SPLENIC CONTRACTION, CATECHOLAMINE RELEASE, AND BLOOD VOLUME
REDISTRIBUTION DURING EXERCISE IN MAN

By

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ABSTRACT

Numerous mammals have the ability to autotransfuse oxygenated red cells from the spleen into circulation during times of stress. This enhancement of the oxygen transport system has benefited mammals capable of heavy work (such as the thoroughbred horse, fox and greyhound) in an improved endurance performance. During times of inactivity the spleen in these mammals can sequester up to 50% of the total red cell volume. This reduces the viscosity of the blood and work of the heart. In humans the spleen is capable of storing considerably less red cells and is primarily a lymphoid organ. Although significant volume reductions have been observed with physical stress, the mechanism responsible remains unidentified and represents a primary objective of this dissertation. To determine the mechanism responsible for splenic volume reduction nine male subjects participated in a two part study. Initially they undertook three cycling bouts of 5, 10, and 15 minute duration at 60% VO_2max . Spleen size and volume was assessed by scintigraphy before and immediately following each cycling bout. Although there was a significant decrease in spleen size and volume with exercise, no significant differences were observed between the three durations. In the second part of the study the same subjects undertook an incremental ride to exhaustion. The spleen size and volume were measured prior to exercise and during one hour of passive recovery. Blood samples were collected in conjunction with spleen imaging and analyzed for plasma catecholamine concentrations. The spleen was restored to its pre-exercise value 20 minutes following the completion of exercise. The volume of the spleen during this time was inversely related to plasma catecholamine levels. It is apparent that the spleen will contract in

response to an intensity dependent signal and that sympathetic stimulation, as reflected in changes in plasma catecholamine concentrations, is involved in this effect. In a simultaneous study, the effect of splenic contraction during exercise on the circulating red cell volume and indirect calculations of plasma volume change (Hb/Hct) was determined. Radio nuclide measurements of red cell and plasma volume were performed, and a blood count taken, prior to and immediately following exercise. The release of 50 mls of red cells from the spleen during exercise accounted for a 2.2% increase in the total circulating red cell volume. The indirect calculations of plasma volume were unaffected by the minor increase in peripheral hematocrit and were equivalent to radio labelled measurements. In conjunction the results of this dissertation imply that the release of red cells during exercise is not a primary function of the human spleen.

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CHAPTER ONE

GENERAL INTRODUCTION

Any organism, with a closed circulatory system, faces the fundamental problem of oxygen delivery to the organs. A large volume of research has been propagated in the animal capable of heavy work, where any enhancement of this oxygen carriage system may be beneficial to performance. The thoroughbred horse, fox, and greyhound all demonstrate a remarkable ability to autotransfuse during exercise a substantial volume of blood from the spleen into the active circulation (Tipton, 1986). This organ also aids in the diving response of aquatic mammals (Hochachka, 1986). In humans the spleen is predominantly a lymphoid organ and traditionally its role as a reservoir to be used during exercise has been dismissed (Johnson et al, 1996; West, 1995). This dissertation examines the effect the human spleen plays during acute exercise.

1.1 ANATOMY

The spleen is located under the eighth through eleventh ribs on the left side of the body, lying between the fundus of the stomach and the diaphragm. Measuring, on average, 5-6 inches in length, 3-4 inches wide, and 1.5 inches thick, the spleen represents the largest lymphoid organ in the human body (Gray, 1977). The spleen is composed of a supporting connective tissue framework comprising its capsule and trabeculae and a unique functional parenchyma made up of red and white pulp. Except at its hilum where the splenic artery, vein, lymphatics, and nerves, enter or leave, a dense connective tissue capsule surrounds the spleen. This capsule is less than 1 mm thick. From the inner aspect

of the capsule, trabeculae of dense connective tissue extend deeply into the substance of the spleen. The splenic trabeculae can take the form of cylinders or plates, which anastomose extensively with each other throughout the substance of the spleen. The parenchyma (pulp) occupies all the spaces between elements of the capsular-trabecular meshwork. The gross colour of the white pulp is derived from its closely packed lymphocytes, and the colour of the red pulp is produced by its high content of erythrocytes. The red pulp contains terminal branches of the central arteries, splenic sinuses, splenic cords separating the sinuses, and pulp veins, all supported by a meshwork of reticular cells and fibres. It is still the matter of some debate whether the arterial capillaries open directly into the pulp reticulum and the blood gradually filters into the venous sinuses (open circulation theory) or if the arterial capillaries empty directly into the venous sinuses (closed circulation theory); a combination of both mechanisms has been suggested (Pinkus et al, 1986).

The spleen serves numerous functions: formation of antibodies; production of lymphocytes and monocytes; filtration, phagocytosis and destruction of red blood cells; as well as storage of iron and viable blood cells. Generally, the storage function is not well developed in the human spleen since on average it is believed to contain only 200-250 ml of blood (Rushmer, 1972). Historically it was thought that the human spleen was incapable of contracting as it had fewer trabecular fibres than the animal models studied (Weiss, 1988). However, recent immunohistochemical staining has identified contractile proteins not only within the walls of the arteries, veins, capsule, trabecular, but also within the reticular cells of the white pulp and sinus lining cells of the red pulp of the

spleen (Pinkus et al, 1986). This anatomical evidence indicates that the human spleen could be capable of contracting and regulating its volume from within.

1.2 SPLENIC VOLUME CHANGES

Clinical

The first reported case of splenic emptying occurred in 1852, when Henle (as cited in Ayers et al, 1972) electrically stimulated the splenic nerve of a decapitated human, both *in situ* and after removal. He observed that the spleen which was originally pale and wrinkled became darker and smoother after removal from the body. This, he suggested, showed that the spleen had contracted. More recently, observations of splenic volume reduction have been recorded in patients suffering from follicular non-Hodgkin lymphoma following administration of corticosteroids (Silagy et al, 1998), drowning victims (Haffner et al, 1994), and in normal subjects after acute insulin-induced hypoglycaemia (Fisher et al, 1990), injection of cocaine (Kaufman et al, 1998) and epinephrine (Knecht et al, 1988). In a comprehensive series of *in vitro* studies Ayers et al. (1972) found that stimulation of the postganglionic sympathetic nerves to the human spleen evoked reductions in spleen volume. The injection of epinephrine and norepinephrine produced equipotent reductions in spleen volume. They concluded that, due to the small magnitude of change observed, these results provided evidence that the normal human spleen does not have a red cell reservoir function. All of the research detailing splenic volume reduction has been associated with an elevation of sympathetic nervous system activity.

Exercise

Exercise, with its related elevations in sympathetic activity, has provided another vehicle for studying splenic volume reductions. Sandler et al. (1984), using technetium-99m (^{99m}Tc) labelled red blood cells, were the first to demonstrate splenic shrinkage after exercise. They found that splenic radioactivity decreased by 49% following 5-6 minutes of maximal, symptom-limited, supine cycling. They suggested that the significant increase in catecholamine concentration found during exercise might induce both splenic and splanchnic vasoconstriction. Their conclusion was that, regardless of the mechanism, it was apparent that the spleen had a reservoir function during supine exercise.

Other studies have also had success in demonstrating a decrease in splenic radioactivity with different types of exercise. Hurford and associates (1990) studied Korean ama to determine if humans exhibited a similar response to that found in diving mammals. They found that during a three hour shift of repetitive diving, splenic volume, measured by ultrasound, decreased 19.5% and hematocrit rose 9.5%. Simulated diving, with face immersion, was associated with a 6.5% and 3.3% increase in hematocrit and hemoglobin, respectively (Schagatay et al, 2001). This response was abolished in the splenectomized control group, who also displayed a shortened breath-hold time. Spleen volume was not measured in this investigation.

Froelich et al. (1988) also used ^{99m}Tc -labelled red blood cell's to determine changes in blood volume of the liver, spleen, kidneys and lungs with graded continuous exercise on a cycle ergometer. The counts for each of the organs were expressed as a percent of activity at zero workload. They found a 39% decrease in splanchnic but no significant decreases in liver or kidney blood volumes. Flamm et al. (1990) utilizing a

similar protocol reported a 46% decrease in splenic activity as well as reductions in kidney and liver counts. The 4.3% increase in hematocrit was significantly correlated to the reduction in spleen activity ($r = 0.80$), leading the authors to suggest "... a role for the spleen in augmenting cardiovascular performance..."

Similarly, Laub et al. (1993) studied five subjects exercising on a bike for three 10 minute periods of different power outputs (75, 150, and 200 watts) to determine the percent reduction in splenic activity. They found that the average hematocrit increased from 44.6 to 48.0% at the highest exercise load. Epinephrine and norepinephrine concentrations also rose gradually with increased levels of activity. During exercise the splenic erythrocyte content linearly decreased to 34.2% of resting value. They concluded that, with increasing exercise levels, splenic contraction and subsequent erythrocyte release was related to hematocrit increase, but "... was not clearly connected to increased adrenergic activity."

Allsop et al. (1992) tracked intra-splenic kinetics of erythrocytes, platelets and granulocytes. Based upon the assumption that suggested that if the spleen was expelling blood cells by active contraction, one would expect expulsion of whole blood, and therefore, to record splenic radioactivity-time curves for the three cell types that had essentially identical time courses. This was not the case as erythrocytes exited the spleen within 60 seconds, while platelets and granulocytes required approximately 10 minutes to traverse the spleen. This would indicate that blood cell expulsion from the spleen is not the result of active splenic contraction but probably the passive result of a decrease in splenic blood flow. The modest decrease seen in the splenic size was attributed to a passive collapse secondary to the fall in splenic blood flow.

Differences in splenic activity between studies can be explained by the differing modes and protocols of exercise used, as well as, the use of only a posterior scan to image the spleen (Allsop et al, 1992; Flamm et al, 1990; Froelich et al, 1988; Laub et al, 1993; Sandler et al, 1984). To make valid conclusions these authors assumed that changes in spleen size due to blood expulsion are uniform in the anterior-posterior plane, a point that is contentious and was easily avoided in the work of Wolski (1998) who scanned both anterior and posterior images of the spleen.

Wolski's (1998) exercise protocol consisted of three groups, two differentiated by training status and a splenectomized control group, undertaking 10 minutes of exercise at three levels of increasing intensity (25, 50 and 75% VO_2 max) under normoxic and hypoxic conditions. Radionuclide labelling was used to quantify red cell, plasma and spleen volume, concurrently. Hematocrit was measured and the spleen was imaged pre-exercise and after each workload. The pre-exercise spleen volume of the normal group was higher than that of the trained group, but when the change in spleen volume was shown as a percentage of original spleen volume, the decrease in spleen volume after each exercise load was identical for both groups in normoxic and hypoxic conditions. The volume of red cells released from the spleen was reported to represent 7-9% of the total circulating red cell volume. The increase in circulating red cell volume (which the author attributed to splenic contraction) had a significant effect on peripheral hematocrit, accounting for 22-33% of the increase associated with exercise. Unfortunately, an unexplainable increase in the total, not just circulating, red cell volume was observed following exercise. This indicates that red cells were released from some source other than the spleen during exercise, as tagged red cells would have been expected to

equilibrate within the spleen prior to exercise (Kowalsky and Perry, 1987). The increase in exercising hematocrit attributable to the spleen is therefore difficult to quantify accurately from this data.

The changes in spleen activity and volume associated with exercise are not insignificant. However, the fact remains that the human spleen contains only 200-250 ml of blood (Rushmer, 1972) and <10% of the total red cell volume (Gray, 1977) compared to ~50% in the racehorse (Persson et al, 1973) and the Weddell seal (Hurford et al, 1996), and closer to 20% in sheep (Turner and Hodgetts, 1959) and dogs (Barcroft and Stephens, 1928), underlies the lack of significance placed on the red cell storage function in humans (Table I).

Animal

Barcroft (1925) was the first to suggest that the spleen may have a red cell storage function in mammals. He detected a reduction in spleen weight, in cats, following rapid movements and having been bled to death. In the same decade, Scheunert and Krywanek (1927; 1929, as cited in Hartwig and Hartwig, 1985) reported that forced exercise in horses and dogs was followed by an increase in hematocrit and hemoglobin, which was not observed in splenectomized animals. With the advancement of technology, Baker and Remington (1960) were able to employ radioactive isotopes to label plasma proteins and red cells in order to calculate their respective volumes in dogs. They found that when the plasma volume and the red cell volume techniques were applied to the same animal, only rarely were the two calculated total blood volumes the same. This disparity was taken to indicate the presence of body reservoirs for both red cells and plasma. Furthermore,

splenic contraction produced a rise in arterial hematocrit, but no significant change in the calculated total red cell volume. This indicated that the tagged red cells reached an equilibrium concentration in the spleen fairly rapidly. Their findings supported Barcroft and Stephens (1928) work suggesting that intact dogs have a significant splenic reservoir capacity for red blood cells. Furthermore, Guntheroth et al. (1967) found that splenic volume in dogs decreased in response to epinephrine, fright, and haemorrhage, as well as exercise.

Building on the work of the dog model, Turner and Hodgetts (1959) studied the role of the spleen in hematocrit changes in sheep. Using ^{51}Cr red cell labelling, they were able to show that red cells accumulated in large numbers in the spleen, and that individual cells were retained there for periods ranging up to 30 minutes or longer. They concluded that a state of dynamic red cell storage existed in sheep, similar to that in dogs. Turner and Hodgetts (1959) were also able to demonstrate that hematocrit increased and ^{51}Cr counts in the splenic region decreased after the sheep were exposed to stressful situations. From this, they concluded that red cells were mobilized from the spleen and were, in part, responsible for the increase in hematocrit.

These observations were supported by the findings of Vatner et al. (1974) who studied the impact of severe, spontaneous exercise before and after splenectomy in six unrestrained, healthy dogs. Before splenectomy, exercise increased hematocrit from 40 to 49% while after splenectomy, hematocrit failed to increase. From their findings, they concluded that the contribution of red cells from the spleen during exercise made a significant contribution to the increased exercise hematocrit. Subsequent research has shown that the spleen contracts during exercise and under stressful situations resulting in

a release of a sequestered pool of red blood cells into the circulation in other animals such as racehorses (Persson et al, 1973) and Weddell seals (Hurford et al, 1996; Qvist et al, 1986).

In all of the animals studied, the Weddell seal has the highest spleen mass of any reported mammal, comprising 0.89% of body mass (Qvist et al, 1986). It is estimated that 20 litres of red blood cells are stored in the spleen of an average 350kg Weddell seal (Hurford et al, 1996). Qvist et al. (1986) found that hemoglobin concentrations increased by nearly 60% during the first 10-12 minutes of both long and short dives and suggested that the source of the influx of oxygenated red blood cells was the spleen. The diving reflex is characterized by a profound sympathetic outflow, in order to cause vasoconstriction of the peripheral vasculature. These authors suggested that this sympathetic stimulation could have been the mechanism for the contraction of the seal's spleen. Continuing research by this group associated increased levels of plasma catecholamines with free diving and intravenous injection of epinephrine at rest produced a 30% decrease in spleen size and increased hemoglobin concentration and hematocrit in the Weddell seal (Hochachka et al, 1995; Hurford et al, 1996).

While benefiting the aquatic mammal with enhanced oxygen availability, the detrimental effect on blood viscosity of an instantaneous rise in circulating red cell mass would be appreciable. However the peak in hematocrit is not observed until at least 10 minutes into the dive (Qvist et al, 1986; Thornton et al, 2001). Observations in Northern elephant seals of a rapid contraction of the spleen and a simultaneous filling of the hepatic sinus explain the time delay (Thornton et al, 2001). These authors' suggest that the seal uses the sphincter-controlled hepatic sinus as a mechanism for the gradual release

of red cells into the general circulation, thereby preventing any detrimental effects of an acute increase in blood viscosity.

In contrast to the seal, the other animals that have demonstrated splenic storage of red cells have relatively smaller spleens (Table 1).

TABLE 1. Relative spleen weight and red cell storage volume of different mammals.

	Spleen Weight	Spleen Volume
	(% Total mass)	(% Total Red Blood Cells)
Weddell Seal	0.89	50
Horse	0.3	54
Sheep	0.2	26
Dog	0.22	20
Human	0.25-0.29	<10

1.3 MECHANISM OF SPLEEN VOLUME CHANGES

Direct neural innervation

Innervation of the spleen originates in the superior mesenteric/celiac ganglion and the nerve fibres enter the spleen around the splenic artery. The splenic nerve contains approximately 98% sympathetic nerve fibres and its greatest density is within the central artery of the white pulp and associated periarterial lymphatic sheath (Williams and Felten, 1981; Felten et al, 1985). The red pulp contains scattered fibres, primarily associated with the plexuses along trabeculae and surrounding tissues.

Stimulation of the splenic nerve of the dog (Green et al, 1960; Ojiri et al, 1993), guinea pig (Digges et al, 1981) and in the human *in situ* (Henle, 1852, as cited in Ayers et al, 1972) and *in vitro* (Ayers, 1972) has resulted in changes in hematological profiles and observations of splenic volume reduction.

Plasma catecholamines

The free plasma concentrations of catecholamines are low at rest, being 1 – 2 nM·L⁻¹ for norepinephrine and 0.2 – 0.8 nM·L⁻¹ for epinephrine (Bray et al, 1992). All of the epinephrine comes from the adrenal medullae and the norepinephrine from the adrenal medullae and spill over from sympathetic nerves. Low levels are maintained at rest because the rate of release from the adrenal medullae is normally low and the half-life of the circulatory catecholamines is relatively short (less than 2 minutes). An increase in catecholamines can be induced by acute stressful situations (generally referred to as the fight/fright/flight response (Cannon, 1929)). Catecholamine concentrations also increase during shock (induced by trauma or sepsis) and experimental infection in animals and man (Lang and Dobrescu, 1989; Benedict and Grahame-Smith, 1978; Groves et al, 1973) and are responsible for many of the characteristic changes observed in these conditions (Lang, 1992).

The effects of catecholamines are mediated via adrenoreceptors, which can be separated into four categories (α_1 , α_2 , β_1 , β_2) based on their different sensitivity to certain agonists. A third β -adrenoreceptor (β_3) has recently been described (Krief et al, 1993; Enocksson et al, 1995). This receptor is exclusively expressed on adipose tissue and is therefore irrelevant in the context of this thesis.

Binding of catecholamines with β_1 - and β_2 -adrenoreceptors causes activation of adenylate cyclase via the G_s protein, with a consequent increase in cyclic adenosine monophosphate (cAMP) as an intracellular messenger. α_1 -adrenoreceptors produce their effects by activating phospholipase C, whereas α_2 -adrenoreceptor stimulation results in inhibition of adenylate cyclase via the G_i protein, thus decreasing intracellular cAMP (McDevitt, 1989). If both receptors are present in a given tissue, α -adrenoreceptors typically dominate (Brodde et al, 1992; McDevitt, 1989).

TABLE 2. Efficacy of catecholamines on adrenoreceptors.

Adrenoceptor	Catecholamine order of efficacy
α_1	Epinephrine > norepinephrine
α_2	Epinephrine > norepinephrine
β_1	Epinephrine = norepinephrine
β_2	Epinephrine > norepinephrine

(Adapted from Bray et al, 1992)

Most tissues have mixed populations of adrenoreceptors and the response depends on the agent, the adrenoreceptors and their location. Evidence of both α - and β -adrenoreceptors have been described in the human splenic vasculature and capsule (Ayers et al, 1972).

Plasma catecholamines and hematological changes

Splenic contraction and increased hemoglobin and hematocrit has been observed following infusion of epinephrine and norepinephrine *in situ* in dogs (Ojiri et al, 1993) and rats (Kuwahira et al, 1999), *in vitro* in hooded and harp seals (Cabanac et al, 1997) and *in vivo* in Weddell seals (Hurford et al, 1996) and correlated with plasma catecholamine levels in the Weddell seal (Hochachka et al, 1995). In humans smaller increases in red cell count have been observed following administration of epinephrine (Benhamou et al, 1929, as cited in Ayers et al, 1972; Ebert and Stead, 1941), which were not apparent in splenectomized subjects (Yang, 1928). Pathologically enlarged spleens have been noted to produce greater changes than the normal human response (Ayers et al, 1972).

Pharmaceutical intervention has assisted in identifying the specific adrenoreceptors responsible for splenic contraction. Phentolamine, a non-specific α -adrenoreceptor blocker, completely abolished the effect of epinephrine-induced splenic volume reduction and hematocrit increase in seals (Cabanac et al, 1997) and hemoglobin increase in rats (Kuwahira et al, 1999). The α_1 -blocker, prazosin, prevented the elevation in hemoglobin and hematocrit in resting dogs induced by splenic nerve stimulation and epinephrine and norepinephrine infusion (Ojiri et al, 1993). In comparison the α_2 -blocker, yohimbine, has produced inconsistent results. Preventing an elevation in hemoglobin in rats with epinephrine infusion (Kuwahira et al, 1999), but having no effect on the splenic diameter or hematological variables of the dog following catecholamine administration (Ojiri et al, 1993). In humans, administration of the α -adrenoreceptor blocking drug, phenoxybenzamine, completely abolished or reduced the vascular

responses of the spleen to norepinephrine and direct neural stimulation (Ayers et al, 1972). Where as the use of a β -blocker, propranolol had no effect on the exercise-induced increase in hemoglobin concentration and hematocrit (McKelvie et al, 1997). The paucity of research makes drawing conclusions difficult but it appears that splenic contraction appears to be mediated through α -adrenoceptor activation. Exactly which isoform of the α -adrenoreceptor remains to be identified.

Plasma catecholamines and immune function

Frey was the first to assign a role of the spleen in catecholamine-induced lymphocytosis in 1914. This author noticed that after splenectomy in rabbits epinephrine-induced lymphocytosis could no longer be observed. The same experiments in guinea pigs, however, showed that in these animals the lymphocyte reaction remained unchanged. Additional experiments in human subjects showed that epinephrine injection immediately after splenectomy did not induce lymphocytosis (Frey, 1914; Frey and Lury, 1914, as cited in Benschop et al, 1996). The responses gradually returned to normal over time, which indicated to Frey that the remaining lymphatic tissue took over the role of the spleen. Based on these investigations, Frey concluded that epinephrine caused a "contraction" of the spleen, resulting in a mechanical efflux of lymphoid cells. This finding was questioned by others who observed epinephrine-induced lymphocytosis in splenectomized animals (Edmunds and Stone, 1924) and humans (Patek and Daland, 1935; Lucia et al, 1937). The observations made by Frey in freshly splenectomized subjects were also challenged by others who observed positive responses in asplenic subjects (Grimm, 1919; Hess, 1922, as cited in Benschop et al, 1996).

Recent investigations have again highlighted the role of the spleen in catecholamine-induced changes in lymphocyte circulation. Van Tits et al. (1990) observed no lymphocytosis in splenectomized subjects after infusion of the β -adrenoceptor agonist isoproterenol. The only observed change was a blunted increase in natural killer cell (large granular lymphocyte) numbers. In normal and splenectomized subjects, changes in natural killer cell numbers were identical, after epinephrine infusion (Schedlowski et al, 1996). Norepinephrine administration, however, resulted in a more pronounced increase in natural killer cell numbers in splenectomized subjects than in normals. Indicating that lymphocytes can be mobilized from sources other than the spleen.

Gader and Cash (1975) infused human subjects with epinephrine and norepinephrine, as well as with the selective β -agonists salbutamol and isoprenaline. Administration of epinephrine and norepinephrine induced increases in granulocytes and lymphocytes, whereas salbutamol and isoprenaline only induced lymphocytes. These results indicate that different mechanisms are involved in lymphocyte and granulocyte redistribution. Pharmacological adrenoceptor-blockade experiments have resolved some of the mysteries of catecholamine-induced leukocytosis.

French et al. (1971) reported that combined α - and β -adrenoreceptor blockade was required to inhibit epinephrine-induced leukocytosis in man. They did not, however, discriminate between lymphocyte and granulocyte changes. Gader (1974) blocked β -adrenoreceptors with propranolol prior to epinephrine administration. The increase in lymphocytes observed 15 to 30 minutes after administration of epinephrine was completely inhibited by this pretreatment, indicating that these increases were mediated

via β -adrenoreceptors. The increase in total lymphocytes and leukocytes was attenuated, but not completely inhibited. Recently, Schedlowski et al. (1996) demonstrated that the increase in natural killer cell numbers upon epinephrine and norepinephrine infusion was inhibited via blockade of β_2 - but not β_1 -adrenoceptors.

Parallel to these injection/infusion studies, the effect of β -blockade on leukocytosis induced by physiological or psychological stress has been evaluated. Even though physical and psychological stresses are accompanied by changes in different endocrine factors, the results are remarkably compatible with those of the infusion studies. Initial experiments showed that blockade of β -adrenoreceptors inhibited exercise-induced leukocytosis (Ahlborg and Ahlborg, 1970). Later it was demonstrated that the exercise-induced lymphocytosis predominantly consists of increases in natural killer cell numbers and that these changes can also be inhibited by propranolol (β -blocker) (Murray et al, 1992). More recently, it was reported that the increases in natural killer cell numbers observed after acute mental or emotional stress are mediated via β -adrenoreceptor stimulation (Benschop et al, 1994; Bachen et al, 1995). However, in accordance with earlier observations (Gader and Cash, 1975), β -blockade could not inhibit the stress-induced alterations in granulocyte numbers (Benschop et al, 1996). Ernstrom and Sandberg (1973) noted that in guinea pigs, although the splenic blood flow did not change, norepinephrine or isoprenaline induced an increased release of lymphocytes and granulocytes from the spleen. These processes could be blocked by either phentolamine (α -blocker) in the case of norepinephrine administration or with propranolol (β -blocker), when isoprenaline was applied.

Taken together, these results suggest that changes in lymphocyte trafficking induced by catecholamine administration, physical exercise, or acute psychological stress are regulated via β -adrenoreceptors. In contrast, data indicate that α -adrenoreceptors are involved in the effects of catecholamines on granulocyte trafficking.

Other vasoactive hormones

Angiotensin II is a peripheral vasoconstrictor and is part of the renin-angiotensin-aldosterone hormone system that acts to regulate blood pressure. Stimulation of the system occurs in response to a decrease in arterial blood pressure detected by the intra-renal baroreceptors. Both renin and angiotensin II plasma levels have been observed to increase up to 10-fold in response to maximal exercise (Wade and Freund, 1990).

Antidiuretic hormone (vasopressin) is a potent vasoconstrictor released from the posterior pituitary gland. It acts on vascular smooth muscle (via V_1 -receptors) to increase the concentration of inositol trisphosphate, which increases intracellular calcium. Vasopressin is released in response to increased osmolality of the extracellular fluid, decreased blood volume and pressure, and during periods of increased sympathetic activity i.e. pain, stress and exercise (Bray et al, 1992).

Angiotensin II and vasopressin have been shown to influence splenic blood flow, via their effect on arterial smooth muscle, but appear to have a negligible effect on spleen volume in cats (Greenway and Stark, 1970) and humans (Ayers et al, 1972).

Intra-abdominal pressure

Increased ventilatory rates associated with exercise result in fluctuating intra-abdominal pressure. No direct research has investigated, to the author's knowledge, exercise-independent elevations in ventilation and spleen volume. Alterations in hematological variables have been indirectly linked to splenic function. Investigations have produced conflicting results, with hematocrit and hemoglobin levels increasing (Straub and Buhlmann, 1970; Staubli et al, 1985; Fairburn et al, 1993), decreasing (Rapoport et al, 1946), or displaying no change (Rowe et al, 1962). In the best designed study to date Fairburn et al. (1993) compared the effect of exercise and exercise-independent increases in ventilation on leukocyte kinetics. Following 20 minutes of incremental cycling hematocrit increased 3%, although not statistically significant due to the low sample size ($n = 6$). During this cycle white blood cell count significantly increased, with corresponding increases in lymphocyte and neutrophil concentrations. When the ventilation was increased to comparable levels, in an exercise-independent condition, white blood cell count and hematocrit did not increase.

The studies reporting increased hematological levels have generally had higher rates of ventilation, which has produced elevated cardiovascular parameters. It is this elevation in central cardiovascular parameters, indirectly indicated by an increased heart rate, which has been suggested as the mechanism behind the reported increases in hematocrit and hemoglobin levels (Fairburn et al, 1993).

The mechanism for the reduction in human spleen volume following exercise is not known and is the focus of the investigation detailed in Chapter Two.

1.4 PHYSIOLOGICAL RELEVANCE OF SPLEEN VOLUME CHANGES

Animal

The release of 20 litres of red cells into circulation increases the concentration of hemoglobin by as much as 60% in the Weddell seal (Qvist et al, 1986). The effect of such a large reserve of oxygenated red blood cells being released into circulation has been suggested to be the primary mechanism behind an extended dive time (Hochachka et al, 1986; Zapol, 1987). However, in the hooded and harp seals, an increase of only 80 – 105 seconds in dive time followed maximal splenic release (Cabanac et al, 1997). This indicated that the primary reason behind the splenic reservoir of red blood cells was to minimize the surface time necessary for respiratory gas exchange between dives and conversely to reduce blood viscosity, during extended periods of time at the surface, when the seal is asleep or resting (Qvist et al, 1986; Elsner and Meiselman, 1995). While benefiting the animal in its ability to transport oxygen, such a large increase in red cell volume also significantly raises the viscosity of the blood and thus increases resistance to flow.

The ergogenic properties of infusion of red blood cells i.e. blood doping or the use of erythropoietin to artificially increase red cell mass has become a hot topic in the last twenty years (Gledhill et al, 1999). Any means by which the hemoglobin concentration and therefore arterial oxygen content can be increased has been shown to be beneficial to aerobic performance. Increases in hematocrit of up to 23% in dogs (Vatner et al, 1974) and in hemoglobin of 48% in ponies (Manohar, 1987) have been reported near maximal

exercise. The size of the human spleen, in comparison, to the animal models studied means that an internal blood doping mechanism is not present in the human (Table I).

Human

The comparatively small volume and the paucity of smooth muscle have placed the human spleen in many physiologists' minds as a non-essential organ during exercise. This is not to say that it does not have any physiological effect.

Viscosity

Exercise causes both acute and chronic changes in plasma volume, hematocrit and total red cell volume. These changes, in turn, have an effect on blood viscosity and oxygen delivery to the body tissues. In order to have optimal oxygen delivery to the body tissues during exercise, a balance between a high hematocrit (increased oxygen carrying capacity per given volume of blood) and blood viscosity is necessary.

Increases in the concentration of cellular components and other constituents of the blood due to decreases in plasma volume produce changes in the flow properties of blood. Stress-hemoconcentration results in the blood becoming thicker or more viscous. The viscosity of blood is a contributor to the total peripheral resistance in the circulatory system, and ultimately to blood pressure. This is evidenced in the Hagan-Poiseuille equation relating flow, pressure, and resistance:

$$V = (\Delta P \pi r^4) / (8 \eta l)$$

V = flow; ΔP = hydrostatic pressure gradient; r = radius of the vessel; η = viscosity of the fluid; l = length of the vessel

Viscosity is defined as shear stress (force per unit area on one fluid layer which produces its movement relative to another layer) divided by shear rate (the velocity gradient between two layers of fluid) (Lowe, 1987). Plasma viscosity is primarily determined by temperature (which is relatively constant *in vivo*) and plasma protein composition (Lowe, 1987). Fluid shifts out of the plasma will concentrate proteins, and thus has a large effect on increasing plasma viscosity. The increase in plasma viscosity is especially influenced by the greater concentration of large proteins such as globulins and fibrinogen, due to both their size and asymmetry (Harkness, 1971). Plasma behaves as a Newtonian fluid, meaning that its viscosity is approximately the same at various shear rates. Thus, the effective viscosity of plasma is approximately equal regardless of the cardiac output.

Whole blood viscosity depends upon cardiac output, or more precisely on the shear rate (Kameneva, 1990). Blood is a non-Newtonian fluid due primarily to the suspension of deformable red cells, and to a much lesser extent, the presence of white cells. At high shear rates ($>200/s$), whole blood reaches an asymptomatic minimum value, which is about 2.5 times the viscosity of plasma, and 5 times that of water (Lowe, 1987). At low shear rates, however, the viscosity of blood can become many times greater than its corresponding viscosity at high shear rates, mainly due to red cell

aggregation (Brooks et al, 1970). Plasma viscosity, temperature, the concentration of suspended particles (especially red cells), the deformability of the red cells, and the extent of cellular aggregation influence the viscosity of whole blood (Lowe, 1987). The influences of red cell deformability changes are complex and depend in large degree upon the cardiac output (Lowe, 1987), but deformability changes would not negate the increased blood viscosity due to increases in plasma viscosity and hematocrit under constant shear conditions. Thus, blood viscosity is a dynamic measure that varies dramatically depending on the cardiac output at which it is measured.

The release of red cells from the spleen enables an increase in oxygen carrying capacity of the blood when high cardiac outputs diminish the effect of the increased viscosity. Whether this is a primary effect of the spleen or a secondary response to the increase in inflammatory mediators that are released during this period of stress, is unknown.

Plasma Volume Measurement

Graded continuous exercise causes a 12 – 22% reduction in plasma volume (El-Sayed, 1999). The fluid shift from the intra- to the extravascular compartment is the result of elevated blood pressure during exercise causing a net increase in filtration over reabsorption across the capillary length in skeletal muscle beds. Other associated mechanisms include increased muscle cell osmolarity and thermoregulatory fluid loss (Selby and Eichner, 1994; Wilkerson et al, 1977).

The most precise methods of measuring plasma volumes in steady-state are plasma-albumin labelling techniques (I-RISA) or Evan's blue-dye analysis. However, an

assumption during these protein based methods is that the protein content remains constant during the measurement period, which is not the case during exercise. Consequently, when calculating a change in plasma volume due to exercise, two independent isotopes must be utilised. Both requiring at least three samples and regression analyses to determine plasma volume at time of injection (International Committee for Standardization in Haematology, 1980).

The percentage change in plasma volume (% Δ PV) can also be calculated from measurements of hematocrit (Hct) and the hemoglobin concentration [Hb] using the equation derived from Dill and Costill (1974).

$$\% \Delta PV = \{ ([Hb]_{pre} / [Hb]_{post}) \times ((100 - Hct_{pre}) / (100 - Hct_{post})) - 1 \} \times 100$$

The equation which was described originally by Elkinton et al. (1945), implicitly assumes that all changes in hematocrit and [hemoglobin] can be attributed to a loss or gain of plasma or red cell water by the intravascular space. This assumption is only valid, however, if the total number of red cells within the intravascular space remains constant.

If the red cell distribution does alter, then correction for the change in the F-cell ratio (whole body hematocrit to peripheral hematocrit) must be made to both the hematocrit and [hemoglobin]. The average hematocrit of the whole body is always lower than venous or arterial hematocrit. In fact, clear cut F-cell shift caused by compensatory mobilization of blood from the micro- to the macrocirculation has been demonstrated in response to bleeding animals (LaForte et al, 1992), suggesting that similar changes might occur in other states of hypovolemia e.g. after transcapillary plasma volume loss. Such

events serve to underestimate, plasma volume changes calculated from [hemoglobin] and hematocrit measurements (Lundvall and Lindgren, 1998). Conversely, [hemoglobin] and hematocrit changes might overestimate plasma volume reductions, since in humans, as in animals, the spleen might release pooled erythrocytes, as indicated by several exercise studies (Flamm et al, 1990; Froelich et al, 1988; Laub et al, 1993; Sandler et al, 1984; Wolski, 1998). These authors suggest that between 25 and 30% of the increase in exercising hematocrit can be attributed to the spleen releasing red cells. This introduces substantial errors into equations calculating changes in plasma volume, which assume that circulating red cell volume remains constant throughout exercise (Dill and Costill, 1974; Strauss et al, 1951; Van Beaumont, 1972). The prevalence of these equations, cited over 1000 times in the last decade alone (Science Citation Index), indicate the importance the spleen has in the field of exercise physiology today.

The effect of splenic volume reduction on circulating red cell volume, peripheral hematocrit and indirect assessment of changes in plasma volume is not known and is the focus of Chapter Three.

CHAPTER TWO

SPLENIC CONTRACTION AND CATECHOLAMINE RELEASE

The spleen is capable of sequestering red blood cells. It is apparent from animal models that the spleen can also expel red blood cells in times of stress. This response involves activation of the sympathetic nervous system which results in contraction of the smooth muscle within the splenic capsule (Williams and Felten, 1981). The observation of a decrease in spleen volume has also been reported in man following psychological and physiological stresses (Allsop et al, 1992; Flamm et al, 1990; Froelich et al, 1988; Haffner et al, 1994; Hurford et al, 1990; Laub et al, 1993; Sandler et al, 1984; Wolski, 1998). The mechanism responsible for this change has not been determined to date, but two possibilities have been suggested. First, the spleen may actively contract (Barcroft, 1925). Until recently this was deemed unlikely as the capsule of the human spleen, in comparison with other mammals, was believed to contain little or no smooth muscle (Weiss, 1988). The second mechanism suggested was that the change in spleen volume may be a passive process, with progressive egress of blood after splanchnic flow is reduced due to sympathetic directed vasoconstriction (Allsop et al, 1992). Thus both mechanisms involve the sympathetic nervous system and its interaction with the spleen.

The splenic nerve can be classified as predominantly sympathetic, but few fibres are associated with the red pulp, the location of red cell storage (Felten et al, 1985; Williams and Felten, 1981). Direct stimulation of the human splenic nerve *in situ* and *in vitro* has revealed minor changes in the appearance of the spleen, but with little alteration in spleen size and volume (Ayers et al, 1972). Identification of smooth muscle through

out the spleen including the sinus lining cells of the red pulp (Pinkus et al, 1986) indicates that the spleen may be capable of contracting in response to a hormonal signal.

To date only one investigation has attempted to identify a mechanism responsible for the change in the size of the human spleen during exercise. Laub et al. (1993) measured the vasoactive hormones epinephrine, norepinephrine and neuropeptide Y, in conjunction with spleen imaging, during graded cycling. A gradual, non-linear, decrease in spleen erythrocyte content was observed with increasing plasma catecholamine concentrations. Elevations in neuropeptide Y was observed only at the highest intensity level, by which time major changes in spleen activity had already occurred. The small sample size ($n=5$) prevented any statistical correlations being made but the authors did propose that the change in spleen size was partially caused by the increased adrenergic activity.

An inverse relationship between exercise intensity and splenic size has been observed (Laub et al, 1993; Wolski, 1988). This reflects the effect of the sympathetic nervous system during exercise. The change in spleen size with exercise could result from either a sympathetic associated humoral signal acting on smooth muscle within the red pulp, or a passive collapse of the spleen as blood flow is redistributed away from the splanchnic area. To investigate the mechanism responsible a constant intensity was maintained for three different durations of exercise. This was assumed to elicit a similar sympathetic response that would result in a consistent blood flow to the spleen.

The two suggested mechanisms for change in spleen volume would have been expected to produce vastly different results. A passive collapse, with no active constriction of the splenic vasculature, would mean that the average red blood cell transit

time would remain constant. This would result in the change in spleen volume being linearly related to the exercise duration. Conversely, if an active constriction of the splenic vasculature was occurring in response to the degree of sympathetic activation, then the change in spleen volume should be identical regardless of the exercise duration.

STATEMENT OF THE PROBLEM

The mechanism for the reduction in spleen volume following exercise is not known. Decrements in spleen volume have been shown to be linearly related to exercise intensity, however the relationship of spleen volume to exercise of different durations has not been determined. The effect of circulating catecholamines on spleen volume during exercise is also not clear.

Two studies were planned. Due to the use of four separate radioisotopes, the portion of the study utilizing nuclear medicine was performed on the same day.

Study One

This maintained exercise intensity but varied the duration of activity. The hypothesis to be tested was:

1. Splenic volume reduction will be equivalent across exercise periods of different duration, indicating an active contraction of the splenic vasculature in response to an intensity dependent signal.

Study Two

This study determined the change in spleen volume following a maximal exercise challenge and also determined the change in spleen volume during an hour of passive recovery. The hypotheses to be tested were:

1. The spleen volume will reach a minimum value immediately after the maximal exercise challenge and return to basal levels within the hour of passive recovery.
2. Plasma catecholamine levels will be inversely related to spleen volumes.

2.1 METHODOLOGY

SUBJECT SELECTION

The subjects consisted of nine males, with no medical history of any cardiovascular, respiratory or musculoskeletal condition that would compromise their ability to perform all of the required testing, and without exposure to radioactive nucleotides within the past three months. Subjects were selected to give a range of aerobic capacities from 40 to 70 ml.kg⁻¹.min⁻¹.

EXPERIMENTAL DESIGN

Subjects participated in three testing sessions. The first day of testing was conducted at the Allan McGavin Sports Medicine Centre on the University of British Columbia campus, while the second and third testing sessions were conducted at the Nuclear Medicine Department of Vancouver General Hospital.

Day One

Prior to any testing the subjects received a verbal description of the experiment, and were required to complete a written consent for this study approved by the University of British Columbia Ethics Committee. Basic anthropometric measurements (height and weight) were then undertaken.

The maximal aerobic power test was performed on an electronically braked cycle ergometer (Quinton Excalibur, Lode, Groningen, Netherlands) utilizing a 30 W.min⁻¹ incremental protocol for the more aerobically trained individuals and 25 W.min⁻¹ for the untrained subjects. Expired gases were collected and analyzed using S-3A oxygen and

carbon dioxide analyzers (Ametek, Pittsburgh, Pennsylvania, USA). Ventilation was measured by a flow transducer (Flo-1B, Physio-Dyne Fitness Instrument Technologies, Quogue, New York, USA). Oxygen consumption, minute ventilation, carbon dioxide production, and respiratory exchange ratio were all measured and recorded at 15 second intervals. Heart rate was recorded by telemetry (Polar Vantage XL, Kempe, Finland) and arterial oxygen saturation, using a pulse ear oximeter (Ohmeda Biox 3740, BOC Health Care Inc., Edison, New Jersey, USA) with data recorded every 15 seconds. Standard indicators for achieving $\text{VO}_{2\text{max}}$ were used: volitional fatigue; a plateau in VO_2 with increasing work rate; heart rate $\geq 90\%$ of age predicted maximum; and a respiratory exchange ratio ≥ 1.15 . All subjects satisfied at least three of the preceding criteria. Values for maximal VO_2 , V_E and R were determined by averaging the four highest consecutive 15 second values.

Day Two

Study One

The results of the maximal aerobic test were used to determine the power output for the first study. The exercise session consisted of three work bouts of 5, 10, and 15 minute duration at a power output requiring 60% of maximal VO_2 . Thirty minutes rest was prescribed between each workload. The order of the work loads was randomized. Steady state performance was maintained by monitoring oxygen consumption (K4b², Cosmed, Rome, Italy) and heart rate (Polar Vantage XL, Kempe, Finland) throughout each work bout and adjusting the power output accordingly. A determination of spleen activity was conducted immediately preceding and following each exercise bout.

Study Two

Following adequate time for the subject to recover from the last exercise workload an incremental exercise test to exhaustion was performed using an identical protocol as that performed on the first day of testing. Blood samples were drawn and spleen activity was determined at 0, 10, 20, 30, 40, 50, and 60 minutes post-exercise. The blood samples were analyzed to determine plasma epinephrine and norepinephrine concentrations.

SPLEEN IMAGING AND PLASMA CATECHOLAMINE DETERMINATION

Subjects were asked to report to the Department of Nuclear Medicine well hydrated (i.e. having had eight glasses of water in the past 12 hours), rested (no exercise 24 hours prior) and six hours post-absorptive. Each subject had a 20g Jelco intravenous catheter, with a heparin lock, placed in both the right and left antecubital fossa, immediately upon arriving in the Department of Nuclear Medicine. This allowed for the ease of blood withdrawal and injection with a minimal number of venipunctures to the subject. Blood was withdrawn from the left arm for ^{99m}Tc labelling. Isotonic saline with iu heparin per ml was used to flush the catheters intermittently to maintain patency.

SPLEEN VOLUME

Five ml of red cells were labelled using 1.5 mg of stannous pyrophosphate followed by 25 mCi of ^{99m}Tc pertechnetate. Labelling yield of red blood cells using this method is greater than 95% (Saha, 1979). Anterior and posterior views of the spleen were

taken using an ultra-high resolution collimator of a gamma camera (MultiSpect 2, Siemens Medical Systems Inc., Illinois, USA). The image was counted for two minutes using a 20% energy window around ^{99m}Tc . In order to determine spleen counts for the anterior and posterior views, a region of interest was drawn around the posterior image, which contained no overlap from surrounding organs, and this was duplicated onto the anterior image. A geometric mean was obtained by averaging the anterior and posterior decay-corrected activity counts. An intraclass coefficient of 0.92 was calculated for the nuclear medicine determined regions of interest.

All spleen image counts were decay-corrected to the time of the initial resting spleen image using the following equation.

$$A_t = A_o e^{-\lambda t}$$

A_t = activity after a period of elapsed time, A_o = activity in the original sample, λ = $0.693/6.02$ hr physical half-life of ^{99m}Tc , and t = the time between when the spleen image was counted and the initial spleen image

A 1 ml sample of red cells was obtained during each of the post-exercise spleen counts in part one and at 40 and 50 minutes following the incremental ride to exhaustion. The decay-corrected count of activity in the known volume (1 ml sample) was used to determine an elution factor (Appendix D). The spleen volume was then calculated from the decay-corrected geometric mean spleen activity counts of the anterior and posterior images at each of the measurement times, using the elution factor. These samples only contained red cells because the hematocrit of the spleen has been estimated to be close to

95-97% (Gray, 1977). Centrifuging the blood will result in a red cell column that is approximately 96% pure red cells (some plasma is trapped in this procedure). Therefore, a sample of red cells only will have a hematocrit reading that is much closer to spleen hematocrit.

SPLEEN SIZE

A two-dimensional area of the spleen was calculated by drawing a region of interest around the spleen using the posterior view. A pixel count of the area selected was determined, with part pixels being counted as whole. One pixel represented an area of 0.24 cm^2 .

PLASMA CATECHOLAMINE CONCENTRATIONS

Plasma epinephrine and norepinephrine concentrations were measured in each of the blood samples drawn (pre-exercise, immediately following the incremental ride to exhaustion and every ten minutes during recovery for the next hour). Two 5 ml samples were collected into pre-cooled EDTA blood collection tubes. Samples were immediately placed on ice and processed within a half hour of collection. Blood samples were spun at $3000 \text{ revs Min}^{-1}$ in a refrigerated centrifuge for 15 minutes. The plasma was then transferred (a minimum of 2.5 ml) into two separate aliquots, which were immediately stored at -70°C until analysis. Catecholamines were measured by high performance liquid chromatography (HPLC) methods using electrochemical detection at Vancouver General Hospital, Vancouver, British Columbia.

STATISTICS

Mean values and measures of variability were determined for anthropometric and descriptive maximal oxygen consumption data obtained during preliminary screening. Data from the 5, 10, and 15 minute rides was compared with a 3 (exercise duration) by 2 (time) two-way factorial analysis of variance with repeated measures on both factors. Data from the recovery from the incremental ride was compared with a one-way analysis of variance with repeated measures. When significant F -ratios were observed, Scheffé's test was applied *post-hoc* to determine where the differences occurred. Pearson-product moment correlations were applied to the semi-log plots of the plasma catecholamine concentrations and spleen volume reduction data. The level of significance was set at $P < 0.01$ for all ANOVA and correlation procedures. Statistical power calculations were performed *a priori* to estimate an appropriate minimum sample size. A sample size of eight was calculated.

2.2 RESULTS

Nine healthy male subjects completed the study (age 24.9 ± 1.0 years, height 176 ± 3.0 cm, mass 75 ± 2.8 kg, and maximal oxygen consumption 54 ± 2.3 ml·kg⁻¹·min⁻¹).

All individual subject data are presented in Appendix A - E.

Study One: Constant intensity exercise of different duration

The effect of the constant intensity exercise of different duration on spleen volume results is reported in Figure 1. There was no significant effect of the different durations on spleen volume. A significant time effect was observed in spleen volume (pre = 85.8 ± 10.1 , post = 59.3 ± 8.1 ml; $p = 0.0006$), indicating a pre- to post-exercise change across all durations.

FIGURE 1. Spleen volume pre and post 5, 10, and 15 minutes cycling at 60 % VO_2 max. Values are means $\pm$ SEM. * indicates significantly different from pre-exercise, $p < 0.01$.

Oxygen consumption, heart rate and power outputs prior to and during the different duration exercise periods are displayed in Table 3. There was no significant effect in pre-exercise heart rate or oxygen consumption. Nor was there any significant difference during the different duration exercise periods for power output, heart rate or oxygen consumption. There was a significant increase in heart rate (pre = 69 ± 3.6 , average exercise = 144 ± 3.9 beats·min⁻¹; $p < 0.0001$) and oxygen consumption (pre = 5.6 ± 0.4 , average exercise = 32.4 ± 1.4 ml·kg⁻¹·min⁻¹; $p < 0.0001$) when comparing pre-exercise to the average exercise values across all exercise durations.

TABLE 3. Heart rate, oxygen consumption (VO₂) and power output data for the 5, 10 and 15 minutes of cycling at 60 % VO_{2 max}. Values are means $\pm$ SEM. * Significantly different than pre-exercise values, $p < 0.0001$.

	5 min	10 min	15 min
Heart Rate (beats·min ⁻¹)			
Pre-exercise	68 ± 4.4	68 ± 3.1	70 ± 3.2
Average exercise value	$140 \pm 2.8^*$	$144 \pm 4.1^*$	$147 \pm 3.3^*$
VO ₂ (ml·kg ⁻¹ ·min ⁻¹)			
Pre-exercise	5.9 ± 0.47	5.3 ± 0.47	5.7 ± 0.38
Average exercise value	$31.9 \pm 1.26^*$	$32.8 \pm 1.46^*$	$32.7 \pm 1.53^*$
Power (W)			
	172 ± 12.2	172 ± 13.4	169 ± 12.7

Study Two: Recovery from the incremental ride to exhaustion

The effect of recovery from maximal exercise on spleen volume is reported in Figure 2. Following the maximal ride there was a significant time effect observed ($p < 0.0001$). Immediately following (TIME 0) and ten minutes after maximal exercise (TIME 10) spleen volumes were significantly reduced compared with pre-exercise (REST = 85.9 ± 9.93 , TIME 0 = 36.4 ± 3.95 , TIME 10 = 61.9 ± 8.51 ml; $p < 0.0001$). There was no significant difference between REST and TIMES 20, 30, 40, 50, and 60. These effects were identical when the decrease in spleen volume was represented as a percentage change from REST (Figure 3).

FIGURE 2. Spleen volume at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion. Values are means $\pm$ SEM. * Significantly different than REST, $p < 0.01$.

FIGURE 3. Spleen volume, as a percentage of rest, at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion. Values are means $\pm$ SEM. * Significantly different than REST, $p < 0.01$.

Plasma norepinephrine and epinephrine concentrations are represented in Figures 4 and 5, respectively. There was a significant time effect for both norepinephrine (REST = 1.51 ± 0.30 , TIME 0 = $29.09 \pm 6.84 \text{ nM}\cdot\text{L}^{-1}$; $p < 0.0001$) and epinephrine (REST = 0.13 ± 0.05 , TIME 0 = $3.17 \pm 0.82 \text{ nM}\cdot\text{L}^{-1}$; $p < 0.0001$). There was no significant difference between REST and TIMES 10, 20, 30, 40, and 60, for norepinephrine or epinephrine concentrations.

The decrease in spleen volume was inversely correlated with both plasma catecholamine concentrations. Figure 6 shows the relationship between spleen volume following maximal exercise and the plasma norepinephrine concentration ([norepinephrine]) (spleen volume = $93.8 - 17.7\ln([\text{norepinephrine}])$; $r = 0.58$, $p < 0.0001$). Figure 7 shows the relationship between spleen volume and the plasma epinephrine concentration ([epinephrine]) (spleen volume = $56.9 - 13.6\ln([\text{epinephrine}])$; $r = 0.46$, $p = 0.0003$).

When the decrease in spleen volume was represented as a percentage change from REST, the correlations became stronger (spleen volume = $7.8 - 19.2\ln([\text{norepinephrine}])$; $r = 0.84$, $p < 0.0001$ and spleen volume = $36.7 - 15.35\ln([\text{epinephrine}])$; $r = 0.70$, $p < 0.0001$).

Spleen size and volume were positively correlated ($r = 0.89$, $p < 0.0001$). Statistical significance was achieved for spleen size at identical comparisons as with spleen volume (Appendix B).

FIGURE 4. Plasma norepinephrine concentration at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion. Values are means $\pm$ SEM. * Significantly different than REST, $p < 0.01$.

FIGURE 5. Plasma epinephrine concentration at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion. Values are means $\pm$ SEM. * Significantly different than REST, $p < 0.01$.

FIGURE 6. Relationship between spleen volume and plasma norepinephrine concentration in all subjects at rest and during recovery from an incremental cycle to exhaustion ($n = 63$, $r = -0.58$, $p < 0.0001$).

FIGURE 7. Relationship between spleen volume and plasma epinephrine concentration in all subjects at rest and during recovery from an incremental cycle to exhaustion ($n = 63$, $r = -0.46$, $p < 0.0001$).

2.3 DISCUSSION

Study One: Constant intensity exercise of different duration

No differences were observed in the decrease in spleen size and volume between the 5, 10, or 15 minute exercise periods. These findings support the theory that the spleen is actively contracting due to an intensity dependent signal. If the process of change were a passive egress, the spleen would have been expected to have a significantly larger decrease in size and volume following the longer bouts of exercise. No differences were observed between resting spleen size and volumes, heart rates or oxygen consumption before each exercise bout. This indicates that the spleen had adequate time to recover from the previous exercise period and that the resting status of the subject was also not compromised by the prior exercise. No differences were observed in the average oxygen consumption, heart rate or power output during each of the different duration exercise protocols. Therefore the author is confident that the intensity of exercise was identical over the three different exercise durations.

A 16 and 31% decrease in spleen size and volume, respectively, was observed following cycling at 60% $\text{VO}_{2\text{ max}}$. Wolski (1998) measured a 35 and 58% decrease in volume with cycling at 50 and 75% $\text{VO}_{2\text{ max}}$, respectively. The present study controlled the intensity of the exercise directly via breath-by-breath oxygen consumption during the rides of different duration; in comparison, Wolski (1998) utilised a constant power output, corresponding to that intensity obtained from an incremental $\text{VO}_{2\text{ max}}$ test. This method has a tendency to overestimate the intensity during a steady-state exercise protocol and therefore helps explain the larger decrease in spleen volumes.

In previous exercise studies', decreases in spleen size and volume have been shown to be linearly correlated with exercise intensity. Wolski (1998) demonstrated that the decrease in spleen volume was identical, in trained and untrained subjects, when initial spleen volume was normalized and that this response was unchanged in a hypoxic environment. Laub et al. (1993) also showed an inverse linear relationship between spleen radioactivity and increasing exercise intensity, although constant power outputs were utilised indicating subjects were exercising at different relative intensities.

Increased activation of the sympathetic nervous system is associated with increased exercise intensity. Laub et al. (1993) were the first to measure plasma catecholamine concentrations in conjunction with spleen imaging. A gradual, not linear, decrease in spleen erythrocyte content during graded exercise was observed with increasing plasma catecholamine concentration. No statistical correlation was attempted but the authors suggested that the decrease in splenic erythrocyte activity with increasing exercise levels was partially explained by the increased adrenergic activity.

Study Two: Recovery from the incremental ride to exhaustion

A 44 and 59% decrease in spleen size (pre = 69.7 ± 3.63 , post = 38.7 ± 2.67 cm²) and volume (pre = 85.94 ± 9.93 , post = 36.35 ± 3.95 ml), respectively, was observed immediately following the incremental ride to exhaustion.

Decrements in splenic activity, ranging from 39 to 54%, have been recorded at exhaustion, following graded and continuous maximal exercise protocols (Allsop et al, 1992; Flamm et al, 1990; Froelich et al, 1988; Sandler et al, 1984). The variation in the activity changes could be explained by methodological limitations. The use of the pre-

exercise region of interest for the post-exercise scan, despite observations of a reduced spleen size and the possibility of the spleen moving within the abdominal cavity, could effect the activity count. As the degree of movement of the spleen observed in the present study was minimal, it is possible to assume that the spleen stayed within the pre-exercise region of interest in the previous studies. However, movement of other organs i.e. the heart and kidneys may effect the region of interest and artificially inflate the spleen activity. In the present study the selection of a new region of interest for each image, accurately portrays the change in spleen size associated with exercise.

This is the first study to quantify spleen volume change following maximal exercise. In sedentary and trained males, Wolski (1998) reported a 58% decrease following 30 minutes of continuous exercise with the final 10 minutes at a power output representing 75% VO_2 max. The similarity between the present study and the Wolski research in the spleen volume decrement, plus the similar reduction in spleen activity following maximal exercise in the different studies, and the small variation in maximal exercise volumes compared with other measurement periods, achieved in the present study, are all suggestive of the spleen having a limit to its minimal sustainable size. This could be indicative of the spleen maximally contracting.

This is the first study to determine spleen size and volume during recovery from an incremental cycle to exhaustion. The spleen returned to pre-exercise size and volume following 20 minutes of supine rest. Allsop et al. (1992) tracked splenic radioactivity for 40 minutes following supra-maximal exercise and indicated that the spleen had not fully recovered in this time period. The small sample size ($n=5$) of Allsop's study and the large

intra-subject variability in radionuclide measurements could account for the conflicting results.

The present investigation and the earlier work by Laub et al. (1993) are the only two *in vivo* studies to measure splenic change and adrenergic activity in humans. The decrease in spleen size and volume in the present investigation correlated with increasing plasma catecholamine concentrations in a semi-logarithmic fashion. Laub et al. (1993) also observed that the steepest rise in catecholamine concentration occurred between heavy and maximal work, which was associated with the smallest change in spleen volume. The variations in plasma catecholamine concentrations do not completely account for the changes in spleen size and volume (r values ranged between 0.68 and 0.84, percent spleen change; and 0.46 and 0.67, absolute spleen change; norepinephrine and epinephrine, respectively). Additional mechanisms undoubtedly assist in regulating splenic size and volume.

The ability of the human splenic capsule to assist in contraction has often been overlooked, mainly due to the scarcity of smooth muscle. However, small volume changes have been recorded following stimulation of the human splenic nerve (Ayers et al, 1972), indicating that, direct neural innervation aids in the decrease in spleen size and volume. The passive collapse of the spleen, in response to splanchnic vasoconstriction may have a small effect on the decrease in spleen size and volume. Attributing a significant role would be foolish as the evidence from Study One argues against a passive collapse. However, it could represent a minor contributing factor, in combination with stimulation of the splenic nerve, during the initial stages of the decrease in spleen volume when plasma catecholamine concentrations remain near basal values.

Summary

The consistent decrease in spleen size and volume following the exercise of different duration, reflects the spleen actively contracting in response to an intensity dependent signal. The small variation in spleen volume measurements following maximal exercise and the semi-logarithmic correlation with plasma catecholamine concentrations, suggests that the spleen is actively contracting to a minimal sustainable size. Plasma catecholamine concentrations cannot account completely for the changes in spleen size and volume, indicating that additional mechanisms, not identified in this study, assist in regulating spleen size and volume.

CHAPTER THREE

EFFECT OF SPLENIC RED CELL EXPULSION ON EXERCISE-INDUCED HEMOCONCENTRATION

Exercise results in hemoconcentration. This has been attributed solely to a reduction in plasma volume that occurs as fluid moves from the intra- to the extravascular compartment in response to elevations in blood pressure, the change in muscle cell osmolarity, and thermoregulatory requirements (Johnson et al, 1996; West, 1995). Changes in plasma volume are generally calculated indirectly from hemoglobin and hematocrit. The gold standard techniques of Evan's blue-dye or radioisotope labelled human serum albumin are seldom used in exercise studies due to their invasiveness and cost. Indirect assessments have become the norm and are currently accepted throughout the world.

In these often used equations (Dill and Costill, 1974; cited ~1000 times in the last decade alone), circulating red cell volume is assumed to be consistent with total red cell volume. The ability of the spleen to release up to 10% of the total red cell volume into the peripheral circulation has been overlooked. Recent investigations have suggested that the spleen could account for 25% of the increase in hematocrit (Laub et al, 1993; Wolski, 1998). This would introduce substantial errors into the indirect assessment methods of plasma volume changes.

STATEMENT OF THE PROBLEM

The effect of splenic volume reduction on circulating red cell volume, peripheral hematocrit and indirect assessment of changes in plasma volume has not been determined. In a study conducted in conjunction with the first two, the following hypotheses were tested.

1. Splenic volume reduction will increase circulating red cell volume and peripheral hematocrit.
2. Indirect assessments of plasma volume reduction will overestimate the magnitude of change compared with radioisotope labelled human serum albumin.

3.1 METHODOLOGY

SUBJECT SELECTION

This study was performed in conjunction with the first two studies utilising the same subjects.

EXPERIMENTAL DESIGN

The subjects performed four exercise bouts (see pages 30 - 31). Prior to the initial exercise bout and immediately following the completion of the maximal exercise challenge, a standard blood count was taken and spleen, red cell and plasma volumes determined.

BLOOD VOLUME DETERMINATION

Red Cell Volume

15 ml of whole blood was withdrawn from the subjects' right antecubital vein into a syringe containing 3 ml acid-citrate-dextrose solution. This sample was then added to a 30 ml evacuated vial using a breather needle. 150 μ Ci of ^{51}Cr sodium chromate was slowly added to the vial and then incubated at room temperature for 20 minutes, with mixing occurring every five minutes. After incubation, 50 mg ascorbic acid was added to the inverted vial and three minutes later 1 ml of the tagged blood was diluted with ~99 ml of water and ~10 mg of sodium chromate crystals to produce a 100 ml volume. A total activity injected count was determined (Cobra II auto-gamma, Packard BioScience Company, Meriden, Connecticut, USA). 10 ml of the ^{51}Cr labelled blood was then injected into the left antecubital vein. The labelling yield of red blood cells using this

technique is greater than 90% (Saha, 1979). After 30 minutes of equilibration time, a 10 ml sample was drawn from the right antecubital vein and a patient activity count determined (Cobra II auto-gamma, Packard BioScience Company, Meriden, Connecticut, USA). Red cell volume was then determined using the following formula:

$$\text{RCV (ml)} = \text{Volume injected} \times (\text{total activity injected} / \text{patient RBC activity})$$

^{51}Cr reaches an equilibrium concentration throughout the body within 15-20 minutes (Kowalsky and Perry, 1987). Baker and Remington (1960) showed that splenic contraction does not cause a significant change in total red cell volume indicating that tagged ^{51}Cr red cells equilibrate in the spleen as well as throughout the peripheral circulation.

Plasma Volume (*Pre-exercise*)

10 μCi of ^{125}I -human serum albumin (^{125}I -RISA) was injected into the subject at the same time as the ^{51}Cr was injected. 10 ml blood samples were then drawn from the opposite arm 10, 20 and 30 minutes following the initial injection. The samples were centrifuged at 3000 rpm for 10 minutes (Sorvall GLC-2, Kendro Laboratory Products, Newton, Connecticut, USA) and a 1 ml plasma sample from each was counted by a multi-channel analyzer (Cobra II auto-gamma, Packard BioScience Company, Meriden, Connecticut, USA). The log of the sample activity was then plotted against time and extrapolated back to zero time to determine the plasma volume (Kowalsky and Perry, 1987). Albumin leaks slowly from the intravascular space, therefore when measuring

plasma volume with ^{125}I -RISHA or ^{131}I -RISHA, a plot of the log sample activity against time is extrapolated back to zero time to obtain the true activity concentration.

Plasma Volume (*Post-exercise*)

^{131}I -RISA was used to determine post-exercise plasma volume following the same procedure as above. The formula for determining plasma volume was:

$$\text{PV (ml)} = \text{Volume injected (ml)} \times [\text{Total activity injected (cpm)} / \text{Patient plasma activity (cpm)}]$$

Plasma Volume Change – Indirect calculations

The change in plasma volume from pre- to post-exercise was also calculated via the following equations:

1. Hemoglobin and Hematocrit (Dill and Costill, 1974)

$$\% \Delta \text{PV} = \{ ([\text{Hb}]_{\text{PRE}} / [\text{Hb}]_{\text{POST}}) \times ((100 - \text{Hct}_{\text{POST}}) / (100 - \text{Hct}_{\text{PRE}})) - 1 \} \times 100$$

2. Hemoglobin and Hematocrit with F-cell correction (Harrison et al, 1982)

As above but with Hct multiplied by 0.91 to correct for F-cell shift

3. Hematocrit (van Beaumont, 1972)

$$\% \Delta \text{PV} = (100 / (100 - \text{Hct}_{\text{PRE}})) \times 100((\text{Hct}_{\text{PRE}} - \text{Hct}_{\text{POST}}) / \text{Hct}_{\text{POST}})$$

Total Blood Volume

Blood volume was calculated pre- and post-exercise using corresponding red cell and plasma volume measurements. Because a relationship between plasma volume and red cell volume can be derived from knowledge of a subject's hematocrit, a single determination of either plasma volume or red cell volume is often used to determine whole blood volume. However, this method for quantifying whole blood volume is potentially erroneous due to variations between central and peripheral hematocrit values. The accurate quantification of total blood volume therefore requires a determination of both the plasma and the red cell volumes (Swanson et al, 1990). The methods employed in calculating red cell and plasma volume are identical to those recommended by the International Committee for Standardization in Haematology and have been shown to have reliability coefficients of 0.977 and 0.642, respectively (Wolski, 1998).

$$BV \text{ (pre-exercise)} = RCV + PV \text{ (pre-exercise)}$$

$$BV \text{ (post-exercise)} = RCV + PV \text{ (post-exercise)}$$

Spleen Volume

Previously described (see pages 31 - 33).

Standard Blood Count

Prior to exercise and immediately following the incremental ride to exhaustion a 5 ml blood sample was collected into sterile vacutainer tubes containing EDTA and stored at room temperature until analysis. Hematological measures - white and red blood cell

counts, hemoglobin, hematocrit, mean cell volume, red cell distribution width, platelet count, neutrophils, eosinophils, basophils, lymphocytes, and monocytes – were determined using standard techniques on a Coulter counter at Vancouver General Hospital, Vancouver, British Columbia.

Plasma Viscosity

At the same time periods as the sample for the standard blood counts were taken, a 7 ml blood sample was drawn. This sample was centrifuged for ten minutes at 3000 rpm (Sorvall GLC-2, Kendro Laboratory Products, Newton, Connecticut, USA). Using a pipette, 0.95 ml samples of plasma were removed and transferred into a clean 7 ml test tube and the mass of the plasma determined (AB104, Mettler-Toledo Inc., Columbus, Ohio, USA). The average mass of the 0.95 ml plasma samples was recorded and the density of the plasma calculated using the following equation:

$$\begin{aligned}\text{Plasma Density } (\rho_s) &= \text{average mass (gm)} / \text{volume (ml)} \\ &= \text{average mass (gm)} / 0.95\end{aligned}$$

The plasma sample was then transferred to a viscometer (Cannon-Manning Semi-Micro Viscometer, Cannon Instruments, Philadelphia, USA) that was placed in a constant temperature water bath (37 degrees Celsius) maintained by a hot plate (Thermix Stirrer Model 120 MR, Fisher Scientific, Nepean, Ontario, Canada). The viscosity of the sample (η_s) was determined by measuring the time required for the sample to pass between two fixed points on the viscometer (t_s) and comparing this to the time required for a sample of

water, with a known density (p_o) and viscosity (η_o), to pass between the same two points (t_o).

$$\begin{aligned}\eta_s \text{ (mPa}\cdot\text{s}^{-1}) &= \eta_o (t_s p_s / t_o p_o) \\ &= 0.6915 (t_s p_s / t_o \times 0.99777)\end{aligned}$$

STATISTICS

Hematological parameters were compared using *t*-tests for dependent samples (pre- vs. post-exercise). Plasma volume reduction was compared with a one-way ANOVA. The level of significance was set at $P < 0.05$. Statistical power calculations were performed *a priori* to estimate an appropriate minimum sample size. A sample size of nine was calculated.

3.2 RESULTS

Maximal Exercise Challenge

The peak workload achieved during the maximal exercise challenge was 368 watts (range 285 – 465). A peak oxygen consumption of $53 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (range 48 – 64) was recorded.

Spleen Volume

There was no significant correlation between the subjects' aerobic capacity and their resting spleen volume ($r = 0.18$, $p = 0.63$) or the change in spleen volume following maximal exercise ($r = 0.16$, $p = 0.68$). There was a significant decrease in spleen volume pre- to post-exercise (pre = 85.9 ± 9.93 , post = 36.4 ± 3.95 ; $p < 0.0001$).

Blood Volume

Blood volume changes are displayed in Figures 8 and 9. Plasma volume showed a significant decrease following exercise when represented as a function of body mass (pre = 46.6 ± 1.44 , post = $38.3 \pm 1.83 \text{ ml} \cdot \text{kg}^{-1}$; $p = 0.002$) as well as an absolute value (pre = 3508 ± 164.9 , post = $2896 \pm 187.4 \text{ ml}$; $p = 0.026$). There was no significant change in red cell volume or total blood volume, although when expressed relative to body mass, total blood volume approached significance (pre = 77.3 ± 2.85 , post = $68.5 \pm 3.14 \text{ ml} \cdot \text{kg}^{-1}$; $p = 0.054$).

FIGURE 8. Plasma, red cell and total blood volume at rest (pre-exercise) and immediately following the incremental cycle to exhaustion (post-exercise). Values are means $\pm$ SEM. * indicates significantly different from pre-exercise, $p < 0.05$.

FIGURE 9. Plasma, red cell and total blood volume, relative to the subject's mass, at rest (pre-exercise) and immediately following the incremental cycle to exhaustion (post-exercise). Values are means $\pm$ SEM. * indicates significantly different from pre-exercise, $p < 0.05$.

Figure 10 shows the calculated change in plasma volume following exercise using equations based around hemoglobin and hematocrit changes compared with radioisotope labelling. There was no significant difference between any of these methods in calculating plasma volume change.

FIGURE 10. Plasma volume change, immediately following the incremental cycle to exhaustion, as a percentage of pre-exercise, measured with radiolabelled albumin (I-RISA), and calculated with hemoglobin (Hb) and hematocrit (Hct) changes with and without F-cell correction. Values are means $\pm$ SEM.

Table 4 illustrates the changes pre- to post-exercise in standard blood count variables. There was a significant increase in hemoglobin, hematocrit, white and red blood cell counts, and platelet count following exercise. Also the leukocytes: neutrophils, basophils, lymphocytes, and monocytes all significantly increased in concentration following exercise. Plasma viscosity was also shown to significantly increase following exercise. Only the eosinophil concentration, mean cell volume and red cell distribution width showed no significant change from pre-exercise values.

TABLE 4. Hematological data at rest (pre-exercise) and immediately following the incremental cycle to exhaustion (post-exercise). Values are means $\pm$ SEM. *

indicates significantly different from pre-exercise, $p < 0.05$.

	Pre - exercise	Post - exercise
White Blood Cell Count ($\times 10^9 \cdot L^{-1}$)	4.975 ± 0.221	$11.709 \pm 0.944^*$
Red Blood Cell Count ($\times 10^{12} \cdot L^{-1}$)	4.751 ± 0.088	$5.369 \pm 0.091^*$
Hemoglobin ($g \cdot L^{-1}$)	143.3 ± 2.52	$161.5 \pm 3.24^*$
Hematocrit	0.417 ± 0.007	$0.474 \pm 0.008^*$
Mean Cell Volume (fL)	87.7 ± 0.492	88.3 ± 0.527
RDW	12.8 ± 0.123	13.1 ± 0.174
Platelet Count ($\times 10^9 \cdot L^{-1}$)	224 ± 10.52	$287 \pm 10.21^*$
Neutrophils ($\times 10^9 \cdot L^{-1}$)	2.713 ± 0.217	$5.938 \pm 0.747^*$
Eosinophils ($\times 10^9 \cdot L^{-1}$)	0.188 ± 0.052	0.250 ± 0.062
Basophils ($\times 10^9 \cdot L^{-1}$)	0.013 ± 0.012	$0.075 \pm 0.015^*$
Lymphocytes ($\times 10^9 \cdot L^{-1}$)	1.575 ± 0.075	$4.550 \pm 0.145^*$
Monocytes ($\times 10^9 \cdot L^{-1}$)	0.450 ± 0.04	$0.950 \pm 0.099^*$
Plasma Viscosity ($mPa \cdot s^{-1}$)	1.166 ± 0.017	$1.353 \pm 0.019^*$

RDW = red cell distribution width (expression of the coefficient of variation of red blood cell volume of distribution)

3.3 DISCUSSION

The present study indicated that prior to exercise the red cell volume was 2325 ± 158 ml (mean $\pm$ SEM) and the splenic pool consisted of 86 ± 10 ml. This represented 3.8% (range 2 – 6.9%) of the total circulating red blood cells. Following exercise the splenic pool shrank to 1.6% (0.7 – 2.7%) or 36 ± 4 ml. The subjects' aerobic capacity was not related to the initial spleen volume, or the magnitude of the exercise-induced decrease.

An autopsy investigation of 539 spleens indicated that the average spleen weighed 168 g (range 60 – 378) (Sprogø-Jakobsen and Sprogø-Jakobsen, 1997), while Ayers et al. (1973) sample of 30 isolated spleens ranged from 60 – 370 g with an average value of 212 g. The spleen has been estimated to have a hematocrit of between 95 and 97% (Gray, 1977). Spleen red cell volumes have been reported to range from 130 to 360 ml and this represents 2 – 9% of the total erythrocyte content of the body (Allsop et al, 1988; Wadenvik and Kutti, 1988; Wolski, 1998).

While the relative change in spleen size and volume are consistent with previous findings, the absolute volumes are significantly smaller. Wolski (1998) reported a release of 142 – 187 ml of red cells from the spleen, while Laub calculated that 200 ml could have been released into the active circulation during maximal exercise. The differences arise from the methodologies employed in calculating the volumes. Laub et al. (1993) measured splenic activity and from the percent change in activity calculated a volume from an assumed "normal" 300 ml spleen. Wolski used a similar methodology to the present study, with the activity of a known volume of red cells being compared to the activity of the spleen. The lower volume calculated in the present investigation was due

to the spleen region of interest being drawn with removal of any influence of overlapping organs i.e. heart and kidneys, which was not the case in the Wolski study.

The effect of splenic contraction on circulating red cell and plasma volumes

The pre-exercise blood volumes were consistent with normal values reported for adults by Sawka and Coyle (1999). Red cell volume did not change, pre- to post-exercise despite the significant decrease in spleen red cell volume, indicating that the tagged cells reached equilibrium within the spleen in the thirty minutes between injection of the cells and the determination of the pre-exercise red cell volume. This is in direct contrast to Wolski (1998) who observed a significant increase in red cell volume following exercise. Wolski (1998) attributed the increase to extraneous pools of red cells being mobilized during exercise from the bone marrow and/or subpopillary plexus of the skin. Red cell swelling was also suggested, although the present study noted a significant increase in red blood cell count, without a concomitant increase in volume, which indicated that red cell shrinkage may have occurred. This is possible as an increase in plasma osmolality, as indicated by the increase in plasma viscosity, could have resulted in a fluid shift from the red cell into the plasma. A more plausible explanation is that the red blood cell count increased simply due to the decrease in plasma volume.

Graded continuous exercise has been reported to cause a 12 – 22% decrease in plasma volume (El-Sayed et al, 1999). The decrease in plasma volume in the present study was within this range when expressed in absolute terms as well as relative to body mass. This was expected as increased blood pressure during exercise results in an increase in filtration over reabsorption across the capillary length in skeletal muscle beds.

Other mechanisms for the decrease in plasma volume include increased muscle cell osmolarity and thermoregulatory fluid loss (Selby and Eichner, 1994; Wilkerson et al, 1977). The fluid shift from the intra- to the extravascular compartment caused an increase in plasma viscosity due to an increased concentration of plasma proteins. While not measured directly, whole blood viscosity would also have been expected to increase.

The role of the spleen as a storage vehicle for red cells has been hypothesised as a way for the body to regulate the viscosity of the blood. As a non-Newtonian fluid whole blood viscosity depends upon flow rate (Kameneva, 1990). Therefore in the seal and horse, where the spleen is capable of containing up to 50% of the red cell volume, the impact on viscosity of a maintained elevation in circulating red cell volume during periods of resting flow rates would place extreme loads on the heart (Elsner and Meiselman, 1995; Fedde and Wood, 1993). While the human spleen is capable of storing significantly less red cells, the role of reducing resting blood viscosity may still be an important function of the human spleen.

A maximal exercise challenge resulted in a 13.6% increase in hematocrit (pre = 0.417 ± 0.007 , post = $0.474 \pm 0.008 \text{ L}\cdot\text{L}^{-1}$). This was larger than the 9% change observed by Laub et al. (1993) and the 8.8% recorded by Wolski (1998), although these studies did not use maximal exercise protocols. Historically the increase in exercising hematocrit has been fully attributed to a decrease in plasma volume, and as such has been used as a way to calculate plasma volume changes. Recently the release of red cells from the spleen has been suggested to account for ~25% of the increase in exercising hematocrit and therefore capable of introducing substantial errors in indirect plasma volume calculations (Laub et al, 1992; Wolski, 1998).

Laub et al. (1992) did not measure red cell or plasma volume, while Wolski (1998) was unable to explain an unexpected increase in red cell volume following exercise. In this study the spleen was not the cause of the increase in peripheral hematocrit as red cell equilibration should have occurred between the spleen and the circulating pool. Allsop et al. (1992) reported no difference in plasma volume changes, following 60 minutes of recovery from supra-maximal exercise, between estimations using hemoglobin and hematocrit changes (Dill and Costill, 1974) and direct measurement with radiolabelled albumin. However the use of only ^{125}I -labelled human serum albumin, for both pre- and post-exercise measures, would have resulted in an overestimation of the decrease in plasma volume, as leakage of albumin from the intravascular space is accelerated during exercise. For accurate quantification another isotope (^{131}I -labelled human serum albumin was used in the present study) is required to measure post-exercise plasma volume.

The red blood cells that were released from the spleen during exercise did not affect the measurement of plasma volume. Whether calculated using hemoglobin and hematocrit (Dill and Costill, 1974), with F-cell correction (Harrison et al, 1982), or with hematocrit alone (van Beaumont, 1972), the decrease in plasma volume did not differ from the measurements obtained using the more direct method of radio-labelled albumin.

Spleen volume was significantly correlated with hematocrit level ($n = 18$, $r = -0.49$, $p = 0.038$), which indicates that the red blood cells in the spleen are in dynamic equilibrium with the circulating pool of red blood cells. However the change in spleen volume was not correlated with the increase in circulating hematocrit ($n = 9$, $r = -0.04$, $p = 0.91$). The small size and volume of the spleens in the present study were not

responsible for the lack of an effect on circulating hematocrit, as the relative change in spleen volume was also not correlated with the increase in hematocrit ($n = 9$, $r = -0.18$, $p = 0.64$). Interestingly, the change in plasma volume could not account for the change in hematocrit either ($n = 9$, $r = -0.50$, $p = 0.17$), although the small sample size undoubtedly affects the lack of a statistically significant correlation.

The effect of splenic contraction on leukocytosis

The exercise-induced leukocytosis, as observed in the present study, was initially reported in the early 20th century (Larrabee, 1902). Since that date the role of the spleen in exercise-induced leukocytosis has been debated.

The spleen is capable of holding approximately one-third of the total circulating pool of platelets (Heyns et al, 1980; Peters et al, 1980) and granulocytes (Peters et al, 1985). The present study showed a 28% increase in circulating platelets following exercise and a 33, 119, and 477% increase in the circulating granulocytes (eosinophils, neutrophils, and basophils, respectively). Since levels of platelets and white cells in the spleen were not undertaken, the exact location of the release into the active circulation cannot be determined from the present data. However platelets are known to be pooled solely in the spleen whereas granulocytes are predominantly released from the bone marrow, lung and marginal zone in the blood vessels (Benschop et al, 1996).

The present study indicated a 189% increase in circulating lymphocytes immediately following maximal exercise. Many have suggested a primary role of the spleen in the increase in lymphocytes following maximal exercise (Knudsen et al, 1995; Nielsen et al, 1997; van Tits et al, 1990), while others have indicated the process is

independent of the spleen (Baum et al, 1996; Iverson et al, 1994). The conflict of opinion has been suggested to result from the ability of other organs to replace the spleen, following removal, in its cell sequestering function (Iverson et al, 1994). Apart from exercise, lymphocytes have also been mobilized following catecholamine administration (Gader and Cash, 1975), predominantly norepinephrine, via β -adrenergic receptors present on the lymphocyte cell. α -adrenergic receptors are responsible for the increase in granulocytes induced by exercise, catecholamine administration, or acute psychological stress (Benschop et al, 1996). Also noradrenergic fibres present in splenic smooth muscle cells, located in the white pulp and sinuses (Pinkus et al, 1988), may be expected to cause contraction of the splenic parenchyma with an increase in circulating leukocytes and platelets.

Summary

The release of red blood cells from the spleen had no significant effect on circulating red cell volume and therefore peripheral hematocrit. This resulted in the indirect calculation of plasma volume being equivalent to radio labelled isotope measurements. The role of the spleen in storing red blood cells during periods of inactivity or low stress may be important in reducing the viscosity of the blood, it does not however appear to have a role in the hemoconcentration observed following acute intense exercise.

CHAPTER FOUR

METHODOLOGICAL LIMITATIONS

An accurate determination of spleen size and volume via radio nucleotide imaging is fraught with difficulties. The anatomical position of the spleen means that isolated views, without other organs overlapping is often impossible. Anterior and posterior views were chosen in the present study after a comparison with oblique views revealed no significant differences. Radioactivity was captured simultaneously over a 2 minute period from the anterior and posterior views. The majority of research, involving technetium labelling of red cells has utilised only a posterior scan of the spleen (Allsop et al, 1992; Flamm et al, 1990; Froelich et al, 1988; Laub et al, 1993; Sandler et al, 1984). Wolski (1998) imaged from both anterior and posterior planes but the images were obtained during consecutive 2 minute periods rather than simultaneously.

In measuring spleen size a region of interest was drawn around the entire spleen. In some individuals this required an estimation of the boundaries of the spleen that overlapped other organs. Any influence of an overlapping organ was removed in the volume calculations. While this resulted in smaller changes, it eliminates the effect of fluctuating activity in other organs i.e. the heart, influencing the spleen volume.

Volume calculations are problematic as a third dimension, depth, is impossible to obtain due to other visceral organs being included in the lateral view. The weighting of the anterior and posterior activity counts evenly is erroneous as the spleen is predominantly a posterior organ. However without a lateral view and the inter-subject

variability in spleen dimensions, the ability to correctly weight each subject's anterior and posterior counts separately is lost.

The measurement of a large number of hematological variables, as undertaken in the present study, required the removal of a significant volume of blood from each subject during the course of the experiment (see Appendix E for details). The majority of the blood removal was required for the determination of plasma catecholamine concentrations. This volume would not have interfered with the determination of pre- to post-exercise responses for red cell, plasma, total blood or spleen volumes, as the catecholamine samples were drawn during the recovery from the maximal exercise test.

CHAPTER FIVE

GENERAL SUMMARY AND CONCLUSIONS

The spleen serves a wide range of functions. Although in mammals it is not irreplaceable, as evidenced by the normal existence of asplenic individuals. The spleen is an organ of stress and one of its roles as a stress organ is in oxygen transport and blood volume homeostasis. The human spleen contains only a small percentage of the total red cell volume and cannot be regarded as providing clinically significant autotransfusion of red cells during times of stress as in some mammals. Regardless, the human spleen can still effectively release this small volume of red cells into circulation.

Chapter two attempted to determine the mechanism responsible for splenic volume reduction observed in humans during exercise. *In vivo* quantification of the spleen's response to acute exercise is rare and small sample sizes often limit significant observations. The present investigation was twice as large as previous radionuclide studies and allowed for statistically significant physiological findings.

No differences were observed in the decrease in spleen size and volume between the 5, 10, or 15 minute exercise periods at 60% VO_2 max. This is the first study to measure the change in splenic radioactivity in response to exercise of different duration. The findings support the theory that the spleen is actively contracting due to an intensity dependent signal.

An inverse relationship between exercise intensity and splenic radioactivity has been observed in humans (Flamm et al, 1990; Froelich et al, 1988; Laub et al, 1993; Wolski, 1998). Increased activation of the sympathetic nervous system is associated with

increased exercise intensity. The present investigation and the earlier work by Laub et al. (1993) are the only two *in vivo* studies to attempt to measure spleen volume change and adrenergic activity in humans. A significant semi-logarithmic correlation between plasma catecholamine concentrations and spleen volume was observed following the maximal exercise challenge. Justification for a causal relationship are warranted, when comparisons are made to animal studies where infusion of catecholamines induced splenic contraction and increased circulating red cell mass (Hochachka et al, 1995; Ojiri et al, 1993). The identification of α -adrenoreceptors in the human splenic vasculature (Ayers et al, 1972) and the role these have been shown to have in contraction of the seal (Cabanac et al, 1997) and dog (Ojiri et al, 1993) spleen in response to catecholamine stimulation indicate a similar mechanism maybe responsible for the human exercise response.

Chapter three investigated a more applied function of the human spleen. The effect of splenic volume reduction on circulating red cell volume, peripheral hematocrit and indirect assessment of changes in plasma volume was determined. At rest, the human spleen has been reported to account for approximately 10% of the total red cell volume (Wolski, 1998). The release of the stored red cells from the spleen has been suggested to contribute 25% of the increase in exercising hematocrit (Laub et al, 1992; Wolski, 1998). This would introduce substantial errors into indirect assessment of plasma volume reduction if proven to be correct.

The release of 50 mls of red blood cells from the spleen during exercise accounted for a 2.2% increase in the total circulating red cell volume. This was substantially smaller

than the 10% increase indicated by Wolski (1998), but comparable to the 2.5% increase in circulating red cell volume reported by Allsop et al. (1988).

The comparison between the indirect methods' of assessing plasma volume reduction and the more direct method of radio labelled albumin based upon the work of Laub et al. (1992) and Wolski (1998) was expected to show significant differences. Whether calculated using hemoglobin and hematocrit (Dill and Costill, 1974), with F-cell correction (Harrison et al, 1982), or with hematocrit alone (van Beaumont, 1972), the decrease in plasma volume did not differ statistically from the measurements obtained using the more direct method of radio labelled albumin. The lack of a significant finding was the result of the spleen having only a minor influence on the circulating red cell volume. The two previous studies, which suggested that the spleen could play a major part in the indirect plasma volume measurements, had significant methodological flaws that seriously influenced their conclusions.

The release of red cells from the mammalian spleen during exercise enables an increase in oxygen carrying capacity of the blood when high cardiac outputs offset the increased viscosity. The small volume of red cells released in comparison to numerous animal models indicates that this is not a primary function of the human spleen but possibly a secondary response to the increase in inflammatory mediators that are released during periods of stress.

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APPENDIX A

**INDIVIDUAL DATA:
SPLENIC VOLUME REDUCTION**

TABLE 5. Individual subject data for age, height, mass, and maximal oxygen consumption ($\text{VO}_{2\text{ max}}$)

Subject	Age (yrs)	Height (cm)	Mass (kg)	$\text{VO}_{2\text{ max}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)
1	28	170	68	59.5
2	22	174	85	49.9
3	21	165	64	46.8
4	22	178	79	47.7
5	29	176	69	52.2
6	26	195	81	68.0
7	24	181	85	53.9
8	27	171	69	55.2
9	24	183	78	57.3
MEAN	24.9	176.3	75.0	54.1
SEM	1.0	3.0	2.8	2.3

TABLE 6. Individual subject data for spleen volume (ml) pre and post 5, 10, and 15 minutes cycling at 60% $\text{VO}_{2\text{ max}}$.

Subject	5 min		10 min		15 min	
	pre	post	pre	post	pre	post
1	97.9	76.5	71.2	53.5	92.1	60.5
2	109.2	84.2	96.2	75.4	93.6	74.7
3	133.7	115.8	140.0	100.1	120.2	102.3
4	71.6	58.4	66.0	55.7	76.0	60.6
5	74.2	53.8	73.5	51.2	76.7	48.5
6	128.6	70.8	141.1	74.4	127.3	61.5
7	47.5	23.3	52.6	31.3	51.8	18.6
8	56.5	40.5	65.9	43.8	67.3	37.6
9	61.9	46.2	65.6	46.6	57.3	34.0
Mean	86.8	63.3	85.8	59.1	84.7	55.4
SEM	10.6	9.1	11.0	6.9	8.7	8.2

TABLE 7. Individual subject data for heart rate (beats·min⁻¹) pre-exercise and averaged across the 5, 10, and 15 minutes of cycling at 60% VO_{2 max}.

Subject	5 min		10 min		15 min	
	pre	average	pre	average	pre	average
1	70	141	64	141	65	150
2	60	150	74	165	78	161
3	64	135	60	133	59	136
4	72	129	68	125	72	134
5	63	148	69	151	60	149
6	70	135	84	147	76	147
7	98	152	76	140	89	155
8	50	141	52	155	63	156
9	64	133	69	137	65	135
Mean	67.9	140.4	68.4	143.6	69.5	147.0
SEM	4.36	2.75	3.06	4.08	3.22	3.30

TABLE 8. Individual subject data for oxygen consumption (VO₂, ml·kg⁻¹·min⁻¹) pre-exercise and averaged across the 5, 10, and 15 minutes of cycling at 60% VO_{2 max}.

Subject	5 min		10 min		15 min	
	pre	average	pre	average	pre	average
1	7.4	35.9	6.6	37.1	5.9	36.6
2	4.9	28.3	4.4	29.2	6.1	28.3
3	5.1	29.2	4.2	28.1	5.4	30.3
4	5.7	26.2	5.9	28.1	6.7	27.0
5	5.8	30.6	6.4	30.9	5.0	31.0
6	8.9	38.2	5.2	41.1	7.2	42.3
7	5.2	32.1	3.6	31.9	3.7	32.3
8	4.4	32.6	3.9	33.7	4.5	33.2
9	5.8	33.5	7.7	34.6	6.8	33.0
Mean	5.90	31.85	5.34	32.75	5.69	32.68
SEM	0.47	1.26	0.47	1.46	0.38	1.53

TABLE 9. Individual subject data for power output (W) averaged across the 5, 10, and 15 minutes of cycling at 60% $\text{VO}_{2\text{ max}}$.

Subject	5 min	10 min	15 min
1	169	171	168
2	189	196	190
3	112	118	113
4	135	122	131
5	147	147	143
6	233	245	238
7	200	195	200
8	174	161	159
9	190	190	181
Mean	172.1	171.5	169.3
SEM	12.22	13.44	12.67

TABLE 10. Individual subject data for spleen volume (ml) at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion.

Subject	REST	0 min	10 min	20 min	30 min	40 min	50 min	60 min
1	88.7	33.3	53.6	69.8	81.6	87.9		100.0
2	99.7	46.4	67.7	89.7	94.6	97.2	95.5	101.9
3	131.3	49.7	113.2	146.7	149.1	151.1	143.1	151.9
4	71.2	27.1	51.4	66.5	71.7	78.6	84.4	85.1
5	74.8	26.1	49.0	57.6	60.6	64.1	72.0	75.1
6	132.3	48.2	82.2	109.5	121.2	130.5	131.2	141.9
7	50.7	15.3	20.3	34.6	41.7	46.1	45.8	49.4
8	63.2	43.9	65.2	69.8	83.5	77.9		84.1
9	61.6	37.2	54.8	63.8	65.3	64.9	70.8	74.5
Mean	85.94	36.35	61.92	78.66	85.49	88.70	91.85	95.99
SEM	9.93	3.95	8.51	10.94	10.90	11.13	11.55	10.94

TABLE 11. Individual subject data for spleen volume, as a percentage of rest (%) every 10 minutes during an hour recovery from an incremental cycle to exhaustion.

Subject	REST	0 min	10 min	20 min	30 min	40 min	50 min	60 min
1	0	-62.5	-39.6	-21.4	-8.0	-0.9		12.7
2	0	-53.4	-32.1	-10.0	-5.1	-2.4	-4.2	2.2
3	0	-62.2	-13.8	11.7	13.6	15.1	9.0	15.7
4	0	-61.9	-27.8	-6.6	0.8	10.3	18.5	19.5
5	0	-65.1	-34.5	-23.0	-19.0	-14.3	-3.7	0.5
6	0	-63.5	-37.9	-17.2	-8.4	-1.4	-0.8	7.2
7	0	-69.8	-60.0	-31.8	-17.7	-8.9	-9.5	-2.4
8	0	-30.6	3.1	10.3	32.0	23.2		33.0
9	0	-39.6	-11.0	3.7	6.1	5.3	15.0	20.9
Mean	0	-56.5	-28.2	-9.4	-0.6	2.9	3.5	12.2
SEM	0	4.4	6.2	5.1	5.4	3.9	3.6	3.8

TABLE 12. Individual subject data for plasma norepinephrine concentration ($\text{nM}\cdot\text{L}^{-1}$) at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion.

Subject	REST	0 min	10 min	20 min	30 min	40 min	60 min
1	2.95	12.62	6.03	4.84	3.38	4.60	2.42
2	2.87	25.31	4.86	2.23	1.86	1.90	1.71
3	1.05	22.47	3.37	1.88	1.29	1.25	1.08
4	0.32	11.66	2.27	0.96	0.73	0.84	1.38
5	0.95	15.41	4.46	2.16	2.54	1.28	2.19
6	1.36	60.17	3.87	1.26	1.19	0.84	1.28
7	1.04	67.60	13.56	6.26	4.86	3.08	2.52
8	1.05	27.26	3.09	1.57	1.06	0.99	1.28
9	2.00	19.27	1.89	1.41	1.36	1.36	1.39
Mean	1.51	29.09	4.82	2.51	2.03	1.79	1.69
SEM	0.30	6.84	1.17	0.60	0.45	0.42	0.18

TABLE 13. Individual subject data for plasma epinephrine concentration ($\text{nM}\cdot\text{L}^{-1}$) at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion.

Subject	REST	0 min	10 min	20 min	30 min	40 min	60 min
1	0.16	0.90	0.18	0.38	0.33	0.30	0.14
2	0.46	6.76	0.22	0.10	0.11	0.11	0.10
3	0.07	2.20	0.10	0.11	0.10	0.08	0.08
4	0.09	0.77	0.24	0.10	0.11	0.08	0.16
5	0.13	2.75	0.09	0.07	0.20	0.09	0.00
6	0.09	7.63	0.49	0.28	0.22	0.25	0.12
7	0.14	3.35	0.17	0.08	0.07	0.07	0.00
8	0.00	3.00	0.11	0.16	0.23	0.12	0.09
9	0.00	1.14	0.21	0.12	0.00	0.00	0.00
Mean	0.13	3.17	0.20	0.16	0.15	0.12	0.08
SEM	0.05	0.82	0.04	0.04	0.03	0.03	0.02

APPENDIX B

INDIVIDUAL DATA: SPLENIC SIZE REDUCTION

TABLE 14. Individual subject data for spleen size (pixels) pre and post 5, 10, and 15 minutes cycling at 60% $\text{VO}_{2\text{ max}}$.

Subject	5 min		10 min		15 min	
	pre	post	pre	post	pre	post
1	1271	1058	1256	1071	1360	994
2	1236	1232	1269	1107	1270	1101
3	1450	1318	1244	1275	1452	1396
4	1121	1193	1158	1029	1103	1117
5	1154	959	1120	855	1173	957
6	1429	1049	1268	985	1367	857
7	854	712	922	683	893	643
8	937	777	1012	818	1039	679
9	1334	1127	1336	1217	1308	978
Mean	1198	1047	1176	1004	1218	969
SEM	68	68	45	64	60	77

TABLE 15. Individual subject data for spleen size (pixels) at rest and every 10 minutes during an hour recovery from an incremental cycle to exhaustion.

Subject	REST	0 min	10 min	20 min	30 min	40 min	50 min	60 min
1	1256	617	752	798	1081	986		914
2	1236	863	1020	1151	1203	1183	1059	1254
3	1452	756	1274	1575	1667	1490	1498	1534
4	1158	605	885	893	1115	1122	1108	967
5	1173	656	1048	1003	1005	1035	1187	1097
6	1429	891	1205	1262	1546	1463	1467	1512
7	922	466	604	728	719	771	733	778
8	937	582	906	997	973	975		1140
9	1336	611	863	1231	1264	1269	1269	1319
Mean	1211	672	951	1071	1175	1144	1189	1168
SEM	63	46	70	88	97	78	87	87

NB. 1 pixel = 0.24cm^2

APPENDIX C

INDIVIDUAL DATA: THE SPLEEN AND THE CIRCULATING BLOOD

TABLE 16. Individual subject data for plasma volume (ml) pre- and post-exercise.

Subject	Pre	Post	% Change
1	3000	2700	-10.0
2	3755	3020	-19.6
3	2965	1995	-32.7
4	3730	3055	-18.1
5	2970	2315	-22.1
6	4320	3790	-12.3
7	3390	2825	-16.7
8	3380	2780	-17.8
9	4070	3590	-11.8
Mean	3508.89	2896.67	-17.88
SEM	164.91	187.37	2.27

TABLE 17. Individual subject data for plasma volume relative to body mass (ml·kg⁻¹) pre- and post-exercise.

Subject	Pre	Post
1	44.12	39.71
2	44.02	35.40
3	46.47	31.27
4	47.22	38.67
5	42.86	33.41
6	53.07	46.56
7	40.02	33.35
8	48.99	40.29
9	52.18	46.03
Mean	46.55	38.30
SEM	1.44	1.83

TABLE 18. Individual subject data for red cell volume (ml) pre- and post-exercise.

Subject	Pre	Post	% Change
1	2020	1935	-4.2
2	2290	2365	3.3
3	1900	1870	-1.6
4	2420	2505	3.5
5	1950	1930	-1.0
6	3030	2980	-1.7
7	2345	2235	-4.7
8	1835	1730	-5.7
9	3135	3015	-3.8
Mean	2325	2285	-1.77
SEM	158.91	158.20	1.11

TABLE 19. Individual subject data for red cell volume relative to body mass ($\text{ml}\cdot\text{kg}^{-1}$) pre- and post-exercise.

Subject	Pre	Post
1	29.71	28.46
2	26.85	27.73
3	29.78	29.31
4	30.63	31.71
5	28.14	27.85
6	37.22	36.61
7	27.69	26.39
8	26.59	25.07
9	40.19	38.65
Mean	30.76	30.20
SEM	1.59	1.54

TABLE 20. Individual subject data for total blood volume (ml) pre- and post-exercise.

Subject	Pre	Post	% Change
1	5020	4635	-7.7
2	6045	5385	-10.9
3	4865	3865	-20.6
4	6150	5560	-9.6
5	4920	4245	-13.7
6	7350	6770	-7.9
7	5735	5060	-11.8
8	5215	4510	-13.5
9	7205	6605	-8.3
Mean	5834	5182	-11.73
SEM	315.18	335.59	1.36

TABLE 21. Individual subject data for total blood volume relative to body mass ($\text{ml} \cdot \text{kg}^{-1}$) pre- and post-exercise.

Subject	Pre	Post
1	73.82	68.16
2	70.87	63.13
3	76.25	60.58
4	77.85	70.38
5	71.00	61.26
6	90.29	83.17
7	67.71	59.74
8	75.58	65.36
9	92.37	84.68
Mean	77.30	68.50
SEM	2.85	3.14

TABLE 22. Individual subject data for plasma volume reduction, immediately following the incremental cycle to exhaustion, as a percentage of pre-exercise (%), measured with radiolabelled albumin (I-RISA), and calculated with hemoglobin (Hb) and hematocrit (Hct) changes with and without F-cell correction.

Subject	I-RISA	Hb & Hct	Hct	Hb & Hct with F-cell correction
1				
2	19.6	15.1	14.5	12.7
3	32.7	25.7	26.4	22.7
4	18.1	25.4	26.0	22.7
5	22.1	23.9	24.5	21.0
6	12.3	14.3	15.6	11.9
7	16.7	19.2	20.6	16.6
8	17.8	23.6	25.2	21.1
9	11.8	11.8	12.3	9.6
Mean	18.9	19.9	20.6	17.3
SEM	2.41	1.83	1.90	1.75

NB. Subject 1 results were removed as post-exercise plasma volume measurements were not conducted until 20 minutes following the maximal exercise protocol.

NB. In Tables 23 – 25, 27 – 33 the results for Subject 1 are missing as a standard blood count was not completed for this individual.

TABLE 23. Individual subject data for white blood cell count ($\times 10^9 \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	5.2	11.8
3	6.0	12.5
4	5.9	14.6
5	4.7	10.3
6	4.3	8.5
7	4.8	16.8
8	4.4	9.4
9	4.5	9.8
Mean	4.975	11.709
SEM	0.221	0.944

TABLE 24. Individual subject data for red blood cell count ($\times 10^{12} \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	4.91	5.33
3	4.95	5.81
4	4.68	5.45
5	4.86	5.64
6	4.96	5.37
7	4.73	5.25
8	4.15	4.94
9	4.77	5.16
Mean	4.751	5.369
SEM	0.088	0.094

TABLE 25. Individual subject data for hemoglobin concentration ($\text{g}\cdot\text{L}^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	146	118
3	150	125
4	138	146
5	151	103
6	148	85
7	146	168
8	129	94
9	138	98
Mean	143.3	161.5
SEM	2.52	3.24

TABLE 26. Individual subject data for hematocrit ($\text{L}\cdot\text{L}^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1	0.402	0.454
2	0.438	0.477
3	0.429	0.505
4	0.405	0.479
5	0.433	0.503
6	0.430	0.472
7	0.417	0.474
8	0.372	0.442
9	0.409	0.441
Mean	0.417	0.474
SEM	0.007	0.008

TABLE 27. Individual subject data for mean cell volume (fL) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	89.1	89.3
3	86.6	86.9
4	86.8	87.8
5	89.0	89.2
6	86.7	87.8
7	88.1	90.2
8	89.7	89.4
9	85.6	85.4
Mean	87.7	88.3
SEM	0.492	0.527

TABLE 28. Individual subject data for red cell distribution width pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	13.1	13.4
3	12.6	12.8
4	13.1	14.0
5	12.7	12.6
6	12.5	12.8
7	12.8	13.1
8	12.1	12.4
9	13.2	13.4
Mean	12.8	13.1
SEM	0.12	0.17

TABLE 29. Individual subject data for platelet count ($\times 10^9 \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	248	298
3	195	293
4	261	296
5	210	290
6	195	236
7	229	270
8	186	270
9	265	343
Mean	224	287
SEM	10.5	10.2

TABLE 30. Individual subject data for neutrophils ($\times 10^9 \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	3.1	5.5
3	3.5	6.3
4	3.5	7.9
5	2.9	4.8
6	2.1	3.5
7	2.7	10.4
8	1.9	4.5
9	2.0	4.6
Mean	2.713	5.938
SEM	0.217	0.747

TABLE 31. Individual subject data for basophils ($\times 10^9 \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	0.0	0.1
3	0.0	0.1
4	0.0	0.0
5	0.0	0.0
6	0.0	0.1
7	0.0	0.1
8	0.0	0.1
9	0.1	0.1
Mean	0.013	0.075
SEM	0.012	0.015

TABLE 32. Individual subject data for lymphocytes ($\times 10^9 \cdot L^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	1.4	4.6
3	1.7	4.8
4	1.4	5.1
5	1.3	4.6
6	1.6	4.0
7	1.5	5.0
8	1.7	3.9
9	2.0	4.4
Mean	1.575	4.550
SEM	0.075	0.145

TABLE 33. Individual subject data for monocytes ($\times 10^9 \cdot \text{L}^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1		
2	0.6	1.4
3	0.4	0.8
4	0.6	1.1
5	0.4	0.9
6	0.5	0.9
7	0.5	1.3
8	0.3	0.6
9	0.3	0.6
Mean	0.45	0.95
SEM	0.04	0.10

TABLE 34. Individual subject data for plasma viscosity ($\text{mPa} \cdot \text{s}^{-1}$) pre- and post-exercise.

Subject	Pre-exercise	Post-exercise
1	1.176	1.320
2	1.086	1.413
3	1.157	1.387
4	1.232	1.387
5	1.182	1.344
6	1.156	1.320
7	1.208	1.366
8	1.144	1.250
9		
Mean	1.166	1.353
SEM	0.017	0.019

NB. Subject 9 results are missing due to technical difficulties experienced during the determination of the measurement.

APPENDIX D

INDIVIDUAL DATA: ELUTION FACTORS

A 1 ml sample of red cells was obtained during each of the post-exercise spleen activity counts in part one and at 40 and 50 minutes following the incremental ride to exhaustion. The decay-corrected count of activity in the known volume (1 ml sample) was used to determine an elution factor. The spleen volume was then calculated from the decay-corrected geometric mean spleen activity counts of the anterior and posterior images at each of the measurement times, using the elution factor.

TABLE 35. Individual subject data for elution factors determined for calculating spleen volume

Subject	Equation	r
1	$Y = 703.76 - 64.998x$	0.88
2	$Y = 510.58 - 18.726x$	0.67
3	$Y = 866.85 - 65.168x$	0.83
4	$Y = 635.58 - 39.204x$	0.94
5	$Y = 955.47 - 51.436x$	0.99
6	$Y = 468.31 - 23.148x$	0.60
7	$Y = 834.18 - 47.201x$	0.97
8	$Y = 979.24 - 107.32x$	0.93
9	$Y = 785.25 - 55.784x$	0.63

Y = activity count of 1 ml of red blood cells; x = time

APPENDIX E

PHLEBOTOMY

TABLE 36. Volume of blood removed per procedure.

Sample	Size	Quantity	Total
Red Cell Volume	7 ml - residual	2 (pre & post-ex)	14
	7 ml - sample	2 (pre & post-ex)	14
Plasma Volume	7 ml - residual	2 (pre & post-ex)	14
	3 x 7 ml - samples	2 (pre & post-ex)	42
Spleen Volume	15 ml - labelling	1	15
	10 ml - returned	1	-10
	7 ml - sample	5	35
Standard Blood Count	5 ml	2	10
Plasma Viscosity	7 ml	2	14
Plasma Catecholamines	2 x 5 ml	7	70
Waste	0.5 ml	22	11
Total			229

APPENDIX F

EXAMPLES OF REGIONS OF INTEREST

FIGURE 11. Anterior and posterior spleen regions of interest prior to exercise.

FIGURE 12. Anterior and posterior spleen regions of interest immediately following the incremental cycle to exhaustion.