The Relationships Between Systemic Hypertension, Proteinuria, and Renal Histopathology in Clinically Healthy Retired Racing Greyhounds

Thesis
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Abstract

Background: As a result of the increasing popularity of Greyhounds as pets, veterinarians are likely to evaluate them more frequently in practice. Clinical experience has identified an increased frequency of certain disease syndromes, including renal disease, more specifically protein losing nephropathy and hypertension. Greyhounds are considered a model for primary hypertension in people. In human medicine, identifying early makers of renal disease such as microalbuminuria and hypertension, and subsequent control, is associated with a decreased rate of progression and increased survival.

Objectives: The objective of this study was to evaluate renal function and hemodynamic status in a population of clinically healthy retired racing Greyhounds (RRG’s). Our goals were to evaluate this group of dogs with respect to blood pressure, but also for any indication of early renal disease based on markers of renal damage including microalbuminuria, overt proteinuria, altered fractional excretion of electrolytes, and structural changes based on histopathology.

Animals: Forty-eight clinically healthy RRG’s that were presented in 2007 and 2008 for participation in a 3rd year student castration and ovariohysterectomy lab.

Methods: Immediately upon presentation, all dogs underwent a complete physical examination, urinalysis and urine profile, urine protein-creatinine ratio, urine culture, HESKA ERD microalbuminuria, blood pressure via Doppler
ultrasonographic and oscillometric method, and pulse rate via digital palpation and the oscillometric monitor. On day 3 of hospitalization, all blood pressure and heart rate testing was repeated using identical methods. Twenty of the female dogs that underwent an exploratory laparotomy for ovariohysterectomy also had renal biopsies performed. Renal tissue was evaluated with light microscopy, immunofluorescence, and electron microscopy.

**Results:** Forty-seven dogs were enrolled in this study. Twenty-six were considered hypertensive (BP > 165mmHg) and 21 normotensive (BP < 165mmHg). There was a statistically significant difference between the systolic BP in these 2 groups. There was good correlation between the Doppler and oscillometric methods on the same day, and between each method on different days. There were no statistically significant differences between the 2 methods on the same day, or between each method on different days. Twenty-two of the dogs were negative for microalbuminuria and twenty-five were positive. There was a statistically significant association between the presence of hypertension and the presence of microalbuminuria, p<0.001. There were no significant associations between hypertension and UPC, fractional excretion of electrolytes, or renal histopathology. There were no significant associations between the presence of microalbuminuria and fractional excretion of electrolytes or renal histopathology. Overall histopathologic changes were mild and nonspecific.

**Conclusions and Clinical Relevance:** There was a high incidence of hypertension in these Greyhounds, and the Doppler and oscillometric methods are both
appropriate for assessing blood pressure status in this breed. Stress did not appear to artificially raise the BP in these dogs as heart rate was generally low and there was no difference between heart rate in the normotensive and hypertensive Greyhounds. Additionally, no change in blood pressure was identified after a 48 hr acclimation period. There was also a fairly high incidence of microalbuminuria in these Greyhounds, and a significant association between the presence of hypertension and microalbuminuria. This may indicate a very early sign of renal dysfunction, and may be secondary to uncontrolled primary hypertension. Further study is warranted to evaluate whether the presence of microalbuminuria is associated with an increased risk of progression to overt proteinuria or chronic kidney disease, and whether blood pressure control impacts this progression.
Dedicated to my parents, Steve and Nickie Surman, and may Aunt Phyllis and Uncle Phil for all their years of support.
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Chapter 1

Hypertension

1.1: Definition of Hypertension

Hypertension is defined as the presence of systolic, mean, or diastolic blood pressure (BP) above the upper range of normal acceptable BP. Systemic hypertension has long been known to be a serious risk factor for end-organ damage, most notably affecting the kidneys, eyes, brain, and cardiovascular system. There is no one cause of systemic hypertension, and it generally can be categorized into 1 of 3 types: a) that caused by measurement artifact (white coat hypertension), b) that associated with other systemic diseases (secondary hypertension), or c) that found in the absence of any other associated diseases (primary or essential hypertension).

1.2: Classification of Hypertension

White coat hypertension is defined by the identification of persistently high BP as measured in a hospital or office setting, with normal self-measured or ambulatory BP. White coat hypertension may occur in children and adults, as well as in veterinary patients. The precise mechanism of white coat hypertension is unknown; however it is thought to occur as a consequence of the effects of stress or excitement on the autonomic nervous system, primarily the sympathetic nervous system. Consequences of white coat hypertension include falsely identifying normotensive individuals as hypertensive and instituting unnecessary treatment. A study in cats using a surgically-implanted telemetry device showed a significant increase in BP (17.6 ±/-.
1.5mmHg) compared to the 24-hour average when cats were subjected to a veterinary clinic setting. This change was highest at the beginning of the visit with the magnitude of increase diminishing over time, but not disappearing completely.\(^2\) In a study of clinically normal dogs in which BP was measured at home and in the clinic, no significant difference was found between these locations. Heart rate was significantly higher in the clinic as compared to the home environment.\(^3\) Another study comparing at-home and in-clinic measurements found that all 14 dogs had a normal BP at home, compared to 5/14 that were classified as hypertensive in the clinic. The differences between these measurements were dependent of the limb used, with metatarsal measurements showing more variability than metacarpal measurements.\(^4\) These findings support an effect of stress or anxiety on BP in dogs, with a more substantial effect on heart rate than BP. Overall, the presence of a “white coat effect” on BP measurements in veterinary medicine has convincingly been shown; however the effects of anxiety and stress on BP are not predictable. Some animals show a very pronounced increase in BP whereas others show no change or in some situations even a decrease.\(^2\)

The clinical relevance of the white coat effect is controversial. In people, several cross-sectional studies have shown that white coat hypertension is likely a true form of hypertension, intermediate between normotension and true sustained hypertension. Studies of people with white coat hypertension have shown as strong an association with adverse cardiovascular events as observed in those people with sustained hypertension. People with white coat hypertension also are documented to have similar changes in left ventricular function, arterial compliance, elasticity, and vascular stiffness as do people with sustained hypertension, compared to normotensive individuals.\(^5\) In
addition, left ventricular mass, carotid intima-media thickness, presence of hypertensive retinopathy, and severity of microalbuminuria in people with white coat hypertension all were intermediate between results in normotensive subjects and those with sustained hypertension. For these reasons, some advocate treatment of white coat hypertension in people with other risk factors for development or progression of renal disease. Regardless of treatment recommendations, identification of white coat hypertension in humans warrants more aggressive monitoring.

Primary (also called essential or idiopathic) hypertension is defined as high BP for which no underlying cause can be identified. Essential hypertension accounts for 95% of all cases of hypertension in humans, and although reported in dogs, the prevalence seems to be much lower than that seen in people. Reported cases in dogs are mostly limited to individuals bred specifically to establish a colony of dogs with this syndrome. This was first shown in the mating of a mixed breed male with primary hypertension and a female Huskie with primary hypertension. This produced 24 offspring of which 12 were considered hypertensive by 24 months of age. Secondary causes of hypertension were ruled out in all 12 dogs based on serum chemistries, electrolytes, glomerular filtration rate (GFR), plasma rennin, aldosterone, and catecholamines, leading to the diagnosis of primary hypertension. This outcome has also been achieved in Huskies after a female with primary hypertension was bred repeatedly over a 5-year period. In total, this dog produced 39 offspring, 30 of which were evaluated repeatedly, and 13 of which were judged to be hypertensive. The hypertensive individuals had clinical findings to support this being true hypertension including significantly increased left ventricular wall thickness in the hypertensive as
compared to the normotensive individuals.\textsuperscript{8} Primary hypertension has been hypothesized to explain the documented higher BP seen in Greyhound dogs. Racing Greyhounds are reported to have higher arterial BP and cardiac output compared to control mixed breed dogs both awake and under light anesthesia.\textsuperscript{9,10}

Secondary hypertension involves high systemic BP associated with a concurrent clinical disease known to cause hypertension. It can also be secondary to administration of drugs known to cause increased BP. In dogs, the most common causes of secondary hypertension are acute and chronic kidney disease CKD (reported to range from 9% to 93% of cases),\textsuperscript{11,12} iatrogenic or naturally-occurring hyperadrenocorticism (80% and 73% of cases, respectively),\textsuperscript{13,14} diabetes mellitus (24 to 46% of cases),\textsuperscript{15,14} and pheochromocytoma (43 to 86% of cases).\textsuperscript{16,17} In cats, the most common causes of secondary hypertension include CKD (19 to 65% of cases),\textsuperscript{18,19} hyperthyroidism (5 to 87% of cases),\textsuperscript{20,21} and primary hyperaldosteronism (50 to 100% of cases).\textsuperscript{22,23} Drugs reported to cause hypertension include glucocorticoids, mineralocorticoids, erythropoietin, sodium chloride, phenylpropanolamine, and non-steroidal anti-inflammatory drugs (NSAIDS).\textsuperscript{24}

1.3: Blood Pressure Measurement

In veterinary medicine, BP is measured both by indirect and direct methods. Blood pressure can be measured directly, intra-arterially, or indirectly by devices that utilize a compressive cuff. Indirect methods generally are acceptable in normal or hypertensive animals, but these techniques are more likely to fail in critically ill patients,
especially those that are hypotensive. In those situations, direct methods are preferable over indirect.

Direct BP monitoring involves placement of an arterial catheter connected to a transducer. The transducer is connected directly to a monitor that indicates systolic, diastolic, and mean pressures, as well as a schematic of the pressure waveform. The dorsal metatarsal artery is the most commonly used site as a result of its superficial location, easily palpable pulse, and limited risk of hemorrhage due to the ease of compression to control bleeding. The femoral artery also can be used but its use carries a much higher risk of serious hemorrhage and requires more intensive monitoring of the catheter site. Advantages of this method include more accurate measurements, continuous monitoring, and painless collection of multiple blood samples that may be required in addition to BP monitoring. Disadvantages include technical difficulty associated with placement of arterial catheters, cost of the equipment, and necessity for well-trained staff and 24-hour monitoring. Direct arterial BP determination is also associated with higher morbidity compared with noninvasive methods. Complications include hematoma formation at the site of arterial puncture, infection, thrombosis of the artery with potential necrosis of tissues distal to the catheter, and severe hemorrhage if the catheter becomes dislodged. Direct monitoring generally is limited to critically ill patients that can benefit from continuous monitoring of BP such as animals with a high anesthetic risk or those in an intensive care unit.

Indirect methods of BP measurement include Doppler ultrasound and oscillometric methods. Indirect methods rely on an occlusive cuff, and indirect measurement of BP in a superficial artery distal to the cuff. The presence of blood flow
distal to the cuff can be identified by palpation of a pulse, auscultation, ultrasonographic detection by Doppler, and measurement of cuff inflation pressure oscillations. Cuffs are inflated to completely occlude the peripheral artery (recognized as loss of pulse distal to the cuff), followed by gradual release of cuff pressure allowing for an estimate of BP based on return of blood flow. Appropriate cuff size is essential and should be approximately 40% of the circumference of the limb (or tail) around which it is to be placed. Cuffs that are too large will lead to artificially low readings and too small a cuff will give falsely high readings. Ideally, cuffs should be placed on a limb that is close to heart level (the level of the right atrium is the zero mark for BP). Limbs well above the heart may give artificially low readings. Legs positioned well below the heart will give falsely high readings. The cuffs are usually marked so the appropriate portion of the cuff is placed directly over the artery. They should not be applied too tightly because doing so may occlude flow and cause inaccurate readings as well as swelling distal to the cuff.

Doppler ultrasonographic BP machines have a transmitting and receiving transducer. This method relies on the Doppler Effect to detect movement of the arterial wall or of red blood cells beneath the transducer. Within the transducer is an ultrasonic piezoelectric probe that produces high frequency energy that is transmitted into the tissue beneath the probe. The presence of blood flow triggers a change in frequency of the ultrasonic waves (i.e. a shift), transforming the reflected wave from the ultrasonic to audible range. This sound then is amplified by the Doppler machine. An inflatable cuff with an aneroid pressure gauge is used to apply pressure to a peripheral artery and the pressure is displayed by the gauge. The cuff is inflated to a pressure higher than the
estimated systolic pressure. Doing so occludes the artery, and wall motion stops so that a signal is no longer produced. The cuff is slowly deflated to a pressure below systolic pressure, a point at which a signal is once again produced, correlating to the return of blood flow; this is equivalent to the systolic pressure. The advantage of Doppler assessment is ease of measurement and the inexpensive nature of the equipment. Major limitations are the inability to discriminate sounds designating systolic and diastolic pressures, and therefore, mean pressures. As a result, the Doppler method only assesses systolic BP and can only identify systolic hypertension. In veterinary medicine, increased systolic BP alone or in combination with the increased diastolic pressure typically is identified; isolated diastolic hypertension is extremely rare.27

The oscillometric technique also utilizes an occluding cuff; however the cuff is attached to an automated transducer that measures these oscillations. The cuff again is inflated to a pressure above the estimated systolic pressure, and gradually deflated in decrements of 5 to 10 mmHg. At each decrement, the processor measures and averages the amplitude of the oscillations. The oscillations increase in amplitude as systolic pressure is approached and then rapidly decline as diastolic pressure approaches. The mean pressure is identified as the point with the lowest cuff pressure that has the greatest amplitude of oscillations.26 The oscillometric method typically is used only in dogs, both awake and under general anesthesia. The poor reliability of the oscillometric technique in cats is the result of the presence of small peripheral arteries in cats that do not generate adequate pressure oscillations to be reliably detected.27
1.4: Reliability of Blood Pressure Measurement

Direct BP measurement through an arterial catheter is the most reliable method to measure BP in domestic animals; however, the cost, difficulty obtaining them, and the associated morbidity preclude their use in veterinary practice; except in the anesthesia and critical care settings. With the increasing importance of diagnosing hypertension and monitoring BP in veterinary medicine, indirect methods are becoming common. Consequently, these methods must be accurate and precise. Recommendations in people for acceptable indirect methods have been made by the Association for the Advancement of Medical Instrumentation (AAMI), and several indirect methods have met these standards for humans. The AAMI recommends that indirect methods differ from the gold standard method (i.e., direct measurement) by less than 5 to 8 mmHg, that 95% of all indirect measurements be within 10 mmHg of the direct measurement, and that 85% of all indirect measurements be within 5 mmHg of the direct measurement. Numerous studies have evaluated the correlation between indirect and direct methods in veterinary medicine. In general, indirect methods consistently underestimate BP. In a recent study comparing oscillometric and Doppler methods with those obtained via a direct radiotelemetric device, both techniques correlated well with direct measurements, with $R^2$ values of 0.786 and 0.753, respectively. These values were significantly increased to 0.886 and 0.810, respectively, when the average of 5 consecutive measurements was used. The results did not meet the AAMI criteria; however, they support the application of these methods for BP estimation. If anything, studies have shown that, in hypertensive individuals, indirect methods, including both Doppler and oscillometric methods, underestimate BP compared to direct methods.
Repeated underestimation of blood pressure by indirect methods suggests that some animals may be more hypertensive than indirect methods indicate.

1.5: Significance of Hypertension

Systemic hypertension has long been recognized as both a cause and consequence of CKD. To complicate matters, hypertension in either setting can be self-perpetuating and lead to a progressive decline in renal function and progressive increase in BP. Unlike the situation in human medicine in which primary or essential hypertension is the most prevalent cause of hypertension, hypertension in dogs more often is secondary, the most common cause being kidney disease. Various studies have identified the prevalence of hypertension secondary to kidney disease ranging from 60% to 93% in dogs with CKD and 87% in dogs with acute renal failure. Primary hypertension in dogs is much less frequently identified, although there are isolated reports in the veterinary literature.

The exact mechanism by which renal disease triggers hypertension is unknown; however, several hypotheses have been suggested including failure to excrete normal amounts of salt and water, loss of venous capacitance due to venous stiffening, activation of the rennin–angiotensin–aldosterone system (RAAS) with associated increase in vascular resistance and salt retention, increased cardiac output secondary to anemia, and stimulation of renopressors with suppression of renodepressors and prostaglandins.

Salt and water retention is a key factor in the pathogenesis of hypertension in renal disease. In the normal kidney, increased renal perfusion leads to an increase in
salt and water excretion through an increase in glomerular capillary pressure (so-called pressure natriuresis). Ensuing dehydration is prevented by renal vasoconstriction and other mechanisms that limit fluid losses and return systemic BP to normal. As kidney disease progresses, the ability to respond appropriately to increased renal perfusion is lost, leading to progressive salt and water retention. This retentive state occurs despite increased systemic BP, and eventually leads to increased cardiac output and increased systemic vascular resistance.⁴¹

A crucial feature in the pathogenesis of hypertension is inappropriate activation of the sympathetic nervous system. This is triggered by afferent signals from the diseased kidney and the effects of these signals on the central nervous system. These afferent signals stimulate the hypothalamus leading to increased sympathetic activation. This effect has been shown in 5/6 nephrectomized rats (i.e. experimentally-induced CKD), in which the rate of secretion and turnover of norepinephrine from the posterior hypothalamus is increased compared to control rats.³⁵ In addition, concentrations of catecholamine-degrading and synthesizing enzymes are increased in people with renal failure.³⁶ In the experimental rats, rhizotomy of nerve roots at T10 – T13 prevents increased norepinephrine synthesis and turnover, systemic hypertension, and subsequent development of renal disease. Reductions in sympathetic nervous system inhibitors such as dopamine, B-endorphin, and B-lipotropin also have been documented.³⁷

The function of the RAAS is also critically important in the pathogenesis of the hypertension of renal disease. Supporting evidence includes high rennin concentrations relative to extracellular fluid volume, the presence of a direct relationship between
plasma rennin activity and systemic BP in human patients undergoing hemodialysis, and an effect of aldosterone on systemic BP and renal injury in the remnant kidney model in rats. In humans with dialysis-refractory hypertension, a response to angiotensin-converting enzyme (ACE) inhibitors or angiotensin antagonists also is seen. These results in humans have also been documented in dogs using the remnant kidney model. In a study of 7/8 renal ablation in dogs, significant increases in BUN and serum creatinine concentrations, BP, as well as plasma rennin, angiotensin I and II, and aldosterone concentrations were identified 1 month after surgery compared to control dogs. These dogs were treated with an ACE inhibitor, at which time BP, as well as angiotensin II and aldosterone concentrations decreased significantly.

Regardless of its cause, hypertension is a well-known cause of progressive renal injury. Normally, the renal microvasculature is protected from episodic or sustained hypertension by autoregulatory mechanisms that cause vasoconstriction of the pre-glomerular vasculature. This protective mechanism allows renal blood flow and glomerular hydrostatic pressure to remain fairly constant despite changes in systemic arterial pressure. This process is the result of two normal mechanisms that are intrinsic to the kidney: a myogenic reflex in the afferent arteriole and tubuloglomerular feedback. The myogenic reflex occurs in the smooth muscle of the afferent arteriole and causes this vessel to either constrict or dilate in response to changes in pressure. An increase in arterial pressure, as sensed by the afferent arteriole causes vasoconstriction whereas decreased arterial pressure leads to vasodilatation. Tubuloglomerular feedback involves the macula densa, specialized tubular cells located at the junction of the thick ascending limb of the loop of Henle and distal convoluted tubule, which senses the
composition of the tubular fluid at this point in the nephron. Increased sodium chloride concentration or transport at the macula densa is indicative of increased glomerular filtration rate (GFR) in the parent nephron whereas decreased sodium chloride concentration or transport is indicative of decreased GFR in the parent nephron. Detection of increased sodium chloride concentration or transport triggers the release of signaling molecules from the macula densa, causing afferent arteriolar constriction and a decrease in GFR in the parent nephron. This effect is thought to be mediated by adenosine. Detection of a decreased sodium chloride concentration or transport at the macula densa is interpreted as an indicator of decreased renal perfusion pressure, which triggers dilatation of the afferent arteriole and constriction of the efferent arteriole. Angiotensin II-mediated constriction of the efferent arteriole provides additional support for maintenance of GFR when renal perfusion pressure decreases.40

Under normal conditions, autoregulation occurs between a mean arterial pressure of approximately 80 mmHg and 180 mmHg, such that with any decrease below 80 mmHg, GFR and renal blood flow will begin to decrease in parallel. Above 180 mmHg, or the upper end of the autoregulatory curve, constriction of the preglomerular vessels is overcome by the high pressure, thus allowing more direct transmission of high systemic pressure into the glomerular circulation. The resultant high intraglomerular pressure can lead to glomerular injury and loss of renal function.40

In patients with chronic hypertension, high arterial pressure can lead to thickening of the walls of the larger arcuate and interlobular arteries, as well as thickening of the smaller afferent arteriole. The thickening occurs primarily in the medial and intimal layers, and is associated with muscular hypertrophy and metaplasia of
smooth muscle cells, and accumulation of additional water, acid mucopolysaccharides, and collagen. In addition, the small vessels, including the afferent arterioles, show evidence of endothelial dysfunction leading to impaired vasodilatation.

These structural changes in the renal vasculature initially are protective; however, the progressive narrowing eventually can become severe enough to cause ischemic injury to the glomeruli and tubules, resulting in glomerulosclerosis, tubular atrophy, and interstitial fibrosis. With progressive injury, intraglomerular pressure begins to directly reflect the systemic arterial pressure, rather than remaining constant. This is due to the impaired autoregulatory capacity of the vasculature. The cause of this impairment is inability of the preglomerular vessels to respond appropriately to changes in renal perfusion; both the ability to constrict and dilate are affected. Prolonged high perfusion pressure can lead to marked vasoconstriction, which can result in localized damage to the glomeruli. This damage can cause glomerular necrosis leading to microalbuminuria, which can in turn lead to the development of marked proteinuria if left untreated. This course of events has been well documented in several models of diabetic and non-diabetic nephropathies in people. A recent study of people with hypertension and proteinuria treated with antihypertensive medications alone, showed an equal reduction in BP compared to people with hypertension but without proteinuria. The GFR however decreased significantly in patients with proteinuric nephropathies, whereas GFR remained stable in the control group. In some patients, this impairment is so severe that any change in mean arterial pressure is matched by a proportional change in GFR. The result of this diminished autoregulatory capacity is that even moderate degrees of hypertension can lead to exaggerated increases in intraglomerular
pressure and subsequent renal injury. The impairment in renal autoregulation may explain why patients with CKD are more susceptible to accelerated loss of renal function if they also have uncontrolled hypertension.

The presence of systemic hypertension in people is highly associated with cardiovascular complications, progression of renal disease, and increased risk of death. For example, in diabetic patients with CKD, the presence of hypertension is associated with more rapid progression of disease, with even small changes in blood pressure leading to marked increases in the rate of decline of renal function.\textsuperscript{43} In these patients, for every 10 mmHg increase in BP, there was a 6.7% increase in the risk of developing end-stage renal disease. In another study of hypertensive patients in whom diet was manipulated to achieve BP control, patients randomized into the category with a target mean BP of < 95 mmHg had significantly less deterioration in GFR than did those randomized to a target mean BP of 110 mmHg.\textsuperscript{44} The importance of hypertension and BP control in veterinary medicine is less well defined; however, several studies support this hypothesis. An analysis of dogs with CKD showed that time to development of a uremic crisis, time to death, and magnitude of deterioration in renal function were worse in dogs classified as hypertensive than in those classified as normotensive.\textsuperscript{45} This study did not evaluate the effect of treatment of hypertension on these variables; however, studies in people with CKD have shown that BP control in hypertensive individuals does have favorable effects on survival and disease progression.

Systemic hypertension, especially primary hypertension, initially leads to an increase in GFR associated with impaired autoregulation and increased renal blood flow, and thus intraglomerular pressure. These changes can cause direct pressure-
induced damage to the renal vasculature as discussed earlier, but also can cause progressive kidney damage as a result of hyperfiltration. As functional nephrons are lost, overall kidney function is maintained by hypertrophy of the blood vessels and glomeruli as well as functional changes in preglomerular and postglomerular arteriolar resistance. These changes lead to increased glomerular capillary flow rate and capillary hydrostatic pressure in the remaining nephrons and an increase in the single nephron glomerular filtration rate (SNGFR). In a study of 3/4 and 7/8 nephrectomy in dogs, renal ablation lead to progressive increases in SNGFR, glomerular plasma flow rate, glomerular capillary pressure, transcapillary hydraulic pressure gradient, and glomerular ultrafiltration coefficient compared to controls. They also identified an increase in glomerular volume in remnant nephrons. Increased intraglomerular pressure associated with hyperfiltration has similar effects on the vasculature, glomeruli, and tubules as does systemic hypertension transmitted to the nephron, and can occur with or without systemic hypertension. Regardless, this hyperfiltration is an important factor in the progression of renal injury. Much of the evidence for the effect of hyperfiltration on progressive renal injury comes from studies on rats. In a study of partially nephrectomized rats, triple antihypertensive therapy with reserpine, hydralazine, and hydrochlorothiazide was effective at lowering systemic blood pressure, but doing so had no effect on glomerular injury as assessed by the presence of proteinuria and glomerulosclerosis. A different group of rats treated with an ACE inhibitor alone showed a reduction in systemic BP, but also decreased intraglomerular capillary pressure and ameliorated proteinuria and glomerular injury. Additional evidence for the effect of hyperfiltration on progressive renal injury, independent of systemic BP,
comes from studies evaluating dietary protein restriction. Dietary protein restriction has been shown to limit the adaptive increase in SNGFR and glomerular capillary hydrostatic pressure in numerous models of experimental renal disease in rodents. Systemic hypertension usually is unaffected by such intervention; however, glomerular injury is consistently ameliorated or halted compared to animals on normal or high protein diets. In a study on Munich-Wistar rats that underwent 11/12 nephrectomy, those rats fed a restricted protein diet maintained a normal SNGFR and lacked the glomerular structural lesions seen in rats with the same reduction in renal mass, but fed a usual protein diet. In another partial nephrectomy model of CKD in rats, dietary protein restriction maintained glomerular blood pressure at near normal levels, stabilized proteinuria, and produced minimal glomerular lesions compared to untreated rats.

Similar research has been performed in people with naturally developing CKD; however, the results are not as conclusive as in experimental CKD in rodents. The modification of diet in renal disease (MDRD) study showed a beneficial effect of blood pressure control in proteinuric patients; however, it failed to identify a conclusive beneficial effect of dietary protein restriction. The results of this are inconclusive, and although the primary analysis showed no beneficial effect, numerous secondary studies using the MDRD database have identified a possible benefit to dietary protein restriction. Many investigators interpret the initial MDRD study as flawed because of a nonlinear decline in GFR between groups, limited duration of follow-up, and variety of renal diseases included in the study. Although the results of secondary analyses cannot
be interpreted as definitive, they suggest a benefit to dietary protein restriction on progression of CKD in patients with moderate and advanced renal disease.\textsuperscript{54,55}

The process of hyperfiltration has been documented in dogs with partial renal ablation, with increases in glomerular volumes and mesangial matrix expansion indicating glomerular hypertrophy as well as increased glomerular capillary pressure and glomerular plasma flow. The effects of dietary protein restriction in dogs with partial renal ablation have yet to be established; however, one study evaluating the effect of protein restriction showed no effect on preventing the development of glomerular hyperfiltration. In this partial nephrectomy model of CKD in dogs, restriction of dietary protein to 16\% had no impact on the adaptive changes in nephron structure or function, with dogs developing similar increases in SNGFR and glomerular hyperfiltration, hypertension, and glomerular hypertrophy as those dogs fed a usual diet of 30\% protein.\textsuperscript{56,57} This study did not evaluate more severely protein restricted diets, and it is still unknown what affect if any this could have on the adaptive changes seen in CKD. More aggressive protein restriction in dogs may be associated with an increased incidence of hypoproteinemia, hypoalbuminemia, and hypercholesterolemia compared to dogs fed a moderately protein restricted diet.\textsuperscript{58}

Although protein restriction in dogs has not been shown to improve SNGFR, hyperfiltration, or hypertension, protein restricted diets in dogs reduce morbidity and mortality associated with uremia. In one study, dogs fed an 8.2\% or 17.2\% protein diet were more active, had improved hair coats, and had lower serum urea concentrations then dogs fed a usual protein diet.\textsuperscript{59} It is unlikely that this benefit is due solely to the protein restriction, as by nature, protein restricted diets are also phosphorous restricted.
In a study to discriminate the benefits of protein and phosphorous restriction, 4 diets (low protein and low phosphorous; low protein and normal phosphorous; normal protein and low phosphorous; normal protein and normal phosphorous) were fed to dogs with induced CKD. The dogs fed phosphorous restricted diets had stabilization of their GFR and longer survival times compared to controls or dogs fed protein restricted diets.\textsuperscript{60}
Chapter 2
Proteinuria

2.1: Introduction to Proteinuria

The glomerulus is a tuft of branching capillaries that serves as a filter of plasma. It is composed of capillary endothelial cells, visceral epithelial cells (podocytes), mesangial cells, mesangial matrix, and capillary basement membranes. There are three main layers that compose the glomerular filtration barrier; the fenestrated endothelium with its associated glycocalyx, the glomerular basement membrane, and the epithelial podocytes and filtration slits. This barrier selects molecules based on their size, shape and charge, almost completely excluding some and freely filtering others. A unique feature of the endothelial cells of the glomerulus is the presence of numerous large pores called fenestrae. Glomerular capillaries are covered by visceral epithelial cells (podocytes) which contain cytoplasmic foot process that cover the glomerular basement membrane (GBM). The spaces between the cytoplasmic foot processes are called slit pores, and the region between the fenestrae of the capillary endothelial cells and the slit pores of the podocytes is where filtration occurs. The slit pores acts as a size-selective filter, and the negatively-charged molecules within the diaphragm also create a charge barrier that acts to repel negatively-charged plasma proteins, such as albumin.\textsuperscript{61,62,63} Mesangial cells are located between the capillary loops, and the matrix that is produced by these cells provides structural support for the glomerulus. The GBM is located between the endothelial and visceral epithelial cells. The permselectivity of the glomerulus to proteins is largely based on charge as a result of the negatively-charged fenestrae, slit pores, and podocytes, but also based on size, shape, and sterical
conformation. The visceral epitheliu

conformation. The visceral epithelium is covered with sialoprotein, which is negatively-charged and tends to inhibit the passage of other negatively-charged macromolecules, including albumin. In general, large negatively-charged molecules are almost completely excluded from the glomerular ultrafiltrate, while smaller, positive or neutral molecules are more easily filtered.\(^6^3\)

An intact molecule of albumin has a size of 69,000 D. In a healthy kidney, molecules larger than 69,000 D are retained within the vasculature, whereas those that are smaller can pass into the urine. The normal ultrafiltrate of healthy dogs contains only 2-3 mg/dL of albumin compared with 4 g/dL albumin in plasma. In a healthy kidney, there is almost complete reabsorption of any smaller plasma proteins or positively-charged larger proteins that happen to pass through the glomerulus. Reabsorption takes place in the proximal convoluted tubule via pinocytosis, after which reabsorbed plasma proteins are broken down to amino acids that are utilized by tubular epithelial cells or returned to the bloodstream. By the time filtered proteins are reabsorbed, the albumin concentration in the urine typically is reduced to less than 1 mg/dL.\(^7^1\) When renal damage is present, alterations in hydrostatic pressure in glomerular vessels or damage to the filtration layers of the glomerulus (e.g. loss of negative charge) can cause leakage of larger molecules into the urine.

Protein can be present in urine because of pre-renal causes, primary-renal causes, or post-renal causes. Pre-renal causes include systemic hypertension, gammopathies or production of large numbers of small proteins that overwhelm the kidney’s ability for reabsorption (e.g., immunoglobulin light chains in multiple myeloma), fever, or hyperthermia. Exercise, which often has been cited as a potential pre-renal
cause of proteinuria did not cause a significant increase in urinary albumin excretion in one study. This was true for dogs regardless of whether or not they were proteinuric before exercise.\textsuperscript{64} Interestingly, multiple studies regarding strenuous exercise have failed to show an association between running/racing and increased urinary protein excretion, whereas this is consistently increased by swimming.\textsuperscript{65,66,67} Although it is generally assumed that pre-renal proteinuria is transient, recent studies have documented pathologic renal damage as a cause of proteinuria in diseases such as lymphoma.\textsuperscript{68} In cases such as this, clinically relevant renal proteinuria can develop that does not always resolve with control of the primary disease.\textsuperscript{69} Post-renal causes include lower urinary tract infection or inflammation, neoplasia, and genital tract diseases such as pyometra or prostatitis; however, the finding of proteinuria in these settings is variable and not consistent.\textsuperscript{70,69} Renal proteinuria includes renal parenchyma diseases such as pyelonephritis and neoplasia, tubular diseases in which the reabsorptive capacity is decreased such as Fanconi syndrome, and primary glomerular disease such as amyloidosis, glomerulonephritis, and glomerulosclerosis.\textsuperscript{76}

2.2: Assessment of proteinuria

In clinical practice, urinary protein most commonly is detected by semiquantitative screening tests. These include traditional dipstick (colorimetric) and sulfosalicylic acid (SSA) turbidimetric test; the dipstick method is readily available and inexpensive, and thus most commonly used. The protein pad on the multireagent dipstick (Multistix \textcopyright) is based on the "protein error of pH indicator dyes." This test relies on the ability of the amino groups present on proteins to bind to and alter the color of
acid-base indicators, even though the pH is unchanged. The reaction is extremely sensitive to albumin, which contains the most amino groups, identifying concentrations as low as 15 mg/dL. Overall, it is much less sensitive to globulins, and insensitive to immunoglobulin light chains (Bence-Jones proteins). Because most causes of clinically relevant renal proteinuria involve albumin, the relative insensitivity to globulins and immunoglobulin light chains is generally not a concern. As sensitive as the dipstick test is, a urinary albumin concentration of less than 15 mg/dL of albumin may be missed altogether despite the presence of renal disease. False-positives are encountered commonly with the dipstick test and may be seen with alkaline urine, highly concentrated urine, presence of an active sediment (e.g., pyuria, hematuria, bacteriuria) or if the sample is left in contact with the reagent pad for too long before interpretation. False-negative results are commonly seen with immunoglobulin light chains, low concentrations of albumin below the sensitivity level of the dipstick, extremely dilute samples, or acidic urine. A recent study comparing sensitivity of tests for urine albumin showed a high percentage of false-positive and -negative results with the dipstick test when compared to a quantitative ELISA, in both dogs and cats.

An alternative to the traditional dipstick, or to confirm findings is the SSA test. This test is performed by mixing an equal volume of urine supernatant with 5% SSA in a glass tube and assigning a grade based on turbidity. The grading scale is from 0 to 4+. This test is often used to confirm positive results from the urine dipstick test, and has added sensitivity because it can identify samples with > 5 mg/dL of protein. This test will also detect globulins and immunoglobulin light chain so is less specific for the presence
of albumin alone. The grading scale based on turbidity is subjective, and results can vary based on the individual performing the test and the laboratory.\(^{71}\)

Protein identified by semi-quantitative methods should be interpreted in light of the urine specific gravity and microscopic evaluation of fresh sediment. Urine sediment has been shown to affect the presence of macroalbuminuria (> 30 mg/dL) which would be categorized as positive on routine urine dipstick or SSA testing. A study evaluating urinary albumin concentrations in dogs with active sediments identified a significantly higher urine albumin concentration in dogs with bacteriuria compared to those without. Interestingly, no significant correlation was identified between hematuria or pyuria and urine albumin concentration.\(^{70,73}\)

As a result of the low specificity and sensitivity of traditional semiquantitative methods to identify clinically relevant proteinuria, newer semiquantitative methods and qualitative methods are becoming available. Microalbuminuria refers to the presence of albumin in the urine at concentrations below the limits of the conventional dipstick (i.e., concentrations < 30 mg/dL). A point-of-care semiquantitative test (ERD HealthScreen Urine Test, Heska Corporation) assigns microalbuminuria to one of 5 categories: negative, low, medium, high, and very high. Urine albumin concentration also can be assessed by quantitative methods. The point-of-care assay has been shown to correlate highly with other methods for assessing urine protein including the urine dipstick, SSA test, and urine protein-creatinine ratio (UPC), but may be advantageous over other methods due to a higher sensitivity, and theorized elevation earlier in the course of disease. For interpretation, the urine albumin concentration must be adjusted for differences in urine concentration. This adjustment can be made by dividing the albumin
concentration by urine creatinine concentration (urine albumin-creatinine ratio) or by
diluting urine samples to a standard specific gravity of 1.010 before performing the
assay. Evidence has shown that normalizing the samples to a specific gravity of 1.010
yielded similar results to the urine albumin-creatinine ratio.\textsuperscript{74}

Proteinuria can also be quantified, as should be done in any patient where
results of screening tests suggest the presence of proteinuria or albuminuria. The UPC
has been the most widely used method to quantify proteinuria in veterinary medicine.
Before development of the UPC, the presence of proteinuria was based on the results
of urine protein concentration after a 24-hour urine collection. Previous studies in dogs
have shown normal 24-hour urine protein excretion to be 10 to 30 mg/kg or less. The
use of 24-hour protein excretion in veterinary medicine was largely limited to clinical
research because of the difficulty in obtaining an accurate 24-hour urine sample, and it
has been largely replaced by the UPC in clinical practice. A study evaluating the
correlation between 24-hour urine protein excretion and UPC using a random voided
urine sample showed extremely high correlation ($r=0.975$, $p<0.01$).\textsuperscript{75} The same study
identified the normal UPC for dogs as 0.2 to 0.3 or less. Also, because the urine protein
concentration is compared to freely filtered creatinine, the UPC eliminates the effect of
alterations in GFR, renal blood flow, and urine specific gravity on evaluation of urinary
protein content. The current recommendation for dogs is that a UPC $<0.5$ be
considered normal and that persistent proteinuria indicative of glomerular or
tubulointerstitial CKD be defined as a UPC $>0.5$ on 3 or more samples collected 2 or
more weeks apart.\textsuperscript{76} Although use of the UPC has facilitated the ability to detect early
renal disease in non-azotemic dogs and to monitor the effect of therapy in these
patients, smaller amounts of protein (i.e., microalbuminuria) still may be missed, which could be identified using more sensitive methods such as screening for microalbuminuria. Additionally, the UPC measures both urinary albumin and globulins, reducing its specificity for albumin which is the protein of prime importance in evaluation of renal disease.

2.3: Clinical Relevance of Proteinuria

Recently, magnitude of proteinuria has been established as one of the most important risk factors for progression of renal disease in both people and animals. In dogs, a UPC greater than 1.0 at the time of initial diagnosis of CKD has been shown to correlate with higher risk for development of uremic crisis and death, compared to those with an initial UPC of less than 1.0 at the time of diagnosis.\textsuperscript{77} Although proteinuria typically is less marked in cats than in dogs, the severity of proteinuria in cats with CKD has been shown to also correlate with survival.\textsuperscript{78} Tubulointerstitial damage induced by proteinuria is hypothesized to be responsible for this relationship.

When the protein concentration in the tubular lumen is increased, nuclear factor kappa B (NF-κB) translocates to the nucleus of the tubular cells, binds to specific receptors, and enhances gene transcription and generation of inflammatory cytokines including angiotensin, endothelin-1, TGF-B, RANTES, monocyte chemoattractant protein-1 (MCP-1), IL-1, plasminogen activator inhibitor-1 (PAI-1), and metalloproteinases.\textsuperscript{79,82} This ultimately leads to the presence of interstitial cellular infiltrates and increased matrix protein deposition, which are common observations in renal biopsies of patients with proteinuric renal disease.\textsuperscript{79}
Increased matrix deposition and fibrosis play an important role in progressive kidney disease and are mediated by up-regulation of several factors related to proteinuria. For example, increased concentrations of IgG and transferrin in the glomerular ultrafiltrate lead to increases in the rate of synthesis of endothelin-1. This is a locally released hormone that stimulates cell proliferation and extracellular matrix protein synthesis. In experimental studies synthesis and secretion of Endothelin-1 tend to occur at the basolateral membranes of tubular cells and leads to the interstitial changes seen. Experimentally, rabbit proximal tubular cells exposed to bovine serum albumin show increases in endothelin-1. In remnant kidney studies in rats where renal mass was reduced by partial nephrectomy or ligation of the renal arteries, or in rats with membranous glomerulopathy, the expression and excretion of renal endothelin-1 was increased, and the extent of this increase correlated with the rate of progression of renal damage. Studies on renal tissue in rats with glomerular disease also have shown an increase in renal mRNA for endothelin-1 in proximal tubular cells as well as in the infiltrating mononuclear cells. Transgenic mice with the gene for human endothelin-1 also show progressive interstitial renal disease with glomerulosclerosis and significant fibrosis.

Other molecules known to play an important role in interstitial fibrosis are the metalloproteinases. The 3 classes of metalloproteinases are capable of degrading virtually all matrix proteins. There are several inhibitors of metalloproteinases that appear to play a role in progressive renal injury. The most extensively studied is TIMP-1 (Tissue Inhibitor of Metalloproteinases-1). Many models of interstitial fibrosis, including protein overload, have been shown to induce significantly high levels of TIMP-1.
mRNA. This would lead to a decrease in the rate of degradation of matrix proteins and contribute to their accumulation in the renal interstitium.

The plasmin-dependant pathway also plays an important role in interstitial fibrosis. Plasmin activates procollagenases as well as degrading matrix proteins such as fibronectin and laminin. Plasmin is activated by tissue plasminogen activator (TPA) and urokinase-type plasminogen activator (UPA), and leads to degradation of matrix proteins. Plasminogen activator inhibitor-1 (PAI-1) prevents plasmin formation by inhibiting TPA and UPA, and thus prevents activation of procollagenases by plasmin, and the subsequent degradation of collagen and other matrix proteins. This situation leads to accumulation of matrix protein and progressive interstitial fibrosis. PAI-1 expression has been shown to increase in various nephropathies as well as cases of proteinuria in rodent models.

Chemokines also play an important role in interstitial inflammation and fibrosis. One such chemokine is RANTES, which is thought to regulate interstitial inflammation by attracting lymphocytes and macrophages to the renal interstitium where they secrete pro-fibrotic molecules including TGF-β1, endothelin-1, angiotensin-II and plasminogen activator inhibitor-1. TGF-β1 is the most extensively studied and seemingly most important pro-fibrotic cytokine. Active TGF-β1 leads to increased matrix synthesis, inhibition of matrix degradation, up-regulation of the matrix adhesion molecules, and chemoattraction of fibroblasts and monocytes. TGF-β1 mRNA levels have been shown to increase by 200–400% in experimental models of kidney injury. Interstitial cells and tubular cells are the source of TGF-β1 production, but fibroblasts and myofibroblasts are also important in the up-regulation of its synthesis. TGF-β1 production in renal
tubular cells and fibroblasts is increased by angiotensin-II, and ACE inhibitors have been shown to decrease levels in various forms of kidney disease, and this treatment has been associated with ameliorated damage to the renal interstitium.\textsuperscript{83} Other chemoattractants that promote inflammation and migration of inflammatory cells include MCP–1 and IL–1.\textsuperscript{79}

Microalbuminuria has been identified as a reliable predictor of developing renal disease in people with diabetic nephropathy and those with renal disease secondary to systemic hypertension. The presence of microalbuminuria predicts worsening of renal disease to overt diabetic nephropathy and increased risk of cardiovascular disease. Studies have shown the presence of abnormally high urine albumin concentrations in up to 30\% of people with newly diagnosed type 2 diabetes. Of these, approximately 75\% of diabetics will have only microalbuminuria and about 25\% will have overt diabetic nephropathy.\textsuperscript{85} The presence of microalbuminuria in this group of patients also has been associated with increased risk for developing end-stage renal disease. In the MICRO-HOPE study, for example, the risk of progression to diabetic nephropathy over a 5 year period was 2\% for normal patients, compared to 20\% for microalbuminuric patients.\textsuperscript{86} In another study of diabetics, those with a high urine albumin concentration at study entry were more likely to develop overt proteinuria, and mortality rate was significantly higher.\textsuperscript{87} Microalbuminuria also is a common finding in people with primary hypertension, and is a marker for developing renal insufficiency in those people. Microalbuminuria also is significantly more prevalent in hypertensive people than in normotensive ones.
Application of these findings to small animal medicine is difficult because essential hypertension and diabetic nephropathy are rarely diagnosed in animals, and when a presumptive diagnosis is made, they are poorly characterized. Microalbuminuria is prevalent in human patients with a variety of chronic inflammatory and neoplastic disorders, tending to be more severe in patients with active, extensive, or severe disease. This association also has been made in veterinary patients as well in that several infectious, inflammatory, neoplastic, and metabolic diseases have been reported in association with microalbuminuria.\(^8^8\) Conversely, microalbuminuria has been found to be a reliable, early marker of developing glomerulopathy in male dogs with X-linked familial nephropathy, a rapidly progressive glomerular disease caused by a defect in Type IV collagen normally present in the GBM. In 36 dogs with this condition, lesions of the glomerular basement membrane were histologically evident by 8 weeks of age, and persistent microalbuminuria was identified between 8 and 23 weeks. Microalbuminuria occurred significantly earlier than did overt proteinuria (based on UPC), which occurred between 14 and 30 weeks.\(^7^4\) A similar finding has been reported in Soft-Coated Wheaten Terriers with a genetic predisposition for developing protein-losing nephropathy. In these dogs, the prevalence of persistent microalbuminuria was 76%, with increasing magnitude of proteinuria over time. This finding preceded overt proteinuria based on UPC, and 43% of the dogs eventually developed increased UPC.\(^8^9\) Finally, similar results were shown in dogs with experimentally-induced heartworm disease. In this setting, the onset of microalbuminuria corresponded with antigenemia, with an increasing severity of microalbuminuria over time, which preceded the
development of overt proteinuria. All dogs eventually were shown to have histologic evidence of glomerular disease.\textsuperscript{90}

Proteinuria has been linked to established hypertension and renal disease, and it also has been determined that it may precede hypertension. In people, higher rates of urinary albumin excretion in non-hypertensive individuals were associated with increased risk of developing hypertension.\textsuperscript{91} A recent study in dogs also identified a link between albuminuria and hypertension in dogs with CKD. This study evaluated 40 dogs with CKD based on BP, UAC, and UPC. They identified microalbuminuria (UAC ratio 0.03–0.3) and macroalbuminuria (UAC > 0.3) in 32% and 50% of dogs, respectively. Of the 40 dogs, 60% were hypertensive (systolic BP > 180 mmHg) and those dogs classified as hypertensive had significantly higher UAC and UPC.\textsuperscript{92} It also is well documented in human patients that control of proteinuria has a positive impact on the rate of decline of renal function as well as survival. Treatment of proteinuric individuals with ACE inhibitors, and the subsequent decrease in the magnitude of proteinuria, decreases the rate of progression of renal disease independent of an effect on BP.\textsuperscript{93}
Chapter 3

Greyhound Relevance and Significance

3.1: Introduction

During the past 10 years, adoption of RRG’s has become increasingly popular. Approximately 20,000 retired greyhounds are adopted each year, and currently approximately 120,000 greyhounds are estimated to live as pets compared with 55,000 living on racetracks.\(^9^4\) As a result of this popularity, veterinarians are likely to evaluate Greyhounds more frequently in their practice. This clinical evaluation is complicated by the presence of hematological peculiarities that veterinarians must be aware of when evaluating the complete blood count or biochemical profile of greyhounds. Many studies have identified these hematologic and biochemical differences, and they should be taken into consideration when evaluating this breed. One study found that mean RBC count, packed cell volume, and mean corpuscular hemoglobin concentration were higher in Greyhounds as compared to mixed breed dogs. In addition, they also were reported to have higher sodium, chloride, and bilirubin concentrations and aspartate transaminase activity.\(^9^5\) Sullivan et al reported similar findings in Greyhounds, in addition to lower platelet counts than those in non-Greyhound dogs.\(^9^6\) Recently, Greyhounds have been reported to have significantly lower basal serum T4 and free T4 concentrations than do non-Greyhound dogs.\(^9^7\)

As clinical experience with Greyhounds grows, veterinarians are observing increased frequency of certain disease processes in this breed. In a recent web-based survey of greyhound owners, 113/747 (15%) of greyhounds evaluated died within the 2-year study period. Of those that died or were euthanized, trends were identified
regarding the most common causes of death. Neoplastic conditions were most common, accounting for 58% of all deaths, and osteosarcoma was the reported cause of death in 42% of these dogs. Neoplastic disease was followed by orthopedic disorders (18%), renal disease (8%), and bleeding disorders (8%). Recognition of a high mortality associated with certain diseases has sparked interest in the pathogenesis and treatment of these disorders in Greyhounds.

Nephrology has been of particular interest in the greyhound community with multiple publications in the last few years relating to renal disorders in the breed. With regard to serum creatinine concentration, Greyhounds have significantly higher serum creatinine concentrations than non-greyhound dogs. Serum creatinine concentrations in Greyhounds have been reported to be 1.2 to 1.9 mg/dL, with a mean concentration of 1.6 mg/dL. In this study, 14/30 dogs evaluated had serum creatinine concentrations above the hospital reference range of 0.6-1.6 mg/dL, and the serum creatinine concentrations in the greyhounds were significantly higher than in non-Greyhound controls. The higher serum creatinine concentrations in Greyhounds are thought to be a result of their high muscle mass and stores of phosphocreatinine, as well as a diet that is normally high in creatinine, essentially a raw meat diet. Decreased GFR could also explain the high serum creatinine concentrations seen in Greyhounds, but the dogs studied were normal and had normal urine specific gravity. Many of these greyhounds were followed for several years without evidence of progressive renal disease. Another study confirmed higher serum creatinine concentrations in Greyhounds, but at the same time documented significantly increased GFR in these dogs (3.0 ml/kg/min) compared to non-Greyhound dogs (2.5 ml/kg/min). Taken together, these results suggest the
high serum creatinine concentration is not renal in origin, and the finding of a serum creatinine concentration up to 1.9 mg/dL likely does not indicate the presence of renal disease.

Greyhounds have not been identified among breeds reported in review articles of glomerulonephritis and other protein-losing nephropathies (PLN), but this may reflect the fact that until 10 to 15 years ago, Greyhound rescue and adoption was still relatively uncommon and the breed was seen infrequently in private practice. In our experience, protein losing nephropathy (PLN) is the most common renal pathology seen in RRG’s.

Another disease leading to acute illness and renal failure has been identified in actively racing Greyhounds. This has been termed “cutaneous and renal glomerular vasculopathy,” or “Alabama rot” as it was first identified in dogs from an Alabama racetrack. It has subsequently been identified in dogs from all states where Greyhound racing is performed. This disease leads to multifocal ulceration of the skin accompanied by limb edema and / or acute renal failure. This is typically identified in young adult Greyhounds living in a racetrack / kennel environment, and affects females and males equally. Four distinct syndromes have been reported for this condition: skin lesions alone, with no evidence of systemic illness and recovery over a 2 to 4 month period; skin lesions with concomitant lethargy and fever, followed by the development of edema and azotemia; cutaneous ulcerations with progression to acute renal failure w/in 10 days; and acute azotemia prior to the onset of cutaneous ulceration. The skin lesions begin as erythematous and tender swellings centered on the tarsus, stifle, or inner thigh. These generally progress becoming ulcerated, with delayed healing. Other clinical findings commonly reported include thrombocytopenia, anemia, azotemia,
hypoalbuminemia, leukocytosis, elevated ALT, and elevated CK.\textsuperscript{101} Renal histopathologic changes endothelial swelling, detachment, and necrosis; membranous whorl formation; and platelet adhesion and aggregation. Fibrinoid necrosis of the afferent arterioles and thrombi composed of fibrin, aggregated platelets, red blood cells, and cellular fragments have also identified in the glomerular capillaries. Evidence of infectious agents or electron dense deposits consistent with immune complexes has not been shown in affected dogs.\textsuperscript{100,102} The etiology of this condition is unknown but resembles hemolytic uremic syndrome in children caused by vero-toxin producing \textit{E. coli}, most notably serotype O157:H7.\textsuperscript{102}

3.2: Objectives

The objectives of this study were to evaluate renal function and hemodynamic status in a population of clinically healthy, RRG’s (RRG’s). Our goals were to evaluate this group of dogs with respect to BP, but also for any indication of early renal disease based on markers of renal damage including microalbuminuria, overt proteinuria, altered fractional excretion of electrolytes, and histopathologic changes.

Hypotheses:

1) Doppler and oscillometric methods for BP measurement in Greyhounds will correlate well and will not be statistically different from each other.

2) Greyhounds will have higher BP measured on the day of initial presentation to the hospital, compared to results obtained after a 48-hour acclimation period, as a consequence of the white coat effect and stress of transport.
3) The population of Greyhounds presented to the hospital will have a high prevalence of hypertension as indicated by systolic BP > 165 mmHg

4) “Hypertensive” Greyhounds will have a significantly higher prevalence of microalbuminuria, as measured by the HESKA point of care ERD kit, compared to “normotensive” Greyhounds.

5) “Hypertensive” Greyhounds will have significantly higher UPC compared to “normotensive” Greyhounds.

6) “Hypertensive” Greyhounds will have significantly higher fractional excretion of electrolytes (Na, K, Cl, Ca, and Phos) compared to “normotensive” Greyhounds.

7) “Hypertensive” Greyhounds will have a higher frequency of abnormal renal histopathologic findings as compared to “normotensive” Greyhounds.
Chapter 4

Materials and Methods

4.1: General Information

RRG’s presented to The Ohio State University Veterinary Medical Center were eligible for inclusion in this study. Dogs were presented to participate in a 3rd year veterinary student spay/neuter program. Dogs traveled to the Veterinary Medical Center from various racetracks. Forty-nine dogs were enrolled in the study, 25 in November 2007 and 24 in February 2008. On initial presentation, all dogs underwent a thorough physical examinations performed by the principal investigator and all abnormalities were recorded. CBC (IDEXX LaserCyte) and serum biochemistry panels (Cobas C501, Roche Diagnostics) also were performed. In total, 40 mL of blood was collected from each dog for use in various studies. Jugular venipuncture was performed using a Safety Lock Blood Collection Set (Becton Dickinson; Sparks, MA) and blood was collected into serum separator and 7.5% EDTA collection tubes (Monoject; Mansfield, MA). Dogs were included in this study based on unremarkable physical examination, normal results of CBC and biochemical profile based on breed standards, and lack of historical health concerns. No dog was excluded on the basis of abnormal physical examination or clinical pathology results.

4.2: Blood Pressure Recording

All dogs were evaluated on day 1 after presentation to the hospital, and on day 3 after a 48-hour acclimation period, before any surgical procedures. Blood pressure data were recorded by a single investigator using 2 standard techniques: ultrasonic (Model
811 Ultrasonic Doppler Flow Detector; Parks Medical Electronics, Aloha, OR) and oscillometric (Cardell Veterinary Monitor 9402; Sharn Veterinary Inc., Tampa, FL). Dogs were placed in right lateral recumbency and allowed a 5-minute acclimation period with minimal restraint. The first several readings were discarded and five additional consecutive measurements were obtained. The arithmetic mean of the five measurements was used for data analysis. For the Doppler measurements, an inflatable cuff was placed directly around the left antebrachium without clipping the hair. Cuff size was chosen to match a width approximately 40 per cent of the circumference of the antebrachium. The occluding cuff, with an aneroid manometer attached, was secured proximally to the Doppler flow detector. The hair on the palmar aspect of the metacarpus was clipped, the skin moistened with alcohol, and an adequate amount of ultrasonography coupling gel applied. The Doppler ultrasound probe was held in a fixed position over the superficial palmar artery until a strong audible signal was achieved, and the probe fixed into position with tape. The cuff was inflated to 40 to 50 mmHg above the point at which the Doppler signal was no longer audible, and the pressure slowly released until the signal became audible; this point was recorded as the systolic pressure. For the oscillometric technique, the cuff again was placed directly around the left antebrachium. Care was taken to prevent the cuff from touching the table or being disturbed during measurements. Any measurements obtained while the dog was moving were discarded. Systolic, diastolic, and mean arterial pressures as well as heart rate were recorded. A manual heart rate was determined by digital femoral pulse palpation to determine if the heart rate obtained by
the oscillometric monitor was accurate. Any pressure measurement with a heart rate that did not match the manual heart rate was excluded.

4.3: Urine Collection and Evaluation

Urine was collected in all dogs on day 1, immediately after arrival, via routine cystocentesis. All dogs had been fasted for 12 hours prior to arrival for transport. Dogs were placed in lateral or dorsal recumbency and the urinary bladder identified by ultrasonography. A 22-gauge, 1.5-inch needle attached to a 6-ml syringe was used for collection. Urine was divided into 5 aliquots for analysis: urinalysis, urine chemistry profile, urine culture, microalbuminuria (MA), and UPC. Samples for urinalysis, urine chemistry profile, urine culture, and UPC were submitted for immediate analysis. Urine was plated for aerobic culture on blood and MacConkey agar, and incubated for 3 days at 35°C. Urine electrolytes were measured on the Cobas C501 automated chemistry analyzer. Serum and urine sodium, chloride, and potassium were measured using ion specific electrodes to generate an electrical potential that correlates with the concentration of the ions in solution. Serum and urine calcium were measured using the o-cresolphthalein complexone (o-CPC) colorimetric/photometric method. Serum and urine phosphorus were measured using the ammonium molybdate/sulfuric acid photometric method. Fractional excretion of electrolytes (expressed as a percentage) was calculated using the following equation:

\[
FE_e = \left[ \frac{U_e}{P_e} / \frac{U_{cr}}{P_{cr}} \right] \times 100
\]

Where,

\( FE_e \) = Fractional excretion of electrolyte
U_e = Urine electrolyte concentration
P_e = Serum electrolyte concentration
U_cr = Urine creatinine concentration
P_cr = Serum creatinine concentration

Reference values for fractional excretion of electrolytes were based on results obtained in a study of clinically normal RRG’s in Australia using the same methodology.\textsuperscript{105}

The UPC was measured on a Cobas C501 automated chemistry analyzer using a turbidmetric method. Both serum and urine creatinine were measured on a Cobas C501 automated chemistry analyzer using the picric acid colorimetric/photometric method. The UPC was determined by dividing the urine protein concentrations by the urine creatinine concentrations. Normal UPC for dogs is less than 0.4.\textsuperscript{71} Aliquots for semiquantitative analysis of MA were stored in individual airtight containers at \(-70^\circ\text{C}\) until analysis. Evidence has shown that urine can be stored at 4°C for one week without any degradation or urine albumin. Long term storage of urine at -20°C can result in degradation of urine albumin; however, samples frozen at -70°C show minimal decreases in albumin or total protein for up to 2.5 years.\textsuperscript{106,107}

4.4: Assessment of Microalbuminuria

Frozen aliquots were removed from the freezer and allowed to thaw and equilibrate at room temperature. All samples from each group were processed together on the same day, within 14 days of collection. Once thawed, samples were gently inverted 3-4 times to resuspend settled particulate matter. MA was determined using a
semiquantitative point-of-care assay immunoassay (Canine ERD – Screen Urine Test, Heska Corporation). This test utilizes an anti-canine albumin antibody which, upon binding to albumin, causes blue lines to appear in the test window. The number and intensity of these lines correlate with the concentration of albumin in the sample. All samples were processed according to the manufacturer’s instructions. Urine specific gravity for each was determined using a manual refractometer, and all samples were normalized to a specific gravity of 1.020 by dilution with distilled water. The test device was inserted into the diluted sample for 5 minutes and the result determined by comparing the intensity of the 2 colored bands in the test window against the manufacturer-provided control results, corresponding to negative, low, medium, high, and very high for MA. All samples were processed and interpreted by Dr. Surman.

4.5: Biopsy Collection

Biopsy samples were collected on all female Greyhounds undergoing routine ovariohysterectomy. All dogs were placed under general anesthesia, induced by intravenous injection of Propfol and maintained with isofluorane and oxygen. Samples were collected by 2nd and 3rd year surgery residents, supervised by a board-certified surgeon. Samples from the first 10 dogs were collected using an 18-gauge automated needle biopsy device with an 11 mm specimen notch (E-Z Core, Products Group International Inc.). The left kidney was stabilized by hand and the biopsy instrument cannula placed through the capsule and lodged in the outermost aspect of the cortex. The biopsy cannula was advanced, and the collected sample removed from the instrument by flushing gently with sterile saline, collecting it in a pool of saline on a glass
slide. An individual core did not provide sufficient tissue for all pathologic techniques; therefore on each occasion three 1-2 cm cores were obtained from each dog. Hemostasis was achieved by direct pressure, gel foam application, and a single suture placed in the capsule when necessary. Samples were immediately processed and divided by the principal investigator. The 3 samples were placed on individual glass slides in a small pool of isotonic sterile saline to rinse the cores. The total biopsy mass was sectioned into 3 samples using a razor blade, and designated for fixation in either 10% formalin (light microscopy), glutaraldehyde (electron microscopy), or Michel’s media (immunofluorescence microscopy). Sectioning was done so that the size of each sample would be appropriate for the specified technique. Light microscopy required the largest sample, approximately 1 cm in length, followed my immunofluorescence, approximately 0.5 cm to 1.0 cm in length, and finally electron microscopy, approximately 0.5 cm in length. All samples were placed in the appropriate media within 5 minutes of collection. Samples were refrigerated overnight, and shipped on ice overnight to Texas A&M University Renal Pathology Center. Due to poor quality samples from 5 of the first 10 dogs, the renal biopsy technique was changed for the next 10 dogs. Wedge biopsies obtained from on the left kidney of the remaining 10 dogs. The capsule and kidney were incised with a number 10 scalpel blade over a region approximately 1 cm long and 0.25 cm deep into the cortex. Hemostasis was achieved with direct pressure and gel foam application, and the capsule sutured closed. The same procedure discussed previously for sample handing and division was used.
4.6: Histologic and Ultrastructural Evaluation:

*Light Microscopy:*

Tissues were fixed in 10% neutral buffered formalin, then routinely processed and embedded in paraffin. Thin (3 micron) sections were cut and stained with the hematoxyline and eosin (HE), periodic acid-Shiff (PAS), and Masson’s trichrome (Tri) methods using standard procedures.

All biopsy specimens were evaluated and scored by the principal investigator and a boarded-certified veterinary internist with experience in renal histopathology. The biopsies were scored using a standardized scale (Table 1). Light microscopic samples were scored based on the distribution and severity of the lesions in 16 categories, 8 relating to the renal interstitium and 8 relating to the glomeruli. Lesions distribution was classified as absent, focal, multifocal, or diffuse with 0, 1, 2, and 3 points assigned for absent, focal, multifocal, and diffuse lesions, respectively. Lesion severity was classified on a 0 to 3 scale, corresponding to absent, mild, moderate, and severe, with the score representing the number of points related to severity. For each type of lesion, the total number of points was determined by adding the points from distribution to the points from severity, for a maximum score of 6 per lesion. For example, an animal with multifocal moderate interstitial fibrosis would be scored 2 points for distribution, and 2 points for severity, for a total of 4 points for the individual variable. The score for all variables was added to give the total histopathologic score (See Table 1).

*Ultrastructural:*
Tissues were fixed in chilled 3% glutaraldehyde. Specimens were post-fixed in 1% osmium tetroxide, dehydrated in a series of graduated alcohols, infiltrated in an acetone/epoxy plastic, and embedded in a plastic mold. Plastic blocks were cut with a Sorvall MT2-B ultramicrotome. Thick sections were stained with toluidine blue. Sections were then evaluated and appropriate areas identified for thin sectioning. Thin sections were cut at silver-grey interference color (65-80 nm) and placed on copper mesh grids. Grids were stained with uranyl acetate and lead citrate and were examined in a JEOL TEM-1230 transmission electron microscope.

**Immunofluorescence:**

Tissues were immersed in chilled Michel’s Transport Media (Newcomer Supply, Middleton, WI, USA) for up to 48 hours during transport to the pathology laboratory where they were washed 3 times in Michel’s Wash (Newcomer Supply, Middleton, WI, USA), placed in plastic cryomolds filled with Tissue-Tek OCT embedding compound (Electron Microscopy Sciences, Fort Washington, PA, USA), and snap-frozen in liquid nitrogen. Blocks were stored at -80°C until sectioned. Thin (4 micron) cryosections were cut on a Leica CM 1850 UV cryostat (Bannockburn, IL, USA) and stored at -80°C until thawed for 1 hour at room temperature for staining. Sections were fixed for 5 minutes in cold 100% acetone, air dried for 1 hour, then rehydrated in phosphate-buffered saline. Direct immunofluorescence immunostaining was performed with fluorescein isothiocyanated-(FITC)-conjugated polyclonal goat anti-dog IgG, IgM, IgA, and C3 antibodies (Bethyl Labs, Montgomery, TX, USA). Sections were incubated for 1 hour with an appropriate dilution of each antibody, then washed with phosphate-buffered...
saline. Sections were coverslipped using a mounting medium that retarded fluorescence quenching (Prolong Gold, Invitrogen, Carlsbad, CA, USA), and were examined with an epifluorescence microscope using appropriate filters (Olympus, Center Valley, PA, USA).

4.7: Statistical Analysis

All statistical analyses were performed by a licensed statistician in The Ohio State University College of Biostatistics. For all testing, significance was defined as a p-value < 0.05.

Bland Altman plots were used to identify bias when evaluating the 2 methods on the same day, and the same method on different days, utilizing the method described by Bland and Altman. For comparing methods on individual days, each systolic Doppler pressure was compared to the corresponding oscillometric pressure. The mean difference was calculated by subtracting the oscillometric pressure from the Doppler pressure. A positive bias would indicate that the Doppler pressure was consistently higher than the oscillometric pressure, and vice versa. For comparing the same method on different days, the day 3 pressures were subtracted from the day 1 values. A positive bias would indicate that the day 1 pressures were consistently higher than the day 3 pressures, and vice versa. Correlation was further assessed using the Pearson Correlation Coefficient (r value), Intraclass Correlation Coefficient (ICC), and the Concordance Correlation Coefficient (CCC). The ICC measures the proportion of the total variance that is due to variability among dogs, rather than variability between the methods of measurement or days. The closer the proportion is to 1, the more the
variability is due to differences among dogs, rather than to disagreement between methods. The CCC determines the degree to which pairs of observations from the 2 methods or 2 days fall on the 45° line of identity. These are preferable over the Pearson Correlation Coefficient because the Pearson method only determines goodness-of-fit to a line of identity, not necessarily the 45° line of identity. Consequently, significant differences could occur between the 2 methods or days, and still have good correlation, and an r value close to 1. These methods were used to assess the following:

1) Differences in the systolic Doppler measurements on day 1 compared to day 3
2) Differences in the systolic oscillometric measurements on day 1 compared to day 3.
3) Differences in the systolic Doppler and oscillometric measurements on day 1.
4) Differences in the systolic Doppler and oscillometric measurements on day 3.

Based on the average systolic Doppler measurements, dogs were classified as “hypertensive” if their systolic BP was greater than 165 mmHg, and “normotensive” if their systolic BP was < 165 mmHg. All blood pressure measurements were assessed for normality with the Kolmogorov-Smirnov (KS) normality test, D’Agostino and Pearson omnibus normality test, and the Shapiro-Wilk normality test, and passed for all data sets. Paired T-tests were used to assess for statistical significance between the following:

1) Significant difference in the overall average systolic BP measured by Doppler and oscillometric methods.

An unpaired t test was used to assess for statistical significance between the following:
1) Significant difference in the systolic Doppler pressure on day 1 compared to day 3.

2) Significant difference in the systolic oscillometric pressure on day 1 compared to day 3.

3) Significant difference in the mean systolic Doppler and oscillometric pressures on day 1.

4) Significant difference in the mean systolic Doppler and oscillometric pressures on day 3.

To assess for a relationship between age and BP status, a non-parametric test (Mann Whitney U) was used because the age of the Greyhounds was not normally distributed. Chi-square analysis was used to evaluate the relationship between sex and BP status. All results except for the relationship between BP status and sex were assessed graphically with a Box and Whiskers plot, a + symbol identifying the mean value, horizontal line identifying the median value, upper and lower borders of the box identifying the interquartile range (IQR), and error bars extending to 1.5 times the IQR in the up and down direction, or to the min/max value whichever was shorter. Outliers were identified by dark circles. For the relationship between BP status and sex, a general grouped bar graph was used. For BP data, outliers were included in the statistical analysis because BP passed the normality tests.

Based on hypertension groupings, associations were evaluated for BP grouping and the presence or absence of MA, UPC, and urinary fractional excretion of electrolytes. Because of the low numbers associated with each category on the MA test, low, medium, and high values all were considered positive for MA, and data was
evaluated on the basis of either negative MA or positive MA. Chi-square analysis was used for the comparison between the BP grouping and MA. Non-parametric testing with the Wilcoxon Rank Sum test was used for the comparison between the BP groups and the fractional excretion of electrolytes and UPC because fractional excretion of electrolytes and UPC were not normally distributed. Outliers with regard to fractional excretion of electrolytes were removed from the analysis.

Renal histopathology also was assessed with respect to all other variables, BP grouping, presence/absence of MA, UPC, and fractional excretion of electrolytes. Pearson correlations were calculated to assess the relationship between biopsy score and fractional excretion of individual electrolytes and UPC. The Wilcoxon Rank Sum test was used to evaluate the association between biopsy score and BP group, and biopsy score and MA group.
Chapter 5

Results

5.1 CBC, Chemistry, Urinalysis

Serum biochemical and CBC data were available from 48 dogs, 24 in the first group and 24 in the second group. Of the 48 dogs, 28 (58%) were female and 20 (42%) were male; all dogs were intact at the time of evaluation. The mean ± SD age was 3.7 ± 1.7 yrs (range, 1 – 9 yrs). Routine serum biochemical analysis generally was unremarkable in all dogs. The mean ± SD serum creatinine concentration was 1.57 ± 0.97 mg/dL (range, 1.1 – 2.2 mg/dL). These results were above the reference limit for the clinical pathology laboratory of 1.6 mg/dL in 17/49 (34.5%) of dogs; however, using 1.9 mg/dL as a cut-off for normal Greyhounds, only 4/49 (8.1%) were considered outside of the normal reference range. The mean ± SD BUN concentration was 15.7 mg/dL ± 3.7 (range, 9 – 25 mg/dL). These results were above the reference limit for the clinical pathology laboratory in 4/49 (8%) of dogs. Additional serum biochemical data are summarized in Table 2. The results of the CBC data are summarized in Table 3. CBC results were mostly within normal limits, but, on average, the Greyhounds had MCHC, HCT, and hemoglobin concentrations at the upper end of the reference range or even above the reference range, and platelet counts at the lower end or below the reference range. Given the results of previous studies of Greyhounds, these results are not unexpected, and generally are considered normal for the breed.

Urine was collected by cystocentesis from 47 dogs and analyzed. Each dog had a routine urinalysis, urine chemistry profile including a UPC, urine culture, and HESKA ERD MA assay performed. In general with the exception of a few outliers, the urine
specific gravity was moderately high with mean ± SD of 1.046 ± 0.15 (range, 1.010 – 1.059). Although the range was extremely wide, only 3 dogs were classified as isosthenuric, all others had specific gravity of greater than 1.030. Looking more closely at the 4 dogs that had serum creatinine concentrations of approximately 1.9 mg/dL (the reported maximum for normal Greyhounds), all four had specific gravity greater than 1.040 indicating that the high serum creatinine concentration was not of primary renal origin. In addition, all four had serum albumin concentrations above 3.5 g/dL and 2/4 had serum albumin concentrations greater than 4.0 g/dL, supporting a pre-renal azotemia. Urine culture was negative in all dogs. Urine profile results are shown in Table 4.

MA was evaluated in 47 dogs. Of these, 22 (47%) were negative, 13 (28%) were low-positive, 10 (21%) were medium-positive, and 2 (4%) were high-positive. Because of the low numbers in many of the groups, the data were collapsed into “negative” and “positive”; dogs measuring low, medium, and high were all considered positive. Overall, 22/47 (47%) dogs were negative, and 25/47 (53%) dogs were positive. Full results can be seen in Table 5.

The mean ± SD for UPC was 0.11 ± 0.16 (range, 0.03 to 1.14). Only one dog had a high UPC > 0.4; this was 1.13 and the dog was “high” on the MA semiquantitative test. Three other dogs had UPC’s of approximately 0.2, which would warrant further monitoring; all 3 dogs tested “medium” on the MA semiquantitative test. All other dogs, including those that were positive for MA had normal UPC’s < 0.2, see Table 4.
5.2 Fractional Excretion of Electrolytes:

The mean ± SD values for urinary fractional excretion of electrolytes are listed in Table 5. Outliers were identified and subsequently removed from statistical analysis. The outliers removed were as follows: 1 for sodium, 1 for chloride, 1 for potassium, 1 for phosphorus, and 3 for calcium.

5.3 Blood Pressure:

Data were collected from all 48 dogs on day 1 and day 3 of the experiment. The mean ± SD for the systolic Doppler pressure was 166.9 ± 17.1 mmHg (range, 134 to 210) and 167.7 ± 14.9 mmHg (range, 142 to 204) on day 1 and day 3, respectively. There was no significant difference between these means, p = 0.53 (Figure 1). The mean ± SD for systolic oscillometric pressure was 160.9 ± 18.6 mmHg (range, 122 to 201 mmHg) and 162.3 ± 16.1 mmHg (range, 126 to 193) on days 1 and 3, respectively. There was no significant difference between these means, p = 0.07 (Figure 2). The overall mean systolic pressure was 167.3 ± 15.5 mmHg (range, 139 to 207) and 161.6 ± 17.1 mmHg (range, 124 to 197) for the Doppler and oscillometric methods, respectively. There was no significant difference in the overall average systolic blood pressure, p = 0.0928 (Figure 3). There was good correlation between Doppler results obtained on days 1 and 3, between oscillometric values obtained on days 1 and 3, between oscillometric and Doppler values obtained on day 1, and between oscillometric and Doppler values obtained on day 3 (Table 6). Correlation curves are presented in Figures 4 through 7. The highest correlations were seen between days using the same methods, ICC and CCC for the Doppler between days 1 and 3 were 0.85 and 0.86, and
ICC and CCC for the oscillometric between days 1 and 3 were 0.94 and 0.94, respectively. These results indicate excellent agreement between days when using the same method. The correlation was slightly lower for different methods on the same day, with the ICC and CCC on day 1 comparing Doppler and oscillometric methods of 0.8 and 0.81, respectively, and on day 3 of 0.71 and 0.73, respectively. These results still indicate acceptable correlation, although not as strong as when using the same method. When evaluated using Bland-Altman plots, the bias and 95% limits of agreement can be determined (Table 8). Bland-Altman plots are presented in Figures 8 through 11.

Of the 47 dogs evaluated, 26 were classified as hypertensive (systolic BP > 165 mmHg) and 21 were classified as normotensive (systolic BP < 165 mmHg). The mean ± SD for blood pressure was 178.4 ± 10.5 mmHg (range, 166 to 204) in the hypertensive group and 154.5 ± 6.4 mmHg (range, 142 to 164) in the normotensive group. The mean blood pressure was significantly different for these two groups, p < 0.01 (Figure 12).

The mean ± SD for heart rate was 86.5 ± 15.9 bpm (range, 59 to 124) and 81.5 ± 14.1 bpm (range, 63 to 113) for the hypertensive and normotensive groups, respectively. The mean HR between these 2 groups was not significantly different, p = 0.25 (Figure 13).

The mean ± SD for age was 3.9 ± 1.4 yrs (range, 1 to 9) and 3.5 ± 1.8 yrs (range, 1.5 to 8.5) for the hypertensive and normotensive groups, respectively. There was no significant difference in age between these two groups, p = 0.1682 (Figure 14). With regard to sex, 12/25 (48%) and 13/25 (52%) of hypertensive dogs were male and female respectively, and 7/22 (32%) and 15/22 (68%) of normotensive dogs were male.
and female respectively (Figure 15). Chi square analysis indicated no significant association between sex and blood pressure, $p = 0.26$.

5.4: Association of Hypertension With Other Variables

Summary data for sex, age, MA, fractional excretion of electrolytes, and UPC are presented in Table 10. When evaluated with respect to blood pressure status, Chi square results identified a highly significant association between the presence MA and blood pressure status, $p < 0.001$. In all, 82% of the normotensive dogs were negative for MA, while 84% of the hypertensive dogs were positive for MA. There were no significant associations between the presence of hypertension and any other variables including urinary fractional excretion of electrolytes (sodium, chloride, potassium, calcium, and phosphorous) or UPC (Table 10). Removing the outliers from the fractional excretion data had no effect on statistical significance.

5.5 Renal Histopathology:

Renal histopathology was available from 20 female dogs. The mean age $±$ SD was $4.1 ± 2.3$ yrs (range, 1 to 9.5). The first 10 dogs underwent needle biopsies of the left kidney at the time of exploratory laparotomy. In all, 5/10 dogs had adequate tissue collection for light microscopy, immunofluorescence, and transmission electron microscopy. In the remaining 5/10 dogs, the biopsy contained only renal medulla and no cortical tissue. Information from these 5 dogs was not included because there were no glomeruli to evaluate. The second 10 dogs all had wedge biopsies performed of the left
kidney, and all 10 samples were considered excellent, with adequate material available for light microscopy, immunofluorescence, and transmission electron microscopy.

*Light Microscopy:*

Light microscopic data was evaluated for the 15 dogs that had acceptable tissue on renal biopsy. The mean ± SD for biopsy score was 15.1 ± 8.8 (range, 4 to 36). The light microscopic biopsy score was evaluated for associations with hypertension, MA, UPC, and fractional excretion of electrolytes. The Pearson correlation coefficients for UPC and fractional excretion of electrolytes are summarized in Table 11. There was no significant correlation between biopsy score and any of the variables evaluated including MA, urinary fractional excretion of electrolytes, and UPC (Table 11). The correlation between biopsy score and UPC was the highest (correlation coefficient 0.51), and graphically a slight upward trend could be seen between increasing UPC and biopsy score. This result approached significance, p = 0.05 (Figure 6). There was no significant association between the presence of hypertension and biopsy score (p = 0.41) or between presence of MA and biopsy score (p = 0.63).

Overall, biopsy scores tended to be fairly low, correlating with mild lesions. Several trends could be identified, however. Evaluating the 5 dogs with the highest renal biopsy scores, 3/5 were had mild mesangial glomerulopathy characterized by an increase in mesangial matrix and mesangial cell proliferation, and the other 2 had mild glomerulosclerosis. The majority of dogs (12/15) had some degree of thickening of Bowman’s capsule and 12/15 had expansion of the mesangial matrix.
Immunofluorescence and electron microscopy:

Immunofluorescence was performed for IgG, IgM, IgA, and C3 in 12/15 dogs that had adequate biopsy samples. The 3 dogs that did not have immunostaining performed had no glomeruli in that tissue section. All 13 dogs had diffuse, segmental to global, mesangial staining for IgM. One dog also was positive for IgG and C3. All other dogs were negative for IgG, IgA, and C3. Electron microscopy confirmed mesangial matrix expansion in those dogs that were positive for this lesion on light microscopy, but failed to identify abnormalities in the glomerular basement membrane or any areas of electron dense deposits. Overall, the findings on immunostaining and electron microscopy were nonspecific and unremarkable.
Chapter 6

Discussion

6.1 Discussion

In clinical practice, measurement of blood pressure is becoming increasingly necessary; the consequences of hypertension are becoming more clinically relevant. The gold standard of direct arterial blood pressure measurement is time consuming, labor intensive, expensive, requires substantial technical skill, is invasive, and carries a higher risk of complications than other methods. Thus, direct arterial measurement of blood pressure generally is not applicable in the routine diagnosis and monitoring of hypertension. Indirect methods are inexpensive and fairly simple to perform with appropriate training; however, their reliability compared to the gold standard is questioned. Numerous studies have reported that, although not perfect, indirect methods including Doppler ultrasonic and oscillometric techniques correlate fairly well with results obtained by direct arterial puncture and with each other. Although no direct arterial measurement was used in this study, two other methods of blood pressure measurement, with known reliability, were used on each dog, on two separate days to assess blood pressure status. There was no significant difference in the average pressure measured between the 2 methods, and the correlation coefficients were fairly high, indicating good agreement between the two methods. Overall, however, there was a higher correlation between the Doppler measurements on day 1 and day 3 compared to the oscillometric methods on day 1 and day 3. This finding may indicate that the Doppler measurements were more reliable, and consequently the average Doppler
pressure was used to classify individuals into the 2 groups, hypertensive (BP > 165 mmHg) and normotensive (BP < 165 mmHg).

There was considerable concern entering into this study about what effect if any the stress of transport and hospitalization may have on hemodynamic status in these dogs. This concern was based mostly on clinical experience with blood pressure measurement in that white coat effect is relatively common in routine in-clinic blood pressure measurements. Complicating the diagnosis and importance of white coat hypertension is the fact that it is unreliable and difficult to predict. There are few studies evaluating white coat effect in veterinary medicine, one of which evaluated dogs in their home environment and in the hospital using oscillometric methods; in that study the authors failed to demonstrate a white coat effect. Results of this study identified a high prevalence of hypertension in a population of otherwise healthy RRG’s; approximately half were classified as hypertensive, BP > 165 mmHg systolic. Most had been shipped a fairly long distance before arriving at the university. For this reason, we hypothesized that blood pressure and heart rate evaluated on day 1, immediately upon arrival, would be higher on average than results obtained after a 48-hr acclimation period. However, this was not the case, as there were no significant differences in blood pressure or heart rate between days 1 and 3. Additionally, none of these dogs were tachycardic based on conventional standards; this provides additional evidence that stress did not play a role in these measurements. In veterinary studies that have identified white coat hypertension, heart rate was also elevated during periods of stress, which was not present in this study. Although heart rate is not a definitive indicator of stress, it does seem to elevate in stressful situations along with BP, whereas in non-stressful
situations, neither heart rate nor BP were elevated. Despite having persistently high blood pressure over a 48-hr acclimation period, it is difficult to completely eliminate a possible white coat effect. Another recent study in dogs did identify the presence of a white coat effect. Using untrained laboratory Beagles, the authors demonstrated that blood pressure decreased significantly from the initial readings as a result of acclimation to the techniques used, with a maximal decrease at about 14 days, after which BP remained stable for 161 days. In contrast, the Greyhounds in this study were from racetracks and accustomed to travel, routine veterinary care, and housing in a kennel environment. The stress of transport and unfamiliar surroundings may have been expected to artificially increase the variables studied; however, given the husbandry of these dogs, the lack of effect of stress is not entirely surprising. Additionally, it is known that Greyhounds as a breed have significantly higher blood pressures compared to other breeds. This has even been theorized as a model for essential hypertension in people. What is still unclear however, is whether this higher blood pressure leads to pathologic changes in Greyhounds and is clinically significant.

Hypertension, even when mild, is a well known risk factor for development of microalbuminuria / proteinuria and kidney disease, as well as progression of pre-existing renal disease. The Greyhounds in this study had no clinical evidence of pre-existing kidney disease. Four of the Greyhounds did have elevated serum creatinine concentration, however all four had urine specific gravity > 1.040 making underlying kidney disease unlikely as the cause of the elevated creatinine. There were 3 dogs classified as isosthenuric, however none of these were azotemic. Many of the dogs did have histopathologic changes; however, these were generally mild and based on
clinical parameters did not appear to affect kidney function. Glomerular filtration rate was not measured in these Greyhounds, which could have provided more definitive evidence that renal function in these dogs was normal.

There is a strong interest in human medicine on the diagnosis of early renal disease before the onset of overt azotemia, and its implications for treatment success. Markers of early renal disease may provide insight into disease status, risk of progression, and the possibility of therapeutic intervention. One such marker is proteinuria. Overt proteinuria of renal origin is a well known marker of kidney dysfunction and in human and veterinary medicine has been shown to be a prognostic indicator and therapeutic target. Unfortunately, overt proteinuria as identified by an increased UPC suggests that clinically relevant renal disease may already be present. This concern has led to investigation of markers of renal dysfunction that may precede the onset of overt proteinuria, especially microalbuminuria. There has been considerable interest in this phenomenon in people, especially in diabetics and patients with primary hypertension, in whom microalbuminuria has been shown to precede the onset of overt proteinuria and predict progression of renal disease, as well as being a risk factor for other complications such as cardiovascular disease. Similar associations have been shown in dogs with X-linked nephropathy and soft-coated Wheaten Terriers with PLN. Therapeutic resolution of microalbuminuria in people has been shown to decrease the risk of cardiovascular and renal complications. Whether or not early identification of microalbuminuria would affect treatment and prognosis in veterinary patients remains to be seen.
In this study, we evaluated healthy RRG’s and identified a high percentage of dogs with hypertension. Greyhounds have been proposed as a model for primary hypertension because many of them have hypertension on routine testing, often in the absence of known secondary causes of hypertension. Currently this is thought to be a breed related train in Greyhounds, however the clinical significance of this is unknown. We identified a significant relationship between microalbuminuria and hypertension, which does support the clinical relevance of this in Greyhounds. We were unable to document any other evidence of underlying renal dysfunction or abnormal renal histology in this population of dogs. Microalbuminuria however is known to be one of the earliest findings in hypertensive nephropathy and other primary glomerular diseases, preceding overt proteinuria, tubular dysfunction, and histopathologic changes in the kidney.

6.2: Limitations of this study

One of the major limitations of this study is the fact that individual Greyhounds could not be followed over time. The risks of white coat hypertension dictate that mild to moderate hypertension without definitive evidence of target organ damage, should not be treated based on a single high blood pressure measurement. The typical recommendation is to repeat BP measurements in 1-2 weeks in animals with borderline hypertension. This recommendation is supported by research in Beagles showing a significant decrease in blood pressure as a result of acclimation to the technique over approximately 14 days.
We hypothesize that our blood pressure measurements in these dogs were reliable and that there is clinical evidence that this was pathologic hypertension; however, white coat effect could not be conclusively excluded, and ideally, BP measurements in these dogs would have been followed over a period of 2-3 weeks to insure that the BP was persistently high. Additionally, no gold standard (i.e., direct arterial measurement) was used in this study to confirm that the indirect methods were accurate. Indirect methods tend to underestimate BP, and so it is likely that direct methods would have confirmed hypertension, possibly even of higher magnitude.

It is also only recommended to treat microalbuminuria after it has been confirmed in multiple urine samples over a period of several weeks. Studies in people and dogs have shown that a large number of systemic inflammatory, infectious, metabolic, and neoplastic diseases can be associated with microalbuminuria. Without being able to follow these dogs, it is impossible to know whether microalbuminuria would have been persistent or not.

With regard to other markers of renal dysfunction, no consistent histopathologic changes were seen, fractional excretion of electrolytes were normal, and most UPC results were normal. These findings may have been a result of the population of animals evaluated. Most of these Greyhounds were young, with an average age of 3.7 years, whereas clinical experience with hypertensive proteinuric Greyhounds involves older dogs. The lack of other findings in this study could indicate that renal dysfunction is slow to develop, and may not be evident until later in life. Long-term longitudinal studies of hypertensive greyhounds would be required to evaluate this possibility.
6.3: Future Avenues of Investigation:

In the future, a similar study performed in a cohort of RRG’s with long-term follow up should allow for more accurate characterization of blood pressure status, microalbuminuria / proteinuria, and long-term changes in these variables. Such a study also would allow for better characterization of the renal histopathologic lesions that may develop in proteinuric Greyhounds, as the decision to biopsy could be based on progression to overt proteinuria rather than microalbuminuria. Microalbuminuria in this study did not correlate well with renal histopathologic changes.

Treatment of microalbuminuria and hypertension also could be evaluated in a long-term longitudinal study because individuals documented to have microalbuminuria and hypertension could be randomized into various treatment groups (e.g., dietary modification, ACE inhibitors, calcium channel blockers, no treatment) to see if various treatments affect the rate of progression to overt proteinuria or a decrease in kidney function including GFR.
APPENDIX A:

TABLES

Table 1: Histopathologic scoring system used for light microscopic evaluation of renal biopsies.

<table>
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<tr>
<th>Lesion</th>
<th>Lesion Severity A</th>
<th>Lesion Distribution B</th>
<th>Total Score C</th>
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<tbody>
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<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
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<td>0-3</td>
<td>0-6</td>
</tr>
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<td>Lymphocytic/Plasmacytic Infiltrates</td>
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<td>0-3</td>
<td>0-6</td>
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<td>Tubular Dilatation</td>
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<td>0-3</td>
<td>0-6</td>
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</tr>
<tr>
<td>Glomerular Atrophy</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Periglomerular fibrosis</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Bowman Capsule Thickenning</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Glomerular Basement Membrane Thickening</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Mesangial Matrix Expansion</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
<tr>
<td>Mesangial Cell Proliferation</td>
<td>0-3</td>
<td>0-3</td>
<td>0-6</td>
</tr>
</tbody>
</table>

A: 0 – absent; 1 – mild; 2 – moderate; 3 – severe
B: 0 – absent; 1 – focal; 2 – multifocal; 3 – diffuse
C: Severity score (0-3) + distribution score (0-3)
Table 2: Biochemical Data in 48 Clinically Normal RRG’s.

<table>
<thead>
<tr>
<th>Chemical Measure</th>
<th>Reference Range</th>
<th>Number of dogs</th>
<th>Mean +/- std. dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Urea Nitrogen (BUN) mg/dL</td>
<td>5-20</td>
<td>48</td>
<td>15.67 ± 3.71</td>
<td>9-25</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>0.6-1.6</td>
<td>48</td>
<td>1.57 ± 0.25</td>
<td>1.1-2.2</td>
</tr>
<tr>
<td>Sodium meq/L</td>
<td>143-153</td>
<td>48</td>
<td>149.16 ± 4.09</td>
<td>144-167</td>
</tr>
<tr>
<td>Chloride meq/L</td>
<td>109-120</td>
<td>48</td>
<td>112.27 ± 3.83</td>
<td>108.5-129.4</td>
</tr>
<tr>
<td>Potassium meq/L</td>
<td>4.2-5.4</td>
<td>48</td>
<td>3.94 ± 0.31</td>
<td>3.4-4.76</td>
</tr>
<tr>
<td>Phosphorous mg/dL</td>
<td>3.2-8.1</td>
<td>48</td>
<td>3.74 ± 0.98</td>
<td>1.8-6.3</td>
</tr>
<tr>
<td>Calcium mg/dL</td>
<td>9.3-11.6</td>
<td>48</td>
<td>10.56 ± 0.58</td>
<td>9.5-12.8</td>
</tr>
<tr>
<td>Bicarb mmol/L</td>
<td>16-25</td>
<td>48</td>
<td>23.9 ± 1.98</td>
<td>19.7-29</td>
</tr>
<tr>
<td>Alanine Aminotransferase IU/L</td>
<td>10-55</td>
<td>48</td>
<td>69.4 ± 33.6</td>
<td>27-184</td>
</tr>
<tr>
<td>Aspartate Aminotransferase IU/L</td>
<td>12-40</td>
<td>48</td>
<td>47.57 ± 22.69</td>
<td>19-167</td>
</tr>
<tr>
<td>Alkaline Phosphatase IU/L</td>
<td>15-120</td>
<td>48</td>
<td>40.26 ± 19.22</td>
<td>13-112</td>
</tr>
<tr>
<td>Cholesterol mg/dL</td>
<td>80-315</td>
<td>48</td>
<td>136.53 ± 26.67</td>
<td>74-199</td>
</tr>
<tr>
<td>Total Bilirubin mg/dL</td>
<td>0.1-0.4</td>
<td>48</td>
<td>0.22 ± 0.07</td>
<td>0.1-0.4</td>
</tr>
<tr>
<td>Total Protein g/dL</td>
<td>5.1-7.1</td>
<td>48</td>
<td>6.08 ± 0.52</td>
<td>4.5-7.2</td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td>2.9-4.2</td>
<td>48</td>
<td>3.78 ± 0.32</td>
<td>2.8-4.7</td>
</tr>
<tr>
<td>Globulins g/dL</td>
<td>2.2-2.9</td>
<td>48</td>
<td>2.29 ± 0.35</td>
<td>1.6-3.7</td>
</tr>
<tr>
<td>Glucose mg/dL</td>
<td>77-126</td>
<td>48</td>
<td>103.39 ± 13.63</td>
<td>69-127</td>
</tr>
</tbody>
</table>
Table 3: CBC Data in 48 Clinically Normal RRG’s.

<table>
<thead>
<tr>
<th>Chemical Measure</th>
<th>Reference Range</th>
<th>Number of dogs</th>
<th>Mean +/- std. dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC K/µL</td>
<td>5.5-16</td>
<td>48</td>
<td>7.63 ± 2.11</td>
<td>4.38-12.67</td>
</tr>
<tr>
<td>Lymphocytes K/µL</td>
<td>0.5-4.9</td>
<td>48</td>
<td>1.62 ± 0.53</td>
<td>0.96-3.49</td>
</tr>
<tr>
<td>Monocytes K/µL</td>
<td>0.3-2</td>
<td>48</td>
<td>0.69 ± 0.25</td>
<td>0.27-1.43</td>
</tr>
<tr>
<td>Neutrophils K/µL</td>
<td>2-12</td>
<td>48</td>
<td>5.11 ± 1.76</td>
<td>2.51-9.41</td>
</tr>
<tr>
<td>Eosinophils K/µL</td>
<td>0.1-1.49</td>
<td>48</td>
<td>0.18 ± 0.27</td>
<td>0.02-1.88</td>
</tr>
<tr>
<td>Basophils K/µL</td>
<td>0-0.1</td>
<td>48</td>
<td>0.02 ± 0.01</td>
<td>0-0.06</td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>37-55</td>
<td>48</td>
<td>50.82 ± 3.7</td>
<td>42.4-60.5</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>12-18</td>
<td>48</td>
<td>18.65 ± 1.8</td>
<td>15.3-24.7</td>
</tr>
<tr>
<td>MCV fL</td>
<td>60-77</td>
<td>48</td>
<td>63.37 ± 2.2</td>
<td>57.9-68.5</td>
</tr>
<tr>
<td>MCHC g/dL</td>
<td>30-37.5</td>
<td>45</td>
<td>36.08 ± 1.67</td>
<td>32.9-39.3</td>
</tr>
<tr>
<td>Platelet K/µL</td>
<td>175-500</td>
<td>48</td>
<td>209.83 ± 54.35</td>
<td>123-393</td>
</tr>
</tbody>
</table>

Table 4: Urine Profile Results in 47 Clinically Healthy RRG’s

<table>
<thead>
<tr>
<th>Chemical Measure</th>
<th>Number of dogs</th>
<th>Mean +/- std. dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Specific Gravity</td>
<td>47</td>
<td>1.046 ± 0.15</td>
<td>1.010-1.059</td>
</tr>
<tr>
<td>Urine Urea mg/dL</td>
<td>47</td>
<td>2795.64 ± 838.7</td>
<td>607-4168</td>
</tr>
<tr>
<td>Urine Creatinine mg/dL</td>
<td>47</td>
<td>427.07 ± 150.7</td>
<td>97.9-938.3</td>
</tr>
<tr>
<td>Urine Sodium meq/L</td>
<td>47</td>
<td>110.28 ± 86.7</td>
<td>9-367</td>
</tr>
<tr>
<td>Urine Potassium meq/L</td>
<td>47</td>
<td>111.05 ± 58.9</td>
<td>12.1-399.2</td>
</tr>
<tr>
<td>Urine Chloride meq/L</td>
<td>47</td>
<td>100.21 ± 75.8</td>
<td>20-360</td>
</tr>
<tr>
<td>Urine Phosphorous meq/L</td>
<td>47</td>
<td>126.21 ± 107.39</td>
<td>0.6-526</td>
</tr>
<tr>
<td>Urine Calcium meq/L</td>
<td>47</td>
<td>3.09 ± 2.93</td>
<td>0.1-13.3</td>
</tr>
<tr>
<td>Urine Protein mg/dL</td>
<td>47</td>
<td>46.21 ± 59.9</td>
<td>6-417</td>
</tr>
<tr>
<td>Urine Protein-Creatinine Ratio</td>
<td>47</td>
<td>0.11 ± 0.16</td>
<td>0.03-1.14</td>
</tr>
</tbody>
</table>
Table 5: Frequency of Microalbuminuria and Breakdown in 47 Clinically Healthy RRG’s.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Mean ± SD (range)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbuminuria</td>
<td>Negative</td>
<td>-</td>
<td>22 (47%)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
<td>13 (28%)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-</td>
<td>10 (21%)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Microalbuminuria (collapsed)</td>
<td>Negative</td>
<td>-</td>
<td>22 (47%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>-</td>
<td>25 (53%)</td>
</tr>
</tbody>
</table>

Table 6: Urinary Fractional Excretion of Electrolytes, Mean ± Std. Deviation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD (range)</th>
<th>Greyhound Specific Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.1% ± 0.1% (0.0% to 0.3%)</td>
<td>≤ 0.13</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.3% ± 0.3% (0.1% to 1.4%)</td>
<td>≤ 0.55</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>11.4% ± 7.2% (0.2% to 28.7%)</td>
<td>≤ 16.5</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.5% ± 4.5% (2.9% to 20.5%)</td>
<td>≤ 12.2%</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.3% ± 0.3% (0.0% to 1.0%)</td>
<td>≤ 0.72%</td>
</tr>
</tbody>
</table>

Table 7: Pearson Correlation Coefficient, ICC, and CCC for Systolic Blood Pressure Measurements Between Days, and Between Methods.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Pearson Correlation</th>
<th>ICC</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1: Oscillometric vs. Doppler</td>
<td>0.86 (0.77, 0.92)</td>
<td>0.80 (0.68, 0.89)</td>
<td>0.81 (0.72, 0.91)</td>
</tr>
<tr>
<td>Day 3: Oscillometric vs. Doppler</td>
<td>0.77 (0.62, 0.86)</td>
<td>0.71 (0.56, 0.83)</td>
<td>0.73 (0.60, 0.86)</td>
</tr>
<tr>
<td>Oscillometric: Day 1 vs. Day 3</td>
<td>0.95 (0.92, 0.97)</td>
<td>0.94 (0.90, 0.97)</td>
<td>0.94 (0.91, 0.98)</td>
</tr>
<tr>
<td>Doppler: Day 1 vs. Day 3</td>
<td>0.86 (0.76, 0.92)</td>
<td>0.85 (0.76, 0.92)</td>
<td>0.86 (0.78, 0.93)</td>
</tr>
</tbody>
</table>
Table 8: Bland-Altman Plots, Bias and 5% and 95% Limits of Agreement.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Bias</th>
<th>95% Limits of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Day 1: Doppler – Oscillometric</td>
<td>6.3</td>
<td>-11.8</td>
</tr>
<tr>
<td>Day 3: Doppler – Oscillometric</td>
<td>5.3</td>
<td>-15.1</td>
</tr>
<tr>
<td>Doppler: Day 1 – Day 3</td>
<td>-0.4</td>
<td>-17.0</td>
</tr>
<tr>
<td>Oscillometric: Day 1 – Day 3</td>
<td>-1.4</td>
<td>-12.6</td>
</tr>
</tbody>
</table>

Table 9: Summary Data For Age, Sex, MA, Fractional Excretion of Electrolytes, and UPC When Broken Down into 2 Groups: Hypertensive and Normotensive.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Not Hypertensive (n=22)</th>
<th>Hypertensive (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>15 (58%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7 (32%)</td>
<td>12 (48%)</td>
</tr>
<tr>
<td>Age</td>
<td>Mean ± SD (range)</td>
<td>4.1 ± 1.9 (1, 9.5)</td>
<td>3.6 ± 1.9 (1.5, 8.5)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>Negative</td>
<td>18 (82%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>3 (14%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0 (0%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1 (5%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Microalbuminuria (collapsed)</td>
<td>Negative</td>
<td>18 (82%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>4 (18%)</td>
<td>21 (84%)</td>
</tr>
<tr>
<td>Calcium</td>
<td>Mean ± SD (range)</td>
<td>0.1% +/- 0.1% (0%, 0.2%)</td>
<td>0.1% +/- 0.1% (0%, 0.3%)</td>
</tr>
<tr>
<td>Chloride</td>
<td>Mean ± SD (range)</td>
<td>0.3% +/- 0.3% (0.1%, 1.1%)</td>
<td>0.4% +/- 0.3% (0.1%, 1.4%)</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>Mean ± SD (range)</td>
<td>9.9% +/- 6.6% (0.2%, 28.7%)</td>
<td>12.8% +/- 7.6% (0.3%, 26.2%)</td>
</tr>
<tr>
<td>Potassium</td>
<td>Mean ± SD (range)</td>
<td>9.5% +/- 3.8% (2.9%, 17.5%)</td>
<td>11.4% +/- 5% (3.7%, 20.5%)</td>
</tr>
<tr>
<td>Sodium</td>
<td>Mean ± SD (range)</td>
<td>0.3% +/- 0.3% (0%, 1%)</td>
<td>0.3% +/- 0.3% (0%, 1%)</td>
</tr>
<tr>
<td>Urine Protein/Creatinine Ratio</td>
<td>Mean ± SD (range)</td>
<td>0.085 ± 0.05 (0.037, 0.242)</td>
<td>0.14 ± 0.219 (0.032, 1.139)</td>
</tr>
</tbody>
</table>
Table 10: Pearson correlation, 95% CI, and p-value for urine protein creatinine ratio and urinary fractional excretion (Fe) of electrolytes compared with renal biopsy score.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine protein/creatinine ratio</td>
<td>0.51</td>
<td>(-0.02, 0.81)</td>
<td>0.0502</td>
</tr>
<tr>
<td>Fe Sodium</td>
<td>-0.14</td>
<td>(-0.62, 0.42)</td>
<td>0.6306</td>
</tr>
<tr>
<td>Fe Phosphorous</td>
<td>0.31</td>
<td>(-0.27, 0.72)</td>
<td>0.2730</td>
</tr>
<tr>
<td>Fe Calcium</td>
<td>-0.17</td>
<td>(-0.64, 0.4 )</td>
<td>0.5608</td>
</tr>
<tr>
<td>Fe Potassium</td>
<td>-0.30</td>
<td>(-0.7, 0.25 )</td>
<td>0.2695</td>
</tr>
<tr>
<td>Fe Chloride</td>
<td>-0.27</td>
<td>(-0.7, 0.31 )</td>
<td>0.3492</td>
</tr>
</tbody>
</table>

Table 11: Pearson Correlation Coefficients, 95% CI, and p-value for the following parameters associated with the renal histopathology score.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine protein/creatinine ratio</td>
<td>0.51</td>
<td>(-0.02, 0.81)</td>
<td>0.0502</td>
</tr>
<tr>
<td>Fe Sodium</td>
<td>-0.14</td>
<td>(-0.62, 0.42)</td>
<td>0.6306</td>
</tr>
<tr>
<td>Fe Phosphorous</td>
<td>0.31</td>
<td>(-0.27, 0.72)</td>
<td>0.2730</td>
</tr>
<tr>
<td>Fe Calcium</td>
<td>-0.17</td>
<td>(-0.64, 0.4 )</td>
<td>0.5608</td>
</tr>
<tr>
<td>Fe Potassium</td>
<td>-0.30</td>
<td>(-0.7, 0.25 )</td>
<td>0.2695</td>
</tr>
<tr>
<td>Fe Chloride</td>
<td>-0.27</td>
<td>(-0.7, 0.31 )</td>
<td>0.3492</td>
</tr>
</tbody>
</table>
APPENDIX B:

FIGURES

Comparison of Systolic BP via Doppler:
Day 1 vs. Day 3

Figure 1: Box and whisker plots of systolic blood pressure in 47 Greyhounds measured via Doppler on Day 1 and Day 3. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. There was no statistical difference between systolic blood pressure on day 1 and day 3, p = 0.53.
Comparison of Systolic BP via Oscillometric Method:
Day 1 vs. Day 3

Figure 2: Box and whisker plots of systolic blood pressure in 47 Greyhounds measured via oscillometric method on Day 1 and Day 3. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. There was no statistical difference between systolic blood pressure on day 1 and day 3, p = 0.07.
Figure 3: Box and whisker plots of systolic blood pressure in 47 Greyhounds measured via Doppler and oscillometric methods. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. There was no statistical difference between systolic blood pressure on day 1 and day 3, p = 0.53.
Figure 4: Correlation curve for the systolic Doppler pressure on day 1 vs. the systolic oscillometric pressure on day 1.

Figure 5: Correlation curve for the systolic Doppler pressure on day 3 vs. the systolic oscillometric pressure on day 3.
Figure 6: Correlation curve for the systolic Doppler pressure on Day 1 vs. the systolic Doppler pressure on day 3.

Figure 7: Correlation curve for the systolic oscillometric pressure on day 1 vs. the systolic oscillometric pressure on day 3.
Figure 8: Bland-Altman plot comparing the Doppler and oscillometric pressures on day 1. Central hashed line indicates the bias, and the upper and lower hashed lines the 95% limits of agreement. Bias and 95% Limits of Agreement are listed in Table 8.

Figure 9: Bland-Altman plot comparing the Doppler and oscillometric pressures on day 3. Central hashed line indicates the bias and the upper and lower hashed lines the 95% limits of agreement. Bias and 95% Limits of Agreement are listed in Table 8.
Figure 10: Bland-Altman plot comparing the Doppler pressures on day 1 and day 3. Central hashed line indicates the bias and the upper and lower hashed lines the 95% limits of agreement. Bias and 95% Limits of Agreement are listed in Table 8.

Figure 11: Bland-Altman plot comparing the oscillometric pressures on day 1 and day 3. Central hashed line indicates the bias and the upper and lower hashed lines the 95% limits of agreement. Bias and 95% Limits of Agreement are listed in Table 8.
Comparison of BP: Hypertensive vs. Normotensive Greyhounds

![Box and whisker plots of mean systolic pressure in the hypertensive (BP > 165mmHg) and normotensive (BP < 165mmHg) groups. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. The stars indicate statistical significance between the groups at p <0.001.](image)

Figure 12: Box and whisker plots of mean systolic pressure in the hypertensive (BP > 165mmHg) and normotensive (BP <165mmHg) groups. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. The stars indicate statistical significance between the groups at p <0.001.
Comparison of Heart Rate: Hypertensive vs. Normotensive Greyhounds

Figure 13: Box and whisker plots of heart rate in 47 Greyhounds. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. There was no statistical difference between mean heart rate in the hypertensive and normotensive groups, p = 0.25.
Comparison of Age:  
Hypertensive vs Normotensive Greyhounds

Figure 14: Box and whisker plots of age in 47 Greyhounds. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent 1.5 x the IQR in the up and down direction range, and circles represent outlying values. There was no statistical difference between mean age in the hypertensive and normotensive groups, p = 0.1682.
Figure 15: Standard bar graph evaluating sex in 47 Greyhounds. Groups are designated as noted in the legend. Chi-square analysis of this showed no significant association between sex and blood pressure status, $p = 0.26$. 
Figure 16: Correlation curve evaluating the association between the UPC and renal biopsy score. A slight upward trend can be seen associating increasing UPC with increasing biopsy score. The r value was low at 0.51; however this did approach significance at p = 0.0502
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