of a metabolite of endoperoxidase which accumulates when thromboxane synthesis is inhibited by PGI$_2$ administration.

Such data regarding platelet interaction in disease provides additional rationale for elucidating the in vivo response of platelets to various levels of exercise in humans.

**Implications for Further Research**

It is evident that further research is necessary to elucidate the effect of exercise on platelet release and subsequently on platelet function. Research data published to date suffer from the following weaknesses:

1. There has been a failure by most researchers to standardize exercise level across subjects in order to insure that each is exercising at the same relative level.

2. Little data have been published regarding the effect of prolonged exercise on platelet release. Data have been primarily focused upon the effects of short duration, exhaustive exercise episodes.

3. Studies thus far have had wide procedural variations particularly with regard to blood specimen collection and laboratory procedures.

4. Many researchers have failed to include thorough physical screening and medication records for subjects. Such data are necessary in order to evaluate the effects of disease and/or medication upon platelet function.

5. Many researchers have not included a suitable control group within the research design.
6. Data from many studies may have been confounded by in vitro platelet release. This error can be minimized by procedural modifications which have been documented to eliminate in vitro release as well as by appropriate controls.

**Summary Statement**

In summary, it is evident that various levels of exercise result in quantitative and qualitative changes in many hemostatic parameters. These changes in vivo are reflected by changes in in vitro hemostatic laboratory assays. Exercise has been documented to bring about alterations in primary and secondary hemostasis and fibrinolysis. Exercise induced changes suggest that hemostatic and fibrinolytic systems are activated during exercise.

Platelets are cellular elements that actively participate in primary hemostasis, and contribute to the mechanism of secondary hemostasis. During the primary hemostatic process, platelets undergo activation. Activation involves shape change, adhesion to subendothelial tissues, aggregation and release of granular contents. Platelet activation in vivo results in the liberation of two platelet specific proteins (BTG and PF4) from platelet alpha granules. The presence of these release substances in plasma is a specific marker of platelet release.

Research data suggest that in vivo platelet activation results in a hemostatic disorder resulting from the circulation of dysfunctional "exhausted" platelets. Platelet activation in vivo has also been implicated in athersclerogenesis and in sudden cardiac death.
It is essential that the effect of exercise on platelet function be elucidated. Further research is necessary in order that the health educator and health care professional have adequate data with which to decide the appropriateness of exercise in various health care/wellness settings.

It was the purpose of this study to address the following research hypotheses:

1. The mean plasma level of BTG of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.

2. The mean plasma level of PF4 of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.
CHAPTER III

METHODS AND PROCEDURES

Statement of Purpose

The purpose of this study was to investigate the effect of a single episode of aerobic exercise on in vivo platelet functional parameters: more specifically this study was designed to determine whether platelets undergo activation and subsequent release of their alpha granule contents during aerobic exercise. Alpha granule release was determined by measuring the immediate post exercise levels of two platelet specific proteins: platelet factor 4 (PF4) and beta-thromboglobulin (BTG).

Research Hypotheses

This study focused on the following research hypotheses:

1. The mean plasma level of BTG of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.

2. The mean plasma level of PF4 of the aerobically exercising test subjects will be significantly higher than the mean of the non-exercising controls.

Subjects

Subjects were moderately trained female volunteers (n=20). For the purpose of this study, moderately trained was defined as those individuals who participated in aerobic levels of exercise for periods
Aerobic exercise was defined as those physical activities involving large muscle groups, and during which the exercise intensity is easily sustained with little variability in heart rate. These activities correspond to the type, frequency and duration of exercise recommended by the American College of Sports Medicine (ACSM) for cardiorespiratory fitness. Subjects were required to meet the following qualifications in order to be included in the study:

1. no medical contraindications to exercise
2. female, 20 to 40 years of age
3. moderately trained
4. free from acute and chronic disease
5. medication free for seven days prior to testing
6. free from the following cardiac risk factors:
   a. smoking
   b. hypertension
   c. history of coronary artery disease

Subjects were limited to an accessible population with the vicinity of The Ohio State University.

**Research Design**

The research design was a post-test only control group design:

\[ R \times 0_1 \]
\[ R \rightarrow 0_2 \]
Statistical Hypotheses

\[ H_0 : u_{test \ BTG} = u_{control \ BTG} \]
\[ H_0 : u_{test \ PF4} = u_{control \ PF4} \]

Alternate Hypotheses

\[ H_1 : u_{test \ BTG} > u_{control \ BTG} \]
\[ H_1 : u_{test \ PF4} > u_{control \ PF4} \]

Subject Screening and Consent Procedures

Subjects for this study were moderately active female volunteers (N=20). Subjects were 24 to 40 years old (X = 30.7 yr.). Subjects were individually informed regarding the nature and significance of the research, the details of the exercise protocol and inherent risks both verbally and in writing (Appendix A). Each subject read and signed a consent form meeting the requirements of The Ohio State University, Human Subjects Research Committee protocol number 85H0256 (Appendix A). Subjects were asked to complete a personal fitness questionnaire. This questionnaire (Appendix A) was modified from similar forms used in The Ohio State University Cardiac Rehabilitation and Faculty/Staff fitness programs, and met recommendations for exercise screening as outlined by the ACSM.4 Screening included a complete medical history, medication history and coronary risk evaluation. Written responses were reviewed during an interview with a licensed physician. Each subject had a limited physical exam by a physician who signed a statement attesting to the suitability of the
subject for the research exercise protocol. In addition, a physician was present during all maximal exercise testing.

All subjects met the following qualifications:

1. female 22-40 years
2. moderately trained
3. free from chronic/acute disease
4. medication free for seven days prior to exercise protocol
5. free from the following cardiac risk factors:
   a. smoking
   b. hypertension
   c. history of coronary artery disease

Maximal Exercise Test

Twenty female volunteers meeting the above criteria were given a graded, symptom-limited maximal exercise test on a bicycle ergometer. The exercise protocol was modified from the maximal cycle ergometer protocol described in the 1986 ACSM guidelines. Protocols were adapted to match the exercise levels of the subject being tested (Appendix A). Beginning workloads were adjusted based on the subject's exercise history, so that subjects would exercise a minimum of five minutes and a maximum of fifteen minutes. After a warmup of approximately two minutes, the initial workload was applied. The workload was increased by a given increment after each two minute exercise period. The following measurements were made each minute for the duration of testing:
1. heart rate (single lead EKG)

2. volume of inspired gases ($V_I$) (spirometer with Microscribe™ 4500 Strip Chart Recorder)

3. percent $O_2$ in expired air ($O_2$ Analyzer, Ametek™ S-3A/I).

4. percent $CO_2$ in expired air ($CO_2$ analyzer, Beckman™, Medical Gas Analyzer LB-2)

$CO_2$ and $O_2$ analyzers were calibrated before each use with a known reference gas having the following assay values: 4.08% $CO_2$ and 16.08% $O_2$. Ambient room temperature ($°C$), relative humidity (%) and barometric pressure (mmHg) were recorded prior to testing. All bicycle ergometers were calibrated with known weights prior to testing. Data from maximal exercise tests were recorded on worksheets designed for this purpose (Appendix B). Using measured variables $V_I$, $FgCO_2$ (%), $FgO_2$ (%) the following parameters were calculated:

- $V_{E, STPD}$ - volume of expired gas corrected for STPD
- $VO_2$ (L/min) - volume $O_2$ used in L/min
- $VO_2$ (ml/kg/min) - volume of $O_2$ used ml/kg/min
- $RQ$ - respiratory quotient = $VCO_2/VO_2$
- $HR_{max}$ - maximal heart rate

The maximal $O_2$ uptake was expressed as the $VO_2$ (ml/kg/min) at maximal effort.

Maximal testing was performed in order to measure the functional capacity or aerobic capacity of each subject. The aerobic capacity was calculated using data recorded during testing, and was expressed as $VO_2$ max (ml/kg/min). This maximal capacity was used to calculate the exercise test workload for each exercising subject, so that each
would be working at the same relative capacity. The exercise test workload was calculated so that subjects would be working at approximately seventy percent of their functional capacity (65-75 percent, \( \bar{x} = 69.6 \)). This level of exercise corresponds to that recommended by the ACSM for cardiorespiratory training.\(^4\)

All maximal testing conformed to the general principles of exercise testing as outlined by the ACSM.\(^4\) The general guidelines were as follows:

1. Initial exercise levels were at an intensity considerably lower than the subjects estimated maximal capacity.
2. The exercise intensity was increased gradually in stages with observations made at each stage.
3. Indications for stopping exercise were closely observed throughout testing by a licensed physician and experienced exercise test technicians.
4. The safety of testing was carefully determined prior to testing.
5. Patient heart rate, EKG, appearance and self-rating of exertion were monitored throughout testing.
6. Subjects were observed for at least ten minutes following maximal testing.
7. Exercise tolerance was calculated directly from oxygen uptake.

Due to lack of sufficient environmental control, it was not possible to maintain a room temperature of 22°C or less during testing as recommended by the ACSM.\(^4\) All subjects but one were exercised at the same time of day under approximately the same ambient conditions. All subjects except for one were maximally tested at an ambient room
temperature of 23–25°C. A single subject was tested approximately three hours earlier at an ambient room temperature of 21°C. Upon completion of the maximal test subjects were given a form listing their max VO₂, a list of normal values by age and the time and date of their return appointment (Appendix A).

A bicycle ergometer was chosen as the exercise modality for the following reasons:

1. Most subjects were unfamiliar with maximal testing and had no experience on a treadmill.

2. The bicycle protocol would limit the degree of orthopedic stress and possible injury.

3. Logistically bicycles were both portable and available in sufficient numbers.

Exercise on the bicycle ergometer was undertaken with the knowledge that ergometer testing will usually underestimate the maximal oxygen capacity by about five to ten percent.63,64

Aerobic Exercise Test Sessions

All subjects were maximally tested within two weeks of the actual experimental test protocol. Subjects were randomly assigned to experimental and control groups. All subjects were instructed to refrain from strenuous physical activity for a minimum of twenty-four hours prior to the final test protocol. Subjects were also instructed to refrain from any medication (over-the-counter or prescription) for seven days prior to the test. All tests were run in the same facility at approximately the same ambient room temperature (23–25°C).
Both exercise and control subjects reported to the test facility. Controls remained sedentary during the twenty minute test period. Experimental subjects exercised for twenty minutes on a calibrated bicycle ergometer at the calculated workload (65 to 75% of VO₂ max, \( \bar{x} = 69.6 \)).

**Specimen Collection and Testing**

Blood specimens were drawn by a qualified technician using an aseptic venipuncture technique. Specimens were drawn with a 21 gauge butterfly needle into a ten milliliter plastic syringe. Two milliliter samples of whole blood were immediately added to a precooled mixture of anticoagulant and platelet inhibitors containing: 0.5 ml 30mM Ethylenediamine tetraacetic acid (EDTA), 5 ul of a 50 ug/ml solution of prostaglandin (PGE₁) in 95% ethanol, and 50 ul of a 36 mg/ml solution of acetylsalicylic acid (ASA) in 95% ethanol. The anticoagulant platelet inhibitory mixture was prepared immediately before the addition of the blood sample in 12 x 75 mm plastic tubes. This mixture was formulated in order to provide an anticoagulant (EDTA) and inhibitors of platelet in vitro release (PGE₁ and ASA). Specimens were placed on melting crushed ice immediately after collection. Within thirty minutes of collection, specimens were centrifuged at 1900 G for one hour at 4°C. Centrifuge speed was calculated using the formula for a horizontal head centrifuge:

\[
\text{RCF(G)} = (11.2 \times 10^{-6}) \times (R) \times (N^2)
\]

(RCF = relative centrifugal force or G; R = radius of head in cm and N = revolutions per minute).
Plasma for the assay was stored at minus 20°C or below until the assay was performed. All assays were run within four weeks of collection. Specimens are known to be stable for this period when stored at minus 20°C or below. All specimens were drawn in duplicate.

PF4 and BTG are both competitive binding radioimmunoassay procedures. In both tests the non-labeled (non-radioactive) antigen in the unknown plasma competes with a constant amount of radiolabeled (¹²⁵ I) antigen for binding sites on a limited amount of known antiserum. The bound antigen-antiserum complex is precipitated with ammonium sulfate. Radioactivity of the precipitate is measured using a gamma counter. The amount of radioactive antigen bound to the antiserum (therefore the gamma count/unit time) is inversely proportional to the amount of PF4 or BTG present in the unknown plasma. The amount of PF4 or BTG present is calculated from a five point standard curve run at the same time as the test specimens (Appendix B). Both standard and unknown values were reported as the mean of duplicate samples.

All specimens and standards were tested using the standard test protocol of the Hematology Department of The Ohio State University Hospitals under the supervision of John Brandt, M.D. (Appendix B) who served as a consultant for this research project.

Normal ranges for PF4 and BTG have been established for this laboratory and are as follows: PF4 (ng/ml) 0.0 to 4.0 and BTG (ng/ml) 6.2 to 26.2.67
Research Design, Statistical Hypothesis and Date Analysis

The research design is the post-test only control group design (Design 6 of Campbell and Stanley). This design can be diagrammed as follows:

\[
R \times O_1 \\
R \quad O_2
\]

Random assignment of subjects to test and control groups assures control for the following threats to internal validity: history, maturation, testing, instrumentation, regression, selection, mortality, and interactions of selection and maturation. This design also controls for the interaction of testing and \( X \) which is a threat to external validity in some designs utilizing pre-testing.

The independent variable in this research protocol is exercise level. The dependent variables being measured are post-exercise levels of PF4 and BTG.

Based on the literature review the research hypotheses are as follows:

1. The mean plasma level of BTG of the exercise test subjects will be significantly higher than the mean of the sedentary control group.

2. The mean plasma level of PF4 of the exercise test subjects will be significantly higher than the mean of the sedentary control group.

Null hypothesis:

\[ H_0: u_{\text{test \ BTG}} = u_{\text{control \ BTG}} \]

\[ H_0: u_{\text{test \ PF4}} = u_{\text{control \ PF4}} \]
Alternate hypothesis:

\[ H_1: u_{\text{test}} \text{ BTG} > u_{\text{control}} \text{ BTG} \]
\[ H_1: u_{\text{test}} \text{ PF4} > u_{\text{control}} \text{ PF4} \]

The data analysis will include:

1. mean
2. standard deviation
3. t-test with independent groups and a directional hypothesis (one-tailed test \( alpha 0.05 \))
4. correlation between PF4 and BTG (Pearson-Product moment)
Chapter IV

RESULTS AND DISCUSSION

Statement of Purpose

The purpose of this study was to investigate the effect of a single episode of aerobic exercise on in vivo platelet functional parameters: more specifically this study was designed to determine whether platelets undergo activation and subsequent release of their alpha granule contents during aerobic exercise. Alpha granule release was determined by measuring the immediate post exercise levels of two platelet specific proteins: platelet factor 4 (PF4) and beta-thromboglobulin (BTG).

Statistical Hypotheses

\[ H_0 : u_{test \ BTG} = u_{control \ BTG} \]
\[ H_0 : u_{test \ PF4} = u_{control \ BTG} \]

Alternate Hypotheses

\[ H_1 : u_{test \ BTG} > u_{control \ BTG} \]
\[ H_1 : u_{test \ PF4} > u_{control \ PF4} \]

Results

Female volunteers (N=20) were randomly assigned to exercise and control groups. Subjects ranged in age from 22 years to 40 years of
age ($\bar{x} = 30.7$ years). Maximal test results were reported in ml/kg/min (Table 1). Maximal $\dot{V}O_2$ or functional capacities of the subjects ranged from 37.0 to 61.0 ml/kg/min ($\bar{x} = 48.3$). Using the classification scale for aerobic capacity based on the work of Katch and McArdle, sixteen of the subjects had aerobic capacities classified as high, one was good and three had average capacities. Of the four subjects with capacities classified below high, two were in the exercise group and two were non-exercising controls. Exercise and control groups were similar with regard to age and functional capacity (Table 2).

Exercisers (n=10) had a mean age of 32.4 years (SD=5.3) while controls (n=10) had a mean age of 28.9 years (SD=5.6). Exercising subjects had a mean $\dot{V}O_2$ maximum of 50.4 ml/kg/min (SD=6.8) and non-exercising controls had a mean $\dot{V}O_2$ maximum of 47.9 ml/kg/min (SD=6.6).

In order to calculate the experimental protocol workload for each exercising subject, the oxygen uptake (ml/kg/min) was plotted against the bicycle ergometer workload on linear graph paper (Appendix A). These graphs were used to calculate workloads for the exercise protocol. All subjects were exercised for twenty minutes at a workload representing 65 to 75% ($\bar{x} = 69.6$) of their maximal functional capacity (Table 3).

Blood specimens were drawn within five minutes of exercise or after twenty minutes of rest for controls.
Table 1
Maximal Ergometer Test Results

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Age</th>
<th>Exercise (E) Control (C)</th>
<th>Max $\text{VO}_2$ (ml/kg/min)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>E</td>
<td>49.4</td>
<td>high</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>C</td>
<td>45.8</td>
<td>high</td>
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<tr>
<td>3</td>
<td>33</td>
<td>C</td>
<td>50.1</td>
<td>high</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>E</td>
<td>48.4</td>
<td>high</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>E</td>
<td>54.1</td>
<td>high</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>E</td>
<td>50.1</td>
<td>high</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>E</td>
<td>43.5</td>
<td>good</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>C</td>
<td>50.7</td>
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<td>33</td>
<td>C</td>
<td>46.6</td>
<td>high</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>E</td>
<td>55.1</td>
<td>high</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>E</td>
<td>61.0</td>
<td>high</td>
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</tr>
<tr>
<td>16</td>
<td>33</td>
<td>C</td>
<td>37.5</td>
<td>average</td>
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<td>17</td>
<td>24</td>
<td>C</td>
<td>56.0</td>
<td>high</td>
</tr>
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<td>18</td>
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<tr>
<td>20</td>
<td>25</td>
<td>C</td>
<td>48.9</td>
<td>high</td>
</tr>
</tbody>
</table>

$\bar{x} = 30.7$ $\quad$ $\bar{x} = 49.2$

SD = 5.6 $\quad$ SD = 6.6

(1) Classification of Katch and McArdle\textsuperscript{71} based on normal values by age and sex. Categories were low, fair, average, good and high (Appendix A).
<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Age</th>
<th>Max ( \dot{V}O_2 ) ml/Kg/min</th>
<th>Test Workload (KPM)</th>
<th>% of Max ( \dot{V}O_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>49.4</td>
<td>750</td>
<td>70</td>
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<td>5</td>
<td>34</td>
<td>54.1</td>
<td>900</td>
<td>72</td>
</tr>
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<td>6</td>
<td>31</td>
<td>50.1</td>
<td>720</td>
<td>70</td>
</tr>
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<td>7</td>
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<td>11</td>
<td>24</td>
<td>61.0</td>
<td>1080</td>
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<td>13</td>
<td>24</td>
<td>37.5</td>
<td>540</td>
<td>69</td>
</tr>
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</tr>
<tr>
<td>18</td>
<td>29</td>
<td>48.2</td>
<td>810</td>
<td>71</td>
</tr>
</tbody>
</table>

\( \bar{x} = 50.4 \) \( SD = 6.8 \) \( \bar{x} = 69.6 \) \( SD = 1.6 \)
BTG and PF4 results were reported as means of duplicate samples. Results of the BTG and PF4 (ng/ml) are illustrated in Table 4. The mean BTG for the control group was 18.23 ng/ml (SD = 9.7). The mean BTG for the exercise group was 38.4 ng/ml (SD = 31.2). The mean PF4 for the control group was 1.7 ng/ml (SD = 2.3) compared with a mean of 9.2 ng/ml (SD = 10.9) for the exercise group. The normal range for these laboratory values reported by The Ohio State University Hospital Laboratory are as follows:

- **BTG:** 6.2 - 25.8 ng/ml
- **PF4:** 0.0 - 4.3 ng/ml

Table 4 illustrates that in the control group, three individuals had elevated levels of BTG and one had an elevated PF4. In these cases the maximum BTG was 31.9 ng/ml. The one elevated PF4 was 6.1 ng/ml. In the exercise group, five individuals had elevated BTG levels. Three of these were more than two times higher than the upper limit of the normal range (greater than 5.16 ng/ml). Among the exercising subjects four had marked elevations in PF4. In each case these results were at least two times greater than the upper limit of normal (greater than 8.6 ng/ml) and three of the four were elevated to levels greater than four times the upper limit of normal (greater than 17.2 ng/ml).

The t-test for independent measurements and Pearson-Product Moment Correlation were performed using the "Statistical Analysis System" (SAS) software package. Data were imput into the system by key
punch cards. The program was run on The Ohio State University IBM 3081 mainframe. The t-test was a one-tailed test due to the directional nature of the research hypothesis. Results of the t-test are illustrated in Table 5.

Table 4

<table>
<thead>
<tr>
<th>Exercise Subjects</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Subject Number</td>
<td>BTG (ng/ml)</td>
</tr>
<tr>
<td>1</td>
<td>11.3</td>
</tr>
<tr>
<td>4</td>
<td>↑84.1</td>
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<tr>
<td>5</td>
<td>18.0</td>
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<td>6</td>
<td>11.9</td>
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<td>7</td>
<td>11.2</td>
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<tr>
<td>10</td>
<td>↑29.4</td>
</tr>
<tr>
<td>11</td>
<td>↑75.3</td>
</tr>
<tr>
<td>13</td>
<td>↑42.6</td>
</tr>
<tr>
<td>15</td>
<td>↑84.2</td>
</tr>
<tr>
<td>18</td>
<td>16.1</td>
</tr>
</tbody>
</table>

\[ \bar{x} = 38.4, \bar{x} = 9.2 \]

\[ SD = 31.2, SD = 10.9 \]

\[ \bar{x} = 18.23, \bar{x} = 1.7 \]

\[ SD = 9.7, SD = 2.3 \]

(1) BTG normal range = 6.2 to 25.8 ng/ml
(2) PF4 normal range = 0 to 4.3 ng/ml
(3) Subject #2 withdrew from experiment therefore no measurements were made.
(4) ↑ elevated above normal ranges for this laboratory.
(5) Test not performed.
Table 5

**t-Test Results**

<table>
<thead>
<tr>
<th>Variable: RIA_j or BTG</th>
<th>Group</th>
<th>N</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>10</td>
<td>-1.8592</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable: RIA_2 or PF4</th>
<th>Group</th>
<th>N</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>10</td>
<td>-2.0131</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

$t_{critical} = \pm 1.74$

The null hypotheses for this research design were stated as:

- $H_0: u_{test BTG} = u_{control BTG}$
- $H_0: u_{test PF4} = u_{control PF4}$

The null hypothesis for the dependent variable BTG (RIA\_j) was:

- $H_0: u_{test BTG} = u_{control BTG}$

The alternate hypothesis for the dependent variable BTG (RIA\_j) was:

- $H_1: u_{test BTG} > u_{control BTG}$

For the dependent variable BTG (RIA\_j), the calculated $t$ or $|t|$ was $-1.8592$ (degrees of freedom = 17). At the a priori alpha level of 0.05, $t$ critical is equal to $\pm 1.74$. Since $|t|$ is greater than $t$ critical, $H_0$ can be rejected.
The null hypothesis for the dependent variable PF4 (RIA₂) was:

\[ H_0: u_{\text{test PF4}} = u_{\text{control PF4}} \]

The alternate hypothesis for the dependent variable PF4 (RIA₂) was:

\[ H_1: u_{\text{test PF4}} > u_{\text{control PF4}} \]

For the dependent variable PF4 (RIA₂) the calculated t or \(|t|\) was -2.0131 (degrees of freedom = 17). At the a priori alpha level of 0.05 t critical is equal to ±1.74. Since \(|t|\) is greater than t critical, \(H_0\) can be rejected.

Therefore, the mean values for the aerobically exercising population were significantly higher for both dependent variables (BTG and PF4) when measured within five minutes of the exercise session. These data support the research hypotheses stated previously:

1. The mean plasma level of BTG of the exercise subjects will be significantly higher than the mean of the resting controls.
2. The mean plasma level of PF4 of the exercise subjects will be significantly higher than the mean of the resting controls.

A Pearson-Product Moment Correlation Coefficient (r) was calculated to determine the degree of association between the BTG and PF4 tests (Table 6). The correlation coefficient was calculated to be 0.77603. This indicates that there was a very strong positive association between the two dependent measurements (BTG and PF4).

Figure 1 illustrates the distribution of test results for both measures for both control and exercise groups.
Platelet counts were run on all subjects to control for the possible effect of abnormal platelet levels on the availability of BTG and PF4. Platelet counts were drawn at the same time that specimens were collected for BTG and PF4 levels. Platelet counts were collected in the same manner described for BTG and PF4. Platelet count results are recorded in Table 7.

Table 6

Correlation Coefficients for the Variables BTG and PF4

<table>
<thead>
<tr>
<th></th>
<th>BTG</th>
<th>PF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTG</td>
<td>1.00000</td>
<td>0.77603</td>
</tr>
<tr>
<td>PF4</td>
<td>0.77603</td>
<td>1.00000</td>
</tr>
</tbody>
</table>

Discussion

The data from this study support the alternative hypothesis stated previously:

\[ H_1: \mu_{\text{test \ BTG}} > \mu_{\text{control \ BTG}} \]
\[ H_1: \mu_{\text{test \ PF4}} > \mu_{\text{control \ PF4}} \]

The means for both dependent variables measured (BTG and PF4) were significantly greater than the means of the resting controls at an alpha level of 0.05. It is evident that, despite the significant differences between group means, there was a great deal of variability in both groups, and for both measures. While it is evident that five members of the exercise group had extremely elevated levels of BTG and
## Table 7

### Platelet Counts for Exercise and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>BTG</th>
<th>PF4</th>
<th>Plt.Ct. (x 10^3)</th>
<th>BTG</th>
<th>PF4</th>
<th>Plt.Ct. (x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.3</td>
<td>0.5</td>
<td>(4) 416</td>
<td>2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>↑84.1</td>
<td>↑26.3</td>
<td>333</td>
<td>3</td>
<td>↑31.9</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>18.0</td>
<td>2.5</td>
<td>↑481</td>
<td>8</td>
<td>13.4</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>11.9</td>
<td>1.1</td>
<td>↑419</td>
<td>9</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>11.2</td>
<td>0.3</td>
<td>347</td>
<td>12</td>
<td>6.5</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>↑29.4</td>
<td>2.1</td>
<td>296</td>
<td>14</td>
<td>12.8</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>↑75.3</td>
<td>↑20.3</td>
<td>283</td>
<td>16</td>
<td>15.5</td>
<td>3.2</td>
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<tr>
<td>13</td>
<td>↑42.6</td>
<td>↑8.7</td>
<td>332</td>
<td>17</td>
<td>14.0</td>
<td>0.3</td>
</tr>
<tr>
<td>15</td>
<td>↑84.2</td>
<td>↑26.4</td>
<td>294</td>
<td>19</td>
<td>↑29.8</td>
<td>1.5</td>
</tr>
<tr>
<td>18</td>
<td>16.1</td>
<td>3.3</td>
<td>---</td>
<td>20</td>
<td>↑30.2</td>
<td>↑6.1</td>
</tr>
</tbody>
</table>

(1) BTG normal range = 6.2 to 25.8 ng/ml
(2) PF4 normal range = 0 to 4.3 ng/ml
(3) Subject #2 withdrew from experiment
(4) ↑ elevated above normal ranges for this laboratory.
(5) Test not performed.
(6) Plt. Ct. normal range = 130,000 to 400,000 per mm^3

Four of these five had extremely elevated levels of PF4, other exercising subjects had BTG and PF4 levels well within normal limits.

Three members of the resting control group had elevated BTG levels, and one of these had an elevated PF4. In the case of the controls the values of BTG were from 4.0 to 6.1/ng/ml (x̄ = 5.0 ng) above the top value of the normal range. The exercisers showed much greater increases in BTG ranging from 3.6 to 58.4 ng (x̄ = 37.3 ng) above the upper limit of the normal range. Similarly, the one control subject whose PF4 level fell above normal had a PF4 of 6.1 which is only
Figure 1

Distribution of BTG and PF4
Results for Exercise and Control Groups
1.8 ng above the upper limit of the normal range. In the exercise group four subjects had elevated PF4 levels ranging from 4.4 ng to 22.1 ng ($\bar{x} = 16.1$) above the upper limit of the normal range.

**Variability in the Control Group**

The reason for three outliers among the control population remains speculative. All subjects were screened by the investigator for medications, lifestyle factors and diseases that are known to effect platelet functional parameters. Upon retrospective analysis it was noted that subject 3 (control) had reported a history of mitral valve prolapse (MVP). The condition was benign, and the subject was not on medication. Since asymptomatic MVP is not a contraindication for exercise in otherwise healthy adults, this subject was included in the study. A subsequent review of the literature on the subject of MVP has uncovered data from a number of sources that link this condition with platelet coagulant hyperactivity.\(^72,73\) MVP is a protrusion of the valve leaflets into the left atrium during contraction or systole. The condition is common, especially in young women, in whom the incidence may be as high as ten percent.\(^72\) It has been estimated that twenty percent of all patients with MVP are symptom free. Other MVP patients may present with a number of symptoms including chest pain, fatigue, dyspnea, palpitations and anxiety.\(^72,73\) Patients with MVP have been reported to experience thromboembolic events such as cerebral and retinal ischemic episodes. Such manifestations have encouraged the investigation of the possible role of platelets in these
thromboembolic episodes. Walsh et al. studied platelet coagulant activity in 85 subjects: 29 patients with MVP; 18 patients with non-vascular, nonthrombotic eye disease and 38 normal subjects. These authors report a high incidence of platelet coagulant hyperactivity in MVP patients when compared with controls. Kostuk et al. suggest that abnormal surface structure on valve leaflets in MVP patients may result in platelet activation and release. Such data could explain the high values for the control subject 3. Further studies are required to substantiate this hypothesized relationship between MVP and elevated levels of BTG and PF4.

Two other control subjects had unexplained elevations in BTG levels at rest. One of these subjects had an elevation of both platelet proteins. A number of hypotheses could be forwarded to explain these aberrant results. It is possible that normal range studies to date have not included members of the subpopulation represented in this study, i.e. moderately trained females. This subpopulation could have different baseline values due to their level of participation in chronic exercise programs. If it were true that moderately trained females had higher baseline values of BTG and PF4 this would not confound the results reported in this study. All subjects were at similar levels of training. Random assignment to exercise and control groups would distribute outliers evenly between groups, allowing for valid interpretation of the post-exercise data. Normal range studies have been performed in other laboratories using identical BTG assay procedures and similar blood collection, processing and storage
protocols. Zahavi\textsuperscript{29} performed a normal range study using 219 apparently healthy subjects. In this study the mean BTG level was 33.9 ng/ml. Dawes, et al\textsuperscript{43} reported a mean BTG of 30.7 ± 13.7 (1 SD). Additional normal range studies are indicated. Such studies should include ranges of values for the normal healthy population as well as for particular subsets of the population. Before additional studies are conducted measuring BTG and PF4 in moderately trained individuals, it would be valuable to include a normal range study for these individuals.

It is unlikely that elevated BTG levels represent residual protein from previous exercise periods prior to testing. All subjects refrained from exercise for twenty-four hours prior to testing. BTG is cleared rapidly from human plasma, and has been reported to have a half-life of 100 minutes at 37°C.\textsuperscript{43} BTG from previous exercise would have been effectively cleared prior to the test procedure.

Variations in BTG and PF4 could be explained by variations in platelet count. Both proteins are specific to platelet alpha granules. The amount of protein present will vary with the number of platelets in circulation. To control for variations due to differences in platelet count, platelet counts were performed on all exercising and resting subjects. The platelet count was drawn at the same time as the specimen for BTG and PF4. Fourteen platelet counts fell within the normal limits of 130,000 to 400,000 per ul.\textsuperscript{75} Four subjects had
slightly elevated platelet counts. There was no demonstrable association between platelet count and plasma levels of BTG or PF4.

Variability in the Exercise Test Group

Although the mean of the exercising subjects was significantly greater than the mean of the subjects at rest, there were notable variations between exercising subjects. Fifty percent of the exercising subjects had marked elevations in one or both platelet specific proteins. Other exercising subjects had BTG and PF4 levels well within established normal limits. These variations could be due to the fact that despite the attempt of the investigator to standardize the level of exercise in the test protocol, there may have been variations in actual work load which might account for variability in these platelet release test parameters. It is possible that a more extended exercise period would have increased the number of subjects with documented platelet release. The study would have been strengthened by the incorporation of additional measures of workload intensity during the test protocol. A recording of perceived exertion may have been a valuable adjunct to the data collected. Other measures of exercise intensity might have been included, such as serum lactate and/or epinephrine levels. Few data are available in the literature to support an association between serum lactate, epinephrine and nor-epinephrine, and platelet release product levels. Levine et al.,\textsuperscript{42} reported no correlation between PF4 and lactate, epinephrine, and nor-epinephrine in CAD patients. Additional studies are necessary to determine
whether exercise level and duration were responsible for the variations observed after twenty minutes of aerobic exercise in this study.

Variations in response to a standard exercise protocol as observed in this study could be accounted for by individual subject variability in platelet responsiveness. In vivo platelet activation involves platelet response to a number of agonists and antagonists. Variations in response could be due to either changes in the circulating levels of agonists and antagonists, or to differences in the ability of the platelets to respond to these substances. Some studies have reported changes in the sensitivity of platelets to prostacyclin (PGI$_2$) after exercise. PGI$_2$ is a potent inhibitor of platelet activation and release. Dix et al.,$^{19}$ studied six marathon runners and six age matched controls. These researchers reported an increase in sensitivity of platelets to PGI$_2$ in runners when specimens were drawn twenty four hours after completion of a marathon race. Runners were reported to be seven to ten times more sensitive to the platelet inhibitory effects of PGI$_2$ on ADP induced platelet aggregation. No conclusions can be drawn from this study due to the fact that the control group was composed of non-runners who were reported to be physically inactive, while the runners were all highly trained athletes. The reported increase in sensitivity to PGI$_2$ may have been a training effect, or it may have been an acute response to the endurance athletic event. It would be necessary to perform a controlled training study in order to establish the relationship between platelet sensitivity to PGI$_2$ and training level. If PGI$_2$ sensitivity varies
with training, such differences could account for some of the variations in platelet release noted in this study. Although individual subjects in this research were moderately active there were significant differences in the type of training, frequency of training and the duration and consistency of individual training programs.

As suggested previously, platelet aggregation and release could be effected by the presence of differing levels of agonists and antagonists. Mehta et al, 76 studied normal controls and CAD patients. These researchers measured plasma levels of thromboxane B $sub 2$ (TXB2) and 6-keto PGF$alpha$ 1 alpha levels at rest and following a standardized treadmill exercise protocol. TXB2 and 6-keto-PGF$alpha$ 1 alpha are stable metabolites of thromboxane A $sub 2$ (TXA2) and PGI2 respectively. TXA2 is a potent platelet activator, and PGI2 is a vessel-wall generated platelet inhibitor. Relative concentrations of these two substances would result in relative changes in platelet reactivity. These authors reported that normal subjects had increased levels of both TXB2 and 6-keto PGF$alpha$ 1 alpha after exercise, and that 6-keto PGF$alpha$ 1 alpha increased to a greater extent than did TXB2. In CAD patients the TXB2 increased more than the 6-keto PGF$alpha$ 1 alpha. Similar changes in agonist-antagonist ratios may account for the variability seen in this study. Such variability may be due to inherent differences among individuals, or it could be due to relative exercise training levels. Further studies are necessary in order to identify the mechanisms by which shifts in agonist-antagonist ratios are initiated.
Summary

The data reported in this study supported the hypothesis that for the population tested, levels of platelet release proteins (BTG and PF4) are significantly elevated after twenty minutes of aerobic exercise when compared with resting controls. Generalizability of these data are restricted to the population studied in this research protocol: i.e., moderately trained females between the ages of 22 and 40 years.

The presence of elevated levels of BTG and PF4 is a specific marker for in vivo platelet activation. As was stated previously, these elevations could not be explained by previous exercise sessions, since all subjects refrained from exercise for twenty-four hours prior to testing. It can be concluded that at the level of exercise studied, a significant number of the exercising subjects exhibited levels of BTG and PF4 associated with platelet activation and release. It could be further concluded that platelet activation and release is associated with aerobic exercise levels that would customarily be prescribed for individuals in programs aimed at cardiorespiratory fitness. The biochemical events that regulate platelet aggregation and release are unknown. A number of mechanisms may interact to bring about platelet activation and release including changes in adenylate cyclate activity in the platelet membrane; fluxes in calcium within the platelet and the interactions of synthesized endoperoxidases and TXA2.29,75 The regulatory pathway and interaction of these
mechanisms is poorly understood. It is evident that exercise stress contributes to platelet activation at least in some responding individuals. The mechanism of this interaction is unclear.
CHAPTER V

SUMMARY, CONCLUSIONS AND IMPLICATIONS

Introductory Statement

A review of the literature has provided experimental data indicating that various levels of exercise result in changes in in vitro laboratory hemostatic values. These changes suggest in vivo activation of primary and secondary hemostasis and fibrinolysis consequent to exercise stress. Among the changes reported in hemostatic and fibrinolytic systems, are many reports of exercise-induced alterations in platelet number, size and function. These data suggest that exercise stress may result in in vivo platelet activation. Platelet activation has been shown to result in a population of circulating platelets with a hemostatic dysfunction similar to that found in some platelet diseases. Furthermore, it has been demonstrated that after activation and release of their granular contents, platelets have a shortened in vivo survival time.

Platelet factor 4 (PF4) and beta-thromboglobulin (BTG) are platelet specific proteins located in platelet alpha granules. The presence of elevated levels of these proteins is a specific marker for in vivo platelet release. Both of these proteins can be measured using a competitive binding radioimmunoassay procedure.
Knowledge regarding exercised induced platelet activation is essential in order that health educators and other health professionals can make knowledgable decisions regarding the appropriate use of exercise prescription in health care and wellness settings.

Statement of Purpose

The purpose of this study was to investigate the effect of a single episode of aerobic exercise on in vivo platelet functional parameters: more specifically this study was designed to determine whether platelets undergo activation and subsequent release of their alpha granule contents during aerobic exercise. Alpha granule release was determined by measuring the immediate post exercise levels of two platelet specific proteins: platelet factor 4 (PF4) and beta-thromboglobulin (BTG).

Subjects

Subjects were moderately trained female volunteers (n=20). For the purpose of this study moderately trained was defined as those individuals who participated in aerobic levels of exercise for periods of at least fifteen minutes, three to seven times per week. Aerobic exercise was defined as those physical activities involving large muscle groups, and during which the exercise intensity is easily sustained with little variability in heart rate. These activities correspond to the type, frequency and duration of exercise recommended by the American College of Sports Medicine (ACSM) for cardiorespiratory
Subjects were required to meet the following qualifications in order to be included in the study:

1. no medical contraindications to exercise
2. female, 20 to 40 years of age
3. moderately trained
4. free from acute and chronic disease
5. medication free for seven days prior to testing
6. free from the following cardiac risk factors:
   a. smoking
   b. hypertension
   c. history of coronary artery disease

Subjects were limited to an accessible population within the vicinity of The Ohio State University.

**Statistical Hypotheses**

\[ H_0: u_{test \ BTG} = u_{control \ BTG} \]
\[ H_0: u_{test \ PF4} = u_{control \ PF4} \]

**Alternate Hypotheses**

\[ H_1: u_{test \ BTG} > u_{control \ BTG} \]
\[ H_1: u_{test \ PF4} > u_{control \ PF4} \]

**Methods**

Female volunteers (n=20) were selected from an accessible population. Subjects were interviewed and screened using a health/exercise questionnaire. Subjects were examined by a licensed physician, and determined to be suitable for the experimental protocol. Subjects
were females, ages 20 to 40 years. All subjects were free from demonstrable acute and chronic disease. Subjects were screened for medication and life style factors that are known to effect platelet function. All subjects met the following criteria:

1. no contraindications to exercise
2. female, 20 to 40 years old
3. moderately trained
4. free from chronic/acute disease
5. medication free for seven days prior to testing
6. free from the following cardiac risk factors:
   a. smoking
   b. hypertension
   c. history of coronary artery disease

Subjects were tested to determine their functional capacity or maximal oxygen uptake (\(\dot{V}O_2\) max). These tests were performed using a symptom limited graded exercise protocol on a bicycle ergometer. Subjects were randomly assigned to exercise test and non-exercise groups. Test workloads were calculated for each exercising subject. Test workloads represented 65 to 75 percent of their measured \(\dot{V}O_2\) max.

On the day of testing exercise test and non-exercising control subjects reported to the test facility. Exercise test subjects exercised at the calculated workload (65 to 75 percent \(\dot{V}O_2\) max) for twenty minutes. Controls remained sedentary. All blood specimens for testing were drawn within five minutes of this twenty minute exercise or sedentary period.

Specimens were assayed for BTG and PF4 using a competitive binding radioimmunoassay procedure. Results were reported as the mean of
duplicate test values. Platelet counts were run on all subjects to control for the effect of abnormal platelet number on BTG and PF4 values.

Results

BTG and PF4 results were reported as means of duplicate samples. The mean BTG for the control group was 18.23 ng/ml (SD = 9.7). The mean for the exercise group was 38.4 ng/ml (SD = 31.2). The mean PF4 for the control group was 1.7 ng/ml (SD = 2.3) compared with a mean of 9.2 ng/ml (SD = 10.9) for the exercise group. The normal range for these laboratory values reported by The Ohio State University Hospital Laboratory are as follows:

BTG:  6.2 - 25.8 ng/ml  
PF4:  0.0 - 4.3 ng/ml

The null hypotheses for this research design were stated as:

\[ H_0: \mu_{test} BTG = \mu_{control} BTG \]
\[ H_0: \mu_{test} PF4 = \mu_{control} PF4 \]

The null hypothesis for the dependent variable BTG (RIA1) was:

\[ H_0: \mu_{test} BTG = \mu_{control} BTG \]

The alternate hypothesis for the dependent variable BTG (RIA1) was:

\[ H_1: \mu_{test} BTG > \mu_{control} BTG \]

For the dependent variable BTG (RIA1) the calculated t or |t| was -1.8592 (degrees of freedom = 17). At the priori alpha level of 0.05 t critical is equal to ± 1.74. Since |t| is greater than t critical, \( H_0 \) can be rejected.
The null hypothesis for the dependent variable PF4 (RIA\textsubscript{2}) was:

$$H_0: \mu_{\text{test PF4}} = \mu_{\text{control PF4}}$$

The alternate hypothesis for the dependent variable PF4 (RIA\textsubscript{2}) was:

$$H_1: \mu_{\text{test PF4}} \neq \mu_{\text{control PF4}}$$

For the dependent variable PF4 (RIA\textsubscript{2}) the calculated $t$ or $|t|$ was -2.0131 (degrees of freedom = 17). At the priori alpha level of 0.05 $t$ critical is equal to $\pm 1.74$. Since $|t|$ is greater than $t$ critical, $H_0$ can be rejected.

Therefore, the mean values for the aerobically exercising population were significantly higher for both dependent variables (BTG and PF4) when measured within five minutes of the exercise session.

These data support the research hypotheses stated previously:

1. The mean plasma level of BTG of the exercise subjects will be significantly higher than the mean of the resting controls.

2. The mean plasma level of PF4 of the exercise subjects will be significantly higher than the mean of the resting controls.

**Conclusions and Implications**

As a result of the data reported in this study it can be concluded that in some individuals twenty minutes of exercise on a bicycle ergometer at 65 to 75 percent VO\textsubscript{2} max resulted in in vivo platelet activation and release.

Due to the conflicting data in the literature, and the variability of response in this protocol, it is evident that further research
is necessary in order to elucidate the relationship between platelet activation and platelet release. Such research should include:

1. Adequate standardization of exercise protocols.
2. Suitable control groups.
3. Expanded normal range studies.
4. Further studies with regard to the relationship of MVP and platelet release.
5. Further studies delineating changes in platelet sensitivity to circulating agonists and antagonists.
6. Studies measuring changes in levels of known platelet agonists and antagonists after exercise.

Implications of Exercise-Induced Platelet Activation in CAD Patients and Sudden Cardiac Death

Platelet responses to exercise stress have a number of implications for exercise physiologists, health educators and physiologists. It is essential to understand not only the mechanism by which platelet activation is elicited, but also to understand the physiological consequences of platelet activation. Such knowledge is necessary in order that we may adequately judge the appropriateness of exercise prescription for various subpopulations.

As described previously, platelet interaction and platelet release products participate in the process of atherogenesis. Although data is mixed, some researchers have associated platelet activation and release with manifestations of myocardial ischemia in CAD patients. Data confirm the active participation of platelets and platelet products in the development of atherosclerotic plaque.
Platelet activation and release have been implicated in the etiology of sudden cardiac ischemic death.\textsuperscript{58,59,60} Further data are necessary in order to elucidate the interactions of exercise stress and in vivo platelet responses. Data implicating short duration exercise stress with platelet activation might suggest that exercise is contraindicated in subjects with ongoing atherosclerotic disease. On the other hand, implications that endurance athletes may have platelets more sensitive to platelet inhibition by prostacyclin may provide evidence to suggest that chronic exercise may prove protective against thrombosis. Further research is necessary to identify whether certain subsets of the population may present with platelet hyperactivity, and if so, whether this altered platelet responsiveness is associated with higher risk for CAD and/or peripheral vascular disease. Such data may provide a basis for treatment with suitable anti-platelet medications, and information regarding the suitability of exercise for these patients.

\textbf{Implications of Exercise-Induced Platelet Release in Health and Physical Education}

The Health and Physical Educator frequently utilizes exercise as a modality in programs of weight loss, smoking cessation and as a suggested life style modification to reduce the risk of cardiovascular disease. Exercise is frequently prescribed as part of a comprehensive treatment and/or rehabilitation program in chronically ill populations. The emphasis has shifted to individual responsibility for life
style changes that will benefit personal health status. Health educators at all levels are incorporating exercise recommendations into programs for wellness and disease prevention.

Because of this increased reliance on physical exercise in programs of wellness, it is essential that researchers fully investigate the physiological consequences of various levels of exercise. It is only as a result of such investigations that we can safely and effectively utilize exercise in various health care and wellness settings.

**Implications of Exercise-Induced Platelet Release on Platelet Donor Screening Procedures**

As described previously, platelets that have undergone activation and subsequent granular release have been demonstrated to have shortened survivals and decreased hemostatic effectiveness. Exercise induced platelet release could result in platelets with decreased hemostatic effectiveness throughout their lifespan. Such data are significant to the transfusion professional. If an ongoing aerobic exercise program results in release, and if such platelets can be demonstrated to have limited hemostatic effectiveness, then aerobically exercising donors may be inappropriate as donors for platelet transfusion products. This would be especially important in the case of single donor platelet pheresis products. Further research is necessary to elucidate the effects of exercise on the hemostatic effectiveness of platelets and on platelet survival times.
Conclusion

Data generated in this study clearly indicate that for the population tested, aerobic levels of exercise result in a significant elevation of plasma levels of PF4 and BTG. Elevated levels of these platelet proteins are reported to be specific markers of in vivo platelet activation and alpha granule release.

This study provides important data regarding platelet responsiveness consequent to quantitated and well standardized levels of aerobic exercise. These data are particularly significant since most previous data were reported using short duration exhaustive exercise or exercise levels that were not standardized across subjects. The levels of exercise tested in this study are more representative of those commonly prescribed for programs of cardiorespiratory fitness.

It can be concluded that the level of exercise tested resulted in substantial in vivo platelet activation and release. Platelet activation and subsequent release has been associated with a number of disease processes: notably atherosclerosis, thrombotic disorders and sudden cardiac death. In addition, some investigators have reported shortened platelet survival and hemostatic dysfunction secondary to platelet release.

Data from this research strongly supports the need for further investigations regarding the nature of this interaction. Further data need be generated elucidating the mechanism of and consequences of exercise induced platelet release.
List of References


3. Cunningham RM: Wellness at work: Not just a passing fancy. JAHA 1982;56:82-86.


Appendix A
THE OHIO STATE UNIVERSITY
BIOMEDICAL SCIENCES
HUMAN SUBJECTS REVIEW COMMITTEE

PRINCIPAL INVESTIGATOR: Sally V. Rudman
(Type name) ___________________________ Signature ___________________________
Type Title: Instructor
Phone No. 422-7303

College: Medicine
Department: SAMP, Medical Technology

Campus Address: 1583 Perry Street, Columbus, Ohio 43210

Co-Investigators:

Signature

Cc: Medical Technology

Cc: Investigators:

PROTOCOL TITLE: The effect of moderately prolonged aerobic exercise on in vivo platelet release: Radioimmunoassay of Platelet Factor 4 and B-thromboglobulin.

DEPARTMENT CHAIRPERSON'S ENDORSEMENT

John E. Snider, Ph. D. ___________________________ Signature ___________________________

PROPOSED PROJECT INVOLVES:

☐ New Drug (IND.) What is IND Number? ________ Issued to: ________________
Generic name ___________________________

☐ Investigational Device. What is IDE Number? ________ Issued to: ________________

☐ Radioactive drugs or Unusual Exposure to External Radiation.
Approval by the Medical Radioisotopic Committee (Tel. 422-0122) is required for final approval by Human Subject Review Committee.
Investigator is responsible for submitting to both committees.

☐ Pregnant Women - Approval by Maternal-Fetal Committee (Tel. 421-8726) is required for final approval by Human Subject Review Committee.
Investigator is responsible for submitting to both committees.

THE PROPOSED ACTIVITY WOULD INVOLVE: (Check at least one.)

☐ Minors ☐ Pregnant Women ☐ Mentally Retarded
☐ Fetuses ☐ Prisoners ☐ Mentally Disabled
☐ Abortuses ☐ None of These

At least one reviewer of this protocol should be knowledgeable about the following disciplines (fields of science): Hematology/Hematology

KS-029A (5/84) Cover Page
1. Abstract
Moderately trained female volunteer subjects (N=15) between the ages of 25 and 40 years will be exercised at approximately eighty percent of their maximal capacity for thirty minutes on a bicycle ergometer. Pre- and post-exercise laboratory data will include platelet factor 4 and beta-thromboglobulin by radioimmunoassay, a whole blood platelet count and hematocrit. Comparison of pre- and post-exercise levels of plasma platelet factor 4 and beta-thromboglobulin will provide data that enables the researchers to determine whether platelets undergo activation and release as a consequence of moderately prolonged aerobic exercise. Data indicate that platelets, having undergone the process of activation and release, may have decreased survival rates and altered functional capacity in vivo. Therefore, such data would be of significance to those determining platelet donor eligibility. Data will assess whether or not an 'exercise history' should be included in the screening protocol for platelet donors.

2. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as prisoners, children, the mentally disabled or groups whose ability to give voluntary informed consent may be in question.

Subjects will be moderately trained female volunteers (ages 25-40 years). Moderately trained is operationally defined as a subject who exercises at levels recommended by the American College of Sports Medicine for cardiorespiratory fitness: 70-80% of maximal, three times per week with exercise bouts at least fifteen minutes in duration.

3. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

Possible risks from venipuncture technique include: bruising, bleeding, inflammation of vein, fainting, infection.

4. Describe consent procedures to be followed, including how and where informed consent will be obtained.
Informed consent will be obtained at the time that the subject initially volunteers for the protocol. The protocol and rationale for the study will be thoroughly explained to each subject by the investigator.
5. Describe procedures (including confidentiality safeguards) for protecting against or minimizing potential risks and an assessment of their likely effectiveness. Potential risks will be minimized by utilization of personnel skilled in the collection of venous blood specimens. Standard aseptic arm preparation techniques will minimize the risk of infection.

6. Assess the potential benefits to be gained by the individual subject, as well as benefits which may accrue to society in general as a result of the planned work.

Data regarding the effects of aerobic exercise on platelet release would be of value in the assessment of the functional status of platelets secured from an exercising donor, as well as contributing to general knowledge concerning the platelet release reaction.

7. Analyze the risk-benefit ratio.

The risk associated with venipuncture is very low. Since many normal healthy blood donors are participating in regular aerobic exercise, assessment of platelet function post-exercise is important in order to assure that donated platelets will function effectively after transfusion.

8. Will the subjects for the study be paid for participating in this study? □ No □ Yes - How much? ______

Will they be paid for only certain parts of the study or for participation for the whole study? __________________________

Is there any other inducement? If so, please describe. □ No □ Yes - Please describe. __________________________
CONSENT TO INVESTIGATIONAL TREATMENT ON SUBJECT

1. I hereby authorize or consent to your seeking, to perform the following treatment or procedures (indicate in general terms):
   - Measurement of platelet count, hemoglobin, platelet factor-4 and beta-thromboglobulin

2. The experimental (research) portion of the treatment or procedure is: The effect of moderately prolonged aerobic exercise on in vivo platelet release. Radioimmunoassay of Platelet Factor-4 and beta-thromboglobulin

This is done as part of an investigation entitled: The effect of moderately prolonged aerobic exercise on in vivo platelet release. Radioimmunoassay of Platelet Factor-4 and beta-thromboglobulin

1. Purpose of the procedure or treatment: to determine if exercise causes platelet release of granules of beta-thromboglobulin and Platelet Factor-4.

2. Possible appropriate alternative method of treatment: None

3. Discomforts and risks reasonably to be expected: bruising, bleeding, inflammation, fainting, infection.

4. Possible benefits for subjects/society: assessment of function of platelets with respect to platelet donation

5. Anticipated duration of subject's participation: 1 hour

I hereby acknowledge that has provided information about the procedure described above, about my rights as a subject, and he/she answered all questions to my satisfaction. I understand that I may contact him/her should I have additional questions. He/She has explained the risks described above and I understand that he/she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study may be made available to the sponsor of this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudice to future care. I have been given to me.

In the unlikely event of injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of the Ohio State University. I also understand that the costs of such treatment will be at my expense and that financial compensation is not available. Questions about this should be directed to the Human Subjects Office at 412-4046.

I have read and fully understood the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: ___________________________ Time ___________________________ Signed ___________________________

Witneses(s) ___________________________ If Required ___________________________

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed ___________________________ (Signature of Project Director or Designated Authorized Representative)

Form RS-02A (Rev. 12/83)
ACTION OF THE REVIEW COMMITTEE

With regard to the employment of human subjects in the proposed research:

85K0256 THE EFFECT OF MODERATELY PROLONGED AEROBIC EXERCISE ON IN VIVO PLATELET RELEASE: RADIOIMMUNOASSAY OF PLATELET FACTOR 4 AND B-THROMBOGLOBULIN, Sally V. Rudmann, Allied Medical Professions

THE BIOMEDICAL SCIENCES REVIEW COMMITTEE HAS TAKEN THE FOLLOWING ACTION:

APPROVED _____ DISAPPROVED

X APPROVED WITH STIPULATIONS* _____ WAIVER OF WRITTEN CONSENT GRANTED

*Stipulations stated by the Committee have been met by the investigator and, therefore, the protocol is APPROVED.

It is the responsibility of the principal investigator to retain a copy of each signed consent form for at least four (4) years beyond the termination of the subject's participation in the proposed activity. Should the principal investigator leave the University, signed consent forms are to be transferred to the Human Subject Review Committee for the required retention period. This application has been approved for the period of one year. You are reminded that you must promptly report any problems to the Review Committee, and that no procedural changes may be made without prior review and approval. You are also reminded that the identity of the research participants must be kept confidential.

Date: September 5, 1985

Signed: [Signature]

Chairperson

MS-025H (Rev. 3/85)
1. **Commencing and terminating times:**

   1. **30 minute exercise on exercise ergometer:**

   The experimental (research) portion of the treatment or procedure is: **measurement of platelet count, hemoglobin, platelet factor-4 and beta-thromboglobulin.**

   This is done as part of an investigation entitled: **The effect of moderately prolonged aerobic exercise on in vivo platelet release; Radioimmunoassay of Platelet Factor-4 and Beta-thromboglobulin.**

   1. **Purpose of the procedure or treatment:** To determine if exercise causes platelet release of cranial source of Beta-thromboglobulin and Platelet Factor-4.

2. **Possible appropriate alternative methods of treatment:** None

3. **Discomforts and risks reasonably to be expected:** Bruising, bleeding, inflammation, fainting, infection.

   Exercise related risks: Muscle Soreness, Fatigue

4. **Possible benefits for subjects/society:** Assessment of function of platelets with respect to platelet donation.

5. **Anticipated duration of subject's participation:** 1 hour

I hereby acknowledge that has provided information about the procedure described above, about my rights as a subject, and have answered all questions to my satisfaction. I understand that I may contact the investigator if I have any questions about the risks described above and I understand these risks have been explained to me. I understand that my participation will remain confidential.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study may be made available to the sponsor of this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time and without prejudice to future care. No permission has been given to be concerning this treatment or procedure.

In the unlikely event of injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospitals of The Ohio State University. I also understand that the costs of such treatment will be at my expense and that financial compensation is not available. Questions about this should be directed to the Human Subject Review Office at 432-9046.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: Time: M: Signed: (Subject)

Witness(es): (Parent, guardian, or legal representative if subject is a minor)

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed: (Signature of Project Director or his/her authorized representative)
PERSONAL FITNESS QUESTIONNAIRE

All information given is personal and confidential. It will enable us to better understand you and your health and fitness habits.

1. GENERAL INFORMATION: Date ____________

Name ___________________________ Age _____ Sex _____

Address __________________________ Zip _______

Phone: Home ___________ Business ___________

Occupation __________________________

Height _______ Weight ___________

In case of emergency, contact:

Name ___________________________ Phone ___________

2. MEDICAL-SURGICAL HISTORY: Check (x) if answer is yes.

Have you ever had (if so indicate date):

( ) Rheumatic heart disease ( ) Accidents
( ) Heart Murmur or unusual ( ) Chest pains
    cardiac findings ( ) Tightness in chest particularly during exercise
( ) High Blood Pressure ( ) Shortness of breath
( ) Gout ( ) Heart palpitations
( ) Varicose Veins ( ) Excessive cough
( ) Injuries to back, etc. ( ) Back pain
( ) Epilepsy ( ) Swollen, stiff or painful joints
( ) Diabetes ( ) Difficulty sleeping
( ) Heart Attack, coronary bypass or other cardiac surgery ( ) Fatigue
( ) Other Operations ( ) Calf pain or cramps with exercise
( ) Kidney Disease ( ) Arthritis
( ) Stomach Ulcers ( ) Nervousness
( ) Arthritis ( ) Hospitalizations
( ) Heart Catherization ( ) Ankle swelling
( ) Phlebitis, embol ( ) Peripheral vascular disease
( ) Phlebitis, emboli ( ) Lightheadedness or fainting
( ) Pulmonary disease including ( ) Abnormal blood lipids
    asthma, emphysema and ( ) Stroke
    bronchitis . ( ) Drug allergies
( ) Orthopedic problems ( ) Other problems

Please explain any positive answers. ________________________________
MEDICATIONS: Please list those you are presently taking. (Include over the counter medications.)

<table>
<thead>
<tr>
<th>DRUG</th>
<th>DOSE</th>
<th>REASON FOR TAKING</th>
</tr>
</thead>
</table>

3. CORONARY RISK FACTOR EVALUATION ITEMS: Circle the appropriate letter.

Cigarette Smoking
- none
- 1/2 pack per day
- 1/2 to 1 pack per day
- 1 1/2 packs per day
- more than 1 1/2 packs per day

Self-rating of stress and tension
- rarely tense or anxious
- calmer than average - feel tense about 3x per week
- about average - feel tense or anxious 2 or 3 times per day
- quite tense - usually rushed
- extremely tense - take a tranquilizer nearly every day

Family history of heart attack/stroke
- none
- yes, over the age of 50 years
- yes, under the age of 50 years
- unknown

Age
- under 30
- 30-39
- 40-49
- 50-59
- over 60

Caffeine (including Cola drinks) Portions/day
- 1 Portion = 1 cup coffee or 1 - 8 oz. cola
- none
- one
- 2-3
- 4-5
- > 5/day
4. PRESENT REGULAR EXERCISE: Briefly describe any regular exercise (at least three times per week for 20 minutes each session) in which you are presently active.

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

SUMMARY IMPRESSION OF PHYSICIAN:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

I have examined the above patient who, to the best of my knowledge, is free from infectious disease and is capable of participating in an exercise program as well as periodic laboratory evaluations, under the guidance of a trained staff.

PHYSICIAN'S SIGNATURE: ____________________________________________

PHYSICIAN'S NAME (print) ____________________________________________

ADDRESS ___________________________________________________________
1. Research Objective: To determine whether platelets undergo activation and release during twenty minutes of aerobic exercise in moderately active females.

2. Research Protocol:
   
   A. First Session: After an initial health screen you will perform an exercise test on a cycle ergometer. The exercise intensity will begin at a level that you can easily accomplish and will be increased at intervals as the test continues. During exercise your heart rate and blood pressure will be measured periodically, an ECG tracing will be recorded, and oxygen uptake measured. We may stop the test at anytime due to indications of fatigue. You may stop when you wish because of personal feelings of fatigue or discomfort.

   This initial exercise test will be used to measure your functional capacity.

   B. Second Session: Each subject will be randomly assigned to an exercise or control group. The control group will remain sedentary during the "test" period. At the end of this period approximately 10 ml of blood will be withdrawn using a clean venipuncture technique performed by a qualified technician. Each subject will be requested to work on the cycle ergometer at 70% of their functional capacity for twenty minutes. Within five minutes of cessation of exercise blood will be withdrawn as described previously.

   All subjects for this second session will:

   1. be free from medications for seven days prior to testing
   2. refrain from strenuous exercise for 24 hours prior to testing

   Inquiries:

   Any questions regarding the exercise procedures are encouraged.

   Freedom of Consent:

   Your permission to perform this exercise test is voluntary.
Normal Values (Bicycle Ergometer)

### Table 6

**Aerobic capacity classification based on sex and age**

<table>
<thead>
<tr>
<th>Age</th>
<th>Low</th>
<th>Fair</th>
<th>Average</th>
<th>Good</th>
<th>High</th>
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<td>Women</td>
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<td>Men</td>
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<td>22-25</td>
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MEASUREMENT OF OXYGEN CONSUMPTION (EXPIRED / INSPIRED)

PERSONAL DATA:
NAME ___________________ AGE _______ HEIGHT _______ in. WEIGHT _______ lb.

AMBIENT DATA:
P0 _______ mmHg. TA _______ °C. PH2O _______ mmHg. RH _______ %
STPD _______ BTPS _______ BELL FACTOR _______ ml./mm. (IF USING SPIROMETER)

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<thead>
<tr>
<th>TIME (MIN:SEC)</th>
<th>INITIAL (L/MIN)</th>
<th>FINAL (L/MIN)</th>
<th>FECO2 (%)</th>
<th>FECO2 (%)</th>
<th>V (L/MIN)</th>
<th>VO2 (L/MIN)</th>
<th>VCO2 (L/MIN)</th>
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<td>FECO₂ (%)</td>
<td>V (L/HR)</td>
<td>V STPD (L/HR)</td>
<td>VO₂ (L/HR)</td>
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<td>1.07</td>
<td>49.89</td>
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</table>

**MAXIMAL TEST RESULTS**

\[ \text{VO}_2 \text{ max} = 49.4 \text{ ml/kg/min} \]
**Measurement of Oxygen Consumption (Expired / Inspired)**

**Personal Data:**
- **Name:** #2
- **Age:** 39
- **Height:** 5'6" in.
- **Weight:** 149 lb.

**Ambient Data:**
- **PB:** 745.8
- **RH:** 33%
- **Ta:** 24.5°C
- **P,H,20:** 23.06

**STPD DTPS BELL FACTOR ml./mm. (IF USING SPIROMETER)**

<table>
<thead>
<tr>
<th>RPM</th>
<th>TIME (MILL)</th>
<th>INITIAL (L)</th>
<th>FINAL (L)</th>
<th>V (L/min)</th>
<th>VS (L/min)</th>
<th>VO2 (L/min)</th>
<th>VCO2 (L/min)</th>
<th>R</th>
<th>VO2 (ML/KG)</th>
<th>ENERGY (KCAL/M)</th>
<th>HR (BPH.)</th>
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**Maximal Test Results**

\[
V_O^2_{max} = 45.84 \text{ ml/kg/min}
\]
### PERSONAL DATA

- **Name**: #3
- **Age**: 33
- **Height**: 5'4"
- **Weight**: 114 lb.

### ANEMETRIC DATA

- **PaO2**: 78.45 mmHg
- **TA**: 20°C
- **PpO2**: 17.53 mmHg

### MEASUREMENT OF OXYGEN CONSUMPTION (EXPIRED / INSPIRED)

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### V̇O₂ max = 50.1 ml/kg/min
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\( \text{VO}_2 \text{ maximum} = 48.4 \text{ ml/kg/min} \)
# Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data**
- **Name:** #5
- **Age:** 34
- **Height:** 5'6"
- **Weight:** 136 lb.
- **Height (cm):**
- **Weight (kg):**

**Ambient Data**
- **PB:** 741.5
- **TA:** 24.5°C
- **PH20:** 22.92
- **Humidity:** 33%

**STPD**
- **BTPS**
- **Bell Factor**
- **ml/cm. (if using spirometer)**

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<th>FECO2 (%)</th>
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**VO2 Max:** 54.1 ml/kg/min
### Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data:**
- **Name:** [Name]
- **Age:** 31
- **Height:** 5'0" in.
- **Weight:** 113 lb.

**Ambient Data:**
- **PB:** 74/15 mmHg
- **TA:** 24.5 °C
- **PH20:** 22.98 mmHg
- **RH:** 53%

---

**60 RPM**

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**VO2 Max = 50.1 mL/kg/min**

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**Measurement of Oxygen Consumption (Expired / Inspired)**

**Personal Data:**
- **Name:** #7
- **Age:** 30
- **Height:** 5'3"
- **Weight:** 113 lb.

**Ambient Data:**
- **PB:** 74.15 mmHg
- **TA:** 24.5°C
- **PhCO2:** 23.92 mmHg
- **HR:** 33

**Maximal Test Results**

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<th>Final (Vt)</th>
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<th>FECO2 (%)</th>
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<th>VSTPD (L/min)</th>
<th>VO2 (L/min)</th>
<th>VCO2 (L/min)</th>
<th>RR</th>
<th>VO2 (Ht/kg)</th>
<th>Energy (KCAL/hr)</th>
<th>HR (BPM)</th>
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\[ VO2 \text{ Max} = 43.5 \text{ ml/kg/min} \]
## Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data: NAME: **

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<tr>
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<th>WEIGHT</th>
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**Ambient Data:**

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<th>PB</th>
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<th>RH</th>
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### 50 RPM

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<th>Final</th>
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<th>FE1O2 (L)</th>
<th>VE (L/Min)</th>
<th>VE1O2 (L/Min)</th>
<th>VO2 (L/Min)</th>
<th>VCO2 (L/Min)</th>
<th>R</th>
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**VO2 Max = 50.7 ml/kg/min**
### Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data:**
- Name: #9
- Age: 33
- Height: 5'7" in.
- Weight: 125 lb.

**Ambient Data:**
- PB: 747 mmHg
- TA: 34.0 °C
- PH20: 22.38 mmHg
- RII: 35%

**60 RPM**

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<th>Final (1/2)</th>
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<th>FEO2 (%)</th>
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<th>V1 37C (L/MIN)</th>
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<th>VCO2 (L/MIN)</th>
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<th>Energy (KCAL/H)</th>
<th>HR (BPM)</th>
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**Maximal Test Results**

\[ VO_2 \text{ max} = 46.6 \text{ ml/kg min} \]
### MAXIMAL TEST RESULTS

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<th>TIME (MINSEC)</th>
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<th>FINAL (L/MIN)</th>
<th>FE02 (%)</th>
<th>FCO2 (%)</th>
<th>V (L/MIN)</th>
<th>VSTPD (L/MIN)</th>
<th>VO2 (L/MIN)</th>
<th>VCO2 (L/MIN)</th>
<th>R</th>
<th>VO2 (ML/KG)</th>
<th>ENERGY (KCAL/H)</th>
<th>HR (BPM)</th>
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\[ VO_2 \text{max} = 55.1 \text{ ml/kg/min} \]
### Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data**
- **Name:** #11
- **Age:** 26
- **Height:** 5'4" In.
- **Weight:** 128 lb.

**Ambient Data**
- **PB:** 74.7 mmHg
- **TA:** 37.0°C
- **P2O2:** 89.38 mmHg
- **RH:** 35%

**STPD**
- **BTPS**
- **Bell Factor** ml./mm. (if using spirometer)

**RPM**

<table>
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<tr>
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<th>TIME (MIN:SEC)</th>
<th>INITIAL</th>
<th>FINAL</th>
<th>FEO2 (L)</th>
<th>FE CO2 (L)</th>
<th>V (L/MIN)</th>
<th>V STPD (L/MIN)</th>
<th>VO2 (L/MIN)</th>
<th>V CO2 (L/MIN)</th>
<th>R</th>
<th>VO2 (ML/ KG)</th>
<th>ENERGY (KCAL/H)</th>
<th>HR (BPM)</th>
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**Maximal Test Results**

VO₂ max = 61.0 ml/kg/min
### Measurement of Oxygen Consumption (Expired / Inspiratory)

**Personal Data:**
- **Name:** S.L.
- **Age:** 31
- **Height:** 5'5" in.
- **Weight:** 141 lb.

**Ambient Data:**
- **PB:** 747 mmHg
- **TA:** 24.0 °C
- **FIH2O:** 22.38 mmHg
- **RH:** 35%
- **STPD:** ______
- **BTPS:** ______
- **Bell Factor:** ml./mm. (if using spirometer) ______

**Maximal Test Results:**

<table>
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<th>Time (MM:SS)</th>
<th>Initial VO2 (L/min)</th>
<th>Final VO2 (L/min)</th>
<th>VO2 Change (L/min)</th>
<th>V (L/min)</th>
<th>VSTPD (L/min)</th>
<th>VO2 (L/min)</th>
<th>VCO2 (L/min)</th>
<th>R</th>
<th>VO2 (H/L/KG)</th>
<th>Energy (KCAL/H)</th>
<th>HR (BPM)</th>
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**VO2 max = 56.18 ml/kg/min**
### Personal Data
- **Name**: #13
- **Age**: 24
- **Height**: 5'6"
- **Weight**: 120 lb.

### Ambient Data
- **PB**: 747 mmHg
- **TA**: 34.0°C
- **FiO2**: 29.38
- **HR**: 35

### Measurement of Oxygen Consumption

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<th>VO2 (L/MIN)</th>
<th>VCO2 (L/MIN)</th>
<th>R</th>
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<th>ENERGY (KCAL/H)</th>
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**VO2 max = 37.5 mL/kg/min**
### PERSONAL DATA:
- **Name:** #14
- **Age:** 22
- **Height:** 5'4½"
- **Weight:** 109 lb.

### AMBIENT DATA:
- **Pb:** 743 mmHg
- **Ta:** 24.5°C
- **RH:** 23.15%
- **PH2O:** 37.5%

### MAXIMAL TEST RESULTS

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Initial (L/min)</th>
<th>Final (1/2) (L/min)</th>
<th>FCO2 (%)</th>
<th>VCO2 (%)</th>
<th>V (L/min)</th>
<th>VSTPD (L/min)</th>
<th>VO2 (L/min)</th>
<th>VCO2 (L/min)</th>
<th>R</th>
<th>VO2 (ml/kg)</th>
<th>Energy (KCAL/Hr)</th>
<th>HR (BPM)</th>
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\[ V_{O2,\text{max}} = 37.0 \text{ ml/kg/min} \]
### Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data:**
- **Name:** #15
- **Age:** 36
- **Height:** 5'6" in.
- **Weight:** 118 lb.

**Ambient Data:**
- **PB:** 143 mmHg
- **TA:** 24.5 °C
- **PH2O:** 23.15 mmHg
- **RH:** 57.5%

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<th>FEO2 (L)</th>
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\[ \text{VO}_2\text{max} = 56.6 \text{ ml/kg/min} \]
### MEASUREMENT OF OXYGEN CONSUMPTION (EXPIRED / INSPIRED)

**PERSONAL DATA**
- **NAME:** #16
- **AGE:** 33
- **HEIGHT:** 5'1'' in.
- **WEIGHT:** 182 lb.

**AMBIENT DATA**
- **PB:** 743 mmHg
- **TA:** 24.5°C
- **pH2O:** 23.15 mmHg
- **Ri:** 37.5%

**STPD**
- **BTPS**
- **BEIL Fctr:** ____________ ml./mm. (IF USING SPHOMETER)

**TIME** (MIN:SEC)
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<th>FINAL (L/MI)</th>
<th>FE02 (%)</th>
<th>FEO2 (%)</th>
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**VO2max = 375 ml/kg/min**
# Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data:**
- **Name:** #17
- **Age:** 24
- **Height:** 5'6" in.
- **Weight:** 114 lb.

**Ambient Data:**
- **PB:** 743 mmHg
- **TA:** 24.5 °C
- **Pulm:** 23.15 mmHg
- **NH:** 625 %

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<th>( \text{FECO}_2 ) (l)</th>
<th>( V ) (L/H)</th>
<th>( V_{500} ) (L/H)</th>
<th>( \text{VO}_2 ) (L/H)</th>
<th>( \text{VCO}_2 ) (L/H)</th>
<th>( R )</th>
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\[ \text{VO}_2\text{max} = 36.0 \text{mL/kg/min} \]

*MAXIMAL TEST RESULTS*
### MEASUREMENT OF OXYGEN CONSUMPTION (EXPIRED / INSPIRED)

**Personal Data:**
- **Name:** [Name]
- **Age:** 29
- **Height:** 5'5" in.
- **Weight:** 122 lb.

**Ambient Data:**
- **PB:** 743 mmHg
- **TA:** 21.5°C
- **PiO2:** 23.15 mmHg
- **RH:** 37.5%

#### 60 RPM

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<th>Final (VE)</th>
<th>FE02 (%)</th>
<th>FECO2 (%)</th>
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<th>V SPO2 (L/Min)</th>
<th>VO2 (L/Min)</th>
<th>VCO2 (L/Min)</th>
<th>VE (L/Min)</th>
<th>O2 (%)</th>
<th>CO2 (%)</th>
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<th>Energy (KCal/Hr)</th>
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* VO2 max = 48.19 ml/kg/min
# Measurement of Oxygen Consumption (Expired / Inspired)

**Personal Data**
- **Name:** #19
- **Age:** 25
- **Height:** 5'4"
- **Weight:** 111 lb.

**Ambient Data**
- **PB:** 743 mmHg
- **TA:** 24.5°C
- **PH2O:** 23.15 mmHg
- **RHI:** 37.5%

**STPD:**
**BTPS:**
**Bell Factor:** ml./mm.

**IF USING SPIROMETER:**

**Maximal Test Results**

<table>
<thead>
<tr>
<th>RPM</th>
<th>TIME (MIN/SEC)</th>
<th>INITIAL (L/SEC)</th>
<th>FINAL (L/SEC)</th>
<th>FE02 (%)</th>
<th>FECO2 (%)</th>
<th>V (L/MIN)</th>
<th>V (L/SEC)</th>
<th>VCO2 (L/MIN)</th>
<th>VCO2 (L/SEC)</th>
<th>R</th>
<th>VO2 (ML/SEC)</th>
<th>ENERGY (KCAL/MIN)</th>
<th>HR</th>
<th>VO2 MAX = 50.3 ML/KG/MIN</th>
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</tbody>
</table>
**Measurement of Oxygen Consumption (Expired / Inspired)**

**Personal Data**
- **Name**: #20
- **Age**: 25
- **Height**: 5'4" in.
- **Weight**: 113 lb.

**Ambient Data**
- **PB**: 754 mmHg
- **TA**: 21.0°C
- **PH20**: 18.65 mmHg
- **RH**: 28%

**60 RPM**
- **STPD**: _____
- **BTPS**: _____
- **Bell Factor**: ml./min. (if using spirometer)

**3/20/86**

<table>
<thead>
<tr>
<th>Time (min/sec)</th>
<th>Initial (L/min)</th>
<th>Final (L/min)</th>
<th>FeO2 (%)</th>
<th>FeCO2 (%)</th>
<th>V (L/min)</th>
<th>V STPD (L/min)</th>
<th>VO2 (L/min)</th>
<th>VCO2 (L/min)</th>
<th>R</th>
<th>V02 (ml/kg)</th>
<th>Energy (KCAL/H)</th>
<th>HR (BPM)</th>
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</table>

* V02 max = 48.93 ml/kg/min
Appendix B
December 26, 1984

Ms. Sally Rudman
Department of Medical Technology
School of Allied Medical Professions
Ohio State University

Dear Sally,

I enjoyed talking with you concerning the effects of exercise on in vivo platelet release. I agree with you that a well-designed study could contribute significantly to this area. I would be happy to serve as a consultant for this project and look forward to working with you.

Sincerely yours,

[Signature]

John Brandt, M.D.
Director, Laboratory Hematology

College of Medicine
**PLATELET FACTOR 4 (PF4)**

**PROCEDURE**

Methodology set up by: Linda Miller, M.T. (HEW) (2/82)

Annual reviewer: John Brandt, M.D.

**Principle:**

When platelets go through their release reaction, proteins normally found within the platelet granules are secreted. Many of these proteins are normally found in plasma. Platelet factor 4, however, is normally found only in the platelet alpha granules. Therefore, an elevation of PF4 in the plasma indicates platelet activation and release.

In order for the measurement of PF4 to be a meaningful indicator of in vivo release, care must be taken to minimize any in vitro activation of the platelets. This is accomplished by using minimal stasis for venipuncture and immediately transferring the blood to sample tubes containing an anticoagulant (EDTA) and platelet inhibitors (in our case, aspirin and prostaglandin E1).

It has been suggested that comparison of BTG and PF4 levels in the same sample will indicate true in vivo platelet activation because of their different routes of clearance. The content of BTG and PF4 within the platelet is quite similar. However, once released from the platelet, PF4 is rapidly cleared through the vascular bed. On the other hand, BTG is cleared through the kidneys resulting in a much longer half-life. An elevated BTG, therefore, when compared to a normal to slightly increased PF4 level would suggest true in vivo release, while an equally increased PF4 would suggest in vitro release.

The following table can be used to compare BTG to PF4 in certain clinical situations:

<table>
<thead>
<tr>
<th>BTG</th>
<th>PF4</th>
<th>Ratio of BTG:PF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal individuals</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>In vivo release</td>
<td>Increased</td>
<td>Normal to slightly increased</td>
</tr>
<tr>
<td>In vitro release</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>In vivo heparin</td>
<td>Normal</td>
<td>Markedly increased</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Increased</td>
<td>Markedly decreased</td>
</tr>
</tbody>
</table>

**Reagents and Equipment:**

1. 12 X 75 mm plastic tubes with caps
2. 5 cc plastic syringe
3. 21 gauge needle
4. 30mM EDTA (1%) diluted in isotonic saline (0.85%)
5. 36 mg/ml ASA (aspirin) prepared in 95% ethanol
6. 100 mg/ml PGE1 (prostaglandin E1) prepared in 95% ethanol
7. 0.85% saline
8. 95% ethanol
9. Melting ice bath
10. 5, 50, and 500 ul pipettors with tips
11. Refrigerated centrifuge
For test procedure:
1. 12 x 75 mm plastic tubes
2. 50,200 and 500 ul pipettors with tips
3. PF₄ standards*
4. 1125 PF₄*
5. PF₄ antiserum*
6. Ammonium sulfate*
7. Dilution Buffer*
8. Vortex mixer
9. Centrifuge
10. Absorbent towels
11. Decantation racks
12. Gamma counter
13. Data sheet

Specimen Collection:
1. Specimens for PF₄ should be part of a blood sampling of no more than 10 ml.
2. Prepare sample tubes by adding 0.5 ml of 30 mM EDTA (H₅EDTA) to 12 x 75 mm plastic tubes with caps.
3. 0.36 g ASA is diluted to 10 ml with 95% ethanol in a volumetric flask (final concentration 36 mg/ml). Store unused portion 2 weeks in the freezer in a tightly sealed container or make fresh daily.
   *Note: Whenever working with 95% ethanol solutions, always try to keep evaporation to a minimum by using a tight fitting seal which is only kept open when necessary. If solutions are left open for a long period of time they must be discarded since evaporation will change the concentration of the solution.
4. 1.0 mg PGE₄ is diluted to 10 ml with 95% ethanol in a volumetric flask (100 µg/ml), tightly sealed, and stored in the freezer.
5. An hour before sampling, thaw ASA and PGE₄. In the meantime, prepare sample tubes as in Step 2 and place into melting ice bath.
6. ASA will precipitate when frozen, so care should be taken if it is completely in solution before use.
7. Mix PGE₄ and make a 1:2 dilution in 95% ethanol as your working solution.
8. Draw 4 ml of blood, using minimal stasis, through a 21 gauge needle.
9. Immediately before adding blood to precooled sample tubes, inject 50 ul ASA solution and 5 ul PGE₄ working solution into the EDTA and mix.
10. Add exactly 2 ml whole blood to each tube, invert twice, and return tube to ice bath.
11. Always take duplicate samples to assure enough plasma in case there is a need for additional testing (see Specimen Handling Step 13).

*Supplied in PF₄ kit by Abbott Laboratories.
Specimen Handling:

1. Within 30 minutes of sampling, centrifuge specimens at 4°C at 1900 g (2800 RPM in RC3) for one hour.

2. Take 0.5 ml aliquot from each of the duplicate samples just below the lipid surface, put into 2 separate plastic tubes, and freeze until ready to test.

3. When ready to assay, thaw tubes 5 minutes at 37°C. Once specimens are thawed they cannot be refrozen.

Procedure:

1. Bring all test kit reagents and specimens to room temperature.

2. Label 12 x 75 mm plastic tubes as follows:
   - Tubes #1 and #2 = Total Count Tubes (TCT)
   - Tubes #3 and #4 = Non-specific Binding Tubes (NSB)
   - Tubes #5 through #14 = for the 5 standards (0, 10, 30, 50, 100) in duplicate.
   - Tubes #15, etc. = unknowns in duplicate.

3. Once tubes are labelled, fill out data sheet with the tube number and the corresponding contents (i.e. TCT, NSB, level of each standard, names of unknowns).

4. Pipet 50 ul Dilution Buffer into the bottoms of tube #3 and #4 (NSB) and #5 and #6 (0 ng standard).

5. Pipet 50 ul of each standard and unknown into the bottom of the appropriately labelled tubes.

6. Pipet 250 ul 125 I into all the tubes.

7. Cap tubes #1 and #2 (TCT) and set aside until ready to put on the gamma counter.

8. Pipet 250 ul Dilution Buffer into tubes #3 and #4 (NSB) and vortex mix the tubes.

9. Pipet 250 ul antiseraum into each of the remaining tubes starting with tube #5. Vortex mix each tube.

10. Incubate all tubes at room temperature for two hours.

11. After incubation, pipet 1000 ul ammonium sulfate into all but TCT tubes and let set for 10 minutes.

12. Centrifuge all but TCT tubes 15 minutes at 1500 g (2300 RPM in RC3) either at room temperature or 4°C.

13. Transfer tubes from centrifuge to decantation racks, making sure tops of tubes extend from the surface of the rack at approximately the same level to ensure even decantation.
14. Invert rack onto absorbent towels, allow to drain about one minute, blot several times, and return to an upright position.

15. Place tubes on racks for the gamma counter in numerical order (be sure to include TCT tubes).

16. Program #4 on the gamma counter is for PF₄. Be sure all parameters are set correctly. These include:
   Copy program: 0
   Type: 2 (% bound)
   Terminators: 1.00 (minute)
   Radionuclide: 1 (I²³)
   # Tubes/samples: 2 (in duplicate)
   Multiplier: 1.00
   Bkg: 2 (NSB)
   Low CPM Reject: A-0
   B-0
   Screening: Level 0
   Outliers: 0 (flag)
   SD Limit: 1.0
   RIA Data Reduction: 1
   Normal Range: Low = 0
   High = 0
   Transforms: Y = 0
   X = 0
   Use current stds: 1 (yes)
   Plot: 1 (yes)
   Stds: enter new values for each new lot # if necessary

Results:

The gamma counter will calculate the level of PF₄ according to % bound radioactivity. It will plot a standard curve from which it will calculate the mean PF₄ level for each patient's duplicate results. Transfer this value to data sheet and report each patient value with the established normal range.

Normal Range:

References:


**BETA-THROMBLOBULIN (B-TG)**

**PROCEDURE**

Methodology set up by: Linda Miller, M.T. (HEW) (2/82)

Annual reviewer: John Brandt, M.D.

**Principle:**

When platelets go through their release reaction, proteins normally found within the platelet granules are secreted. Many of these proteins are also commonly found in normal plasma. Beta-thromboglobulin, however, is normally found only in the platelet alpha granules. Therefore, an elevation of B-TG in the plasma indicates platelet activation and release.

In order for the measurement of B-TG to be a meaningful indicator of in vivo release, care must be taken to minimize any in vitro activation of the platelets. This is accomplished by using minimal stasis for venipuncture and immediately transferring the blood to sample tubes containing an anticoagulant (EDTA) and platelet inhibitors (in our case, aspirin and prostaglandin E1).

It has been suggested that comparison of B-TG and PF4 levels in the same sample will indicate true in vivo platelet activation because of their different routes of clearance. The content of B-TG and PF4 within the platelet is quite similar. However, once released from the platelet, PF4 is rapidly cleared through the vascular bed. On the other hand, B-TG is cleared through the kidneys resulting in a much longer half-life. An elevated B-TG, therefore, when compared to a normal to slightly increased PF4 level would suggest true in vivo release, while an equally increased PF4 would suggest in vitro release.

The following table can be used to compare B-TG to PF4 in certain clinical situations:

<table>
<thead>
<tr>
<th>Condition</th>
<th>B-TG</th>
<th>PF4</th>
<th>Ratio of B-TG:PF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal individuals</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>In vivo release</td>
<td>Increased</td>
<td>Normal to slightly increased</td>
<td>Normal</td>
</tr>
<tr>
<td>In vitro release</td>
<td>Increased</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>In vivo heparin</td>
<td>Normal</td>
<td>Markedly increased</td>
<td>Markedly decreased</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Increased</td>
<td>Normal</td>
<td>Increased</td>
</tr>
</tbody>
</table>

**Reagents and Equipment:**

For sample collection:

1. 12 x 75 mm plastic tubes with caps
2. 5 cc plastic syringe
3. 21 gauge needle
4. 30mM EDTA (1X) diluted in isotonic saline (0.85%)
5. 36 mg/ml ASA (aspirin) prepared in 95% ethanol
6. 100 μg/ml PGE1 (prostaglandin E1) prepared in 95% ethanol
7. 0.85% saline
8. 95% ethanol
9. Melting ice bath
10. 5, 50, and 500 μl pipettes with tips
11. Refrigerated centrifuge
For test procedure:
1. 12 x 75 mm plastic tubes
2. 50,200 and 500 ul pipettors with tips
3. PF4 standards
4. 1125 PF4
5. PF4 antiserum
6. Ammonium sulfate
7. Dilution Buffer
8. Vortex mixer
9. Centrifuge
10. Absorbent towels
11. Decantation racks
12. Gamma counter
13. Data sheet

Specimen Collection:
1. Specimens for PF4 should be part of a blood sampling of no more than 10 ml.
2. Prepare sample tubes by adding 0.5 ml of 30 mM EDTA to 12 x 75 mm plastic tubes with caps.
3. 0.36 g ASA is diluted to 10 ml with 95% ethanol in a volumetric flask (final concentration 36 mg/ml). Store unused portion 2 weeks in the freezer in a tightly sealed container or make fresh daily. Note: Whenever working with 95% ethanol solutions, always try to keep evaporation to a minimum by using a tight fitting seal which is only kept open when necessary. If solutions are left open for a long period of time they must be discarded since evaporation will change the concentration of the solution.
4. 1.0 mg PGE1 is diluted to 10 ml with 95% ethanol in a volumetric flask (100 ug/ml), tightly sealed, and stored in the freezer.
5. An hour before sampling, thaw ASA and PGE1. In the meantime, prepare sample tubes as in Step #2 and place into melting ice bath.
6. ASA will precipitate when frozen, so care should be taken it is completely in solution before use.
7. Mix PGE1 and make a 1:2 dilution in 95% ethanol as your working solution.
8. Draw 4 ml of blood, using minimal stasis, through a 21 gauge needle.
9. Immediately before adding blood to precooled sample tubes, inject 50 ul ASA solution and 5 ul PGE1 working solution into the EDTA and mix.
10. Add exactly 2 ml whole blood to each tube, invert twice, and return tube to ice bath.
11. Always take duplicate samples to assure enough plasma in case there is a need for additional testing (see Specimen Handling Step #3)

*Supplied in PF4 kit by Abbott Laboratories.
Specimen Handling:

1. Within 30 minutes of sampling, centrifuge specimens at 4°C at 1900 g (2800 RPM in RC3) for one hour.

2. Take 0.5 ml aliquot from each of the duplicate samples just below the lipid surface, put into 2 separate plastic tubes, and freeze until ready to test.

3. When ready to assay, thaw tubes 5 minutes at 37°C. Once specimens are thawed they cannot be refrozen.

Procedure:

1. Reconstitute lyophilized reagents per manufacturer's instructions. These are stable for one week when stored in refrigerator, bring to room temperature before proceeding.

2. Label 12 x 75 mm plastic tubes as follows:
   - Tubes #1 and #2 = Total Count Tubes (TCT)
   - Tubes #3 and #4 = Non-specific Binding Tubes (NSB)
   - Tubes #5 through #14 = for the 5 standards (0, 10, 30, 50, 100) in duplicate.
   - Tubes #15, etc. = unknowns in duplicate

3. Once tubes are labelled, fill out data sheet with the tube number and the corresponding contents (i.e. TCT, NSB, level of each standard, names of unknowns).

4. Pipet 50 ul saline into the bottom of tube #3 and #4 (NSB).

5. Pipet 50 ul of each standard and unknown into the bottom of the appropriately labelled tubes.

6. Pipet 200 ul 1125 ^125 I into all the tubes.

7. Cap tubes #1 and #2 (TCT) and set aside until ready to put on the gamma counter.

8. Pipet 200 ul saline into tubes #3 and #4 (NSB) and vortex mix the tubes.

9. Pipet 200 ul ^125 I antiserum into each of the remaining tubes starting with tube #3. Vortex mix each tube.

10. Incubate all tubes at room temperature for one hour.

11. After incubation, pipet 500 ul ammonium sulfate into all but TCT tubes and let stand for 10 minutes.

12. Centrifuge all but TCT tubes 15 minutes at 1500 g (2300 RPM in RC3) either at room temperature or 4°C.

13. Transfer tubes from centrifuge to decantation racks, making sure tops of tubes extend from the surface of the rack at approximately the same level to ensure even decantation.
14. Invert rack onto absorbent towels, allow to drain about one minute, blot several times, and return to an upright position.

15. Place tubes on racks for the gamma counter in numerical order (be sure to include TCT tubes).

16. Program #3 on the gamma counter is for BTC. Be sure all parameters are set correctly. These include:
   - Copy program: 0
   - Type: 2 (2 bound)
   - Terminators: 1.00 (minute)
   - Radionuclide: 1-(125)
   - # Tubes/samples: 2 (in duplicate)
   - Multiplier: 1.00
   - Bkg: 2 (NSB)
   - Low CPM Reject: A-0
   - Screening: Level 0
   - Outliers: 1 (flag)
   - SD Limit: 1.0
   - RIA Data Reduction: 1 (yes)
   - Normal Range: Lo = 0
   - High = 0
   - Transforms: Y = 0
   - X = 0
   - Use current stds: 1 (yes)
   - Plot: 1 (yes)
   - Stds: enter new values for each new lot # if necessary

Results:

The gamma counter will calculate the level of BTC according to 2 bound radioactivity. It will plot a standard curve from which it will calculate the mean BTC level for each patient's duplicate results. Transfer this value to data sheet and report each patient value with the established normal range.

Normal Range:

References:


DOSE-RESPONSE CURVE - BTG

STANDARD CURVE
BTG

4/14/86

STANDARDS (ng/ml)

9
18
44
88
205

DOSE
DOSE-RESPONSE CURVE - BTG

STANDARD CURVE

STANDARDS (ng/ml)

4/29/86

DOSAGE
DOSE-RESPONSE CURVE - PF4

STANDARD CURVE

PF4

4/29/86

STANDARDS (ng/ml)

0
10
30
50
100

DOSE
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# Laboratory Worksheet

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