LAKEHEAD UNIVERSITY

MECHANISMS OF EXERCISE HEMATURIA

A THESIS SUBMITTED TO

THE SCHOOL OF PHYSICAL EDUCATION AND ATHLETICS

IN CANDIDACY FOR THE DEGREE OF MASTERS OF APPLIED SPORTS SCIENCE AND COACHING

BY

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ABSTRACT

The purpose of this study was to establish the prevalence of hematuria in a group of otherwise healthy male runners aged 23 to 54 years (n = 10), and to compare the occurrence of hematuria under four different exercise conditions. The four exercise protocols chosen for the study were: a 60 minute treadmill run (run) at 90% of anaerobic threshold (AT), a 60 minute cycle (bike) ergometer ride at 90% of AT, three 400 meter sprints (sprint) at maximum effort, each followed by a four minute rest consisting of light walking, and three 60 second Wingate cycle ergometery (Wingate) at maximum effort, each followed by a four minute rest consisting of light cycling. The study employed a 3 by 4 (time by protocol) within-subjects design. The dependent variables were measured before, four minutes post and one hour post exercise, and included: hematuria, proteinuria, urinary pH, serum haptoglobin (Hp), serum creatine kinase (CPK), plasma lactate (pLa), and hemoglobin (Hb). It was hoped that by observing the occurrence of hematuria under the various conditions, and by cross-examining the dependent variables, further understanding into the mechanisms of hematuria would be gained. Repeated 400 meter sprinting at maximal effort was found to significantly increase both hematuria and proteinuria ($p < .01$). In addition, post exercise hematuria for the sprint protocol was significantly different than both the bike ($p < .01$) and run ($p < .01$) protocols. Significant correlations were observed between both hematuria ($p < .01$) and proteinuria ($p < .001$), with plasma lactate (pLa) and with urinary pH ($p < .001$). Due to

the significant increase in hematuria and proteinuria following the sprint protocol it was concluded that intensity related changes in renal function were the responsible mechanisms.

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DEFINITIONS

Anaerobic threshold (AT) - the subjective prediction of anaerobic threshold during the maximal oxygen consumption tests was assessed via a non-linear increase in ventilation in concert with an excessive CO_2 production. Consequently, exercise intensity between the 60 minute bicycle test and the 60 minute running test may vary slightly.

Cast - masses of red blood cells molded by the renal tubules, originating from the glomeruli. Abnormal microscopic blood in the urine composed of coagulated serum covered with red blood cells (Thomas, 1989).

Cytoscopy - microscopic examination of cells for purposes of diagnosis (Thomas, 1989).

Creatine Kinase - enzyme present in skeletal and cardiac muscle and the brain. Serum level is increased following trauma to skeletal muscle. Normal expected range for creatine kinase-MM (muscle type) 5-70 U/l (Harold et al. 1991).

Dysmorphic - not normal in form (Thomas, 1989).

Erythropoietin - a hormone that stimulates bed blood cell production (Thomas, 1989).

Filtration fraction - used to describe the size of particle which can pass through the glomeruli of the kidney.

Glomerular filtration rate - the amount of fluids passing through the kidney. The rate of filtration is mainly dependent upon blood pressure in the glomeruli. The amount can vary depending on: water intake, nature of diet, degree of body activity, environment and body temperature, age, and blood pressure (Thomas, 1989).

Glomeruli - capillary blood vessels in the kidney designed to filter urine.

Haptoglobin - a mucoprotein to which hemoglobin released into plasma is bound. It is increased in certain inflammatory conditions and decreased in hemolytic disorders (Thomas, 1989). Normal levels: .38 to 2.7 g/l (Harold et al. 1991).

Hematuria - blood in the urine (Thomas, 1989). Normal findings in an average healthy population: 0 to 3 red blood cells/high power field (Harold et al. 1991).

Hemoglobin - a protein composed of four globular subunits, each bound to a single molecule of heme; the protein found in the red blood cells that gives them the ability to transport oxygen in the blood. Normal values: men - 140-180 g/l, women - 120-160 g/l (Harold et al. 1991).

Hemoglobinuria - presence of hemoglobin in the urine, but free from red blood cells. Occurs when hemoglobin from damaged red blood cells exceeds the binding ability of the blood protein (haptoglobin) (Thomas, 1989). Normally, hemoglobin is not found in the urine (Harold et al. 1991).

Hemolysis - the destruction of red blood cells with the liberation of hemoglobin, which diffuses into the fluid surrounding them (Thomas, 1989).

Lactic Acid - an intermediate product of glucose metabolism in the glycolytic pathway. The chemical formula for lactic acid is $C_3H_6O_3$. Although lactate is the salt of lactic acid, lactic acid and lactate will be used interchangeably throughout this thesis. Normal resting values: 0.93 to 1.65 mmol/l (Harold et al. 1991).

Myoglobinuria - myoglobin in the urine. It may occur following muscular activity, trauma, or as a result of a deficiency of muscle phosphorylase (Thomas, 1989).

Nephron - the structural and functional unit of the kidney. Urine is formed by filtration in renal corpuscles, and selective reabsorption and secretion by cells of the renal tubule (Thomas, 1989).

Proteinuria - protein, usually albumin in the urine (Thomas, 1989).

Pseudonephritis - a false indication of nephritis - nephritis is an abnormal function of the kidney (Glanze et al. 1990). It is a term used in the literature to describe proteinuria.

Serum ferritin - a major iron storage protein found in reticuloendothelial cells, a small portion is usually found in the watery portion of the blood serum. Serum ferritin relates directly with the amount of iron stored in the body and can be accurately measured by radioimmunoassay. Used to screen for iron deficiency or overload. Normal values: men - .20 to 3.0 µg/l, women - .20 to 1.2 µg/l (Harold et al. 1991).

$\dot{V}O_2max$ - the highest oxygen uptake obtainable for a given form of ergometery despite further work rate increases and effort by the subject. This is characterized by a plateau of oxygen uptake despite further increases in work rate (Wasserman et al. 1987). Identified in this study as the highest 15 second value obtained during the test.

CHAPTER 1

INTRODUCTION

Modern societies are showing an increasing awareness towards exercise and fitness (Stephens and Craig, 1990). Accompanying this trend is a developing concern of sport specific medical issues such as the occurrence of blood in the urine (hematuria). Gross hematuria (visible blood in the urine) is less frequent but is easily diagnosed and therefore effectively treated. Microscopic hematuria (invisible) on the other hand is a common clinical problem which can easily go unnoticed (Mariani, Mariani, Macchioni, Hariharan, & Moriera, 1989). Red blood cells (RBC) are commonly found in the urine, but three or more cells per high power field is considered unusual (Harold et al. 1991). The mechanisms of microscopic hematuria are varied, thus making it difficult to establish the cause.

Nevertheless, based on research, it is appears that the amount of red blood cells in the urine are proportional to the intensity and duration of exercise (Poortmans, 1984; Poortmans & Henrist, 1989; Eichner, 1990; Cianflocco, 1992). The literature has indicated many possible mechanisms of hematuria which include: cancer (Eichner, 1990; Sutton, 1990; Elliot, Goldberg, & Eichner, 1991), infections (Schramek, Schuster, Georgopoulos,

Porpaczy, & Maier, 1989; Sutton, 1990), footstrike hemolysis (Eichner, 1985, 1986; Egan, Watts, & Silta, 1987; Miller, Pate, & Burgess, 1988; Miller, 1990; Dressendorfer et al. 1991), increased catecholamines (Yoshimura, 1970; Lindemann, Ekanger, Opstad, & Nummestad, 1978; Poortmans, 1984; Cianflocco, 1992), hypoxic damage to the nephrons in the kidney (Abarbanel, Benet, Lask, & Kimche, 1990; Eichner, 1990; Cianflocco, 1992), dehydration (Poortmans, 1984; Helzer, Latin, Mellion, Berg, & Langan, 1988; York, 1990), non-steroidal anti-inflammatory agents (Kraus, Siroky, Babayan & Krane, 1984), muscular tissue damage (Milne, 1988; Schiff, Macsearraigh, & Kallmeyer, 1978; Lijnen, Hespel, Lysens, Goris, Vanden-Eynde, Fagard, & Amery, 1987), bladder or renal trauma (Reid, Hosking, & Ramsey 1987; York, 1990), or mechanical muscular trauma (Schobersberger, Tschann, Hasibeder, Steidl, Herold, Nachbauer, & Koller, 1990). However, for the purposes of this study, the focus was on those mechanisms specifically related to exercise.

Purpose

The purpose of this study therefore was to establish the prevalence of hematuria in a group of otherwise healthy male runners, and to compare the occurrence of hematuria under maximal and moderate intensities for short and long durations following cycling and running. To achieve this goal, the study employed a 3 by 4 (time by protocol) within-subjects design. The dependent variables were measured before, four minutes post and one hour post exercise, and included: hematuria, proteinuria, urinary pH, serum haptoglobin (Hp), serum creatine kinase (CPK), plasma lactate (pLa), and hemoglobin

(Hb). The four exercise protocols chosen for the study were: a 60 minute treadmill run at 90% of anaerobic threshold (AT), a 60 minute cycle ergometer ride at 90% of AT, three 400 meter sprints at maximum effort, each followed by a four minute rest consisting of light walking, and three 60 second Wingate bike rides at maximum effort, each followed by a four minute rest consisting of light cycling. It was hoped that by observing the occurrence of hematuria under the various conditions, and by cross-examining the dependent variables, further understanding into the mechanisms of hematuria would be gained.

Exercise related hematuria can be divided into three basic groups based on the location of the body where it occurs. (1) Hematuria may occur intravascularly, where footstrike or mechanical muscular trauma are indicated. Footstrike hemolysis and mechanical muscular trauma can be identified by a marked drop in Hp with a subsequent increase in hematuria (Miller, 1988; Eichner, 1985, 1990; Harold et al. 1991). If mechanical muscular trauma is responsible for the intravascular hemolysis, hematuria should occur during both the run and bike protocols. Whereas if footstrike hemolysis is responsible, hematuria should occur during the run and not the bike protocol. (2) Hematuria may occur at the level of the nephrons in the kidney, where altered renal function is the mechanism. Dysmorphic red blood cells will occur in the urine when abnormal glomerular permeability allow red blood cells to pass through the nephrons in the kidney (Reid et al. 1987). Their presence implicates altered function of the kidney (Schramek et al. 1989; Abarbanel et al. 1990). Additionally, urinary red blood cell casts, particularly hyaline, which originate at the renal cortex provide evidence of

glomular origin (Stransinger, 1989). (3) Finally, hematuria may occur post nephrons, where jostling of the bladder from running may result in bleeding due to microscopic lesions in the interior wall (Blacklock, 1977; York, 1990). Bladder or kidney trauma will be identified by cytoscopic analysis of the urine. The presence of whole and normal bio-concave red blood cells in the urine indicates post glomerular bleeding (Reid et al. 1987).

Hematuria and proteinuria have been shown to exhibit a positive relationship with exercise intensity (Poortmans, 1984; Poortmans et al. 1981; Helzer et al. 1988). Therefore, pLa was measured following exercise to provide an indication of exercise intensity. Additionally, pLa was measured as a means of observing differences in exercise intensities prescribed between the cycling and running protocols.

Serum CPK was measured as a direct indication of muscle membrane injury and as an indirect measurement of associated cellular release of myoglobin. Myoglobin may have a toxic effect on the kidney thereby effecting their function (Poortmans, 1984; Milne, 1988). A significant increase in CPK combined with hematuria, may suggests muscular tissue damage as the mechanism (Schobersberger et al. 1990). Reagent strip analysis is unable to differentiate between hematuria and myoglobinuria. Therefore, microscopic analysis of positive hematuria tests was used to differentiate between urinary myoglobin and or red blood cells.

Significance of Study

It is important for today's physicians to understand and identify transient physiological abnormalities caused by exercise, one example is hematuria which may have been caused by exercise. Although not completely understood, blood in the urine (hematuria), be it gross or microscopic, may provide a clue to a serious underlying disease (Sutton, 1990; Reid et al. 1987). Consequently, hematuria induced by exercise can be a major diagnostic problem for doctors and a concern for runners. Understanding the mechanisms of exercise hematuria will enhance the clinician's ability to differentiate between normal and abnormal urinary findings which may facilitate the early diagnosis of disease. Nevertheless, the general consensus is that exercise related hematuria is unusual and that the degree of hemolysis during exercise would only effect a very small percentage of the overall red blood cell pool (Balaban, 1992). It is also believed that the erythropoesis response would be fairly rapid (Lindermann, et al. 1978; Schmidt, Maassen, Tegtbur, & Braumann, 1989). Still, it is important to recognize that hematuria in concert with poor dietary iron intake may be one of several factors leading to reduced iron stores or iron-deficiency anemia, (Colt & Heyman, 1984; Wishnitzer, Berrebi, Hurwitz, Vorst, & Eliraz, 1986; Dallongeville, Ledoux, & Brisson, 1989; Seiler, Nagel, Franz, Hellstern, Leitzmann, & Jung, 1989) and possibly reduced physiological performance (Eichner, 1985; 1986; Prudhomme, & Hudgins, 1990; Dressendorfer et al. 1991).

Limitations

1. Analysis of blood and urine was limited to the accuracy of measurement assays and equipment.
2. The microscopic analysis of urine was limited to the subjective judgment of the laboratory technician.
3. Collection of urine was limited to the ability of the subjects to provide a sample following exercise.
4. Maximal oxygen consumption ($\dot{V}O_2max$) relies on the accuracy of the Beckman Metabolic Measurement Cart® and the willingness of the subjects to exert themselves to maximal exhaustion. A percentage of $\dot{V}O_2max$ was used to equate relative workloads for the 60 minute run and the 60 minute bike test.
5. The study was limited to the willingness of subjects to abstain from vigorous exercise one day prior to each session.
6. The subjective prediction of AT during the $\dot{V}O_2max$ tests was assessed via a non-linear increase in ventilation in concert with an excessive CO_2 production as well as a respiratory exchange ratio (RER) consistently above 1.00. Consequently, the intensity of exercise during the 60 minute bicycle and the 60 minute running protocols may vary slightly.
7. Although both the 400 meter sprints and repeated wingate tests are maximal efforts the relative intensities could vary.
8. Hydration was not strictly controlled for and may therefore impose limits on the findings of this study.

9. Following the Wingate protocol subjects may not be willing or able to walk. Therefore, the prescribed recovery activity of walking may vary between subjects and therefore alter the findings of the study.
10. Due to time constraints, some of the subjects only had one day of rest between exercise protocols. This may have not provided adequate recovery.

Delimitations

1. The study was delimited to physically active male runners aged 23 to 54 years of age in the Thunder Bay, Ontario region having no history of genitourinary disease.
2. The only blood measurements taken were: Hb, Hp, pLa, and CPK.
3. The only urine measurements taken were: hematuria, proteinuria, urinary cytoscopy (positive tests only), and urinary pH.
4. The only exercise protocols to be studied were: the 60 minute bicycle test, the 60 minute treadmill run, a 3 by 60 second Wingate bike ride, and a 3 by 400 meter maximal sprint.
5. The significance level for the analysis of variance was set at the 0.05 level while all post hoc analysis were changed to the 0.01 level of significance.

CHAPTER 2

REVIEW OF LITERATURE

Historic Perspective and Prevalence

Early theories of exercise induced hematuria date back to 1793, when Bernardini Ramazzini, an Italian physician, noted bloody urine in runners. It was his belief that a small vein had burst in the kidney (cited in Eichner, 1990). Epidemiological studies that have examined hematuria have shown the incidence of hematuria to vary in normal healthy adults (Mariani et al. 1989; Mohr, Offord, Owen, & Melton, 1986; Froom, Ribak, & Benbassat, 1984; Eichner, 1990). A study examining the annual medical records of 1,000 asymptomatic servicemen over a 15 year period (12.2 visits per year) found a 38% cumulative incidence of microhematuria (Froom, Ribak and Benbasset, 1984). Another study conducted by the Mayo Clinic found 13% of men (aged 35 and up) and of postmenopausal women (aged 55 and up) to have displayed hematuria (Mohr et al. 1986). Similar findings were presented by the Kaiser Medical Center in Honolulu with 15% of the medical students (n=1,346) studied presenting hematuria (Marriani et al. 1989). Vehaskari, (1979) found that 4% of the 8,954 Finnish students aged 8 to 15 years to present hematuria. Similar findings in a population of Israeli air force recruits aged 18 to 25 (5%) have been reported (Froom et al. 1984).

Hematuria has been observed in the general population and to a greater extent among athletes (Eichner, 1990). Recent studies on marathon runners

have reported hematuria to occur in 21% of those participating in the half marathon, and 22% for the full marathon (Reid, Hosking, & Ramsey, 1987), while Eichner, (1990) reported the occurrence of hematuria in ultramarathoners to range from 50% to 70% (Eichner, 1990). Alvarez & Mir, (1987) studied 26 male runners during a 100 km race and found 19.2% to present macroscopic hematuria and 34.6% to present microscopic hematuria. Still, based on these findings it is difficult to determine the exercise intensity or duration responsible for hematuria. Thus it is difficult to formulate any conclusions. As such, the review will focus mainly on those studies that quantify exercise intensity and duration.

Proposed Causes of Sports Hematuria

a) Intravascular hemolysis

Footstrike hemolysis has been studied as a common mechanism of hematuria. At the moment of heel-strike, RBCs traveling through the capillary beds in the foot become compressed and damaged, thus allowing Hb to leak into the plasma (Miller, 1988). Haptoglobin is a blood protein which binds with free Hb released from damaged RBCs, with the resulting complex being removed by the liver. A decrease in Hp is a good indicator of intravascular hemolysis (Eichner, 1985; 1990; Miller et al. 1988; Harold et al. 1991). If the RBC damage is slight, haptoglobin is able to control and resynthesize damaged red blood cells through hepatocytes (Figure 1.).

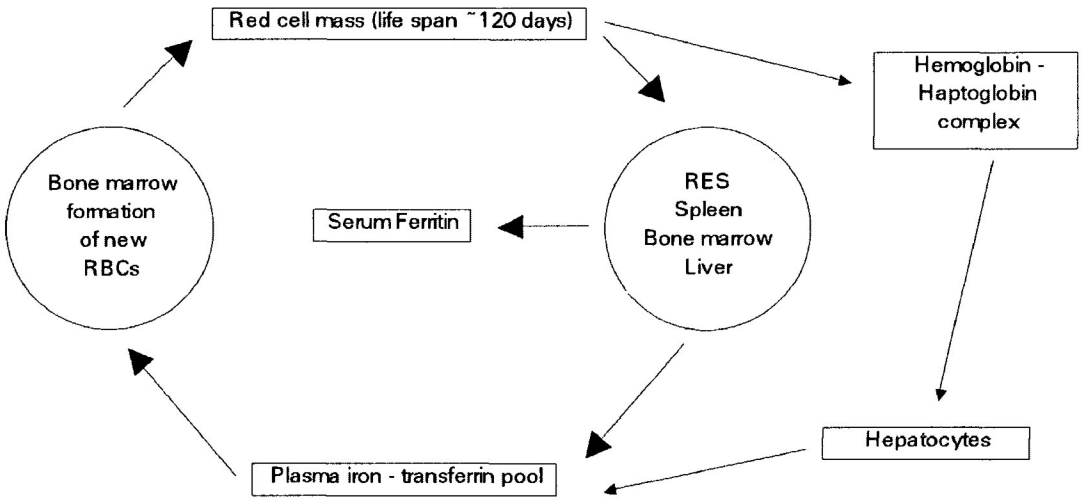

Figure 1. Normal red blood cell formation and break down in the reticuloendothelial system (RES). Footstrike hemolysis in runners will cause RBC resynthesis to increase via the hemoglobin-haptoglobin complex (Magnusson, Hallberg, Rossander, & Swolin, 1984).

Consequently, hematuria is not observed. In contrast, if the damage is more severe, and the available pool of haptoglobin becomes saturated, Hb will appear in the urine via the kidneys (Buckle, 1965; Streeton, 1970; Pare, 1954;

Poortmans, 1984; Abarbanel et al. 1990; Eichner, 1990; Cianflocco, 1992). Research that supports footstrike hemolysis has demonstrated a greater occurrence of intravascular hemolysis in downhill versus uphill running (Miller et al. 1988). Miller et al. (1988) employed 14 male subjects in three testing sessions: (1) resting control, (2) +6% grade treadmill run, and (3) -6% grade treadmill run. Following downhill running haptoglobin significantly decreased and plasma free Hb significantly increased. The conclusion was that mechanical trauma to RBCs occurred at footstrike. Dufaux, Hoederath, Streitberger, Hollmann, & Assmann, (1981) investigated the iron status among middle and long distance runners, elite rowers, and professional racing cyclists. The results showed that the runners had significantly lower ferritin, iron, and haptoglobin than a group of non-exercising subjects. The authors concluded that decreased haptoglobin for the runners was due to an increased hemolysis, and that the diminished iron stores was in part caused by hematuria. Lindemann et al. (1978) studied hematological changes in normal men during prolonged severe military training. Results showed Hb, and haptoglobin dropped 18% to 24%, and 40% to 72% respectively. Interestingly, following exercise, a significant increase in erythropoietin activity (70%) was observed in response to the intravascular hemolysis. This may suggest that intravascular hemolysis will promote or aid in the development of a younger RBC pool. Falsetti, Burke, Feld, Frederick, & Ratering, (1983) studied hematological variations after endurance running with hard-soled and soft-soled running shoes. All subjects completed a 15 mile run on an asphalt surface. The hard-soled group showed a significant decrease in haptoglobin, and a significant increase in plasma Hb (+37.1%).

The authors concluded that (1) material properties of training or racing shoes appear to be correlated with physiological measurements, and (2) appropriate cushioning in running shoes may reduce the RBC abnormalities experienced in long-distance running.

Clearly, increased impact force at the moment of heel contact increases the degree of intravascular hemolysis. However, a major limitation is the failure to demonstrate hematuria or hemoglobinuria in conjunction with increased intravascular hemolysis. Current research which has focused on the mechanism of footstrike has reported intravascular hemolysis, but has failed to demonstrate hematuria or hemoglobinuria. As such, one of the goals of this project will be to clarify the relationship between increased intravascular hemolysis and hematuria.

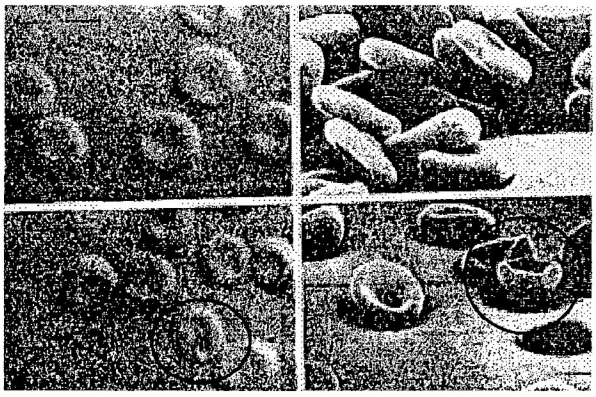

Figure 2. Red blood cells before (top, × 3420) & immediately after (bottom × 3150) a marathon race (Balaban, 1992).

b) Intravascular muscular trauma

Intravascular hemolysis caused by mechanical muscular trauma has been cited as a possible cause of intravascular RBC damage. In a study by Schobersberger et al. (1990) a significant reduction of Hb, mean cell Hb concentration, and mean corpuscular Hb was observed following 6 weeks of strength training. Serum ferritin and Hp also decreased significantly by 35% and 30.5% respectively. The authors concluded that the mechanical stress on red cells due to strength training led to increased intravascular hemolysis and possibly an initial depletion of the body's iron stores.

c) Non-traumatic intravascular hemolysis

Yoshimura, (1970) reviewed anemia during physical activity. In that article he cited the following: Brown, (1922) stated that an increased rate of circulation in the capillary beds caused mechanical damage to the red blood cell, while Singer, (1941) pointed out that lysolecithin which is released from the spleen will increase RBC fragility. Furthermore, Shiraki, (1968) stated that during exercise, increased levels of adrenaline may stimulate the spleen to release a hemolysing factor. Ohtsuka, (1966) indicated that the factor which is associated with increased RBC fragility is located in the plasma, and that injections of 0.1 mg of adrenaline increased RBC fragility. Hiramatsu, (1960) found that the life span of RBCs in exercising rats was 40% shorter than control rats. Also, that resynthesized isotope ^{59}heme iron appeared in spleen, bone marrow, skeletal, and heart muscle; while serum protein re-appeared in the liver. Yoshimura, (1966) concluded that the RBC break down was an adaptive reaction which would promote or aid in the adaptation in skeletal muscle.

d) Renal function and physiology

Exercise has been shown to place extreme stress on renal function (Poortmans, 1984; Abarbanel et al. 1990; Cianflocco, 1992). During exercise, neural responses cause a shunting of the blood flow away from the body's core resulting in an increased blood flow to the working muscles and this facilitates oxygen delivery (McArdle Katch & Katch., 1986). Poortmans, (1984) indicated that renal blood flow falls to 15% - 67% of resting values during light to heavy exercise respectfully. The decreased blood flow can result in a hypoxic state which may result in nephron damage and increased glomerular permeability (Abarbanel et al. 1990). Javitt & Miller, (1952) suggested renal ischemia as the cause of increased permeability of the intercellular cement. Furthermore, renal vasoconstriction caused by sympathetic and adrenaline and noradrenaline responses is more pronounced in the efferent glomerular arteriole which causes both an increased glomerular filtration rate (GFR) and filtration fraction (FF) (Castenfors, 1977; Poortmans, 1984; Poortmans, & Henrist, 1989; Poortmans, Jourdain, Heyters, & Reardon, 1990; Cianflocco, 1992). The result of an increased GFR and FF is an increase in hematuria and proteinuria (Poortmans, 1984; Poortmans, & Henrist, 1989; Poortmans et al. 1990). In addition, a dehydration insult to the nephrons of the kidney has been shown to increase the occurrence of hematuria (Poortmans, 1984; York, 1990). Helzer et al. (1988) studied the effect of exercise intensity and hydration on athletic pseudonephritis. Treatments consisted of four 60 minute runs of varying intensities and levels of hydration. The results showed that high exercise intensity ($p \leq .05$) and

dehydration ($p \leq .06$) were significant factors in producing increases in proteinuria and hematuria. Accordingly, exertion induced renal vasoconstriction and ischemia causing an altered GFR and FF, and a combination of exercise conditions such as dehydration, hypoxic and acidity may be the mechanism of hematuria and proteinuria.

e) Mechanical bladder trauma

Bloody urine, in some male runners, has been due to repeated impact of the posterior bladder wall against the bladder base causing vascular lesions (Abarbanel et al., 1990). Siegel, Hennekens, Solomon, & Van Boeckel, (1979) suggested this may be more common in males than in females due to anatomical differences in the outlet tubule. Blacklock, (1977) noted gross hematuria following strenuous running or marathons and coined the term *runner's bladder.* The author reported *kissing lesions* on the posterior bladder wall and postulated that the repeated trauma of running caused these contusions. This mode of injury presumes that the bladder is empty, thus allowing for opposition of the two surfaces (Abarbanel et al. 1990). Cianflocco, (1992) states that athletes who usually void prior to competition may have an increased risk of bladder trauma.

f) Mechanical kidney trauma

Renal injuries can include contusions, lacerations, shattered kidneys, and pedicle injuries (York, 1990). York, (1990) stated that children are particularly prone due to the relatively larger size of the kidney. Nevertheless, he states that the incidence of renal trauma increases with age.

Although not mentioned, this type of injury is likely due to changes in activity patterns and attitudes towards aggressiveness in older athletes.

g) Muscular tissue damage

During extreme exertion muscle membrane injury may occur resulting in the release of cell contents, such as myoglobin and CPK into the blood. Myoglobin may have a toxic effect on the kidney, thereby effecting their function (Poortmans, 1984; Milne, 1988). Schiff et al. (1978) found that 57% of the 44 ultramarathoners he examined displayed an increased post race blood myoglobin concentration. In addition, muscle cell injury is increased with eccentric exercise, a hot environment, or if there is any preceding infectious disease, drug ingestion or underlying metabolic disorder (Milne, 1988).

h) Free radical damage

The formation of free radicals has been linked to muscle soreness and tissue damage (Sjodin, Westing, and Apple, 1990). During normal cellular respiration a small but significant amount (1% to 2%) of highly reactive oxygen intermediates (free radicals) are formed (Rhoades & Saunders, 1992). This process is markedly enhanced during high intensity aerobic exercise (Sjodin et al. 1990). However, the jury is still out regarding free radical formation during highly anaerobic sprint type exercise (Brooks, Gillam, Kanter, Packer 1992). Free radicals have also been shown to play a role in organ tissue damage, including the kidney (Rhoades & Saunders, 1992). Therefore, the formation of free radicals may play a duel role as a mechanism for hematuria. The primary role being direct tissue damage to the kidney

thereby altering it's function (Rhoades and Pflanzer ??). The secondary role being a release of myoglobin caused by muscular tissue damage resulting in a toxic effect on the kidneys (Milne, 1988; Poortmans, 1984).

CHAPTER 3

METHODS AND PROCEDURES

PART I. SCREENING

Purpose

In order to investigate the mechanisms related to hematuria, without using large populations, identification of individuals who display hematuria was essential. The purpose of the screening was to establish the prevalence of hematuria in a group of healthy male runners following either a 5 km, a 10 km, or a 21 km run, and to recruit subjects for further study.

Subjects

Seventy healthy male runners aged 16 to 66 years were tested for hematuria during the Heart of Thunder Bay Road Race on September 25, 1992 which consisted of a 5 km, a 10 km, and a 21.1 km run. The subjects reported an average weight and height of 177.9 cm and 75.4 kg respectively. These tests were used to recruit subjects who displayed exercise related hematuria. All subjects were volunteers residing in the Thunder Bay, Ontario area and written consent was obtained prior to admission into the study.

Procedures

Initial contact was made via a letter sent out with pre-registration kits. Race participants were asked to fill out a questionnaire concerning personal

training habits, to not participate in vigorous exercise two days prior to running the race, and to provide 1 urine sample of at least 15 ml. immediately following the race. Urine samples were collected immediately following the race and were placed on ice until transport for reagent strip analysis.

Hematuria, proteinuria and urinary pH were measured by reagent strips for urinalysis (Hemastix: Miles Canada Inc.) using an automated reagent strip analyzer (Ames Clinitea 200). Negative tests were randomly retested to evaluate the reliability of reagent strip method. Reports have shown that reagent strip analysis has a 97.5% sensitivity and specificity for detection of hematuria (Mee, & McAninch, 1989). Urine that was positive for hematuria was centrifuged for 5 minutes at 1500 rpm before examination of RBC distribution by an independent laboratory technician using a microscopic high powered field (hpf, × 40). If more than 30 red blood cells were observed, counting was stopped and a value of 30 was recorded. Urine samples that were not analyzed within one hour of voiding were refrigerated (Harold et al. 1991).

PART II. EXERCISE PROTOCOLS

Purpose

The purpose of the exercise protocols was to investigate the mechanisms of hematuria by evaluating the effect of running and cycling at high and low intensities on male runners.

Subjects

Twelve physically-fit male recreational runners, aged 23 to 54 years residing in the Thunder Bay, Ontario area volunteered to participate in the study. The subjects included six male athletes who displayed post race hematuria following the screening tests in September, two subjects who reportedly displayed hematuria following exercise, and four subjects who were not known to display hematuria following exercise. All subjects gave written informed consent prior to beginning the study. The subjects who displayed hematuria on post race samples were required to receive medical examination before admittance into the study in order to rule out genitourinary disease. Two of the subjects were excluded from the study, one due to lack of compliance, and the other due to positive pre-test urinanalysis. The subject who tested positive on pre-test samples was encouraged to seek medical advice. He was later found to have a urinary tract infection, and follow up treatment was successful.

Criteria for Exclusion from the Study

1. Females were not included in the study due to the potential for false positive hematuria because of menstrual bleeding.
2. Runners with a history of genitourinary disease.
3. Failure to adhere to protocols. Subjects were required:
   a) To give maximal effort for anaerobic tests.
   b) To not exercise 24 hours preceding tests.
   c) To provide both urine and blood samples.
4. Positive outcome for hematuria on any pre-test sample.

Procedures

All testing sessions were administered between 4 and 9 p.m., and subjects (n=10) were instructed to wear their normal running clothing and shoes for all tests. Subjects were instructed to refrain from exercise for at least 24 hours prior to testing, and to eat a high carbohydrate meal the night before testing. Two days of rest separated each of the 4 exercise protocols as well as the treadmill and bicycle $\dot{V}O_2max$ tests.

The first two testing sessions were used to determine the subjects $\dot{V}O_2max$ and AT. These were obtained using both cycle and treadmill protocols. The subject's height and weight were determined immediately prior to the first laboratory session, with height measured to the nearest 0.5 centimeter, and weight to the nearest 0.1 kilogram.

A standardized warm up consisting of three minutes of light jogging or cycling, and five minutes of stretching preceded all sessions. Prior to and during exercise, subjects were encouraged to drink water *ad libitum.*

Initial speed for the treadmill $\dot{V}O_2max$ test was set at 2.22 meters/sec (8 mph) and was then increased by 0.22 meters/sec (½ mph) every minute. The treadmill maintained a grade of 0° until 11 mph was achieved, at which time treadmill grade was increased 2° every minute. A Monarch cycle ergometer was used for the cycle $\dot{V}O_2max$ test. Initial tension was set at 2 kp and was then increased 0.5 kp every 2 minutes, and subjects were instructed to maintain a cadence of 60 revolutions per minute. The $\dot{V}O_2max$ tests were terminated upon voluntary exhaustion. The $\dot{V}O_2max$ tests were used to determine AT which was then used to equate matching workloads for the bike and run protocols. The subjective prediction of AT during the maximal oxygen consumption tests was assessed via a non-linear increase in ventilation in concert with an excessive CO_2 production. Consequently, exercise intensity between the 60 minute bicycle test and the 60 minute running test may have varied slightly. Additionally, a respiratory exchange ratio (RER) at or consistently over 1.00 was used to judge the point of AT. The highest 15 second value (ml/kg/min) observed was judged as $\dot{V}O_2max$. Gas exchange measurements for the $\dot{V}O_2max$ tests were analyzed using a Beckman Metabolic Measurement Cart® (Horizon model). Heart rates for both tests were monitored via direct chest lead electrocardiogram (Mortara Instruments, ELI-XR). These sessions also served as a subject familiarization period to laboratory settings and equipment. .

The four exercise protocols were randomized and counterbalanced among the subjects. The workload for the 60 minute bike (bike) and 60 minute run (run) was obtained by calculating 90% of the workload corresponding to AT on the $\dot{V}O_2max$ tests. The result was then prescribed for

the bike and run protocols. The run test was performed on a treadmill while the bike test utilized a Monarch cycle ergometer. The subjects continued to exercise at the prescribed intensity until 60 minutes elapsed. The sprint (sprint) protocol consisted of three 400 meter sprints, each separated by four minutes of light walking. Subjects were instructed to run each 400 meters as fast as possible. A 167 meter indoor banked mondo® rubberized track was utilized for all sprints. The Wingate protocol consisted of three 60 second Wingate cycle ergometer tests, each separated by four minutes of light cycling or walking. A modified Monarch cycle ergometer was used for the test. Modifications included: an additional counter weight added to speed the time to correct belt tension; and an on-line microprocessor designed to monitor revolutions and perform calculations. Resistance was calculated by multiplying 0.075 · body weight (kg).

Blood samples were collected via the venipuncture Vacutainer method from an antecubital vein 5 minutes pre-exercise (pre), 4 minutes post (post) and 1 hour post (1hr post) exercise in a 2.5 ml Vacutainer containing ethylenediaminetetacetic acid (EDTA) anticoagulant for hematological study, a 10 ml *red top* Vacutainer for CPK and Hp determination, and in a 5 ml Vacutainer containing sodium fluoride and potassium oxalate for pLa determination. Blood samples were centrifuged at 3000 rpm for 5 minutes until transport to MDS laboratories for analysis. Serum ferritin was measured using a automatic chemiluminescence system (ACS) with Ciba Corning Instruments and Reagents. Determination of Hb and hematocrit was assessed using a Coulter 5880 system, Hp using rate nephelometry, pLa using EDTA, and CPK was assayed via Kodak Ektachem Clinical Chemistry

Slides. Prior to statistical analysis, all data except Hb measurements were corrected for changes in plasma volume using the method of Dill & Costill (1974).

Urine specimens of at least 15 ml each were collected approximately 5 minutes before, and 5 minutes and 1 hour post exercise. Urine analysis during the laboratory portion of the study utilized the same procedures employed during the screening portion of the study.

All of the exercise tests except the 400 meter sprints took place in the Exercise Physiology Laboratory, C. J. Sanders Fieldhouse, Lakehead University, Thunder Bay, Ontario. The 400 meter sprints took place at Confederation College Campus, Thunder Bay, Ontario.

Statistical Analysis

The study employed a 3 by 4 (time by protocol) within-subjects design to analyze within subject differences for Hb, Hp, pLa, plasma CPK, hematuria, proteinuria and urinary pH. The level of significance for the MANOVA was set at $p \leq 0.05$. In an effort to simplify these results and to create a clearer picture, post hoc paired t-tests were performed when significant differences were observed. The criterion level of significance was then changed to $p \leq 0.01$ for all post hoc tests. Additionally, descriptive statistics for protocol and time, as well as correlations on the dependent variables were performed.

CHAPTER 4

RESULTS

The subjects in the screening had an average age of 38.5 ± 11.5 years (*M* ± *SD*). The average weight and height as reported on the consent form was 75.4 ± 8.4 kg and 177.9 ± 6.3 cm, respectfully. In total seventy subjects participated in the screening, with 23%, 40%, and 37% of the male runners participating in the 5 km, 10 km, and 21 km races respectfully.

Table 1. Urinary findings for the variable hematuria following the screening (n = 70). Values represent the occurrence of hematuria and not the amount present in the urine.

	EVENT		
	5 km race (n=16)	10 km race (n=28)	21 km race (n=26)
Negative	12(75%)	25(90%)	26(100%)
Positive	4(25%)	3(10%)	0(0%)

Table 2. Urinary findings for the variable proteinuria following the screening (n = 70). Values represent the occurrence of proteinuria and not the amount presented.

	EVENT		
	5 km race (n=16)	10 km race (n=28)	21 km race (n=26)
Negative	5(32%)	6(22%)	8(31%)
Positive	11(68%)	22(78%)	18(69%)

Table 3. Characteristics of the subjects (n = 10) who participated in the exercise protocols. Values shown are *M* ± *SD*. P. B.: personal best.

	Mean	*SD*	Range
Age (yrs)	38	± 11.2	23 - 54
Weight (kg)	76.6	± 8.7	63 - 95
Height (cm)	177.2	± 6.8	167 - 188
Serum ferritin (μg/l)	110	± 89.5	28 - 259
P.B. 5 km time (min)	18.4	± 3.3	15 - 25
P.B. 10 km time (min)	39.3	± 6.3	32.5 - 52.0
Max VO2 bike(ml/kg/min)	47.2	± 6.7	38.9 - 57.5
Max VO2 treadmill(ml/kg/min)	53.1	± 8.9	39.8 - 70.3

Table 4. Work capacities for the four exercise protocols (n = 10).

	Mean	*SD*	Range
60 minute Run (mph) @ 90% AT	8.2(74%)	± 1.0	6.8 - 9.5
60 minute Bike (kpm) @ 90% AT	2.7(59%)	± 0.6	1.75 - 3.5
Average Sprint time (sec) for 3 trials.	73.5	± 9.9	56 - 93
Peak power @ 5 seconds on Wingate (Watts/kg) for 3 trials.	7.7	± 1.6	4.7 - 10.6
Mean power @ 60 seconds on Wingate (Joules/kg) for 3 trials.	338.4	± 45.3	255 - 410

Table 5. Hematological measurements taken at rest, 4 minutes following, and 1 hour following exercise. Values shown represent mean ± standard deviations (n = 10).

Hb (g/l)	Wingate	Run	Sprint	Bike
pre	150 ± 8.9	152 ± 5.7	151 ± 5.8	155 ± 5.3
post	162 ± 9.6	161 ± 5.6	158 ± 9.9	161 ± 7.0
1hr post	150 ± 11.4	155 ± 8.3	148 ± 8.1**	154 ± 4.8**
Hp (g/l)	**Wingate**	**Run**	**Sprint**	**Bike**
pre	0.68 ± .28	0.60 ± .16	0.56 ± .22	0.58 ± .27
post	0.64 ± .28	0.50 ± .17*	0.60 ± .24	0.54 ± .24
1hr post	0.68 ± .26	0.45 ± .17*	0.53 ± .22	0.54 ± .24
pLa (mmol/l)	**Wingate**	**Run**	**Sprint**	**Bike**
pre	1.3 ± 1.0	0.8 ± 0.3	1.4 ± 0.9	1.0 ± 0.4
post	14.7 ± 1.5*	1.8 ± 0.4*	15.2 ± 3.1*	2.2 ± 1.2
1hr post	2.2 ± 1.1	0.9 ± 0.5	2.1 ± 0.7	0.8 ± 0.3**
CPK (µg/l)	**Wingate**	**Run**	**Sprint**	**Bike**
pre	356 ± 334	335 ± 330	364 ± 307	299 ± 228
post	417 ± 402	428 ± 418	459 ± 369*	313 ± 238
1hr post	374 ± 353	455 ± 458	410 ± 320*	344 ± 247

***significantly different than pre sample, **significantly different than post sample $p \leq .01$.**

Table 6. Urinary findings for pH, proteinuria, and hematuria. Tests were determined using the reagent strip method. Values represent means ± standard deviations.

Urinary pH	Wingate	Run	Sprint	Bike
pre	5.8 ± 1.0	6.3 ± 0.9	5.9 ± 0.7	5.9 ± 0.8
post	5.4 ± 0.7	5.9 ± 0.6♠	5.1 ± 0.3	5.8 ± 0.8
1hr post	5.2 ± 0.4	6.0 ± 1.2	5.0 ± 0.0	5.9 ± 0.8
Proteinuria (g/l)	**Wingate**	**Run**	**Sprint**	**Bike**
pre	.00± .00	.00 ± .00	.00 ± .00	.00 ± .00
post	.12 ± .16	.15 ± .16	.89 ± .84*	.03 ± .10
1hr post	.12 ± .33	.07± .13	.57 ± .97	.03 ± .10
Hematuria (cells/hpf)	**Wingate**	**Run**	**Sprint**	**Bike**
pre	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
post	0.9 ± 1.4	0.0 ± 0.0♠	6.5 ± 6.0*	0.3 ± 1.0♠
1hr post	0.4 ± 1.3	0.0 ± 0.0	4.8 ± 10.9	0.3 ± 1.0

*** significantly different than pre sample $p \leq .01$. ♠ significantly different than the sprint protocol $p \leq .01$.**

Table 7. Correlations for the variables: Hb, hematuria, proteinuria, hematocrit (Hct.), urinary pH (pH), and lactate.

	Hb	Hematuria	Proteinuria	Hct.	pH
Lactate	.3790**	.2714*	.3947**	.5351**	.2217ns
Proteinuria	.0577	.5134**		.0981	.2335*

(ns: not significant, **: significantly correlated at 0.001 level, *: significantly correlated at 0.01 level).

The subjects who participated in the exercise protocols ranged in age from 23 to 54 years, had an average weight and height of 76.8 kg and 177.0 cm, and an average aerobic capacity of 52.97 ml/kg/min and 46.65 ml/kg/min on the treadmill and bike tests respectively. These values were within the normal expected age range for a similar group of Canadians (Canadian Standardized Test of Fitness, 1987). Additional descriptive data can be seen in table 3. The iron status of the 10 subjects tested was considered normal, with serum ferritin values measured at the start of the study ranging from 28 to 259, and having a mean score of 110 ± 89.5 μg/l ($M \pm SD$).

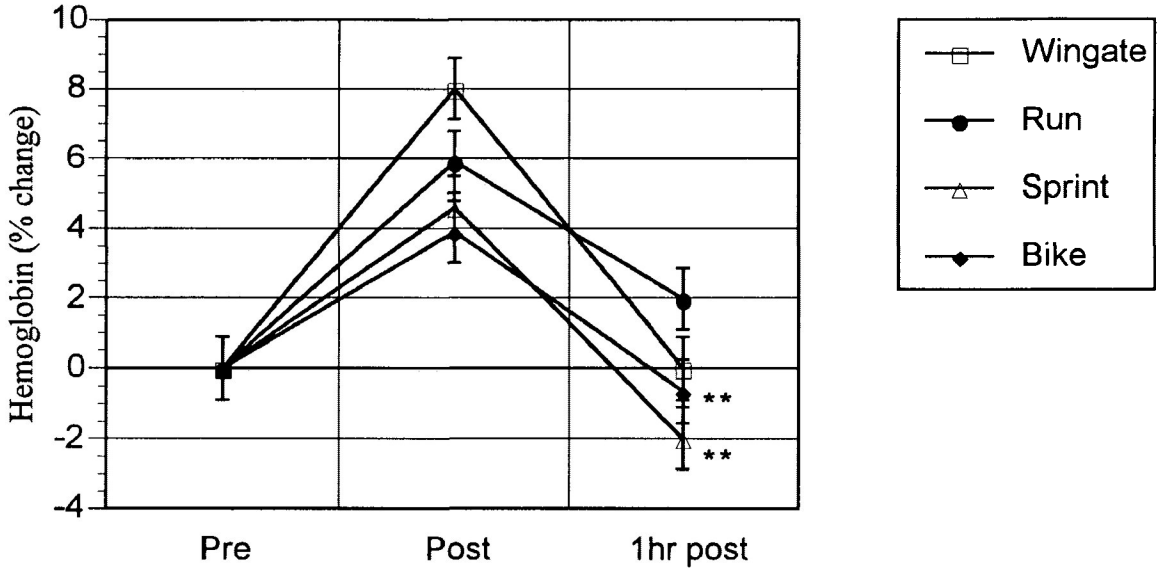

Figure 3. Hemoglobin values taken from the antecubital vein immediately before (pre), 4 minutes after (post), and 60 minutes following exercise (1hr post). Values shown are average percent change and standard error of the mean (*SEM*) and have not been corrected for changes in plasma volume. ** Significantly different than post sample $p < .01$.

The MANOVA revealed a significant difference for Hb values as a function of time $F(2,18) = 6.6, p < .01$. Whereas, within subject differences between protocols were not significant $F(3,27) = .39, p = .762$. The paired t-test indicated that the post sample was significantly different than the 1hr post sample for the sprint $t(9) = 6.21, p < .01$ and bike protocols $t(9) = 7.71, p < .01$. Figure 3. shows the average percent change in Hb values for all protocols.

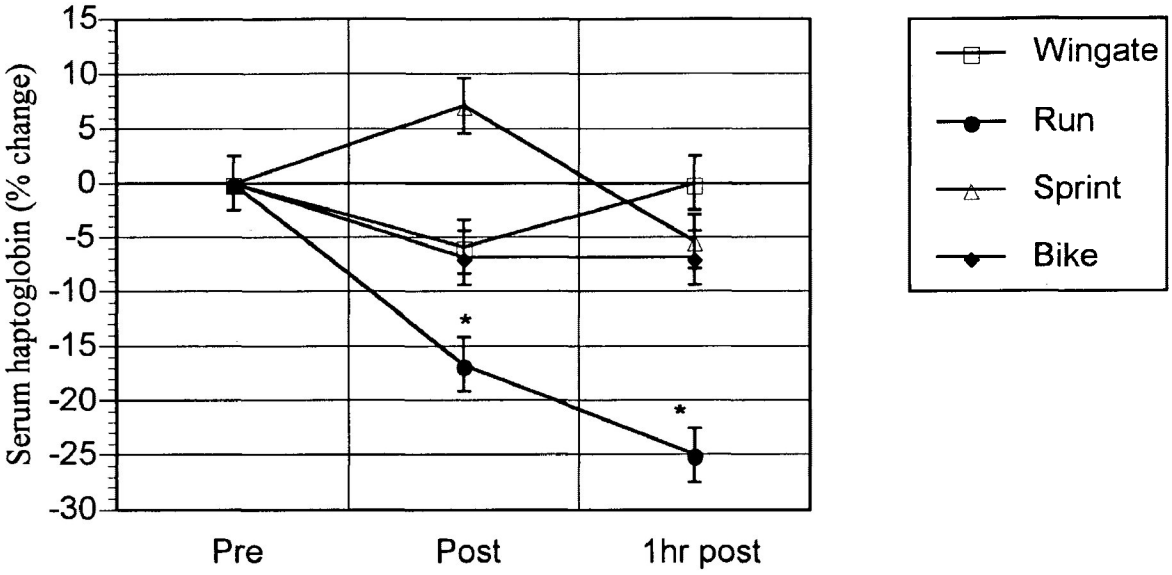

Figure 4. Haptoglobin taken from antecubital vein immediately before (pre), 4 minutes following (post), and 60 minutes after exercise (1hr post). Values are average percent change± *SEM* which have been corrected for changes in plasma volume. Significant within subject interactions were observed for the 4 exercise protocols and the 3 blood sampling times F (6,48) = 4.05, $p < .01$. * Significantly different than pre, ** significantly different than post $p < .01$.

The haptoglobin concentrations showed a significant within subject interaction effect F (6,48) = 4.05, $p < .01$. The paired t-test revealed that haptoglobin values for the run protocol significantly decreased from pre to post t (9) = 4.21, $p < .01$, from pre to 1hr post t (9) = 4.77, $p < .01$, but not from post to 1hr post t (9) = 3.02, $p > .01$. In addition, only the Wingate and run protocol significantly differed for the 1hr post sample. Figure 4. illustrates the average percent change in haptoglobin to the four protocols. Statistical means and standard deviations can be seen in table 5.

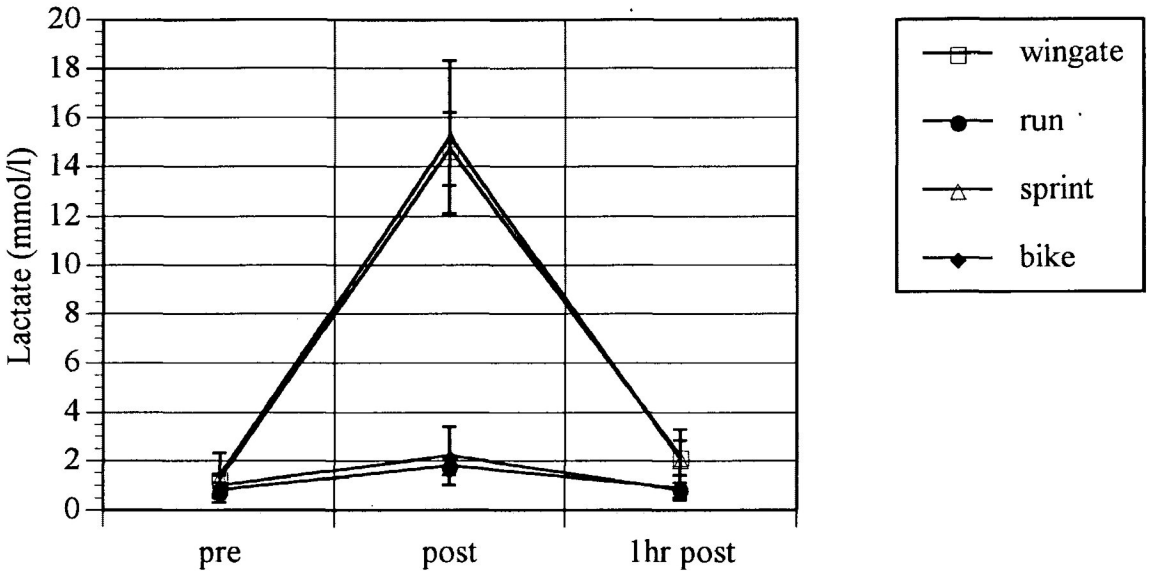

Figure 5. Plasma lactate values taken from antecubital vein immediately before (pre), 4 minutes following (post), and 60 minutes after exercise (1hr post). Values represent $M \pm S.D.$ which have been corrected for changes in plasma volume. Significant within subject interaction effects were observed between the 4 exercise protocols and the 3 blood sampling times $F(6,48) = 45.6, p < .001$.

Within subject treatment by time interaction effects were significant for the variable lactate $F(6,48) = 45.6, p < .001$. The paired t-test revealed significant differences between: the Wingate and run $t(7) = 14.33$ $p < .001$, the Wingate and bike $t(8) = 14.01, p < .001$, the sprint and bike $t(9) = 5.84$, $p < .001$, and the sprint and run $t(8) = 5.22, p < .01$, but not between the Wingate and sprint $t(8) = .05, p \geq .01$, or the bike and run $t(8) = -.83, p \geq .01$. In figure 5. average pLa values during the anaerobic protocols show an expected increase from the pre to post test with values returning to normal within one hour following exercise. Lactate values for the pre, post, and 1 hr post tests can be seen in table 5.

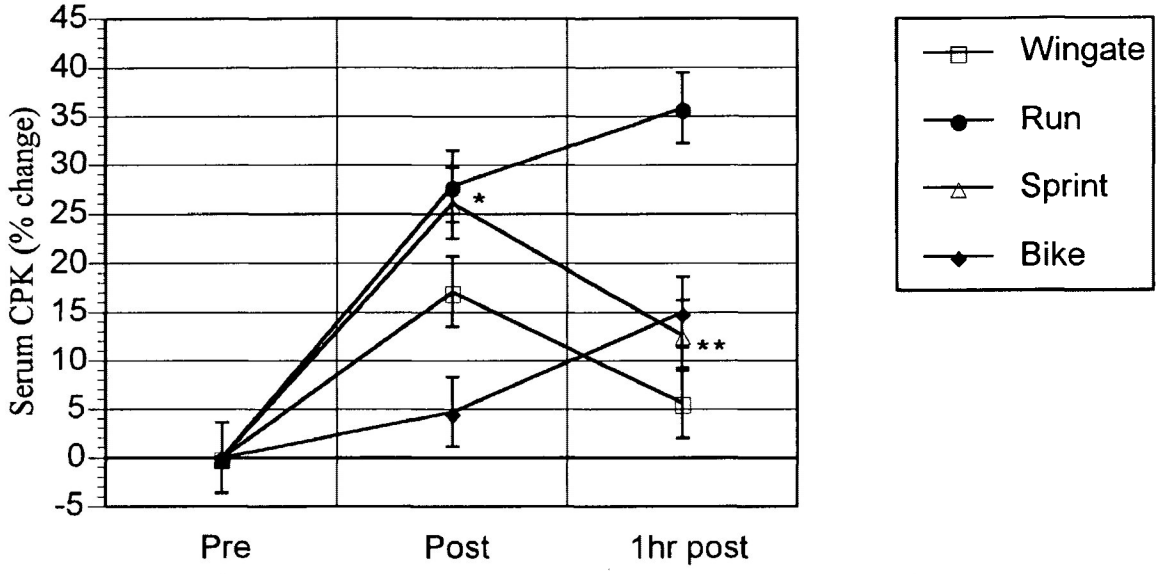

Figure 6. Serum CPK values taken from the antecubital vein immediately before (pre), 4 minutes after (post), and 60 minutes following exercise (1hr post). Values shown are average percent change ± *SEM* which have been corrected for changes in plasma volume. Significant within subject interaction effects were observed for the 4 exercise protocols and the 3 blood sampling times F (6,54) = 2,79, $p < .05$. * Significantly different than pre sample, ** significantly different than post sample $p < .01$.

Concentrations of CPK displayed a significant within subject interaction effect F (6,54) = 2.79, $p < .05$. Figure 6. shows the average percent change in CPK values during the aerobic and anaerobic protocols. The paired t-test exposed significant difference on the sprint protocol between pre and post samples t (9) = -3.89, $p < .01$, and between pre and 1hr post samples t (9) = 5.81, $p < .01$ (table 5.). However, the paired t-test failed to reveal any significant difference between the four exercise protocols at the .01 level of significance.

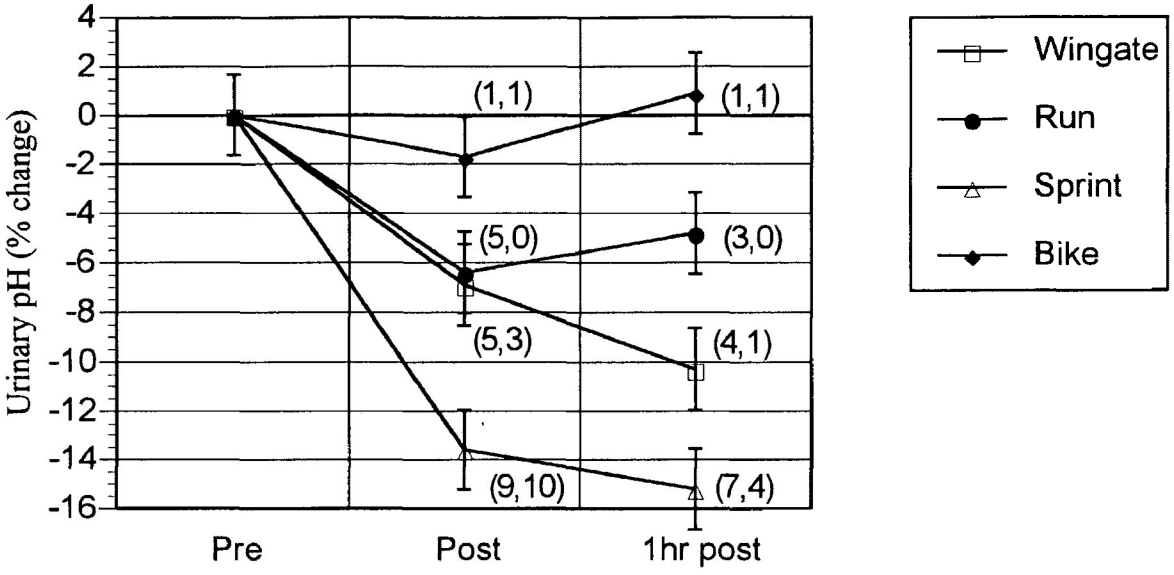

Figure 7. Urinary pH values for the 4 exercise protocols take immediately before (pre), immediately following (post), and 1 hour after exercise (1hr post). Values in brackets are the occurrence of proteinuria and hematuria and suggest a relationship between urinary pH and urinary sediments. Significant within subject differences were observed between the 4 exercise protocols F (3,21) = 5.02, p < .01.

The MANOVA revealed a significant within subject variation between protocols for urinary pH F (3,21) = 5.02, p <.01, but not for time F (2,14) = 2,45, p = .123. The paired t-test indicated that post exercise urinary pH for the sprint protocol was statistically different than the run protocol t (8) = -4.71, p < .01, but not the bike t (7) = -2.94, p > .01 or Wingate protocols t (8) -1.89, p > .01. All other comparisons between protocols were not statistically different at the .01 level of significance. Figure 7 shows the average percent change in urinary pH included in brackets are the occurrence of proteinuria and hematuria respectively.

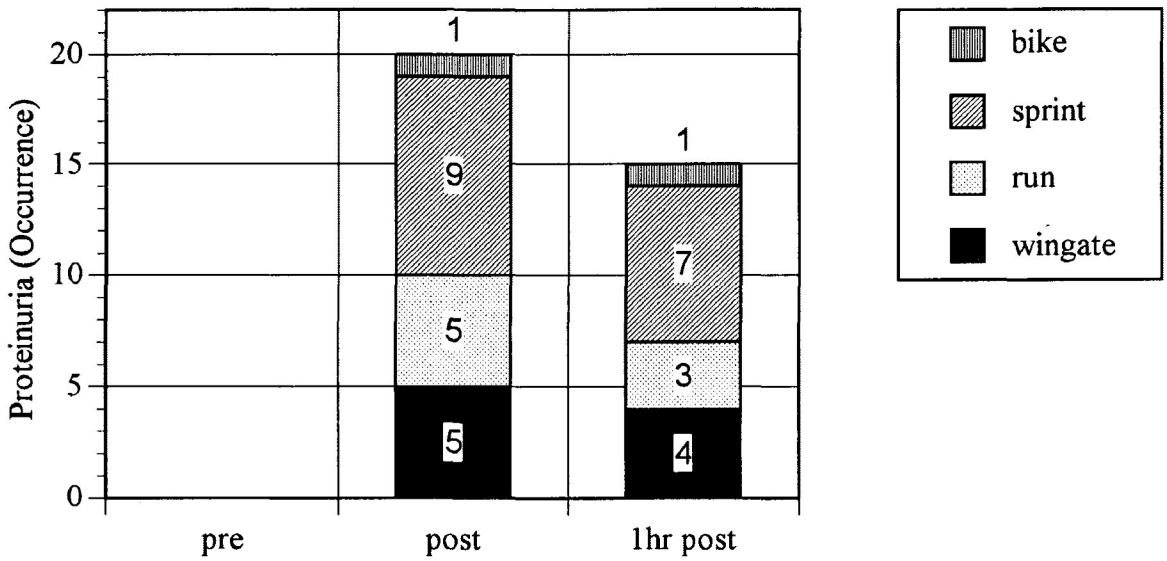

Figure 8. The occurrence of proteinuria determined by reagent strip analysis (n = 10). Significant within subject differences are observed between the three blood sampling times F (2,14) = 4.01, p < .05 and the four protocols F (3,21) = 9.01, p < .001.

The dependent variable proteinuria was significantly different for both the three sampling times F (2,14) = 4.01, p < .05 and for the four exercise protocols F (3,21) = 9.01, p < .001; though, no interaction effect was observed. The paired t-test revealed that only the pre and post sampling times were significantly different for the sprint protocol t (9) = -3.35, p > .01. The mean and standard deviation for proteinuria are presented in figure 8. All subjects tested negative for proteinuria on the pre exercise samples. Table 6 presents the statistical means and standard deviations for the proteinuria.

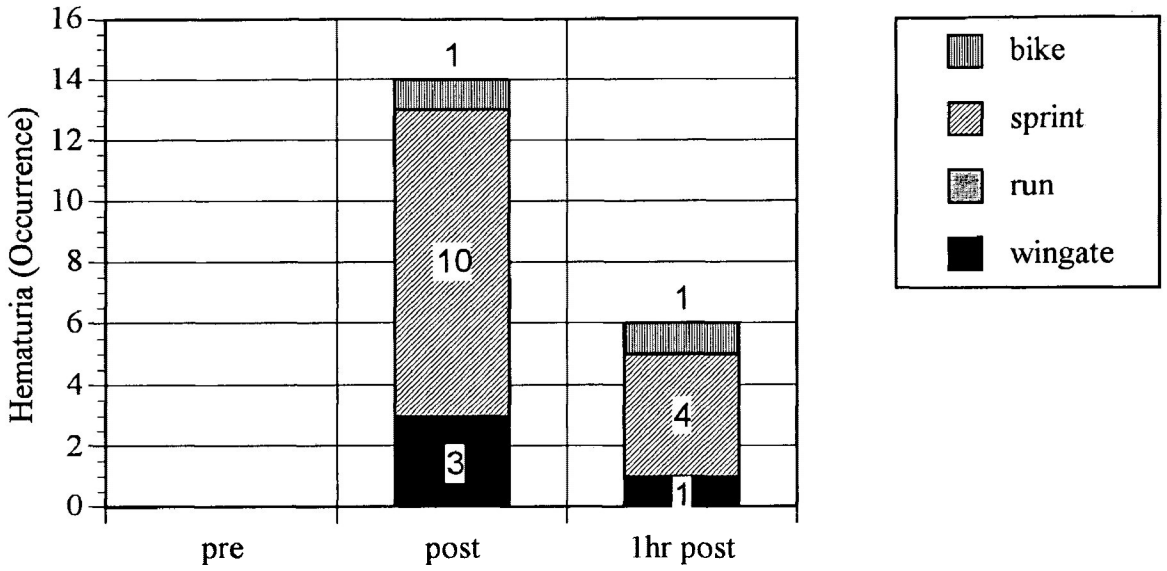

Figure 9. The occurrence of hematuria determined using reagent strip method followed by microscopic analysis for positive tests (n = 10).

Within subject treatment by time interaction effects were significant for the variable hematuria F (6,54) = 3.5, $p < .05$. The post hoc paired t-test exposed significant post sample differences between the sprint and run t (9) = 3.40, $p < .01$, sprint and bike t (9) 3.48, $p < .01$, but not the Wingate and sprint t (9) 3.11, $p = .012$ protocols. In addition, hematuria for the sprint protocol significantly increased from pre to post t (9) -3.40, $p < .01$. Table 6 presents the statistical means and standard deviations for the variable hematuria. Urinary casts, both granular and hyaline were observed for the sprint and Wingate protocols. Immediately following the sprint protocol, 7 urine samples presented granular casts and 2 hyaline, while 1hr post samples decreased to 3 and 1 respectively. Urinary samples following the Wingate protocol presented 2 cases of granular and 1 case of hyaline casts which decreased to 1 case of granular within 1hr of recovery.

Significant correlations were observed between the variable lactate and Hb ($r = .379$, $p < .001$), proteinuria ($r = .3947$, $p < .001$), hematocrit ($r =$

.5351, $p < .001$), and hematuria ($r = .2714$, $p < .01$), but not urinary pH ($r = .2217$, $p \geq .05$). However, a significant linear relationship was also observed between proteinuria and hematuria ($r = .5134$, $p < .001$) and urinary pH ($r = .2335$, $p < .01$).

CHAPTER 5

DISCUSSION

Serum Ferritin

Among the 10 subjects tested in this study, all serum ferritin levels were above the criterion of < 20 μg/l demarking a potential iron deficient state (Harold, 1991). The average serum ferritin displayed by the subjects in this study was 110 and had a range of 28 to 259 μg/l. These data are similar to that observed in the current literature (Dickson, Wilkinson, & Noakes, 1982; Magnusson et al. 1984). Dickson et al. (1982) reported 21 ultramarathoners, 12 swimmers, and 52 controls to have resting serum ferritin levels of 118.6, 169.2, and 88.7 μg/l respectively. Magnusson, et al. (1984) also found elite runners (n = 43) to have an average serum ferritin level of 64.3 (range = 15 - 215) μg/l and controls (n = 100) to have 81.5 (range = 23 - 260) μg/l. The subjects in this study can therefore be considered to have normal iron status. Consequently, no evidence is found to suggest an iron deficiency predisposition related to hematuria.

Hemoglobin

The Hb levels at rest among the 10 male subjects averaged 151.7 g/l. The normal range is defined as 140 to 180 g/l (Harold, 1991). All of the subjects in this study fell within the normal range suggesting normal

physiological status. Some researchers have noted trained athletes are at the lower end of the range subjecting them to a possible physiological handicap (Clement & Asmundson, 1982; Dufaux et al. 1981). Brotherhood et al. (1975) found below average Hb concentrations in middle and long distance runners, but higher plasma volumes and total Hb levels were observed. The typical hemodilution effect caused by an increased plasma volume may cause the hemoconcentration to appear depressed in athletes. Average post exercise Hb values significantly increased to 160.5 g/l and returned to pre exercise levels within one hour following exercise. These data are consistent with observations made by Falsetti et al. (1983) and Poortmans & Henrist, (1989). The observed hemoconcentration may have been caused by a plasma volume shift following the anaerobic protocols, and/or water loss due to sweating following the aerobic protocols. Significant differences in Hb concentration between protocols were not observed.

Haptoglobin

Researchers have recognized haptoglobin as the hematological marker which provides evidence of hemolysis in endurance athletes (Dufaux et al. 1980; Eichner, 1985; 1990; Falsetti et al. 1983; Harold et al. 1991; Miller et al. 1988). Pre exercise Hp levels, among the 10 subjects tested, averaged .60 g/l. Normally, Hp concentrations, measured in terms of the protein's Hb binding capacity, are .38 to 2.7 g/l (Harold, 1991). A significant within subject interaction effect was observed for the variable Hp ($p < .01$). Figure 4 presents the average percent change in Hp and effectively shows the interaction effect. The bike and Wingate protocols show a similar but non-

significant decrease from pre to post of -9.5% and -6.9% respectively. The response of Hp for the Wingate protocol then returned to normal within one hour following exercise, whereas it remained the same for the bike protocol. Similarly, although more pronounced, Hp significantly decreased from pre to post (-16%) and from post to 1 hour post (-25%) for the run protocol. Levels of Hp for the sprint protocol showed an unexplained mirror image with the Wingate protocol.

A decrease in haptoglobin supports the assumption that footstrike hemolysis is the cause of hematuria (Eichner, 1985; 1986; Egan et al. 1987; Miller et al. 1988; Dressendorfer et al. 1991). The results of our study demonstrate the characteristic drop in Hp following exercise. Therefore, it can be concluded that intravascular hemolysis occurred as a result of the protocols. The data presented in figure 4 shows a significant decrease from pre to post and from pre to 1 hour post for the 60 minute run protocol ($p \leq .01$). Nevertheless, the suggestion that intravascular hemolysis and hematuria have a cause and effect relationship is not supported by our data. On the contrary, because hematuria occurred more frequently following the anaerobic protocols, alteration of kidney function is indicated. The data presented in table 6 supports this contention with a 100% occurrence of hematuria following the sprint protocol. Conversely, following the run, where footstrike hemolysis is suspected, hematuria was not observed. Therefore, 60 minutes of running at the prescribed intensity may not have been sufficient to produce hematuria. Still, a more severe bout of exercise may be required to exhaust the capacity of haptoglobin and thereby cause hematuria. Thus,

based on treadmill running for 60 minutes at 90% of AT, footstrike hemolysis is not identified as the mechanism of hematuria.

Plasma Lactate

Normal pLa values at rest range from 0.93 to 1.65 mmol/l (Harold, 1991). Subjects in this study fell well within the normal expected range with average pre-exercise lactates of 1.12 mmol/l. An expected interaction effect for pLa can be seen in figure 5, where responses between the aerobic and anaerobic protocols show an expected difference. Post exercise lactates for the Wingate and sprint exercise protocols increased to 14.7 and 15.2 mmol/l respectively. These values confirm high intensity anaerobic output for the anaerobic protocols as well as demonstrating similar average intensities for the sprint and Wingate protocols. Post exercise values for the run and bike protocols were 1.8 and 2.2 mmol/l, with no significant differences seen between cycling and running ($p \leq .01$). Thus, the respiratory exchange ratio (RER) and the corresponding workload taken from the metabolic cart seems to provide a valid method for prescribing similar sub-AT intensities. Plasma lactate values for the bike and run protocols returned to normal within one hour following exercise, while lactate values for the Wingate (2.2 mmol/l) and Sprint (2.1 mmol/l) had not fully recovered one hour following exercise, however.

Serum Creatine Kinase

Normal reference values for CPK are 5 to 70 U/l (Harold, 1991). A significant interaction effect for CPK can be viewed in figure 6. Muscular tissue damage resulting in a significant increase in post exercise CPK for the

sprint protocol was observed, whereas CPK for the bike, run and Wingate protocols did not significantly increase.

The presence of CPK is a good indicator of muscular tissue damage and therefore should be a reliable indicator of myoglobin in the blood (Lijnen et al. 1988; Harold et al. 1991). Milne, (1988) suggested that kidney function would be effected by the release of myoglobin during muscular tissue damage. Although a significant increase in CPK occurred from pre to post and pre to 1hr post for the sprint protocol, non-significant correlations were reported for proteinuria ($r = .1138$, $p > .01$), and hematuria ($r = .038$, $p > .01$). Perhaps the direct measurement of myoglobin would clarify the issue. Therefore, if the presence of myoglobin was effecting renal function, the current data does not support this relationship. Maxwell & Bloor, (1981) studied myoglobin and CPK levels in runners following a 14 mile run at 8 miles per hour. Myoglobin levels returned to normal within 3 days of rest whereas CPK remained elevated for the same period. Possibly, because of differences in rest days between protocols, recovery of CPK may not have been adequate. This may explain the higher than normal values observed in our study. Additionally, if CPK remained elevated for several days it may not have provided a valid indication of cellular damage or myoglobin leakeage. Thus, it cannot be concluded that myoglobin is the casual mechanism for hematuria in any protocols employed in our study. Still, myoglobin in concert with other exercise conditions may aggravate renal function and help with the onset of hematuria.

Urinary pH

Harold et al. (1991) reported that normal findings for urinary pH range from 4.5 to 8.0. Urinary pH findings of our subjects fall within the normal expected range and indicate no caution (table 6). The exercise protocols significantly differed in their effect on urinary pH ($p < .01$). No significant difference was detected for the three sampling times, or between the aerobic protocols ($p \geq .05$).

Typically not reported in the literature, urinary pH has received little attention, but as a cost effective measurement urinary pH warrants further investigation. Interestingly, a significant correlation was observed between proteinuria and urinary pH ($r = .2335, p < .01$). This relationship most likely reflects the intensity of exercise as the kidney buffers lactate from the blood (McArdel Katch & Katch, 1986). However, it is unclear why pLa and urinary pH were not significantly correlated ($r = .2217, p > .01$). Possibly, an increased buffering capacity of the kidney during high intensity exercise allows for the loss of plasma proteins. If the kidney does increase it's buffering capacity during high intensity exercise, it could in part be responsible for the enhanced active recovery process; and the loss of plasma proteins and red blood cells may only be a physiological compromise. Figure 7 demonstrates the relationship between the percent change in urinary pH and the occurrence of proteinuria and hematuria.

Proteinuria

Normally up to 150 mg of protein are excreted in a 24 hour period (Harold et al. 1991). Proteinuria, which may indicate disease, may result from glomerular leakage, overflow of low weight molecular filtered proteins, and/or impaired tubular reabsorption (Harold et al. 1991). Gardner, (1956) first described post-exercise proteinuria with the phrase *athletic pseudonephritis* to differentiate it from the nephrotic syndrome. Post-exercise proteinuria is relatively common and has been reported following such sports as rowing, running and cycling (Poortmans, 1984; Poortmans & Henrist, 1989; Poortmans et al. 1990). Alyea, Parish, & Durham, (1958) observed proteinuria in 70% to 80% of the athletes they studied. The results of our screening showed similar occurrences with 68%, 76%, and 69% of the subjects displaying proteinuria following the 5 km, 10 km, and 21 km races respectively.

However, post-exercise proteinuria is most often associated with short term exhaustive effort (Poortmans, 1984). Helzer et al. (1988) reported exercise intensity to be a significant factor in producing proteinuria and hematuria. The data in our study concurs with these findings with a significant correlation observed between lactate and proteinuria ($p < .001$). Poortmans et al. (1981) also found post-exercise proteinuria and lactate to be significantly correlated ($r = 0.87$). Proteinuria in our study showed an increase from pre to post exercise and ranged in occurrence from 90% following the sprint, to 50% following the Wingate and run, and 10% following the bike. Helzer et al. (1988) studied 13 male runners while exercising for 60 minutes at 9 and 6.6 miles per hour and also found a

significant increase in proteinuria for both intensities. The subjects in our study averaged 8.2 miles per hour during the run protocol and also showed a significant increase in proteinuria. Although it is difficult to compare relative intensities, the subjects in both studies had reported 10 km running times (minutes) of 35:16 for Helzer's group and 39:18 for our group. The reported race time for Helzer's group, which can be considered to closely relate to AT, reveals that 9.0 mph and 6.6 mph equates to approximately 86% and 63% of AT. Similarly, the subjects in our study exercised at 90% of AT.

Poortmans & Henrist (1989) used progressive treadmill and cycle ergometer tests to study the effect of intensity on proteinuria. The subjects in that study, who's average post exercise pLas were 10.98 and 11.35 for the cycle and treadmill tests, showed a significant increase in proteinuria following both tests. Poortmans et al. (1990) reported similar findings following progressive maximal tests, where elevated venous lactates while cycling (12.25 mmol/l) and rowing (11.49 mmol/l) were associated with a significant increase in proteinuria. Their data agrees with our findings, where pLas increased to 15.2 for the sprint and 14.7 mmol/l for the Wingate protocols. However, pLa of 1.8 for the run and 2.2 mmol/l for the bike protocols also significantly increased proteinuria. Still, proteinuria significantly differed on the post sample for the sprint and bike protocols ($p \leq .01$). Our data supports the assumption that a relationship exists between exercise intensity and proteinuria, but that the threshold for the onset of proteinuria is below 90% of AT as prescribed in our study.

Post exercise proteinuria does not appear to be a normal physiological response to exercise. It's appearance seems to be related to exercise

intensity, with significant correlations observed between proteinuria and pLa (table 7). The mechanisms of proteinuria appear to be increased glomerular permeability and impaired tubular reabsorption resulting from decreased renal flow and limitations of the renal system (Poortmans, 1984). Clinically, post exercise proteinuria is expected to subside within 24 to 48 hours (Mariani et al. 1989). Our data is in agreement with these findings. All pre exercise urine samples tested within a 48 hour period were confirmed as being negative for proteinuria.

Hematuria

Exercise hematuria has been reported following a variety of activities, with incidences ranging from 5% of Israeli air force recruits to 70% in ultramarathoners (Froom et al. 1986; Eichner, 1990). The current assumption is that hematuria is related to both the aerobic demand and duration of the event (Eichner, 1990; Cianflocco, 1992).

However, the findings of our study implicate exercise intensity and not duration as the causal mechanism of hematuria. The results of the exercise protocols show a significant interaction effect for the variable hematuria ($p <$ .01). Follow up post-hoc analysis using a paired t-test revealed that the sprint protocol was significantly different than both the bike and run protocols on the post exercise sample ($p < .01$), but was not significantly different than the Wingate protocol ($p = .012$). Furthermore, data presented following the screening showed a similar trend towards exercise intensity with 25% of the runners in the 5 km race displaying hematuria, 10% in the 10 km race and none following the half marathon (table 2). Under the conditions of the four

exercise protocols, 10% of the subjects displayed hematuria following the one hour bike, 30% following the Wingate sprints, and 100% following the 400 meter sprints (figure 8). Further evidence which suggests glomular bleeding as the source of blood is seen by the number of urinary red blood cell casts observed following the sprint and Wingate protocols. Hyaline and granular casts, which originate in the lumen of the distal convoluted tubule and collecting duct provide a microscopic view of conditions within the nephron (Strasinger, 1989). Strenuous exercise, dehydration, heat exposure, and emotional stress have all been associated with increased urinary casts (Strasinger, 1989). The post urinary samples for the sprint protocol produced 7 granular casts and 2 hyaline, while 1hr post samples decreased to 3 and 1 respectively. Urinary samples following the Wingate protocol presented 2 cases of granular casts and 1 case of hyaline casts which decreased to 1 case of granular casts within 1hr of recovery.

Helzer et al. (1988) exercised male subjects on a treadmill at 6.6 (63% of AT) and 9.0 (86% of AT) mph for 60 minutes under hydrated and dehydrated conditions. They found intensity of exercise and hydration to be significant factors in the production of hematuria. Conditions which produced hematuria were: low intensity dehydration, high intensity hydration and high intensity dehydration. The subjects in our study, who were hydrated, did not significantly produce hematuria while exercising at 90% of AT. If exercise intensity is related to hematuria, it is unclear why our subjects, who exercised at a higher intensity, did not significantly produce hematuria following the aerobic protocols. Differences between studies include the fact that our subjects ran on a treadmill which highly controls the running pace, whereas

Helzer's group ran on a track where intensity may vary. Therefore, the subjects in Helzer's study may have periodically varied their running intensity thereby causing the observed hematuria. This may also help to explain why the low intensity dehydrated group displayed hematuria while exercising at a lower percent of the reported AT.

Increased pLa levels have been associated with significant increases in proteinuria but not hematuria (Poortmans et al. 1981; Poortmans & Henrist 1989; Poortmans, Jourdain, Heyters, & Reardon, 1990). Significant correlations between hematuria ($p < 0.01$) and lactate, and proteinuria ($p < 0.001$) and lactate provide support for this association. Although relationship does not establish cause, the incidence of hematuria on post samples of 100% for the sprint ($p < .01$), 30% for the Wingate ($p = .087$), 10% for the bike ($p = .343$), and 0% for the run cannot be ignored. Clearly, something associated with higher exercise intensity and or muscular involvement is increasing the occurrence of hematuria.

Plasma LAs of 10.98 and 11.35 mmol/l following maximal cycling and treadmill running (Poortmans & Henrist, 1989), and 12.25 and 11.49 mmol/l following maximal cycling and rowing (Poortmans et al. 1990) did not produce hematuria. Conversely, in our study hematuria did occur with pLas of 14.7 and 15.2 mmol/l for the Wingate and sprint protocols. Differences seen between our study and previous findings may be related to the degree of acidic insult and or the duration of exposure. The subjects in our study performed three repeated bouts of exercise each separated by four minutes of rest. This was done to allow for a maximal accumulation of pLa. Additionally, the subjects in our study were exposed to maximal lactate

values for approximately 11 minutes (exercise and rest periods). Whereas, during a progressive maximal test, lactate values would not reach peak values until the final minutes. This may explain differences in hematuria observed between our anaerobic protocols and reports in the literature.

If increased exercise intensity results in hematuria and proteinuria, as the results of the current study suggest, then alteration of renal function is the mechanism. A vast number of studies support this conclusion (Poortmans, 1981; 1984; Poortmans, & Henrist, 1989; Poortmans et al. 1990). Poortmans, (1977) suggests post-exercise proteinuria occurs as a result of increased glomerular permeability, and that if exercise is continued, the process will allow the loss of red blood cells into the urine. In addition, a reduction in plasma flow to the kidney occurs proportionally to the intensity of the exercise, with values ranging from 30% during moderate exercise (50% of VO2max) to 75% during heavy exercise (65% of VO2max) (Poortmans, 1984). This drop in pressure causes an increased glomerular filtration rate (GFR) and filtration fraction (FF), both of which increase the passage of red blood cells and proteins into the urine (Castenfors, 1977; Poortmans, 1984; Poortmans & Henrist, 1989; and Poortmans et al. 1990).

The reduction in renal blood flow during exercise is caused by vasoconstriction which will result in an ischemic insult as well as altered kidney function (Abarbanel et al. 1990; Helzer et al. 1988; Javitt & Miller, 1952). The mechanism controlling vasoconstriction in the renal artery is circulating norepinephrine (Guyton, 1991). An increased circulation of norepinephrine will lead to a more severe vasoconstriction of afferent and efferent renal arterials (Cianflocco, 1992; Lindermann et al. 1978; Poortmans,

1984; Yoshimura, 1970). Although norepinephrine was not measured, the assumption can be made that the anaerobic activities produced a greater norepinephrine response because of an increased muscular involvement. A study by Schneider, McGuiggin, & Kamimori, (1992) investigated the relationship between blood lactate threshold and plasma epinephrine and norepinephrine threshold. Findings demonstrated that plasma epinephrine and norepinephrine threshold occurred after blood lactate threshold. Thus, if increased epinephrine and norepinephrine are the causal mechanisms for hematuria, 90% of AT prescribed in this study for the bike and run protocols would not be sufficient to bring about the epinephrine or norepinephrine threshold. This may explain the increased occurrence of hematuria following the anaerobic and not the aerobic protocols as well as the differences seen between cycling and running.

CHAPTER 6

SUMMARY

Under the conditions of our experiment, traumatic and non-traumatic intravascular hemolysis could not be identified as a mechanism of either hemoglobinuria or hematuria. Failure of the 60 minute run protocol to produce hematuria in light of significant decreases in Hp suggest that another mechanism is functioning. It was concluded that 60 minutes of treadmill running at 90% of AT was not sufficient to exhaust Hp's binding capacity, but that a more severe bout of exercise may be needed to result in exercise hematuria.

Kidney or bladder mechanical trauma resulting in red blood cells in the urine cannot be supported based on the results of this study. The evidence of hematuria, or lack thereof for the 60 minute run protocol suggests that: either, the repeated impact of the posterior bladder wall causing vascular lesions did not occur, or that the bout of exercise was not severe enough to cause hematuria via kidney or bladder trauma. In any case, under the conditions of our experiment mechanical trauma to the kidney or bladder are not indicated.

Muscular tissue damage, or free radical formation resulting in the release of myoglobin does not appear to be related to the onset of hematuria seen in our study. Although not a direct measurement, CPK was found to have a non-significant relationship with hematuria. In future research direct

measurement of plasma myoglobin and free radicals may provide a better understanding of their role in exercise hematuria.

In summary, both hematuria and proteinuria appear to be intensity related. In this author's opinion, alteration of renal function is the mechanism responsible for the hematuria and proteinuria observed in our study. Specifically, the mechanisms appear to be related to a decrease in renal plasma flow caused by vasoconstriction, which results in an increased FF and GFR. Although lactate and urinary pH were significantly correlated, it is difficult to tell to what degree, if any, lactate played a role in the onset of proteinuria or hematuria. Still, the intensity relationship observed in our study is consistent with findings presented in the literature. It is hypothesized that the relationship between intensity and increased renal flow of proteins and red blood cells may be associated to an increased buffering mechanism of the kidney. Evidence for this relationship may be demonstrated by the significant correlation between both hematuria and proteinuria, and urinary pH.

Table 8. Summary of findings.

Mechanisms?	Supportive data	Non-supportive data
Intravascular hemolysis.		No hematuria following the run protocol.
	(↑) hemolysis following the run - but no hematuria/proteinuria.	
		Intact dysmorphic RBC found in urine. Hemaglobinuria not indicated.
Altered Renal Function.	(↑) hematuria & proteinuria following anaerobic protocols. Significant correlation between lactate & hematuria/proteinuria. Hylaine & granular casts found in urine.	
Mechanical trauma.		(↓) incidence of hematuria & proteinuria following prolonged upright running.
Muscular tissue damage.		(↑) in CPK not linked to hematuria.
Free radical damage.		(↑) in CPK not linked to hematuria. Hematuria not observed following aerobic protocols.

Recommendations for Future Study

1. Dependent variables for future study should include plasma free hemoglobin, epinephrine and norepinephrine.
2. Larger sample size and randomized selection where possible would help in the statistical analysis.

3. An attempt should be made to identify an intensity threshold for the production of hematuria and proteinuria by examining subjects at varying percents of anaerobic threshold.
4. Examine the effects of renal function (hematuria and proteinuria) following the infusion of norepinephrine and epinephrine. Levels could be determined by quantifying levels following anaerobic exercise.

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APPENDIX A: Screening Recruitment Letter

Dear , Runner.

We are interested in identifying male athletes who display hematuria (blood in the urine). Present research suggests that following intense exercise hematuria occurs in about 13%-38% of the population. Although its occurrence in runners may be a clue to an underlying disease; most often it is believed to be caused by repeated foot-strike trauma and is not a serious problem. The red blood cell (RBC) that is being damaged is also responsible for carrying oxygen to and carbon-dioxide away from the working muscles. Consequently, a reduction, may reduce running performance. The severity of hematuria can range from microscopic to macroscopic the former case being the most common. A runner may have microscopic hematuria but not know it because it is undetectable to the human eye. Hematuria may also contribute to the onset of "runners-anemia" (low iron); which, if left undetected, may lead to iron deficiency.

The intent of this research project is to: (a) identify runners who display hematuria, (b) investigate the relationship between running distance (5km, 10km, and 1/2 marathon) and incidence of hematuria, and (c) to screen potential subjects for future research. If you consent to participate, you will be asked to fill out a questionnaire concerning personal training habits, to not participate in vigorous exercise two days prior to running the race, and to provide 2 urine samples, one immediately before the race, and one immediately after the race. On September 26th during the pre-registration and on race day a hematuria registration booth will be set up and any questions you may have will be answered. If you are interested in taking part in the study please fill out the case record form on the reverse side and return it with your registration. Participation kits will be handed out during registration, i.e. consent form and equipment (urine cups, labels, etc.). If you have any immediate questions you can contact myself at (H) 622-2888 or Dr. Newhouse at (W) 343-8074 / (H) 344-0786.

All information you provide will remain confidential. However, upon completion of the project the findings will be available to you at your request.

Yours in fitness and health,

Mark McInnis
Ian Newhouse Ph.D.

APPENDIX B: Case Record Form

Date:_____.
Name:_____Age:_____Ht:____(cm) Wt:_____(kg)
Address:_____ Phone:_____(home)
_____ _____(work)
_____.
Date of Birth:_____.

Training Background
Primary sport(s)
Winter_____Summer_____.
Years of experience in sport(s)_____.
Hrs/week presently training_____.
Level of performance: (circle one)
purely recreational, local competitions, provincial, national, international.
Best times in:
5km,_____Yr. 10km,_____Yr. 1/2 marathon_____Yr._____.

Medical History
- please note that all information provided will be confidential
OHIP number_____.
Family Doctor_____phone_____.
Allergies_____.
Medications_____.

please check any medical condition you have experienced

_____athsma	_____high blood pressure	_____epilepsy
_____anemia	_____diabetes	_____heart trouble
_____kidney/bladder disorder	_____syncope (fainting)	_____hematuria

use this space to elaborate on any of the above conditions:
_____.

are there any other conditions that you have that may be of relevance?

_____.

APPENDIX C: Screening & Laboratory Consent Form

The purpose of this study is to:

A) examine the mechanisms of hematuria in athletic populations following a 5km, 10km, and a 21km road race.

As a subject you will be asked to do or undergo the following:

B) fill out a questionnaire concerning training, and medical history.

C) Urinalysis: urine specimens of at least 15 ml each will be collected before and or immediately following the race. Containers will be provided, when filled, you will return the labeled containers to the laboratory technician for analysis.

Publication of the results will not reveal subject identity as subjects will be reference by number. Subjects will be notified of positive urinalysis and will be responsible for follow up with their personal physician.

I have read and understand the above explanations of the purpose and procedures for this study and agree to participate. I also understand that I am free to withdraw my consent at any time.

Signature_____ Witness_____

Date_____

The purpose of this study is to:

A) establish the prevalence of hematuria after various exercise protocols

B) examine the mechanisms of hematuria in athletic populations

As a subject you will be asked to do or undergo the following:

A) fill out a questionnaire concerning training, and medical history

B) anthropometric tests: height and weight

C) six exercise tests.

For each visit to the laboratory you will be asked to refrain from hard exercise in the preceding 48 hours and to consume a light, mainly carbohydrate, meal prior to the exercise test. Each test will be preceded by a standardized warm up involving 3 minutes of easy jogging, or cycling, and 5 minutes of stretching.

The exercise tests will be:

1) A $\dot{V}O_2max$ test on the treadmill. During this progressive workload test to exhaustion your expired air will be continuously collected for gas analysis. The test will start at an easy pace and will progressively become harder. The treadmill speed will be increased 1/2 mph every minute until you are no longer able to maintain the pace. At this time you will grab the hand rails and straddle the belt. Your anaerobic threshold will be estimated based on the results and will be used to calculate exercise pace for the remaining running tests.

2) A $\dot{V}O_2max$ test on the bicycle ergometer. During this progressive workload test to exhaustion your expired air will be continuously collected for gas analysis. The test will start at an easy pace and will progressively become harder. The bicycle ergometer tension will be increased 2 kp every 2 minutes until you are no longer

able to maintain a cadence of 60 rpm. Your anaerobic threshold will be estimated based on the results and will be used to calculate exercise pace for the remaining cycling tests.

3) A running sprint - 3 by 400 meters sprint on an indoor track to the best of your ability. Each 400 meters will have a 4 minute rest interval.

4) A cycling sprint - 3 by 60 second Wingate. Belt tension will be calculated as 0.07 times body weight (kg). Each cycling sprint will have a 4 minute rest interval.

5) A 60 minute treadmill run at a pace just below your anaerobic threshold.

6) A 60 minute bicycle at a pace just below your anaerobic threshold.

D) Electrocardiogram: during the $\dot{V}O_2max$ test and the two 60 minute exercise bouts there will be 5 electrodes taped to you chest so that your heart rate can be monitored.

E) Blood samples will be collected via venipuncture technique from an antecubital vein 5 minutes pre-exercise, and 5 minutes and 1 hour post exercise for tests 3-6.

F) Urinalysis: urine specimens will be collected before, immediately and 1 hour after the four exercise tests. Containers will be provided, when filled, you will return the labeled containers to the laboratory technician for analysis.

You will be asked to maintain a training diary for the duration of the study. Training and diet should be kept consistent with pre-study habits.

Blood sampling will be conducted by professionally trained technicians. The amount of blood taken will be small and there should be little discomfort during the procedure. Slight bruising may result but is considered normal. Except for temporary exhaustion during the tests, very little risk is involved if you are a healthy normal individual.

Publication of the results will not reveal subject identity as subjects will be referenced by number.

I have read and understand the above explanations of the purpose and procedures for this study and agree to participate. I also understand that I am free to withdraw my consent at any time.

Signature_____Witness_____

Date_____