INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

THE EFFECTS OF FUROSEMIDE ADMINISTRATION ON GLUCOSE HOMEOSTASIS IN HEALTHY DOGS AND DOGS WITH ALLOXAN-INDUCED INSULINOPENIC DIABETES MELLITUS

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the Department of Companion Animals

Faculty of Veterinary Medicine

University of Prince Edward Island

Kristi L. Graham

Charlottetown, PEI

June, 1999

The author has agreed that the Library, University of Prince Edward Island, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Chairman of the Department or the Dean of the Faculty in which the thesis work was done. It is understood that due recognition will be given to the author of this thesis and to the University of Prince Edward Island in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Prince Edward Island and the author's written permission is prohibited.

Requests for permission to copy or to make any other use of material in this thesis in whole or in part should be addressed to:

Chairman of the Department of Companion Animals

Faculty of Veterinary Medicine

University of Prince Edward Island

Charlottetown, PEI

Canada C1A 4P3

SIGNATURE PAGES

ii-iii

REMOVED

ABSTRACT

Diuretic therapy is an essential element in the therapeutic plan for cardiac disease and other conditions characterized by fluid retention. Although considered medications with few side effects, several diuretics have been implicated in the development of glucose intolerance. In human medicine, sporadic reports have linked furosemide, a loop diuretic, to glucose intolerance in euglycemic patients and to reduced glycemic control in diabetic patients. Proposed mechanisms of impaired glucose tolerance following diuretic administration include decreased peripheral glucose effectiveness and inhibition of insulin secretion. Insulin secretion may be inhibited by hypokalemia, increased endogenous prostaglandin concentrations, or decreased calcium and chloride ion fluxes in the pancreatic beta cells.

Recent advances have improved glycemic control in animals with diabetes mellitus. As a result, diabetic canine and feline patients are enjoying living longer. However, the likelihood of developing other diseases, some of which may require diuretic therapy, has increased. Understanding the potential side effects a medication is important in devising an efficacious and safe therapeutic plan, especially for those patients with multiple disorders. Although the effects of furosemide on glucose metabolism have been evaluated in rodents and humans, no studies have evaluated glucose tolerance during furosemide administration in dogs. This study was designed to monitor glucose homeostasis in healthy and diabetic dogs receiving furosemide.

In the first part of the study (Phase I), the glycemic and selected biochemical effects of clinically relevant doses of furosemide in healthy beagle dogs was evaluated.

Acute trials evaluated intravenous furosemide administration at 0 mg/kg (saline), 2 mg/kg, and 6 mg/kg every 8 hours for a 24-hour period. Dose levels were organized in a randomized crossover fashion so that each dog received each dose one time. Chronic trials evaluated oral furosemide administration at 2 mg/kg and 4 mg/kg every 12 hours for 28 days. Dose levels were again organized in a randomized crossover fashion. In both trials, glycemic parameters, including fasting serum glucose (acute: $p = 0.3$; chronic: $p > 0.05$) and insulin (acute: $p = 0.2$; chronic: $p = 0.4$) concentrations, insulin sensitivity (acute: $p = 0.07$; chronic: $p = 0.12$), and glucose effectiveness (acute: $p = 0.9$; chronic: $p = 0.86$), were not significantly affected.

In the second part of the study (Phase II), alloxan was administered to the same group of beagles to induce insulinopenic diabetes mellitus. Following stabilization with exogenous insulin therapy, the trials were repeated as in Phase I with dose levels again organized in a randomized, crossover fashion. Acute trials evaluated intravenous furosemide administration at 0 mg/kg (saline), 2 mg/kg, and 6 mg/kg every 8 hours for a 24-hour period. Chronic trials evaluated oral furosemide administration at 0 mg/kg (empty inert capsule) and 2 mg/kg every 12 hours for 28 days. No significant changes were noted in either trial for fasting serum glucose (acute: $p = 0.8$; chronic: $p = 0.72$) and insulin (acute: $p = 0.33$; chronic: $p = 0.78$) concentrations, insulin sensitivity (acute: $p = 0.33$; chronic: $p = 0.82$), and glucose effectiveness (acute: $p = 0.93$; chronic: $p = 0.63$).

Furosemide was found to have no effect on glucose tolerance at the doses administered to these groups of dogs. Although a prospective clinical trial with larger sample size is necessary to confirm these observations, it would appear that it is safe to include furosemide in the therapeutic plan of dogs with diabetes mellitus.

ACKNOWLEDGMENTS

The author is grateful for the support and guidance provided in so many different ways by all those individuals involved in the completion of this project. Specifically, I would like to extend my gratitude to:

Sherri L. Ihle, my Graduate Advisor and Supervising Diplomate;

My Supervisory Committee: Cathy Chan, James Miller, Alastair Cribb, Brian Hill, and India Lane;

Carole McCarville, for all her help and expertise with the "Beagle Brigade";

Michael Brimacombe, for support with statistical analysis;

AVC Companion Animal Trust, for providing the financial support for this project;

John Daniel Barbee Educational Trust, for providing an equipment grant allowing the preparation of this manuscript and associated presentations;

And always: Bob, Mom, Dad, and Tammy, for everything.

TABLE OF CONTENTS

TABLE OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CNS	Central nervous system
C-peptide	Cleavage peptide
DNA	Deoxyribonucleic acid
FSIGT	Frequently sampled intravenous glucose tolerance test
GLUT-4	Insulin-sensitive glucose transport protein 4
GLUT-2	Insulin-independent glucose transport protein 2
IDDM	Insulin-dependent diabetes mellitus
IVGTT	Intravenous glucose tolerance test
NIDDM	Noninsulin-dependent diabetes mellitus
OGTT	Oral glucose tolerance test
PGE_2	Prostaglandin E_2
RNA	Ribonucleic acid

GENERAL INTRODUCTION

In humans and companion animals, thiazide and loop diuretics are integral components of therapeutic protocols for many commonly diagnosed disorders. In companion animals, the use of furosemide, a loop diuretic, is widespread. It is most frequently used in the treatment of congestive heart failure, but is also used for noncardiogenic pulmonary edema, non-inflammatory edematous conditions, hypercalcemic nephropathy, acute renal failure, and, occasionally, hypertension (1-3).

Diuretics are efficacious and safe when used in the appropriate setting. Side effects include mild hypovolemia, hypokalemia, hypercholesterolemia, hyperuricemia, and, occasionally, ototoxicity (furosemide) (1-3). In addition, both thiazide and loop diuretics have been reported to cause glucose intolerance. Sporadic reports in humans have linked various diuretics, such as chlorothiazide, hydrochlorothiazide, and furosemide, with spontaneous hyperglycemia in previously euglycemic patients or with the loss of glycemic control in diabetic patients (4-7). This potential side effect presents a therapeutic dilemma because conditions requiring diuretics may coexist with diabetes mellitus.

Heart disease and diabetes mellitus are common clinical disorders in companion animals. It is important for clinicians to recognize and understand potential side effects or drug interactions when developing a safe and efficacious therapeutic plan. Despite recommendations in the veterinary literature to use diuretics judiciously and with caution in diabetic patients (1,8), no clinical *in vivo* studies have been performed in dogs to document the presence or absence of hyperglycemic effects associated with diuretic administration (8).

This study was designed to determine if administration of furosemide would alter glucose tolerance and glycemic control in healthy and diabetic dogs. This knowledge will be beneficial to clinicians developing therapeutic protocols for patients at risk for developing diabetes mellitus or for patients with diabetes mellitus and concurrent diseases such as heart failure.

1. LITERATURE REVIEW

1.1. Glucose homeostasis

1.1.1. Introduction

Glucose is the most important energy substrate available to mammals. The metabolism of glucose through glycolysis and the citric acid cycle provides energy in the form of adenosine triphosphate (ATP) to all cells of the body (9-13). Glucose is vital because it is the only source of energy for cells of the central nervous system (CNS) under normal conditions. Neurons are unable to synthesize glucose and only have a small reserve of glucose readily available for metabolism (10). This small reserve capacity of the CNS mandates that a constant supply of glucose must be available from the systemic circulation. For this reason, many of the body's homeostatic mechanisms are designed to maintain the blood glucose concentration within a narrow range.

1.1.2. Role of carbohydrate metabolism

Exogenous glucose is supplied to the body primarily through gastrointestinal digestion of carbohydrates. Breakdown of carbohydrates to monosaccharides results in approximately 80% glucose, with the remaining 20% consisting of fructose and galactose. Once absorbed into the portal circulation, the majority of the fructose and galactose are transported to the liver and converted to glucose. Thus, greater than 95% of carbohydrate metabolism results in the formation of glucose (9).

Although carbohydrate metabolism is the primary source of glucose, gluconeogenesis in liver and kidneys can synthesize glucose from non-carbohydrate precursors. These precursors include glycerol, lactate, and certain glucogenic amino acids, especially alanine (14). These sources of glucose are most important during periods of starvation when oral carbohydrates are not available.

1.1.3. Cellular transport and storage of glucose

Once the body acquires glucose, it must either be delivered to individual cells to satisfy their energy requirements or be converted into a form that can be stored by the body for later use. Glucose transport throughout the body is accomplished via the systemic circulation. Because most cells are not readily permeable to glucose, passive intracellular diffusion of glucose does not occur in spite of a favorable concentration gradient. Glucose is primarily delivered intracellularly by facilitated diffusion. Facilitated diffusion occurs along a concentration gradient, but it also a membrane-bound carrier protein. Several carrier proteins have been identified. One such protein is the insulin-sensitive glucose carrier protein (GLUT-4) found primarily in skeletal muscle and adipose tissue. Once stimulated by insulin, the carrier protein transports glucose from the plasma into the cell (15,16). Enterocytes, renal tubular epithelial cells, erythrocytes and leukocytes utilize another process. In these cells, glucose is co-transported with sodium ions into the cell. The active transport of sodium ions by ATPase facilitates and maintains the gradient which encourages the facilitated diffusion of glucose intracellularly. The transport of glucose is in this way coupled to the transport of sodium ions. A glucose carrier molecule, the insulin-independent transporter protein (GLUT-2),

also exists on the serosal surface of gastrointestinal epithelial cells where it acts to transport glucose to the portal circulation (15,16).

Once within the cytosol, glucose is immediately converted to glucose-6-phosphate (9). This is an irreversible process in all cells except hepatocytes, gastrointestinal cells, and renal tubular epithelial cells. These three cell types contain the enzyme glucose-6-phosphatase which is necessary for conversion of glucose-6-phosphate back to glucose. Once glucose-6-phosphate has been formed, it either enters the glycolytic pathway for ATP production or is converted into glycogen, a large glucose polymer, for storage purposes. Glycolysis generates two molecules of pyruvate from one glucose-6-phosphate molecule resulting in the net production of two ATP molecules. Pyruvate is then transported into the mitochondrial matrix where it is decarboxylated to acetyl Coenzyme A (acetyl CoA). Acetyl CoA reacts with oxaloacetate and water to produce citrate. Through the entry of citrate into the citric acid cycle and subsequent completion of this cycle two more ATP molecules are generated. The remainder of the ATP molecules are generated through electron transfer during oxidative phosphorylation. In total, glycolysis, the citric acid cycle, and oxidative phosphorylation generate 36 molecules of ATP from the oxidation of one glucose molecule (9,11-13).

When additional energy is needed, catecholamines or glucagon activate the enzyme phosphorylase. Phosphorylase catalyzes the phosphorylation of stored glycogen (glycogenolysis) in hepatocytes and skeletal muscle liberating glucose-6-phosphate for entry into the glycolytic pathway (14).

1.1.4. Hormonal regulation of glucose homeostasis: the role of insulin

The narrow range of blood glucose concentration is maintained primarily through hormonal regulation, although minor neuronal control does exist (14). Because significant hypoglycemia is life-threatening, nearly all homeostatic hormones increase blood glucose. Examples of these glucogenic hormones include catecholamines (epinephrine and norepinephrine), somatotropin, glucagon, and glucocorticoids (specifically cortisol). In contrast, insulin causes a rapid decrease in the blood glucose concentration. Insulin is essential to avoid hyperglycemia. Chronic hyperglycemia is detrimental and eventually leads to beta cell exhaustion, glucose toxicity, and other abnormalities (10,17,18). The blood glucose concentration is the result of a complex interaction between oral carbohydrate intake, hormonal regulation, and the body's need for energy.

1.1.4.1. Insulin synthesis and storage

Insulin is synthesized in the beta cells of the islets of Langerhans, a component of the endocrine pancreas. It is first transcribed as a 12 kDa protein known as pre-proinsulin. Pre-proinsulin is transported into the rough endoplasmic reticulum where it is cleaved to form the 9 kDa protein proinsulin. Proinsulin spontaneously folds upon itself and forms two disulfide bonds. It then travels to the Golgi apparatus and is packaged into secretory granules. Within the granules, proinsulin is cleaved into the final product of insulin and a biologically inactive protein known as cleavage peptide (C-peptide). Insulin is comprised of an alpha and beta chain held together by disulfide bonds. Insulin

and C-peptide are stored in equimolar amounts within the secretory granules and are released together. At any one time, pancreatic beta cells have approximately ten times the normal daily insulin requirement in storage (16,19).

1.1.4.2. Secretion of insulin

Insulin is secreted from pancreatic beta cells by calcium-dependent exocytosis. There are two types of insulin secretion. Constitutive, or unregulated, secretion occurs without stimulation even in the presence of low blood glucose concentration. Stimulated insulin secretion occurs in response to specific stimuli (20). Various stimuli of insulin secretion have been documented, but increasing blood glucose concentration is considered to be the primary stimulus. Elevated blood glucose concentration will increase glucose metabolism in beta cells. The ATP formed initiate closure of potassium channels located within the beta cell membrane (21-23). The decrease in the potassium ion efflux from the cell causes depolarization of the cell membrane and allows entry of calcium ions. The increased intracellular calcium concentration is believed to activate a microtubular network which brings the secretory granules to the cell leading to membrane fusion and exocytosis of granular contents (16,21-23).

Stimulated insulin secretion is biphasic. The initial phase of secretion is immediate, occurring within seconds and lasting approximately one minute. This phase likely results from release of a stored pool of insulin in the secretory granules located near the cell membrane. After the first phase of secretion, a resting period occurs which lasts about five to seven minutes. This is followed by the second phase of secretion which occurs more gradually, eventually reaching a sustained peak. This second peak of

insulin secretion likely represents both the less available intracellular storage pool of insulin as well as newly synthesized insulin (21,22).

Other stimuli for increased insulin secretion include gastrointestinal hormones (e.g. glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1)), parasympathetic nervous system activation, glucagon, and certain amino acids (i.e. leucine, arginine). Glucocorticoids, estrogen, and progesterone will increase insulin secretion indirectly by decreasing the responsiveness of peripheral tissues to insulin. As a result, the blood glucose increases and stimulates insulin secretion. The two main inhibitors of insulin secretion are somatostatin and increased sympathetic nervous system activation (16).

1.1.4.3. Actions of insulin

The effects of insulin are widespread and involve many different tissues. Its overall effect is to promote storage of excess energy (i.e. glucose) as either glycogen or triglyceride molecules. Insulin does this via several actions. It stimulates the insulin-sensitive GLUT-4 carrier protein in skeletal muscle and adipocytes, promoting a net intracellular movement of glucose. It stimulates glucokinase in hepatocytes resulting in the formation of glucose-6-phosphate. It also stimulates the enzyme glycogen synthase, encouraging glycogenesis, and inhibits glucose-6-phosphatase, suppressing glycogenolysis. The final result is an increase in total body glycogen content (14-16).

Insulin increases intracellular transport of amino acids and fatty acids. It promotes lipogenesis and inhibits lipolysis resulting in increased triglyceride storage, adipose tissue formation, and decreased ketogenesis. Increased RNA, DNA, and protein

synthesis stimulated by insulin contribute to the overall "protein-sparing" effect of insulin and to growth and development of the entire animal (10).

Another effect of insulin is the maintenance of the intracellular potassium concentration. This is a result of increased cellular uptake of potassium and magnesium ions and increased activity of the Na^+/K^+ ATPase pump in response to insulin. The significance of this action is currently unknown (10).

1.1.5. Diabetes mellitus in companion animals

The importance of the role of insulin in the control of glucose homeostasis and the maintenance of body energy stores is best demonstrated when a deficiency of insulin exists. A relative or absolute deficiency of insulin is the hallmark of diabetes mellitus, one of the most common canine and feline endocrine disorders recognized in veterinary medicine (24).

The term diabetes means "running through", describing the increase in water intake and urination noted in patients with the disease. Mellitus is Latin for "honey", or sweet, and describes the increase in urine glucose characteristic of the condition. Diabetes mellitus is characterized clinically by polyuria, polydipsia, polyphagia, and weight loss. Insulin deficiency or insulin resistance results in persistent hyperglycemia. Glycosuria develops when the blood glucose concentration exceeds the renal threshold. The hypertonicity of the urine promotes a diuresis accounting for the clinical signs of polyuria and the compensatory polydipsia. The perceived energy deficiency maintains a catabolic state resulting in polyphagia and muscle wasting. Polyphagia may also result

from reduced inhibitionof the satiety center. Insulin mediates the movement of glucose into cells of the hypothalamus in the region of the satiety, or feeding, center. Without insulin, extracellular glucose cannot inhibit the satiety center and the patient continues to experience hunger (17,18,25).

In the dog and cat, diabetes is usually classified as either insulin-dependent diabetes mellitus (IDDM) or noninsulin-dependent diabetes mellitus (NIDDM). Pancreatic beta cell destruction resulting in a decrease or absence of insulin secretion is characteristic of IDDM. By contrast, NIDDM is characterized by beta cell dysfunction and insulin resistance. Thus, administration of exogenous insulin is a requirement in IDDM but not in NIDDM. Diabetes mellitus may be either primary or secondary to concurrent disease or medications. Secondary diabetes may result from conditions that induce an increase in serum progesterone concentration such as diestrus and pregnancy, or those which induce insulin resistance such as obesity or hyperadrenocorticism (17,18,25).

1.1.6. The role of drugs in glucose homeostasis

Drugs may affect glucose homeostasis directly or indirectly. Conflicting information makes it difficult to interpret the clinical significance of these interactions. Still, it is important to realize that glucose homeostasis is a delicate balance that may be altered by drug administration.

The most common drug-induced glucose intolerance is caused by glucocorticoid administration. They promote protein catabolism and liberate amino acids, mobilizing them into circulation. The liver deaminates the amino acids and converts them into

glucose. Glucocorticoids also decrease the response of the peripheral tissues to insulin producing an insulin resistant state (26). Hyperadrenocorticism caused by endogenous glucocorticoid excess, is the most common condition where clinical insulin resistance is encountered. However, exogenous administration of glucocorticoids can also cause insulin resistance (26,27).

In humans, combined estrogen and progesterone oral contraceptives can cause decreased insulin sensitivity in peripheral tissues and reduce glucose tolerance. This effect may be the result of alterations in insulin receptor concentrations or of the receptor itself (28). Progesterone compounds, such as megesterol acetate, have been implicated to cause glucose intolerance in dogs (29,30). This may be the result of progesterone-induced growth hormone secretion and decreased insulin sensitivity in the peripheral tissues (29,30).

Calcium channel antagonists, specifically nifedipine, have been associated with decreased glucose tolerance in some hypertensive human patients (31,32). Calcium channel blocking agents may decrease the influx of calcium ions into the beta cell, thereby inhibiting insulin secretion. One clinical trial on human patients with NIDDM found no significant changes in results of oral glucose tolerance tests (OGTT) between placebo and treatment groups (33). Thus, the significance of calcium channel antagonists on glucose intolerance is unclear.

Although the mechanisms of drug-induced glucose intolerance remain to be elucidated, it is obvious that many drugs alter the complex homeostatic mechanisms governing glucose tolerance. The clinical importance of these interactions is not known.

However significant disease may result if certain drugs precipitate diabetes mellitus, affect insulin secretion or produce insulin resistance.

1.2. Furosemide

1.2.1. Introduction

Diuresis, the process of increasing urine production, is useful for many diseases. There are many classes of diuretics, each with a different site of action within the nephron. Furosemide is a high-ceiling, or loop, diuretic. Loop diuretics act by inhibiting reabsorption of sodium and chloride ions in the thick ascending limb of the loop of Henle. Specifically, they inhibit the active co-transport of sodium, chloride, and potassium ions from the luminal side of the cell and decrease NaCl reabsorption. The loss of NaCl into the urine decreases the ability of the kidney to maintain a hypertonic interstitium. Thus, the favorable concentration gradient for water reabsorption is decreased and water cannot be conserved. Loop diuretics are the most potent diuretics with the greatest peak effect and the most rapid onset of action. Furosemide, as well as the other loop diuretics, is potassium-wasting, and chronic use may lead to hypokalemia (34). Increased urinary excretion of potassium results from decreased reabsorption and increased secretion of K^+. Inhibition of K^+ reabsorption in the thick ascending limb occurs in association with the inhibition of Na^+ and Cl^- reabsorption as a result of the primary action of furosemide. Several mechanisms may enhance K^+ secretion in the distal tubule. Diuresis decreases medullary osmolality thus resulting in increased urine flow; increased distal tubule potassium secretion is directly proportional to the increase in

urine flow (2,3,34). Volume contraction induced by the diuresis activates the renin-angiotensin-aldosterone system, increasing aldosterone concentrations, and subsequently stimulating K^+ secretion (2,3).

1.2.2. Other actions of furosemide

In addition to diuresis, furosemide is believed to have other effects. Furosemide may increase endogenous prostaglandin concentrations. Studies have shown that furosemide will increase urinary prostaglandin excretion (35-38). Other studies have demonstrated an increase in renal medullary synthesis of PGE_2, which may be calcium-dependent (39-41). This may occur through inhibition of one or both of the enzymes that degrade active prostaglandins, 9-ketoprostaglandin dehydrogenase and 15-hydroxy-prostaglandin dehydrogenase (36). Increased prostaglandin activity stimulates renin release and increases renal blood flow (3). Prostaglandins also increase peripheral venous capacitance, which decreases left atrial pressure. Furosemide may increase renal blood flow through hypovolemia-induced activation of the renin-angiotensin system. Vascular dilation around the juxtaglomerular apparatus and increased sodium content near the macula densa are proposed mechanisms for stimulating renin release (2,3,42). At the cellular level, furosemide can inhibit membrane transport ATPase, mitochondrial respiration, glycolysis, and the microsomal calcium pump (43-48).

Furosemide administration can alter serum lipid and lipoprotein concentrations in humans. Significant increases in serum lipid and lipoprotein concentrations were seen within three hours following furosemide administration (49). This may have resulted

from diuretic-induced hemoconcentration as because of the concurrent increases in serum protein concentration and decreases in body weight (49).

1.3. Furosemide-induced hyperglycemia

1.3.1. Introduction

Both thiazide and loop diuretics have been implicated in glucose intolerance in humans. Diuretics commonly used as antihypertensive agents, such as chlorothiazide and hydrochlorothiazide, have been associated with spontaneous hyperglycemia (4,50). Diazoxide, a nondiuretic thiazide originally used only for emergency therapy of hypertension, causes profound hyperglycemia. It is currently the preferred medical adjunctive therapy for the hypoglycemia associated with pancreatic beta cell insulinomas (51-53).

1.3.2. Clinical case reports in the human literature

A 67-year-old woman diagnosed with congestive heart failure developed glycosuria and a diabetic OGTT after one month of hydrochlorothiazide therapy. Her OGTT returned to normal following discontinuation of the diuretic. Because continued diuretic therapy was deemed necessary, she then began receiving 40-80 mg/day of furosemide. After seven months of furosemide therapy, she again developed glycosuria and a diabetic OGTT. Four months after cessation of furosemide, her OGTT was near normal. Although an OGTT was not performed prior to initiating therapy, the return of

the OGTT to near normal following the discontinuation of both the hydrochlorothiazide and furosemide suggests that the diuretics were the cause of her glucose intolerance (4).

In 1966, Toivonen presented a case of suspected furosemide-induced hyperglycemia in a 61-year-old woman in congestive heart failure. Prior to initiating medical therapy, the patient had a normal OGTT. Following four weeks of therapy with 0.08 g/day of furosemide and 0.25 mg/day of digoxin, she developed glycosuria and an abnormal OGTT supporting a diagnosis of glucose intolerance (5). An OGTT was not performed after discontinuing the furosemide, but would have been useful to decide if furosemide precipitated the glucose intolerance. However, a normal OGTT was present prior to therapy and no familial history of diabetes mellitus was documented. In light of these facts, and that other diuretics have been implicated in causing hyperglycemia (50), it seems likely that the furosemide was associated with the glucose intolerance in this patient.

1.3.3. Studies in nonhuman species

Although there are only two reports of furosemide-induced hyperglycemia in humans, numerous studies have evaluated the effect of furosemide administration on glucose homeostasis in rodents. It is difficult to make direct comparisons among studies due to widely variable doses, routes of administration, and duration of treatment. However, all of the studies discovered significant alterations in either glucose metabolism or glucose tolerance.

Furosemide has been repeatedly shown to significantly increase blood glucose concentrations in the rat (54-57) and the mouse (58-60). In a study evaluating ten

different diuretics administered to rats, seven produced significant hyperglycemia (56). Intraperitoneal administration of 200 mg/kg furosemide resulted in a two-hour post treatment blood glucose concentration that was 49.6% greater than the immediate pretreatment value ($p<0.01$) (56). Other acute experiments in rats have evaluated the effects of intravenous furosemide administration at a much lower dose (1-2 mg/kg) (54). In normal rats, a significant decrease in plasma insulin concentration was noted between one and ten minutes following furosemide injection. At 20 minutes post injection, there was a small, but statistically significant elevation in blood glucose concentration (54). An intravenous glucose tolerance test (IVGTT) revealed a decrease in the glucose disappearance rate (54). Interestingly, when diabetic rats were similarly tested, there were no changes in either blood glucose or plasma insulin concentrations (54).

Acute experiments in mice have utilized doses between 100 and 200 mg/kg of furosemide administered intraperitoneally. In normal mice, only a transient rise in blood glucose concentration was noted at 30 minutes post injection in one study. This elevation returned to normal within two hours and was not statistically significant (61). However, a significant rise in the glucose/insulin ratio was found, suggesting a decrease in insulin secretion (61). Furosemide administration to *ob/ob* mice, a strain of mice characterized by genetic obesity, hyperinsulinemia, hyperglycemia, and polyphagia, caused a significant increase in blood glucose concentrations within 30 minutes (58). Although control *ob/ob* mice also demonstrated an increase in blood glucose concentrations, presumably due to the stress of handling, this was statistically less than the increase noted in the furosemide-treated mice. The furosemide-induced hyperglycemia was shown to persist for up to two days (58). As in normal mice, these studies also demonstrated an

increase in the glucose/insulin ratio (58). Another study evaluating IVGTTs in *ob/ob* mice following furosemide administration found consistent increases in blood glucose but variable IVGTT results between individual mice (62). Two distinct groups of *ob/ob* mice were utilized in this study. The first group consisted of three-month-old mice. Genetically obese mice at this age have not yet developed obesity or insulin resistance (62). At 180 minutes following furosemide administration, this group developed significant hyperglycemia that resolved within two days (62). The second group consisted of eight-month-old mice. At this age, all *ob/ob* mice have developed obesity and clinical diabetes mellitus (62). After furosemide administration, hyperglycemia occurred within 180 minutes and was sustained for at least two days (62). The IVGTTs performed on both groups of mice were widely variable, with some demonstrating glucose intolerance while others, in the eight-month-old group only, had improved glucose tolerance (62).

Chronic administration of furosemide to rats and mice has also been investigated. In one study, furosemide was administered subcutaneously to normoglycemic Sprague-Dawley rats, an animal model of arteriosclerosis (55). After receiving 1 mg/kg every 12 hours for four weeks, the rats developed several significant biochemical changes, including hyperglycemia. Further evaluations of glucose metabolism were not performed on these rats (55). Two weeks of oral furosemide (100 mg/kg/day) administration to normal rats resulted in significantly altered OGTTs consistent with glucose intolerance (55). Normal mice receiving furosemide at a dosage of 100mg/kg/day for 14 days developed hyperglycemia, increased hepatic glycogen content, and an abnormal OGTT

consistent with glucose intolerance. However, there was no effect on IVGTT or insulin sensitivity in these mice (59).

The differences in dosages and duration of furosemide treatment make it difficult to directly compare these results. However, they do suggest that furosemide has the ability to alter glucose metabolism in rodent models. The doses used often exceed the usual clinical doses in human and companion animal medicine, making it difficult to predict the clinical significance of these observations.

1.3.4. Clinical studies in human patients

Although data from rodent studies appears to support the concept of diuretic-induced glucose intolerance, the clinical significance in humans and companion animals must be evaluated directly. Clinical trials have been performed evaluating both thiazide diuretics (6,7,63-66) and furosemide (7,65-68) in human patients.

The effect of long term oral diuretic therapy was evaluated in 34 hypertensive patients receiving uninterrupted oral thiazide therapy for 14 years (6). No significant change was noted in their OGTT during the first year (6). After six years, a significant decrease in glucose tolerance was noted and this deterioration was even greater by the end of the 14-year-period (6). In ten patients, thiazide therapy was discontinued for seven months and an OGTT was repeated. Withdrawal of thiazide therapy resulted in a 10% reduction in the fasting serum glucose concentration and a 25% reduction in the two-hour serum glucose concentration, suggesting an improvement in glucose tolerance but not a return to normal (6). There was no correlation between the presence of glucose intolerance and decreased serum potassium concentration. However, if persistent

hypokalemia was present, glucose intolerance was more severe than if the patient was normokalemic (6). Comparison of hydrochlorothiazide, propranolol, and combined drug therapy in hypertensive men with NIDDM revealed similar findings (64). Hydrochlorothiazide for three weeks produced significant hyperglycemia and increased glycosylated hemoglobin while propranolol had no significant effects on glucose parameters (64). Therapy with both agents produced a more profound hyperglycemia than did hydrochlorothiazide alone (64). No relationship was found between the presence of hyperglycemia and endogenous serum insulin concentration or serum potassium concentration (64).

The glucose tolerance of hypertensive patients treated with benzothiadiazine diuretics for at least three years was evaluated utilizing OGTTs (7). Out of 40 patients, 12 (30%) had results consistent with diabetes mellitus. Seven of the 12 patients discontinued diuretic therapy, but only one patient had improved OGTT (7). Four of the 12 patients were switched to furosemide therapy. Three of these patients had an OGTT and two had a decreased glucose tolerance when compared to their OGTT prior to furosemide (7). As glucose tolerance was not evaluated just prior to initiating furosemide therapy, the possibility of pre-existing diabetes mellitus could not be ruled out (7). A trend toward hyperglycemia was noted in 17 diabetic, hypertensive humans following four weeks of furosemide therapy (68). This trend disappeared by three months and was never statistically significant (68).

Two studies have failed to document a worsening of glucose intolerance following thiazide diuretic or furosemide therapy in NIDDM patients (66,67). A double-blind comparison between furosemide and piretanide was performed in 24 human

patients with congestive heart failure and NIDDM (67). No statistically significant changes were seen between treatment groups. As well, no changes in serum potassium concentrations or oral antidiabetic therapy were noted (67). Another double-blind crossover study compared furosemide to hydrochlorothiazide therapy in 24 patients with NIDDM (66). Nine of these patients had concurrent hypertension and 15 had concurrent congestive heart failure. No difference was noted in blood glucose concentration between treatment groups (66).

Another study compared a regimen of furosemide for three weeks, chlorothiazide for three weeks, followed again by furosemide at the initial dose for three months (65). Although a trend toward improved glucose tolerance during furosemide therapy was noted in these 19 nondiabetic hypertensive patients, statistical significance was not reached (65).

These contradictory results make it difficult to draw conclusions regarding diuretic therapy and its effect on glucose tolerance. Negative results may have occurred due to an inadequate dose or duration of therapy. Individual patient variability in response to furosemide administration may also explain the conflicting responses in clinical trials.

1.3.5. Suggested mechanisms of action underlying furosemide-induced hyperglycemia

Several mechanisms have been proposed for furosemide-induced glucose intolerance. They relate to either alteration in glucose metabolism or inhibition of insulin secretion. Alterations in glucose metabolism that have been documented with furosemide

administration include inhibition of glycolysis and inhibition of glucose utilization by the peripheral tissues (43-46,69).

Inhibition of glycolysis by furosemide has been demonstrated in several studies (43,46,69). A dose-dependent inhibition of lactate production, an indicator of glycolysis, was noted in cell-free preparations of turtle urinary bladder, human erythrocytes, and renal medulla and cortex of various species (43). Although direct enzymatic studies were not performed, the authors suggested that a direct inhibition of glycolysis through inhibition of glyceraldehyde-3-phosphate dehydrogenase had occurred (43). Studies with soleus muscle harvested from rats given high doses of furosemide (100 and 1000 mg/kg) also demonstrated a decrease in lactate formation (69). In skeletal muscle and liver, furosemide inhibited activities of several glycolytic enzymes including hexokinase, phosphofructokinase and pyruvate kinase (46). Inhibition of these enzymes slows or stops glycolysis and decreases glucose utilization (46). Further studies have documented a noncompetitive, noninsulin-dependent decrease in glucose transport in muscle and erythrocytes (44,45). Decreases in glucose transport will decrease glucose utilization (44). The documented decrease in glucose transport has been suggested to be the result of furosemide-induced alterations within the microenvironment surrounding the carrier molecules (45).

The most investigated possible mechanism of furosemide-induced glucose intolerance has been inhibition of insulin secretion. Proposed causes of reduced insulin secretion include hypokalemia, reduced pancreatic blood flow, and increased prostaglandin concentrations (70,71). Hypokalemia is a well-accepted side effect of furosemide administration, but one that is rarely recognized clinically in the dog.

However, due to the intracellular nature of potassium, serum concentrations may be misleading. Total body potassium depletion can be present with normal serum potassium concentrations.

Hypokalemia can impair glucose tolerance (72,73). Potassium depletion was induced in seven healthy men utilizing a potassium-reduced diet. Glucose tolerance was evaluated by the hyperglycemic clamp technique (72). Following potassium depletion, the amount of glucose metabolized was significantly decreased when compared to pre-diet values (72). The plasma insulin response to hyperglycemia was significantly decreased once hypokalemia was established (72).

Aggressive diuretic therapy can induce a hypovolemic state. Reduction in pancreatic blood flow could alter pancreatic islet function. A study on anesthetized dogs receiving intravenous furosemide (2 mg/kg) showed a significant decrease in the pancreatic blood flow with no change in peripheral blood pressure (74). Blood flow was decreased in all areas of the pancreas. If volume replacement was maintained with intravenous fluid therapy, no change in blood flow was noted (74). There was no change in the plasma glucose or insulin concentration (74). An interesting, but difficult to explain observation, was that intravenous diazoxide (10 mg/kg) in these dogs resulted in an increase in pancreatic blood flow (74).

As noted earlier, furosemide increases renal and urinary prostaglandin concentrations (3). Information regarding prostaglandins and insulin secretion is contradictory. Prostaglandin E_2 (PGE_2), has been shown to decrease insulin secretion. Intravenous infusion of PGE_2 in human patients with NIDDM inhibited the acute insulin response to a glucose bolus (75). In support of these observations, infusion of sodium

salicylate, a prostaglandin antagonist, increased the insulin response (75). In 30 healthy human patients, intravenous furosemide decreased the insulin response to two glucose boluses (76). Lysine acetylsalicylate, another prostaglandin antagonist, reversed this inhibitory effect (76). Reversal of the insulin inhibition with a prostaglandin antagonist would suggest that the furosemide-induced reduction in insulin secretion is mediated by an increase in endogenous prostaglandin concentrations. However, prostaglandins (specifically PGE_2) have also been shown to have no effect on *in vitro* insulin secretion (77). Addition of exogenous PGE_2 to isolated, perfused rat pancreas had no effect on either basal or glucose-stimulated insulin secretion (77). Surprisingly, when furosemide was utilized to increase endogenous PGE_2 concentrations, insulin secretion was augmented suggesting that tissue prostaglandins may be involved or another non-prostaglandin mediated mechanism occurs (77).

1.4. Purpose of this study

There is both experimental and clinical evidence to suggest that furosemide administration may alter glucose tolerance in rodents and humans. Due to species differences in drug metabolism, it is difficult and often dangerous to extrapolate from one species to another regarding potential side effects. This study was designed to evaluate the effects of furosemide administration on glucose tolerance and glycemic control in healthy and diabetic dogs. No other clinical studies have been performed in dogs to evaluate these effects.

2. MATERIALS AND METHODS

2.1. Subject Selection

Six healthy beagle dogs, three intact males and three spayed females, were used for the study. Each dog was identified as apparently healthy following a physical examination, complete blood count, and serum biochemical profile. Each dog had free access to fresh water for the duration of the study. The diet composition (Science Diet canine maintenance dry, Hill's Pet Products, Topeka, Kanasas) was kept constant, but infrequent small changes in the amount fed were made to maintain body weight. The dogs were housed and cared for in accordance with guidelines established by the Canadian Council on Animal Care in the "Guide to the Care and Use of Experimental Animals" and the Animal Care Committee of the University of Prince Edward Island.

2.2. Experimental Design

The study was comprised of two phases. Phase I evaluated the effects of furosemide administration in healthy beagle dogs. Phase II evaluated these effects in dogs with alloxan-induced insulinopenic diabetes mellitus. Each phase consisted of an acute and a chronic trial.

2.2.1. Phase I – Acute Trial

The acute trial was designed to simulate an emergency clinical setting (e.g. severe cardiogenic pulmonary edema) in which a wide dose range of furosemide administration may be necessary.

Dogs were randomly assigned to a control group, a low-dose group and a high-dose group in a crossover manner (Table 1). A seven-day "washout period" was used between dose trials. Furosemide (Lasix^{R} 5% solution, Hoechst Roussel Vet, Regina, SK Canada) was administered intravenously at 2 mg/kg body weight (low-dose) and 6 mg/kg body weight (high-dose) every eight hours for a 24-hour period. One milliliter of 0.9% sodium chloride (Baxter, Toronto, Ontario Canada) was administered intravenously to control dogs. Food was removed 12 hours into the trial.

After the 24-hour period, samples were collected for a serum electrolyte profile. An insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) was performed. The complete procedure for the FSIGT has been described elsewhere (78-84). Briefly, an indwelling catheter was aseptically placed in the jugular vein to facilitate collection of blood samples and allow for intravenous administration of glucose and insulin. At time 0 minutes, glucose at 0.3 g/kg body weight was administered intravenously over 60 seconds. Regular crystalline insulin (Eli Lilly, Indianapolis, IN USA) was infused at 0.03 U/kg body weight from time 20-25 minutes. A total of 26 blood samples were collected for serum glucose and insulin concentrations at times –1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. Approximately one half a milliliter of heparinized saline was flushed into the jugular catheter in between sample collection to prevent the formation of blood clots

within the catheter lumen (85). The samples were centrifuged within 15 minutes of collection and the serum harvested and stored frozen at -20° C for a maximum of nine months until the assays were performed.

Table 1 – Assignment of Normal Dogs to Treatment Groups during the Acute Trial

SUBJECT	WEEK 1	WEEK 2	WEEK 3
Dog 1	6 mg/kg	0 mg/kg	2 mg/kg
Dog 2	0 mg/kg	6 mg/kg	2 mg/kg
Dog 3	2 mg/kg	6 mg/kg	0 mg/kg
Dog 4	6 mg/kg	2 mg/kg	0 mg/kg
Dog 5	2 mg/kg	0 mg/kg	6 mg/kg
Dog 6	0 mg/kg	2 mg/kg	6 mg/kg

2.2.2. Phase I – Chronic Trial

The chronic trial was designed to simulate a clinical situation in which low dose oral furosemide administration would be necessary for maintenance medical therapy. Four days following the acute trial, the dogs were randomly assigned to two groups in a crossover manner (Table 2). Furosemide (Apo-furosemide, Apotex Inc, Toronto, ON Canada) was administered orally at 2 mg/kg body weight (low-dose) and 4 mg/kg body

weight (high-dose) every 12 hours for 28 days. A 14-day "washout period" was used between the dose trials.

Table 2 – Assignment of Normal Dogs to Treatment Groups during the Chronic Trial

SUBJECT	WEEK 0	WEEK 6
Dog 1	4 mg/kg	2 mg/kg
Dog 2	2 mg/kg	4 mg/kg
Dog 3	4 mg/kg	2 mg/kg
Dog 4	4 mg/kg	2 mg/kg
Dog 5	2 mg/kg	4 mg/kg
Dog 6	2 mg/kg	4 mg/kg

To monitor for development of dehydration, body weight and blood samples collected for packed cell volume and serum total protein concentration were assessed on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, and 71. On days 29 and 71, blood samples were collected for a serum electrolyte profile, packed cell volume, and serum total protein concentration. An insulin-modified FSIGT was performed on days 29 and 71 as previously described.

2.2.3. Induction of Diabetes Mellitus

Three days after completion of Phase I, all dogs entered Phase II of the study. 5,6-Dioxyuracil monohydrate (Alloxan^{R}, Sigma Chemical Co, St. Louis, MO USA) was administered intravenously at 65 mg/kg body weight to induce permanent insulinopenia via pancreatic beta cell destruction (86). To minimize the risk of nephrotoxicity, diuresis with Lactated Ringers solution (Baxter, Toronto, ON Canada) was performed for 2 ½ hours prior and 3 ½ hours following the administration of alloxan.

Diabetes mellitus was confirmed within 48 hours of alloxan administration by persistant hypergylcemia (i.e. blood glucose > 15 mmol/L; Accu-Chek III blood glucose monitor, Boehringer Mannheim Canada LTD., Laval, QC Canada) and glycosuria (documented by urine reagent dipstick, Multistix^{R} 8 SG, Bayer, Inc Healthcare Division, Etobicoke, ON Canada).

Four weeks were allowed for establishment of glycemic control prior to initiating Phase II. Subcutaneous NPH insulin (Eli Lilly, Indianapolis, IN USA) once daily was used to treat the diabetes. Blood glucose concentrations and a urine reagent dipstick were evaluated every 7 days. Glycemic control was defined as the ability to maintain blood glucose between 6 and 14 mmol/L with minimal glycosuria.

2.2.4. Phase II – Acute Trial

The dogs were randomly reassigned to treatment groups in a crossover design (Table 3). The acute trial was performed as described for Phase I. Exogenous insulin treatment was withheld each day a FSIGT was performed.

Table 3 – Assignment of Diabetic Dogs to Treatment Groups during the Acute Trial

SUBJECT	WEEK 1	WEEK 2	WEEK 3
Dog 1	6 mg/kg	2 mg/kg	0 mg/kg
Dog 2	6 mg/kg	0 mg/kg	2 mg/kg
Dog 3	2 mg/kg	6 mg/kg	0 mg/kg
Dog 4	0 mg/kg	6 mg/kg	2 mg/kg
Dog 5	2 mg/kg	0 mg/kg	6 mg/kg
Dog 6	0 mg/kg	2 mg/kg	6 mg/kg

2.2.5. Phase II – Chronic Trial

The dogs were randomly reassigned to treatment groups (Table 4). Fourteen days following the acute trial, the chronic trial was performed as described for Phase I with one exception. The dose groups in Phase II consisted of a control (empty capsule) dose and furosemide at a dosage of 2 mg/kg body weight (tablet plus empty capsule). This change was deemed necessary due to the variable condition of diabetes mellitus. It was thought that the pre-trial values would not be representative of the entire trial due to the on-going disease process, and therefore these values could not act as controls. It was noted in Phase I that the difference between the doses was minimal and thus the 2 mg/kg body weight dose was chosen as the most clinically applicable oral dose. As in the acute trial, exogenous insulin treatment was withheld each day that the FSIGT was performed.

Table 4 – Assignment of Diabetic Dogs to Treatment Groups during the Chronic Trial

SUBJECT	WEEK 0	WEEK 6
Dog 1	2 mg/kg	0 mg/kg
Dog 2	0 mg/kg	2 mg/kg
Dog 3	2 mg/kg	0 mg/kg
Dog 4	0 mg/kg	2 mg/kg
Dog 5	2 mg/kg	0 mg/kg
Dog 6	0 mg/kg	2 mg/kg

2.3. Analytical Methods

Initial biochemical parameters (Appendix A) and subsequent electrolyte measurements (Appendix B) were determined using a wet chemistry analyzer (Hitachi 911, Roche Diagnostics, Laval, QC Canada). The normal range utilized was established for adult dogs by the diagnostic services laboratory of the Atlantic Veterinary College. From samples obtained during the FSIGT, serum glucose concentrations were determined using the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Inc., Galway, Ireland). Serum insulin concentrations were determined using solid phase ^{125}I radioimmunoassay (Coat-a-Count Insulin, Diagnostic Products Corp, Los Angeles, CA USA) previously validated for canine serum (87).

Using minimal model analysis (MINMOD computer program, copyright R.N. Bergman), glucose effectiveness and insulin sensitivity were calculated from the glucose

and insulin values of the FSIGT (88). Glucose effectiveness is a measurement of the ability of glucose per se to promote its own disposal. Insulin sensitivity is defined as the measurement of the sensitivity of the peripheral tissues to the effects of insulin (88).

2.4. Statistical Analysis

All variables in the acute trial of Phase I and both trials of Phase II were evaluated with analysis of variance (ANOVA) using a general linear model, incorporating the crossover design and the effect of time. Significant findings were further evaluated utilizing Duncan's Multiple Range Test to identify differences between individual doses. In the chronic trial of Phase I, a Student's paired t-test was used to evaluate the variables as compared to pre-trial values. In Phase II, the variables associated with glucose homeostasis (fasting serum glucose, fasting endogenous serum insulin, insulin sensitivity, and glucose effectiveness) were also analyzed with ANOVA using a general linear model incorporating exogenous insulin dose as a covariant. In these models, a p-value less than 0.05 was considered significant.

3. RESULTS

3.1. Phase I

The dogs were of similar age and weight. The average age was three years and eight months; range: three years, four months - four years of age. The average body weight was 12.5 kilograms (kg); range: 10.5 – 14.5 kg. The dogs were fed a constant amount of an isocaloric diet during the entirety of Phase I.

3.1.1. Acute trial

No adverse reactions to intravenous furosemide administration were noted. Having constant access to fresh water, the dogs were able to maintain normal hydration with one exception. Dog 5 demonstrated mild dehydration (mild skin tenting and dry mucus membranes on physical examination and elevated packed cell volume, serum total protein, urea, and creatinine concentrations) eight hours following the third administration of the high dose of furosemide (6 mg/kg).

3.1.1.1. Serum electrolytes and selected renal parameters

No significant differences in serum sodium ($p = 0.2$), chloride ($p = 0.1$), potassium ($p = 0.08$), or calcium ($p = 0.08$) concentrations were noted when dogs received the placebo versus either furosemide dose (Figures 1A-D). Serum phosphorus

concentrations were not significantly different in the overall model ($p = 0.05$). However, because of the proximity of this p-value to statistical significance, multiple comparisons were performed to further compare the phosphorus values between dose trials. These analyses showed that serum phosphorus concentrations at the high dose were significantly higher than at the low dose of furosemide, but neither dose was significantly different from the placebo (Figure 1E).

Serum potassium, calcium, and phosphorus concentrations at all doses were within the established normal range for adult dogs (Diagnostic Services laboratory, Atlantic Veterinary College). Two dogs demonstrated hyponatremia when receiving the placebo. Two different dogs demonstrated hyponatremia when receiving the high dose of furosemide. Three dogs demonstrated hypochloremia when receiving the placebo. Two dogs demonstrated hypochloremia when receiving low dose furosemide and four dogs demonstrated hypochloremia when receiving high dose furosemide.

Serum urea ($p = 0.4$) and serum creatinine ($p = 0.3$) concentrations were not significantly different when dogs received placebo versus either dose of furosemide. When receiving the placebo, one dog had a serum urea concentration below the normal range. Another dog had a serum urea concentration below the normal range on the high dose furosemide. One dog had a serum urea concentration above the normal range when receiving the high dose furosemide. None of the serum creatinine concentrations for any dose were outside the established normal range for adult dogs (Figures 1F & 1G).

Figure 1 – Effect of intravenous furosemide on serum electrolytes and biochemical parameters in healthy dogs
All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of established normal range for adult dogs.

Figure 1A
Phase I - Acute Trial
Sodium

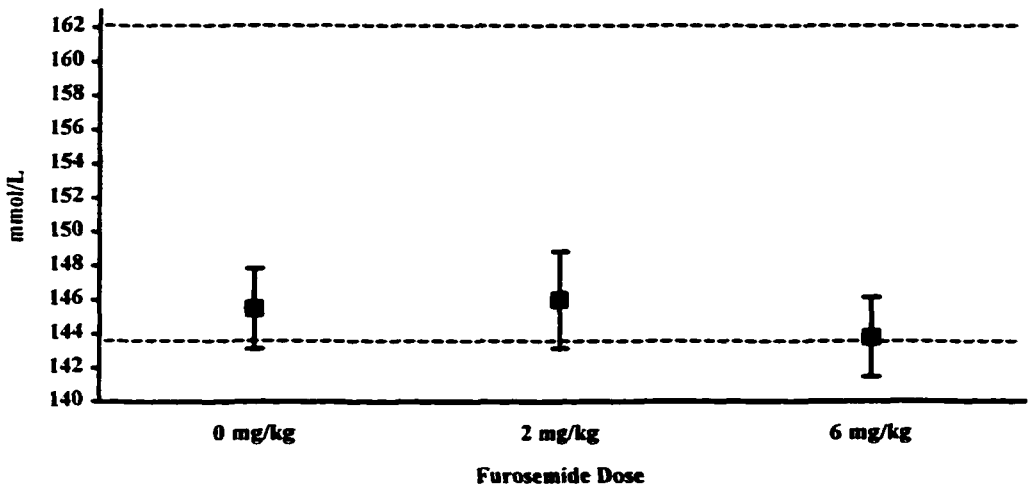

Fig 1A - 0 mg/kg = 145.5 +/- 2.36; 2 mg/kg = 146 +/- 2.84; 6 mg/kg = 143.8 +/- 2.36 (mean +/- 2SD)

Figure 1B
Phase I - Acute Trial
Chloride

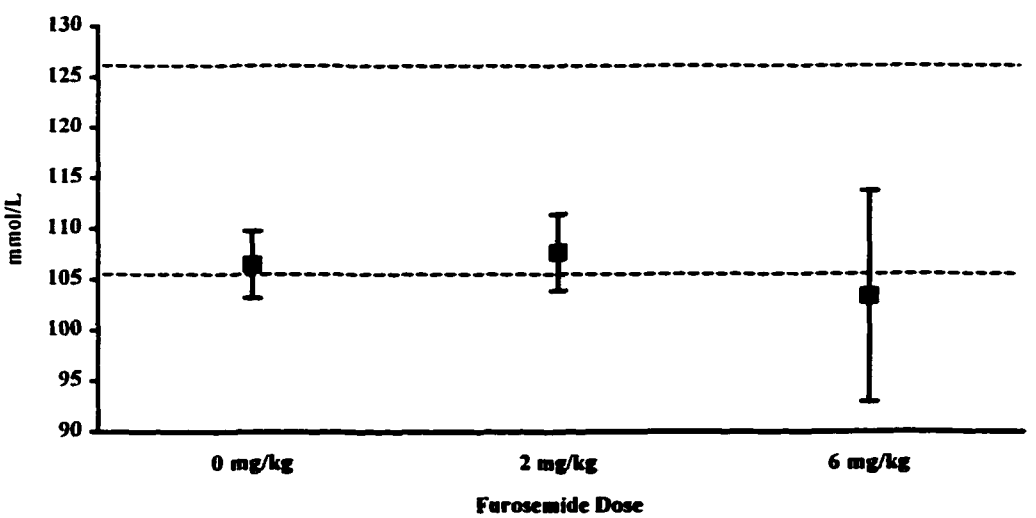

Fig 1B – 0 mg/kg = 106.5 +/- 3.5; 2 mg/kg = 107.7 +/- 3.76; 6 mg/kg = 103.3 +/- 10.38 (mean +/ 2SD)

Figure 1C
Phase I - Acute Trial
Potassium

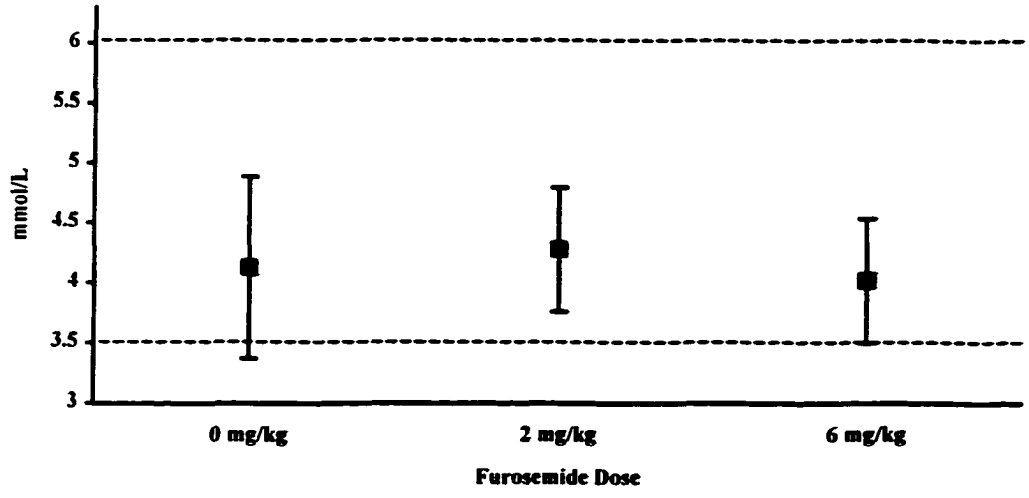

Fig 1C – 0 mg/kg = 4.13 +/- 0.76; 2 mg/kg = 4.28 +/- 0.52; 6 mg/kg = 4.02 +/- 0.52 (mean +/- 2SD)

Figure 1D
Phase I - Acute Trial
Calcium

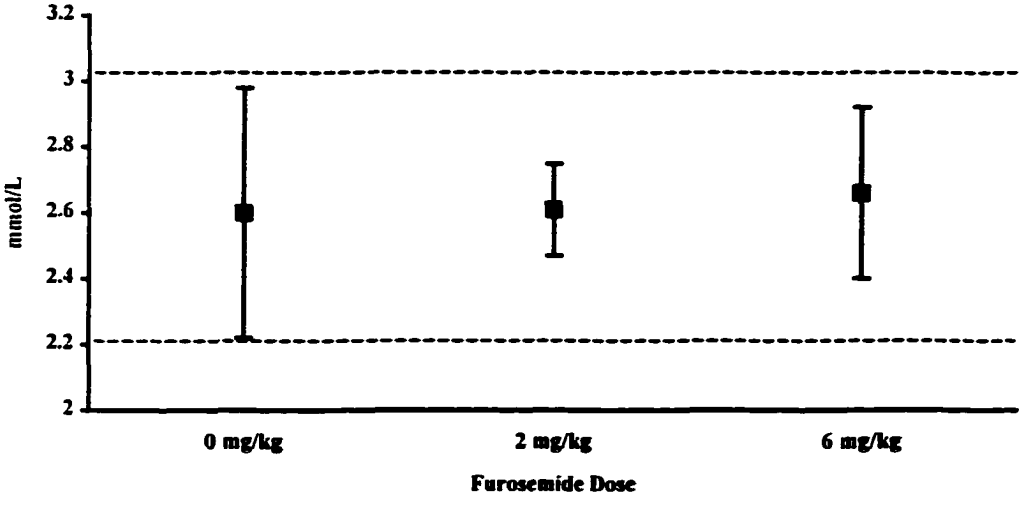

Fig 1D – 0 mg/kg = 2.6 +/- 0.38; 2 mg/kg = 2.61 +/- 0.14; 6 mg/kg 2.66 +/- 0.26 (mean +/- 2SD)

Figure 1E
Phase I - Acute Trial
Phosphorus

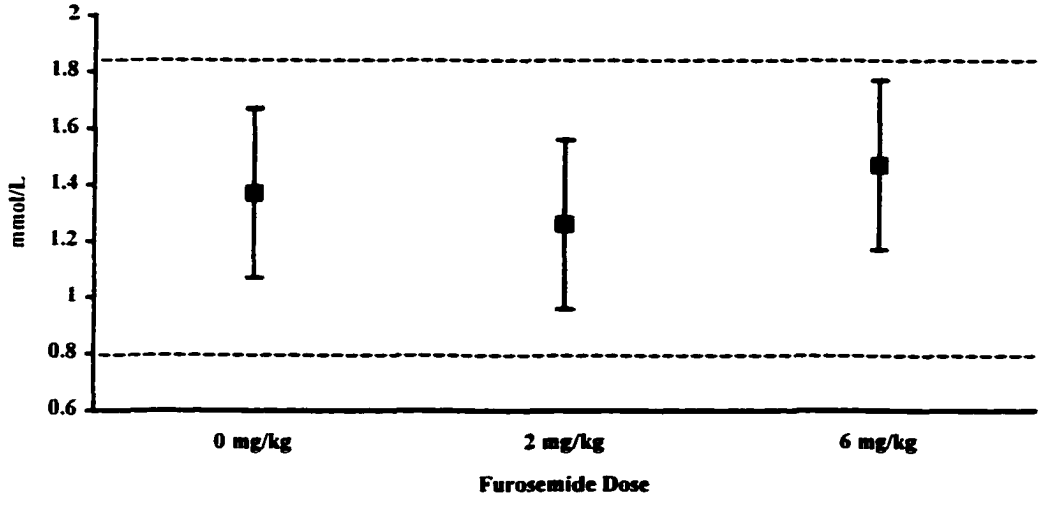

Fig 1E – 0 mg/kg = 1.37 +/- 0.3; 2 mg/kg = 1.26 +/- 0.3; 6 mg/kg = 1.47 +/- 0.3 (mean +/- 2SD)

Figure 1F
Phase I - Acute Trial
Serum Urea

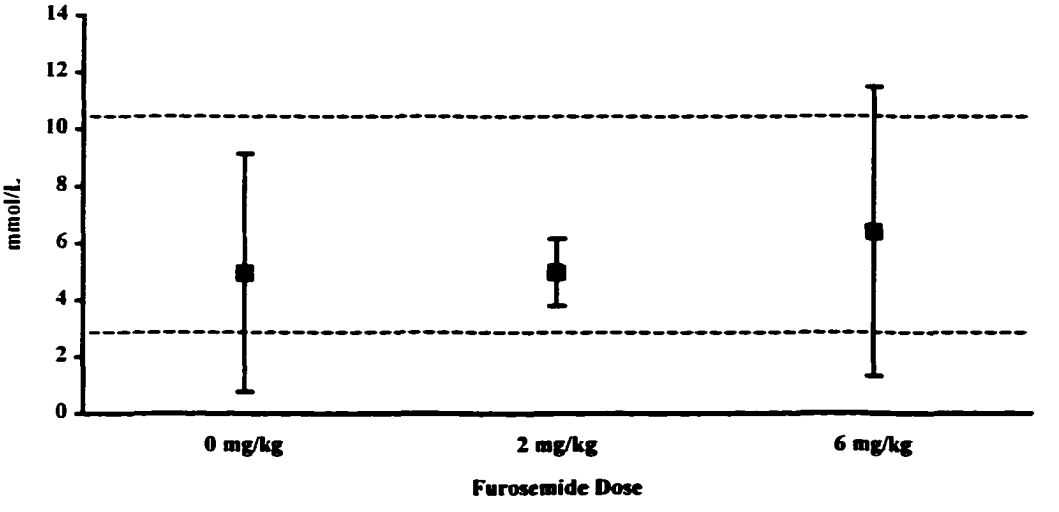

Fig 1F – 0 mg/kg = 4.95 +/- 4.18; 2 mg/kg = 4.98 +/- 1.18; 6 mg/kg = 6.4 +/- 5.08 (mean +/- 2SD)

Figure 1G
Phase I - Acute Trial
Serum Creatinine

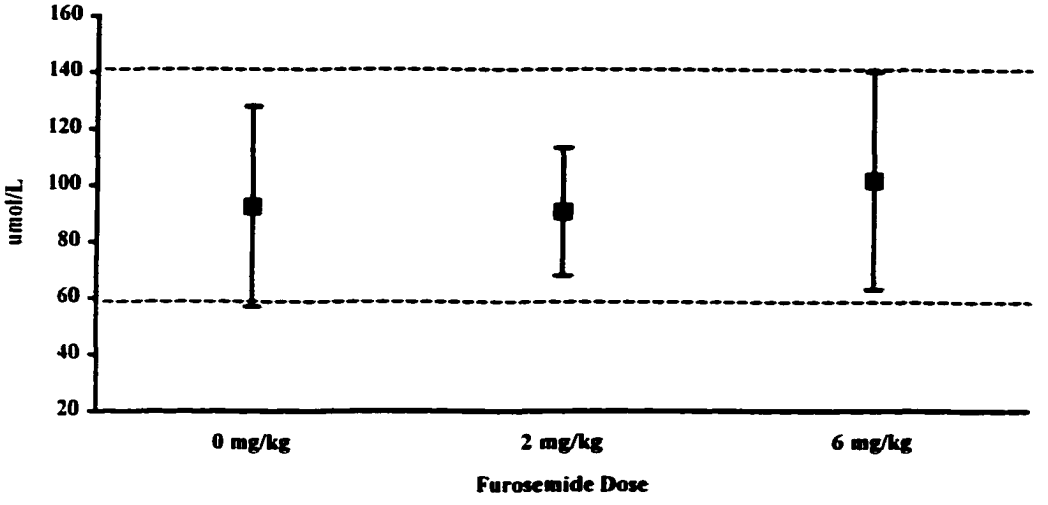

Fig 1G – 0 mg/kg = 92.5 +/- 35.36; 2 mg/kg = 90.67 +/- 22.62; 6 mg/kg = 101.67 +/- 38.66 (mean +/- 2SD)

3.1.1.2. Parameters of glucose homeostasis

No significant differences were found when dogs received placebo versus either furosemide dose for fasting serum glucose concentrations ($p = 0.3$), fasting serum insulin concentrations ($p = 0.2$), glucose effectiveness ($p = 0.9$), and insulin sensitivity ($p = 0.07$) (Figures 2A-D).

One dog had mild hyperglycemia when receiving the placebo and another dog had mild hyperglycemia when receiving the low furosemide dose. Three dogs had mild hyperglycemia when receiving the high furosemide dose.

Figure 2 – Effect of intravenous furosemide on serum glycemic parameters in healthy dogs
All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of the established normal range for adult dogs.

Figure 2A
Phase I - Acute Trial
Fasting Serum Glucose

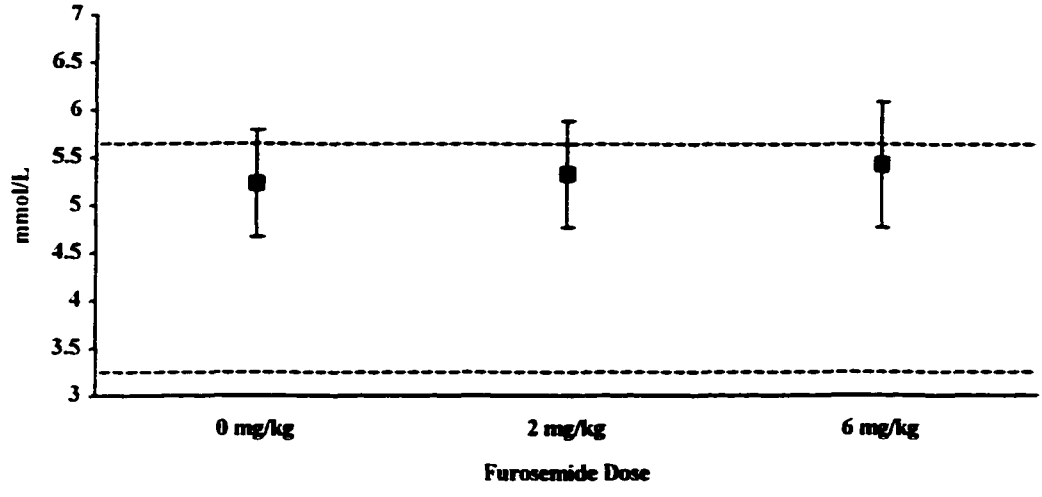

Fig 2A – 0 mg/kg = 5.23 +/- 0.56; 2 mg/kg = 5.33 +/- 0.56; 6 mg/kg = 5.43 +/- 0.66 (mean +/- 2SD)

Figure 2B
Phase I - Acute Trial
Fasting Serum Insulin

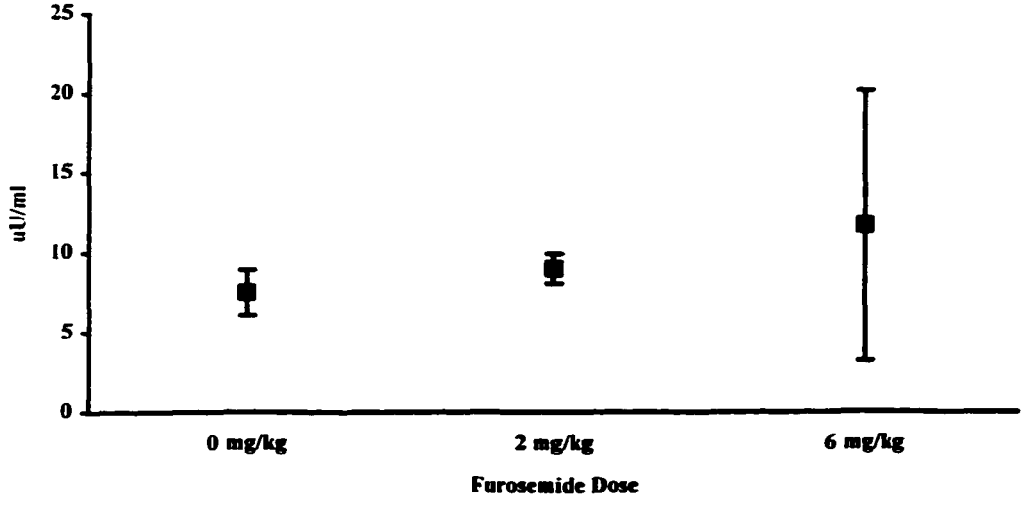

Fig 2B – 0 mg/kg = 7.5 +/- 1.42; 2 mg/kg = 9 +/- 0.94; 6 mg/kg = 11.67 +/- 8.48 (mean +/- 2SD)

Figure 2C
Phase I - Acute Trial
Glucose Effectiveness

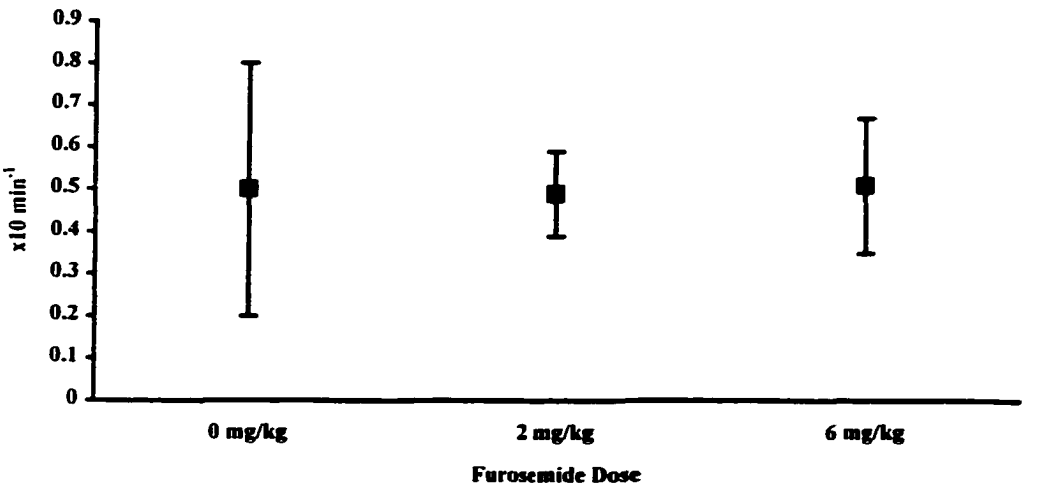

Fig 2C – 0 mg/kg = 0.5 +/- 0.3; 2 mg/kg = 0.49 +/- 0.1; 6 mg/kg = 0.51 +/- 0.16 (mean +/- 2SD)

Figure 2D
Phase I - Acute Trial
Insulin Sensitivity

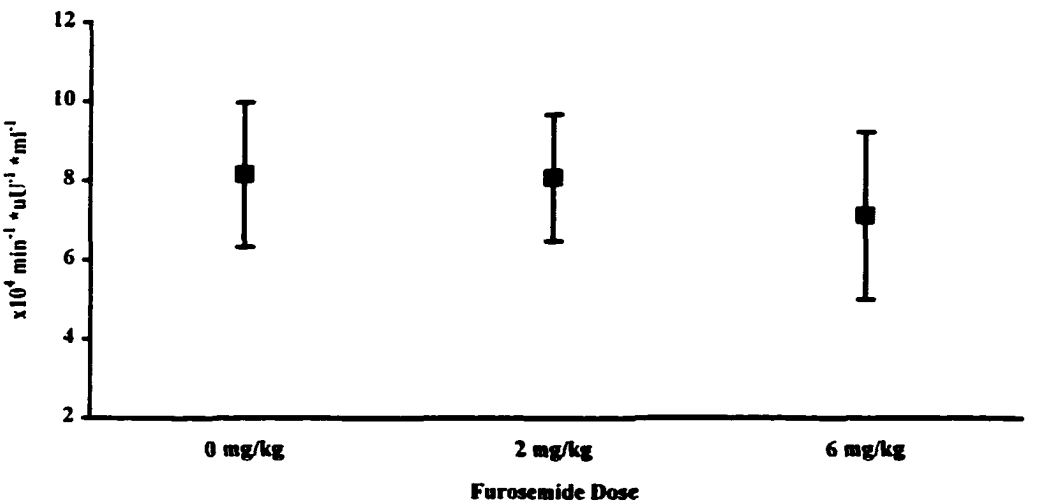

Fig 2D – 0 mg/kg = 8.16 +/- 1.82; 2 mg/kg = 8.09 +/- 1.6; 6 mg/kg = 7.12 +/- 2.12 (mean +/- 2SD)

3.1.2. Chronic trial

No adverse reactions to oral furosemide administration were seen. Body weight, packed cell volume, and serum protein concentrations were evaluated weekly to monitor for dehydration. No significant changes in these parameters were seen during the trial. None of the dogs demonstrated any clinical evidence of dehydration during the trial.

3.1.2.1. Serum electrolytes and selected renal parameters

There were no differences between pre-trial serum sodium, chloride, and calcium concentrations and values found after dogs received either oral furosemide dose (Figures 3A-C). The serum potassium concentrations significantly decreased with administration of both the low dose ($p<0.01$) and high dose ($p<0.01$) of furosemide (Figure 3D). The serum phosphorus concentrations significantly increased with administration of both the low dose ($p<0.01$) and high dose ($p<0.01$) of furosemide (Figure 3E).

Serum sodium and calcium concentrations remained within the established normal range for adult dogs throughout the trial. One dog had a pre-trial serum chloride concentration below the normal range. One dog had mild hyperphosphatemia when receiving the high dose of furosemide. One dog demonstrated hypokalemia during the low furosemide dose. This dog and two others demonstrated hypokalemia when receiving the high furosemide dose.

Serum urea (low: $p=0.01$; high: $p=0.04$) and creatinine (low: $p<0.01$; high: $p<0.01$) concentrations were significantly higher with both doses of furosemide when compared to pre-trial concentrations. There were no differences between the two doses

for either variable. However, all serum urea and serum creatinine concentrations remained within the normal range throughout the trial (Figures 3F & 3G).

Figure 3 – Effects of oral furosemide on serum electrolyte and biochemical parameters in healthy dogs
All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of established normal range for adult dogs. Asterisks represent statistical significance compared to pretrial values ($p < 0.05$).

Figure 3A
Phase I - Chronic Trial
Sodium

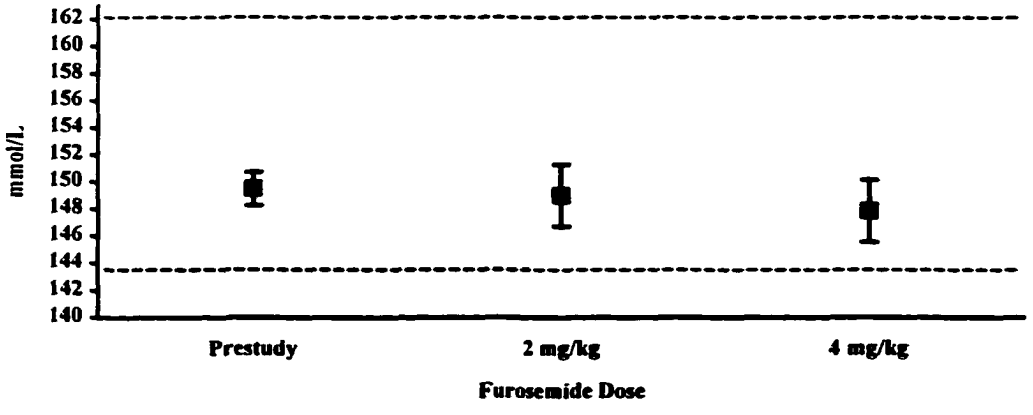

Fig 3A – Pre-study = 149.5 +/- 1.24; 2 mg/kg = 149 +/- 2.3; 4 mg/kg = 147.83 +/- 2.3 (mean +/- 2SD)

Figure 3B
Phase I - Chronic Trial
Chloride

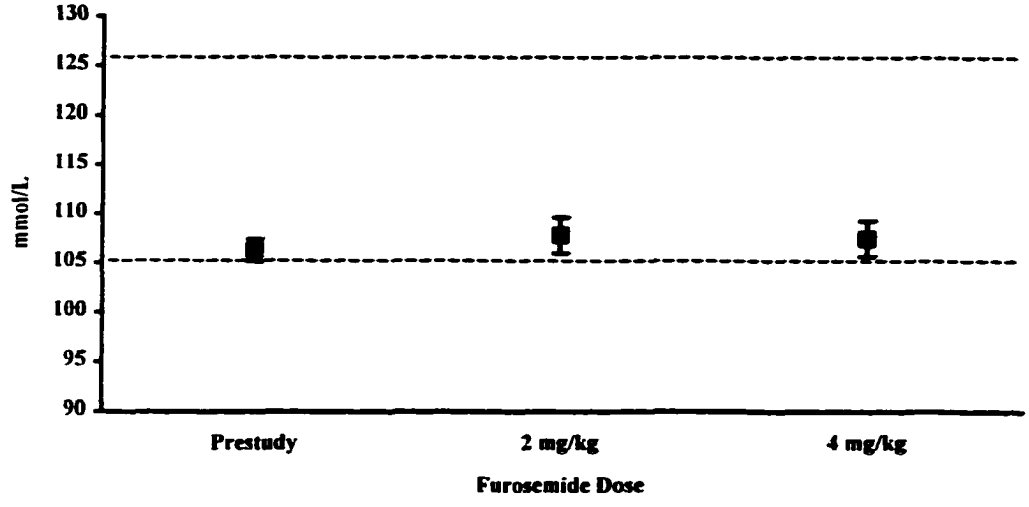

Fig 3B – Pre-study = 106.3 +/- 1.12; 2 mg/kg = 107.8 +/- 1.82; 4 mg/kg = 107.5 +/- 1.82 (mean +/- 2SD)

Figure 3C
Phase I - Chronic Trial
Calcium

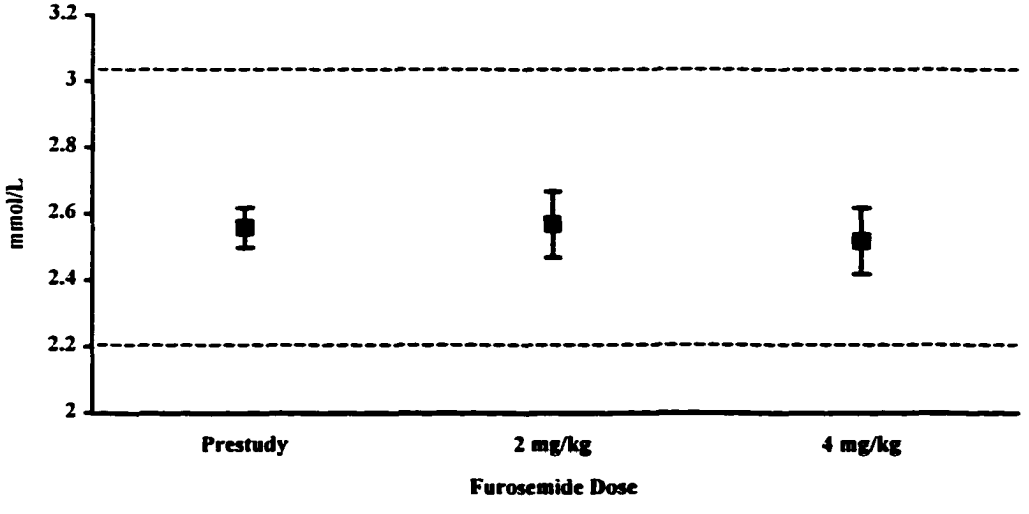

Fig 3C – Pre-study = 2.56 +/- 0.06; 2 mg/kg = 2.57 +/- 0.1; 4 mg/kg = 2.52 +/- 0.1 (mean +/- 2SD)

Figure 3D
Phase I - Chronic Trial
Potassium

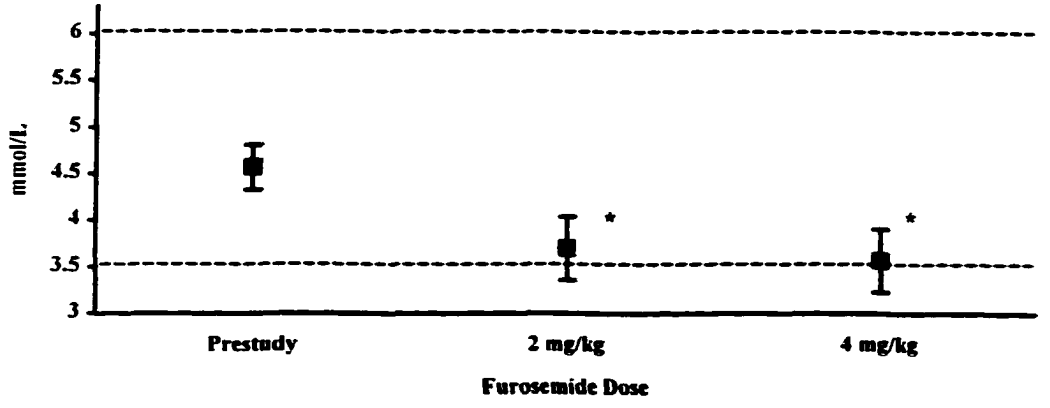

Fig 3D – Pre-study = 4.57 +/- 0.24; 2 mg/kg = 3.7 +/- 0.34; 4 mg/kg = 3.57 +/- 0.34 (mean +/- 2SD)

Figure 3E
Phase I - Chronic Trial
Phosphorus

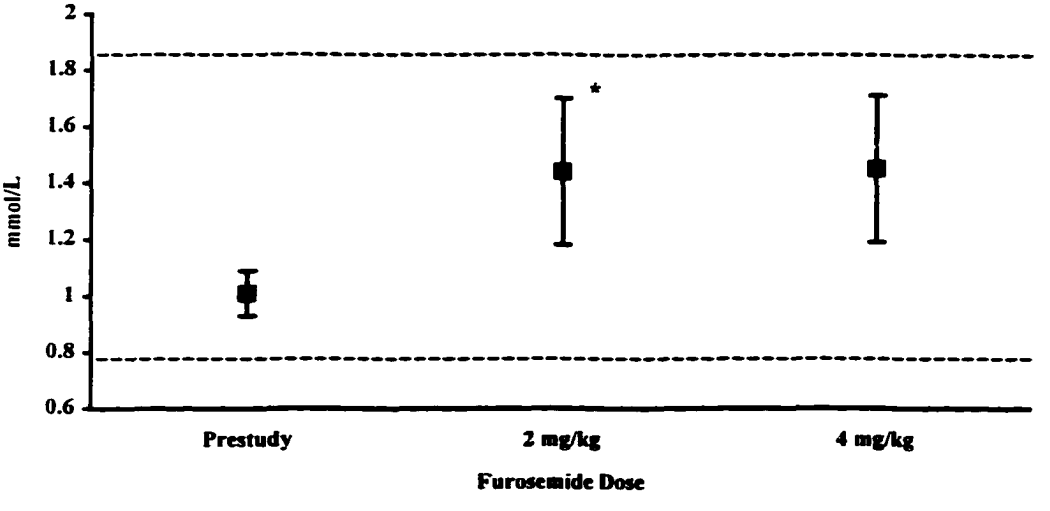

Fig 3E – Pre-study = 1.01 +/- 0.08; 2 mg/kg = 1.44 +/- 0.26; 4 mg/kg = 1.45 +/- 0.26 (mean +/- 2SD)

Figure 3F
Phase I - Chronic Trial
Serum Urea

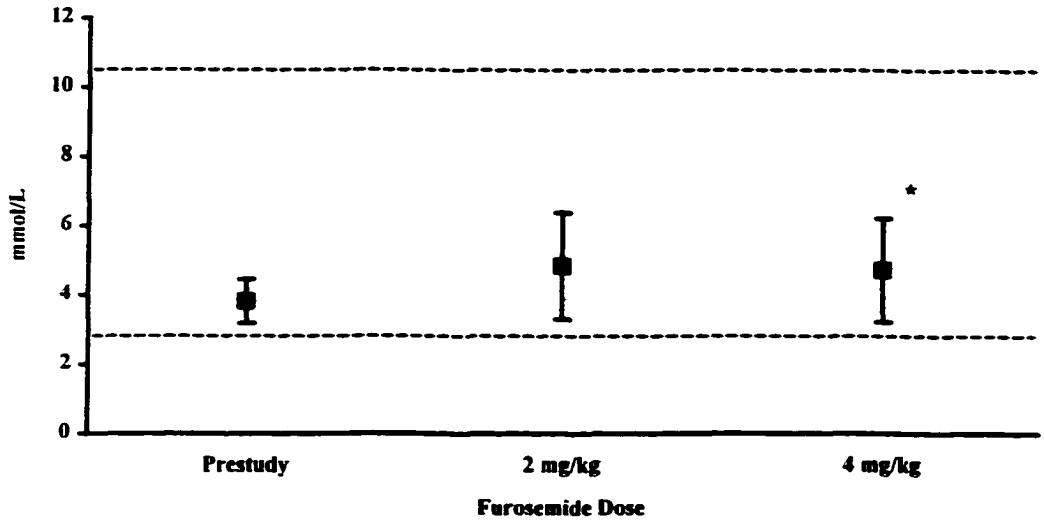

Fig 3F – Pre-study = 3.82 +/- 0.64; 2 mg/kg = 4.85 +/- 1.54; 4 mg/kg = 4.73 +/- 1.5 (mean +/- 2SD)

Figure 3G
Phase I - Chronic Trial
Serum Creatinine

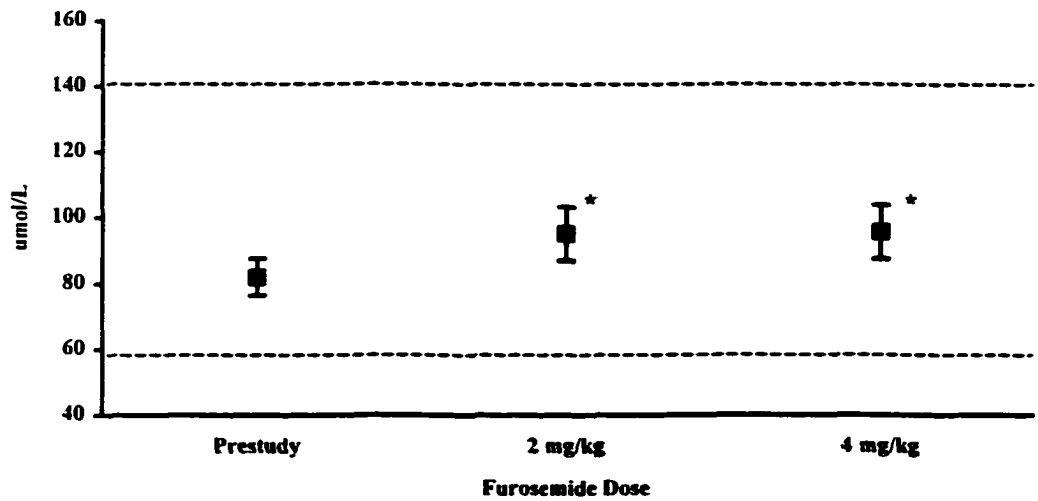

Fig 3G – Pre-study = 82 +/- 5.5; 2 mg/kg = 95.17 +/- 8.16; 4 mg/kg = 95.67 +/- 8.16 (mean +/- 2SD)

3.1.2.2. Parameters monitoring glucose homeostasis

No significant differences were noted when comparing pretrial fasting serum glucose concentrations ($p > 0.05$), fasting serum insulin concentrations ($p = 0.4$), insulin sensitivity ($p = 0.12$), and glucose effectiveness ($p = 0.86$) to either furosemide dose (Figures 4A-D).

Three dogs had pretrial fasting serum glucose concentrations that were above the laboratory reference range for adult dogs. One dog demonstrated mild hyperglycemia when receiving the low dose of furosemide. All dogs receiving the high dose of furosemide had serum glucose concentrations within the established normal range.

Figure 4 – Effects of oral furosemide on serum glycemic parameters in healthy dogs
All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of established normal range for adult dogs.

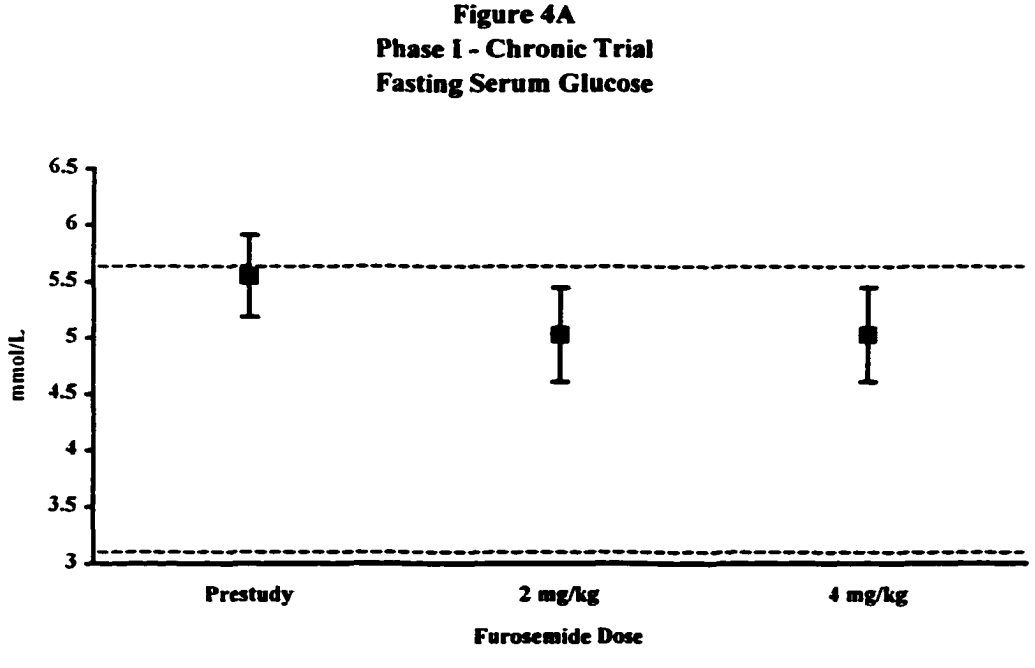

Fig 4A – Pre-study = 5.55 +/- 0.36; 2 mg/kg = 5.03 +/- 0.42; 4 mg/kg = 5.03 +/- 0.42 (mean +/- 2SD)

Figure 4B
Phase I - Chronic Trial
Fasting Serum Insulin

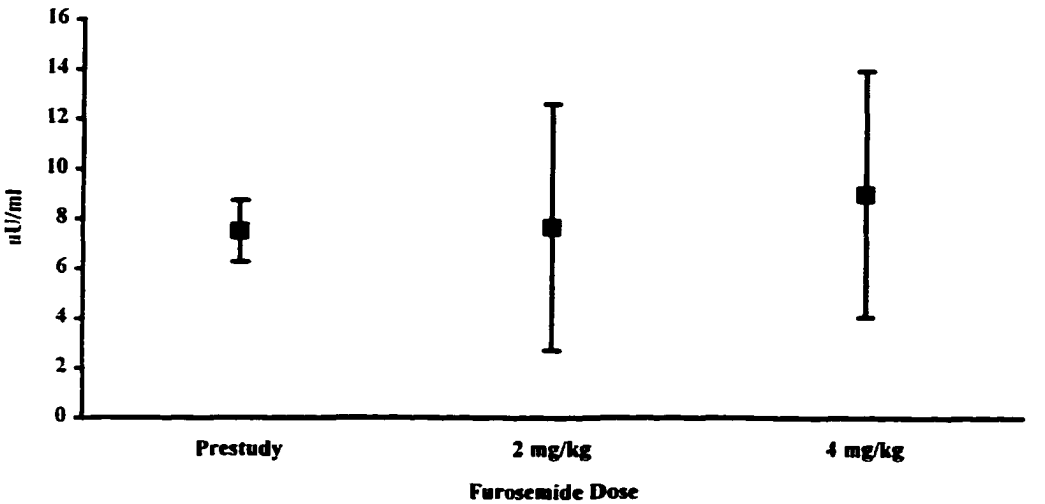

Fig 4B – Pre-study = 7.5 +/- 1.24; 2 mg/kg = 7.67 +/- 4.94; 4 mg/kg = 9 +/- 4.94 (mean +/- 2SD)

Figure 4C
Phase I - Chronic Trial
Insulin Sensitivity

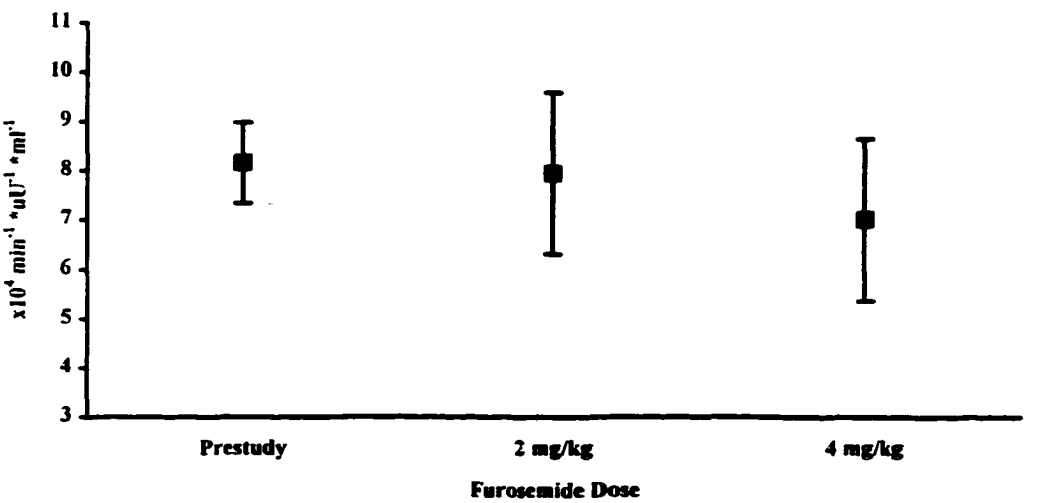

Fig 4C – Pre-study = 8.16 +/- 0.82; 2 mg/kg = 7.95 +/- 1.64; 4 mg/kg = 7.01 +/- 1.64 (mean +/- 2SD)

Figure 4D
Phase I - Chronic Trial
Glucose Effectiveness

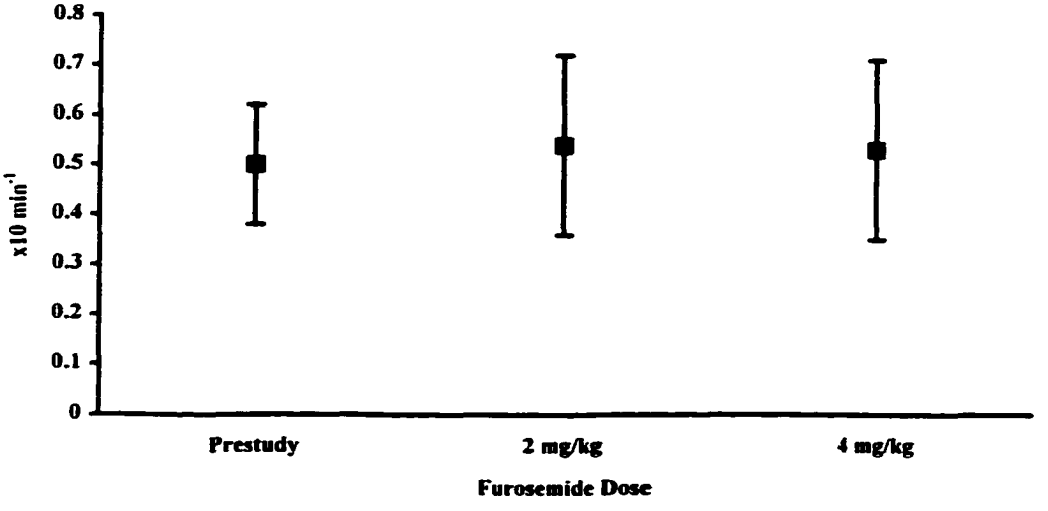

Fig 4D – Pre-study = 0.5 +/- 0.12; 2 mg/kg = 0.54 +/- 0.18; 4 mg/kg = 0.53 +/- 0.18 (mean +/- 2SD)

3.2. Phase II

All dogs developed hyperglycemia and glycosuria by 56 hours following intravenous alloxan administration. No unexpected adverse effects associated with alloxan administration were noted. Glycemic control was established with exogenous NPH insulin therapy within 32 days.

3.2.1. Acute trial

No adverse effects associated with intravenous furosemide administration were seen. All dogs were able to maintain normal hydration throughout the trial. One dog required a 1 unit increase in exogenous insulin dose (9 units to 10 units q24 hours), and

two dogs required a 1 unit decrease in their exogenous insulin doses (9 units to 8 units; 11 units to 10 units q24 hours) during the acute trial.

3.2.1.1. Serum electrolytes and selected renal parameters

There were no significant differences between serum sodium ($p = 0.41$) or phosphorus ($p = 0.78$) concentrations when dogs received placebo versus either furosemide dose (Figures 5A & 5B). Serum potassium concentration decreased significantly when dogs received furosemide versus placebo (low: $p = 0.04$; high: $p = 0.02$); no difference was noted between doses (Figure 5C). Serum chloride concentrations also decreased significantly from placebo when either furosemide dose was administered ($p = 0.04$); no difference was noted between doses (Figure 5D). When compared to placebo, serum calcium concentrations were significantly increased with either furosemide dose ($p = 0.02$); no difference was noted between doses (Figure 5E).

Four of six dogs were hyponatremic following placebo, low, and high dose furosemide administration. The two exceptions were dogs receiving the placebo dose. These dogs had serum sodium concentrations within the normal range. Three dogs receiving the placebo dose had serum chloride concentrations within the normal range. The other three dogs and both the low and high dose groups were hypochloremic. One dog receiving the placebo dose was hyperphosphatemic. The concentrations for serum potassium and calcium were within the normal range for all doses of furosemide.

Serum urea ($p = 0.15$) and creatinine ($p = 0.08$) concentrations were not significantly changed by either furosemide dose. All samples for each dose remained

within the established normal range for both serum urea and serum creatinine (Figures 5F & 5G).

Figure 5 – Effect of intravenous furosemide on serum electrolytes and biochemical parameters in diabetic dogs.
All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of established normal range for adult dogs. Asterisks represent statistical significance compared to placebo ($p < 0.05$).

Figure 5A
Phase II - Acute Trial
Sodium

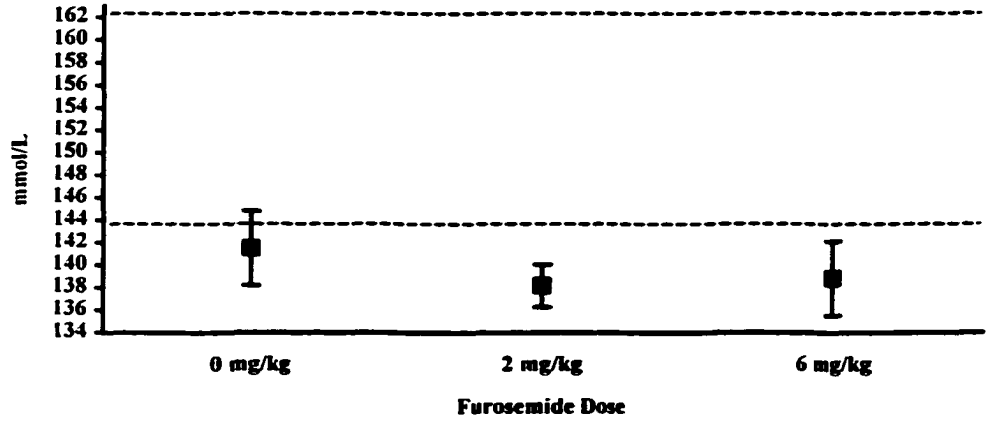

Fig 5A – 0 mg/kg = 141.5 +/- 3.3; 2 mg/kg = 138.17 +/- 1.88; 6 mg/kg = 138.83+/- 3.3 (mean +/- 2SD)

Figure 5B
Phase II - Acute Trial
Phosphorus

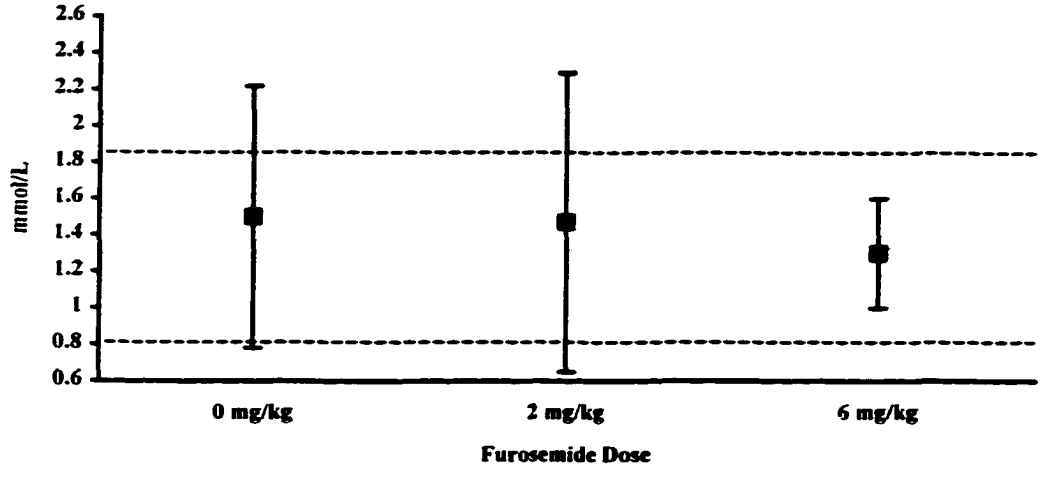

Fig 5B – 0 mg/kg = 1.5 +/- 0.72; 2 mg/kg = 1.47 +/- 0.82; 6 mg/kg = 1.3 +/- 0.3 (mean +/- 2SD)

Figure 5C
Phase II - Acute Trial
Potassium

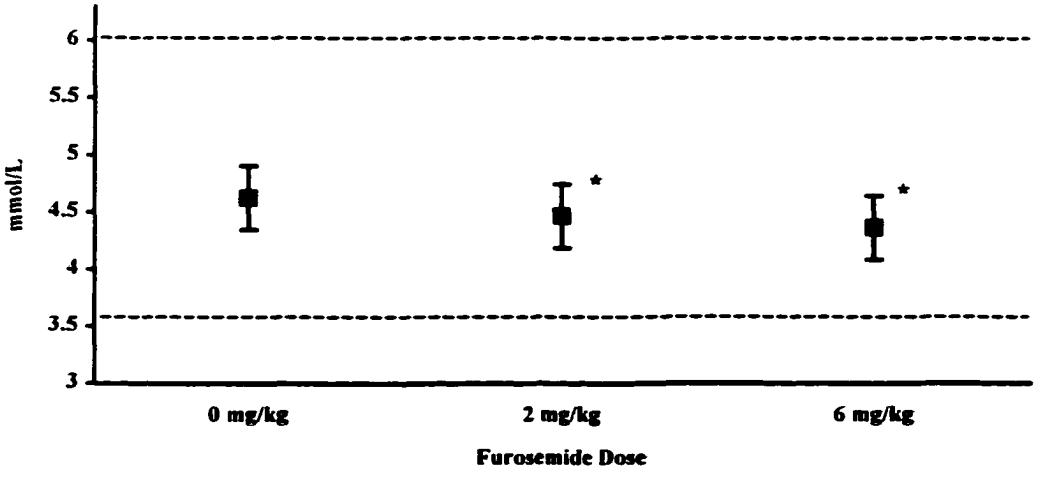

Fig 5C – 0 mg/kg – 4.63 +/- 0.28; 2 mg/kg = 4.47 +/- 0.28; 6 mg/kg = 4.37 +/- 0.28 (mean +/- 2SD)

Figure 5D
Phase II - Acute Trial
Chloride

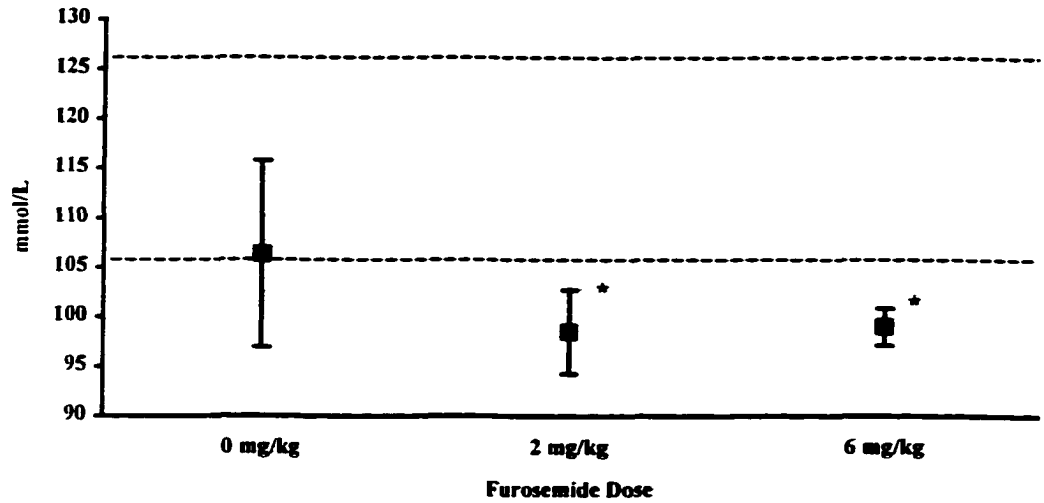

Fig 5D – 0 mg/kg = 106.33 +/- 9.42; 2 mg/kg = 98.5 +/- 4.24; 6 mg/kg = 99 +/- 1.88 (mean +/- 2SD)

Figure 5E
Phase II - Acute Trial
Calcium

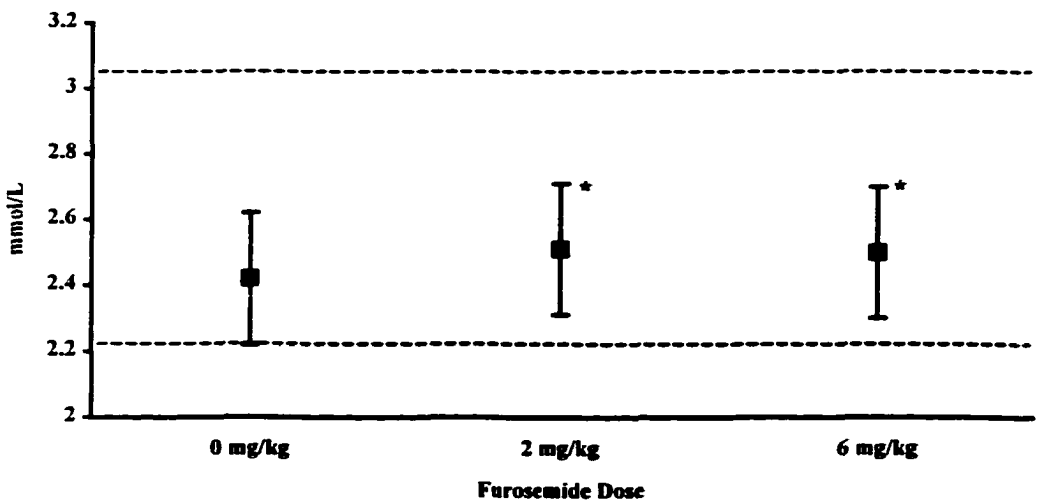

Fig 5E – 0 mg/kg = 2.42 +/- 0.2; 2 mg/kg = 2.51 +/- 0.2; 6 mg/kg = 2.5 +/- 0.2 (mean +/- 2SD)

Figure 5F
Phase II - Acute Trial
Serum Urea

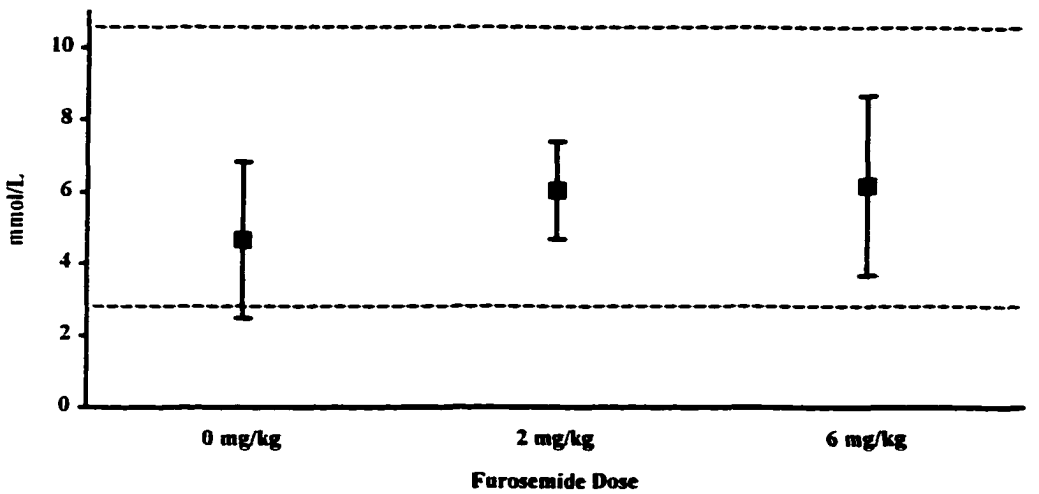

Fig 5F – 0 mg/kg = 4.67 +/- 2.18; 2 mg/kg = 6.05 +/- 1.36; 6 mg/kg = 6.18 +/- 2.5 (mean +/- 2SD)

Figure 5G
Phase II - Acute Trial
Serum Creatinine

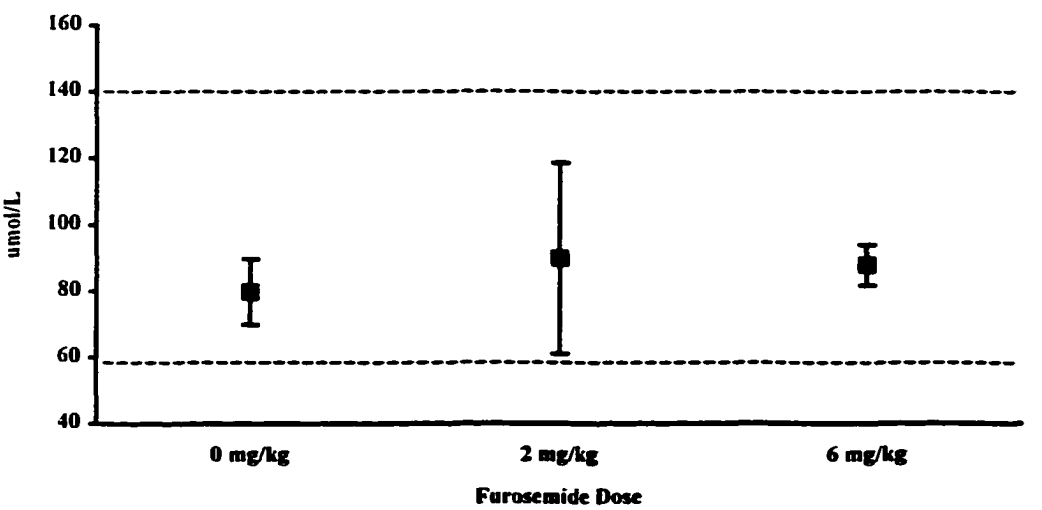

Fig 5G – 0 mg/kg – 79.83 +/- 9.9; 2 mg/kg = 89.83 +/- 28.76; 6 mg/kg = 87.83 +/- 6.12 (mean +/- 2SD)

3.2.1.2. Parameters associated with glucose homeostasis

No significant differences were seen when dogs received placebo versus either furosemide dose for fasting glucose concentrations ($p = 0.80$), fasting endogenous serum insulin concentrations ($p = 0.33$), insulin sensitivity ($p = 0.33$), or glucose effectiveness ($p = 0.93$) (Figures 6A-D).

Figure 6 – Effect of intravenous furosemide on serum glycemic parameters in diabetic dogs
All graphs show data as mean +/- 2SD.

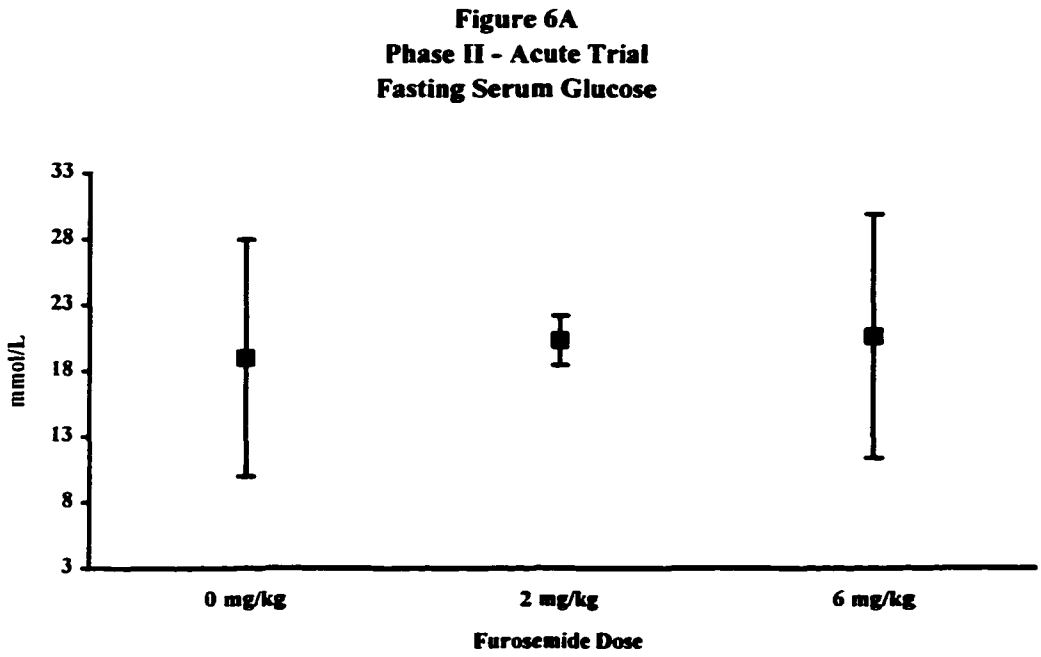

Fig 6A – 0 mg/kg = 18.98 +/- 9; 2 mg/kg = 20.33 +/- 1.88; 6 mg/kg = 20.65 +/- 9.28 (mean +/- 2SD)

Figure 6B
Phase II - Acute Trial
Fasting Serum Insulin

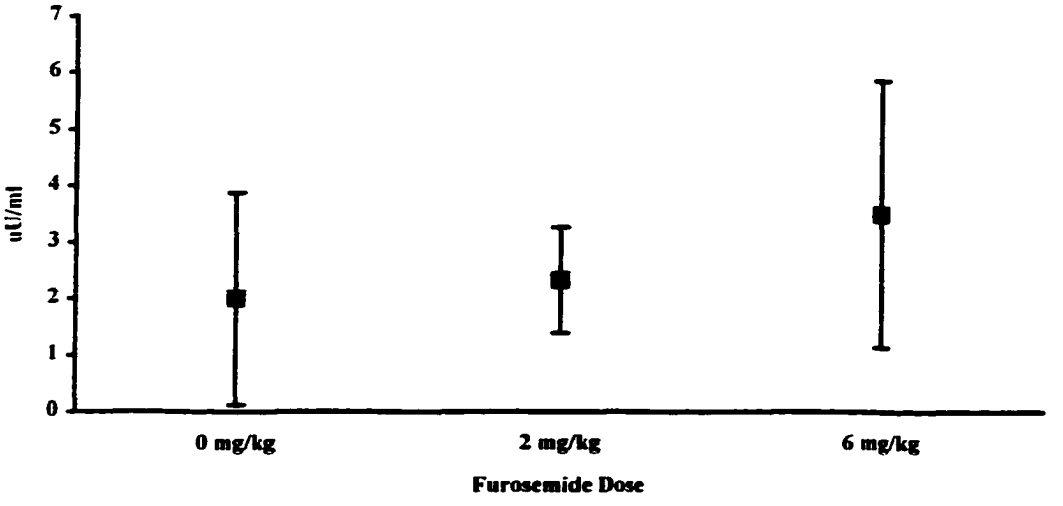

Fig 6B – 0 mg/kg = 2 +/- 1.88; 2 mg/kg = 2.33 +/- 0.94; 6 mg/kg = 3.5 +/- 2.36 (mean +/- 2SD)

Figure 6C
Phase II - Acute Trial
Insulin Sensitivity

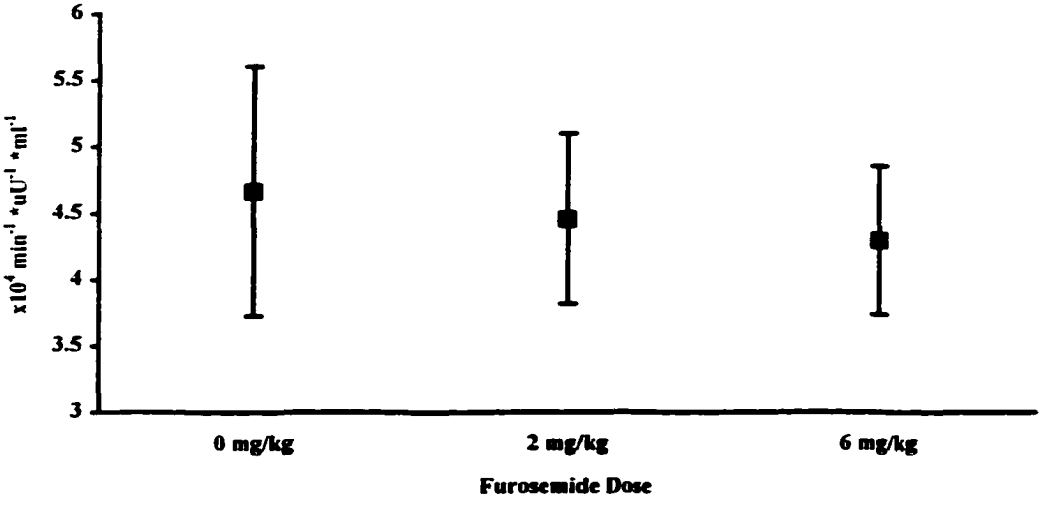

Fig 6C – 0 mg/kg = 4.67 +/- 0.94; 2 mg/kg = 4.46 +/- 0.64; 6 mg/kg = 4.3 +/- 0.56 (mean +/- 2SD)

Figure 6D
Phase II - Acute Trial
Glucose Effectiveness

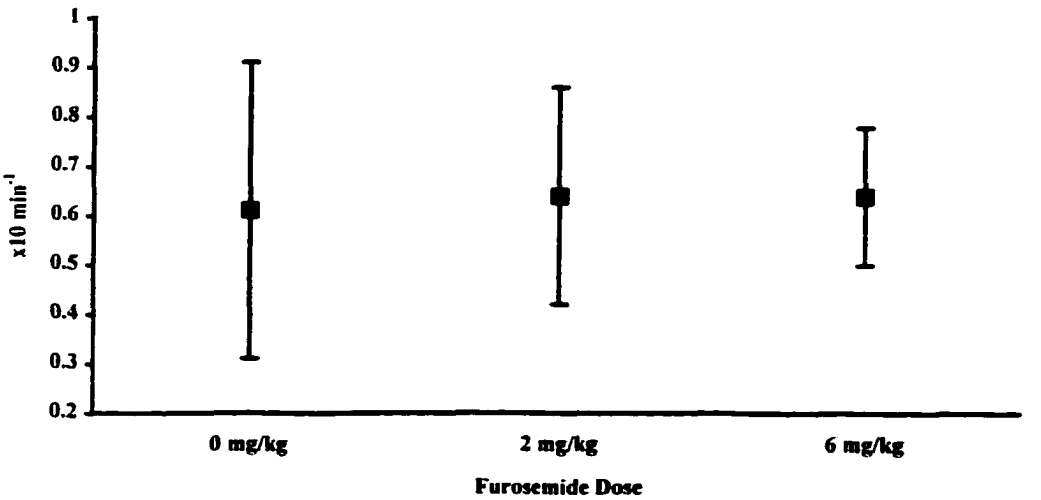

Fig 6D – 0 mg/kg = 0.61 +/- 0.3; 2 mg/kg = 0.64 +/- 0.22; 6 mg/kg = 0.64 +/- 0.14 (mean +/- 2SD)

3.2.2. Chronic trial

No adverse reactions to oral furosemide administration were noted. Body weight, packed cell volume, and serum protein concentrations were evaluated weekly to monitor for dehydration. No significant changes were noted among these parameters. Clinically, the dogs were able to maintain normal hydration with constant access to fresh water. Two dogs required a 1 unit increase in their exogenous insulin dose (8 units to 9 units; 9 units to 10 units q24 hours) during the trial.

3.2.2.1. Serum electrolytes and selected renal parameters

When dogs received placebo versus furosemide, no significant differences were noted for serum sodium ($p = 0.32$), chloride ($p = 0.06$), or phosphorus ($p = 0.58$) concentrations (Figures 7A-C). Serum potassium concentrations were significantly decreased when furosemide was administered compared to placebo ($p = 0.03$) (Figure 7D). Serum calcium concentrations were significantly increased when furosemide was administered compared to placebo ($p = 0.03$) (Figure 7E).

One dog was hyponatremic during placebo and two dogs were hyponatremic when receiving furosemide. Only one dog was hypochloremic when receiving placebo; however, four dogs were hypochloremic when receiving furosemide. One dog was hyperphosphatemic when receiving furosemide. All dogs had serum potassium and calcium concentrations within the normal range when receiving either placebo or furosemide.

No significant differences in serum urea ($p = 0.27$) or serum creatinine ($p = 0.19$) concentrations were noted during placebo or furosemide administration. All values were within the established normal range for both serum urea and serum creatinine (Figures 7F & 7G).

Figure 7 – Effect of oral furosemide on serum electrolytes and biochemical parameters in diabetic dogs All graphs show data as mean +/- 2SD. Dashed lines represent upper and lower limits of established normal range for adult dogs. Asterisks represent statistical significance when compared to placebo ($p < 0.05$).

Figure 7A
Phase II - Chronic Trial
Sodium

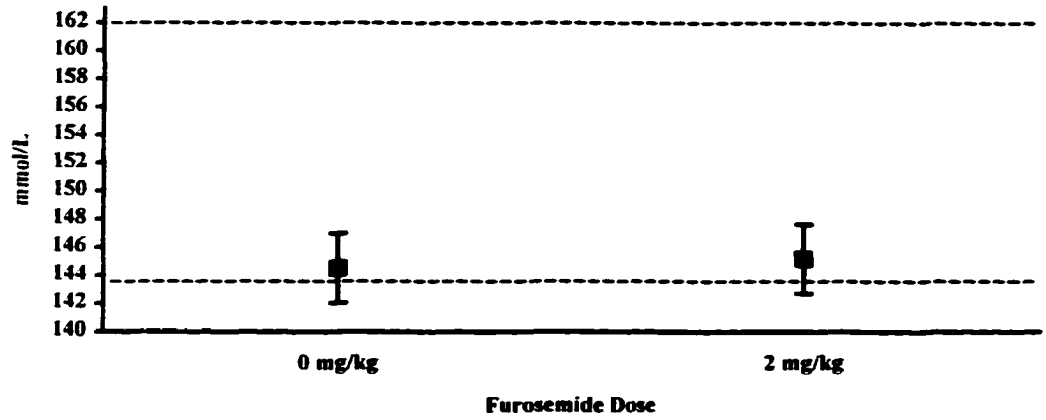

Fig 7A – 0 mg/kg = 144.5 +/- 2.48; 2 mg/kg = 145.17 +/- 2.48 (mean +/- 2SD)

Figure 7B
Phase II - Chronic Trial
Chloride

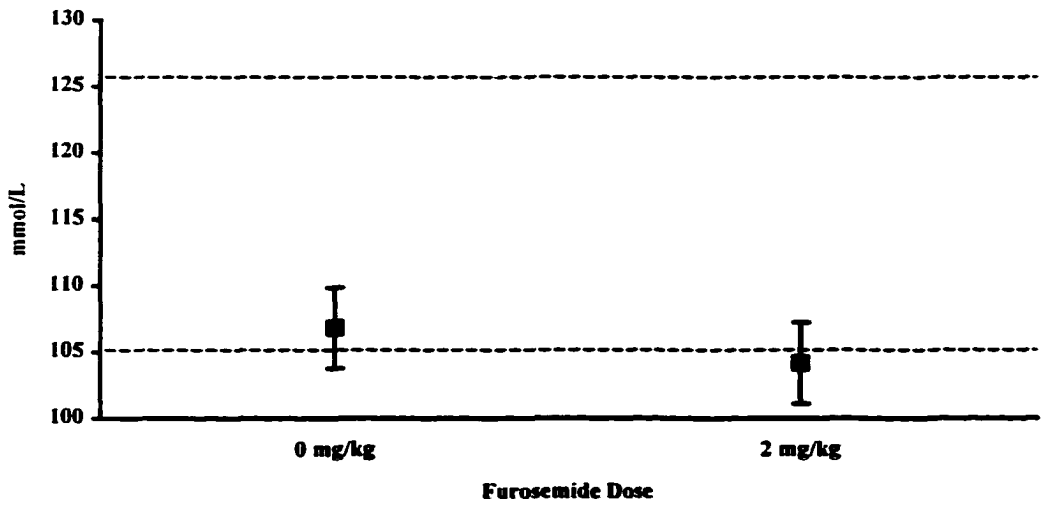

Fig 7B – 0 mg/kg = 106.83 +/- 3.06; 2 mg/kg = 104.17 +/- 3.06 (mean +/- 2SD)

Figure 7C
Phase II - Chronic Trial
Phosphorus

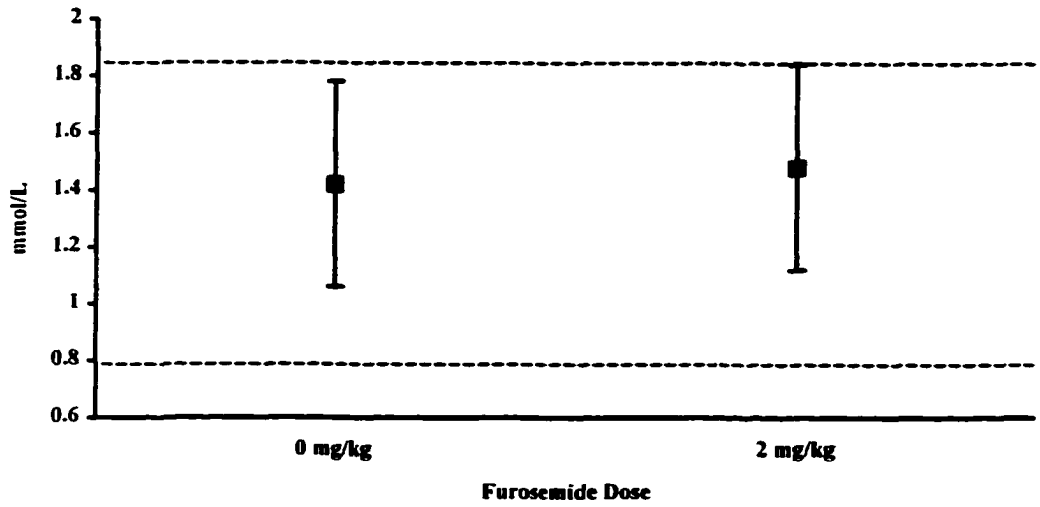

Fig 7C – 0 mg/kg = 1.42 +/- 0.36; 2 mg/kg = 1.48 +/- 0.36 (mean +/- 2SD)

Figure 7D
Phase II - Chronic Trial
Potassium

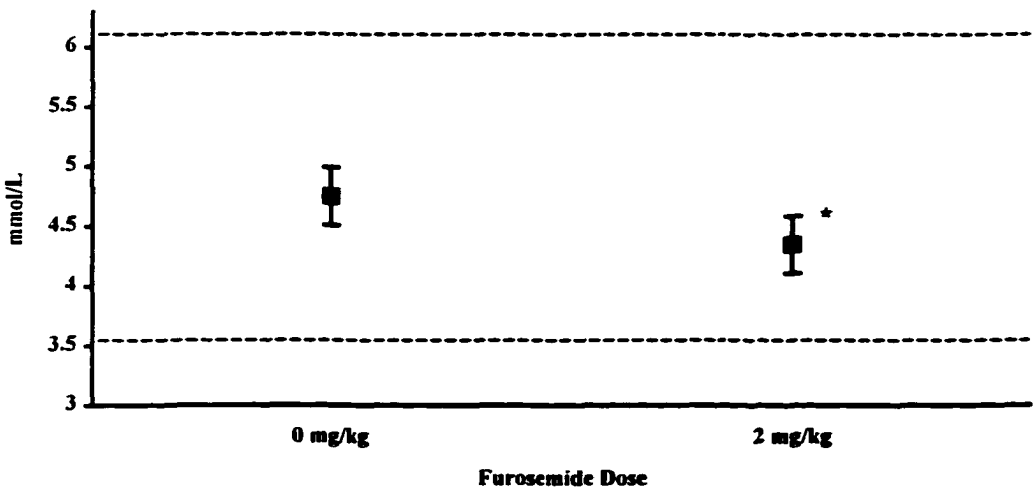

Fig 7D – 0 mg/kg = 4.75 +/- 0.24; 2 mg/kg = 4.35 +/- 0.24 (mean +/- 2SD)

Figure 7E
Phase II - Chronic Trial
Calcium

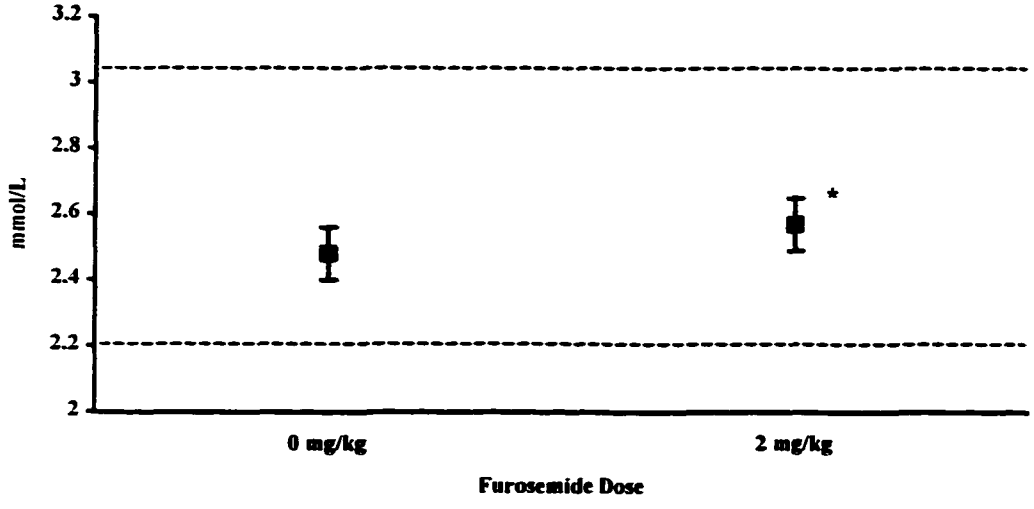

Fig 7E – 0 mg/kg = 2.48 +/- 0.08; 2 mg/kg = 2.57 +/- 0.08 (mean +/- 2SD)

Figure 7F
Phase II - Chronic Trial
Serum Urea

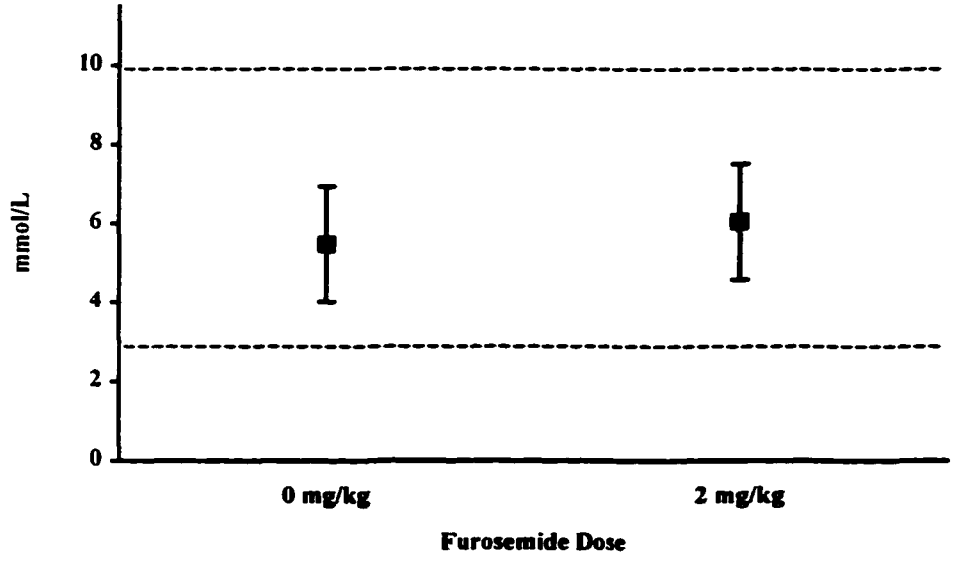

Fig 7F – 0 mg/kg = 5.48 +/- 1.46; 2 mg/kg = 6.05 +/- 1.46 (mean +/- 2SD)

Figure 7G
Phase II - Chronic Trial
Serum Creatinine

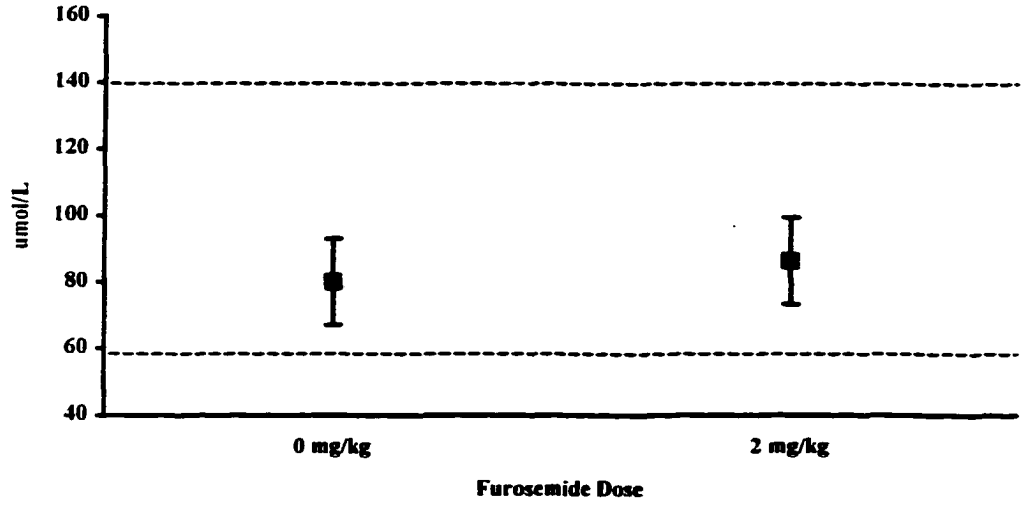

Fig 7G – 0 mg/kg = 80 +/- 13.06; 2 mg/kg = 86.33 +/- 13.06 (mean +/- 2SD)

3.2.2.2. Parameters associated with glucose homeostasis

No significant differences were noted between placebo and furosemide administration for fasting serum glucose concentrations ($p = 0.72$), fasting endogenous serum insulin concentrations ($p = 0.78$), insulin sensitivity ($p = 0.82$), or glucose effectiveness ($p = 0.63$) (Figures 8A-D).

Figure 8 – Effect of oral furosemide on serum glycemic parameters in diabetic dogs
All graphs show data as mean +/- 2SD.

Figure 8A
Phase II - Chronic Trial
Fasting Serum Glucose

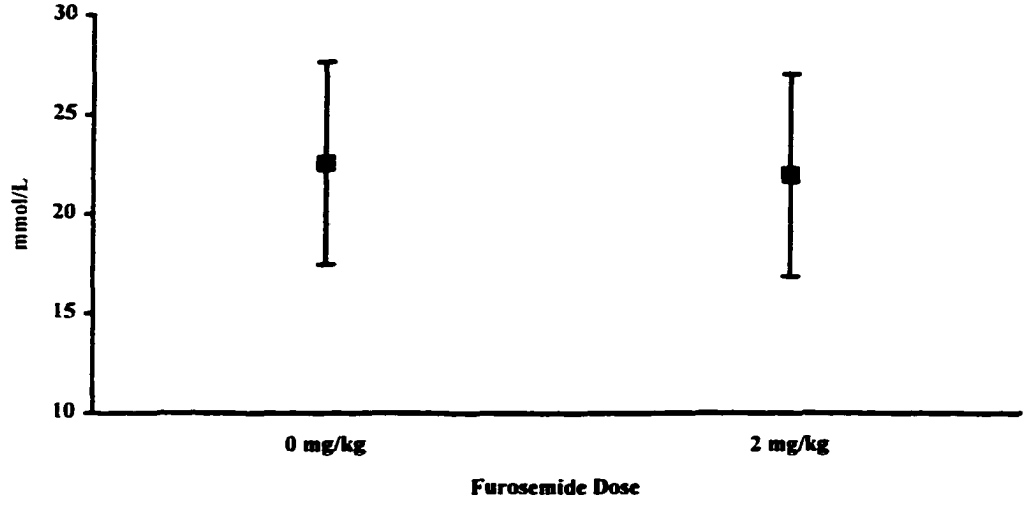

Fig 8A – 0 mg/kg = 22.57 +/-5.1; 2 mg/kg = 21.98 +/- 5.1 (mean +/- 2SD)

Figure 8B
Phase II - Chronic Trial
Fasting Serum Insulin

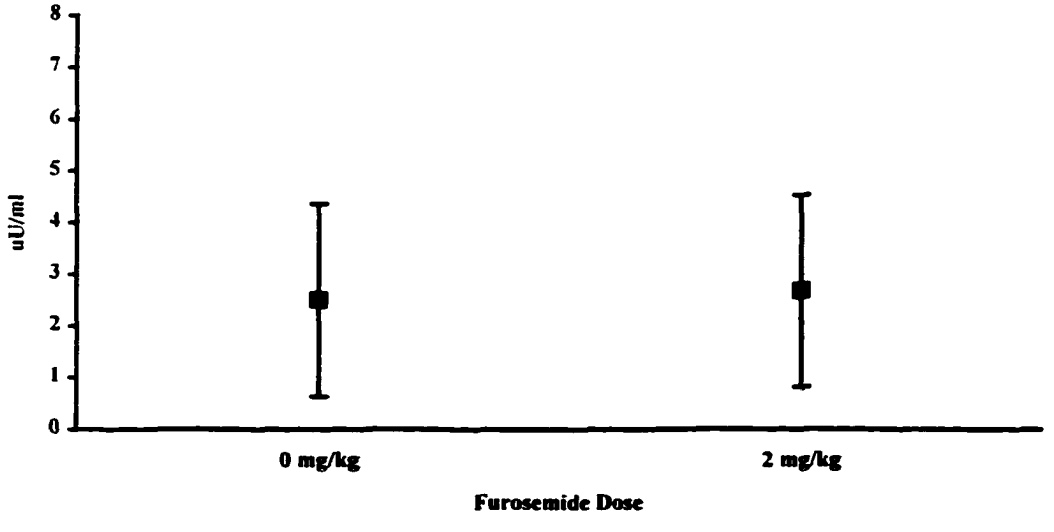

Fig 8B – 0 mg/kg = 2.5 +/- 1.86; 2 mg/kg = 2.67 +/- 1.86 (mean +/- 2SD)

Figure 8C
Phase II - Chronic Trial
Insulin Sensitivity

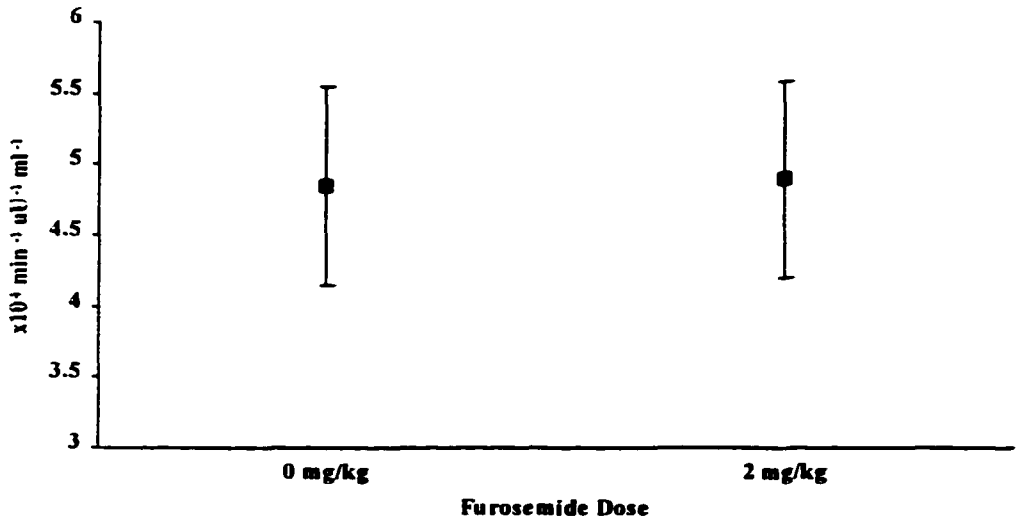

Fig 8C – 0 mg/kg = 4.85 +/- 0.7; 2 mg/kg = 4.9 +/- 0.7 (mean +/- 2SD)

Figure 8D
Phase II - Chronic Trial
Glucose Effectiveness

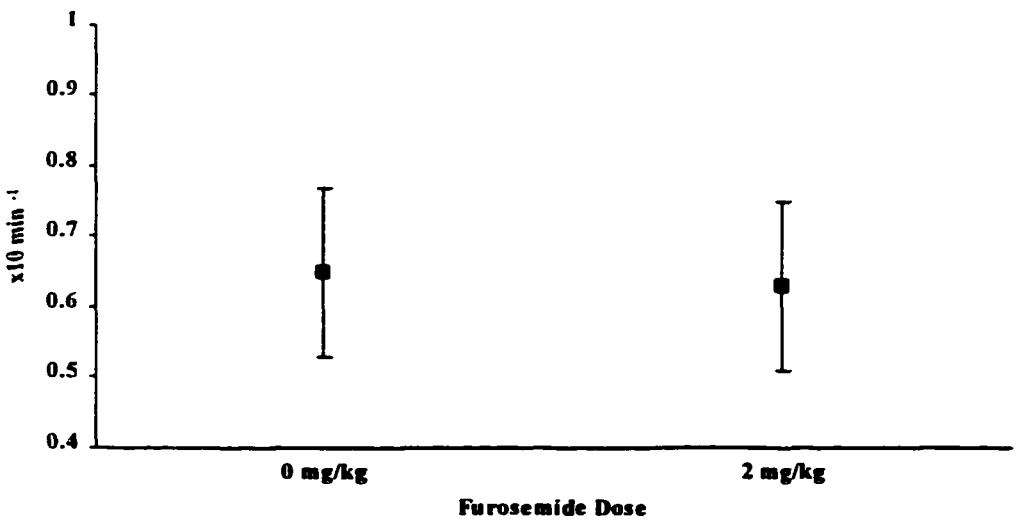

Fig 8D – 0 mg/kg = 0.65 +/- 0.12; 2 mg/kg = 0.63 +/- 0.12 (mean +/- 2SD)

4. DISCUSSION

4.1. Introduction

This study was designed to evaluate the effects of oral and intravenous furosemide administration on glycemic parameters in healthy (Phase I) and diabetic (Phase II) dogs. Our hypothesis was that the administration of furosemide would negatively alter glucose tolerance in dogs. This hypothesis was not supported by the results of this study. Administration of either oral or intravenous furosemide did not significantly alter the measured parameters of glucose metabolism in this group of dogs.

Although the value of statistics for evaluating scientific studies cannot be disputed, veterinary clinicians are also interested in the clinical implications of a study. While a scientific observation may be statistically significant, it may not be clinically relevant. In other words, a statistically significant increase or decrease in a parameter following administration of a medication may not alter that parameter enough to cause a significant clinical change in a patient. The doses of furosemide chosen for this study were designed to evaluate the clinical relevance of any resulting alterations. A standard value for a clinically significant increase in blood glucose concentration has not been reported to the authors' knowledge. For the purposes of this study, an approximate 1.5 to two fold increase in the fasting serum glucose concentration was considered clinically significant for healthy dogs. A lower percentage increase could be significant in diabetic dogs. The significance of the fasting serum insulin concentration is based upon its relation to the concurrent serum glucose measurement. If the serum insulin concentration is inappropriately low for the magnitude of hyperglycemia, it may be considered

clinically significant. Clinically, as well as statistically, furosemide did not significantly alter serum glucose or insulin concentrations in this group of dogs.

The validity of using purpose-bred laboratory beagles may be questioned, in part due to their lack of genetic diversity. They were used in this study to compare the effects of furosemide administration both prior to and following the onset of diabetes mellitus in the same group of dogs. In humans, the development of glucose intolerance, specifically in NIDDM, has been linked to genetics. A family history of NIDDM increases the risk of an individual developing glucose intolerance (89-91). However, an association between genetics and glucose intolerance or diabetes mellitus has not been established in most breed of dog although some breed predispositions do exist (17). Thus, glucose regulation and the effects of furosemide should not be markedly different between laboratory-bred beagles and the general dog population.

The insulin-modified frequently sampled glucose tolerance test was chosen as an efficient, noninvasive method of evaluating glucose tolerance (80-82,92). Previous studies have demonstrated positive correlation of the FSIGT with glucose clamp techniques, accepted methods of estimating glucose tolerance (80,82,93,94). When data obtained from the FSIGT is analyzed utilizing the minimal model method, the parameters of insulin sensitivity and glucose effectiveness are derived (88). These parameters give a more complete picture of the glucose tolerance and metabolic profile of an individual patient (80-84,92).

An alloxan-induced model of insulin-dependent diabetes mellitus was chosen over other experimental models for several reasons. It is noninvasive, requiring only an intravenous injection. By selectively destroying beta cells, alloxan produces an insulin-

dependent state, but leaves exocrine pancreatic function intact similar to the naturally occurring disease. Total pancreatectomy requires an invasive surgical procedure and results in loss of both exocrine and endocrine pancreatic functions, making it a less desirable model. Streptozotocin selectively destroys beta cells (86), but it has been associated with acute renal failure and death (86). Alloxan can also cause acute renal failure, but it is a dose-dependent nephropathy that is potentially reversible with appropriate medical therapy (86). It also appears to occur less frequently in the dog compared to streptozotocin (86).

4.2. Phase I – healthy dogs

The administration of furosemide did not result in any significant statistical or clinical changes in glycemic parameters during Phase I. Those dogs demonstrating hyperglycemia during the pre-study, placebo, low-, and high-dose samplings had only mild elevations in serum glucose. This magnitude of elevation was consistent with the hyperglycemia often noted with stress or excitement and was considered an appropriate physiologic response (17). At the doses used, furosemide does not appear to alter glucose metabolism in healthy dogs. This result may differ from observations made in human and rodent studies for several reasons. The clinically applicable doses of furosemide used in this study were much lower than those doses required to elicit glucose intolerance in rodent models (55-62). It is possible that the doses routinely used are not high enough to alter glucose metabolism in normal dogs. In this study, the effects of furosemide administration were evaluated after 24 hours of intravenous administration and 28 days of oral administration. Based on previous studies in other species, these durations should be adequate for glucose intolerance to develop (54-59,61,62,68).

Several studies have implicated decreased glucose utilization or glucose transport in peripheral tissues as a cause of furosemide-induced glucose intolerance (44-46,57). Other studies have suggested a decrease in insulin secretion (48,58,71). The results of this study did not support either of these observations. There were no changes in glucose effectiveness or insulin sensitivity. These are indirect measurements of glucose utilization and the ability of the peripheral tissues to respond to insulin. Insulin secretion did not change. Fasting serum insulin concentration following furosemide administration did not change from placebo or pre-trial values. This would suggest that the dogs retained their ability to secrete insulin during furosemide administration.

Another reason for the failure of furosemide to cause hyperglycemia in these dogs may be due to the absence of significant clinical hypokalemia during the trials. Hypokalemia has been suggested as a mechanism for decreased insulin secretion although this has not always been supported (72,73). The development of hypokalemia is a potentially dangerous side effect of furosemide administration and is due to decreased potassium ion reabsorption and increased urine flow (1-3,34,95). During the acute trial, hypokalemia was not a significant problem. Although serum potassium concentration was significantly lower after high-dose compared to low-dose furosemide, neither dose resulted in values significantly different from placebo. All serum potassium concentrations remained within the normal range during this trial. Conversely, in the chronic trial, hypokalemia was a statistically significant finding but was not clinically significant. Both doses of furosemide resulted in lower serum potassium concentrations when compared to pre-trial concentrations. Two dogs in each dosing group had hypokalemia when compared to the normal range. However, these decreases in serum

potassium concentrations were mild and no clinical signs of significant hypokalemia, such as muscular weakness, were noted at any time during the chronic trial.

The dogs demonstrated expected clinical responses to furosemide administration including a subjective increase in urination with a subsequent increase in water intake. With one exception in the acute trial, all dogs were able to maintain adequate hydration with constant access to fresh water. The exception, Dog 5, had a moderate elevation in serum urea and creatinine concentrations following administration of the high dose of furosemide. These elevations were suspected to be due to the excitement that the dog demonstrated on this sampling day which lead to inadequate water intake. In the chronic trial, serum urea and creatinine concentrations were significantly higher following administration of both doses of furosemide when compared to pre-trial values. However, none of the dogs demonstrated clinical evidence of dehydration during the trial and all values remained within their normal range. Thus, these changes were not considered clinically significant.

The ability of furosemide to cause hyponatremia and hypochloremia is well documented (1-3,34,95). As previously discussed, the sodium and chloride abnormalities are the result of diminished reabsorption of these ions from the urine and increased urinary losses. This was not a problem in the acute or chronic trial. Although several dogs had hyponatremia and hypochloremia in the acute trial, these decreases were not severe enough to be clinically important and the differences were not statistically different from placebo. These findings were not unexpected. If an isocaloric diet with adequate sodium and chloride content is provided and access to fresh water is

unrestricted, the risk of clinically significant hyponatremia and hypochloremia in normal dogs is low.

During the chronic trial, serum phosphorus concentrations significantly increased following furosemide administration. This was unexpected as furosemide is not usually associated with increases in serum phosphorus concentrations. Furosemide would be expected to decrease serum phosphorus concentration through increased urine flow and decreased ion reabsorption. Thus, the increases in serum phosphorus concentration cannot be explained. However, hyperphosphatemia did not develop as all values remained within the normal range. Therefore, the increases in serum phosphorus concentration were not considered clinically significant and may be the result of individual dog variation rather than furosemide administration.

4.3. Phase II – diabetic dogs

Furosemide administration did not cause significant statistical or clinical alterations in glycemic parameters in diabetic dogs. All dogs demonstrated hyperglycemia consistent with diabetes mellitus during both trials (25). The severity of the increased serum glucose concentrations can be explained by the routine established for the sampling days. Exogenous insulin therapy was administered on a daily basis each morning throughout each trial. However, on each sampling day, NPH insulin administration was withheld until the fsIVGTT was completed. Only half the normal NPH insulin was then administered to avoid an insulin overdose and subsequent hypoglycemia. The lack of statistically significant change in any glycemic parameter

following furosemide administration when compared to placebo indicates that furosemide does not cause further deterioration in glucose tolerance in insulin dependent diabetic dogs. Only one dog in the acute trial (17%) and two dogs in the chronic trial (30%) required a one unit increase in the exogenous insulin doses to maintain clinical control of the diabetes mellitus. These dose changes were not considered clinically significant because they were minor changes made in a small number of dogs.

In Phase II, insulin sensitivity and glucose effectiveness with administration of the placebo dose were slightly decreased when compared to administration of placebo during Phase I. This observation was consistent with the development of diabetes mellitus. No further deterioration in glucose effectiveness or insulin sensitivity was noted following furosemide administration. This provides further support that furosemide does not alter glycemic control in diabetic dogs. It is not possible to monitor endogenous insulin secretion in this model as alloxan destroys the pancreatic beta cells leading to insulinopenic diabetes mellitus. Thus, the presence of low serum insulin concentrations observed in Phase II is consistent with the alloxan administration.

Unlike Phase I, the administration of furosemide caused a statistically significant decrease in serum potassium concentration when compared to placebo in both trials of Phase II. However, hypokalemia did not develop and these decreases were not considered clinically significant. Serum potassium concentrations remained within the normal range and may explain why furosemide failed to alter glycemic control during either trial.

Administration of furosemide to diabetic dogs resulted in the same effects as in normal dogs. The combined effects of diabetes mellitus and furosemide administration

could have placed the dogs at greater risk for developing dehydration. However, despite subjectively observed increases in urination and water intake, all dogs were able to maintain normal hydration. As well, serum urea, serum creatinine, packed cell volume, and serum total protein values remained within the normal range indicating that dehydration did not occur. Constant access to fresh water could account for their ability to compensate and maintain adequate hydration.

The serum sodium and creatinine concentrations were lower than expected for all trials. The placebo doses resulted in concentrations at the low end of the normal range and below. Statistically significant differences following furosemide administration versus placebo were noted only in serum chloride concentrations during the acute trial. All other serum sodium and chloride concentrations were not different from placebo. The unexpected presence of hyponatremia and hypochloremia may be the result of diuresis associated with the diabetes mellitus. It is unlikely to be associated solely with the administration of furosemide because the placebo resulted in hyponatremia and hypochloremia as well.

The significant increase in serum calcium concentration noted after furosemide administration is difficult to explain. Hypercalcemia did not occur during either trial, thus the clinical significance of this increase is minimal. Similar to the serum phosphorus results in Phase I, furosemide administration should result in a decrease in serum calcium concentration due to decreased ion reabsorption. The changes in the serum calcium concentrations may be due to individual dog variation.

4.4. Conclusions

Furosemide administration at the doses utilized did not alter glucose tolerance or glycemic control in healthy or insulin dependent diabetic dogs. There was no evidence of clinically significant hyperglycemia, decreased glucose utilization, or decreased insulin secretion in the healthy dogs or further deterioration of glucose tolerance in the diabetic dogs. Furosemide may affect glycemic parameters in normal dogs with either a predisposition towards diabetes mellitus or previously unidentified glucose intolerance. However, further studies utilizing a larger sample size would be necessary to evaluate this. Furosemide appears safe to use in dogs with diabetes mellitus.

APPENDIX A

COMPONENTS OF THE SERUM BIOCHEMICAL PROFILE	
Sodium concentration (Na)	144 – 162 mmol/L
Potassium concentration (K)	3.6 - 6.0 mmol/L
Na:K ratio	
Chloride concentration (Cl)	106 - 126 mmol/L
Calcium concentration (Ca)	2.24 – 3.04 mmol/L
Phosphorus concentration (P)	0.82 – 1.87 mmol/L
Urea concentration	3.0 – 10.5 mmol/L
Creatinine concentration (Cr)	60 – 140 umol/L
Glucose concentration (BG)	3.3 – 5.6 mmol/L
Cholesterol concentration (Chol)	2.5 – 7 mmol/L
Total bilirubin concentration (T. Bili)	0 – 17 umol/L
Amylase concentration	300 – 1100 U/L
Lipase concentration	<280 U/L
Creatine kinase concentration (CK)	<300 IU/L
Alkaline phosphatase (SAP)	23 – 87 IU/L
Aspartate tranferase (AST)	20 - 50 IU/L
Alanine transferase (ALT)	5 - 69 IU/L
Gamma glutamyl transpeptidase (GGT)	<8 IU/L
Total protein concentration (TP)	51 – 71 g/L
Albumin concentration	22 – 38 g/L
Albumin:Globulin ratio	0.6 – 1.5

APPENDIX B

COMPONENTS OF THE SERUM RENAL/ELECTROLYTE PROFILE	
Sodium concentration (Na)	144 – 162 mmol/L
Potassium concentration (K)	3.6 – 6.0 mmol/L
Na:K ratio	
Chloride concentration (Cl)	106 – 126 mmol/L
Phosphorus concentration (P)	0.82 – 1.87 mmol/L
Urea concentration	3.0 – 10.5 mmol/L
Creatinine concentration (Cr)	60 – 140 umol/L
Glucose concentration (BG)	3.3 – 5.6 mmol/L

REFERENCES

1. PLUMB DC. Furosemide. In: Plumb DC, editor. Veterinary Drug Handbook. 2nd ed. Ames, Iowa: Iowa State University Press, 1995:285-287.

2. MUDGE GH. Diuretics and other agents employed in the mobilization of edema fluid. In: Gilman AG, Goodman LS, Gilman A, editors. The Pharmacological Basis of Therapeutics. Sixth ed. New York: MacMillan Publising Co., 1980:892-915.

3. QUAMME G. Loop diuretics. In: Dirks J, Sutton R, editors. Diuretics: physiology, pharmacology & clinical use. Philadelphia: W.B. Saunders, 1986:86-116.

4. JONES IG, PICKENS PT. Diabetes mellitus following oral diuretics. Practitioner. 1967; 199:209-210.

5. TOIVONEN S, MUSTALA O. Diabetogenic action of frusemide. Br.Med.J. 1966; 9:920-921.

6. MURPHY MB, LEWIS PJ, KOHNER E, SCHUMER B, DOLLERY CT. Glucose intolerance in hypertensive patients treated with diuretics; a fourteen-year follow-up. Lancet 1982; 2:1293-1295.

7. BRECKENRIDGE A, WELBORN TA, DOLLERY CT, FRASER R. Glucose tolerance in hypertensive patients on long-term diuretic therapy. Lancet 1967; 1:61-64.

8. PLUMB DC. Insulin. In: Plumb DC, editor. Veterinary Drug Handbook. 2nd ed. Ames, Iowa: Iowa State University Press, 1995:321-326.

9. GUYTON AC, HALL JE. Metabolism of carbohydrates and formation of adenosine triphosphate. In: Guyton AC, editor. Textbook of Medical Physiology. 9 ed. Philadelphia: W.B. Saunders, 1996:855-864.

10. LAYCOCK JF, WISE PH. The pancreas (1): control of metabolism, diabetes, and hypoglycaemia. In: Laycock JF, Wise PH, editors. Essential Endocrinology. Third ed. Oxford: Oxford University Press, 1996:274-314.

11. STRYER L. Glycolysis. In: Kudlacik A, editor. Biochemistry. 3rd ed. New York: W.H. Freeman and Company, 1988:349-372.

12. STRYER L. Citric Acid Cycle. In: Kudlacik A, editor. Biochemistry. 3rd ed. NewYork: W.H. Freeman and Company, 1988:373-396.

13. LODISH H, BALTIMORE D, BERK A, ZIPURSKY SL, MATSUDAIRA P, DARNELL J. Cellular energetics: Formation of ATP by glycolysis and oxidative phosphorylation. In: Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J, editors. Molecular Cell Biology. 3rd ed. New York: Scientific American Books, Inc, 1995:739-777.

14. CHERRINGTON AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999; 48:1198-1214.

15. KAHN BB. Lilly lecture 1995. Glucose transport: pivotal step in insulin action. Diabetes 1996; 45:1644-1654.

16. ENGELKING LR. Physiology of the endocrine pancreas. Seminars in Veterinary Medicine and Surgery (Small Animal) 1997; 12:224-229.

17. FELDMAN EC, NELSON RW. Diabetes mellitus. In: Feldman EC, Nelson EW, editors. Canine and Feline Endocrinology and Reproduction. 2nd ed. Philadelphia: W.B.Saunders, 1996:339-391.

18. GUYTON AC, HALL JE. Insulin, glucagon, and diabetes mellitus. In:Guyton AC, editor. Textbook of Medical Phyisology. 9 ed. Philadelphia: W.B. Saunders, 1996:971-983.

19. ESPINAL J. Understanding insulin action. 1st ed. Chichester, England: Ellis Horwood Limited, 1989.

20. MALAISSE WJ. Physiology, pathology and pharmacology of insulin secretion: recent acquisitions. Diabetes Metab. 1997; 23 Suppl 3:6-15:6-15.

21. SATO Y, HENQUIN JC. The K+-ATP channel-independent pathway of regulation of insulin secretion by glucose: in search of the underlying mechanism. Diabetes 1998; 47:1713-1721.

22. KOMATSU M, SCHERMERHORN T, NODA M, STRAUB SG, AIZAWA T, SHARP GWG. Augmentation of insulin release by glucose in the absence of extracellular Ca2+; New insights into stimulus-secretion coupling. Diabetes 1997; 46:1928-1938.

23. DUKES ID, PHILIPSON LH. K+ channels: Generating excitement in pancreatic B-cells. Diabetes 1996; 45:845-853.

24. MARMOR M, WILLEBERG P, GLICKMAN LT, PRIESTER WA, CYPESS RH, HURVITZ AI. Epizootiologic patterns of diabetes mellitus in dogs. Am.J Vet.Res. 1982; 43:465-470.

25. NELSON RW. Disorders of the endocrine pancreas. In: Ettinger SJ, editor. Textbook of Veterinary Medicine. 3rd ed. Philadelphia: W.B. Saunders, 1989:1676-1720.

26. PETERSON E, ALTSZULER N, NICHOLS CE. Decreased insulin sensitivity and glucose tolerance in spontaneous canine hyperadrenocorticism. Research in Veterinary Science 1984; 36:177-182.

27. PAGANO G, BRUNO A, CAVALLO-PERIN P, CESCO L, IMBIMBO B. Glucose intolerance after short-term administration of corticosteroids in healthy subjects. Arch.Intern.Med 1989; 149:1093-1101.

28. DE PIRRO R, FORTE F, BERTOLI A, GRECO AV, LAURO R. Changes in insulin receptors during oral contraception. J.Clin.Endocrinol.Metab. 1981; 52:29-33.

29. SELMAN PJ, MOL JA, RUTTEMAN GR, RIJNBERK A. Progestin treatment in the dog. I. Effects on growth hormone, insulin- like growth factor I and glucose homeostasis. Eur.J Endocrinol. 1994; 131:413-421.

30. EIGENMANN JE, EIGENMANN RY, RIJNBERK A, VAN DER GAAG I, ZAPF J, FROESCH ER. Progesterone-controlled growth hormone overproduction and naturally occurring canine diabetes and acromegaly. Acta Endocrinol.(Copenh.) 1983; 104:167-176.

31. BHATNAGAR SK, AMIN MM, AL-YUSUF AR. Diabetogenic effects of nifedipine. Br.Med.J.(Clin.Res.Ed.) 1984; 289:19

32. ZEZULKA AV, GILL JS, BEEVERS DG. Diabetogenic effects of nifedipine. Br.Med.J. 1984; 289:437-438.

33. ABADIE E, PASSA P. Diabetogenic effects of nifedipine. Br.Med.J. 1984; 289:438-438.

34. PRICHARD BN, OWENS CW, WOOLF AS. Adverse reactions to diuretics. Eur.Heart J. 1992; 13 Suppl G:96-103:96-103.

35. SREENIVASAN V, WALKER B, KRASNEY J, MOOKERJEE B, VENUTO R. Role of endogenous prostaglandins in volume expansion and during furosemide infusion in conscious dogs. Hypertension 1981; 3:59-66.

36. RATHAUS M, BAUMINGER S, BERNHEIM J. Effect of furosemide on renal prostaglandins E2 and F2 alpha in normal subjects and in patients with essential hypertension. Isr.J Med Sci. 1980; 16:106-110.

37. MACDONALD TM, CRAIG K, WATSON ML. Frusemide, ACE inhibition, renal dopamine and prostaglandins: acute interactions in normal man. Br.J Clin.Pharmacol. 1989; 28:683-694.

38. ABE K, YASUJIMA M, CHIBA S, IROKAWA N, ITO T, YOSHINAGA K, et al. Effect of furosemide on urinary excretion of prostaglandin E in normal volunteers and patients with essential hypertension. Prostaglandins 1977; 14:513-521.

39. MIYANOSHITA A, TERADA M, ENDOU H. Furosemide directly stimulates prostaglandin E2 production in the thick ascending limb of Henle's loop. J Pharmacol.Exp.Ther. 1989; 251:1155-1159.

40. KATAYAMA S, ATTALLAH AA, STAHL RA, BLOCH DL, LEE JB. Mechanism of furosemide-induced natriuresis by direct stimulation of renal prostaglandin E2. Am.J Physiol. 1984; 247:F555-F561

41. CRAVEN PA, DERUBERTIS FR. Calcium-dependent stimulation of renal medullary prostaglandin synthesis by furosemide. J Pharmacol.Exp.Ther. 1982; 222:306-314.

42. BAGGOT JD. Mechanisms of drug action. In: Booth NH, McDonald LE, editors. Veterinary Pharmacology and Therapeutics. Fifth ed. Ames, Iowa: Iowa State University Press, 1982:23-35.

43. KLAHR S, YATES J, BOURGOIGNIE J. Inhibition of glycolysis by ethacrynic acid and furosemide. Am.J.Physiol. 1971; 221:1038-1043.

44. JUNG CY, MOOKERJEE BK. Inhibitory effect of furosemide on glucose transport. J.Lab.Clin.Med. 1976; 87:960-966.

45. JACOBS DB, MOOKERJEE BK, JUNG CY. Furosemide inhibits glucose transport in isolated rat adipocytes via direct inactivation of carrier proteins. J.Clin.Invest. 1984; 74:1679-1685.

46. DIMITRIADIS G, TEGOS C, GOLFINOPOULOU L, ROBOTI C, RAPTIS S. Furosemide-induced hyperglycaemia: the implication of glycolytic kinases. Horm.Metab.Res. 1993; 25:557-559.

47. DIMITRIADIS G, LEIGHTON B, PARRY-BILLINGS M, TOUNTAS C, RAPTIS S, NEWSHOLME EA. Furosemide decreases the sensitivity of glucose transport to insulin in skeletal muscle in vitro. Eur.J Endocrinol. 1998; 139:118-122.

48. SANDSTROM PE, SEHLIN J. Furosemide reduces insulin release by inhibition of Cl- and Ca2+ fluxes in beta-cells. Am.J.Physiol. 1988; 255:E591-E596

49. CAMPBELL NRC, WICKERT WA, SHUMAK SL. Frusemide causes acute increases in lipid concentrations. Br.J.Clin.Pharmac 1993; 36:607-609.

50. DAWSON KG, SUTTON R. Metabolic disorders. In: Dirks J, Sutton R, editors. Diuretics: Physiology, Pharmacology, and Clinical Use. 1st ed. Philadelphia: W.B. Saunders, 1986:341-361.

51. GILL GV, RAUF O, MACFARLANE IA. Diazoxide treatment for insulinoma: a national UK survey. Postgrad.Med J 1997; 73:640-641.

52. SKRHA J, SVACINA S, SRAMKOVA J, PAV J. Use of euglycaemic clamping in evaluation of diazoxide treatment of insulinoma. Eur.J Clin.Pharmacol. 1989; 36:199-201.

53. LEIFER CE, PETERSON ME, MATUS RE. Insulin-secreting tumor: diagnosis and medical and surgical management in 55 dogs. J Am.Vet.Med Assoc. 1986; 188:60-64.

54. AYNSLEY-GREEN A, ALBERTI KG. Diuretics and carbohydrate metabolism: the effects of furosemide and amiloride on blood glucose, plasma insulin and cations in the rat. Diabetologia 1973; 9:34-42.

55. WEXLER BC. Furosemide-induced hyperuricemia, hyperglycemia, hypertension and arterial lesions in nonarteriosclerotic and arteriosclerotic rats. Atherosclerosis 1981; 39:253-266.

56. FOY JM. Acute diuretic induced hyperglycaemia in rats. Life Sci. 1967; 6:897-902.

57. WELLER JM, BORONDY M. Effect of furosemide on glucose metabolism. Metabolism 1967; 16:532-536.

58. SANDSTROM PE, SEHLIN J. Furosemide-induced glucose intolerance in mice is associated with reduced insulin secretion. Eur.J.Pharmacol. 1988; 147:403-409.

59. FOY JM, FURMAN BL. The effect of fourteen day treatment with diuretics on mouse blood sugar and glucose tolerance. J.Pharm.Pharmacol. 1972; 24:390-395.

60. SANDSTROM PE. Probenecid potentiates the hyperglycaemic effect but reduces the diuretic effect of frusemide in mice. Br.J.Pharmacol. 1986; 89:307-312.

61. SANDSTROM PE, SEHLIN J. Furosemide causes acute and long-term hyperglycaemia and reduces glucose tolerance in mice. Acta Physiol.Scand. 1988; 132:75-81.

62. SANDSTROM PE, SEHLIN J, AMARK K. Furosemide treatment causes age-dependent glucose intolerance and islet damage in obese-hyperglycaemic mice. Pharmacol.Toxicol. 1993; 72:304-309.

63. SUNDBERG S, SALO H, GORDIN A, MELAMIES L, LAMMINSIVU U, NUOTTO E, et al. Effect of low dose diuretics on plasma and blood cell electrolytes, plasma uric acid and blood glucose. Acta Med.Scand.Suppl. 1982; 668:95-101:95-101.

64. DORNHORST A, POWELL SH, PENSKY J. Aggravation by propranolol of hyperglycaemic effect of hydrochlorothiazide in type II diabetics without alteration of insulin secretion. Lancet 1985; 1:123-126.

65. JACKSON WP, NELLEN M. Effect of frusemide on carbohydrate metabolism, blood-pressure, and other modalities. A comparison with chlorothiazide. Br.Med.J. 1966; 2:333-336.

66. MOLLER A, SCHMITZ A, PEDERSEN MM, CHRISTIANSEN JS. Lipoproteins and diuretics in type II diabetes. J.Diabet.Complications. 1991; 5:13-17.

67. CAR N, SKRABALO Z, VERHO M. The effects of piretanide in patients with congestive heart failure and diabetes mellitus: a double-blind comparison with furosemide. Curr.Med.Res.Opin. 1988; 11:133-141.

68. WALSH CH, O'SULLIVAN DJ. A study of the effect of frusemide on carbohydrate metabolism in diabetic subjects. J.Ir.Med.Assoc. 1974; 67:187-190.

69. DIMITRIADIS G, LEIGHTON B, PARRY-BILLINGS M, NEWSHOLME EA. Effects of the diuretic furosemide on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat. Diabetologia 1988; 31:58-61.

70. GIUGLIANO D, TORELLA R, SCHEEN AJ, LEFEBVRE PJ, D'ONOFRIO F. [Prostaglandins, insulin secretion and diabetes mellitus]. Diabete.Metab. 1988; 14:721-727.

71. HERMANSEN K, SCHMITZ O, ARNFRED J, MOGENSEN CE. Effects of furosemide and indapamide upon pancreatic insulin and somatostatin secretion in vitro. Diabetes Res. 1986; 3:221-223.

72. ROWE JW, TOBIN JD, ROSA RM, ANDRES R. Effect of experimental potassium deficiency on glucose and insulin metabolism. Metabolism 1980; 29:498-502.

73. SAGILD U, ANDERSON V. Further studies on glucose metabolism on experimental potassium depletion: Effect of insulin, glucagon, and muscular exercise. Acta Medica Scandiavica 1964; 175:681-685.

74. HOLLAND SD, WILLIAMSON HE. Acute effects of high ceiling diuretics on pancreatic blood flow and function. J.Pharmacol.Exp.Ther. 1984; 229:440-446.

75. ROBERTSON RP, CHEN M. A role for prostaglandin E in defective insulin secretion and carbohydrate intolerance in diabetes mellitus. J.Clin.Invest. 1977; 60:747-753.

76. GIUGLIANO D, TORELLA R, SGAMBATO S, D'ONOFRIO F. Acetylsalicyclic acid restores acute insulin response reduced by furosemide in man. Diabetes 1979; 28:841-845.

77. LANDGRAF R, LANDGRAF-LEURS MM. The prostaglandins system and insulin release. Studies with the isolated perfused rat pancreas. Prostaglandins 1979; 17:599-613.

78. YANG YJ, YOUN JH, BERGMAN RN. Modified protocols improve insulin sensitivity estimation using the minimal model. Am.J Physiol. 1987; 253:E595-E602

79. TOBIN BL, FINEGOOD DT. Reduced insulin secretion by repeated low doses of STZ impairs glucose effectiveness but does not induce insulin resistance in dogs. Diabetes 1993; 42:474-483.

80. SAAD MF, STEIL GM, KADES WW, AYAD MF, ELSEWAFY WA, BOYADJIAN R, et al. Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. Diabetes 1997; 46:1167-1171.

81. STEIL GM, MURRAY J, BERGMAN RN, BUCHANAN TA. Repeatability of insulin sensitivity and glucose effectiveness from the minimal model. Implications for study design. Diabetes 1994; 43:1365-1371.

82. SAAD MF, ANDERSON RL, LAWS A, WATANABE RM, KADES WW, CHEN YD, et al. A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. Diabetes 1994; 43:1114-1121.

83. ADER M, PACINI G, YANG YJ, BERGMAN RN. Importance of glucose per se to intravenous glucose tolerance. Comparison of the minimal-model prediction with direct measurements. Diabetes 1985; 34:1092-1103.

84. YANG YJ, YOUN JH, BERGMAN RN. Modified protocols improve insulin sensitivity estimation using the minimal model. Am.J.Physiol. 1987; 253:E595-E602

85. ABBATE SL, FUJIMOTO WY, BRUNZELL JD, KAHN SE. Effect of heparin on insulin-glucose interactions measured by the minimal model technique: implications for reproducibility using this method. Metabolism 1993; 42:353-357.

86. FELDMAN EC, NELSON RW. Beta-cell neoplasia: insulinoma. In: Feldman EC, Nelson RW, editors. Canine and feline endocrinology and reproduction. 2nd edition ed. Philadelphia: W.B. Saunders, 1999:422-441.

87. HOLSTE LC, NELSON RW, FELDMAN EC, BOTTOMS GD. Effect of dry, soft moist, and canned dog foods on postprandial blood glucose and insulin concentrations in healthy dogs. Am.J Vet.Res. 1989; 50:984-989.

88. PACINI G, BERGMAN RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput.Methods Programs Biomed. 1986; 23:113-122.

89. TAYLOR SI. Lilly Lecture: molecular mechanisms of insulin resistance. Lessons from patients with mutations in the insulin-receptor gene. Diabetes 1992; 41:1473-1490.

90. MORRIS RD, RIMM DL, HARTZ AJ, KALKHOFF RK, RIMM AA. Obesity and heredity in the etiology of non-insulin-dependent diabetes mellitus in 32,662 adult white women. Am.J Epidemiol. 1989; 130:112-121.

91. BERNTORP K, ERIKSSON KF, LINDGARDE F. The importance of diabetes heredity in lean subjects on insulin secretion, blood lipids and oxygen uptake in the pathogenesis of glucose intolerance. Diabetes Res. 1986; 3:231-236.

92. QUON MJ, COCHRAN C, TAYLOR SI, EASTMAN RC. Direct comparison of standard and insulin modified protocols for minimal model estimation of insulin sensitivity in normal subjects. Diabetes Res. 1994; 25:139-149.

93. COATES PA, LUZIO SD, BRUNEL P, OWENS DR. Comparison of estimates of insulin sensitivity from minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test and the isoglycemic hyperinsulinemic clamp in subjects with NIDDM. Diabetes 1995; 44:631-635.

94. KORYTKOWSKI MT, BERGA SL, HORWITZ MJ. Comparison of the minimal model and the hyperglycemic clamp for measuring insulin sensitivity and acute insulin response to glucose. Metabolism 1995; 44:1121-1125.

95. FORMANEK K, KENNER T. Special features of the action of a new diuretic. Br.J.Pharmacol. 1966; 26:27-33.