CLINICAL AND EPIDEMIOLOGICAL STUDIES ON CANINE DIABETES MELLITUS

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Thesis submitted for the degree of DOCTOR OF PHILOSOPHY

UNIVERSITY
of
GLASGOW

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May 1995

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Abstract

The aim of the work presented in this thesis was to investigate aspects of canine diabetes mellitus in order to improve understanding of the condition and the welfare of dogs affected by it. The epidemiology, clinical chemistry and survival data of diabetic dogs treated by the author during the period October 1989 to August 1994 were studied. In addition, studies on specific insulin and dietary formulations were undertaken.

Epidemiological studies employing statistical comparisons with a time-matched hospital derived control population revealed a complicated relationship between age and gender associated with diabetes mellitus, in that females in the older age groups were at greatest risk but in the younger age groups they were not. Predisposed breeds were terriers (particularly Tibetan, Cairn and Jack Russell), collie crossbreeds and Rottweilers. There were differences between the epidemiological characteristics of diabetic dogs of high risk breeds and those of normal or low risk breeds suggesting a possible difference in pathophysiology.

Investigation of diabetic dogs with associated syndromes revealed differences in age distribution between those with hypothyroidism, those with hyperadrenocorticism and 'normal' diabetic dogs and that basal plasma insulin analysis prior to ovariohysterectomy may be a useful prognostic indicator in metoestrus-associated diabetes mellitus.

'New' tests, fructosamine and glycated haemoglobin, validated for use with canine samples, were determined to be useful in the monitoring of canine diabetes mellitus by relative operating characteristic curve analysis. Alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were not useful tests for this purpose.

A highly purified porcine insulin zinc suspension had two reasonably predictable times of peak activity following subcutaneous injection at around 4 and 11 hours and an overall duration of activity of 14 to 24 hours. The efficacy of the same product, studied during a stabilisation and follow-up period in 19 diabetic dogs, was good in both the short and long term. Stabilisation was achieved in 10 - 14 days resulting in a final dose of between 0.8 and 2.0 IU/kg. The rates of bacterial infections and blindness associated with the long term use of this product on a single daily basis were 1 per 1.11 and 1 per 2.23 diabetic-dog-years, respectively.

The feeding of a commercially produced 'high fibre' diet reduced mean 24 hour plasma glucose concentration and the fluctuation in afternoon post-prandial glycaemia. Feeding this diet also improved demeanour and activity scores and plasma concentrations of afternoon glucose, fructosamine and alkaline phosphatase. In addition, reductions were observed in plasma concentrations of total cholesterol, LDL cholesterol, free glycerol and non-esterified fatty acids. There was an association between feeding this diet and weight loss, reduced body condition scores and increased faecal volume score. In the very long term (> 4 months) there was a subjectively high prevalence of unexpected weight loss, recurrent diarrhoea and small intestinal bacterial overgrowth.

Median survival time for diabetic dogs treated with single daily injections of insulin was 2.71 years. Median remaining lifetime increased to 3.11 years for those dogs which survived the first 5 months of therapy. There was no effect on survival of gender or of the type of intermediate duration insulin preparation, but diabetic dogs with hyperadrenocorticism had a much poorer prognosis. Survival rates for diabetic dogs were close to or greater than those of an age and gender-matched general canine population, using a necropsy based modified cohort general life table.

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Acknowledgements

Financial support for this work was provided by the University of Glasgow, Waltham Centre for Pet Nutrition and Intervet UK Ltd. and I am grateful to Professor Norman Wright, Mr Peter Markwell, Dr Ian Maskell, Mr John Watson and Dr Aart Coert for their support in obtaining this funding.

This thesis would certainly not exist if it had not been for the guidance of Professor Andrew Nash and his efforts in my supervision but particularly since he was responsible for initiating my interest in canine diabetes mellitus. The support of Professor Max Murray, in whose department this work was performed, has been unfailing and the existence of this thesis also owes much to Mr Brian Wright and his 'magic wand' for ensuring that I had the necessary equipment and supplies to complete my work.

Technical assistance was gratefully received from Ronnie Barron and Kenny Williamson who performed the haematological analyses; Anne Galbraith who performed the insulin analyses and Christine Marshall, Chris McComb, Jim Harvey, David Moffat and David French who performed routine clinical chemistry analyses. June Downs conducted the plasma glycerol measurements and, along with Kathryn McBride, some of the glycated haemoglobin analyses. John Armstrong, Linda Forrest, Arlene Macrae, June and Kathryn all contributed to my 'laboratory education' ensuring that I quickly learned one end of an air displacement pipette from the other and the meaning of the word microlitre.

Drs. Tim Watson, Joan Barrie and Roger Clampitt kindly helped me through the complexities of clinical lipidology and biochemistry and Joan also provided me with the know how for performing quantitative analysis of canine lipoproteins.

I received advice on statistical methods from Professor George Gettinby and Dr Stuart Reid but could not have performed the analyses or ventured into the hospital computer network without patient tutoring from David Irvine.

Expert nursing care for the dogs featured in this thesis was provided by Pamela Gillan, Mairi Austin, Susan Fitzpatrick, Carole Anderson and Janice Moffat all of whom along with Chris McComb, Callum Blair, Kathryn McBride and Andrew Nash ensured there was someone to help me obtain blood glucose samples after normal working hours.

I have benefited much from association with clinical colleagues including Drs Carmel Mooney, Sean Callanan, Chris Little, Ms Jo Dukes McEwan and houseofficers past and present and I owe them many thanks. The assistance of staff in radiography, clinical records, photography and veterinary pathology in the pursuance of my clinical work must also be acknowledged.

Combinations of friendship, support, encouragement and advice were provided during the completion of this work by: my family; Drs Sandy Love, Richard McCulloch, Heather Spence, Chris Hunter, Jo Burke and Lubna Nasir; Jean Rodgers, Tom Irwin, Carl Mills, Chris Rawlinson, Ian Scott, Tim Harvey, David Murphy, Janice Lloyd, Michael Wilkinson, and Juan Escala in addition to that by many of those already mentioned.

Author's declaration

The work presented in this thesis was performed solely by the author except where the assistance of others has been acknowledged.

The details of diabetic dogs presented in this thesis relate to cases treated by the author in the Department of Veterinary Medicine, University of Glasgow during the period October 1989 to August 1994.

PETER A. GRAHAM, May 1995

Some of the work in this thesis has been the subject of the following publications or presentations:

Graham PA, Maskell IE and Nash AS (1994) Canned high fiber diet and postprandial glycemia in dogs with naturally occurring diabetes mellitus. *Journal of Nutrition* **124**: 2712S -2715S

Graham PA, McKellar QA and Nash AS (1995) The pharmacokinetics of a highly purified porcine insulin zinc suspension (IZS-P) in dogs with naturally occurring diabetes mellitus. *Journal of Small Animal Practice* (Submitted for publication, May 1994).

Graham PA and Nash AS (1995) How long will my diabetic dog live? *British Small Animal Veterinary Association Annual Congress Proceedings* p 217.

Graham PA The Modern Management of Canine Diabetes Mellitus, *Proceedings of the 1st Congress of the Federation of European Companion Animal Veterinary Associations* Paris, November 1994. p599

Graham PA, Maskell IE, Markwell PJ and Nash AS (1994). The long term effects of feeding a commercially produced high-fibre diet to dogs with naturally-occurring diabetes mellitus. Association of Veterinary Teachers and Research Workers Annual Scientific Meeting, Scarborough.

Graham PA, Maskell IE and Nash AS (1993) The effects of feeding a commercially produced high fibre diet on post prandial glycaemia in naturally-occurring diabetic dogs. Waltham Symposium on the Nutrition of Companion Animals at 15th International Congress of Nutrition, Adelaide, Australia.

Graham PA, Maskell IE and Nash AS (1993) The effects of feeding a commercially produced high fibre diet on post prandial glycaemia in naturally-occurring diabetic dogs. *British Small Animal Veterinary Association Annual Congress Proceedings* p 195

Graham PA, and Nash AS (1992) The long-term use of a highly purified porcine insulin zinc suspension (IZS-P) in dogs with naturally-occurring diabetes mellitus. *British Small Animal Veterinary Association Annual Congress Proceedings* p 230

Graham PA, McKellar QA and Nash AS (1992) The pharmacokinetics of a highly purified porcine insulin zinc suspension (IZS-P) in dogs with naturally-occurring diabetes mellitus. *British Small Animal Veterinary Association Annual Congress Proceedings* p 167

Chapter 1:
General introduction and review

This chapter is a brief review of the background, pathophysiology, diagnosis and management of canine diabetes mellitus and an outline of the objectives of the thesis as a whole. More detailed reviews of certain specific aspects of canine diabetes mellitus are presented within relevant chapters.

Definition

Diabetes mellitus is not a single disease but is a clinically and aetiologically heterogeneous group of hyperglycaemic diseases and conditions (Harris and Zimmet, 1992; Unger and Foster, 1985). Hyperglycaemia results from an absolute or relative deficiency of insulin combined with an absolute or relative excess of glucagon.

History

Diabetes mellitus has been recognised in man and animals for many centuries. The earliest reports of the condition in man are from Egypt (c1500 BC), India (c600 BC) and Greece (300 BC) (Tunbridge and Home, 1991; Wilkinson 1957; Harris and Zimmet, 1992) and although diabetic urine had been described as 'sweet' for many centuries it was not until 1674 that an English physician Willis proposed that sweet urine was likely to be secondary to a sweetness of blood. This theory was proven in the late 1700's and some years later glucose was discovered to be the relevant sugar. In veterinary literature the first mention of sugar in the urine of a polyuric animal appeared in Blaine's *Veterinary Art* of 1802 (Wilkinson, 1957) with a description of diabetes mellitus in the horse and the suggestion was that it was not an infrequent disease. Reports of cases of spontaneous canine diabetes mellitus did not appear until the very late 19th and early 20th century (Foster, 1975) but an identical condition was experimentally induced in dogs following pancreatectomy by Von Mehring and Minkowski in 1889 (Wilkinson, 1957), a discovery which paved the way for the elucidation of the underlying mechanisms of the disease in the early 20th century.

Pathophysiology

The hormone insulin is produced by β-cells of the pancreatic islets of Langerhans and is secreted into the blood in response to a rise in the plasma concentration of glucose or

amino acids (Chastain and Ganjam, 1986a). Its principal action is to facilitate the transport of glucose, potassium and phosphate into cells (Chastain and Ganjam, 1986a). Insulin is antagonised by the hormone glucagon which is secreted by the α-cells of the pancreatic islets in response to an increase in plasma concentrations of amino acids such that its role may be to protect against hypoglycaemia during protein stimulation of insulin secretion (Chastain and Ganjam, 1986a). Insulin normally suppresses glucagon driven glycogenolysis and mobilisation of peripheral fat and protein and their subsequent conversion to glucose (gluconeogenesis) whilst promoting the storage of fat, protein and carbohydrate (Chastain and Ganjam, 1986a). When there is insufficient insulin to suppress the effects of glucagon these processes proceed uncontrolled and raise blood glucose concentrations (Chastain and Ganjam, 1986b). This situation is further aggravated because insulin is also required to transport glucose into cells from plasma. When plasma glucose concentration is greater than 10-12mmol/l, the renal threshold is exceeded resulting in glycosuria and an osmotic diuresis occurs (Feldman and Nelson, 1987), with compensatory polydipsia. Bodyweight is reduced because of the mobilisation and metabolism of fat and protein stores (Chastain and Ganjam, 1986b).

The energy deficit created in peripheral tissues by the reduced uptake of glucose is met by the mobilisation of fat stores and their metabolism in the liver to produce ketones which can be used as an alternative energy source (Chastain and Ganjam, 1986b). Ketogenesis is suppressed by insulin in the normal animal. In addition, insulin normally has an inhibitory action on hormone sensitive lipase in adipose tissue (Allen, 1987) but when this inhibition is lost there is increased lipolysis and movement of fatty acids from fat stores to the liver and other tissues. Concentrations of circulating triglycerides are increased in diabetes mellitus because lipoprotein lipase (LPL) - dependent clearance is reduced, insulin being permissive for the efficient synthesis and activity of lipoprotein lipase. Hepatic synthesis of triglyceride is also increased as a result of the increased concentration, and therefore, uptake of non-esterified fatty acids (Nikkila, 1984). As a result, many diabetics have raised circulating concentrations of triglyceride, non-esterified fatty acids and cholesterol. The structure and composition of the lipoproteins, particularly very low density lipoprotein (VLDL), is also seriously altered in diabetes mellitus (Abbate and Brunzell, 1990). The consequence of the mobilisation of peripheral fat and hepatic triglyceride synthesis is fatty infiltration of the liver (hepatic lipidosis) which in turn induces elevations in plasma concentrations of alanine aminotransferase (ALT), alkaline phosphatase (ALKP) (Nelson, 1989a; Allen, 1987) and occasionally gamma-glutamyl transferase (GGT). Plasma concentrations of aspartate aminotransferase may be elevated in unstable canine diabetics because of the catabolism of protein stores (muscle) for gluconeogenesis. Prolonged elevations of plasma glucose concentrations increase the degree of non-enzymatic glycation of plasma proteins and haemoglobin. The

concentrations of glycated plasma proteins (fructosamine) and glycated haemoglobin, therefore reflect the mean concentration of plasma glucose which has prevailed in the preceding weeks or months (Dolhofer and Weiland, 1979; Wood and Smith, 1980; Bunn, 1981; Mahaffrey and Cornelius, 1982; Higgins *et al*, 1982; Reusch *et al*, 1993).

When insulin deficiency is absolute or when glucagon excess is very large, diabetic animals are prone to become ketoacidotic. There is no negative feedback mechanism for ketogenesis and so ketone production, which is regulated by the insulin:glucagon ratio, can exceed requirements (Unger and Foster, 1985: Chastain and Ganjam, 1986b). When overproduction of ketones exceeds the body's buffering capacity, ketoacidosis results (Chastain and Ganjam, 1986b).

Aetiology

In human medicine, diabetes mellitus has been broadly classified as being idiopathic or secondary in origin (Table 1). Idiopathic diabetes mellitus includes insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) (World Health Organisation 1985). Secondary diabetes mellitus can result from a number of causes such as generalised pancreatic disease or injury, effects of antagonistic hormones or drug toxicities. The aetiopathologically descriptive terms Type I and Type II have been used synonymously with the clinically descriptive terms IDDM and NIDDM, respectively, by many in human medicine (Unger and Foster, 1985). However, some authors restrict the use of the term Type I diabetes mellitus to refer solely to diabetes mellitus resulting from immune-mediated islet cell cytotoxicity and the term Type II diabetes mellitus to refer to those diabetics who do not have immune-mediated islet cell toxicity nor any other known cause of diabetes mellitus (Unger and Foster, 1985). This has led to confusion in applying classifications of human diabetes mellitus to the veterinary field and some veterinary diabetologists have exacerbated this confusion by classifying canine diabetes mellitus into Type I, Type II and Type III based on plasma concentrations of insulin and glucose without regard for aetiopathogenesis (Kaneko *et al*, 1978). Nearly all canine diabetics require insulin therapy and are therefore insulin dependent. However, it does not appear that all diabetic dogs fit the human classification of IDDM/Type I because most canine diabetes mellitus appears to be secondary in origin *and* insulin dependent (Chastain and Ganjam, 1986b; Eigenmann 1989).

Idiopathic	
	IDDM
	NIDDM
Secondary	
	Generalised pancreatic disease
	Hormonal antagonism
	Drug toxicity
	Insulin receptor abnormality
Gestational	
Impaired glucose tolerance	
Malnutrition associated	
Previous or potential abnormality of glucose tolerance	

Table 1. Classification of diabetes mellitus in humans

Canine diabetes mellitus can be caused (or exacerbated) by any factors which interfere with insulin production, transport or activity in target tissues (Table 2). For example, failure of insulin production could be the result of immune-mediated islet cell destruction (Sai *et al* 1984) or chronic pancreatitis (Dixon and Sanford, 1962). Peripheral insulin insensitivity can result from obesity, hypercortisolaemia of hyperadrenocorticism or other hormonal antagonism. Failure of insulin transport e.g. by the formation of insulin antibodies is possible but rarely occurs as a recognisable clinical syndrome in dogs (Feldman *et al*, 1983; Feldman and Nelson, 1987; Ihle and Nelson, 1991). Dogs which are, or are about to become, diabetic primarily as a result of insulin insensitivity initially exhibit compensatory elevations in insulin production (Kaneko *et al*, 1978; Mattheeuws *et al*, 1984a; Mattheeuws *et al*, 1984b; Eigenmann, 1989). However, after a sustained period of increased output, which may last months, insulin secreting β-cells may become exhausted (Kaneko *et al*, 1978; Mattheeuws *et al*, 1984a; Mattheeuws *et al*, 1984b; Eigenmann 1989), leaving a diabetic which has a combination of failed insulin production and target tissue insensitivity. In such cases, if the primary cause of insulin resistance can be identified and eliminated early, the dog need not be diabetic for the rest of its life (Campbell and Latimer, 1984; Eigenmann, 1989; Blaxter, 1990). The most common example of a 'cured' diabetic dog is the bitch with metoestrous diabetes, associated with progesterone excess and consequent induction of elevated concentrations of growth hormone which is spayed before β-cell exhaustion occurs (Eigenmann, 1989; Blaxter, 1990).

The relative prevalence of canine diabetes mellitus resulting from each of the known causes is difficult to ascertain. Dixon and Sanford (1962) found as many as 4 out of 8 diabetic dogs to have evidence of pancreatic inflammation at post-mortem examination. It has since been suggested (Chastain and Ganjam, 1986b) that pancreatitis is a relatively unimportant cause of canine diabetes mellitus. Hormonal antagonism, especially by

progesterone induced growth hormone secretion, progestagens or cortisol, is thought to be a much more common aetiology (Nelson and Feldman, 1987; Eigenman, 1989). Islet auto-immunity has also been recorded in some diabetic dogs (Sai *et al*, 1984; Haines and Penhale, 1985) and a condition resembling human Type II diabetes has been reported in about 10% of diabetic dogs, but in which insulin replacement therapy was necessary (Mattheeuws *et al*, 1984b). It is likely that autoimmunity, pancreatic inflammation, hormonal antagonism and Type II disease all have a role in the development of canine diabetes mellitus. However, there are no reported studies which accurately define their relative importance.

Failure of:		
Insulin production	**Insulin transit**	**Target tissue sensitivity**
autoimmunity	insulin antibodies	obesity
pancreatitis		hormonal antagonism
islet cell hypoplasia		glucagon
chemical toxicity		growth hormone
pancreatic neoplasia		progesterone/agens
pancreatectomy		glucocorticoids
senile degeneration		catecholamines
		autoimmunity?

Table 2. Factors which cause or exacerbate diabetes mellitus in dogs

Diagnosis

Diagnosis of canine diabetes mellitus is based on the documentation of a persistent fasting hyperglycaemia (blood glucose > 12-14mmol/l) with glucosuria.

Management

The management of canine diabetes mellitus is based on insulin replacement therapy and dietary manipulation (Chastain and Ganjam, 1986b; Feldman and Nelson, 1987). Only rarely can diabetic dogs be managed without insulin replacement. In these instances, oral hypoglycaemic or antihyperglycaemic agents (sulphonylureas and biguanides) can be used to control hyperglycaemia. These agents depend on the presence of functional β-cells for their action (Hermann and Melander, 1992; Lebovitz and Melander, 1992). They are therefore unlikely to be useful where there is little or no reserve insulin production capacity. In the long-term, sulphonylureas may even reduce insulin production capacity (Lebovitz and Melander, 1992). Insulin replacement therapy is by once or twice daily injection of intermediate or long duration insulin preparations (see Chapter 6). Dietary management is by provision of consistency of composition, quantity and times of meals from day to day. In addition, modifying the composition of the diet can improve the degree of glycaemic control (see Chapter 7). Generally, a period of

'stabilisation' is required during which an ideal treatment regime is sought and this is followed by a maintenance period. Therapeutic success is monitored during the stabilisation period by measurement of blood glucose concentrations and in the maintenance period by measurement of plasma concentrations of 'hepatic' enzymes in addition to nadir blood glucose concentrations.

Concurrent illness

When diabetes mellitus coexists with other disease, insulin requirements are frequently increased because of insulin insensitivity. Bacteraemia and uraemia in man and experimental animals are known to promote insulin insensitivity via an increase in glucagon levels such that sick and dehydrated diabetics are at greater risk of becoming unstable on treatment and of becoming ketoacidotic (Ihle and Nelson, 1991). Similarly dogs with concurrent hyperadrenocorticism (Peterson *et al*, 1981), acromegaly or phaeochromocytoma (Ihle and Nelson, 1991) and entire bitches in metoestrus (Eigenmann, 1981) can have marked insulin insensitivity because of hormonal insulin antagonism. Recently, hypothyroidism has been associated with insulin insensitivity but the underlying mechanisms are less clear (Ford *et al*, 1993).

Complications of diabetes mellitus

Longstanding canine diabetes mellitus, is associated with relatively few complications. The most common of these is the formation of ocular cataracts and loss of vision. Cataracts are believed to form because of the osmotic effects of intralenticular polyols which result from high blood sugar concentrations and increased activity of aldose reductase in the sorbitol pathway (Wyman *et al*, 1988). Terminal hepatic failure and cirrhosis are also possible sequelae to diabetes mellitus which arise as a result of chronic hepatic fatty infiltration common in poorly controlled diabetics (Nelson, 1989a). In human diabetic medicine, microvascular and neuropathic complications are of great concern (Borch-Johnsen and Deckert, 1992) and although neuropathy has been recorded in canine diabetes mellitus (Katherman and Braund, 1983; Anderson *et al*, 1986; Misselbrook, 1987; Haining, 1993), microvascular complications including retinopathy and nephropathy are rare (Nelson, 1989). Macrovascular complications, principally atherosclerosis, common in diabetic humans (Borch-Johnsen and Deckert, 1992; Uusitupa *et al*, 1992) are reported only rarely in canine diabetics (Hargis *et al*, 1981). Pancreatitis can occur in dogs as a consequence as well as a cause of diabetes mellitus and in man an increased risk of pancreatitis has been associated with elevated concentrations of circulating triglycerides (European Atherosclerosis Society, 1988) such as those common in poorly controlled diabetics.

Study objectives

The ultimate aim of the work presented in this thesis is to improve the welfare of dogs with spontaneously occurring diabetes mellitus. Principally the potential for improvement in canine diabetic management has been explored through studies on specific dietary and insulin preparations and through the appraisal of the most useful tools for long-term monitoring. In addition, epidemiological techniques have been applied to a large group of diabetic dogs to identify any changes in the nature or pattern of the disease and to generate useful indicators of therapeutic success and longevity for use in future studies. This study has required a multidisciplinary approach including the application of clinical veterinary medicine, endocrinology, pharmacokinetics, nutrition, gastroenterology and numerous facets of biochemistry, medical computing and biostatistics but it has permitted the cultivation of a more comprehensive understanding of canine diabetes mellitus and provided the opportunity to develop many skills applicable to the study of animal disease.

Chapter 2:
General materials and methods

Additional specific materials & methods are presented within each relevant chapter

Animals

All dogs described within this thesis were referred to the Department of Veterinary Medicine, University of Glasgow by veterinary surgeons in general practice (general veterinary practitioners) between 1989 and 1994. Most referrals came from West Central Scotland but there were some from as far as the Western Isles, Cumbria and Co Durham. The reasons for referral were varied. Most were referred immediately following diagnosis for initial diabetic stabilisation; some for futher investigation of their apparently complicated condition and some before a diagnosis had been made.

Clinical management

Stabilisation

Following referral, untreated and previously treated but unstable cases underwent a stabilisation period for which they were hospitalised. During this period a suitable insulin dose was determined. Prior to stabilisation the diagnostic work up included a thorough clinical examination and a full routine plasma biochemical and haematological analysis. Other diagnostic procedures such as radiography, urinalysis and dynamic endocrine testing were only performed when there was a strong clinical indication.

Non-ketotic dogs were immediately begun on a single daily insulin injection and twice daily feeding stabilisation regimen following laboratory confirmation of diagnosis. The insulin preparations used in this regimen were of the intermediate duration type and were either isophane (Evan's Isophane, Hypurin Isophane) or mixed insulin zinc suspension (lente) (Insuvet Lente, Hypurin Lente, Lentard MC, Caninsulin). Daily insulin injections were administered with 1/3 to 1/2 of the dogs' daily dietary ration and the second portion of the ration was offered at 6 hours post-injection. Diets consisted of commercial canned dog foods (Chappie or Pedigree Chum; Pedigree Petfoods) fed alone or in

combination with bread or commercial mixer biscuit (Mixer, Pedigree Petfoods; Winalot, Spillers). The ration was fed at a rate of 40-80kcal/kg based on a subjective assessment of body size and condition.

The initial dose of insulin administered to an untreated case was between 0.5 and 1.0 IU/kg and subsequent adjustments to dose were made on a daily basis according to the plasma glucose concentration in a blood sample taken just before the second meal on the previous afternoon. It was assumed that the plasma concentration of glucose just before the second meal was likely to be the lowest concentration (nadir) in the day. Adjustments to insulin dose were made until nadir concentrations of plasma glucose were consistently in the range 3.5-7.5 mmol/l. The size of increments or decrements in insulin dose from day to day depended both on the size of the dog and on the concentration of plasma glucose the previous afternoon such that very small dogs may have had their doses adjusted by only 1-2 IU/day but in very large dogs this adjustment may have been 4-6 IU/day. Afternoon plasma glucose concentrations >20 mmol/l would have meant larger increases in dose than plasma glucose concentrations between 7 and 10 mmol/l. Urine samples for glucose estimation were not taken routinely.

Once diabetic stability had been acheived, ovariohysterectomy was performed on entire bitches subject to owner consent. However, bitches which had metoestrus-associated diabetes were, if possible, spayed after only a brief period of insulin replacement therapy and before stability had been achieved.

Ketoacidotic dogs which had reasonable appetite and were not vomiting were treated in a similar way to non-ketotic dogs but they were treated much more intensely if their appetite began to wane. The treatment protocol for those ketoacidotic dogs which were anorexic or vomiting included the administration of intravenous fluid therapy supplemented with potassium chloride and soluble insulin therapy. Initially the intravenous fluid would have been physiological saline but this would have been replaced by 4% dextrose saline once blood glucose began to fall. Potassium supplementation was by the addition of approximately 10 mmol potassium chloride to each litre of intravenous fluids and soluble insulin therapy was administered at a rate equivalent to 0.1 IU/kg/hr either by continuous infusion or regular bolus. When there was difficulty in providing overnight care, a low dose of approximately 0.25 IU/kg isophane insulin was administered subcutaneously last thing at night to provide insulin until the morning. Once

appetite returned and vomiting ceased, the standard stabilistation protocol outlined for non-ketotic dogs was followed.

Maintenance

Once stabilisation had been achieved, dogs were discharged from the hospital to be managed by their owners at home following a prolonged consultation during which the owners were instructed in the administration of insulin and the recognition and importance of insulin-induced hypoglycaemia.

Owners were requested to return with their dogs at one and three weeks after they were discharged from the hospital. Thereafter, a one to two month interval was recommended between follow-up visits. At each visit, blood samples were taken for routine plasma biochemical and haematological analyses (outlined below) and a general clinical examination was performed. A brief distant direct ophthalmological examination was carried out on most of these occasions to record the development of cataracts and a 'glycaemic control score' (1 to 7; see chapter 5) was allocated to each dog on each visit based on the owner's description of their dog's demeanour, clinical condition and an afternoon plasma glucose concentration. A body condition score (1 to 6) was also allocated at each visit.

Computing

Hospital system

The hospital computing system operated by the Departments of Veterinary Medicine and Pathology at University of Glasgow was used to record animal details and the results of biochemical analyses of all the diabetic dogs in this study and to provide details of a control group of dogs referred to the hospital over the period of the study. This hospital system uses multiuser, relational database software (Dataflex, Data Access Corporation, Miami, USA) and a CP/M operating system which runs on a Jarogate Sprite 386 (Jarogate, UK) as the central or host processer. There are terminals for data entry situated within the case reception office and the results of biochemical analyses are imported from a separate IBM-PC compatible processer which is linked to an automated analyser (Cobas Mira, Roche) and is situated within the Department of Veterinary Medicine Clinical Biochemistry Laboratory. In addition, network connections exist with a processor within the Department of Veterinary Pathology which allows access to files concerning necropsy, biopsy and bacteriological examinations performed within that

department. Data generated by interrogation of the hospital computer system can be transferred from the Jarogate Sprite in the form of a textfile via a 5¼ inch floppy disk drive.

The animal file is the central file to many others but it contains only limited information on each animal. Only hospital number, animal name, gender, date of birth and date first seen are directly recorded in this file but it contains numeric references to other files which contain data relating to species, breed, owner, staff member and referring veterinary surgeon. In addition there are 'counter' fields within the animal file which record the number of biochemistry, bacteriology, biopsy, necropsy (maximum one) and clinical summary reports generated for each animal. Figure 1 summarises the relationship of files within the hospital system and Tables 3 and 4 list the fields in the animal and main biochemistry files.

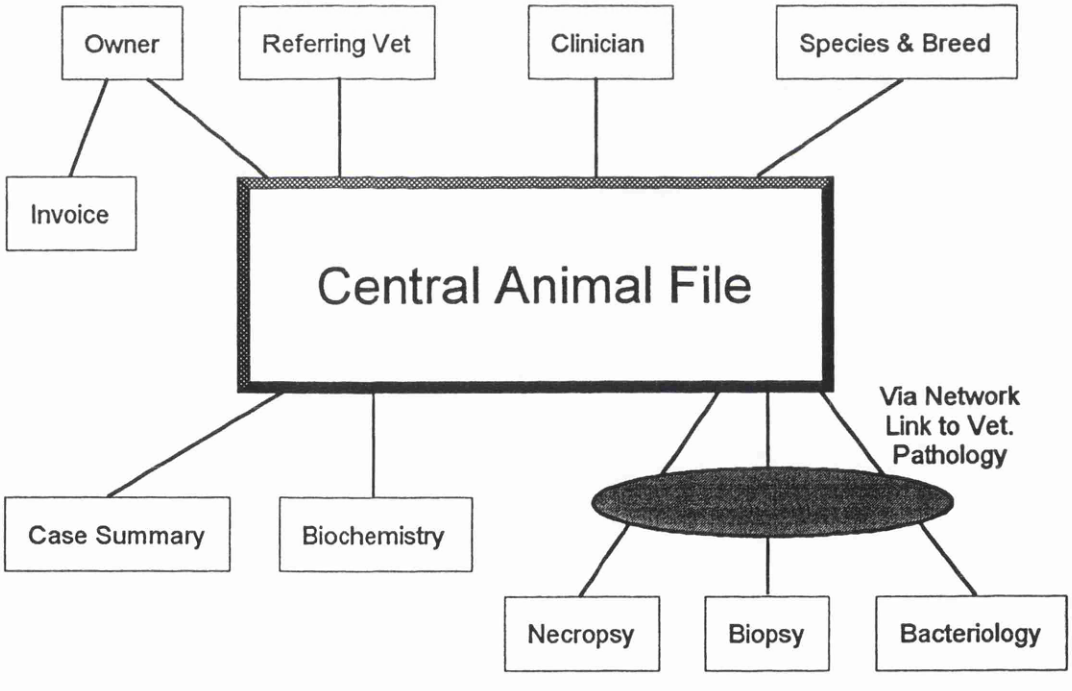

Figure 1. Relationships of files which form the clinical database within the Departments of Veterinary Medicine and Pathology, University of Glasgow.

Smart II system

To enable the further manipulation of the data relating to diabetic dogs and the addition of data not recorded on the hospital system a separate set of database files were created

on an IBM-PC compatible computer. Database files were designed using a commercial software package (Smart II; Informix Software, Lenexa, Kansas, USA). Four main files were created and these contained animal details (ANIMAL), biochemistry results (BIOCHEM), glycated haemoglobin results (GLYTEK) and fructosamine results (FRUCT). Each diabetic animal had only one ANIMAL record but had many records in the BIOCHEM, GLYTEK and FRUCT data files. Records in the ANIMAL file and the other three files were related by unique hospital numbers and records in the BIOCHEM, GLYTEK and FRUCT data files were related using an 'OLAP' field which contained a number generated from a combination of the animal's case number and the date of the sample. In this way biochemistry data could be matched up with glycated haemoglobin and fructosamine data for any particular animal on any particular day. The BIOCHEM file contained additional information obtained at follow up examinations such as body weight, insulin dose and clinical comments. A numeric scoring system for glycaemic control and the progression of cataracts was employed and fields to contain this information were included in the BIOCHEM file. The structure and relationships of the Smart II data files are represented in Table 5.

Data manipulation and statistics

Data manipulation and presentation was facilitated by use of a number of computer software packages including: Word for Windows 2.0 (Microsoft Corporation, USA), Excel 4.0 (Microsoft Corporation, USA), Lotus 123 for Windows (Lotus Development Corporation, Cambridge, MA, USA) and Harvard Graphics for Windows 2.0 (Software Publishing Corporation, Santa Clara, CA, USA). Statistical analyses were performed using MINITAB release 8, MINITAB for Windows release 9 (Minitab Incorporated, State College, PA, USA), EpiInfo 5.01b (Centers for Disease Control and World Health Organisation), Stata release 3.1 (Stata Corporation, College Station, Texas) and Excel 4.0 (Microsoft Corporation, USA). Statistical methods were based on those descibed in the *MINITAB Handbook* (Ryan *et al*, 1985), *MINITAB Software Reference Manuals* (Minitab Incorporated, State College, PA, USA), *Principles of Biostatistics* (Pagano and Gauvreau, 1992) and *Statistical Methods for Survival Data Analysis* (Lee, 1992) and those taught in the short course *Statistics for Bioscientists*, University of Strathclyde.

Clinical biochemistry

Clinical biochemistry analyses were performed in the Department of Veterinary Medicine Clinical Biochemistry Laboratory with the exception of glycated haemoglobin and

glycerol measurements, which were performed in the Department of Veterinary Medicine Research Laboratory. The following analytes were measured in what will be referred to as a routine biochemical profile: urea, sodium, potassium, chloride, phosphate, calcium, bilirubin, cholesterol, glucose, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin and globulin. Table 6 lists the common clinical chemistry methodologies used. Additional methodologies which were used as part of specific studies are described within relevant chapters.

Plasma and blood glucose analyses were also carried out at times when laboratory facilities were not available using either the Glucometer and Glucostix System (Ames) or the Vettest analyser (IDDEX Laboratories). Some clinical chemistry analyses relevant to this thesis were performed outwith the University of Glasgow. These included plasma determinations of trypsin-like immunoreactivity, folate and cobalamin which were performed in the Department of Veterinary Pathology, University of Liverpool, some insulin and some thyroxine estimations which were performed at Serono Clinical Laboratories, Cambridge and a growth hormone analysis performed in the Faculty of Veterinary Medicine, Utrecht University.

Clinical haematology

A routine haematological profile was generally generated on the same occasions as routine biochemical profiles and included a white blood cell count (WBCC), red blood cell count (RBCC), mean red cell volume (MCV), haematocrit, mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count and differential white cell count. This was performed in the Department of Veterinary Pathology, University of Glasgow using an ABX Minos Vet automated cell counter (Roche).

Field	Referenced File	Comment
HOSP_NO		
NAME		
OLAP1		used for indexing purposes
SPECIES	Species & Breed	species code
BREED1	Species & Breed	e.g. retriever, terrier, etc
BREED2	Species & Breed	e.g. golden, cairn, etc
DOB		date of birth
SEX		
ALIVE		not used
SAMPLE_ONLY		not used
VET_REF	Referring Vet	
STAFF1_REF	Clinician	
DATE_FIRST_SEEN		
DATE_LAST_SEEN		
VISITS		number of hospital visits
SPARES		not used
LAST_SIGN		not used
LAST_HAEM		not used
LAST_BIOP		
LAST_NECRO		
LAST_BIOCH		
LAST_DIAG		not used
LAST_SUMMARY		
LAST_BACT		

Table 3. File structure of the Animal file on the Jarogate hospital computer system

Field	Comment
OLAP1	REF1 and REF2 combined
REF1	hospital number
REF2	number of records for this animal
LAB_NO	laboratory number
DATE_COLL	date collected
DATE_RECV	date received
DATE_REPORT	date analysed
SAMPLE	sample type (plasma/urine/CSF etc)
HOSP_NO	hospital number
DAYS	age
WEEKS	age
MONTHS	age
YEARS	age
ALIVE	not used
BIOCHEMIST	not used
COST	cost of analysis
CHARGE	not used
STATUS	not used
UREA	
SODIUM	
POTAS	potassium
CHLOR	chloride
CAL	calcium
MAGNES	magnesium
PHOS	phosphate
SUGAR	glucose
CHOL	cholesterol
CREAT	creatinine
BIL	bilirubin
S_ALK_PHOS	alkaline phosphatase
AST	aspartate aminotransferase
ALT	alanine aminotransferase
TOT_PROT	total protein
ALB	albumin
GLOB	globulin
CPK	creatine phosphokinase
GGT	gamma-glutamyl transferase
LDH	lactate dehydrogenase
AMY	amylase
COMMENT	

Table 4. File structure of the 'Biochem' file on the Jarogate hospital computer system

ANIMAL	BIOCHEM	GLYTEK	FRUCT
Hospital number	Hospital number	Hospital number	Hospital number
Owner name	Date collected	Date collected	Date collected
Animal name	OLAP	OLAP	OLAP
Species	Urea	Species	Species
Date of Birth	Sodium	Date assay	Date assay
Original gender	Potassium	Optical density 1	Fructosamine
Present gender	Chloride	Optical density 2	Diagnosis
Date neutered	Calcium	Glycated HB (%)	
Breed 1	Phosphate	Diagnosis	
Breed 2	Glucose		
Alive/Dead	Cholesterol		
Date first diagnosed	Creatinine		
Date first presented	Bilirubin		
Date died	Alkaline phosphatase		
Weeks of polydipsia	AST		
Other disease 1	ALT		
Date diagnosed 1	Total protein		
Other disease 2	Albumin		
Date diagnosed 2	Globulin		
Other disease 3	GGT		
Date diagnosed 3	Amylase		
Current insulin type	Triglyceride		
Current insulin since	Plasma insulin		
Injection frequency	Cortisol		
Previous insulin type	Cortisol post-ACTH1		
Previous insulin since	Cortisol post-ACTH2		
Meal/Injection time 1	Thyroxin		
Meal 1 content	Thyroxin post TSH		
Meal/Injection time 2	Fasting/post prandial		
Meal 2 content	Glycaemic control score		
Meal/Injection time 3	Clinical comment		
Meal 3 content	Insulin dose (IU)		
Meal/Injection time 4	Bodyweight		
Meal 4 content	Obesity score		
	Cataract score (right)		
	Cataract score (left)		

Table 5. Lists of fields in Smart II database files ANIMAL, BIOCHEM, GLYTEK and FRUCT. Double bordered boxes signify reference fields between data files.

Analyte	Reagent/Kit	Manufacturer	Principle	Instrumentation
Urea	UREA	Roche	urease and glutamate dehydrogenase	Roche Cobas Mira
Sodium		Instrumentation Laboratories	flame photometry	Flame Photometer 543
Potassium		Instrumentation Laboratories	flame photometry	Flame Photometer 543
Chloride		Corning	coulometric analysis	Choride Analyser 925
Calcium		Instrumentation Laboratories	atomic absorption	Atomic absorption spectrophotometer 257
Cholesterol	CHOL	Roche	cholesterol esterase, cholesterol oxidase and peroxidase	Roche Cobas Mira
Glucose	GLUC-PAP	Roche	glucose oxidase and peroxidase	Roche Cobas Mira
Glucose	GLUCOSE	Roche	glucose hexokinase and glucose-6-phosphate dehydrogenase	Roche Cobas Mira
Creatinine	Creatinine Reagent	Bayer (Technicon Series)	Jaffe method, Picric acid and sodium hydroxide	Roche Cobas Mira
Inorganic phosphorus	PHOS	Roche	ammonium molybdate to phosphomolybdate	Roche Cobas Mira
Bilirubin	Bilirubin Test	Roche	Caffeine accelerator (Jendrassik/Grof reaction, 1938)	Roche Cobas Mira
Alkaline phosphatase	ALP-DGKC	Roche	4-nitrophenyl phosphate substrate in diethanolamine buffer	Roche Cobas Mira
Aspartate aminotransferase	AST	Roche	L-aspartate, 2-oxoglutarate, malate and lactate dehydrogenase (IFCC)	Roche Cobas Mira
Alanine aminotransferase	ALT	Roche	L-alanine, 2-oxoglutarate, lactate dehydrogenase (IFCC) (pyridoxal phosphate activated)	Roche Cobas Mira
Total protein	TP	Roche	biuret method	Roche Cobas Mira
Albumin		On-site	bromocresol green binding in succinate buffer	Roche Cobas Mira
Globulin			Arithmetic subtraction (total protein - albumin)	
Gamma-glutamyl transferase	GGT plus	Roche	carboxy-GLUPA substrate	Roche Cobas Mira

Table 6. Reagents, principles and instrumentation used in the commonly measured clinical chemistry analytes

Analyte	Reagent/Kit	Manufacturer	Principle	Instrumentation
Triglycerides	TRIG	Roche	lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase	Roche Cobas Mira
Non-esterified fatty acids	NEFA-C (ACS-ACOD)	Wako distributed through Alpha Laboratories	acyl-CoA synthetase, acyl-CoA oxidase, peroxidase, 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline and 4-aminoantipyrine	Roche Cobas Mira
Glycerol	Glycerol	Randox laboratories	quinoneimine chromogen system utilising glycerol kinase, glycerol phosphate oxidase and peroxidase	Manual and Beckman DU65 Spectrophotometer
Amylase	AMYL	Boehringer Mannheim	*p*-nitrophenyl-α,D-malto-heptaoside substrate with α-glucosidase	Roche Cobas Mira
Lipase	LIPASE	IDDEX laboratories		Vettest analyser
Ammonia	NH4+	IDDEX laboratories		Vettest analyser
Fructosamine	FRUC	Roche	nitroblue tetrazolium	Roche Cobas Mira
Glycated haemoglobin	GLYCO-Tek Affinity Columns	Helena Laboratories	affinity chromatography using dihyroxyboryl column and magnesium chloride and sorbitol eluents	Manual & Beckman DU65 Spectrophotometer
Cortisol	Magic Cortisol	Ciba Corning	radioimmunoassay using magnetic separation and ^{131}I tracer	Canberra Packard Cobra II gamma counter
Insulin	Coat-a Count Insulin	Diagnostic Products Corporation	solid phase radio-immunoassay with ^{131}I tracer	Canberra Packard Cobra II gamma counter
Thyroxin	Magic T4	Ciba Corning	radioimmunoassay using magnetic separation, 8-anilino-1-naphthalene-sulfonic acid and ^{131}I tracer.	Canberra Packard Cobra II gamma counter

Table 6 (cont). Reagents, principles and instrumentation used in the commonly measured clinical chemistry analytes.

Chapter 3:
The epidemiology of canine diabetes mellitus

The accurate description of the pattern of a disease within a population is essential to the understanding of that disease. Principally, such a description can help identify factors which may be associated with predisposition to or initiation of the disease under study and in special circumstances may result in the identification of a single 'cause'. Knowledge of predisposing factors for a given disease also allows for the identification of 'high-risk' groups which can be selected for further, more detailed, investigation of the disease and which may act as 'models' for the disease in a different species.

The epidemiological features of canine diabetes mellitus which have been previously reported are based on a number of large case series. In the United Kingdom, the largest of such reports are by Wilkinson (1960) who reported on 56 cases from The Royal Veterinary College London and Lauder (1972) who reported 97 cases from the University of Glasgow. Neither of these reports made use of control populations for comparison with the diabetic population. Foster (1975) reported on case details from 103 diabetic dogs collected by questionnaire survey of 47 veterinary practices in Kent and generated a non-diabetic 'control' population from cases seen at a single veterinary practice. Foster was able to demonstrate differences between diabetic and control populations but did not apply statistical methods to determine their significance. Only one British report of 43 diabetic dogs from the Royal (Dick) School of Veterinary Studies, Edinburgh (Doxey *et al*, 1985) has used statistical methods for comparison with a referral hospital population. There have also been large case series published from abroad; Marmor *et al* (1982) reported on 1,468 cases from 14 universities in United States of America and Canada, Ling *et al* (1977) reported on 75 cases from California and Krook *et al* (1960) reported on 167 cases in a post-mortem survey in Sweden. These reports generally agree that elderly, entire bitches are most likely to be affected and each report provides a list of 'high-risk' breeds.

The epidemiology of canine diabetes mellitus in the West of Scotland was last reported more than 20 years ago (Lauder, 1972) and it would be incorrect to assume that the pattern of canine diabetes mellitus has remained the same or that it is the same elsewhere in the world. Indeed, medical journals dedicated to the subject of diabetes mellitus

frequently contain papers indicating that the epidemiological patterns of human diabetes mellitus in many different countries and localities throughout the world are not uniform or constant.

The aim of this part of the study was to determine whether any of breed, age or gender were important factors predisposing to the development of canine diabetes mellitus based on a group of 89 diabetic dogs referred to the University of Glasgow and whether any predisposing factors identified agreed with those reported previously for the condition. To facilitate this aim it was necessary to generate a time-matched control population from non-diabetic dogs referred to the University of Glasgow with which comparisons could be made. There were, therefore, two distinct components to this investigation. Firstly, because the control population was derived from a referred hospital population and not from a normal or healthy population it was necessary to characterise its structure in terms of age, gender and breed and to assess its suitability as a control population from which to draw accurate conclusions about the structure of the diabetic population. The second part of the study was to identify differences between the structure of the control and diabetic populations so that predisposing factors for canine diabetes mellitus could be determined.

The control population

Introduction

It is most unlikely that the structure of the population of dogs referred to a specialist centre such as the University of Glasgow Veterinary School will be exactly the same as the general or healthy dog population, however that may be defined, and therefore a control population drawn from such a hospital population might not be suitable for the purposes of investigating the epidemiology of a particular disease. In order to assess the suitability of a hospital derived control population for epidemiological purposes it is necessary to consider the individual factors which determine its structure.

There are three broad levels or categories of factors which determine the structure of a canine referred hospital population in Western society:

- **The source population.** The structure of the overall local canine population will be determined by the sociology, psychology and economics of the local society which will affect the size, breeds and life expectancy of dogs within that locale. In addition, the local genetic pool will affect the nature of the cross-bred component of the population.

- **The reasons for referral.** The rationale behind the decision made by general veterinary practitioners and owners to refer a particular animal is similarly heavily based on local psychology and socio-economics. For example, it is a commonly suggested but unsupported comment that young pedigree dogs are more likely to be referred to a specialist centre than old or cross bred dogs.

- **The referral clinician and disease effects.** The specific disease interests of clinicians working within a referral centre will influence the kinds of dogs which they see because many canine diseases are age, gender and breed related. If the number of referrals seen within any particular clinical speciality is large this will ultimately affect the overall referral population.

In attempting to determine the validity of a hospital derived control population for epidemiological purposes, the contribution of each of the three points above should be considered. It could be suggested that in order to be valid, a control population must accurately reflect the local general canine population. Such a requirement would not only be difficult to test but also might be irrelevant. If conclusions about a disease based on a control population are to be comparable with those generated at other referral centres, then the use of a referral population instead of a local general population as the control would be more correct.

The likelihood of referral based on 'the reasons for referral' should be similar for the disease under study and the remainder of the hospital population, so that using a referral control population rather than a source control population will provide a more relevant comparison. In terms of 'reasons for referral', animals within the disease and control groups will have experienced a similar chance of selection to be part of the hospital population.

Consequently, for the valid comparison of epidemiological data among referral centres, it is less important that a hospital control population is an accurate reflection of the local general population and more important that it reflects its source population to the same degree as other referral centres reflect theirs. Again such a requirement is difficult to test without a large scale multi-centre census study. Ideally, the factors affecting 'the reasons for referral' would be uniform between referral centres but it would be naive to believe that they were. However, differences in factors affecting 'the reasons for referral' between localities are more likely to result in a difference in the total number of referrals (referrals per capita) than in the degree to which the structure of the referral population reflects that of its source population, since the relative likelihood of referral for a young,

pedigree or valuable dog compared with an old, cross-bred or inexpensive one is likely to be roughly uniform between centres in Western society.

In consideration of 'clinician and disease effects' it is possible that these may cause considerable bias in the structure of the hospital population and alter the degree to which it reflects its source population. One way to account for such bias would be to make repeat epidemiological analyses of a disease group following exclusion of individual referral groups from the control population which are known to heavily bias its structure. In that way a more rounded appreciation of the factors important in the disease under study can be developed free from the bias of particular referral groups or clinicians and which is more likely to be based on a population determined by the factors which influence 'the source population' and 'the reasons for referral' above.

It was accepted that in developing a control population at the University of Glasgow for the present study it would not be possible to test how closely the hospital population reflected the source population or whether it reflected its source to the same degree as other referral centres. However, it was possible to investigate 'the clinician and disease effects'. This was done with the aim of determining if the control population was uniform across referral groups or whether referral groups were sources of bias in the control population which would have to be accounted for when using the control population for comparison with the diabetic population. Dogs were grouped according to likely reasons for referral and each group was investigated for differences in age, breed and gender distribution from the remainder of the control group

There were two possible approaches to investigating potential bias from referral groups. The one used was a comparison of each referral group with the remainder of the referral groups. There were disadvantages to this approach. Mainly, any referral groups which caused bias would be present in the 'control' group when another referral group was being tested and potentially disrupt the conclusions drawn about the group under study. However, if there were sufficient groups the pooling approach should have diluted any such disruption. An alternative approach would have been to compare each referral group to a 'benchmark' group. This method would not have suffered the disadvantages of the former but the question as to the most suitable group to be the 'benchmark' could not be resolved.

Methods

Dogs

All diabetic dogs referred to the hospital between January 1989 and April 1994 for which there was a complete set of information regarding date of birth, breed, gender and date of first referral were used as the study group (n=89)(Appendix 2). A time-matched control population was generated by selecting the subsequent 10 dogs which presented to the hospital for the first time following each diabetic dog. Dogs with incomplete records were excluded and these were replaced by the first dog after the group of 10 which did have a complete record. This generated a time matched control population of 890 dogs which represented approximately 10% (890 of 8831) of all non-diabetic dogs referred to the hospital for the first time between January 1989 and April 1994. The dogs in the control population were allocated a most likely reason for referral based on the veterinary surgeon to whom the dog had been referred. The categories were General Medicine, General Surgery, Orthopaedics, Ophthalmology, Neurology and Reproduction. A summary of the age, breed and gender distribution of dogs among referral groups is given in Appendices 3 and 4.

Statistics

Chi-square tables (r x c contingency tables) were used to determine differences in age, gender and breed distributions between each referral group and the remainder of the control population. Where there was a statistically significant higher or lower proportion of a particular age, gender or breed category within a test group compared to that expected based on a control group, that category was referred to as being over- or under-represented as appropriate. Gender was categorised as entire female, neutered female, entire male and neutered male and 4 x 2 contingency tables used. Age was divided into biennial categories (0-2, 2-4, 4-6 .....14-16 years) and an 8 x 2 table used. To test for differences in breed distribution the 10 most commonly represented breeds within each referral group were tested for over-representation when compared to the remainder of the control population. This method was used to give an impression of the differences of breed distributions between the referral groups without exhaustive testing of all breeds in all groups. It was acknowledged that this approach to estimating breed differences could not provide information on the under-represented breeds.

All calculations were performed using computer software[1]. Differences between distributions were tested for statistical significance using the Chi-square method or the

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[1] Epi Info, Version 5.01b (1991); Centers for Disease Control, Epidemiology Program Office, Atlanta, Georgia and World Health Organisation, Geneva, Switzerland

two-tailed Fisher's exact test when appropriate. Significance levels (p values) of less than 5% were considered statistically significant.

Results

Reasons for referral

The distributions of the referral groups are represented in Figure 1. The majority of non-diabetic canine referrals was for surgical reasons and within the surgical disciplines, general surgery and orthopaedics were the most common. The largest, and therefore most important referral groups were General Medicine in which there were 254 cases representing 29% of the control population; General Surgery which consisted of 253 cases and represented 28.4% and Orthopaedics in which there were 215 cases and which represented 24.1%.

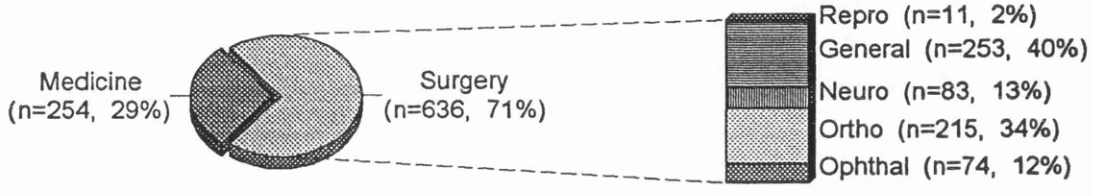

Figure 2. Distribution of hospital control population according to reason for referral.

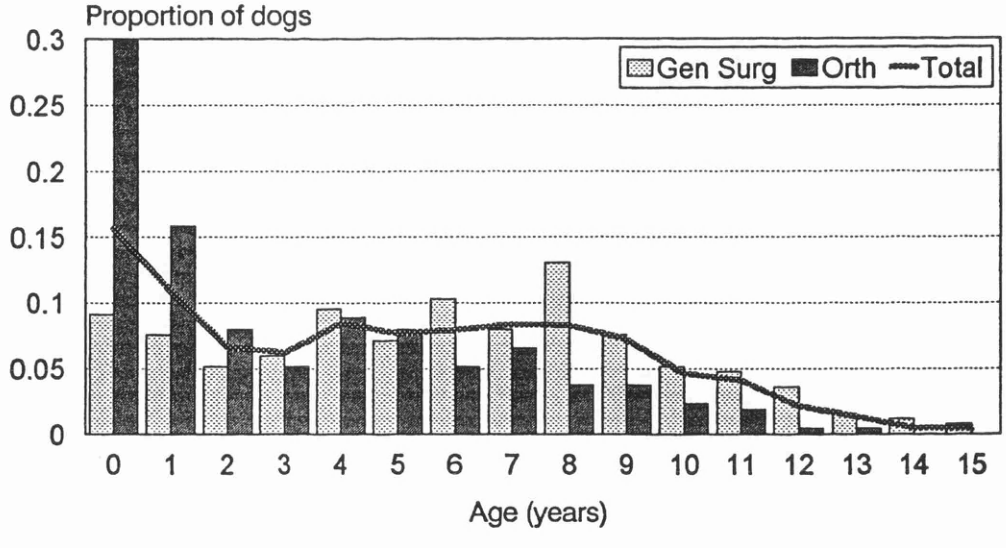

Figure 3. Age distributions of referral groups General Surgery (Gen Surg) and Orthopaedics (Orth) and the age distribution of the entire control population of dogs.

Age distribution

The General Surgery, Orthopaedic and Ophthalmology referral groups had highly significant differences in age distribution from the remainder of the referral groups ($p < 0.005$). In the General Surgery group, there was a high proportion of adult dogs, whereas in the Orthopaedic group there was a high proportion of very young dogs. The Ophthalmology group had an unusually large number of both very young and middle aged dogs. The age distributions of the General Surgery and Orthopaedic groups are represented in Figure 3 along with the age distribution of the total control population.

Gender distribution

Only the Neurology referral group had a significantly different gender distribution from the remainder of the control population ($p = 0.020$).

Breed distribution

It is known that certain breeds are prone to the development of particular diseases. To test whether this was reflected in the breed distribution of the referral groups the 10 most commonly represented breeds within each referral group were tested for over-representation when compared to the remainder of the control population. In the Medicine group, there were high numbers of Golden retrievers, Soft Coated Wheaten terriers and Bull terriers. In General Surgery, there were high numbers of German shepherd dogs and collie cross dogs but unusually few Golden retrievers. Yorkshire terriers were in high numbers in the Neurology group and there were high numbers of Cocker spaniels, Jack Russell terriers, Wiemaraners and Miniature Poodles in the Ophthalmology group. The Orthopaedic group contained significantly higher proportions of Labrador retrievers, Rottweilers and Greyhounds than the remainder of the control population.

Discussion

There were three large referral groups: General Medicine, General Surgery and Orthopaedics. These were the groups most likely to bias the control population. However, because the General Medicine and General Surgery groups were, by definition, very broad clinical categories it is unlikely that these groups contributed greatly to any bias away from a general or normal referral population. Of greater concern was the Orthopaedic referral group, which although large, represented a narrow clinical discipline. There are breed predispositions to orthopaedic conditions, particularly racing injuries, osteochondrosis and hip dysplasia and younger dogs are more likely to suffer developmental skeletal diseases or be involved in traumatic incidents. These observations are supported by the findings of a significantly different age distribution of the

Orthopaedic referral group and that 3 of the 10 most commonly represented breeds within the Orthopaedic referral group were over-represented when compared to the remainder of the control population. The potential for bias in the age and breed distributions of the control population is increased by the inclusion of the Orthopaedic referral group. However, the age distribution of the General Surgery referral group goes some way to compensate for the bias in the control population age distribution arising from the Orthopaedic group.

The gender distribution of the Neurology referral group was different from the remainder of the control population but because of the low numbers of dogs in this group it was unlikely that the gender distribution of the total control group was biased by the inclusion of this referral group.

In conclusion, the structure of a time-matched control population generated for analysis of the epidemiology of a group of diabetic dogs was affected by the specialist interests of hospital clinicians and the age, gender and breed predilection of the diseases that they treat. This was particularly because of the large proportion of dogs referred to the University of Glasgow for orthopaedic reasons. It was therefore reasonable to investigate the group of diabetic dogs for predisposing factors using both the total time-matched control population and also control populations from which individual referral groups which are sources of bias have been removed.

The diabetic population

Methods

All 89 diabetic dogs were included in the epidemiology study and diabetes mellitus was treated as a single disease. Special consideration is given to diabetes mellitus in association with other syndromes in Chapter 4.

Statistics

Statistical testing for association between the presence of a factor (e.g. being female) and the presence of the disease was performed using the chi-square test. Crude odds ratios (OR's) were calculated to measure the strength of any association (Cornfield, 1951 cited by Lee, 1992) and 95% confidence intervals (CL's) were calculated (Lee, 1992). Fisher's exact test was used where numbers or expected numbers in one or more cells were less than 5 in which case 95% exact confidence limits were calculated (Mehta *et al* 1985). When testing for the effect of gender, the data were stratified into 2 year age groups (0-2, 2-4, 4-6 .....14-16 years) and a Mantel-Haenzel weighted odds ratio calculated if odds ratios across all 8 tables were uniform (Pagano and Gauvreau, 1992). A two sample *t*-

test was used to test for differences in age between diabetic and control groups and an odds ratio 'profile' was generated for the diabetics according to age using the 8-10 year age group as the reference group. This was repeated after dividing the diabetic dogs according to whether or not their breed was considered to be at 'high-risk'. The effect of breed was tested using a 2 x 2 Chi-squared table whereby the proportion of a particular breed within the diabetic group was compared with the proportion of that breed within the control population. Those breeds which were over-represented in the diabetic population were classified as 'high-risk' breeds.

An alternative method to assess the effect of breed would have been to make comparisons for individual breeds with the risk of being diabetic associated with a particular 'benchmark' breed e.g. Labrador retrievers. This method was rejected, despite some of its advantages, because it would prevent simple comparison with breed dispositions at other referral centres (particularly on an international level) and because of the difficulties in selecting a suitable breed as the 'benchmark'.

Results

Age distribution

Diabetic dogs were older than the dogs in the time-matched control population ($p=0.0000$). The mean age of the diabetic dogs was 8.7 years (SD 2.72) and that of the control population was 5.17 years (SD 3.65). Following exclusion of the Orthopaedic referral group from the control population because of its bias towards young dogs, diabetic dogs were older than the new control population ($p=0.0000$). The mean age of the control population without the Orthopaedic group was 5.72 years (SD 3.63). The age distributions of diabetic and control populations are represented in Figure 4.

Gender

Odds ratios for the presence of diabetes mellitus by gender stratified for age are represented in Figure 5 for male versus female and in Figure 6 for entire female versus neutered female. There was a statistically significant odds ratio of 3.47 ($p = 0.010$, 95% CLs $1.19<OR<10.38$) in favour of females when compared to males within the 10-12 years age group. The odds ratios for the other age groups were not statistically significant. A test for homogeneity of odds ratios across 8 tables for female versus male indicated that they were uniform enough for a summary odds ratio to be valid. The summary odds ratio gave a close to statistically significant value ($p = 0.075$) of 1.54 (95% CLs $0.96<OR<2.55$). This can be interpreted as females being at 1.5 times greater risk of developing canine diabetes mellitus than males. Closer inspection of the age stratified tables suggests that the association between gender and diabetes mellitus was

age dependent. Indeed, if the diabetic population is divided into those under 7 years of age and those over, there was an odds ratio in the under 7 years group of 0.41 ($p = 0.09$) which if it was statistically significant would indicate that males were at 2.5 times greater risk than females. In the over 7 years group, females were at greater risk ($p=0.002$, OR = 2.25, 95% CLs $1.28<OR<3.96$).

There was no overall effect of neutering within the female group. The summary odds ratio across 8 contingency tables was not statistically significant. However, within the 8-10 year age group entire females were at a much greater risk of developing diabetes mellitus than the neutered females ($p = 0.032$, Odds ratio = 3.60, 95% CLs $0.93 < OR < 15.24$).

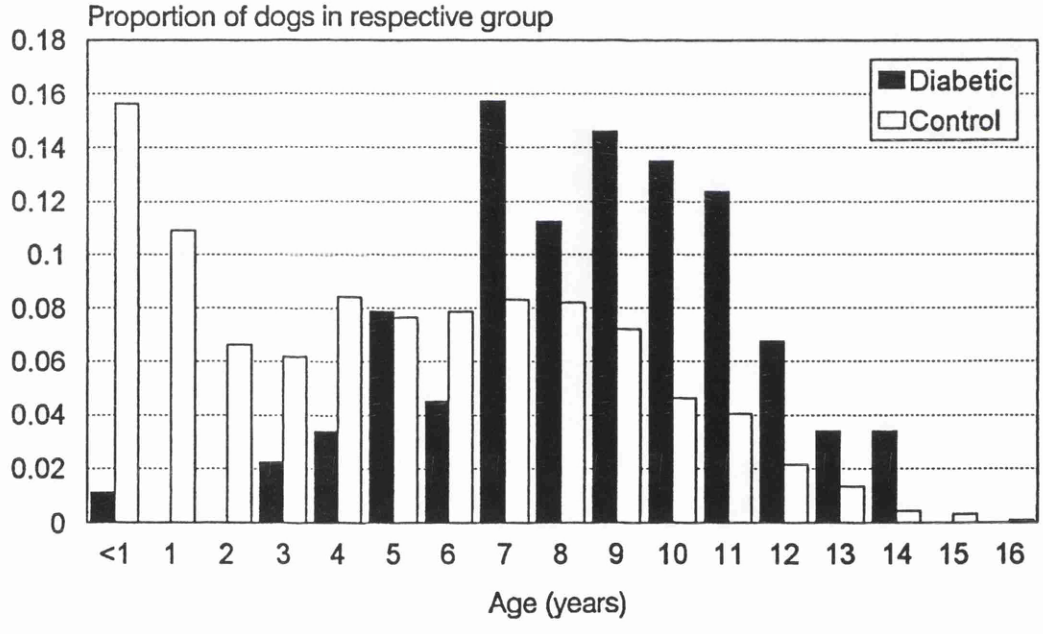

Figure 4. The age distributions of diabetic and time matched control populations (diabetic n=89, control n=890).

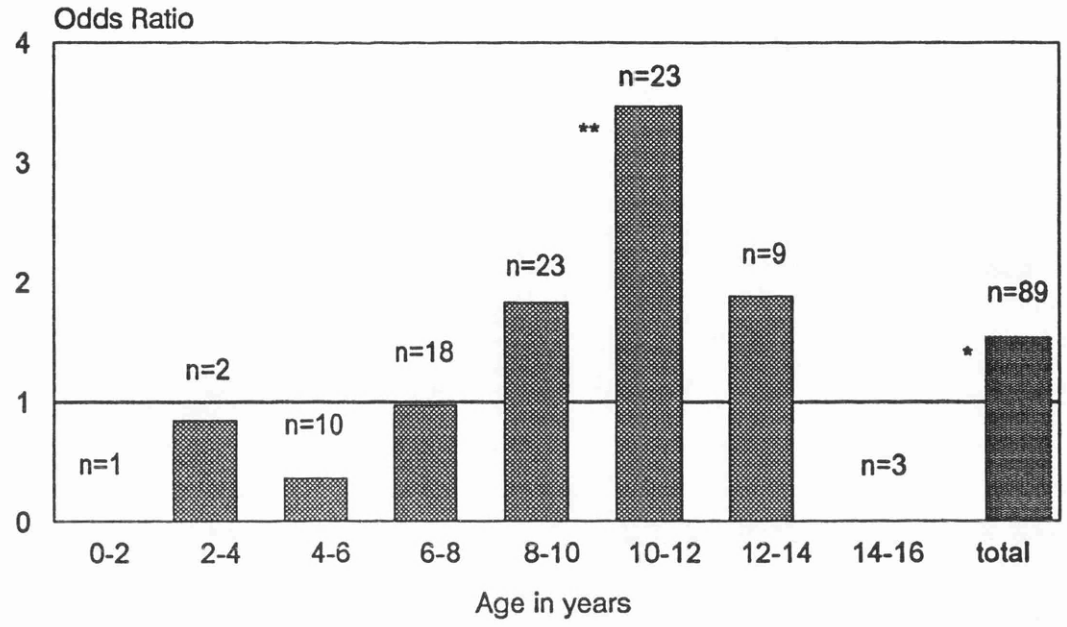

Figure 5. Odds ratios for the presence of canine diabetes mellitus by gender (female versus male) stratified by age. Odds ratio (OR) >1 means females 'at greater risk' than males, * p=0.075, ** p = 0.010.

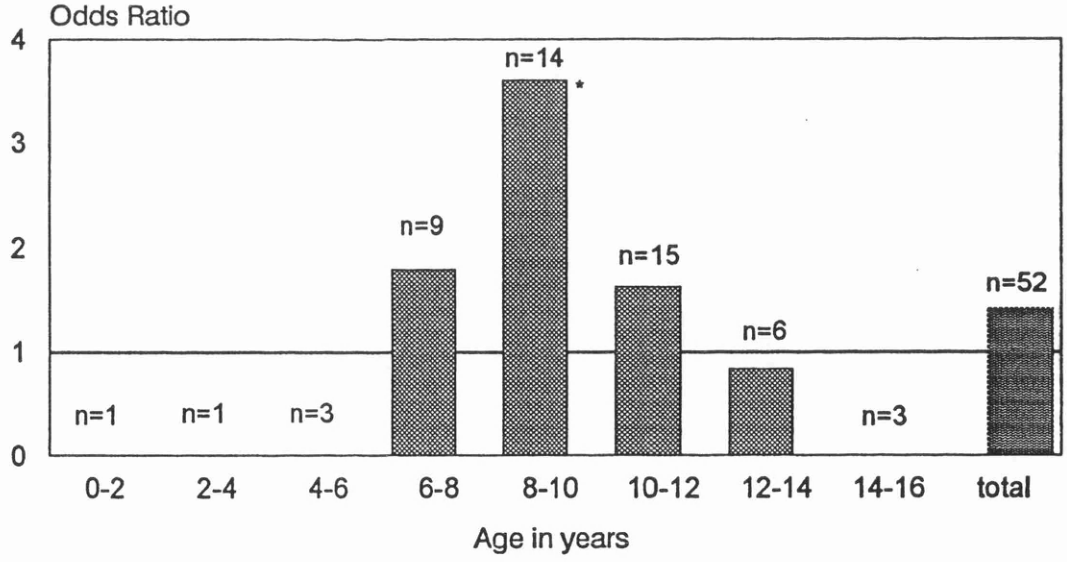

Figure 6. Odds ratios for the presence of diabetes mellitus according to effect of neutering (entire female versus neutered female) stratified by age. Odds ratio >1 means entire females 'at greater risk' than neuters, * p = 0.032.

Breed predisposition

A list of breeds which were significantly over- or under-represented within the group of diabetic dogs when compared to the control population is given in Table 7. Because of the known influence of the breed distribution of the control population by some referral groups, analyses were repeated following the exclusion of individual referral groups.

Additionally, Miniature Smooth-haired Dachshunds, Whippets, Border terriers, and Australian terriers were over-represented but not to a statistically significant degree. English Springer spaniels were similarly under-represented.

Seasonality

There was no apparent seasonal pattern to the number of referrals of diabetic dogs. However, this could not be tested statistically because a time-matched control population had been generated.

Breed	Odds Ratio	Significance (p)	Exact 95% Confidence Limits
Over-represented breeds			
Miniature Poodle	6.2	0.029	0.94 - 32
English setter	20.4	0.023	1.05 - 1206
Cairn terrier	10.8	0.000	3.40 - 34
Jack Russell terrier	3.7	0.013	1.16 - 10
Tibetan terrier	20.0	0.023	1.04 - 1206
All terriers	3.2	0.000*	1.87 - 5
Underrepresented breeds			
German shepherd dog	0.3	0.015*	0.05 - 0.81
Golden retriever	0.0	0.017	0.00 - 0.79
Over-represented breeds following exclusion of single referral groups			
Collie X†	3.7	0.048	0.80 - 14
Rottweiler‡	4.0	0.022	1.03 - 13

Table 7. Breeds over and under-represented within the diabetic population. *p value for Chi statistic, all others refer to Fisher's exact test, † following exclusion of the General Surgery referral group, ‡ following exclusion of the Orthopaedic referral group.

Further observations

Following analyses of breed dispositions to the development of diabetes mellitus, the diabetic dogs were classified into two groups: those belonging to high-risk breeds (all terriers, English setters, Rottweilers, collie crosses and Miniature Poodles) and those belonging to normal or low risk breeds. The age distribution of dogs was different

between the two risk categories (8 x 2 Chi-square, p = 0.012). To further examine this difference and to help determine if the relationship between the development of diabetes mellitus and age differed according to risk category, odds ratios by 2 year age group profiles were generated for the two risk categories and for the overall diabetic population by using the 8-10 year age group (the largest 2 year age group) as the reference age group (OR=1) within each category. Odds ratio profiles for the two risk categories and the overall diabetic population are represented in Figure 7.

Analyses for differences in gender distribution between the two risk categories were also performed. There was a close to statistically significant difference between the gender distribution of the two categories (4 x 2 Chi-square, p = 0.066), no difference between male versus female, but a statistically significant difference in entire female versus neutered female (2 x 2 Chi-square, p = 0.029, OR = 3.56, Exact 95% confidence limits 0.98 - 13.22). There was a higher proportion of entire females in the low or normal-risk category than in the high-risk category.

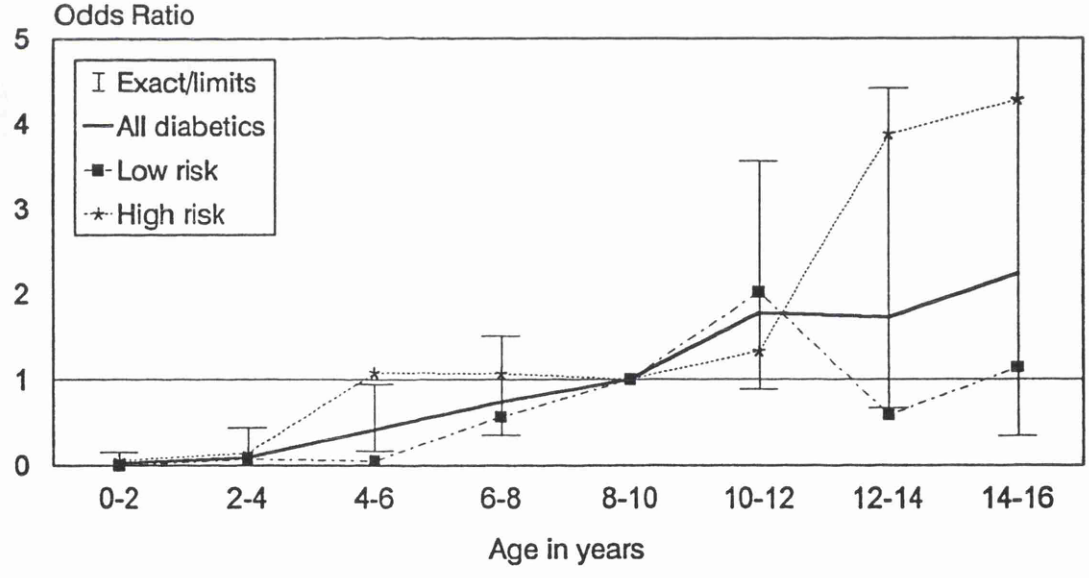

Figure 7. Odds ratio profiles for the development of diabetes mellitus by age compared to the referent age group 8-10 years for all diabetics and following subdivision into low and high risk categories. Odds ratios > 1 mean 'at greater risk' dogs than those in the referent age group. Exact limits are for all diabetics. Low risk n = 46; High risk n = 43.

Discussion

Based on 89 cases of canine diabetes mellitus seen at the University of Glasgow Veterinary School, the condition affected all ages of dogs but middle aged and aged dogs

were most prone. Gender had a complicated association with the occurrence of diabetes mellitus, in that, young males were at equal or greater risk of developing the condition compared to young females but in the older age groups, females were at greater risk. Neutering history did not seem to be an important risk factor for diabetes within females except in the 8-10 year age group where it was associated with a greatly increased risk for entire females. Some breeds of dog were predisposed to diabetes mellitus (Miniature Poodles, English setters, terriers, especially, Cairn, Tibetan and Jack Russell, collie cross breeds and Rottweilers) and some were unlikely to be diabetic (German shepherd dogs and Golden retrievers). The age and gender distribution of diabetes mellitus in the dogs belonging to the high-risk breed category was different from that in those whose breed was associated with a normal or low-risk. In the high-risk category, the odds ratios for the presence of diabetes mellitus for both young and very old dogs were greater than would be expected from a population of diabetic dogs of normal or low risk breeds and there were fewer entire females in the high-risk category.

Previous case series of canine diabetes mellitus in Britain which did not feature statistical comparisons with control populations (Wilkinson, 1960; Lauder, 1972 and Foster 1975) reported that the disease affects dogs between 3 and 14 years old, is most common in 8-12 year olds and that between 66 and 75% of cases are female. The breeds of dog which they considered predisposed were principally Dachshunds and Poodles but also included Samoyeds, King Charles spaniels, mongrels and Cairn terriers. Doxey *et al* (1985) reported an age range for diabetic dogs of 2.33 to 14 years (mean 9.83 ± SD 2.25 years) and after statistical comparison with a referral hospital group concluded that there were significantly more entire females and significantly less entire males in the diabetic group and that cross-bred terriers, Cairn terries and Poodles were significantly over-represented.

Case series of canine diabetes from the United States of America (Ling *et al*, 1977 and Marmor *et al*, 1982) reported that the condition can occur in any age of dog but agreed that dogs older than 7 years were most at risk. Females were at greater risk than males except, according to Marmor *et al* (1982), in the very young age groups where males were at equivalent or greater risk than females. Dachshunds and Poodles were reported as the predisposed breeds in the USA, along with Cairn terriers and a short list of breeds which are unpopular in Britain. Breeds identified by Marmor *et al* (1982) as resistant to diabetes mellitus included German shepherd dogs, collies, Pekingese, Boxers and Cocker spaniels.

In Sweden (Krook *et al*, 1960), there was a similar picture; the mean age of diabetic dogs was 8 years (± SD 0.19) and females were more commonly affected than males. Predisposed breeds included Rottweilers, Dachsbraches, Cocker spaniels, Swedish hounds and mongrels and the resistant breeds were German shepherd dogs and Boxers.

In the present study of diabetic dogs referred to the University of Glasgow there is some agreement with the findings of previous reports of the condition. The age at which diabetes mellitus is most likely to occur does appear to be within the age range of 8-12 years and this is in accord with the findings of the other large case series. However, to quote this age range is misleading and disguises the fact that approximately 20% of diabetic dogs in this study were less than 7 years of age. It is difficult to ascertain whether there has been a change in the age distribution of canine diabetes mellitus in Britain because of the methods of reporting age in the previous British case series but in the USA (Ling *et al*, 1977 and Marmor *et al*, 1982) up to 30% of diabetic dogs fell into this younger age group.

This study shows that in Scotland the association between gender and the development of canine diabetes mellitus is not as simple as 'females are at greater risk' (Wilkinson, 1960; Krook *et al*, 1960; Lauder, 1972; Foster, 1975; Ling *et al*, 1977; Doxey *et al*, 1985) but that, as described by Marmor *et al* (1982) in California, gender-associated risk is age-dependent. The effect of neutering on the development of canine diabetes mellitus is also age-dependent. The explanation for increased risk for entire females in the 8-10 age group probably lies in the diabetogenic effects of progesterone and growth hormone. These hormones are found in increased concentrations during metoestrus in the bitch (Eigenmann and Venker-van Haagen, 1981; Eigenmann, 1981, Eigenmann, 1989) and are believed to promote the exhaustion of the pancreatic islets by their antagonistic effects on insulin. It is likely that the degree of metoestrus-associated islet cell exhaustion which results in clinical diabetes mellitus (either on its own or in combination with other processes) will take many oestrus cycles to develop and result in a late middle age onset of the disease. Neoplastic mammary tissue may be responsible for even greater production of growth hormone (a powerful insulin antagonist) during metoestrus than is normal (Selman *et al*, 1994c) such that the epidemiology of diabetes mellitus in this group of dogs may to some extent be a reflection of the epidemiology of canine mammary neoplasia, a condition which is less likely to occur in young or neutered bitches.

It is interesting to note that there are some similarities in the predisposed and resistant breeds reported in this study and those in previous reports. Poodles, Cairn terriers,

collies and Rottweilers which are over-represented in this study have been mentioned by others (Wilkinson, 1960; Krook *et al*, 1960; Foster, 1975; Ling *et al*, 1977; Marmor *et al*, 1982; Doxey *et al*, 1985). This report also agrees with the findings of Krook *et al* (1960) and Marmor *et al* (1982) that German shepherd dogs are at an unusually low risk of diabetes mellitus. There are some differences in the list of high-risk and resistant breeds in this report from those in previous ones. This is to be expected after consideration of the likelihood that the genetic makeup of the dog population will be the same in many countries or indeed that it will be static. The list of predisposed breeds reported by Lauder (1972) also from the University of Glasgow does not contain any breeds considered at increased risk by the present study (with the exception of the statistically insignificant over-representation of Miniature Smooth haired Dachshunds). This difference between the two reports highlights the potentially dynamic nature of the epidemiology of canine diabetes mellitus.

Further analyses of the characteristics of diabetics belonging to high-risk breeds have not previously been reported. It seems that dogs in the high-risk category have an increased risk of diabetes mellitus both when they are young and when they are very old. In addition, being an entire female is less important in the development of the disease in this group than in the normal or low-risk category. The genetic or family basis of predispostion to canine diabetes mellitus has been highlighted by familial cases reported by Gepts and Toussaint (1960) and Eigenman *et al* (1984).

In conclusion, all of age, gender and breed are important predisposing factors in the development of canine diabetes mellitus based on a group of 89 diabetic dogs referred to the University of Glasgow when compared to a time-matched referred population. There is general agreement between the present study and previously published case series on the ages, gender and some of the breeds most commonly affected. However, this report highlights some aspects which have not been previously emphasised, namely: the large proportion of young dogs affected; the relationship between age and gender-associated risks; the dynamic nature of the epidemiological characteristics of the condition and the possible differences in epidemiological characteristics (and therefore perhaps pathophysiology) between the disease in high-risk breeds and that in normal or low-risk breeds.

Chapter 4:
Diabetes mellitus in association with other syndromes

Introduction

Diabetes mellitus is a group of disorders and syndromes within which chronic hyperglycaemia features. Factors which interfere with the production of insulin, its transport to, or action in, target tissues can cause or exacerbate diabetes mellitus (Unger and Foster, 1985; Stogdale, 1986). A number of syndromes have been associated with the presence of diabetes mellitus in dogs, including autoimmune islet cell destruction (Sai *et al*, 1984; Haines and Penhale, 1985); hormonal antagonism, particularly by endogenous or exogenous progestagens (Eigenmann, 1981; Eigenmann and Venker-van Haagen, 1981; Eigenmann, 1989) or endogenous or exogenous glucocorticoids (Peterson *et al*, 1981; Blaxter and Gruffydd-Jones, 1990; Walker, 1962; Campbell and Latimer, 1984); pancreatic inflammation (Dixon and Sanford, 1961) and a syndrome resembling Type II human diabetes mellitus (Mattheeuws *et al*, 1984b). In addition, there are less common hormonal antagonisms associated with canine diabetes mellitus such as spontaneous acromegaly (Eigenmann, 1981), phaeochromocytoma and glucagonoma (Chastain and Ganjam, 1986b). The association of progestagens or metoestrus and diabetes mellitus in dogs is mediated through the production of growth hormone which is a powerful insulin antagonist (Eigenmann, 1981). Using a reverse transcriptase polymerase chain reaction and other techniques the source of this growth hormone production has been identified as the mammary glands, particularly in the presence of mammary neoplasia (A. Rijnberk, Faculty of Veterinary Medicine, Utrecht University, personal communication; Selman *et al*, 1994c).

In addition, the occurrence of diabetes mellitus in association with other endocrine dysfunction has also been recognised in dogs (Milne and Hayes, 1981; Hargis *et al*, 1981; Eigenmann *et al*, 1984; Chastain, 1992; Kintzer, 1992; Ford *et al*, 1993) and in humans (Carpenter *et al*, 1964; Ganz and Kozak, 1974; Mouradian and Abourizk, 1983). In humans, such multiple endocrine failure syndromes are considered to have an autoimmune basis (Eisenbarth, 1985) and this may also be the case in dogs (Kintzer, 1992). In the dog, the most common co-existent endocrine failures are hypoadrenocorticism and hypothyroidism (Schmidt' syndrome) (Bowen *et al*, 1986; Kintzer, 1992).

Islet cell hypoplasia has been recognised in dogs (Anderson *et al*, 1986, Atkins *et al*, 1988) and in man (Dodge and Lawrence, 1977). Such a condition has, however, not been reported in a British dog. In this condition there are either no recognisable islets of Langerhans or they are shrunken, low in number or contain degenerate vacuolated cells (Atkins *et al*, 1988).

The aims of this part of the study were to identify those diabetic dogs in which a syndrome associated with their diabetic condition could be found and to investigate the clinical features of these dogs for evidence of differences in clinical presentation from those dogs in which no other identifiable syndromes were present.

Materials and methods

The clinical records and laboratory findings of 94 diabetic dogs were reviewed. Fifty dogs had no identifiable associated syndrome and this group formed the control group. Of the remaining 44 dogs, three had received progestagen therapy shortly before developing diabetes mellitus, 18 had concurrent hyperadrenocorticism, 8 were hypothyroid, 14 had an onset of diabetes mellitus during or shortly after a metoestrous phase of the reproductive cycle and one dog had islet cell hypoplasia. The low numbers in the progestagen related and islet cell hypoplasia categories meant that these cases could not be included in statistical analyses as separate groups.

The diagnosis of hyperadrenocorticism was based on the combination of clinical signs and the results of an adrenocorticotrophic hormone (ACTH) stimulation test. Plasma cortisol concentrations were measured before, and 45 or 60 minutes after, an intramuscular injection of 250μg of an ACTH analogue (tetracosactrin, Synacthen; Ciba). In those dogs in which a 45 minute sample was taken a further sample was taken at 90 minutes. A post-ACTH plasma cortisol concentration of close to or greater than 600 nmol/l in association with a consistent clinical presentation was considered to indicate the presence of hyperadrenocorticism.

The diagnosis of hypothyroidism was based on either an extremely low basal total serum thyroxine concentration or the results of a thyrotropin stimulation test. Serum concentration of thyroxine were measured before and 6 hours after an intravenous injection of bovine thyroid stimulating hormone (TSH; Sigma). Dogs which failed to increase serum thyroxine concentration by 150% or to exceed a post TSH cut-off point of 26 nmol/l were diagnosed hypothyroid.

Following a diagnosis of hyperadrenocorticism a course of adrenocorticolytic therapy was instituted if the owners agreed. The protocol used was a low dose mitotane (o,p'-DDD, Lysodren; Kingston Animal Health) regimen of 25mg/kg for an initial loading period of 10 days with glucocorticoid supplementation at 0.4mg/kg of prednisolone. Following this initial period, weekly doses of mitotane were continued at 25 or 50 mg/kg and the frequency of these doses were further adjusted according to response.

Dogs which were diagnosed hypothyroid began thyroid replacement therapy using L-thyroxine (Soloxine, Vet-2-Vet Marketing) at 10 μg/kg once or, if necessary, twice daily.

Bitches with metoestrus-associated diabetes mellitus underwent ovariohysterectomy as soon as was reasonably possible. Anaesthesia was by low dose intravenous induction (thiopentone or propofol) and maintenance by inhalational anaesthesia (halothane, nitrous oxide and oxygen). Fine diabetic stabilisation was not attempted in these dogs until after ovariohysterectomy unless it was going to be some time before surgery could be performed. Sufficient insulin therapy was, however, administered to allow the documentation of a glycaemic response.

The clinical features, breed, age, gender and relevant laboratory test results for dogs with each associated syndrome were reviewed, tabulated and subjectively evaluated.

To investigate the possibility of certain plasma biochemical characteristics being useful for the differentiation of the types of diabetes mellitus, one-way analysis of variance was used to test for differences in the mean initial concentrations of plasma glucose, cholesterol, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and basal insulin between the groups (concurrent hyperadrenocorticism, (HAC); concurrent hypothyroidism (HypoT4); metoestrus-associated (Meto) and no identified associated syndrome (Norm)). The same technique was used to attempt to identify differences in the pre-treatment body condition score between groups. Body conditon was scored as follows: 1 - very thin; 2 - thin; 3 - good; 4 - overweight; 5 - very overweight and 6 - grossly obese.

For those dogs in which an ACTH stimulation test was performed, one-way analysis of variance was used to test for differences between the groups with respect to mean cortisol concentrations before, 45/60 minutes and 90 minutes after the administration of ACTH. A two-sample *t*-test was used to compare pre- and post TSH concentrations of thyroxine between the hypothyroid group and the 'normal' group.

One-way analysis of variance was used to test for differences among groups in the mean insulin dose (IU/kg) required to treat 'normal' diabetic dogs and that required by dogs with concurrent hypothyroidism or hyperadrenocorticism before therapy with thyroxine or mitotane was begun.

Differences in the mean age of dog in each group were tested using one-way analyses of variance and differences between an associated syndrome and the 'normal' group were further investigated by means of a Chi-square 2x2 analysis and relative risk calculation using statistical software (Epi Info 5.01b). Differences in gender distribution between the disease groups were also investigated using the Chi-square technique (except for metoestrus-associated diabetes mellitus).

$$\text{Relative risk} = \frac{\text{(number diseased/number exposed to risk factor)}}{\text{(number diseased/ number unexposed to risk factor)}}$$

Where one-way analysis of variance revealed a difference in means between groups, Tukey's pairwise comparison method (MINITAB 9) was used to elucidate which groups were different from which.

Results

Progestagen-associated diabetes mellitus

It was not possible to determine in these cases whether the administration of progestagen (proligestone) by injection was directly connected with the presence of diabetes mellitus but the known association of these products and diabetes mellitus prevented the inclusion of these dogs in any other analysis group. A summary of the clinical features of these dogs is presented in Table 8. All three dogs had received proligestone by repeated injection for a number of years and all three required continuous insulin therapy following diagnosis of diabetes mellitus. One dog, no. 122551, required high doses of insulin which suggested the presence of insulin resistance. However, this dog also had histologically confirmed cirrhosis and a low growth hormone concentration indicating that proligestone was unlikely to be directly responsible for the insulin resistance at that time.

Hospital number	Breed	Age	Gender	last progestagen before diabetes diagnosis (weeks)	Insulin required
112598	Samoyed	9.0	F	3.6	continuous
115131	Jack Russell terrier	8.0	F	4.4	continuous
122551	Labrador retriever	7.0	F	9.4 and -13.2	continuous

Hospital number	Mean insulin dose (IU/kg)	Minimum insulin dose	Maximum insulin dose	Comments
112598	0.76	0.70	0.83	pyometra 63 weeks after diabetes diagnosis
115131	1.16	0.93	1.34	mammary neoplasia
122551	2.67	1.58	3.45	cirrhosis

Table 8. Clinical features of three diabetic dogs which received long acting progestagen by injection shortly before developing overt diabetes mellitus.

Hyperadrenocorticism-associated diabetes mellitus

The clinical features of 18 dogs with concurrent hyperadrenocorticism and diabetes mellitus are presented in Tables 9 and 10. There was no apparent gender predisposition (8 male, 9 neutered female and 1 entire female) and a wide variety of breeds were affected. Subjectively, Poodles and terriers were most commonly affected. Two dogs had received therapy for previously diagnosed hyperadrenocorticism for some time prior to developing diabetes mellitus and in the others the diagnosis of hyperadrenocorticism was made either at the time of diabetes diagnosis or up to 2.6 years afterwards. The time between the diagnosis of diabetes mellitus and that of hyperadrenocorticism was related to the mean insulin dose pre-mitotane therapy in those dogs in which the diagnosis of diabetes mellitus came first (simple regression, $p = 0.023$, $r = 0.58$, mean insulin = 2.17 + 0.016 x weeks diabetic, $n = 15$). Basal insulin concentration was measured in 8 dogs and varied from 3.6 to 41 μIU/ml. There was no apparent relationship between basal insulin and prognosis in this small group of dogs. In 9 dogs, the institution of mitotane therapy resulted in a substantial decrease in insulin dose and in two cases this was to less than 0.5 IU/kg. In two dogs, nos. 113936 and 116318, the post-ACTH cortisol concentration did not exceed 600 nmol/l. However, there were strong clinical signs present in these dogs, which along with the presence of high insulin requirements (> 2 IU/kg) supported the diagnosis of hyperadrenocorticism. In one other dog, no. 118626, the diagnosis of hyperadrenocorticism was made on the basis of clinical and necropsy findings.

There were a number of unusual clinical presentations in the dogs with concurrent hyperadrenocorticism and diabetes (Table 10). The most significant of these were

neuropathies, displayed by three dogs, and emphysematous cystitis in two others. Neuropathies were rarely seen in the 'normal' diabetic group and emphysematous cystitis was not seen in any of the other groups. Figure 8 shows the radiographic appearance of emphysematous cystitis in dog no. 122026.

The survival data for dogs with concurrent hyperadrenocorticism and diabetes mellitus are analysed statistically in Chapter 8.

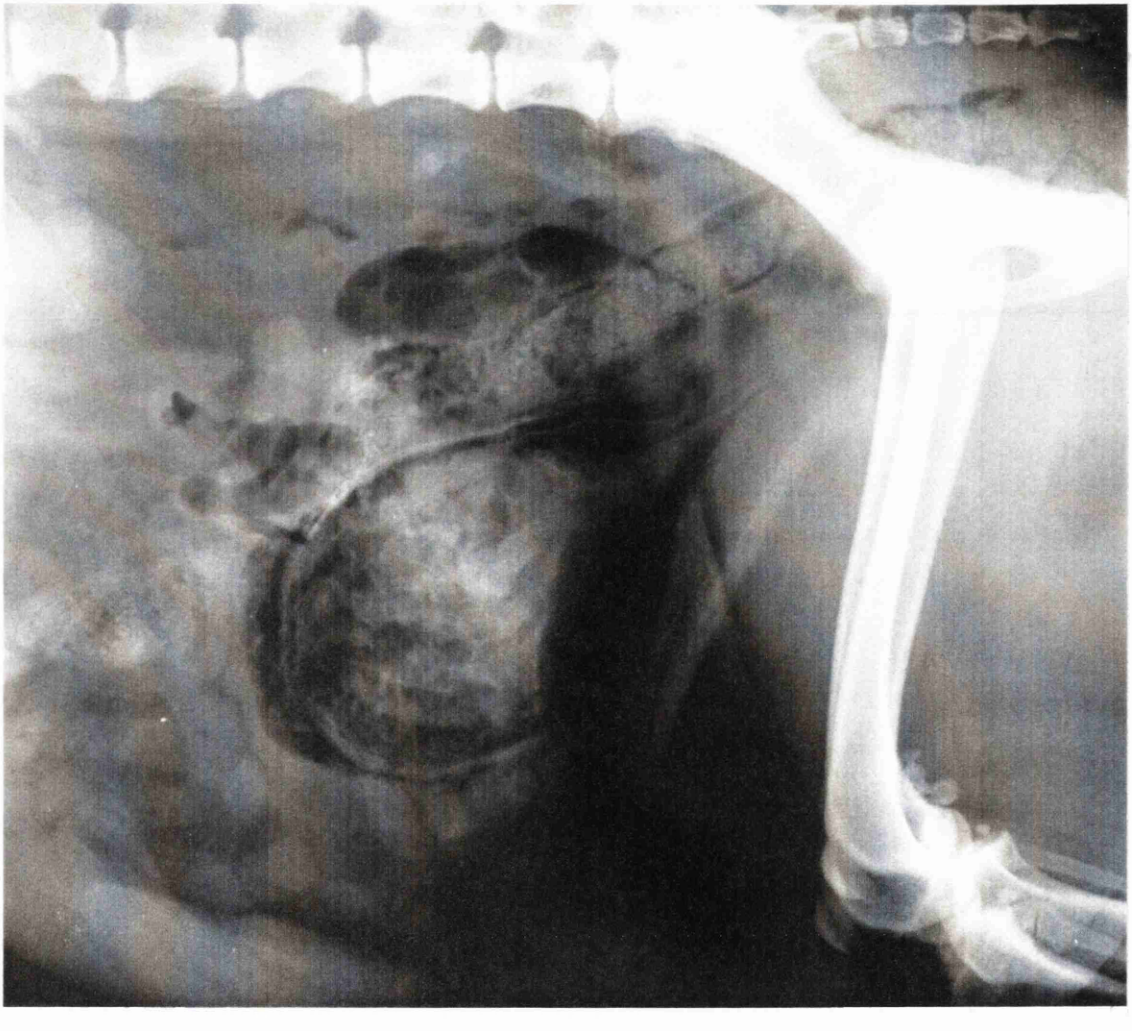

Figure 8. Lateral radiograph of the caudal abdomen of case no. 122026. There is extensive gas lying within the wall of the bladder consistent with a diagnosis of emphysematous cystitis.

Hospital number	Breed	Age (years)	Gender	Number of weeks diabetic before HAC	Basal plasma insulin (μIU/ml)	mitotane therapy	mean insulin dose pre-HAC treatment	Minimum insulin dose	Maximum insulin dose	Basal cortisol (nmol/l)	Post-ACTH cortisol at 45 or 60 mins	Post-ACTH cortisol at 90 mins
109434	Miniature Poodle	12.3	FN	-223	9.2	Yes	*	1.73	2.40	82	648	
109826	West Highland White terrier	13.0	F	58	*	Yes	2.70	1.25	3.75	181	2358	
113105	Cross breed	12.0	M	0	*	No	1.21	*	*	187	668	
113498	Cross breed	8.0	FN	104	*	Yes	2.34	1.25	2.34	97	687	
113936	Cairn terrier	14.0	FN	3	*	Yes	2.33	0.83	5.18	96	352	566
114895	Bull terrier	6.7	M	15	*	Yes	1.25	0.87	1.25	189	871	
116315	West Highland White terrier	14.5	FN	0	*	No	1.32	1.14	2.08	118	453	633
116318	Labrador retriever	5.9	M	4	*	Yes	2.42	1.15	2.42	115	595	586
116744	Miniature Poodle	9.0	M	18	4.1	Yes	2.34	1.31	2.67	86	658	796
117369	Cross breed	9.8	M	-40	5.8	Yes	*	1.89	2.17	61	1196	1482
117705	Cross breed	13.8	FN	11	27.0	No?	2.51	2.02	3.00	91	416	621
118388	Labrador retriever	10.0	M	0	3.6	No	1.78	1.72	1.83	105	481	617
118626	Jack Russell terrier	11.0	M	0	17.2	No	*	*	*	*	*	*
119010	Jack Russell terrier	9.2	FN	41	*	No?	4.33	*	*	195	601	640
122026	Bearded collie	10.8	FN	2	*	Yes	2.24	1.00	2.24	210	1300	
122150	Yorkshire terrier	9.4	FN	137	*	Yes	5.24	0.00	5.26	111	728	901
122970	Jack Russell terrier	11.6	M	0	41	Yes	3.79	0.46	3.79	145	900	1115
122973	Labrador retriever	10.0	FN	0	41	Yes	2.93	2.93	2.93	70	1090	1095

Table 9. Clinical features of 18 dogs with concurrent diabetes mellitus and hyperadrenocorticism (HAC) I. * - not done or not applicable.

Hospital number	Unusual presentations	Comments	Alive/ Dead/ lost	survival (years)
109434	superficial pyoderma	euthanased for age	Dead	0.30
109826	colitis	euthanased for age	Dead	1.66
113105	ketoacidotic on presentation	therapy failed	Dead	0.02
113498	Horner's syndrome both eyes at separate times, ? gastric neuropathy	euthanased for persistent vomiting	Dead	3.86
113936	chronic gingivitis, colitis		Dead	1.89
114895	KCS	died during hypoglycaemia	Dead	0.36
116315		euthanased for aggression	Dead	0.24
116318	Horner's syndrome one eye		Alive	3.75
116744	normal ACTH results at 0 days		Lost	0.84
117369	facial nerve paralysis, resistant *E coli* cystitis and haematuria		Dead	0.31
117705	hepatocutaneous syndrome	lost to follow up	Lost	0.31
118388	osteopenia on radiography		Dead	0.39
118626	hyperosmolar syndrome	treatment ineffective euthanased at 24 hours	Dead	0.00
119010		lost to follow up	Dead	3.17
122026	resistant *E coli* emphysematous cystitis		Alive	1.60
122150		euthanased because of acute pancreatitis	Dead	2.77
122970			Alive	1.00
122973		owner unwilling to continue therapy	Dead	0.10

Table 10. Clinical features of 18 dogs with concurrent diabetes mellitus and hyperadrenocorticism II: unusual presentations, comments, outcome and survival times. KCS- keratoconjuctivitis sicca

Concurrent hypothyroidism and diabetes mellitus

The clinical features of 8 dogs with concurrent hypothyroidism and diabetes mellitus are presented in Tables 11 and 12. In all cases the diagnosis of hypothyroidism was made at the time of or after the diagnosis of diabetes mellitus. There were two Tibetan terriers and two English setters in this group suggesting a breed predisposition. Unfortunately, pedigrees were not available for these dogs for analysis. There were more males (5) than females (3) affected but this was not significant (Chi-square analysis). Four of the 6 dogs which were stabilised on insulin therapy prior to treatment with thyroxine had a high insulin requirement (> 2 IU/kg) which was reduced by thyroxine therapy. There was no relationship between the number of weeks of diabetes prior to the diagnosis of hypothyroidism and the mean pre-thyroxine insulin requirement. Basal insulin analysis was performed in three dogs and ranged from 4.8 to 11.5 nmol/l. No association could be made with these results to any other parameters because of their low number. Neuropathies, pyodermas and pancreatitis were among the unusual presentations in the hypothyroid dogs. None of these presentations were commonly seen in the 'normal'

diabetic group. Seven of the 8 dogs underwent ACTH stimulation testing (Appendix 5) and none had hypoadrenocorticism. The survival data of these dogs are evaluated in detail in Chapter 8.

Hospital number	Breed	Age (years)	Gender	Number of weeks diabetic before HypoT4	Basal thyroxine nmol/l	Post-TSH thyroxine at 6 hrs	Basal plasma insulin µIU/ml	Thyroxine therapy
107303	Collie cross	5.7	M	107	2.0		*	Yes
112714	Tibetan terrier	4.0	FN	14	20.4	19.6	*	Yes
113648	Scottish terrier	5.0	M	123	9.4	7.9	5.0	Yes
116146	English setter	6.0	M	54	5.6	2.0	*	Yes
119136	English setter	5.4	M	0	1.0	1.0	4.8	Yes
120811	Tibetan terrier	6.9	FN	6	8.0	11.0	*	Yes
120931	Cairn terrier	2.5	M	109	8.8	23.8	*	No
124684	Cross breed	8.0	FN	0	5.8	8.7	11.5	Yes

Table 11. Clinical features of 8 dogs with concurrent diabetes mellitus and hypothyroidism I. * - not done.

Hospital number	Mean insulin dose pre-thyroxine (IU/kg)	Minimum insulin dose	Maximum insulin dose	Unusual presentations	Comments	Alive/ Dead/ Lost	survival (years)
107303	1.72	1.52	1.72	Alopecia, superficial pyoderma	Euthanased because of age	Dead	7.73
112714	2.51	1.6	3.18	Extreme hyper-cholesterolaemia, ?pancreatitis		Alive	4.90
113648	2.32	1.45	3.04	KCS, pyoderma		Alive	4.57
116146	0.76	0.4	0.76	RHL neuropathy	Euthanased because of aggression	Dead	2.17
119136	*	0.516	1.32	RHL neuropathy		Alive	2.68
120811	2.73	2.14	4.00		Euthanased because of pancreatitis	Dead	0.57
120931	3.24	*	*	? pancreatitis		Lost	2.13
124684	*	0.862	1.65			Alive	0.32

Table 12. Clinical features of 8 dogs with concurrent diabetes mellitus and hypothyroidism II: unusual presentations, comments, outcome and survival times. * - not done or not applicable, KCS - keratoconjunctivitis sicca, RHL - right hindlimb.

Metoestrus-associated diabetes mellitus

The clinical features of 14 dogs with metoestrus associated diabetes mellitus are presented in Table 13. A variety of breeds of dog were represented and all had been in oestrus recently (mean 6.28 weeks ± SD 1.90). Ovariohysterectomy was performed within a few weeks of the diagnosis of diabetes mellitus except in two dogs (nos. 112324 and 121143) which had been managed in practice prior to referral to the University

clinic, in one dog (no. 111353) which the owners had been reluctant to spay until the dog experienced fluctuating insulin requirements and one dog (no. 114460) which was included on a surgical waiting list in order that cervical spondylopathy investigation and ovariohysterectomy could be performed under one general anaesthetic. Five dogs required continuous insulin therapy after diagnosis, 4 dogs experienced temporary remission of insulin requirement (in two dogs this was prior to ovariohysterectomy) and 4 dogs had no requirement for insulin therapy after ovariohysterectomy although in one of these, insulin therapy was necessary for a few weeks following surgery. One dog failed to recover from ovariohysterectomy and was not made available for necropsy. Basal plasma insulin analysis was performed in 10 dogs and the results of 9 of these are represented graphically in Figure 9 according to whether insulin requirement was continuous or whether there was temporary or indefinite remission. The numbers of observations in each group make statistical analyses inappropriate, but all three 'cured' dogs had basal insulin concentrations greater than 40 μIU/ml and all those with continuing insulin requirement or temporary remission had basal insulin concentrations of less than 20 μIU/ml.

Although insulin resistance is a feature of metoestrus-associated diabetes mellitus (Eigenmann, 1981), mean insulin dose pre-ovariohysterectomy was not calculated since it was unlikely to reflect the true insulin requirement because therapeutic policy in the case of these dogs was to arrange for ovariohysterectomy before attempting to achieve fine glycaemic control. Only one dog had classical clinical signs of canine acromegaly including soft tissue swelling of the head (Eigenmann, 1981) and even in this dog it was only a very temporary feature. In 4 dogs, there was mammary neoplasia and two suffered pseudopregnancy. The survival data of these dogs are included in Chapter 8 among the 'normal' group but not for those dogs which had remission of insulin requirement.

Islet cell hypoplasia

One female Cairn terrier was presented with islet cell hypoplasia at 5 months of age. This dog was stunted and had been polydipsic for 2-3 weeks. An intravenous glucose tolerance test confirmed that this dog had no insulin response. Plasma insulin concentrations remained < 2.5μIU/ml for 2 hours following an intravenous glucose load of 1g/kg. Pancreatic biopsy was performed within one month of diagnosis and histopathological examination revealed no normal islets with only a few small foci of degenerate, vacuolated cells and connective tissue. The exocrine pancreas was normal. The dog was managed on single daily injections of isophane insulin at doses ranging from 0.89 to 2.08 IU/kg (mean 1.42) and was still alive at the end of the study period (3.14 years).

Hospital number	Breed	Age	Gender	Time from oestrus to diagnosis (weeks)	Time from diagnosis to OVH (weeks)	Initial insulin therapy (weeks)	Duration of remission (weeks)	Basal plasma insulin (μIU/ml)	minimum insulin dose (IU/kg)	maximum insulin dose (IU/kg)	Unusual presentations	Alive/ Dead/ Lost	survival (years)
103348	Border collie	10.0	F	8	2	continuous	*	3.2	0.47	1.20		Alive	3.09+
109899	German shepherd	8.0	F	8	1	16.1	32.0	*	0.00	1.06	anal furunculosis	Dead	1.11
110113	whippet	10.0	F	5	1	5.7	indefinite	*	0.00	0.25		Lost	5.64+
110364	English springer spaniel	6.9	F	8	3	continuous	*	*	0.68	1.52	mammary neoplasia	Alive	5.62+
111353	Labrador retriever	11.0	F	6	26	continuous	*	1.0	0.44	1.51	? intermittent EPI	Dead	3.08
112324	Labrador retriever	10.7	F	4	16	8.6	2.7	*	0.92	0.92	remission before OVH	Lost	0.39+
114460	Dobermann	7.5	F	8	7	continuous	*	6.0	0.53	1.65	pseudopregnant, lymphadenopathy	Dead	1.58
114653	cross breed	11.0	F	3	4	pre-OVH	indefinite	45.0	1.14	2.82	mammary neoplasia	Lost	4.24+
117056	Jack Russell terrier	11.0	F	5	2	pre-OVH	indefinite	93.0	0.00	3.20		Lost	3.40+
118568	Greyhound	9.0	F	8	3	pre-OVH	indefinite	189.0	0.00	1.77	soft tissue swelling over head, bilateral 3rd eyelid protrusion, pseudopregnant	Lost	2.90+
119821	miniature poodle	12.0	F	9	3	pre-OVH	died	16.2	0.00	1.85	mammary mass, failed to recover form OVH	Dead	0.08
120899	Yorkshire terrier	8.3	F	4	4	2.6	8.9	6.2	0.83	1.93	pyometritis, mammary masses, remission before OVH	Alive	2.16+
121143	Border collie	9.9	F	6	6	continuous	*	10.7	0.54	0.66		Alive	1.96+
122200	collie cross	11.9	F	6	2	5.9	12.9	6.5	0.00	1.46		Dead	0.90

Table 13. Clinical features of 14 dogs diabetes mellitus believed to be associated with metoestrus. * - not done or not applicable, OVH - ovariohysterectomy, EPI - exocrine pancreatic insufficiency, + - indicates a minimum survival time.

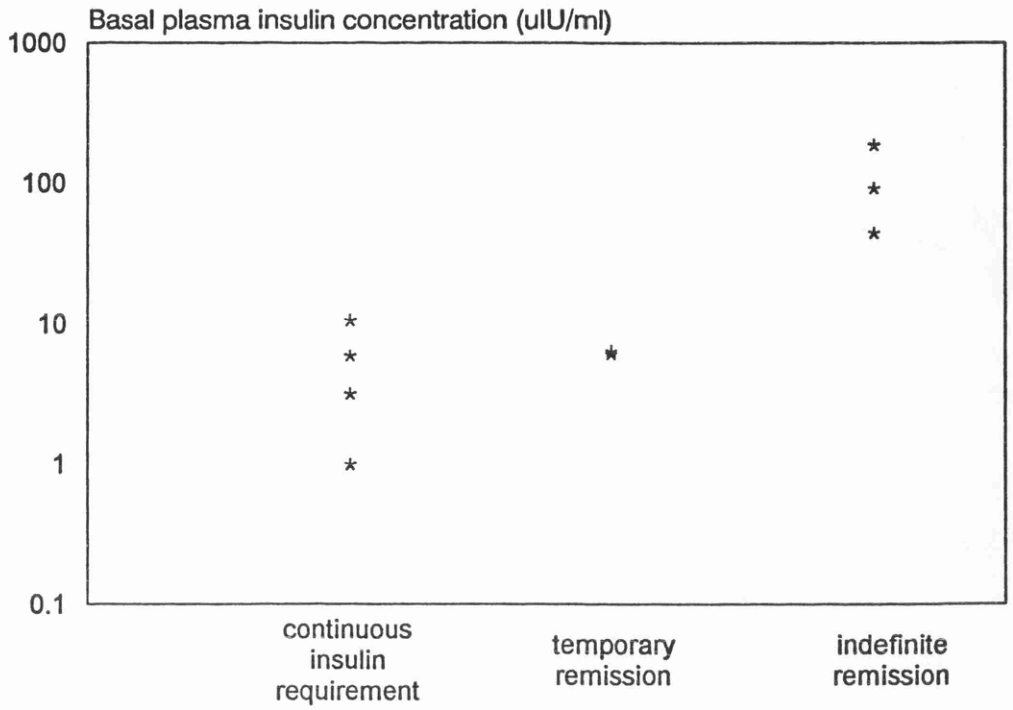

Figure 9. Basal plasma insulin concentration μIU/ml (logarithmic scale) in bitches with metoestrus-associated diabetes mellitus categorised according to duration of insulin requirement.

Age at diagnosis of diabetes mellitus

The age at diagnosis of diabetes mellitus was significantly different between the groups ($p = 0.000$). Dogs with concurrent hyperadrenocorticism were significantly older than those with hypothyroidism and the 'normal' group. The dogs with concurrent hypothyroidism were significantly younger than those with metoestrus-associated diabetes mellitus and the 'normal' group. There was no statistically significant difference between the mean ages in the metoestrus-associated group and those in the hyperadrenocorticism or 'normal' groups. The mean (± standard deviation) ages for each group are presented in Table 14 and Figure 10 represents the age distribution of the 4 groups.

Further analysis of the age distribution of dogs with concurrent hyperadrenocorticism by the Chi-square 2 x 2 method revealed that dogs which were 8 years of age or older when diagnosed diabetic were approximately 5 times more likely to have concurrent hyperadrenocorticism than those diagnosed at less than 8 years of age (relative risk = 4.65, Taylor series 95% confidence limits 1.16 and 18.58, $p = 0.009$). In the case of hypothyroidism, dogs diagnosed diabetic at less than 7 years of age were approximately

15 times more likely to develop concurrent hypothyroidism than those which did not become diabetic until 7 years of age or older (relative risk = 15.56, Taylor series 95% confidence limits 2.06 and 117.28, p = 0.000).

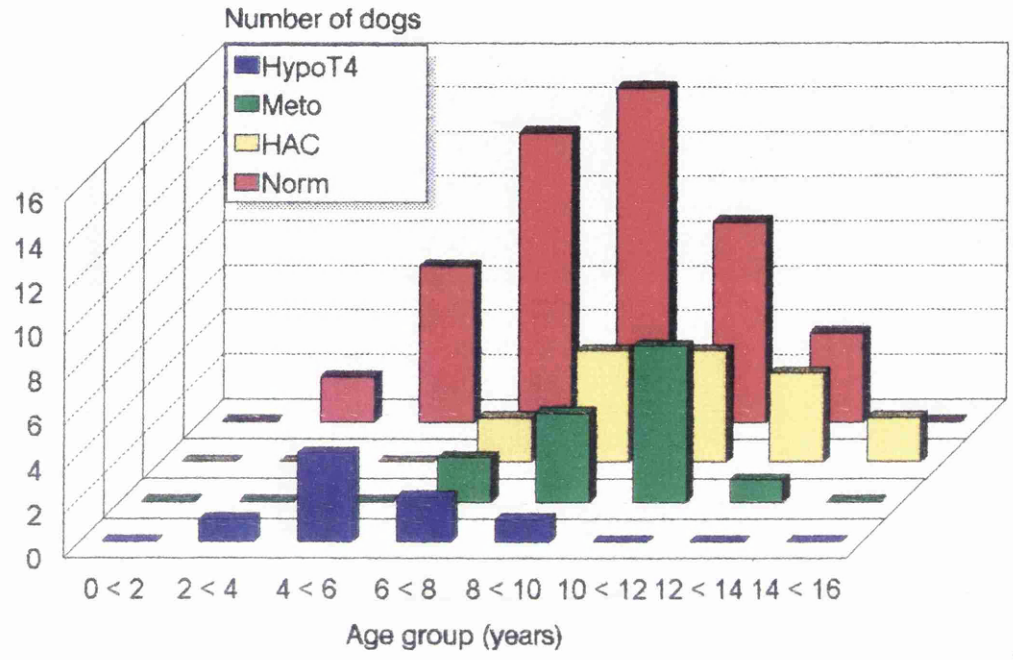

Figure 10. Age distributions of dogs with diabetes mellitus categorised according to disease type.

Group	Number of dogs	Mean age (years)	Standard deviation
Hyperadrenocorticism	18	10.61	2.41
Hypothyroidism	8	5.43	1.68
Metoestrus	14	9.82	1.61
'Normal'	50	8.14	2.40

Table 14. Mean ages of dogs at diagnosis of 4 types of diabetes mellitus.

Gender

There were no significant differences in gender distribution among the groups of diabetic dogs tested (HAC, HypoT4 and Norm).

Initial plasma biochemistry and condition score

The initial results of plasma biochemical analyses and body condition scores for those dogs presented to the University of Glasgow clinic before insulin therapy had begun are presented in Appendix 6. There were no statistically significant differences in initial plasma glucose, cholesterol, alkaline phosphatase, alanine aminotransferase, aspartate

aminotransferase, basal insulin or body condition score between the four groups of diabetic type.

Dynamic endocrine tests

The results of 15 ACTH stimulation tests and 13 thyroid investigations in 'normal' diabetic dogs are presented in Appendix 7 and those from 7 ACTH stimulation tests in diabetic dogs with hypothyroidism are presented in Appendix 5. Dynamic endocrine tests were not performed in metoestrus-associated diabetes mellitus. There was a significant difference between the groups (HAC, HypoT4 and Norm) in basal cortisol concentration ($p = 0.017$). Basal cortisol was significantly higher in the HAC group than in the HypoT4 or Norm groups. There was no difference in basal cortisol between the HypoT4 and Norm groups. This pattern was repeated for the 45/60 minute post-ACTH cortisol results ($p = 0.000$) and the 90 minute results ($p = 0.002$). These results are summarised in Figure 11. A two-sample *t*-test revealed a significant difference in basal thyroxine concentration between the hypothyroid and 'normal' groups ($p = 0.000$) and this difference was also evident in the 6 hours post-TSH thyroxine concentration ($p = 0.000$). These results are summarised in Figure 12.

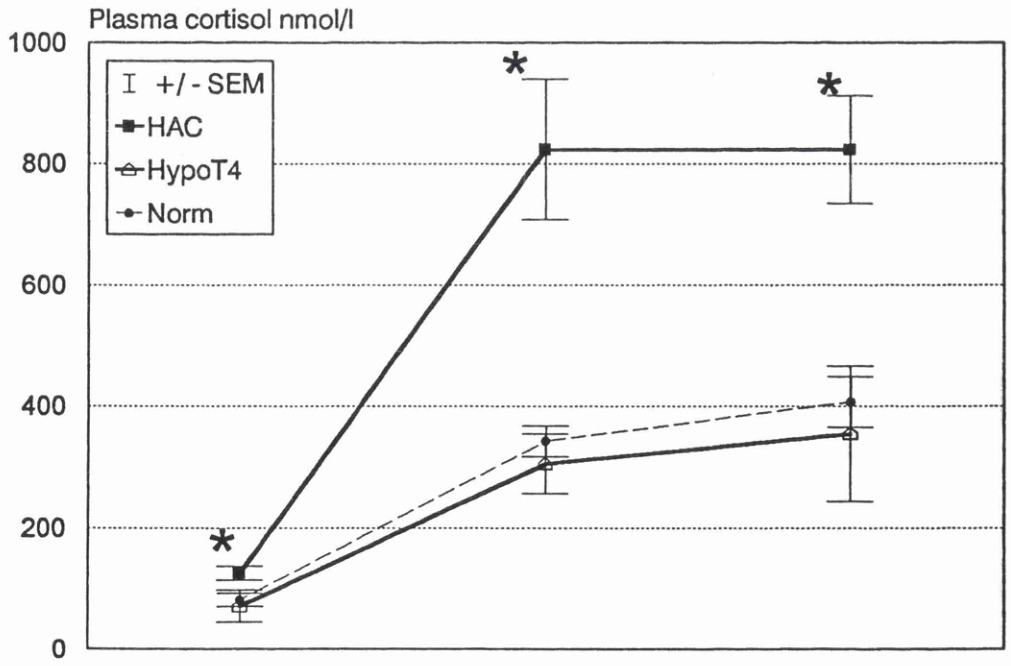

Figure 11. Plasma cortisol responses (mean ± SEM) to intramuscular injections of a synthetic ACTH in diabetic dogs with concurrent hyperadrenocorticism (HAC) or hypothyroidism (HypoT4) or no concurrent illness (Norm). * - significantly different from the Norm group.

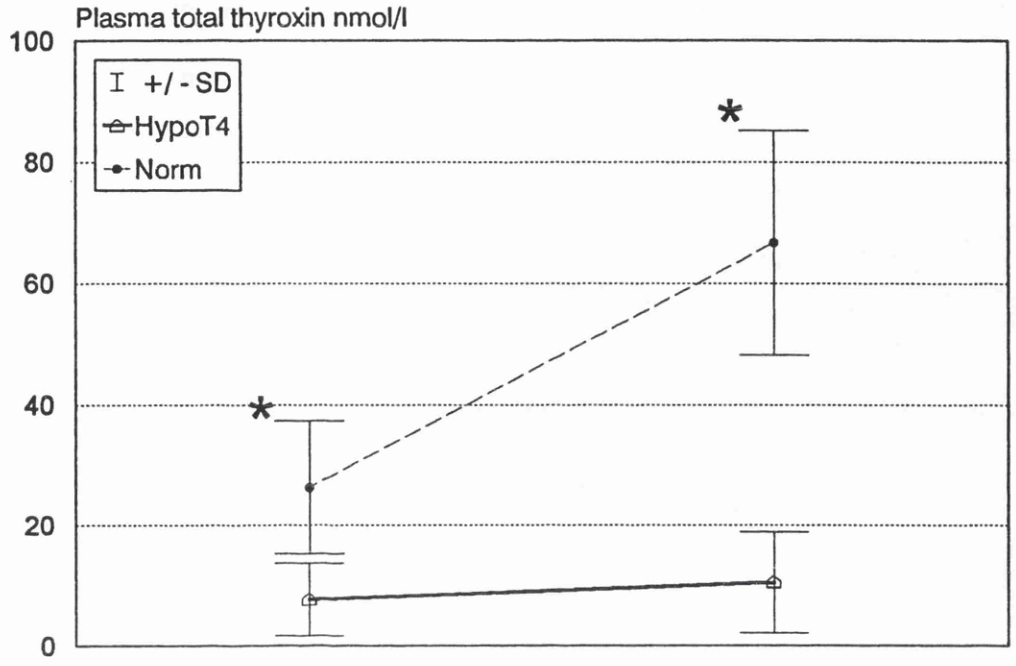

Figure 12. Serum thyroxine concentration responses to intravenous bovine TSH in diabetic dogs with hypothyroidism (HypoT4) and those with no other illness (Norm).

Insulin requirement

One-way analyses of variance of mean insulin doses administered to diabetic dogs (excluding those during mitotane or thyroxine therapy and those for 'cured' metoestrus-associated diabetes mellitus) revealed a significant difference between the groups HAC, HypoT4, Meto and Norm ($p = 0.000$). The data used for this analysis are presented in Appendix 8. Tukey's paired comparisons revealed the mean insulin requirement of the HAC group to be higher than the Meto and Norm groups. The mean insulin requirements of the HypoT4 and Norm groups were also higher than those in the Meto group. Mean insulin doses (and standard deviations) are presented for each group in Table 15. Further analyses were also performed, including doses administered during mitotane or thyroxine therapy, and the same pattern emerged ($p = 0.001$) with the exception of the lack of significant difference between the HAC and Norm groups. This analysis was principally included so that the mean range in insulin doses for each group could be tested as a measure of fluctuating requirement and, by inference, of difficulty in management. There was no significant difference in the range of insulin doses administered to each group ($p = 0.36$).

Group	Number of dogs	Mean insulin requirement (IU/kg)	Standard deviation
Hyperadrenocorticism	15	2.58	1.13
Hypothyroidism	6	2.21	0.87
Metoestrus	11	0.86	0.32
'Normal'	45	1.58	0.69

Table 15. Mean insulin requirement in 4 types of canine diabetes mellitus (excluding doses administered during mitotane or thyroxine therapy).

Discussion

The clinical features and laboratory test results of dogs with diabetes mellitus in the presence of associated syndromes were investigated. Of a referred population of 94 diabetic dogs, 3.2% developed diabetes mellitus following a recent injection of long acting progestagen (proligestone), 19.1% had hyperadrenocorticism, 8.5% had concurrent hypothyroidism, 14.9% developed diabetes mellitus following a recent oestrus and 1.1% had islet cell hypoplasia. The remaining 50 dogs had no identifiable diabetes associated syndromes.

Based on only a small sample, diabetes mellitus associated with proligestone administration was not transient but required continuous insulin therapy. Insulin insensitivity (high insulin requirement) was not a consistent feature of this condition.

In the case of concurrent hyperadrenocorticism and diabetes mellitus, small breeds appeared to be more commonly affected and the degree of insulin insensitivity (insulin requirement) was related to the time between the diagnosis of diabetes mellitus and that of hyperadrenocorticism. In 89% of cases the diagnosis of diabetes mellitus preceded the diagnosis of hyperadrenocorticism and the association between insulin insensitivity and glucocorticoid excess was exemplified by the amelioration of insulin requirement following subsequent adrenocorticolytic therapy.

In all cases of concurrent hypothyroidism and diabetes mellitus, the diagnosis of hypothyroidism was made following the diagnosis of diabetes mellitus. There was unusual breed representation within this group with two each of Tibetan terriers and English setters - breeds of dog not represented in any other diabetic dog group. Insulin insensitivity (high insulin requirement) was an important feature of this condition and there was a subjectively high prevalence of co-existing neuropathy, pyoderma and pancreatitis. The condition observed in these dogs did not fit the classical description of Schmidt's syndrome in man because of the absence of hypoadrenocorticism.

Metoestrus-associated diabetes mellitus was diagnosed approximately 6 weeks post-oestrus on average and varying degrees of reduced insulin requirement were observed along with the requirement for continued insulin therapy. Basal plasma insulin analyses at the time of diagnosis may be a useful prognostic indicator. Classical signs of canine acromegaly were a rare finding present in only one of these dogs.

The distribution of age at diagnosis of diabetes mellitus was different between the groups of diabetic dogs. In general, hypothyroid dogs were younger, and dogs with hyperadrenocorticism were older, than 'normal' diabetic dogs. However, there was no difference in gender distribution between the groups (with the exception of the metoestrus-associated group). There was no difference in initial concentration of plasma biochemical analytes, nor in body condition score between the groups but there was a difference in insulin requirement.

In no previously reported large case series of diabetic dogs has there been an attempt to estimate the relative frequency of different types of diabetes mellitus within the study population (Lauder, 1972; Foster, 1975; Ling *et al*, 1977; Marmor *et al*, 1982; Doxey *et al*, 1985), However, Lauder (1972) recorded one case of hyperadrenalism in a group of diabetic dogs which underwent post-mortem examination and suggested that 40% of diabetic dogs were entire bitches which developed the condition shortly after oestrus. In addition, Foster (1975) recorded three cases of diabetes mellitus and concurrent (unspecified) endocrine disease and observed that 29% of diabetic dogs were entire bitches which developed diabetes mellitus within 10 weeks of the last oestrus. There have been reports of the proportion of dogs with hyperadrenocorticism which have co-existent diabetes mellitus (22% - Peterson *et al*, 1981) and the proportion of dogs with hypothyroidism that have concurrent diabetes mellitus (1.2% - Milne and Hayes, 1981) but not of the proportion of diabetic dogs with either of these two conditions.

The subject of progestagen (proligestone)-associated diabetes mellitus was a topic of great debate in the veterinary literature during the late 1980's (Milne, 1987; Milne, 1988; Evans and Sutton, 1988a; Rutteman *et al*, 1988; Evans and Sutton, 1988b) as a result of suggestions by the distributors that proligestone was not diabetogenic and could be safely used in diabetic bitches for prevention of oestrus. Rutteman *et al* (1988) saw an unspecified number of dogs which developed diabetes mellitus while under proligestone therapy. Evans and Sutton (1988) claimed that no such cases had been reported to the manufacturers or the Veterinary Medicines Directorate in the UK during the period 1978-1988. It has since become apparent that proligestone can induce growth hormone

secretion and that it can therefore be considered to be diabetogenic (Selman *et al*, 1994c) and as a consequence is contraindicated in dogs with diabetes mellitus.

A similar breed predisposition for concurrent diabetes mellitus and hyperadrenocorticism to that observed in the present study was reported by Peterson *et al* (1981). Within their group of 30 dogs, 56% were Poodles and 13% terriers. Of the group of 8 dogs with concurrent hyperadrenocorticism and diabetes mellitus reported by Blaxter and Gruffydd-Jones (1990), 37% were poodles and 12% terriers. A greater proportion of the cases reported by Peterson *et al* (1981) had hyperadrenocorticism diagnosed before diabetes mellitus (23%) than in the present study but in the group of dogs reported by Blaxter and Gruffydd-Jones (1990), all diagnoses of hyperadrenocorticism were made after the diagnosis of diabetes mellitus.

Previously published basal plasma insulin analysis in three dogs with concurrent hyperadrenocorticism and diabetes mellitus (Blaxter and Gruffydd-Jones, 1990) revealed very high concentrations, contrary to the findings of the present study of normal to low concentrations (Serono Clinical Laboratories, Cambridge canine insulin reference range 5-40 μIU/ml). The low basal insulin concentrations observed may be indicative of β-cell exhaustion due to chronic stimulation by glucocorticoid induced insulin insensitivity and may explain the requirement for continuous insulin replacement therapy despite adrenocorticolytic therapy seen in the present study and in that of Peterson *et al* (1981). Blaxter and Gruffydd-Jones (1990) noted a reduction in insulin insensitivity associated with a more aggressive adrenocorticolytic therapy regimen (mitotane at 50mg/kg/day) than that used in the present study but which resulted in the requirement for intensive therapy for hypoglycaemia in 3/8 cases. Hypoglycaemia occurred in only one dog in the present study but unfortunately this episode was fatal. Peterson *et al* (1981) recorded reduced (but not abolished) insulin requirement within 3 weeks of adrenocorticolytic therapy. There have been no previous reports relating the duration of diabetes mellitus prior to a diagnosis of hyperadrenocorticism and the level of insulin requirement before adrenocorticolytic therapy. However, the finding in the present study of a direct correlation emphasises the dynamic nature of these concurrent illnesses. It may be that early in the course of concurrent diabetes mellitus and perhaps unrecognised or sub-clinical hyperadrenocorticism there may be considerable β-cell function but which is insufficient to overcome the insulin insensitivity induced by glucocorticoid excess, meaning that only a low dose of exogenous insulin is required to make up the shortfall. Later in the course of the condition, β-cell function may wane, and glucocorticoid induced insulin insensitivity may increase, necessitating higher doses of exogenous insulin to achieve glycaemic control. Interestingly, both the present study and that of Blaxter

and Gruffydd-Jones (1990) include a single case of bilateral keratoconjunctivitis sicca (KCS). It may be that this represents a rare complication of the two conditions when they occur in combination.

None of the cases of diabetes mellitus in the present study were induced by exogenous glucocorticoid administration.

Five dogs in the USA with concurrent hypothyroidism and diabetes mellitus have been the subject of detailed reports (Eigenmann *et al*, 1984; Ford *et al*, 1993) and less extensive reports have stated the occurrence of the condition in a further 48 dogs (Milne and Hayes, 1981, Hargis *et al*, 1981). Ford *et al*, (1993) noted insulin insensitivity in the form of high insulin requirement in the three dogs featured in their report, consistent with that observed in 4/8 dogs in the present study. In man (Ganz and Kozak, 1974) and some dogs (Eigenmann *et al*, 1984), however, the combination of hypothyroidism and diabetes mellitus leads to a reduced insulin requirement. The familial or genetic basis of the condition is highlighted by the two breed clusters in the present study, the occurrence of the syndrome in two littermates (Eigenmann *et al*, 1984) and its occurrence in a colony of beagles (Hargis *et al*, 1981) in which there was clustering of the syndrome within litters.

In all cases of concurrent hypothyroidism and diabetes mellitus reported by Hargis *et al* (1981), hypothyroidism was diagnosed prior to the development of diabetes mellitus, contrary to the findings of the present study. All necropsy reports of concurrent hypothyroidism and diabetes mellitus have revealed islet vacuolation without inflammatory infiltration (Hargis *et al* 1981; Eigenmann *et al*, 1984). Unfortunately, the one diabetic dog with hypothyroidism made available for post-mortem examination in the present study was suffering from active pancreatitis at the time of euthanasia. A case series of 66 hypothyroid dogs (Panciera, 1994) recorded 19 with neurological abnormalities including peripheral neuropathy so the finding of peripheral neuropathies in 2/8 dogs in the present study is not surprising. However, it is interesting that the two English setters had very similar neuropathies affecting the same limb.

Polyendocrine failures in man have been associated with autoimmune syndromes (Eisenbarth, 1985; Ganz and Kozak, 1974) and a similar association has been suggested in dogs (Bowen *et al*, 1986; Kintzer, 1992). Future studies will be directed at discovering what, if any, autoimmune processes underlie concurrent hypothyroidism and diabetes mellitus in dogs.

The association between metoestrus and diabetes mellitus has been established for a number of years (Lauder, 1972; Foster, 1975; Eigenmann, 1981; Eigenmann and Venker-van Haagen, 1981). The lower prevalence of metoestrus-associated diabetes mellitus in the present study than that of Lauder, (1972) and Foster (1975) may be a reflection of improved veterinary education and a reduced number of this kind of referral or reflection of an increase in elective ovariohysterectomy in dogs over the last 20 or so years.

In previously published reports, metoestrus-associated diabetes mellitus has been completely 'cured' by ovariohysterectomy (Eigenmann, 1981; Eigenmann, 1989, Reusch *et al*, 1993) or has required continued insulin replacement therapy despite ovariohysterectomy (Eigenmann, 1981). There are no previous reports of temporary remission of diabetes mellitus nor of the use of basal plasma insulin concentration as a prognostic indicator. Based on the small number of cases in the present study, a cut-off value for basal plasma insulin concentration in the range 20 to 40 μIU/ml might be very useful in determining which dogs are likely to be completely 'cured' by ovariohysterectomy. Based on the experience of the present study, classical findings of canine acromegaly (Eigenmann and Venker-van Haagen, 1981) are not a common feature of this condition.

Islet cell hypoplasia in the dog has not been previously reported in Britain and the presentation of the one dog in this study was consistent with those reported in the USA (Anderson *et al*, 1986; Atkins *et al*, 1988). It is noteworthy that Cairn terriers are among those breeds identified in Chapter 3 of this thesis as being at high risk for the development of diabetes mellitus.

The difference in age at diagnosis of diabetes mellitus between the 4 groups studied is an interesting finding which has not been previously reported in other case series. The younger age of the hypothyroid dogs suggests a genetic (Milne and Hayes, 1981) or immunological disease process. The older age of dogs with concurrent hyperadrenocorticism is consistent with the age distribution of canine hyperadrenocorticism (Chastain and Ganjam, 1986c) and agrees with the finding of Peterson *et al* (1981), of 87% of cases being 8 years of age or more at diagnosis (88% in the present study).

With the exception of minimum and maximum values for alkaline phosphatase concentration from a group of 43 'normal' diabetic dogs for comparison with results from 8 diabetic dogs with hyperadrenocorticism (Blaxter and Gruffyyd-Jones, 1990),

comparisons of initial biochemical results between different types of diabetic dog have not been previously reported. The lack of difference between the groups in concentrations of alkaline phosphatase and cholesterol suggests that, in diabetic dogs, these analytes, which are traditionally used as an aid to diagnosis of hyperadrenocorticism and hypothyroidism, respectively are likely to be of little value in identifying these endocrinopathies.

Although a difference in basal insulin concentration might have been expected between those conditions associated with insulin insensitivity and those which are not, the fact that there was no difference highlights the dynamic nature of these conditions, the possibility of β-cell exhaustion and the presence of (perhaps obesity-related) insulin insensitivity in a group of 'normal' diabetic dogs. By the time some diabetic dogs, even those with insulin insensitivity as the primary mechanism for their diabetes mellitus were sampled, some degree of β-cell exhaustion may have occurred.

The present study confirms that ACTH and TSH stimulation tests were valid methods of diagnosis of concurrent endocrinopathy in diabetic dogs. Close examination of cortisol results in normal and hypothyroid dogs (Appendices 5 and 7) revealed certain cases where there are post-ACTH cortisol concentrations close to those in the hyperadrenocorticism group. The ACTH stimulation test results were interpreted cautiously and always with regard to signalment and clinical presentation, as is necessary when this test is used in non-diabetic dogs.

The analysis of insulin requirement confirmed that concurrent hyperadrenocorticism and diabetes mellitus was associated with a high insulin requirement consistent with the findings of Peterson *et al* (1981) and Blaxter and Gruffyyd-Jones (1990) and suggested that dogs with metoestrus-associated diabetes mellitus remaining on insulin therapy following ovariohysterectomy have unusually low requirements.

In conclusion, the relative occurrence of recognisable types of canine diabetes mellitus in a referred population of dogs has been documented. Some of the findings of previous reports of diabetes mellitus in association with other syndromes were confirmed, such as the predisposition of Poodles and terriers to hyperadrenocorticism-associated diabetes mellitus, the reduction of insulin requirements in affected dogs by adrenocorticolytic therapy, the occurrence of keratoconjunctivitis and neuropathies in diabetic dogs with concurrent endocrinopathies, the possibility of insulin insensitivity in concurrent hypothyroidism and the clinical and histopathological presentation of canine islet cell hypoplasia. Original observations included: normal or low basal plasma insulin

concentrations in diabetic dogs with hyperadrenocorticism; a relationship between duration of diabetes mellitus and degree of insulin requirement in dogs with hyperadrenocorticism; a difference in the average ages of 'normal' diabetic dogs and those with hypothyroidism or hyperadrenocorticism; ovariohysterectomy in dogs with metoestrus-associated diabetes mellitus may result in temporary remission of insulin requirement and that basal insulin analyses may be a useful prognostic tool in these dogs.

In summary, there are distinct presentations of canine diabetes mellitus and knowledge of these can only improve their recognition and therefore the accuracy of prognoses offered.

Future studies will be directed at elucidating the underlying immunologic or genetic basis of concurrent hypothyroidism and diabetes mellitus and the validation of the basal insulin observations in metoestrus-associated diabetes mellitus by accumulation of additional cases.

Chapter 5:
Long-term monitoring of glycaemic control

Introduction

The ultimate aim in diabetic management is to restore euglycaemia by utilising therapeutic measures to compensate for the dysfunction of mechanisms responsible for glucose homeostasis. In the design, implementation and adjustment of therapeutic protocols, the ability to determine the degree of success in achieving this aim is imperative.

The ideal system for monitoring glycaemic control in treated diabetic people or animals would be continuous blood glucose monitoring. Experimentally, this has been acheived in human diabetic patients in the form of an 'artificial pancreas' which took measurements of blood glucose concentration every minute (1440 samples per day) to enable adjustments in insulin infusion rate (Albisser *et al*, 1974). Unfortunately, technical problems of miniaturisation of such systems and of reliability of glucose sensors has meant that they have not become a viable option. Currently, in human diabetic medicine the most common practical alternative to continuous monitoring is repeated thumb-prick capillary blood glucose testing, often performed between 5 and 10 times daily (Albisser, 1992) using a dedicated portable glucometer and reagent strips. In the veterinary situation, however, this degree of blood glucose monitoring is impractical and prohibitively costly. A less invasive and expensive method than repeated serial blood glucose analyses is required for the assessment of glycaemic control in diabetic dogs. Ideally, such a method would be based on the inexpensive measurement of a single analyte on a single sample obtained at any time of day to gain an accurate assessment of glycaemic control achieved by a particular therapeutic regimen or insulin dose.

There are a number of candidate blood biochemical analytes which could be considered to fulfil this role in the dog, namely: blood concentrations of nadir glucose, alkaline phosphatase, alanine aminotransferase, asparatate aminotransferase, fructosamine and glycated haemoglobin.

Nadir blood glucose concentrations can be used to give an accurate assessment of glycaemic control at only a very specific and ephemeral point in the day and therefore

can be thought to have little value as indicators of overall glycaemic control. However, if used for the purposes of monitoring patients in which the daily effective duration of the regimen has already been assessed by the creation of a 24 hour serial glucose curve then single daily nadir blood glucose concentrations may have a place.

The next most likely candidate analytes are the so-called 'liver enzymes' (alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase) since their measurement is inexpensive and already features as part of the general or routine biochemical health screen. Their potential value as indicators of glycaemic control lies in the fact that the plasma concentrations of these analytes are often grossly elevated in untreated and unstable diabetic dogs (Nelson, 1989a). It is possible that treated and stable diabetic dogs would have normal or near normal plasma concentrations.

Alkaline phosphatase is an enzyme which has a number of iso-forms originating from bone, intestine and liver including a specific steroid-induced form of hepatic origin. Conditions associated with cholestasis induce increased production of hepatic alkaline phosphatase (by enhanced RNA transcription) and its consequent release into the circulation (Cornelius, 1989). Alanine aminotransferase is an enzyme whose elevation in plasma of small animals is associated with conditions of hepatocellular necrosis (Cornelius, 1989). In uncontrolled diabetic animals there is generally hepatomegaly consequent upon the mobilisation of lipid stores. This hepatic fatty infiltration leads to hepatocellular death and to increased intrahepatic pressure on the biliary tree, and therefore cholestasis, resulting in elevated plasma concentrations of these enzymes. Aspartate aminotransferase is much less 'liver specific' in dogs than alanine aminotransferase and raised plasma concentrations are more often associated with damage to many other soft tissues, particularly muscle (Kramer, 1989). Concentrations of plasma aspartate aminotransferase are elevated in unstable diabetics probably because of protein (muscle) catabolism for gluconeogenesis in addition to the contribution made by hepatic aspartate aminotransferase.

In human diabetic medicine, the measurement of glycated proteins, including haemoglobin, has been used to assess glycaemic control. The non-enzymatic chemical combination of sugars and proteins to form stable ketoamine products has been known of for many years. Emil Fischer synthesised isoglucosamine (fructosamine) in 1886 (Kruse-Jarres *et al*, 1989), and in 1912, Maillard described the reaction responsible and noted its potential importance to medical pathology (Dominiczak, 1991). Food technologists have exploited the Maillard reaction for many years because the products are brown in colour and it is for this reason that the process is commonly referred to as

the 'browning reaction'. The first stage in the Maillard reaction is the condensation of the free aldehyde group of a sugar with the amino group of the protein. This results in an unstable Schiff-base compound (aldimine) which can freely dissociate back into sugar and protein and whose concentration is dependent on prevailing glucose concentration. Alternatively, such compounds can undergo molecular transformation by Amadori rearrangement into much more stable ketoamines (Dominiczak, 1991) (Figure 13).

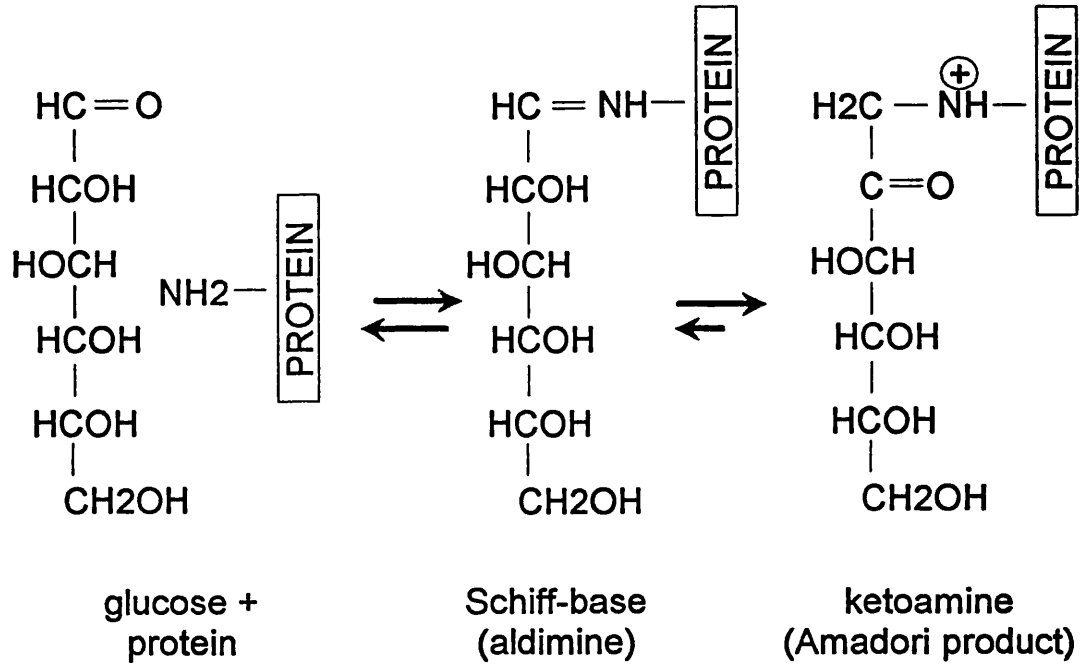

Figure 13. The glycation reaction.

Glycosylated haemoglobin, or more correctly, glycated haemoglobin (Roth, 1983), was the first glycation product to be used by medical diabetologists to monitor diabetic control. The minor components of human haemoglobin were discovered by cation exchange chromatography in 1958 and termed HbA1a, HbA1b and HbA1c. Collectively these haemoglobin variants accounted for less than 10% of total haemoglobin (Allen *et al*, 1958). The association between elevated concentrations of particularly HbA1c and the presence of diabetes mellitus in humans was recognised by the early 1970's but not understood until 1976 when it was discovered that HbA1c could be generated by incubation of haemoglobin or whole blood with glucose (Fluckiger and Winterhalter, 1976). Human HbA1c is haemoglobin (HbA) modified by glucose at the amino terminus of the β chain. This modification generates a negative charge on the new molecule and this is the basis for the separation and measurement of HbA1c by ion exchange

chromatography, isoelectric focusing, high performance/pressure liquid chromatography and agarose gel electrophoresis. HbA1c is the principal human glycated haemoglobin fraction but glycation occurs at a number of loci along both the α and β chains of the haemoglobin molecule (principally at lysine sites). The measurement of all glycated haemoglobin, and not just that which is responsible for large alterations in charge can be achieved using affinity chromatography. The basis for this separation technique is the affinity of boronic acid for *cis*-diol groups. Boronate immobilised on a gel matrix retains glycated haemoglobins and the non-glycated component passes through. The adsorbed glycated products can then be displaced by a high concentration of a competing ligand such as sorbitol. It is likely that affinity choromatography methods would be more applicable to the measurement of glycated haemoglobin in domestic animals where the structural forms of glycated haemoglobin and their ionic properties are not known. Glycated haemoglobin concentrations have been used routinely in human diabetic monitoring since the early 1980's and they are believed to reflect integrated blood glucose concentrations over the preceding 7-8 weeks (approximately the average half-life of human red blood cells). The measurement of glycated haemoglobin in dogs has been reported by Wood and Smith (1980) using macro-column ion-exchange chromatography, by Mahaffey and Cornelius (1982) using a mini-column ion-exchange method and by Smith *et al*, (1982) using a colorimetric assay as an alternative to column methods. Higgins *et al*, (1982) compared the concentrations of glycated haemoglobin in a number of species commonly used in diabetes research, including dogs, using cation exchange chromatography, agarose gel electrophoresis and affinity chromatography. One of the conclusions of this work was that despite the similar red cell life span between dogs and humans (120 days), glycated haemglobin concentrations in dogs were likely to be lower than in humans because the permeability to glucose of canine red cells is approximately half that of human red blood cells.

Shortly after the introduction of glycated haemoglobin measurement in human diabetes clinics, attention shifted to the possibility of using other glycation products such as serum albumin and other proteins. The first reports of glycated albumin synthesis *in vitro* and its isolation from human serum were published in 1979 (Dolhofer and Wieland, 1979; Day *et al*, 1979) and these used chromatographic techniques and a colorimetric thiobarbituric acid method which measured 5-hydroxymethylfurfural (5-HMF) released by acid hydrolysis of ketoamine-protein complexes. A very much more convenient simple colorimetric test for glycated proteins in serum was described by Johnson *et al* (1982) and was termed the fructosamine test. This test was further modified to limit interference from sample matrix and lipaemia and included a new glycated polylysine standard (Kruse-Jarres *et al*, 1989), and became available in a simple-to-automate kit form. The

main drawback of the fructosamine assay is that about 90% of fructosamine activity measured in human serum is glycated albumin (Kennedy, 1992). This makes the test sensitive to fluctuations in serum albumin concentration and a number of workers have advocated the use of albumin or protein corrected values (McCance *et al*, 1987) whilst others have suggested that they are unneccessary except in severely protein deficient states (Kruse-Jarres *et al* 1989; Rowe and Dominiczak, 1989). The measurement of fructosamine in humans has now been reported and critically reviewed in many publications. The use of the assay in veterinary medicine has also started to receive attention not only as an indicator of diabetic control in dogs and cats (Reusch *et al*, 1993; Kawamoto *et al*, 1992; Jensen, 1992; Jensen and Aaes, 1992; Jensen 1993) but also as an indicator of protein turnover (Heath and Connan, 1991) and malnutrition (Cantley *et al*, 1991) in sheep.

When using a laboratory test in the diagnosis or monitoring of a disease condition it is essential to regard such a test as an aid. The confidence with which decisions regarding diagnosis or prognosis can be made when based on laboratory tests depends greatly on the quality of the performance characteristics of such tests.

There are a number of components to the assessment of the quality of a new laboratory test or of an old test used for a new purpose. The initial stage in the evaluation of a laboratory test is to define the performance characteristics of the test within the analytical laboratory. Important factors such as consistency of results, smallest concentration of analyte detectable and whether the test measures the analyte for which it is designed are normally assessed by the performance characteristics: intra and inter-assay coefficients of variation, sensitivity (limit of detection) and accuracy. For the test to be useful when applied to the clinical setting, there must be a difference between the results generated from the healthy and diseased populations on which a reference range can be based and the test must be able to distinguish between healthy and diseased animals within a target population of animals suspected of being diseased.

To assess the usefulness of a laboratory test based on the last criterion of distinguishing between healthy and diseased animals in a target population, indices have been developed based on the rate (ratio) at which a laboratory test gives true (as opposed to false) positive and true negative predictions of the presence of a disease. These indices have been termed the sensitivity (true postive ratio) and specificity (true negative ratio) of the assay (Youden, 1950; Ryan, 1991; Jensen and Poulsen, 1992) and are calculated according to Table 16. Normally such indices would be developed on a 'test' target

population in which the diagnosis of the presence of disease could be determined by a different and independent means.

	Diseased	Healthy
Test Postive	A	B
Test Negative	C	D

Sensitivity (true positive ratio) = A/(A+C)

Specificity (true negative ratio) = D/(B+D)

Table 16. Calculation of sensitivity and specificity of a laboratory test.

The ideal laboratory test would give no false positives or false negatives and so sensitivity and specficity would both equal 1. Certainly, to be useful, a laboratory test must give more true positive than false positive results and more true negative than false negative ones. In effect, this means that the sensitivity and specificity must both be greater than 0.5 to make the test more useful than tossing a coin. Youden (1950) proposed the creation of a performance index (J = Sensitivity + Specificity - 1) such that a perfect test would have a performance index, J = 1 and that a worthless test would have a performance index, J = 0.

The use of the method outlined above to assess laboratory test performance depends on a dichotomous test result. This works well for some tests, particularly serological and parasitological tests in which the antibodies or parasites are detectable or they are not. However, many biochemical and haematological test methods give rise to a continuous scale of possible results and a dichotomous outcome has to be artifically created. This is achieved by selecting a cut-off point above or below which a disease can be said to be present. For example, anaemia is usually deemed to be present when a packed cell volume result is below an arbitrary cut-off value. Commonly cut-off values are selected from the limits of the traditional reference range (mean ± 2SD's or 5 and 95 percentiles of results obtained from a reference population) and sensitivity and specificity indices calculated on that basis. However, the selection of a different cut-off point will alter the ratio of true positives and true negatives and therefore the sensitivity and specificity, particularly if the ranges of results generated from diseased and healthy animals overlap greatly.

As a general rule, the specificity of a laboratory test will increase, but sensitivity decreases, as the cut-off value is shifted towards the affected animals and sensitivity will increase, but specificity decreases as the cut-off value migrates towards non-affected

animals. This means that as the cut-off value is varied across a continuous range of test results an associated range of pairs of sensitivity and specificity values is created. This range of sensitivity and specificity pairs form the basis of the relative (or receiver) operating characteristic (ROC) curve. The generation of an ROC curve means that the usefulness of a laboratory test can be assessed across the whole range of possible results and in a way which is independent of a particular cut-off value. ROC curves have been used in human laboratory medicine since the late 1970's but in veterinary laboratory medicine only recently and rarely (Jensen, 1994). The ROC curve is created by plotting sensitivity (true positive ratio) on the ordinate (y-axis) against the false positive ratio (1-specificity) on the abscissa (x-axis) for each of a range of cut-off values. In an ideal laboratory test which could perfectly distinguish between healthy and diseased individuals the ROC curve would be drawn from the origin (0,0) to (0,1), the top left hand corner and then horizontally across to (1,1), the top right hand corner of the plot. A test which has no value and produces true and false positive results at an equal rate will have an ROC curve which is the diagonal (0,0) to (1,1). Therefore, the more useful the laboratory test, the more the ROC curve will extend into the top left hand corner of the plot. This can be measured more accurately in terms of the area under the ROC curve. A perfect test will have an area under the ROC curve of 1 and a useless test an area under the curve of 0.5 (Hanley and McNeil, 1982). Thus it is possible to have a single value (the area under the ROC curve) with which to determine the usefulness of a laboratory test for a particular purpose, independent of cut-off values, and with which direct comparisons can be made with other candidate laboratory tests designed for that same clinical purpose. Statistical methods have also been developed to determine the significance of a difference between the area under an ROC curve and 0.5 with which the assessment of the laboratory test can be supported (Hanley and McNeil, 1982).

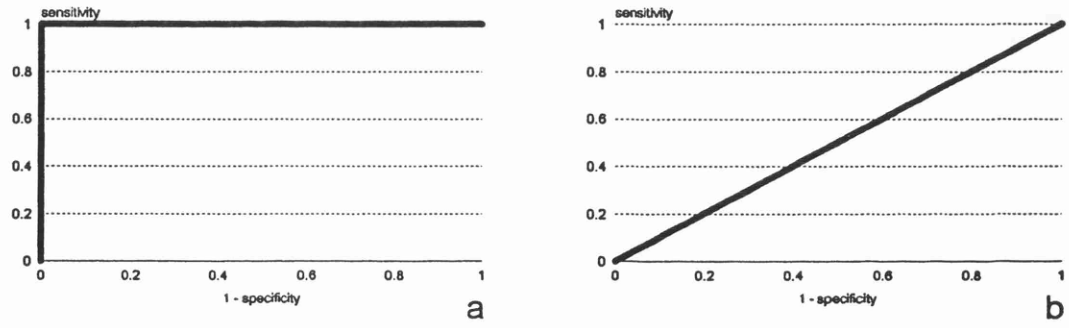

Figure 14. Idealised ROC curves for (a) a perfect laboratory test and (b) a useless laboratory test.

Once a laboratory test has been determined to be useful for a particular purpose by the ROC curve method, the next stage is to determine what the cut-off value needs to be to make the test the most 'efficient'. This can be achieved by an alternative representation of the data included in the ROC curve. The differential positive rate (DPR) can be calculated from the pairs of sensitivity and specificity values generated across a range of cut-off values for creation of the ROC curve and it is the difference between the true positive and false positive ratios (sensitivity - (1 - specificity)) (Ward, 1986). The optimal cut-off value is that at which this difference (DPR) is greatest. DPR is a linear transformation of Youden's performance index (J = sensitivity + specificity - 1) and so the same result can be achieved by calculating a series of Youden's indices across the range of cut-off values (Jensen, 1994). The maximum DPR value can be used in a similar way to the area under the ROC curve when comparing laboratory tests designed for the same purpose. A maximum DPR value of 1 would indicate a perfect test and of 0 a worthless test.

The aims of this chapter were to validate the use of two new laboratory tests (fructosamine and glycated haemoglobin) in the University of Glasgow Veterinary School and to assess the usefulness of measuring plasma concentrations of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and the concentration of glycated haemoglobin in the short and long term monitoring of canine diabetes mellitus using advanced techniques of laboratory test evaluation.

Materials and methods

Fructosamine

Plasma fructosamine concentrations were measured using a commercial colorimetric test kit based on the ability of keto-amines to reduce nitroblue tetrazolium (NBT) in alkaline conditions (Unimate Fructosamine, Roche). The production of formazan forms of NBT is proportional to the concentration of fructosamine and was measured photometrically using an autoanalyser. The autoanalyser protocol was supplied by the kit manufacturers. The reaction pH and the time course ensure negligible interference from other reducing substances such as glucose and creatinine (Johnson *et al*, 1982). Using this method fructosamine measurements are made against a calibrator solution based on glycated polylysine supplied by the kit manufacturer (Fructosamine Calibrator, Roche). To confirm the correct function of this test in canine plasma samples a number of validation procedures were performed. Assay precision was assessed on the basis of intra- and inter-assay coefficients of variation. Intra-assay coefficient of variation was estimated at 4 different concentrations of fructosamine using pooled plasma on three occasions and plasma from an individual animal on the fourth (Case no. 122739). Inter-assay

coefficients of variation were estimated at two concentrations of fructosamine over 8 occasions.

$$CV\% = \frac{SD}{Mean}$$

The sensitivity, or limit of detection, of the assay kit was estimated by analysis of 20 replicates of physiological saline for fructosamine concentration. Linearity of the method was assessed by measurement of fructosamine concentrations in serial dilutions of a plasma pool of high fructosamine concentration as follows: 1 in 1.5, 1 in 2, 1 in 3, 1 in 4, 1 in 6 and 1 in 8. The effect of lipaemia on fructosamine results was studied because plasma taken from diabetic dogs is frequently lipaemic. This was performed by adding 15µl of varying concentrations of a commercial lipid based enteral nutrition product (Intralipid - soya bean oil and glycerol emulsion, Kabi Pharmaceuticals) to 200µl aliquots of plasma pools containing high and low concentrations of fructosamine, thus creating a range of samples from no visible lipaemia through to gross lipaemia similar to that seen in some diabetic plasma samples.

Samples of whole blood from 4 dogs (nos. 97218, 115678, 115980, 120899) were stored on a laboratory bench for 48 hours and aliquots removed at 24 hour intervals for analysis of plasma fructosamine concentration to simulate delayed transport to the analytical laboratory.

A conventional reference range for non-diabetic dogs was created by the analysis of fructosamine concentration in 20 samples of surplus dog plasma submitted for routine clinical chemistry analysis.

Glycated haemoglobin

Glycated haemoglobin concentrations were measured using a commercially available mini affinity column chromatography kit (GLYCO-Tek Affinity Columns; Helena Laboratories). This method separates all glycated haemoglobins from non-glycated haemoglobin in lysed red blood cells based on the the affinity of dihydroxyboryl groups (bound to a celluose resin) for *cis*-diol groups present in simple sugars (including glucose) incorporated in glycated haemoglobin. A basic eluent removes all non-glycated haemoglobin, labile glycated haemoglobin and carbamylated haemoglobin from the chromatography column but leaves the glycated forms which are eluted using a sorbitol buffer. A comparison of the concentrations of the glycated and non-glycated haemoglobins is made using photometric determination of haemoglobin concentration at

415 nm and a percentage concentration of glycated haemoglobin calculated using the following formula:

$$GlycHB\% = \frac{Abs.GlycHB}{Abs.GlycHB + (5 \times Abs.NonGlycHB)} \times 100\%$$

Volume of Non-Glycated haemoglobin eluent = 15ml
Volume of Glycated haemoglobin eluent = 3ml

To assess the precision of this method, intra-assay coefficients of variation were calculated for 4 samples of canine blood: three diabetic and one non-diabetic. Inter-assay coefficients of variation were calculated for 8 replicate analyses of glycated haemoglobin in two blood samples (one diabetic and one non-diabetic) over a period of three weeks. This information was also used to assess the long term effects of refrigerated storage of samples intended for glycated haemoglobin analysis. The effects of sample quality and sample storage conditions were examined by repeated analyses of 5 aliquots of a single canine sample stored in a refrigerator over two weeks or stored at room temperature for one week. Two aliquots were haemolysed by repeated passage through a fine (23 gauge) hypodermic needle at the beginning of the study.

To verify that glycated haemoglobin was being measured, an incubation experiment was conducted. A solution containing 4% dextrose saline solution was added to three whole blood samples with initial glycated haemoglobin concentrations of 2.2, 3.3 and 2.1%, in the proportions 9 parts whole blood to 1 part dextrose saline. This gave an approximate final concentration of glucose in each sample of approximately 27 mmol/l which is close to the physiological maximum plasma glucose concentrations seen in diabetic patients with good renal perfusion and function.

Comparison with two other methods of analysis of glycated haemoglobin were made. The first was with a commercially available cation exchange kit (Glycohaemoglobin HbA1 Test; Bio-Stat Diagnostics). This kit binds non-glycated haemoglobin to a weak binding cation exchange resin and allows photometric determination of glycated haemoglobin in the supernatant. The concentration of glycated haemoglobin is then calculated from the absorbances of total and glycated haemoglobin in the sample and those of a standard glycated haemoglobin solution. This kit measures HbA1c which is the principal component of human glycated haemoglobin and consists of haemoglobin with glucose bound at the terminal valine sites of the two β chains. Glucose attached at this site alters the isoelectric point of the haemoglobin molecule and allows for separation

from non-glycated haemoglobin by cation exchange chromatography. Ten whole blood samples from 7 diabetic dogs were used to make the comparison between the cation exchange and affinity column methods over two analysis days. Simple scatter plot and linear regression were used to assess the relationship between results obtained by the two methods.

The second method of analysis for comparison with affinity column chromatography was agarose gel electrophoresis. A commercial agarose gel kit (Diatrac, Beckman) and scanning densitometer (Appraise, Beckman) were used to analyse 16 whole blood samples from 15 diabetic dogs and one dog with a functional β-cell tumour over two analysis days. This kit measures HbA1c based on the difference in charge, and therefore electrophoretic mobility, between non-glycated haemoglobin and HbA1c.

To assess the effect of anticoagulant on the assay, 9 whole blood samples from 8 diabetic dogs and one non-diabetic dog were analysed over three analysis days. Simple regression analysis was used to determine the relationship between the results of both types of samples and a paired *t*-test was used to test for differences between the methods.

A conventional reference range for non-diabetic dogs was created by analysis of glycated haemoglobin concentration in 26 samples of surplus canine whole blood submitted for routine haematological analysis.

Daily variation

The measurement of plasma glucose is useful in monitoring diabetic therapy. However, in a diabetic therapy regime using only single daily injections of an intermediate acting insulin preparation there can be wide variations in glucose concentration over a 24 hour period, severely restricting the time of day at which a useful determination can be made. Before assessing the usefulness of alkaline phosphatase, alanine aminotransferase and fructosamine for diabetic monitoring, it was important to determine whether these analytes were subject to fluctuations during the day in a manner similar to plasma glucose concentrations. Because of the manual and expensive nature of glycated haemoglobin analyses, an equivalent study of this parameter was not performed. The serial analysis of aspartate aminotransferase was also not performed. Three dogs were bled at regular intervals over 24 hours as part of a study of the effects of diet on glycaemic control (Chapter 7) and plasma concentrations of alkaline phosphatase, alanine aminotransferase and fructosamine were determined in surplus plasma. Plasma concentrations of alkaline phosphatase, alanine aminotransferase and fructosamine were transformed to percentage changes from basal for graphing purposes and general linear

model analysis (two way analysis of variance using the model: analyte = dog time) was used to test for the effect of sampling time on plasma concentrations of these analytes.

Monitoring early diabetic therapy

To compare the usefulness of measuring the plasma concentrations of afternoon glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and the concentration of glycated haemoglobin for monitoring the early response to diabetic therapy, three early sampling periods were chosen. These sampling periods were 0-7 days (Period A), 8-21 days (Period B) and 22-42 days after the start of treatment (Period C) and they were chosen to include samples obtained before treatment and those obtained at the first two follow up examinations post-stabilisation. After the first 6 week period owners did not always return with their pets with sufficient regularity to continue this type of monitoring. Only the biochemical results of 20 dogs which had fructosamine concentrations measured within at least two of the early sampling periods were used for analysis in order to reduce the number of missing results, to improve the reliability of the statistical analyses and to allow for a valid comparison between the parameters. Only 15 of these dogs had glycated haemoglobin measurements performed. Statistical analysis was performed using general linear model analysis (two way-analysis of variance model) to determine whether there was a significant change in the concentration of the parameter. For those parameters in which there was a significant change, a Newman-Keuls multiple range test ('macro' for MINITAB by DR Irvine) was performed to determine how the sampling periods differed.

The effect of the duration of diabetes mellitus prior to treatment (based on the number of weeks of polydipsia) on pre-treatment concentrations of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin concentration was investigated using simple regression analysis.

Long-term diabetic monitoring

To assess the usefulness of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin concentrations as aids to monitoring long-term diabetic control, the results of many hundreds of follow-up examinations were coded according to the degree of glycaemic control which was believed to prevail at that time. This was achieved according to afternoon blood glucose concentrations and clinical information provided by the owner or discovered on clinical examination. The group codings were defined as follows:

Group 1	untreated diabetics;
Group 2	very poor control (high afternoon blood glucose concentrations and clinical signs such as polyuria/dipsia);
Group 3	poor control (high afternoon blood glucose concentrations but without clinical signs of diabetic instability or good afternoon blood glucose concentrations but recent history of significant clinical signs of instability);
Group 4	fair control (moderately elevated afternoon blood glucose concentrations and no history of clinical signs of instability or good afternoon blood glucose concentrations but with very occasional signs of moderate instability);
Group 5	good control (good afternoon plasma glucose concentrations and no clinical signs of diabetic instability);
Group 6	excellent control (afternoon blood glucoses in the range 3 - 5 mmol/l and serial blood glucose evidence of good 24 hour diabetic control);
Group 7	excessive insulin dose (generally, afternoon blood glucose concentrations below 3mmol/l and/or clinical signs of hypoglycaemia)
Group 8	non-diabetic dogs for fructosamine (n = 20) and glycated haemoglobin (n = 26) only

One way analysis of variance was used to determine the presence of statistically significant differences between the means of these groups for concentrations of afternoon plasma glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin and Tukey's family error rate method of multiple comparisons (MINITAB 9) was used to determine how the groups differed.

In order to get a mathematical assessment of the usefulness of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin concentrations in determining the degree of glycaemic control achieved in a diabetic dog, a dichotomous outcome of the test results was necessary. The ability of these biochemical tests to differentiate between poorly controlled diabetic dogs (follow-up examinations in glycaemic control Groups 2 and 3) and fair to well controlled dogs (follow-up examinations in glycaemic control Groups 4, 5 and 6) was assessed using ROC curves generated for each parameter including afternoon plasma glucose. For those tests determined by the ROC curves to be useful, differential positive rate (DPR) curves

were created to determine the optimum cut-off value between the poorly controlled dogs and those with some degree of control.

The methods used to generate ROC curves and differential positive rates were those recommended by Jensen and Poulsen (1992) based on those of Hanley and McNeil (1982). For demonstration purposes, details of the first and last 10 sensitivity, specificity and DPR values used in the evaluation of the fructosamine assay are recorded in Table 17. The methods used to calculate the area under the ROC curve and determine its statistical difference from 0.5 are also those recommended by Jensen and Poulsen (1992) based on Hanley and McNeil (1982) and these are detailed by example in Appendix 9.

Value number	Cut-off value µmol/l	Number observations in each glycaemic control group					Sensitivity	Specificity	1 - specificity	DPR
		2	3	4	5	6				
1	190	0	0	0	1	0	1.000	0.000	1.000	0.000
2	193	0	0	0	1	0	1.000	0.004	0.996	0.004
3	195	0	0	0	1	0	1.000	0.008	0.992	0.008
4	200	0	0	0	0	0	1.000	0.013	0.987	0.013
5	208	0	0	0	0	0	1.000	0.013	0.987	0.013
6	223	0	0	0	1	0	1.000	0.013	0.987	0.013
7	228	1	0	0	0	0	1.000	0.017	0.983	0.017
8	229	0	0	0	0	0	0.989	0.017	0.983	0.006
9	232	0	0	1	1	0	0.989	0.017	0.983	0.006
10	236	0	0	1	1	0	0.989	0.025	0.975	0.015
208	633	0	1	0	0	0	0.096	0.996	0.004	0.092
209	636	0	0	1	0	0	0.085	0.996	0.004	0.081
210	639	0	1	0	0	0	0.085	1.000	0.000	0.085
211	648	0	1	0	0	0	0.074	1.000	0.000	0.074
212	676	1	0	0	0	0	0.064	1.000	0.000	0.064
213	692	0	1	0	0	0	0.053	1.000	0.000	0.053
214	711	1	0	0	0	0	0.043	1.000	0.000	0.043
215	713	1	0	0	0	0	0.032	1.000	0.000	0.032
216	771	1	0	0	0	0	0.021	1.000	0.000	0.021
217	781	1	0	0	0	0	0.011	1.000	0.000	0.011

- *Sensitivity:* the proportion of dogs with poor control (glycaemic control groups 2 and 3) with fructosamine concentrations at or above the cut-off value
- *Specificity:* the proportion of dogs with fair to good control (glycaemic control groups 4,5 and 6) with fructosamine concentrations below the cut-off value
- *DPR* = Sensitivity - (1 - Specificity)

Table 17. The calculation of sensitivity, specificity and differential positive rate for the first and last 10 cut-off values for fructosamine concentrations used in distinguishing between poorly and fair to well controlled diabetic dogs.

Results

Fructosamine

Intra-assay coefficients of variation ranged from 0.83 to 2.26 % across the 4 levels of fructosamine concentration tested (Table 18) making the mean intra-assay CV 1.38 %. The inter-assay C's of V assessed at two levels of fructosamine concentration were 3.11 and 3.23 % (mean 3.17 %) and are given in Table 19.

	low pool	high pool 1	122739	high pool 2
Replicates	10	10	10	10
Mean	260.0	331.7	408.1	643.7
SD	3.77	7.50	4.12	5.31
CV%	1.45	2.26	1.01	0.83

Table 18. Intra-assay coefficients of variation for 4 concentrations of fructosamine.

	low pool	high pool 1
Replicates	8	8
Mean	248.5	341.7
SD	8.0	10.6
CV%	3.23	3.11

Table 19. Inter-assay coefficients of variation for two concentrations of fructosamine.

The analyses of 20 samples of physiological saline yielded results with a mean of 2.85 μmol/l and a standard deviation of 1.31 which, according to the recommendations of Buttner *et al* (1980) (mean + 2.6SD), creates a limit of detection for the fructosamine assay of 6.26 μmol/l.

The linearity of the method was excellent across the range 100 to 640 μmol/l with no obvious deviation even at the highest dilution of 1 in 8 (Figure 15).

There were no obvious trends in measured fructosamine concentration upwards or downwards as a result of progressive lipaemia at two levels of fructosamine concentration (Figure 16).

The maximum deviation from basal concentrations of fructosamine over 48 hours of storage of whole blood at room temperature was ± 3% (Figure 17).

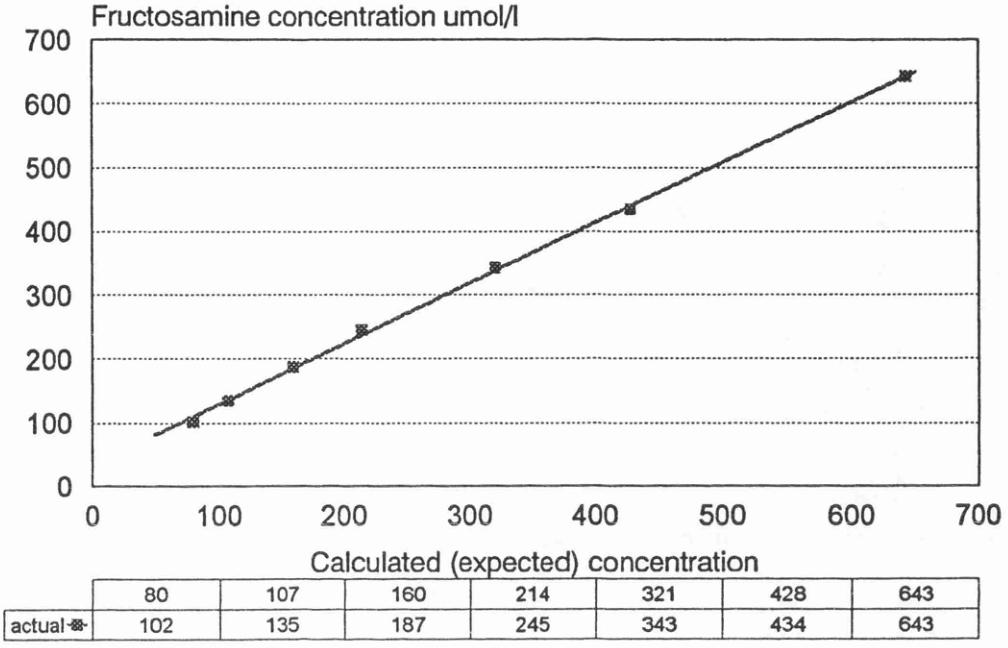

Figure 15. Linearity of fructosamine method at 6 dilutions of a plasma pool of high fructosamine concentration.

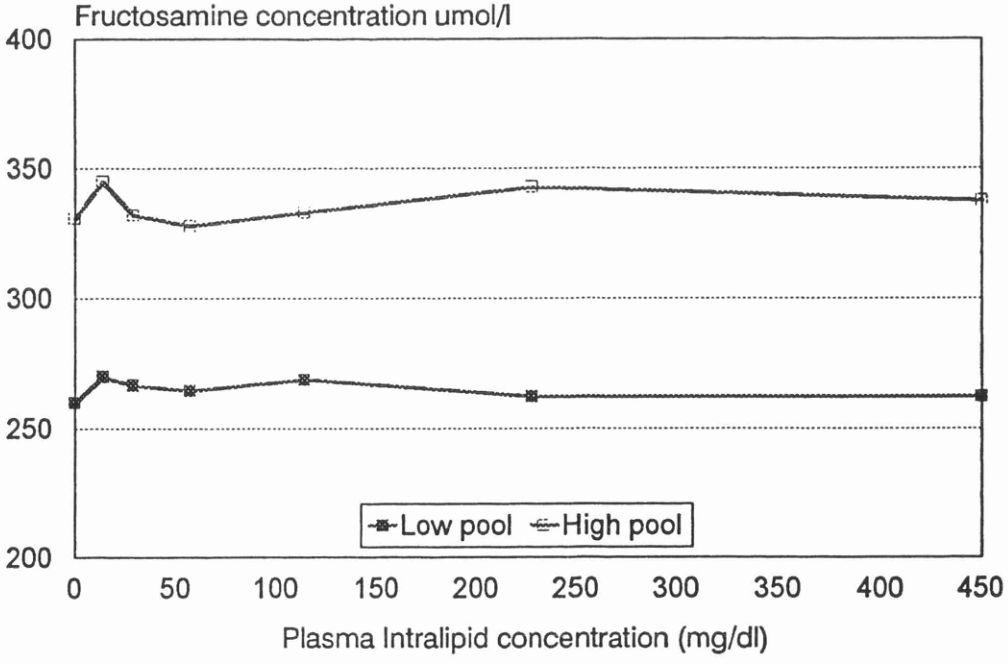

Figure 16. Effects of lipaemia on fructosamine concentration following the addition of Intralipid to two plasma pools.

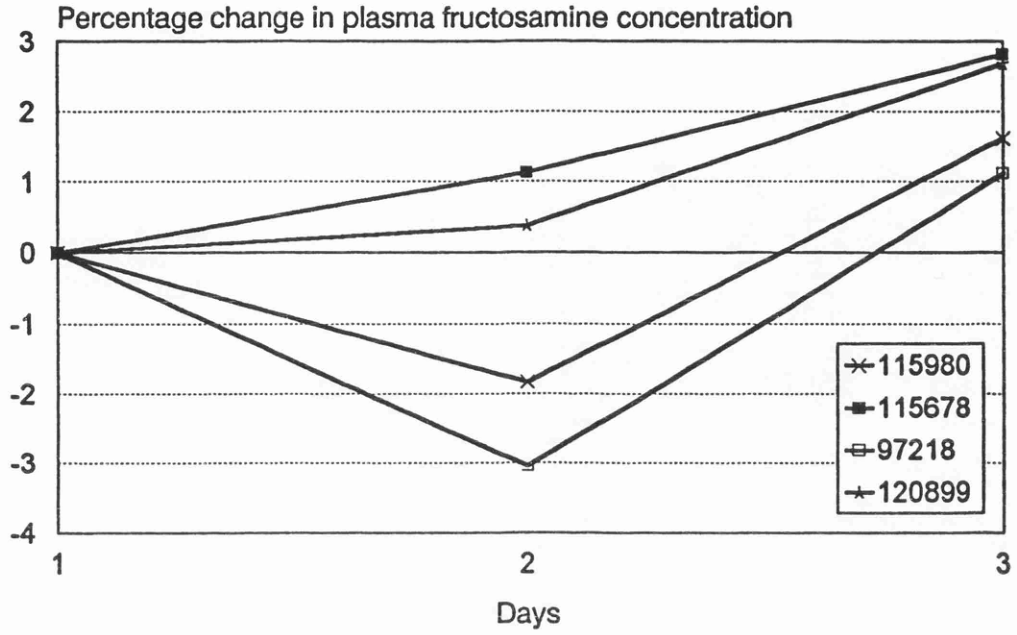

Figure 17. Effects of short term storage of 4 samples of whole blood at room temperature on plasma fructosamine concentration (percentage change).

The reference range for plasma fructosamine concentration in non-diabetic dogs (mean ± 2SD) was 162 to 310 µmol/l (Table 20) based on results from 20 non-diabetic dogs.

Fructosamine concentration µmol/l	
278	219
303	180
259	212
285	172
225	196
213	260
287	216
233	256
200	267
223	243
Mean	236.4
SD	37.0
Mean + 2 SD	310.3
Mean - 2 SD	162.4

Table 20. Plasma fructosamine concentrations in 20 non-diabetic dogs.

Glycated haemoglobin

The intra-assay coefficient of variation for glycated haemoglobin over the 4 levels tested ranged from 3.5 to 4.5 % (mean 3.84%) (Table 21). The mean inter-assay CV was 7.31% and details of the two levels tested are given in Table 22.

	non diabetic	115131	122739	113648
Replicates	10	5	10	5
Mean	3	4.4	6.42	9.11
SD	0.10	0.159	0.293	0.34
CV%	3.5	3.6	4.5	3.76

Table 21. Intra-assay coefficients of variation for 4 concentrations of glycated haemoglobin.

	non-diabetic	121228
Replicates	8	8
Mean	3.11	5.97
SD	0.25	0.38
CV%	8.13	6.49

Table 22. Inter-assay coefficients of variation for two concentrations of glycated haemoglobin.

There was no obvious trend in glycated haemoglobin concentration over three weeks of refrigerated storage of whole blood. At the higher initial concentration there was an apparent initial rise over the first week (Figure 18). The significance of this rise is difficult to ascertain.

The effects of sample haemolysis and storage at room temperature caused large fluctuations in measured glycated haemoglobin concentration. Only the concentration of glycated haemoglobin stored without haemolysis in a refrigerator remained stable over the two week study period (Figure 19).

The incubation of whole blood with high concentrations of glucose caused an increase in glycated haemoglobin concentration over a one week study period and this increase was greatest in those samples stored at 37 °C (Figure 20).

There was no significant correlation ($p = 0.438$) between concentrations of glycated haemoglobin measured by affinity chromatography and cation exchange chromatography (Figure 21) but there was good correlation between the affinity column and agarose gel electrophoresis methods ($p = 0.005$, $r = 0.666$, $n = 16$, Affinity = 1.77 + 0.588 Electrophoresis) (Figure 22).

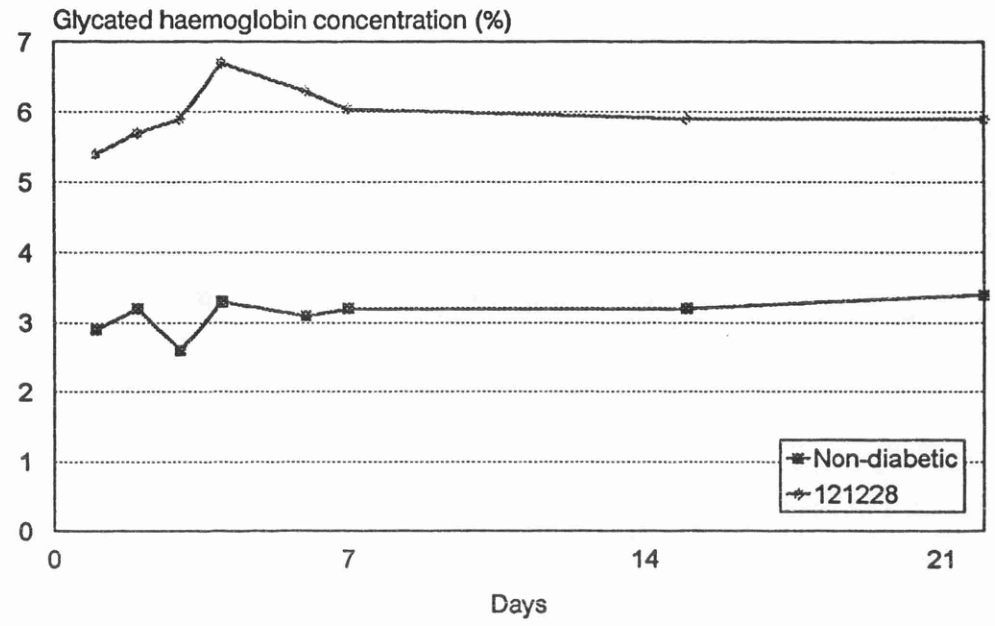

Figure 18. Interassay CV and effects of 3 weeks refrigerated storage of whole blood samples on glycated haemoglobin concentration.

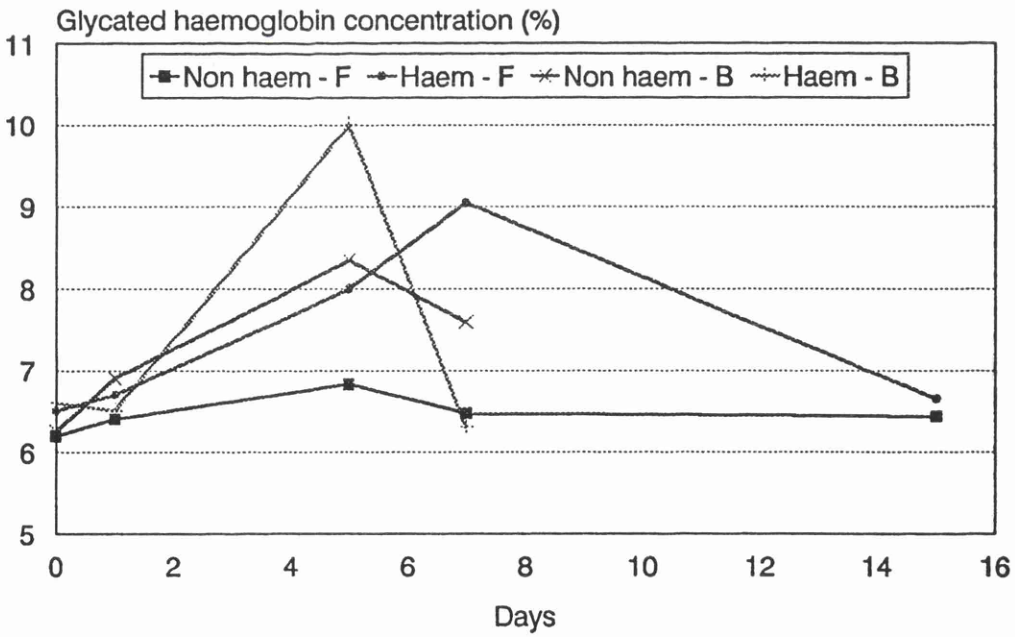

Figure 19. Changes in glycated haemoglobin concentration due to haemolysis and storage conditions (Haem - haemolysed, B - stored on laboratory bench, F - stored in a refrigerator).

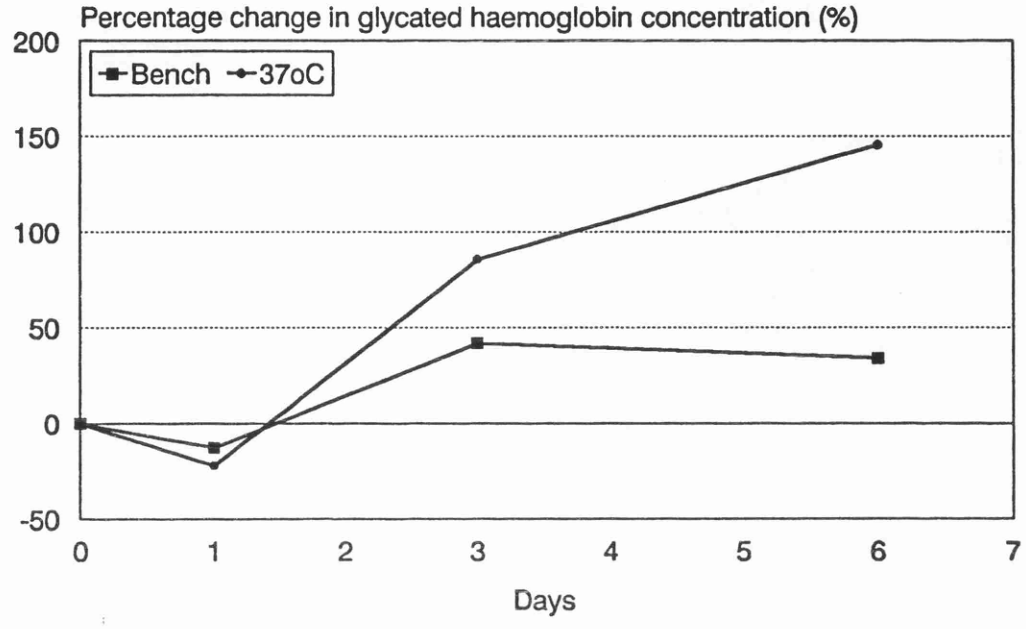

Figure 20. Mean percentage change in glycated haemoglobin concentration in three samples incubated with an initial glucose concentration of approximately 27 mmol/l and stored at either room temperature or 37°C.

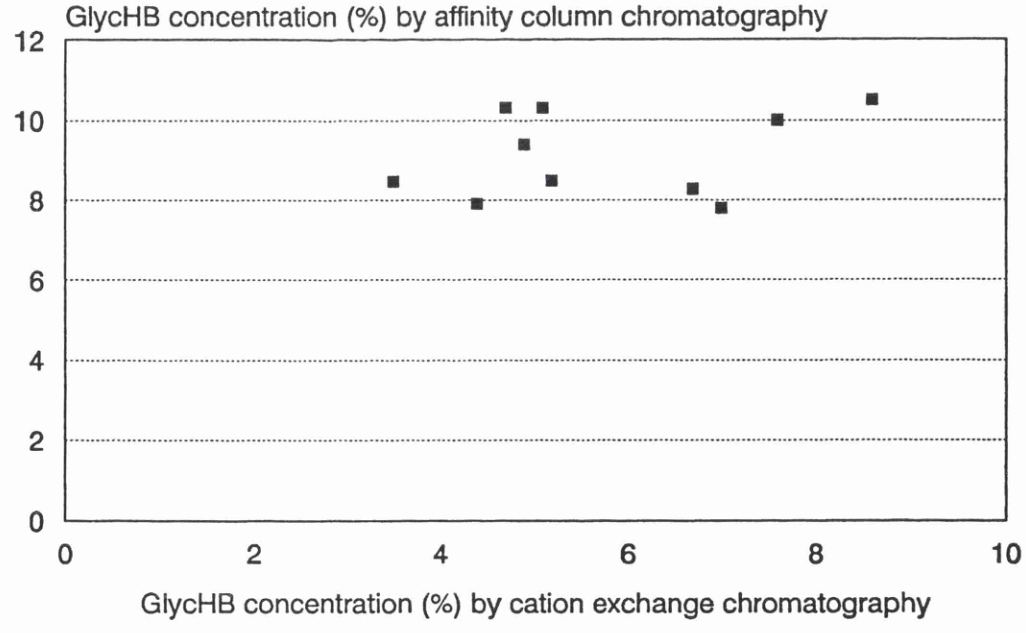

Figure 21. Comparison of glycated haemoglobin concentrations obtained using affinity column or cation exchange chromatography techniques.

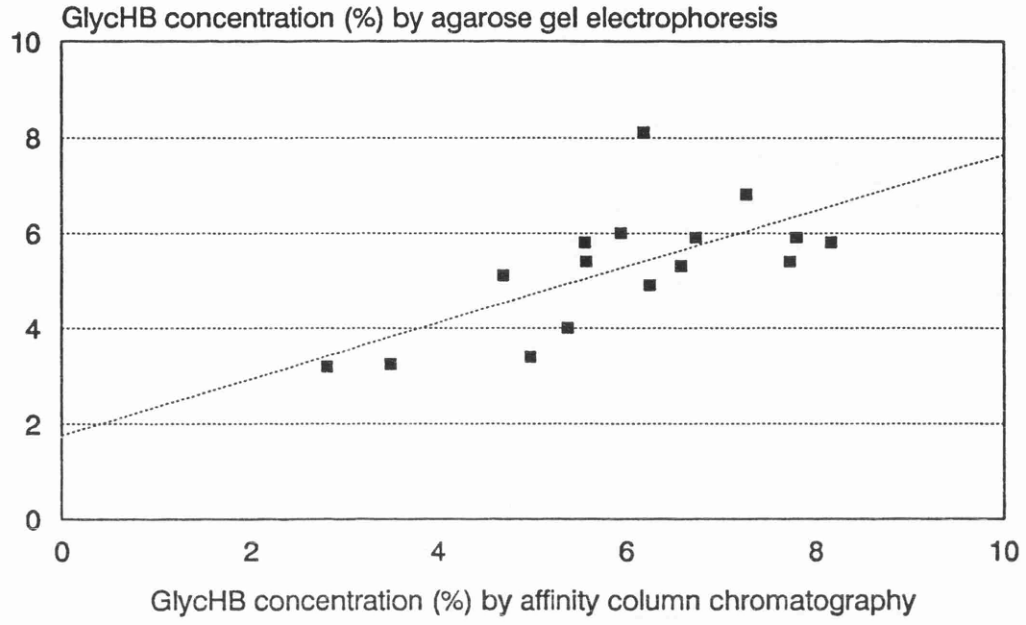

Figure 22. Comparison of glycated haemoglobin concentrations obtained using affinity column chromatography or agarose gel electrophoresis.

The choice of anticoagulant did not affect the assay. There was excellent correlation between results from lithium heparin and those from EDTA samples ($p = 0.000$, $r = 0.979$, $n = 9$, EDTA = - 0.077 + 1.01 LH) and no significant difference between the results obtained ($p = 0.85$) (Figure 23).

The reference range for glycated haemoglobin concentration in non-diabetic dogs based on samples from 26 animals (mean ± 2SD) was 1.7 to 4.9% (Table 23).

Glycated haemoglobin concentration (%)			
3.48	3.88	2.91	3.39
3.39	4.26	3.99	3.37
3.69	5.09	2.71	3.61
3.11	2.85	2.88	5.48
3.23	1.75	3.12	2.18
3.18	3.24	2.33	2.83
		3.02	3.13
	Mean	3.31	
	SD	0.80	
	Mean + 2 SD	4.9	
	Mean - 2 SD	1.7	

Table 23. Glycated haemoglobin concentrations in 26 non-diabetic dogs.

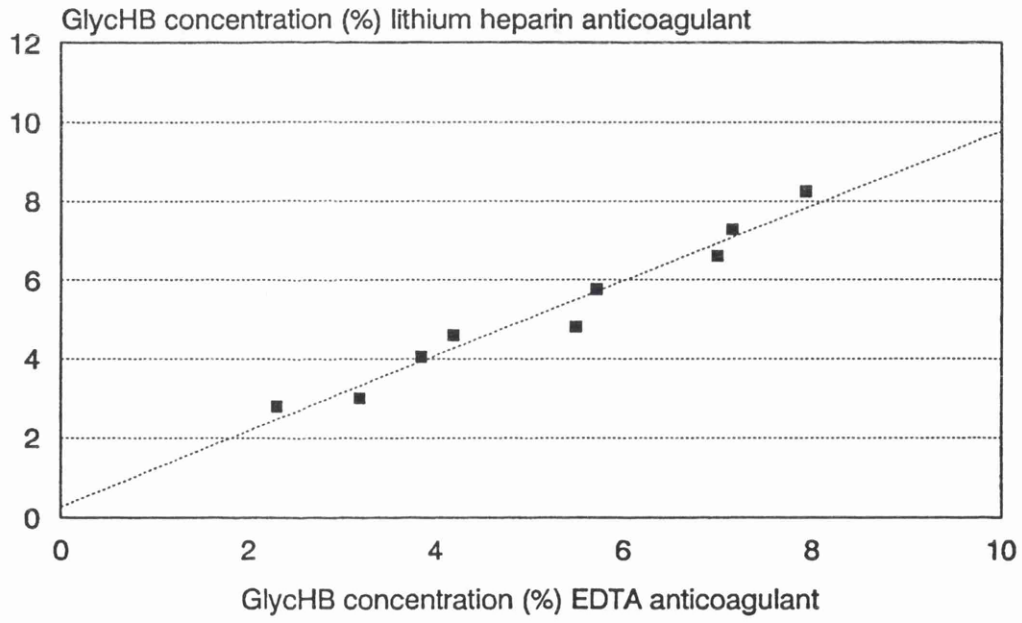

Figure 23. Comparison of glycated haemoglobin concentrations by affinity column chromatography measured in samples containing EDTA or lithium heparin anticoagulant.

Daily variation

The results of 24 hour profiles for plasma concentrations of alkaline phosphatase, alanine aminotransferase, glucose and fructosamine in three dogs are presented in Appendix 10 and represented in Figure 24 as mean percentage changes from basal concentrations. Sampling time had no significant effect on the plasma concentrations of alkaline phosphatase, alanine aminotransferase or fructosamine but there was a significant effect

of sampling time on plasma glucose concentrations (p = 0.039). As expected there were significant inter-dog differences in the concentrations of all of these analytes (p = 0.000).

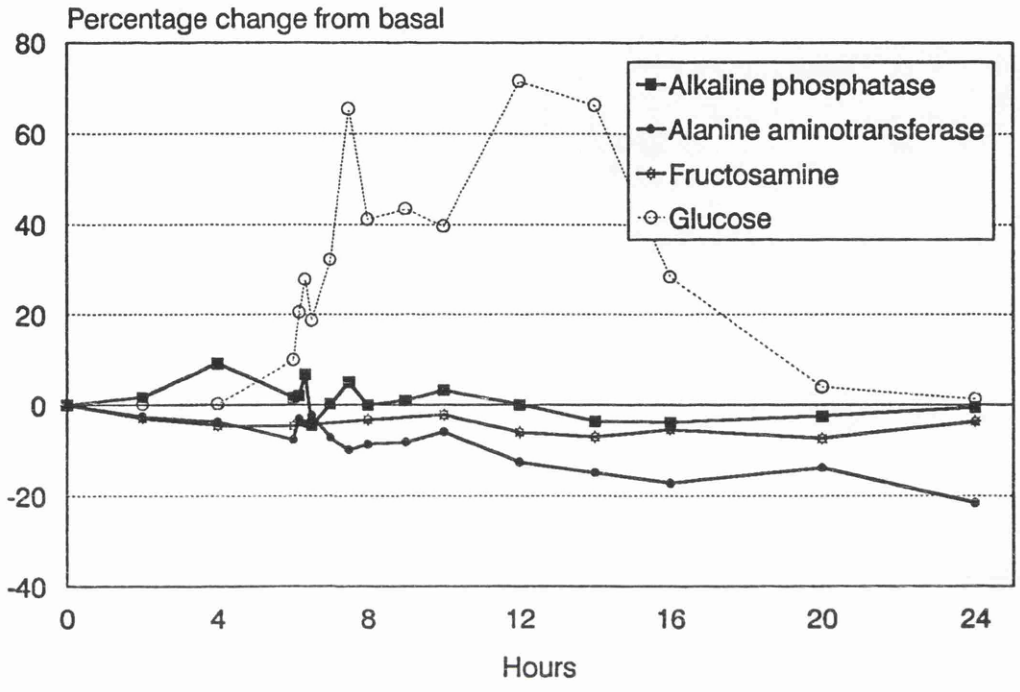

Figure 24. Mean percentage change from basal concentrations plasma concentrations of alkaline phosphatase, alanine aminotransferase, fructosamine and glucose in three diabetic dogs over 24 hours.

Monitoring early diabetic therapy

There was a significant change in afternoon plasma glucose concentrations over the three early sampling periods (p = 0.000). Since the aim in diabetic stabilisation is to reduce this parameter into the 'normal' range this is not surprising. Newman-Keuls multiple range test revealed significant differences between Period A and Period B, and between Period A and Period C but not between Period B and C. The statistical picture was the same for fructosamine (p = 0.000) and glycated haemoglobin (p = 0.000). There were no significant changes in alkaline phosphatase concentration (p = 0.134) nor in aspartate aminotransferase (p = 0.373). There was a significant change in alanine aminotransferase concentration (p = 0.028) by general linear model analysis but Newman-Keuls Multiple range testing revealed only close to significant differences between Periods A and B and between Periods B and C. The principal trend in the concentrations of afternoon glucose, fructosamine and glycated haemoglobin was downwards and this is represented graphically in Figures 25 to 27. However, the concentrations of alanine aminotransferase and (although not significantly) alkaline phosphatase appeared to rise in the early post

stabilisation period. This is apparent in Figures 28 and 29 in which the concentrations of these analytes have been represented by percentages of their pretreatment concentrations because of their wide variation between dogs.

There was no significant correlation between the duration of polydipsia prior to diagnosis and the pre-treatment concentrations of any of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase or glycated haemoglobin. However, there was a significant correlation for fructosamine concentration ($p = 0.022$, $r = 0.43$, $n = 28$, fruc = 375 + 36.4 weeks) which is represented in Figure 30.

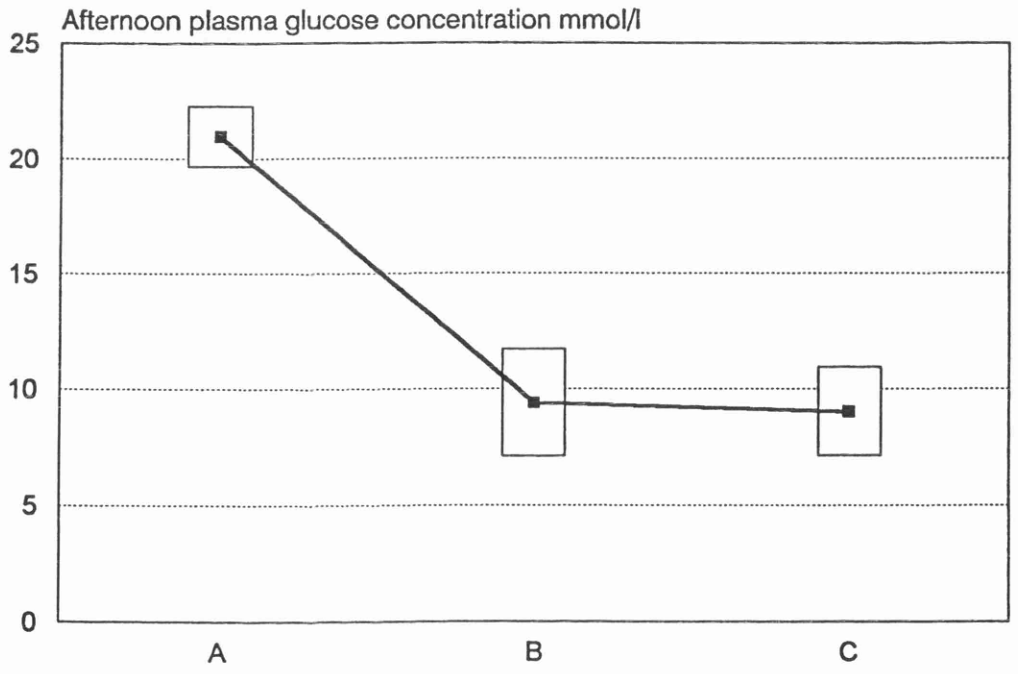

Figure 25. Mean afternoon plasma glucose concentration (± SEM) in 20 dogs during sampling periods A, B and C.

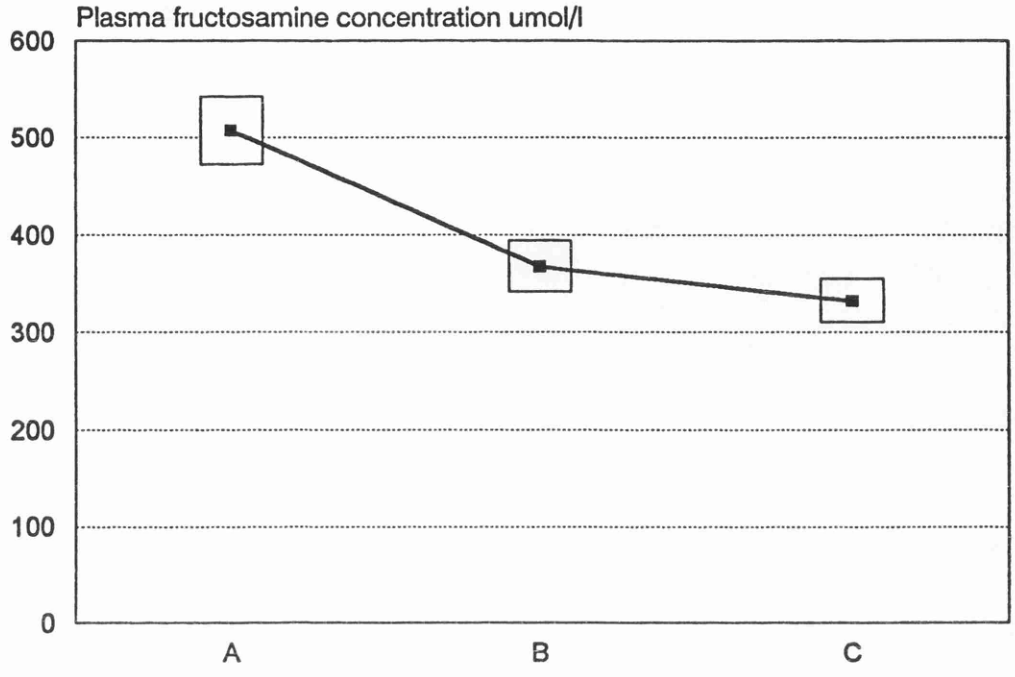

Figure 26. Mean fructosamine concentration (± SEM) in 20 dogs during sampling periods A, B and C.

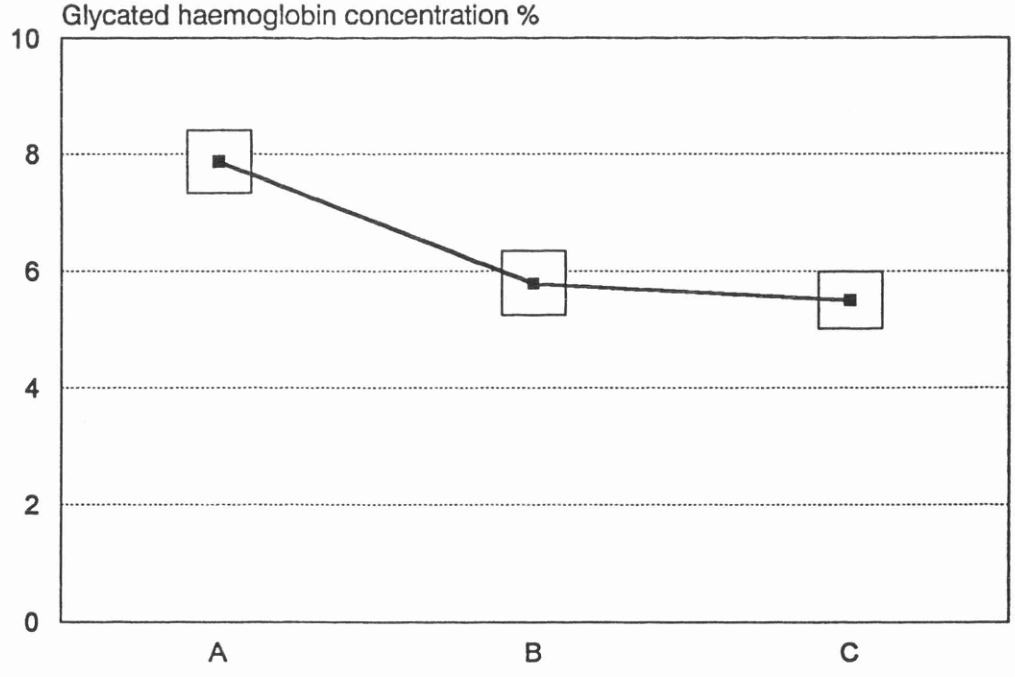

Figure 27. Mean glycated haemoglobin concentration (± SEM) in 15 dogs during sampling periods A, B and C.

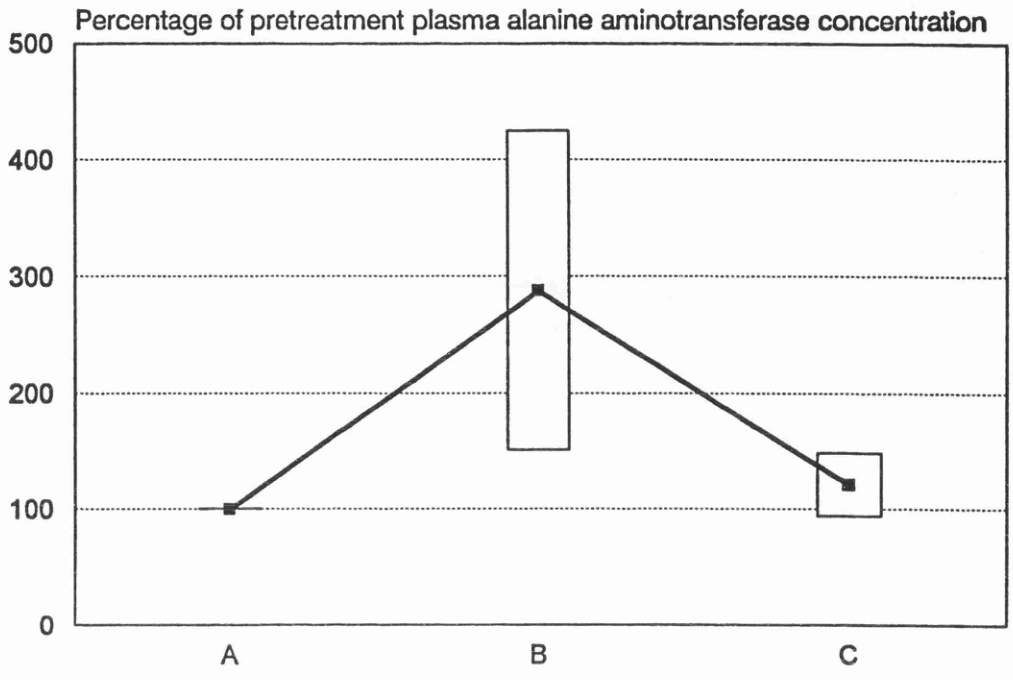

Figure 28. Mean percentage (± SEM) of pretreatment plasma alanine aminotransferase concentration in 20 dogs during sampling periods A, B and C.

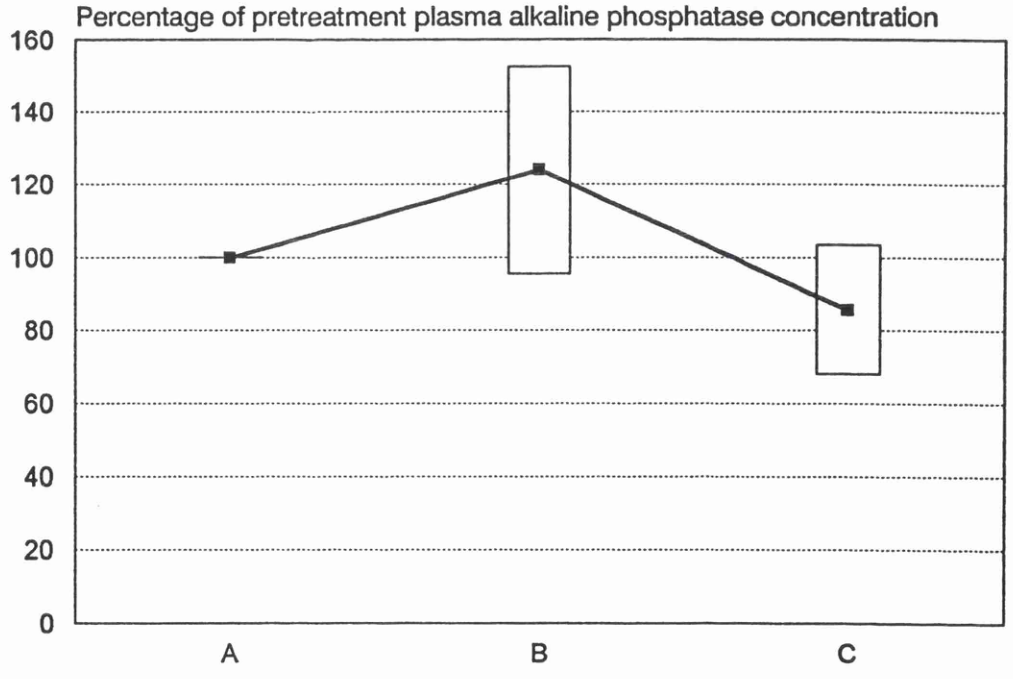

Figure 29. Mean percentage (± SEM) of pretreatment plasma alkaline phosphatase concentration in 20 dogs during sampling periods A, B and C.

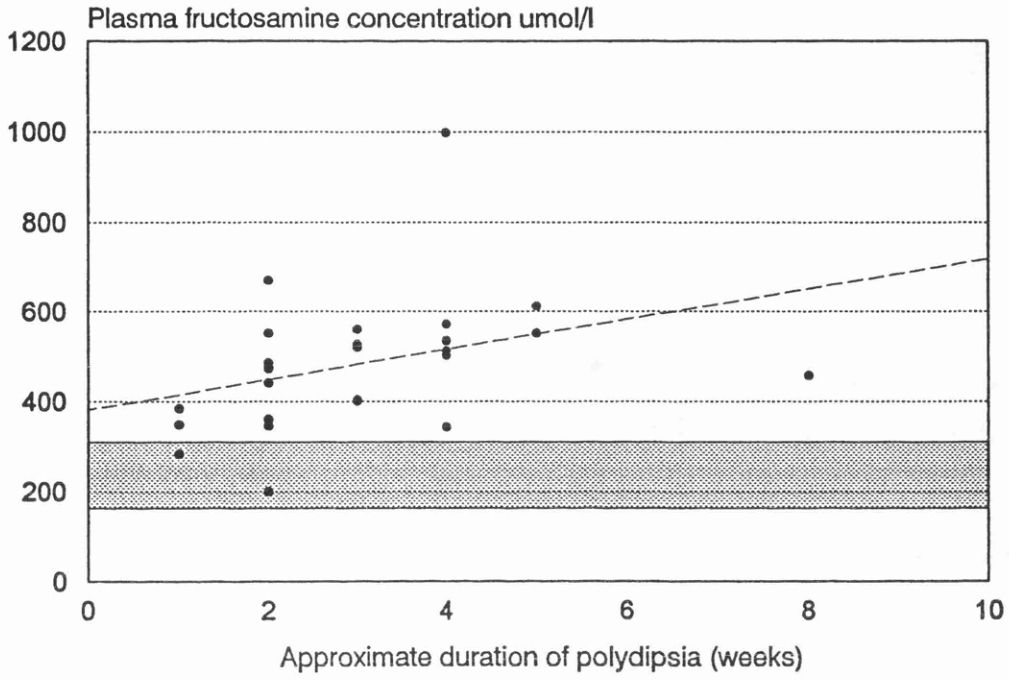

Figure 30. Pretreatment plasma fructosamine concentration versus duration of pretreatment polydipsia (shaded area is reference range 162 to 310 umol/l).

Long-term diabetic monitoring

The results of many hundreds of follow-up examinations were coded according to the degree of glycaemic control believed to prevail at the time. The distribution of these observations for each analyte among the 7 glycaemic control groups along with the numbers of dogs from which these samples originated are detailed in Table 24.

There were significant differences between the 7 glycaemic control groups in the mean concentrations of nadir (afternoon) plasma glucose ($p = 0.000$), plasma alkaline phosphatase (0.005), plasma aspartate aminotransferase ($p = 0.000$) and between the 8 groups in plasma fructosamine ($p = 0.000$) and glycated haemoglobin ($p = 0.000$). There were no significant differences in mean plasma alanine aminotransferase concentration between the glycaemic control groups. Results of Tukey's family error rate multiple comparisons for nadir plasma glucose, alkaline phosphatase, aspartate aminotransferase, fructosamine and glycated haemoglobin are summarised in Tables 25 to 29.

	Glycaemic control group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Afternoon plasma glucose	74	53	163	193	261	8	18
	(68)	(34)	(59)	(64)	(65)	(4)	(13)
Alkaline phosphatase	73	52	162	190	258	7	17
	(66)	(35)	(59)	(64)	(65)	(4)	(13)
Alanine aminotransferase	74	52	153	180	253	7	17
	(67)	(35)	(55)	(63)	(65)	(4)	(13)
Asparate aminotransferase	73	51	143	178	254	7	17
	(67)	(35)	(54)	(63)	(64)	(4)	(13)
Fructosamine	28	23	71	105	130	2	9
	(27)	(18)	(33)	(42)	(37)	(1)	(7)
Glycated haemoglobin	18	21	66	92	124	2	8
	(17)	(17)	(31)	(38)	(37)	(1)	(7)

Table 24. Numbers of observations of each analyte in each glycaemic control group with the number of dogs sampled in brackets.

There were significant differences in mean nadir glucose concentration between the untreated, very poorly and poorly controlled groups and all of the others except between the untreated and very poorly controlled groups. There were also differences between the fairly controlled group and the good and overdosed groups. There were no differences between the good control group and the excellent and overdose control groups. Figure 31 represents the plasma glucose concentrations in each of the glycaemic control groups.

Glycaemic control group	1	2	3	4	5	6
2	-					
3	+	+				
4	+	+	+			
5	+	+	+	+		
6	+	+	+	-	-	
7	+	+	+	+	-	-

Table 25. Statistically significant ($p < 0.05$) differences between glycaemic control groups for nadir plasma glucose concentration (+ = significantly different between groups).

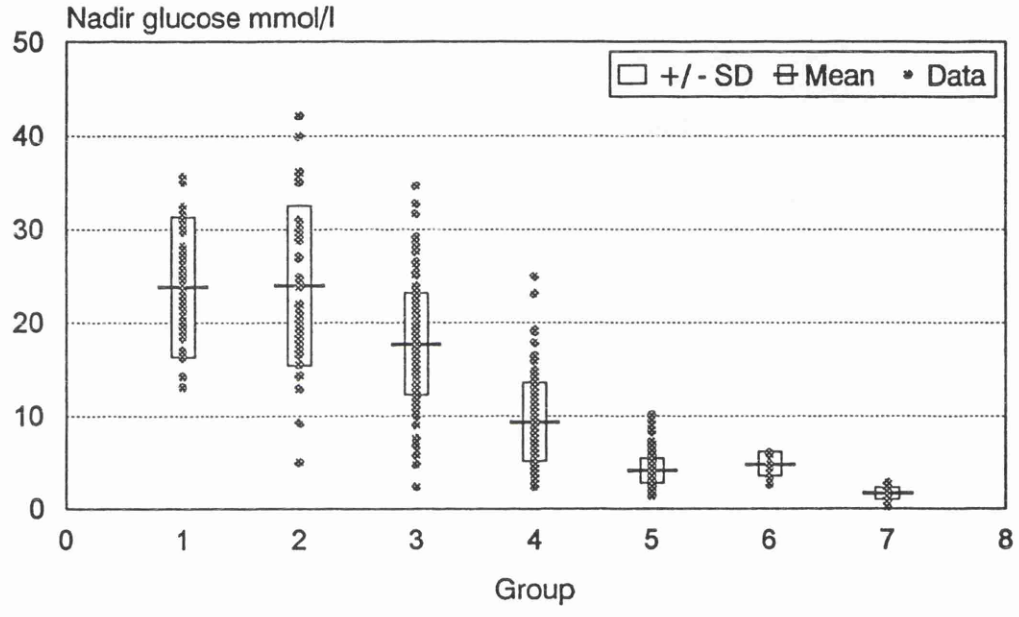

Figure 31. Nadir plasma glucose concentrations (mmol/l) for 7 groups of glycaemic control (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

In the case of alkaline phosphatase concentrations, the untreated diabetic group had significantly higher mean concentration than those under poor, fair or good control but not those under excellent or excessive control. There were no differences between any of the treated diabetic groups. The wide variation in alkaline phosphatase concentrations is demonstrated graphically in Figure 32 in which the data have been plotted on a logarithmic scale and with means and standard error of the mean (SEM), rather than standard deviations (SD), because this variation was so extreme.

Glycaemic control group	1	2	3	4	5	6
2	-					
3	+	-				
4	+	-	-			
5	+	-	-	-		
6	-	-	-	-	-	
7	-	-	-	-	-	-

Table 26. Statistically significant ($p < 0.05$) differences between glycaemic control groups for plasma alkaline phosphatase concentration (+ = significantly different between groups).

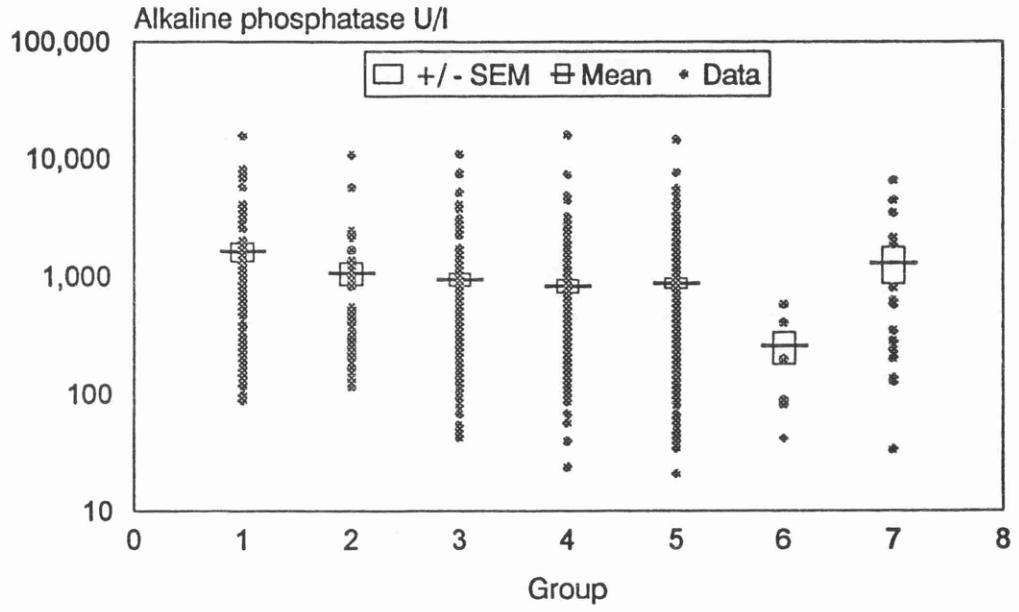

Figure 32. Plasma alkaline phosphatase concentrations (U/l - logarithmic scale) for 7 groups of glycaemic control (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

Mean aspartate aminotransferase concentrations were significantly higher for the untreated and very poorly controlled groups than for the poor, fair and good glycaemic control groups. There were no differences between the untreated and very poor control groups nor between any of the poor through to excessive control groups. The aspartate aminotranferase concentrations of all 7 glycaemic control groups are represented in Figure 33 along with the mean and SEM using a logarithmic scale because of the wide variation in concentration within groups.

Glycaemic control group	1	2	3	4	5	6
2	-					
3	+	+				
4	+	+	-			
5	+	+	-	-		
6	-	-	-	-	-	
7	-	-	-	-	-	-

Table 27. Statistically significant ($p < 0.05$) differences between glycaemic control groups for aspartate aminotransferase concentration (+ = significantly different between groups).

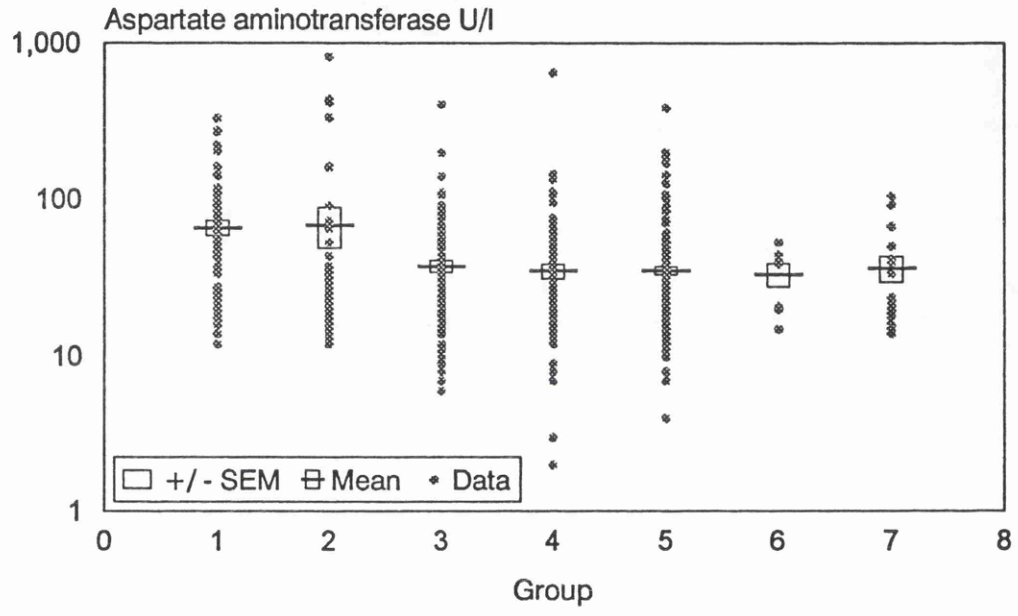

Figure 33. Plasma aspartate aminotransferase concentrations (U/l - logarithmic scale) for 7 groups of glycaemic control (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

Mean fructosamine concentrations were significantly higher for the untreated and very poorly controlled groups than for the fair, good, excessive control and non-diabetic groups but there were no differences between the untreated, very poor and poor control groups. The mean fructosamine concentration was also greater for the poorly controlled group than for the fair, good, excessive control and non-diabetic groups and for the fair control group than for the good, excessive control and non-diabetic groups. The good glycaemic control group had a significantly greater mean fructosamine concentration than the non-diabetic group but there was no difference between the non-diabetic groups and those results from those dogs under excessive diabetic control. There were no significant differences between the excellent glycaemic control group and any of the

others because of the small number of observations. The fructosamine concentrations for the 7 glycaemic control groups are represented in Figure 34.

Glycaemic control group	1	2	3	4	5	6	7
2	-						
3	-	-					
4	+	+	+				
5	+	+	+	+			
6	-	-	-	-	-		
7	+	+	+	+	+	-	
(non-diabetic) 8	+	+	+	+	+	-	-

Table 28. Statistically significant ($p < 0.05$) differences between glycaemic control groups for plasma fructosamine concentration (+ = significantly different between groups).

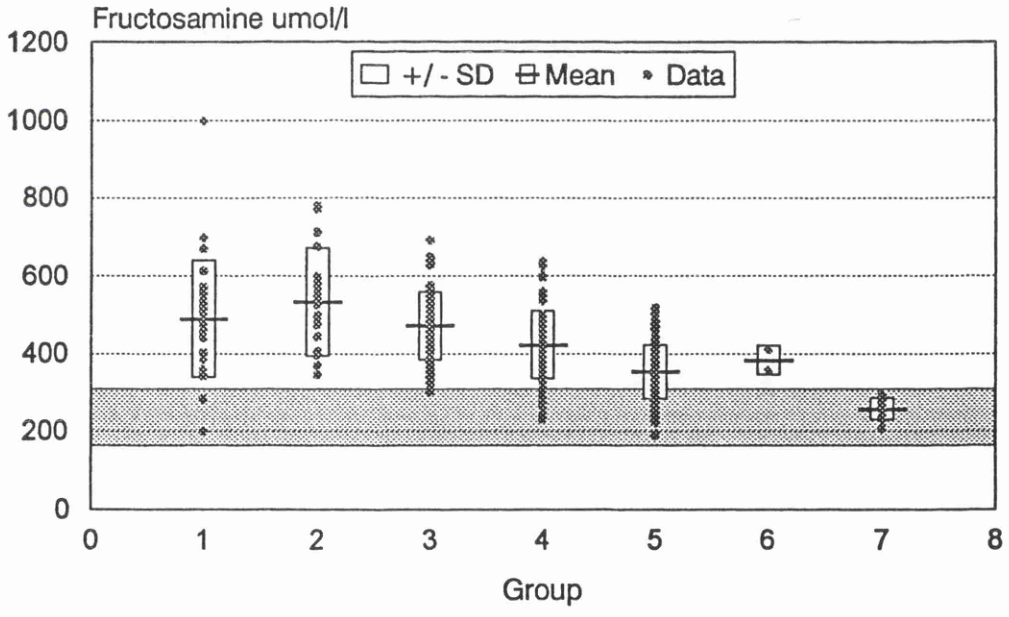

Figure 34. Plasma fructosamine concentrations (μmol/l) for 7 groups of glycaemic control; shaded area is reference range 162 to 310 μmol/l (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

Mean glycated haemoglobin concentrations were greater for the untreated and very poorly controlled groups than for the fair, good, excessive control and non-diabetic groups. Additionally, in the untreated group mean glycated haemoglobin was greater than in the poorly controlled group. There were also significant differences in mean glycated haemoglobin concentration between the poorly and fairly controlled groups and the good, excessive control and non-diabetic groups. The good glycaemic control group

had a higher mean glycated haemoglobin concentration than the non-diabetic group but there were no differences between good control and excessive control groups or between the excessive control and non-diabetic groups. There were no significant differences between the excellent control group and any of the others because of the small number of observations in this group. Glycated haemoglobin concentrations for all 7 groups are represented in Figure 35.

Glycaemic control group	1	2	3	4	5	6	7
2	-						
3	+	-					
4	+	+	-				
5	+	+	+	+			
6	-	-	-	-	-		
7	+	+	+	+	-	-	
(non-diabetic) 8	+	+	+	+	+	-	-

Table 29. Statistically significant ($p < 0.05$) differences between glycaemic control groups for glycated haemoglobin concentration (+ = significantly different between groups).

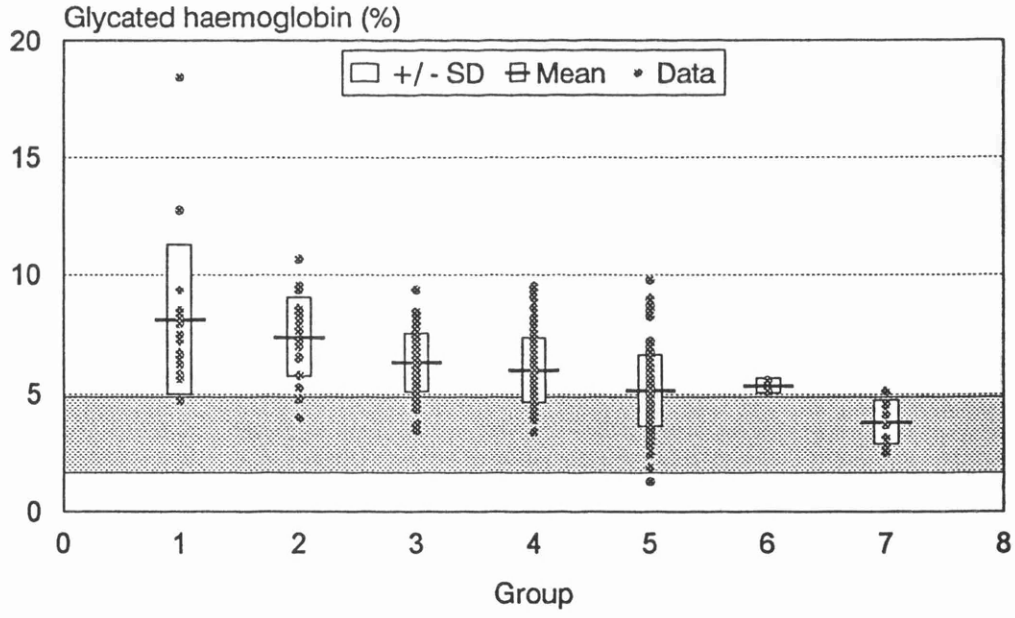

Figure 35. Glycated haemoglobin concentrations (%) for 7 groups of glycaemic control; shaded area is reference range 1.7 to 4.9% (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

There were no significant differences in mean alanine aminotransferase concentrations between the 7 glycaemic control groups. This is demonstrated in Figure 36.

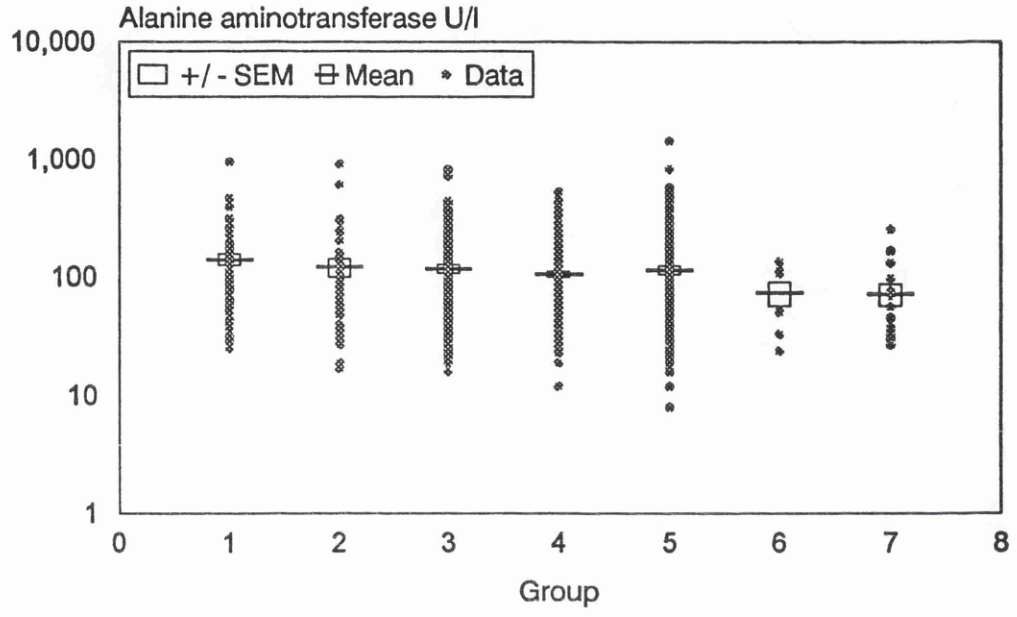

Figure 36. Plasma alanine aminotransferase concentrations (U/l - logarithmic scale) for 7 groups of glycaemic control (Group 1 - untreated, Group 2 - very poor, Group 3 - poor, Group 4 - fair, Group 5 - good, Group 6 - excellent and Group 7 - insulin overdosage).

The areas under the ROC curves (W) for nadir plasma glucose, plasma fructosamine and glycated haemoglobin concentrations were all significantly greater than 0.5, meaning that they were all useful tests for distinguishing between samples from animals with very poor or poor glycaemic control and those from animals with fair, good or excellent control (Table 30). The nadir plasma glucose concentration test was superior to fructosamine and glycated haemoglobin concentrations, based on a higher area under the ROC curve. The areas under the ROC curves for alkaline phosphatase, alanine aminotransferase and alanine aminotransferase were not significantly different from 0.5 (Table 30), meaning that these tests could not distinguish between the two groups of glycaemic control and were therefore worthless in this context. The ROC curves of nadir glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin are represented in Figures 37 to 42, demonstrating the relative merits of these tests.

Analyte	Number of cut-off values	Number of observations	Area under the ROC curve (W) ± SE(W)	p value (probability that W = 0.5)
Nadir glucose	230	678	0.956 ± 0.009	0.000*
Alkaline phosphatase	545	669	0.538 ± 0.024	0.053
Alanine aminotransferase	236	645	0.512 ± 0.025	0.319
Aspartate aminotransferase	102	633	0.508 ± 0.026	0.382
Fructosamine	217	331	0.780 ± 0.028	0.000*
Glycated haemoglobin	295	304	0.705 ± 0.032	0.000*

Table 30. Areas under the ROC curve for nadir glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and glycated haemoglobin including numbers of cut-off values and observations used.

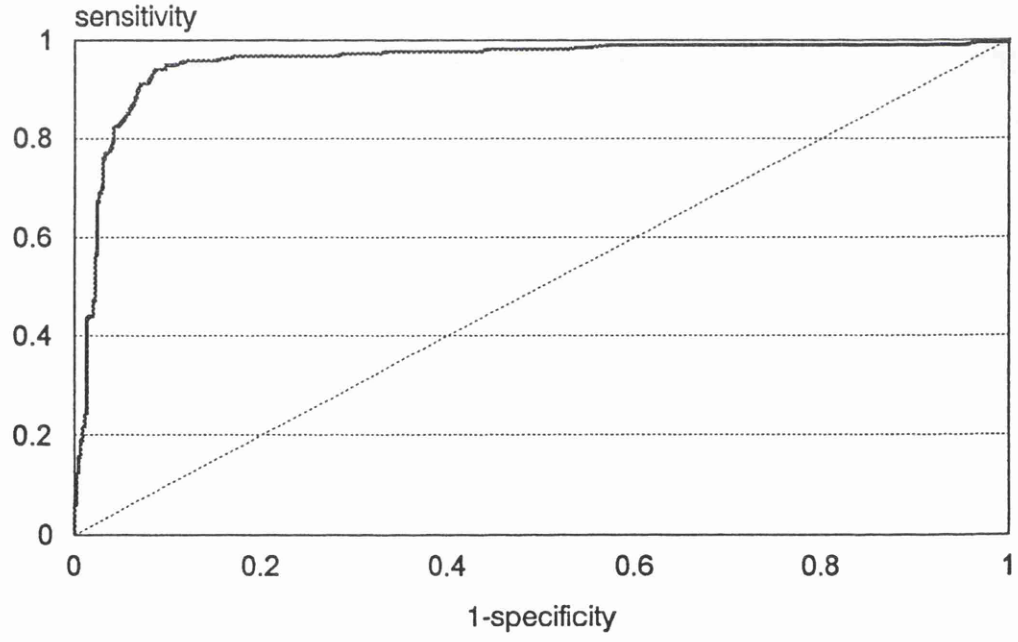

Figure 37. ROC curve for nadir glucose concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

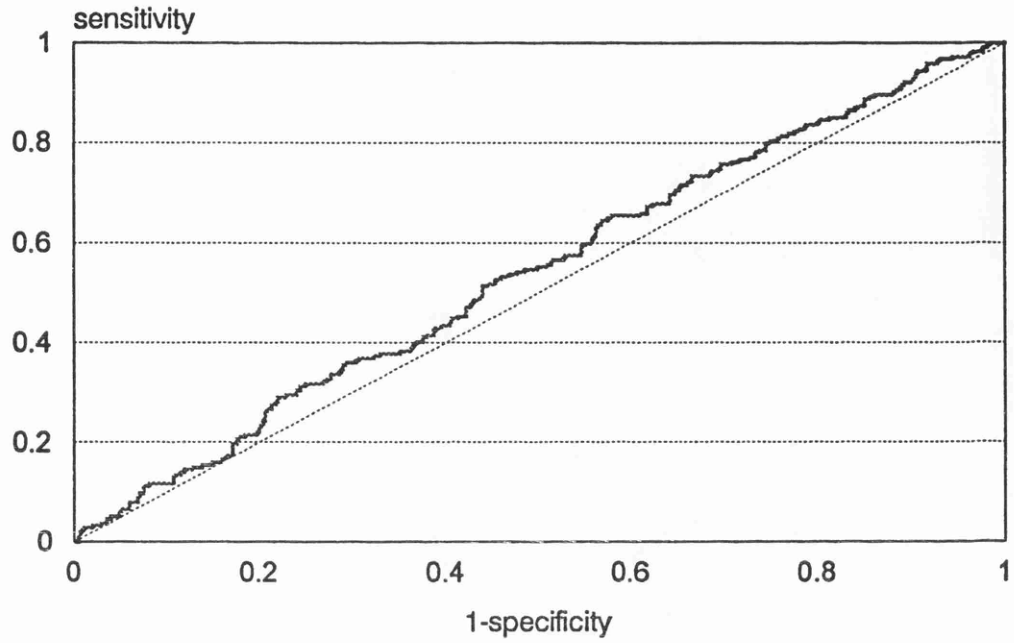

Figure 38. ROC curve for plasma alkaline phosphatase concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

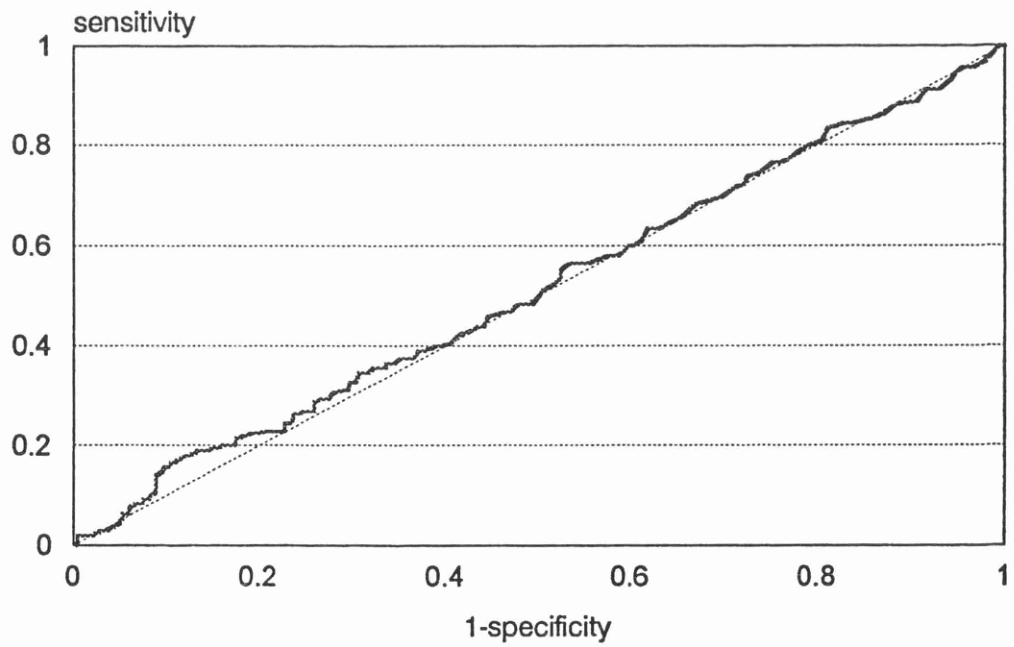

Figure 39. ROC curve for plasma alanine aminotransferase concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

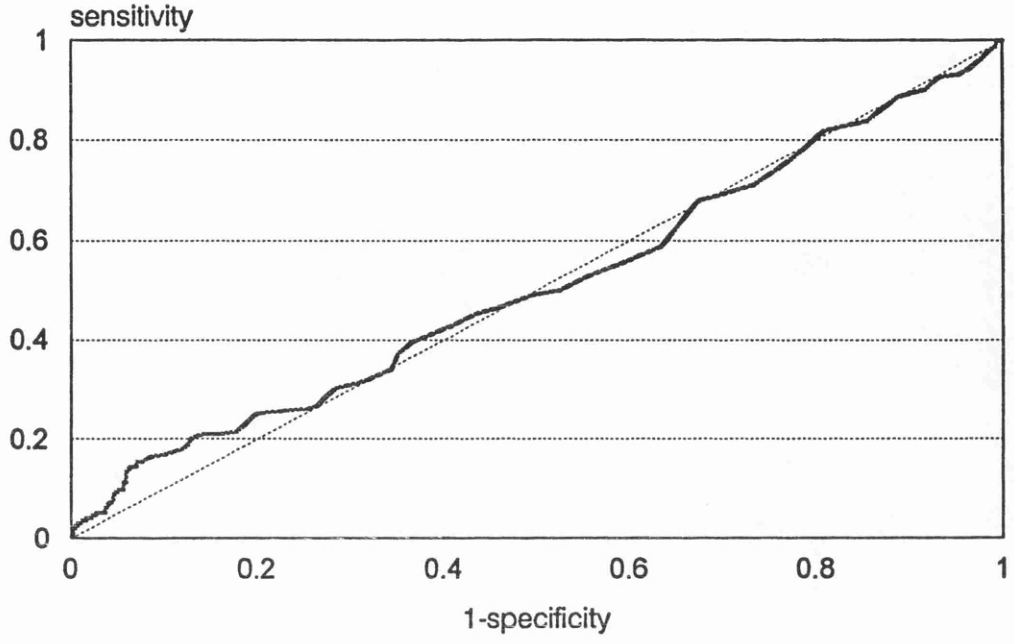

Figure 40. ROC curve for plasma aspartate aminotransferase concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

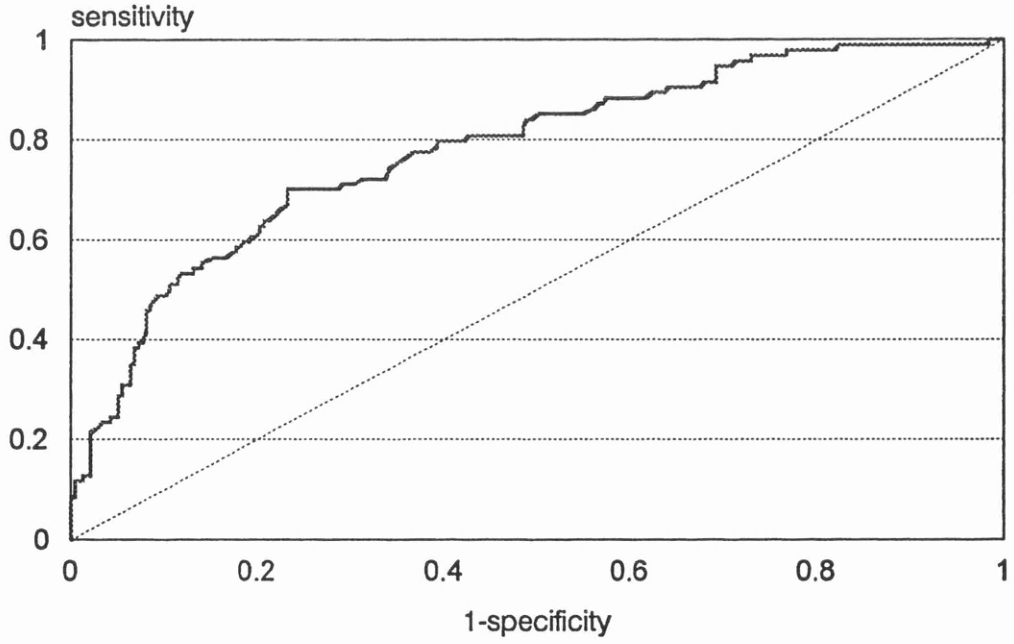

Figure 41. ROC curve for plasma fructosamine concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

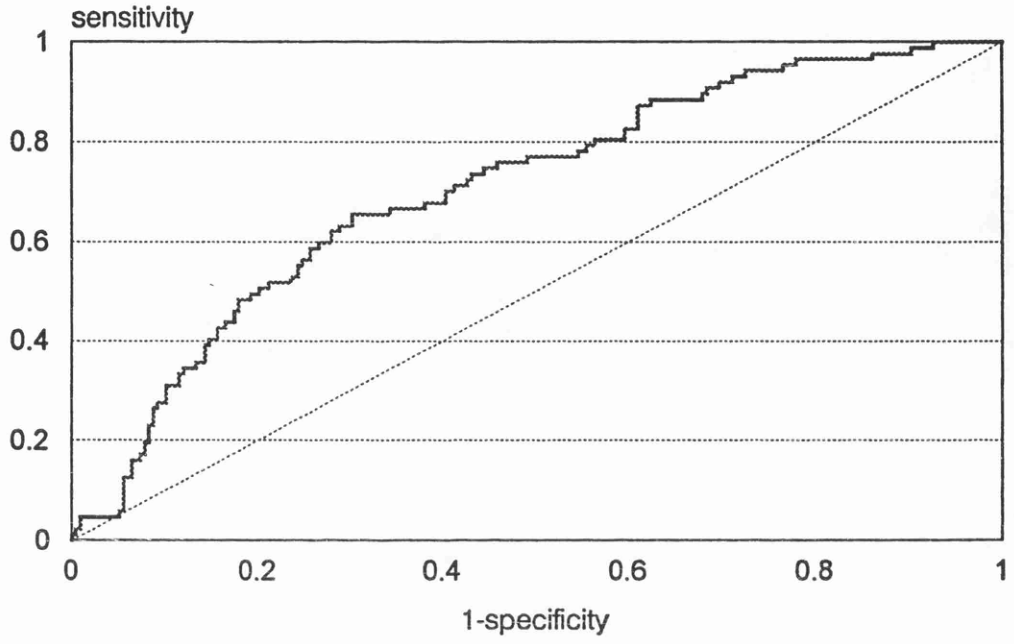

Figure 42. ROC curve for glycated haemoglobin concentration in distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

Differential positive rates were calculated for nadir plasma glucose, fructosamine and glycated haemoglobin because these were the tests determined by the ROC curves to be the most useful. The optimal cut-off values for deciding between very poor or poor glycaemic control and fair, good or excellent glycaemic control were 11.0 mmol/l, 435 μmol/l and 6.13%, respectively, for nadir glucose, fructosamine and glycated haemoglobin. These values along with the associated DPR's are given in Table 31. The DPR curves for each of the three useful analytes are represented in Figures 43 to 45.

Analyte	Maximum DPR	Cut-off value
Nadir glucose	0.853	11.0 mmol/l
Fructosamine	0.470	435 μmol/l
Glycated haemoglobin	0.352	6.13 %

Table 31. Maximum differential positive rates (DPR) and associated cut-off values for nadir glucose, fructosamine and glycated haemoglobin when used for distinguishing between very poor or poor glycaemic control and fair, good or excellent glycaemic control.

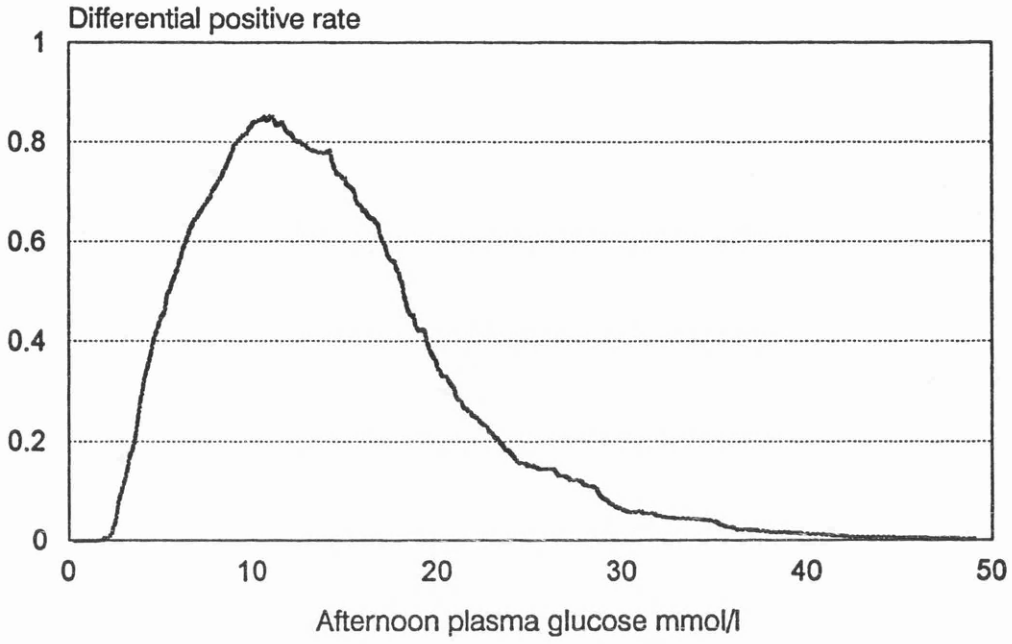

Figure 43. Differential positive rate curve for nadir plasma glucose when used for distinguishing between very poor or poor and fair, good or excellent glycaemic control (maximum DPR is 0.853 at 11.0 mmol/l).

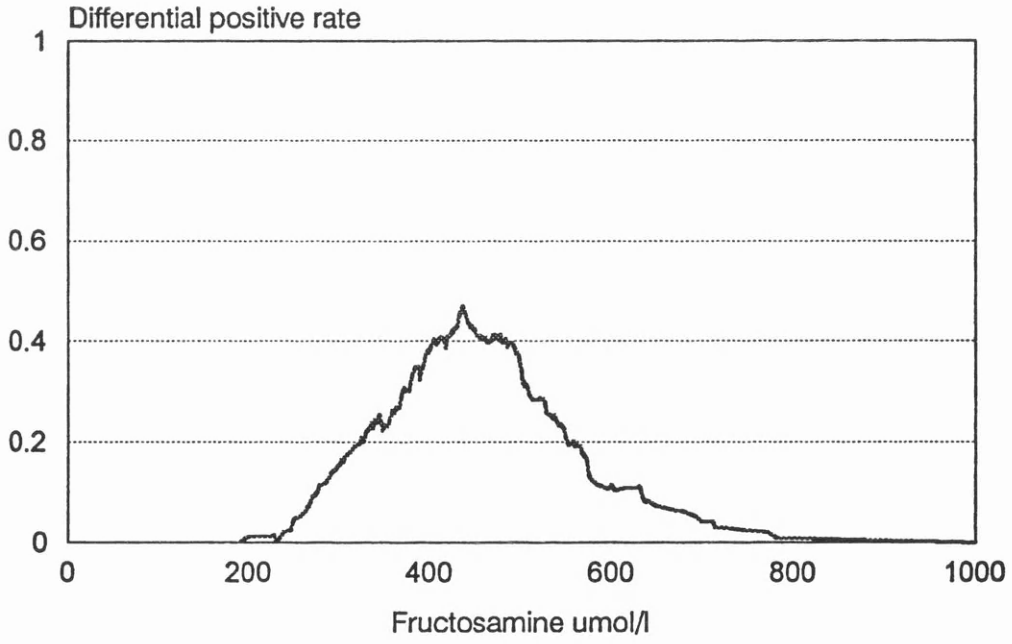

Figure 44. Differential positive rate curve for fructosamine concentration when used for distinguishing between very poor or poor and fair, good or excellent glycaemic control (maximum DPR is 0.470 at 435 μmol/l).

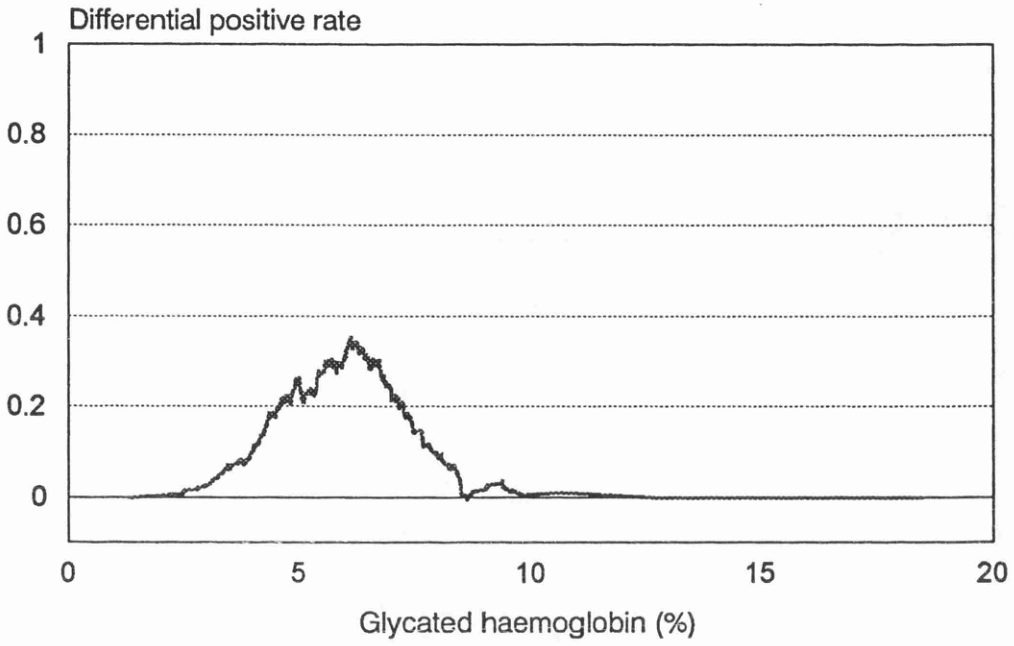

Figure 45. Differential positive rate curve for glycated haemoglobin when used for distinguishing between very poor or poor and fair, good or excellent glycaemic control (maximum DPR is 0.352 at 6.13 %).

Discussion

The validity of laboratory methods for the measurement of fructosamine and glycated haemoglobin in canine samples was studied along with the potential use of plasma concentrations of afternoon glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, fructosamine and concentrations of glycated haemoglobin as indicators of glycaemic control, including the early effects of diabetic stabilisation on the concentration of these analytes.

The fructosamine assay was accurate (as assessed by linearity), precise (mean intra and inter-assay CV's 1.38 and 3.17 respectively) and sensitive (limit of detection 6.26 μmol/l) when applied to canine plasma samples. The performance of the assay was not affected by significant lipaemia and fructosamine was stable in whole blood at room temperature for at least 48 hours.

The glycated haemoglobin assay was less precise than the fructosamine assay (mean intra and inter-assay CV's 3.84 and 7.31 respectively) in keeping with the manual nature of the test. Glycated haemoglobin was stable in whole blood at 4 °C for at least three weeks but storage of haemolysed samples or samples at room temperature greatly affected test

results. Incubation of whole blood with high concentrations of glucose at 37 °C resulted in a dramatic increase in glycated haemoglobin within one week. When the affinity chromatography method was compared to others it was discovered that cation exchange chromatography appeared to be an inappropriate test for canine glycated haemoglobin but that agarose gel electrophoresis might be an acceptable alternative. The choice of anticoagulant between lithium heparin and EDTA had no effect on the results of the test.

The concentrations of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and fructosamine did not vary over 24 hours in contrast to marked fluctuations in plasma glucose over the same time period in insulin treated diabetic dogs. During the early post-stabilisation period, concentrations of afternoon glucose, fructosamine and glycated haemoglobin were reduced by therapy but the plasma concentrations of alkaline phosphatase and alanine aminotransferase increased. The duration of clinical signs of diabetes prior to presentation for treatment was not related to the severity of abnormality in the pre-treatment concentrations of glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase or glycated haemoglobin but was related to the pre-treatment concentration of fructosamine.

Afternoon plasma glucose, fructosamine and glycated haemoglobin were determined to be useful tests in the long term monitoring of canine diabetes mellitus both by comparison of mean concentrations between glycaemic control groups and ROC curve analysis. However, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were shown to be worthless tests for long term diabetic monitoring although there was a significantly higher mean concentration of alkaline phosphatase in samples from untreated diabetic dogs than in samples from diabetic dogs under poor to good glycaemic control and significantly higher mean concentrations of aspartate aminotransferase in samples from untreated and very poorly controlled diabetic dogs than in those from dogs under poor to good control. These statistically significant differences would be irrelevant clinically because of the wide range of alkaline phosphatase and aspartate aminotransferase concentrations within each glycaemic control group.

The results of this study show that fructosamine and glycated haemoglobin assays are valid and useful tests in the monitoring of canine diabetes mellitus and that the 'liver enzymes' - alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase - have no role in this context.

The results of this study compare very favourably with previously published reports of the precision of the fructosamine assay used for both human and animal samples. Kruse-Jarres *et al* (1989) reported the range of C'sV obtained by 12 analytical laboratories using three human serum pools across a range of fructosamine concentrations (200 to 500 μmol/l) as 0.5 to 3.0 and 0.9 to 4.8 for intra and inter-assay C'sV respectively. Sobel and Abbassi (1991) reported intra and inter-assay C'sV of 3.1 and 4.5 % when the assay was used on a similar analyser to the present study to measure fructosamine in diabetic children. In dogs, Kawamoto *et al* (1992) reported mean intra and inter-assay C'sV of 5.6 and 5.7 using a manual adaptation of the method. Jensen (1992), using a similar autoanalyser method to the one in the present study was only able to achieve a mean intra assay CV of 3.5% despite analysing 30 replicates. The mean inter-assay CV achieved by his method was better than in the present study at 3 %. Reusch *et al* (1993) reported intra and inter-assay C'sV of 2.4 and 3.4 %, respectively, using an autoanalyser method and these are also very similar to the results of the present study. The present study was also in agreement with Kruse-Jarres *et al* (1989) findings in human serum of no interference by lipaemia (up to triglyceride concentrations of at least 25 mmol/), that the method was linear (up to 1000 μmol/l in their study) and that the limit of detection of the assay was 7 μmol/l). Only Jensen (1992) has previously reported the linearity and limit of detection of the fructosamine assay in dog samples and he suggested that there were some problems with linearity in his laboratory and that the limit of detection was 9.11 μmol/l. There are no previous reports of the effect of lipaemia on fructosamine measurement in canine samples. In the present study the fructosamine assay performed well and was improved upon when compared with previously published reports of its use for canine samples.

Jensen (1992) and Reusch *et al* (1993) demonstrated that fructosamine was stable in serum at 4 and 25°C for 5 days, and the present study shows that it is stable in whole blood at room temperature for sufficient time to transport samples to the laboratory even by post. The canine reference range for fructosamine of this study is similar to that published by Kawamoto *et al* (1992) of 170 to 338 μmol/l but slightly lower than that published by Jensen and Aaes (1992) of 258 to 343μmol/l and that of Reusch *et al* (1993) of 249 to 374 μmol/l using 29 and 48 dogs, respectively.

There are no previous reports of the use of mini affinity chromatography columns for the measurement of glycated haemoglobin in dog samples with which to compare the performance of the assay used in this study. The manufacturers of the assay used suggest that a mean intra-assay CV of 2.4 % can be obtained in human samples using their kit and independent reports cited by Kennedy (1992) agree that C'Vs of between 2.0 and

2.6% have been achieved in human samples using affinity chromatography methods. The kit manufacturer also claims a mean inter-assay CV of 2.9% but this is based on very large numbers of replicate samples (n = 14 to 45) and it is unwise to make a comparison with a precision study using only 8 replicate analyses. Higgins *et al* (1982), using a different affinity chromatography method for canine samples, reported the results of only two analyses and precision was not investigated. The assay used in the present study was clearly more precise than the colorimetric method of Smith *et al* (1982), when used in canine samples, which had a mean intra assay CV of 9.9%. The precision of the cation exchange methods used by Wood and Smith (1980) and Mahaffrey and Cornelius (1982) were not reported.

The importance of a long-term storage study for glycated haemoglobin was greater than for fructosamine because the manual nature of the glycated haemoglobin method meant that it was most suited to batch analysis. The findings of this study concur with the recommendations of the kit manufacturer, that accurate test results can be obtained from refrigerated human blood samples for up to 15 days after collection and that storage of samples at room temperature for more than 12 hours can cause erratic results. Again, there are no previous detailed reports of affinity chromatography methods applied to canine samples with which to compare this data. The study in which whole blood was incubated at a high glucose concentration confirmed that glycated haemoglobin was the product being measured by the affinity chromatography method and that the production of glycated haemoglobin is temperature dependent as reported by Dolhofer, Stadele and Wieland (1977) (cited by Kennedy, 1992). In addition, this part of the study suggested that an increase in glycated haemoglobin concentration could be expected within one week of clinical hyperglycaemia although the change *in vivo* would be less dramatic because of the contribution of new haemoglobin constantly being introduced to the circulating pool.

The use of cation exchange chromatography for glycated haemoglobin measurement in canine samples was inappropriate, based on the trial of one commercially available kit. This is not surprising since the structure and ionic properties of the principal glycated haemoglobins of the dog are not known. To improve separation of the glycated and non-glycated components with this kind of methodology would involve adjusting buffer pH on a trial and error basis to discover that most suited to canine haemoglobin. It seems imprudent to make in-house modifications to a relatively expensive commercial assay when there are more appropriate and less expensive methods available. Higgins *et al* (1982) utilised three different pH buffers for a cation exchange method in 6 species but interestingly, in the light of the findings in the present study, the same pH for human and

canine samples was chosen. Agarose gel electrophoresis gave comparable results to the affinity column method but each gel has 10 tracks, making the requirement to batch samples greater for cost effectiveness. This approach is, however, compromised by the much shorter stability of samples destined for this type of analysis. In addition, the accurate measurement of very small concentrations of glycated haemoglobin in normal dogs was difficult even at 500% magnification of the densitometric scan.

The excellent correlation between lithium heparin and EDTA samples means that a single whole blood sample can be submitted to the analytical laboratory for analyses of both fructosamine and glycated haemoglobin along with the 'routine' plasma biochemical analyses.

The use of an affinity chromatography method for canine glycated haemoglobin seems more appropriate than the other methods investigated in this study. Kennedy (1992) proposed that this method will become the most popular for the measurement of human glycated haemoglobin because of its high degree of specificity and reproducibility. Even as long ago as 1982, Higgins *et al* were very impressed by the power and versatility of this technique, particularly in relation to the measurement of glycated haemoglobin in animal samples.

Although it has been recognised that the 'liver' - enzymes alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase - are elevated in untreated and unstable diabetic dogs (Chastain and Ganjam, 1986b; Nelson 1989a), reports of the effects of stabilisation on the 24 hour profile of these enzymes are extremely difficult to find. The finding of increased alanine aminotransferase and possibly alkaline phosphatase activity in the early post-stabilisation period is unexpected. It suggests that stabilisation itself is a form of hepatic insult. Such an insult may be a consequence of the sudden turnaround in hepatic metabolism which must occur following the institution of insulin therapy in previously untreated diabetic dogs. Certainly, this study has shown that the measurement of plasma concentrations of alanine aminotransferase or alkaline phosphatase in the early post-stabilisation period should not be used to make judgements concerning the progress of therapy. Reductions in fructosamine and glycated haemoglobin concentrations in a manner mirroring glucose concentrations following diabetic stabilisation or improved control have been reported in human medicine (Dolhofer and Weiland, 1980; Cefalu *et al*, 1988; Sobel and Abbassi, 1991) and similar reduction in fructosamine has previously been reported in diabetic dogs by Reusch *et al* (1993), but there are no reports on the effect of stabilisation on canine glycated haemoglobin concentrations.

The fact that fructosamine concentration can be within the reference range in untreated diabetic dogs was also noted by Reusch *et al* (1993) who cited the example of two dogs with short duration of clinical signs. This is not a surprising finding considering the rate of the glycation reaction and the half life of plasma proteins. There was a similar but not statistically significant trend in the relationship between glycated haemoglobin and duration of clinical signs. One dog, presented after one week of overt clinical signs, had a glycated haemoglobin result which fell within the reference range but all dogs presenting after two weeks of clinical signs had glycated haemoglobin concentrations outwith that range. Plasma concentrations of glucose, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in untreated diabetics cannot be used to assess the duration or severity of the condition based on the results of this study.

A comparison between the biochemical characteristics of different groups of glycaemic control in diabetic dogs has not previously been reported (except by Reusch *et al* (1993) for fructosamine). Such a comparison highlights the potential benefits of fructosamine and glycated haemoglobin analyses in canine diabetic monitoring and was useful as a preliminary investigation prior to embarking on more sophisticated evaluative techniques.

The use of ROC curves to determine the usefulness of a laboratory test have only rarely been used in veterinary medicine (White *et al*, 1986; McNab *et al*, 1991; Jensen and Poulsen 1992; Jensen, 1993). In this study the 'near perfect test result' of the ROC curve analysis of afternoon (nadir) plasma glucose concentration in the differentiation of poor and fair to excellent control must be interpreted with caution because the allocation of observations to glycaemic control groups was not independent of this result. However, even although the contribution of the afternoon glucose concentration to the estimation of glycaemic control was great, it still allowed the creation of a benchmark (if not perfect) test by which the usefulness of the other analytes could be compared to one another. It is likely that fructosamine is a much more accurate test in determining the degree of glycaemic control than a single estimate of blood glucose (Kennedy, 1992; Reusch *et al*, 1993) and that fructosamine concentrations would have been a much more representative classification method for glycaemic control. However, in assessing the performance of the 'new' tests (fructosamine and glycated haemoglobin) it was important to have a method of classification independent of those tests and the choice of afternoon plasma glucose as the benchmark test was not expected to affect the comparison between them. The ideal cut-off point for afternoon glucose concentration of 11.0 mmol/l fits well with clinical practice and is not surprising considering the contribution of this value in the classification system used. Jensen (1993) used an ROC curve method to determine that the upper limit of his reference range was an ideal cut-off

value for fructosamine concentration when used as in the diagnosis of canine diabetes mellitus. Also, using an ROC curve method, that study suggested that there was no advantage to making protein or albumin-corrected fructosamine concentrations for diagnostic purposes. Reusch *et al* (1993) grouped observations of fructosamine concentration from treated diabetic dogs into those with fully satisfactory glycaemic control, moderately satisfactory glycaemic control and poor glycaemic control, based on fasting blood glucose concentrations, and suggested a cut-off value of 400 μmol/l to differentiate between dogs with satisfactory control and those under insufficient control. Interestingly, this value is not dissimilar to the cut-off value of 435 μmol/l suggested by the present study for differentiation of poor control and those with some degree of glycaemic control. There are no previous reports of a suggested cut-off value for glycated haemoglobin in the differentiation of poor and good glycaemic control in treated diabetic dogs.

In conclusion, the 'new' tests fructosamine and glycated haemoglobin are valid when applied to canine samples within the University of Glasgow Veterinary School and are useful tests in monitoring diabetes mellitus in dogs. Alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase have no role in monitoring therapy in diabetic dogs despite the common finding of elevated concentrations of these enzymes in the plasma of untreated and unstable diabetic dogs.

Chapter 6:
Pharmacokinetics and efficacy of a mixed insulin zinc suspension

Part I: Pharmacokinetics

Introduction

Insulin is a large peptide with a molecular weight of around 5800 which consists of an α and a β chain connected by two disulphide bridges. It is formed by the removal of a connecting peptide (C-peptide) from between the two chains of its precursor molecule pro-insulin. Peptides are digested in the intestinal tract, so insulin cannot be administered orally and the large size of insulin prevents trans-mucosal administration, leaving injection as the only feasible insulin delivery route. However, unmodified insulin has only a very short half life (Laurence and Bennett, 1987) and many repeated injections or continuous infusion would be necessary for therapeutic effect. Since the discovery of insulin and its subsequent purification for use as a therapeutic product, many attempts have been made to prolong the duration of action of insulin preparations (Home and Alberti, 1992). This has resulted in three main types of prolonged action insulin preparations: isophane (NPH), lente (insulin zinc suspension) and protamine zinc insulin (PZI).

Protamine zinc and isophane insulin preparations have a longer duration of activity when administered subcutaneously because insulin has been combined with the basic protein protamine. Protamine is found in large quantities in fish sperm nuclei and it has a smooth sheet like structure which is poorly immunogenic. Insulin-protaminate complexes are acted upon by tissue proteinases causing insulin to be released slowly. Protamine zinc insulin contains a greater proportion of protamine than isophane to slow absorption further and zinc which may inhibit the action of tissue proteinases (Home and Alberti, 1992).

Insulin zinc suspensions have prolonged activity because of the reduced solubility of insulin in the presence of large concentrations of zinc (Home and Alberti, 1992). By altering the pH at which insulin zinc precipitates are formed, insulin preparations with differing properties can be generated. Ultralente insulin is a crystalline form which is first

generated at a pH of 5.5 and is then adjusted to a pH of 7.4. This process alters the crystalline structure of the zinc-insulin and creates a preparation which is absorbed very slowly from subcutaneous sites. Semilente insulin is an amorphous zinc-insulin precipitate formed between insulin and excess zinc at a pH of 7.4 when there is no initial crystallisation stage. Semilente insulin is much more rapidly absorbed from subcutaneous sites and 'lente' insulin preparations are a mixture of 30% amorphous semilente and 70% crystalline ultralente and are therefore mixed insulin zinc suspensions (Feldman and Nelson, 1987; Home and Alberti, 1992).

In human diabetic medicine there is a noticeable difference in duration of modified insulin preparations which is dependent on the species of origin of the insulin. Bovine insulin is more slowly absorbed and is more immunogenic than either porcine or genetically engineered human insulins which differ from one another by only one amino-acid residue at the end of the β chain (B30) (Home and Alberti, 1992).

The intermediate duration types, isophane (NPH) and mixed insulin zinc suspension (lente) are the most commonly used in the management of canine diabetes mellitus. Data are available on the absorption kinetics of isophane insulin in the normal dog (Goeders *et al* 1987) but not for the lente group. Previous studies of the peak and duration of activity of porcine mixed insulin zinc suspension (IZS-P) were based solely on plasma glucose responses (Church 1981).

The aim of this study was to determine the times of peak and duration of activity for an IZS-P (Caninsulin; Intervet UK) in dogs with naturally-occurring diabetes mellitus by serial measurement of plasma insulin concentrations following injection.

Materials and methods

Dogs

Ten dogs of varying breed with naturally-occurring diabetes mellitus, referred for diabetic stabilisation, were included in the study. Individual animal details are summarised in Table 32. Three of the 5 females and two of the 5 males had been neutered. The average age was 8.6 years (SD 2.7; range 5-12 years) and average body weight was 16.25 kg (SD 11.9; range 5.5-41.5). For the purposes of this study and the subsequent efficacy study, dogs were assigned a trial number in addition to their hospital number. Dogs are referred to by their trial number throughout this chapter.

Dog No.	Hospital number	Breed	Bweight (kg)	Age (years)	Sex
C3	113648	Scottish terrier	10.0	5	M
C4	109126	West Highland White terrier	10.5	12	MN
C5	111513	Labrador X	14.0	12	FN
C6	114460	Dobermann	31.0	7	F
C7	114653	Spaniel X	24.0	11	F
C8	114695	Terrier X	10.5	9	FN
C9	115009	Dachshund	5.5	10	FN
C14	115678	Rottweiler	41.5	5	M
C18	116594	Cairn terrier	10.3	6	M
C19	116744	Miniature Poodle	6.3	9	MN

Table 32. Details of dogs included in the insulin pharmacokinetic study (M: male, F: female, N: neutered).

Management

During stabilisation of their diabetic state, all animals received a diet consisting of commercial canned food and bread or biscuit divided evenly into two meals. One meal was fed at the time of a single daily insulin injection and the other 7.5 to 8 hours later. The diet fed was based on 40-80 kcal/kg depending on a subjective assessment of size and body condition. Insulin dosages were adjusted according to the previous afternoon plasma glucose concentrations.

On the morning of the pharmacokinetic study, each dog received a subcutaneous injection of IZS-P at a dose according to individual expected requirement and was offered the morning meal. The afternoon meal was fed 7.5 hours later. Immediately prior to the insulin injection and at two hourly intervals over the following 24 hour period blood samples were obtained by jugular venepuncture and aliquots transferred into fluoride/oxalate and lithium heparin coated containers (Sarstedt). All samples were centrifuged immediately and plasma drawn into plastic containers. The fluoride/oxalate plasma samples were stored at 4°C until analysis at the end of the 24 hour study period and the lithium heparin plasma samples were stored at -20°C.

The clinical condition of each animal was carefully monitored and particular attention was paid to any changes in attitude or demeanour suggestive of hypoglycaemia or adverse reaction.

Laboratory analyses

Plasma glucose analyses were carried out using a glucose hexokinase method (Roche) with a Cobas Mira analyser (mean intra-assay coefficient of variation 3.4 per cent).

Plasma insulin analyses were performed using a commercial solid phase radioimmunoassay kit (Coat-a-Count® Insulin; Diagnostic Products Corporation). The mean intra-assay coefficient of variation for the insulin assay was 6.8 per cent. Recovery of known amounts of porcine insulin (Velosulin; Novo Nordisk) added to a plasma pool of low insulin concentration was between 90 and 120 per cent. Dilutional parallelism of this kit was also good.

Derivation of results

Plasma insulin versus time curves were examined visually to identify times of peak insulin concentration.

Persistence, i.e., the time taken for plasma insulin concentration to return to its pre-injection level was estimated as the sample time preceding the first one at which plasma insulin concentration was within 15% of the preinjection concentration or lowest assay standard. Persistence greater than 24 hours (dogs C3 and C14) was arbitrarily designated a value of 24 hours.

Plasma insulin concentrations were converted to incremental values by subtraction of the lowest plasma insulin concentration for each dog and areas under the plasma concentration versus time curves (AUC) for insulin were calculated using the trapezoidal rule. Mean residence times (MRT) calculated from the area under the moment curves (AUMC or AUC x t) are commonly used measures of the persistence of a drug within the body when that drug is administered as a bolus and its disposition is by first order kinetics. When the absorption of a drug follows first order kinetics, the rate of its absorption is proportional to its concentration and its fractional absorption rate is constant. These processes can be described using a half life value which will be constant at all concentrations of the drug. In zero order or rate limited processes the rate of absorption is constant regardless of the concentration of the drug. In absorption kinetics, if the percentage of dose of a drug remaining to be absorbed is plotted against time on a plain (Cartesian) graph, zero order absorption is represented by a straight line and first order absorption by a progressively decreasing gradient with time. On a semilogarithmic plot, first order absorption processes are represented by a straight line and zero order by a progressively increasing gradient (Rowland and Tozer, 1989).

In a first order process, the MRT (AUMC/AUC) is equivalent to the time taken to eliminate 63.2 per cent of a given dose and in zero order processes it is the time taken to eliminate 50 per cent. Examination of the percentage remaining to be absorbed versus

time curves generated from the AUC data showed that the absorption and elimination of IZS-P did not follow first or zero order kinetics (Figures 46 and 47) and so MRT was not calculated.

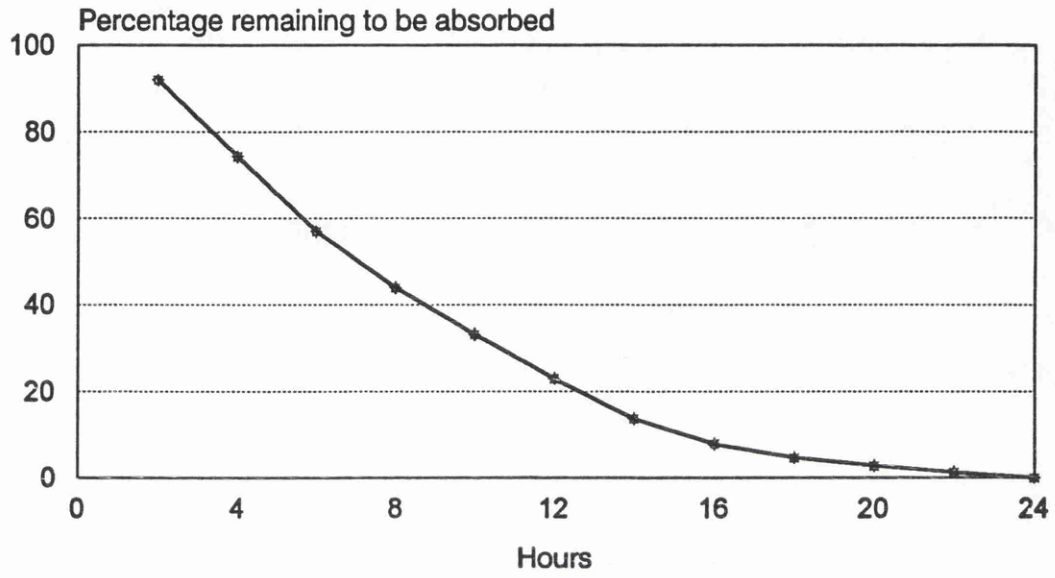

Figure 46. Plain (Cartesian) plot of percentage of IZS-P remaining to be absorbed versus time (based on area under the mean percentage of maximum plasma insulin concentration curve).

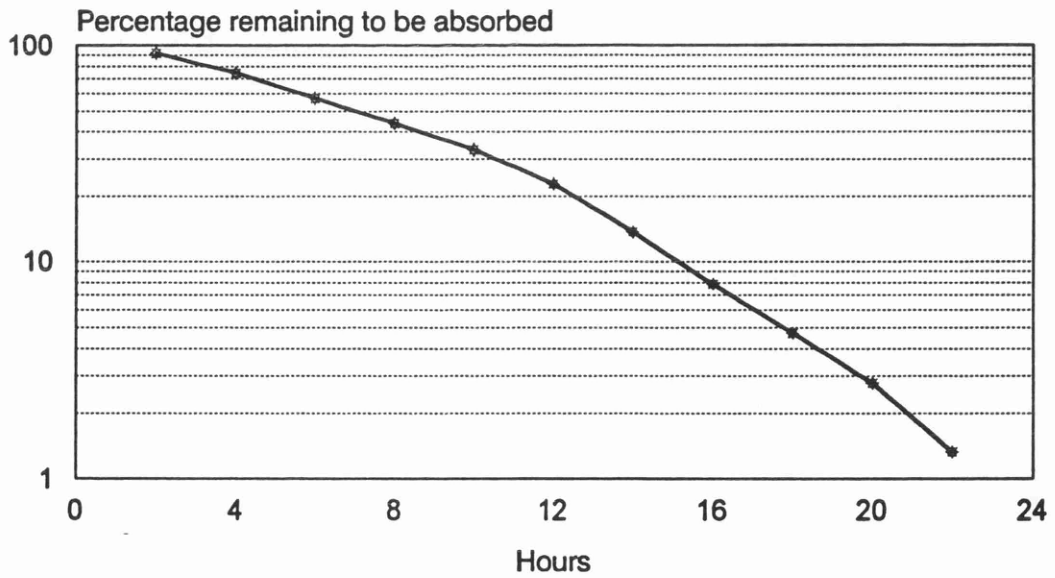

Figure 47. Semilogarithmic plot of percentage of IZS-P remaining to be absorbed versus time (based on area under the mean percentage of maximum plasma insulin concentration curve).

For the purposes of comparison with other preparations an equivalent residence time ($RT_{63.2}$, i.e., approximate time taken for absorption of 63.2 per cent of dose) was calculated from the percentage remaining to be absorbed versus time curves. Because of the short half life of insulin, residence time would have been mostly dependent on absorption time.

To allow the graphical representation of the mean insulin absorption curve, the variation in dose administered was compensated for by transforming plasma insulin concentrations into percentages of the maximum plasma insulin concentration reached in each dog. Plasma glucose concentrations were manipulated in the same way to allow a mean glucose response curve to be generated.

Results

Plasma insulin concentrations following injection are given in Table 33 and represented in Figure 48. In all dogs there was an initial peak in plasma insulin concentration between 2 and 6 hours (mean 4.3 +/- SD 1.3) post-injection (Table 34). As expected, peak plasma concentration of insulin varied between dogs (mean 150μIU/ml +/- SD 64.5; range 85-288) because each dog received a different dose (Table 34). In dogs nos. C3, C14, C18 and C19, the plasma concentration of plasma insulin peaked again after the second meal, in another (no. C4), there were two further peaks of plasma insulin at 8 hours and at around 12 hours. In dogs nos. C14, C18 and C19 the second peak was the greater of the two. In three dogs (nos. C6, C7 and C8) the rate of reduction in plasma insulin concentration appeared less around the time of and for up to three hours after the second meal, forming a 'shoulder' to the plasma insulin curve. When present, the second peak or 'shoulder' in insulin concentration occurred at 11 hours (mean 11 +/- SD 1.85; range 8-14) post-injection (Table 34). Values for Persistence, AUC and $RT_{63.2}$ are given in Table 34. Persistence ranged between 14 hours and 24 hours (mean 17.4 +/- SD 3.65) (Table 34).

The mean insulin absorption curve, based on plasma insulin concentrations transformed into percentages of maximum concentration is represented graphically in Figure 49.

There were statistically significant correlations (Pearson's r, $p < 0.05$) between the time of the second peak and AUC and $RT_{63.2}$; between AUC and Persistence and $RT_{63.2}$ and between Persistence and $RT_{63.2}$. There were no statistically significant correlations between insulin dose (IU/kg) and any of AUC, Persistence or $RT_{63.2}$.

Hours post-injection	Dog C3	C4	C5	C6	C7	C8	C9	C14	C18	C19
0	5	44	3	11	45	14	7	27	9	23
2	154	109	70	43	137	170	65	81	85	85
4	155	288	85	91	183	171	71	148	77	98
6	91	81	57	51	148	187	92	122	35	100
8	79	126	41	49	131	107	44	127	41	97
10	99	83	37	34	117	99	25	102	35	80
12	88	180	29	31	62	41	17	115	100	130
14	80	110	16	20	60	27	9	194	22	96
16	54	82	9	15	65	18	5	111	16	21
18	25	54	3	11	47	16	7	109	7	5
20	16	38	3	6	57	17	4	80	6	0
22	15	25	0	8	50	16	7	86	0	3
24	9	15	0	8	53	13	6	91	8	0

Table 33. Plasma concentrations of insulin (μIU/ml) following subcutaneous injection of IZS-P in 10 dogs with naturally-occurring diabetes mellitus.

Dog No.	Dose (IU/kg)	1st Peak (hours)	2nd Peak (hours)	A.U.C. (IUhr/ml)	Persistence (hours)	$RT_{63.2}$ (hours)
C3	2.80	3	10	1606	24	10.03
C4	2.47	4	12	2051	18	10.95
C5	1.35	4	-	703	16	7.51
C6	1.16	4	8^S	593	16	7.83
C7	2.41	4	10^S	1132	16	7.61
C8	1.61	6	10^S	1453	16	6.84
C9	2.10	6	-	609	14	6.59
C14	1.01	4	14	2020	24	14.34
C18	1.56	2	12	865	16	10.39
C19	2.56	6	12	1453	14	10.52

Table 34. Insulin dose, times of peak concentration, area under plasma insulin versus time curve (AUC), persistence and residence time of 63.2 per cent of dose ($RT_{63.2}$) in 10 dogs with naturally-occurring diabetes mellitus following subcutaneous injection of IZS-P.
^{S} Denotes 'shoulder' rather than distinct peak

The plasma glucose responses to injection of IZS-P are represented in Figure 48. Plasma glucose concentration were reduced in all animals following injection of IZS-P. In 5 dogs (nos. C3, C6-C9), this decrease continued only to the feeding of the second meal, after which plasma glucose concentration continued to rise to preinjection concentrations or higher. In two dogs (nos. C5, C18), plasma glucose was dramatically reduced following injection and remained at a low level (<5mmol/l) between 4 and 18 hours despite the feeding of a second meal. In another dog (no. C4), plasma glucose was also low (<5mmol/l) between 4 and 24 hours post-injection with only a transient increase in response to the second meal. In dog no. C19, plasma glucose concentration fell after an initial post prandial peak and remained at an acceptable level until 18 hours post injection. A similar effect was seen in dog no. C14 but with a further post prandial peak after the second meal. The mean glucose response curve is represented in Figure 50.

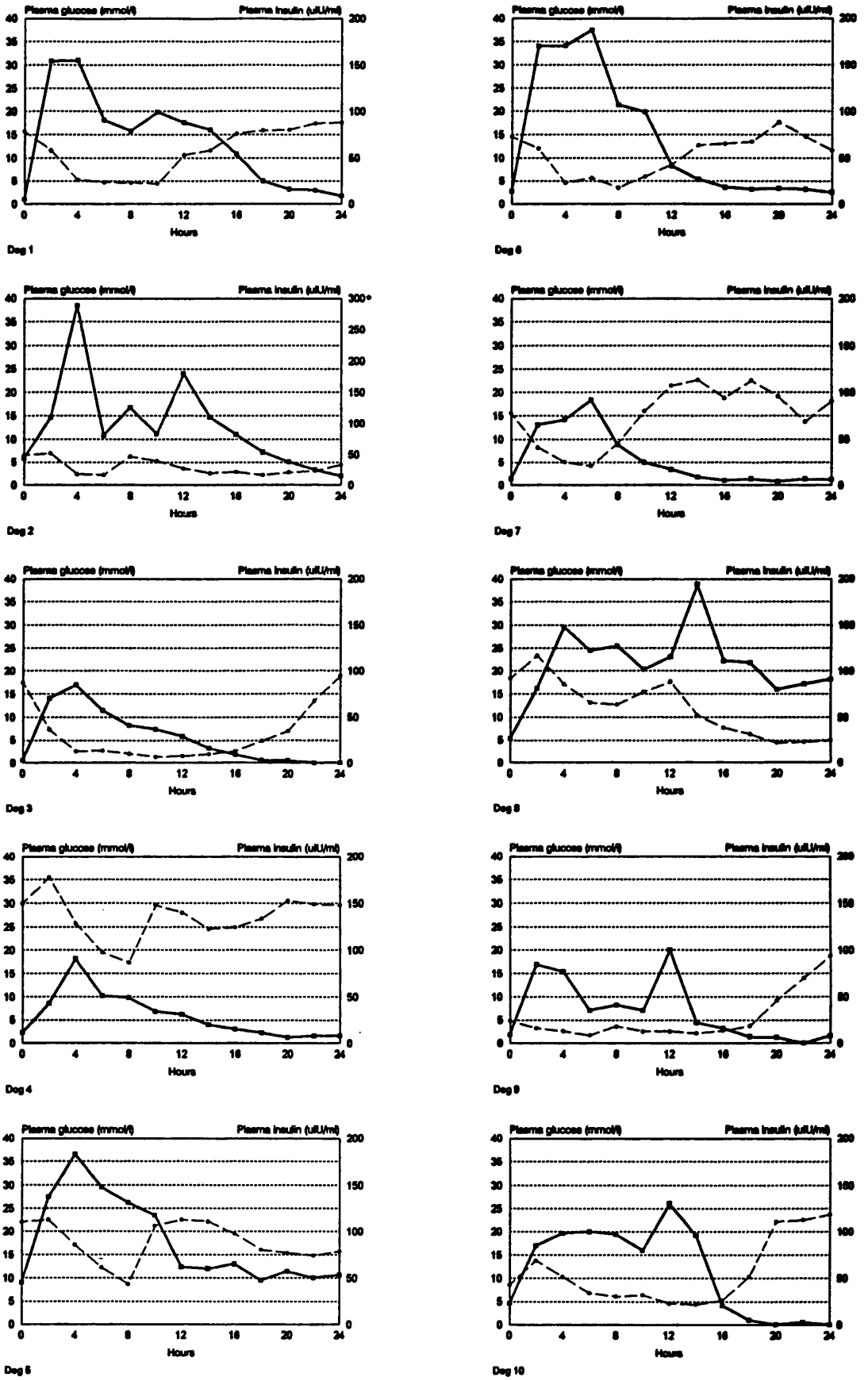

Figure 48. Plasma insulin and glucose concentrations in 10 dogs following injection of IZS-P. Solid lines are plasma insulin concentrations, dashed lines are glucose concentrations.

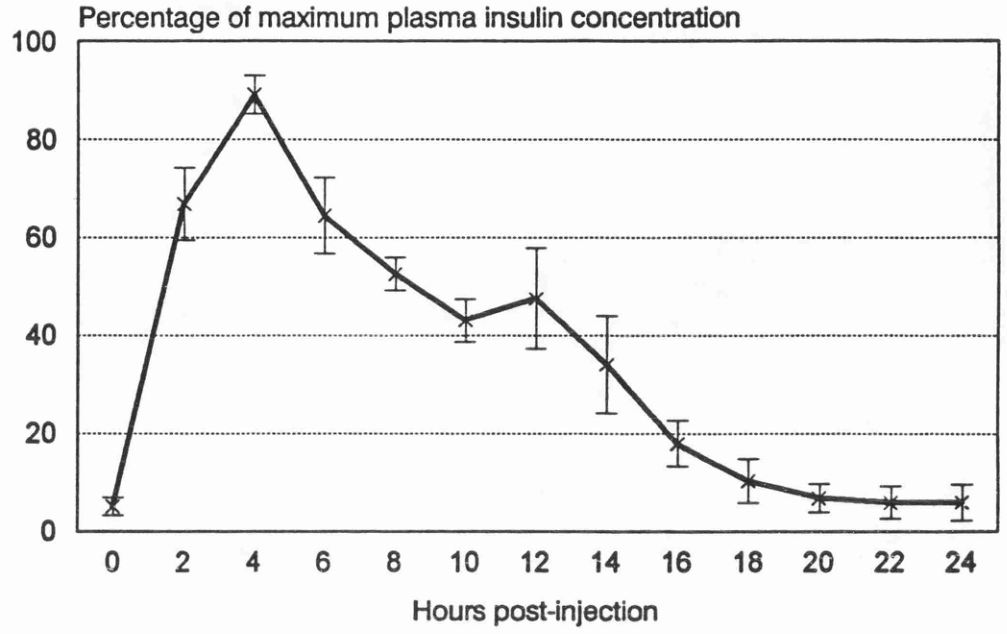

Figure 49. Mean percentage of maximum plasma insulin concentration reached following subcutaneous injection of IZS-P in 10 dogs (±SEM).

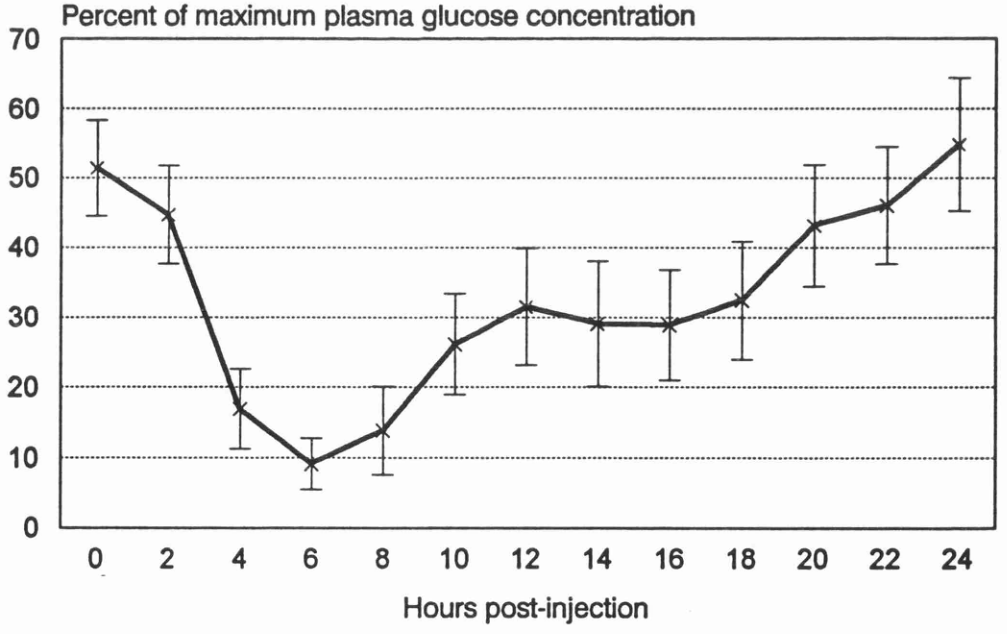

Figure 50. Mean percentage of maximum plasma glucose concentration reached following subcutaneous injection of IZS-P in 10 dogs (±SEM).

Discussion

In this study, porcine insulin zinc suspension had two peaks of activity following subcutaneous administration (the first at around 4 hours and the second at around 11 hours) and a duration of activity of between 14 and 24 hours.

The secondary peaks in plasma insulin concentration seen in dogs nos. C3, C14, C18 and C19 and the 'shoulders' created by the slower rate of decrease of plasma insulin in dogs nos. C6, C7 and C8 are likely to represent the absorption of the crystalline component of the zinc insulin suspension. Alternatively, they could represent endogenous insulin release in response to feeding (as would be seen in normal dogs under the same feeding schedule). However, the presence of exogenous insulin and some degree of hypoglycaemia might be expected to suppress endogenous insulin release, thus rendering this explanation less likely.

The area under the plasma insulin versus time curve results did not correlate directly with insulin dose (IU/kg) administered. This is likely to be the result of the variation between dogs with regard to age, sex, breed, body condition, endogenous insulin production and endogenous insulin response to feeding since it is known, or can be assumed, that these were not constant between dogs. It is, however, apparent that IZS-P is well absorbed following subcutaneous injection in naturally-occurring diabetic dogs.

It could be argued that, all dogs should have received the same dose of insulin or perhaps have been starved. However, it was considered of greater practical value to assess the effects of IZS-P under the conditions for which it was designed, i.e., in naturally-occurring diabetic dogs receiving the particular dose of insulin required and a calculated ration of food at the recommended times post-injection. Moreover, this study was performed during the normal clinical management of these dogs and was designed so as not to interfere with the progress of their treatment.

In the 10 dogs studied, the time of the initial peak plasma insulin concentration and the time of the second peak (when present) have a much narrower distribution than that previously reported for peak activities of lente insulins in dogs. Feldman and Nelson (1987) suggested a wide range of peak activity of lente and IZS-P of 2-10 hours, but these values were based on plasma glucose concentrations and not insulin concentrations. The wide range was probably due to the fact that mixed insulin zinc suspensions should have two peaks of activity and that these were regarded as one.

Church (1981) reported a range of 4-8 hours for peak activity of IZS-P in diabetic dogs, also based on plasma glucose concentrations. Contrary to the impression given previously, the narrow distribution of times of peak plasma insulin concentration in the present study indicates that the timings of peak activities for IZS-P are reasonably predictable. There are obvious clinical benefits of this feature of the pharmacokinetics of IZS-P.

The relatively wide range in duration of activity of 14 to 24 hours is similar to the 8-20 hours reported by Church (1981) and suggests that in some dogs this insulin preparation would be most useful when administered twice daily.

The choice of a porcine insulin preparation for use in dogs may have some benefits principally because it is antigenically identical to canine insulin (Smith, 1966). In the dog, exogenous insulin therapy using purified porcine insulin preparations has induced less immunological response than using insulins of bovine or mixed origin (Feldman *et al* 1983), reducing the chances of immunological interference or of unpredictable release of insulin from insulin/antibody complexes (Bolli *et al* 1984). However, the presence of antibodies to exogenous insulin could be advantageous because they may prolong the duration of action of the preparation and prevent sudden fluctuations in plasma insulin concentrations by the creation of an 'insulin reservoir' (Bolli *et al*, 1984; Feldman and Nelson, 1987). This absence of antibodies to porcine insulin preparations may explain the clinical impression of a more rapid onset and shorter duration of action of porcine insulin preparations than those of bovine or mixed origin (Feldman and Nelson, 1987).

This study has shown that IZS-P is well absorbed following subcutaneous injection and is capable of lowering plasma glucose concentration in dogs with naturally-occurring diabetes mellitus. It has an intermediate duration of action and reasonably predictable times of peak activity. A single daily injection regimen and 7.5 hour feeding schedule works well in many dogs but twice daily injection would be necessary in some to achieve optimal glycaemic control.

Part II: Efficacy

Objective

The purpose of this study was to monitor the effects of a mixed porcine insulin zinc suspension (IZS-P, Caninsulin; Intervet UK) on plasma and urine glucose and clinical condition when used in dogs with naturally occurring diabetes mellitus over an initial 'stabilisation' period. The effects of long-term use of this product were also studied.

Materials and methods

Dogs

Nineteen diabetic dogs were used in this study. Three of these had been stabilised and maintained using an isophane insulin (Evans or Hypurin) before being changed to IZS-P for the purposes of this study. The remaining dogs received only IZS-P except dog C15 which was given regular insulin (Hypurin Neutral) as part of the management of an initial ketoacidosis.

Ten of these dogs were included in a study of the pharmacokinetics of IZS-P (Chapter 6, Part I).

There were 12 female and 7 male dogs. Five females were speyed prior to first presentation and 6 were speyed as part of stabilisation management or soon after stabilisation. One of the males had been castrated before first presentation and one other was castrated during treatment on suspicion of testicular neoplasia. A variety of breeds were represented in the study group. Nine were terrier types, three mongrels, one Rottweiler, one Dobermann, one Poodle, one Dachshund, one Beagle, one Labrador retriever and one Samoyed. The average age was 8.72 years ± 2.75 SD, range 4 - 15 years.

Management

Each dog was given a single daily injection of IZS-P and received a diet of commercial canned dog food mixed with bread or mixer, divided evenly into two meals. One meal was fed at the time of the insulin injection at 9.30am and the other 7.5 hrs later. Owners were advised to continue this regimen when the dog returned home.

The diet fed was based on 40-80 kcal/kg bodyweight depending on a subjective assessment of size and body condition. Details of animals used and diets fed are summarised in Table 35.

All Caninsulin used in this study was from batch number 9405, expiry date October 1991 refrigerated at 4°C following receipt from Intervet UK Ltd. Caninsulin supplied to owners after June 1991 was from batch 13, expiry date March 1993.

Dog nos. C4, C5 and C10 had been previously stabilised on an isophane insulin at University of Glasgow Veterinary School. Dog C8 had been partially stabilised on isophane insulin by the owner and C9 had undergone a degree of stabilisation by the referring veterinary surgeon using Lentard MC (Novo-Nordisk Pharmaceutical Ltd). Both were restabilised on IZS-P when they were returned to the hospital for boarding and they were not subjected to thorough re-investigation prior to conversion to IZS-P. The other 15 dogs were all assessed on initial presentation for suitability for inclusion in the study. Diabetes mellitus was confirmed by plasma glucose analysis and routine haematological and plasma biochemical analyses were performed. Lateral thoracic and abdominal radiographs were taken and suspected concurrent endocrinopathies or other diseases were investigated.

On admission to the study, each dog received a starting daily dose of IZS-P according to their expected requirement. Each morning thereafter, a 1 ml blood sample was taken into a fluoride oxalate collection tube prior to insulin injection and feeding. At this time each dog was also taken for a short walk to enable collection of a voluntarily voided urine sample. If this was unsuccessful, urine was not collected, as collection by catheterisation or cystocentesis were considered to be unnecessarily invasive. Blood and urine sampling were repeated 12 hours after the insulin injection. A further 1 ml blood sample was taken prior to the second meal. The results of glucose analyses on these samples were used to calculate the following day's dose. However, technical difficulties occasionally meant that these results were not available in time to be included in this decision. All blood samples were taken from the jugular veins using 20 or 21 gauge hypodermic needles and 2ml syringes (Terumo).

During the stabilisation period most dogs (depending on owner consent) were sampled for plasma glucose at frequent intervals over a 24 hour period to generate a plasma glucose curve.

Careful monitoring of the clinical condition of each dog was carried out each day whilst hospitalised. Particular attention was paid to changes in attitude, demeanour and willingness to exercise which might have suggested hypoglycaemia or adverse reaction. Dogs were weighed regularly.

Generally speaking, when a consistent and acceptable afternoon plasma glucose concentration was achieved using a specific dose of IZS-P, the dog was discharged from the hospital and the owners were instructed concerning insulin dose, diet and exercise. On occasion, dogs were discharged before this 'ideal' stabilisation because of holiday periods (C15 especially). Owners of dogs in which this 'steady state' had been difficult or impossible to achieve were supplied with urine testing sticks (Keto-diastix, Ames) and instruction was given in adjusting insulin dose according to urine glucose concentrations.

Owners were requested to return with their animals for follow-up checks approximately 7 and 14 days after discharge and for once monthly checks thereafter. Unfortunately, only a few owners were able to return this frequently but the others were encouraged to allow follow-up checks as often as possible. At each follow-up visit a clinical examination was performed and blood samples were taken for routine biochemical and haematological analyses.

Dog no.	Hospital Number	Previously Stabilised	Age (years)	Gender	Body-weight	Breed	kcal/kg
C3	113648	NO	5	M	10.0	Scottish terrier	75
C4	109126	YES	12	MN	10.5	WHW terrier	70
C5	111353	YES	12	FN	14.0	Labrador X	85
C6	114460	NO	7	F	31.0	Dobermann	50
C7	114653	NO	11	F	24.5	Spaniel X	50
C8	114695	NO	9	FN	10.5	Terrier X	75
C9	115009	NO	10	FN	5.5	Dachshund	90
C10	112598	YES	8	F	29.0	Samoyed	45
C11	115131	NO	8	F	5.8	Jack Russell terrier	65
C12	115164	NO	4	FN	8.5	Cairn terrier	70
C13	107674	NO	7	F	11.5	Beagle	70
C14	115678	NO	5	M	40.0	Rottweiler	60
C15	115980	NO	8	F	30.0	Labrador	55
C16	116315	NO	15	FN	12.0	WHW terrier	70
C17	116334	NO	11	M	19.0	Collie crossbred	60
C18	116594	NO	6	M	12.0	Cairn terrier	60
C19	116744	NO	9	MN	6.0	Miniature Poodle	80
C20	116825	NO	8	M	8.7	Australian terrier	75
C21	117056	NO	11	F	6.3	Jack Russell terrier	70

Table 35. Signalment of diabetic dogs used in IZS-P study, including initial bodyweight and daily caloric intake.

Analyses

Plasma glucose analysis was carried out in the Department of Veterinary Medicine Clinical Biochemistry Laboratory as detailed in Chapter 6, Part I. Occasionally at weekends, plasma glucose concentrations were measured using a Vettest 8008 dry chemistry autoanalyser (IDDEX).

Urinanalysis was carried out in the Department of Veterinary Medicine Research Laboratory using Labstix urine reagent stips (Ames) and Clinitest reagent tablets (Ames) in accordance with manufacturer's instructions. The maximum possible urine glucose concentration measurable using these methods, was 2% (2g/100mls) although urine glucose concentrations were often much higher.

Results

Detailed case reports of the stabilisation of 19 diabetic dogs included in the study are contained within Appendix 11.

Hospitalisation

Dogs which were bright and lively on admission to the hospital remained so throughout the hospitalisation period, except for C9 and C13 which became diarrhoeic and dull for a few days. Dogs which were ill on admission (C3, C14, C16 and C20) became bright and alert within a few days of starting IZS-P therapy and continued to be so for the remainder of the hospitalisation period. Dogs C6, 7 and 21 had recently been in oestrus prior to joining the study and were believed to be suffering from metoestrus-associated diabetes mellitus.

Nine dogs were ketonuric at the start of stabilisation and in all cases but one this resolved within 6 days. One dog still had 'trace' or 'small' quantities of ketones until 11 days after the start of stabilisation.

Dogs were considered to be 'stable', in terms of their plasma glucose response to IZS-P injection, when afternoon (5pm) plasma glucose concentrations fell within the range 3 - 8.5 mmol/l. All dogs, except C5 and C15, achieved this target afternoon plasma glucose concentration by 2 or 3 days prior to discharge. Dog C5 was discharged with relatively high afternoon plasma glucose concentrations because increases in insulin dose had to be made cautiously in the light of previously exaggerated responses to lower insulin doses and an episode of clinical hypoglycaemia. Dog C15 was discharged early for owner convenience.

Dogs C3, C5, C7, C12, C14, C16 and C18-21 were polydipsic (water intake >50ml/kg/day) at the beginning of treatment with IZS-P. Of these dogs, C5 (for reasons explained above) and C16 were polydipsic at discharge. Nevertheless, the daily water consumption of dog C16 was approximately halved by IZS-P therapy. Dog C4 was also polydipsic at discharge although plasma glucose concentrations in this dog appeared to be controlled.

Dog	Hospital Number	Days Hospitalised	Initial Dose (IU/kg)	Final Dose (IU/kg)	Change in Body-weight (% of initial)
C3	113648	12	0.45	2.36	0.00
C4	109126	15	2.20	1.57	-2.54
C5	111353	20	1.21	1.25	-14.29
C6	114460	23	0.53	1.68	-19.71
C7	114653	24	1.14	0.00	-7.35
C8	114695	13	1.43	1.33	0.00
C9	115009	14	2.50	2.09	-4.17
C10	112598	6	0.83	0.83	0.00
C11	115131	16	1.21	1.23	12.07
C12	115164	10	1.29	1.70	3.53
C13	107674	13	1.13	1.04	8.70
C14	115678	14	1.00	1.20	2.50
C15	115980	12	0.83	1.45	10.00
C16	116315	10	1.00	1.17	0.00
C17	116334	11	1.00	1.47	0.00
C18	116594	1	1.00	1.58	0.00
C19	116744	10	1.00	2.67	0.00
C20	116825	11	1.15	0.92	0.00
C21	117056	17	0.95	0.00	4.76

Table 36. Stabilisation details of 19 dogs treated with IZS-P including initial and final insulin doses and change in body-weight.

Fourteen dogs (73.6%) maintained or gained weight whilst hospitalised (Table 36). Significant weight loss (>5%) was seen in C7 and considerable weight loss (>10%) was seen in dogs C5 and C6. This can be explained by resistance to insulin and, therefore, therapy associated with metoestrus, seen in dogs C6 and C7, and by a steatorrhoeic and hypoglycaemic episode seen in dog C5.

Starting doses of IZS-P varied between 0.45 IU/kg and 2.50 IU/kg (Table 36). The high starting doses in dogs C4 (2.20 IU/kg) and C9 (2.50 IU/kg) were based on previous responses to insulin therapy. Dogs C3 (0.45IU/kg) and C6 (0.53IU/kg) started at a low dose. The remainder of the starting doses of IZS-P fell within the range 0.80 to 1.50 IU/kg. Final insulin doses were mostly between 1 and 2 IU/kg but zero in dogs C7 and C21 (Table 36). Dogs C3 and C19 were discharged on relatively high doses (>2IU/kg). These two dogs were later discovered to have been suffering from hypothyroidism and hyperadrenocorticism, respectively. Clinical signs of hypothyroidism had not been apparent in dog C3 at the beginning of the study and so a TSH stimulation test was not carried out and in dog C19 the results of initial ACTH stimulation tests did not indicate the presence of hyperadrenocorticism.

The duration of the hospitalisation period depended not only on the dogs' responses to IZS-P but was very strongly dependent on the choice of increment/decrement in insulin

dose made from day to day. The size of the increment/decrement used was subjective in all cases and was not consistently based on any particular protocol. The duration of hospitalisation varied between 6 days (C10) and 24 days (C7) (Table 36). The mean duration of hospitalisation for all dogs was 13.95 days (± SD 4.54 days). The exceptionally long hospitalisation times seen in dogs C6 and C7 were because of the time taken to overcome insulin resistance, i.e., for levels of antagonistic hormones to fall sufficiently for their effects to be overcome by IZS-P (dog C6) or to allow resolution of the problem (dog C7). In dog C5 the prolonged hospitalisation period was due to a steatorrhoeic and hypoglycaemic episode and the short hospitalisation time in C10 was because of previous stabilisation. The mean duration of hospitalisation for new cases, not previously stabilised and which did not have metoestrus associated diabetes mellitus, was 12.31 days ± SD 1.89 days (n=13).

Follow-up

The following results are based on follow-up information obtained during the study period March 1990 to September 1991. No comparisons with other insulin preparations were made in this study.

Of the 19 dogs which started IZS-P therapy, 12 continued, one was withdrawn by its owner, two no longer required insulin therapy and 4 died. Of those which died, C4 was euthanased upon diagnosis of a cerebral tumour, C9 was euthanased 33 days after the start of treatment when showing signs of hepatic encephalopathy, C16 was euthanased for aggression and C17 was euthanased for domestic reasons.

Those dogs which were alive and continuing on IZS-P therapy at the end of the study period were monitored for between 125 and 448 days after the start of treatment with IZS-P (Table 37).

During the follow-up period the dogs which remained alive at the end of the study period and 3 of the dogs euthanased later (C4, C16 and C17) were generally healthy. Dog C14 returned at day 47 when its condition became unstable. Bacterial infections were the most common subsidiary problems encountered in these dogs. Infected interdigital cysts/abcesses occurred in dogs C6 (day 337), C8 (day 254) and twice in C14 (days 92, 114). Dog C5 developed cystitis around day 88, C8 suffered bronchiectasis around day 17, C18 had conjunctivitis at day 62 and C12 suffered an undiagnosed but probable bacterial infection at day 11. Dog C17 had small intestinal bacterial overgrowth from day 34. These diagnoses represent a rate of illness associated with bacterial infection in this

group of diabetic dogs of 1 incident per 1.1 diabetic-dog-years (i.e. 9 incidents in 9.96 years of risk).

Dog	Hospital Number	Duration of follow-up (days)	Status at end of study period	Change in dose required to maintain stability (% of maximum)
C3	113648	349	Alive	0.0
C4	109126	146	Dead	0.0
C5	111353	224	Alive	18.8
C6	114460	448	Alive	33.3
C7	114653	24	Cured	
C8	114695	431	Alive	6.7
C9	115009	33	Dead	14.3
C10	112598	30	Withdrawn	
C11	115131	280	Alive	0.0
C12	115164	176	Alive	25.0
C13	107674	213	Alive	53.3
C14	115678	271	Alive	34.5
C15	115980	242	Alive	40.0
C16	116315	85	Dead	0.0
C17	116334	158	Dead	28.6
C18	116594	166	Alive	19.0
C19	116744	127	Alive	25.0
C20	116825	125	Alive	42.9
C21	117056	22	Cured	

Table 37. Follow up details of 19 dogs treated with IZS-P including duration of follow-up, status at end of study period and range of insulin dose adjustment required to maintain stability ('cured' refers to dogs which had indefinite remission of insulin requirement).

Ocular cataracts, a common complication of canine diabetes mellitus, were present in both eyes of dogs C5 and C16 and in one eye of dog C18 before starting treatment with IZS-P. Cataracts sufficiently severe to significantly affect vision developed in 4 other dogs, C3, C13, C15 and in the other eye of C18 at days 99, 187, 220, and 62 respectively, representing a blindness rate of 1 per 2.23 diabetic-dog-years (4 in 9.11 years of risk). Significant cataracts were observed in dog C11 at day 131 but there was no noticeable visual impairment by the end of the study period.

Adjustments in insulin dose were necessary to maintain glycaemic stability in those dogs which continued on IZS-P therapy (Table 37). The size of these adjustments varied greatly between dogs. The average range of insulin doses each dog received was 21% of their maximum dose (± SD 17%). In 7 dogs less than 20% adjustment was necessary and in 4 others, no adjustment in insulin dose was required to maintain stability. Dogs C13, C14 and C15 were notable exceptions as they required large adjustments in dose. In dog

C14, this was mainly because of changes in diet but the increased requirements could not be explained in dogs C13 and C15.

Discussion

There are no previous comprehensive reports of the stabilisation of naturally occurring diabetic dogs with exogenous insulin. This study has shown that stabilisation of diabetic dogs with a mixed insulin zinc suspension can be achieved in 10 to 14 days of insulin dose adjustment to a final insulin dose of between 0.80 and 2.0 IU/kg. In the majority of cases, weight loss can be arrested or reversed and polydipsa controlled by a single daily insulin injection regimen. Ketonuria can also be controlled by this protocol. In the long-term, diabetic dogs can be managed with single daily injections of mixed insulin zinc suspension and twice daily feeding for periods greater than 4 months.

Many veterinary textbooks and articles outline a stabilisation protocol but do not indicate the expected duration of stabilisation nor the expected short term response to insulin treatment. Such guidance is even difficult to find within medical literature. In addition, there are very few previously published reports on the long-term management of canine diabetics with which to compare the findings of the present efficacy study using a mixed insulin zinc suspension. However, one report (Marmor *et al*, 1977) details the complications encountered in 39 diabetic dogs managed with single daily injections of isophane (NPH) insulin. In that report 94% of dogs which did not have visual impairment following stabilisation subsequently developed cataracts and 33% of dogs suffered bacterial infections of the urinary tract or skin. Unfortunately, Marmor *et al* did not provide information on the duration of monitoring applied to these animals. A direct comparison with rates of blindness and bacterial infection reported in the present study cannot therefore be made.

This study provides unique information of use to veterinary surgeons when advising owners of diabetic dogs on the expectations of treatment, duration of hospitalisation for stabilisation and long-term prognosis and chances of blindness when a single daily insulin injection regimen using a mixed insulin zinc suspension is proposed. Furthermore, the results of the stabilisation and follow-up of 19 diabetic dogs suggest that IZS-P used once daily in conjunction with a twice daily feeding schedule is effective in the management of canine diabetes mellitus in the long term (>4months).

Chapter 7:
High fibre diet In the management of diabetes mellitus

Objective

The aim of this study was to evaluate the short and long-term effects of feeding a specific high fibre diet (Pedigree Canine High Fibre Diet® (CHF)) to dogs with naturally occurring diabetes mellitus.

Background

Alterations to diet are of major importance in the management of diabetes mellitus. In the obese cat and the Type II diabetic human, dietary control can be the primary therapeutic approach, obviating the requirement for insulin or oral hypoglycaemic therapy (Mann and Lewis-Barned, 1992). Dietary manipulation is also important in the management of canine diabetes mellitus and is often employed. Consistency of meal times, content and size are the primary dietary recommendations for insulin treated diabetic dogs. In the past, recommendations centred on feeding diets low in easily digested carbohydrate. More recently, however, there has been a shift in emphasis, following changes in human diabetic management, towards increased complex carbohydrate intake including dietary fibre. Increasing dietary fibre reduces post prandial hyperglycaemia, improves lipid profiles and possibly has a direct effect on modifying insulin receptor activity (Anderson, 1979; Hjollund *et al*, 1983; Nelson, 1988; Nelson, 1989b; Fuessel *et al*, 1987; Simpson *et al*, 1981; British Diabetic Association, 1982). Since most human diabetics suffer from type II diabetes mellitus which is best managed by reduction in calorie intake and weight control, many dietary recommendations for human diabetics include calorie restriction. Weight control is also important in Type I diabetics as obesity leads to decreased tissue sensitivity to insulin. The mechanisms by which increased dietary fibre and complex carbohydrates might reduce post-prandial hyperglycaemia include: delayed gastric emptying, slowed starch hydrolysis, mechanical interference with glucose absorption and altered intestinal transit time. The mechanisms by which plasma cholesterol and triglyceride might be reduced after feeding diets high in fibre and complex carbohydrates may be similar to those which reduce post-prandial hyperglycaemia. However, the ways in which dietary fibre might alter the fractionation of plasma cholesterol between the lipoprotein classes is less clearly understood.

There is much scope for study of dietary aspects of the management of canine diabetes as, currently, most recommendations are based on human data. There are only two published studies on the effects of diets containing increased amounts of complex carbohydrates including fibre in diabetic dogs. Nelson *et al* (1991) studied the effect of canned diets high in soluble and insoluble fibre in 6 experimentally induced diabetic dogs. In that study, the mean concentration and fluctuation of plasma glucose, urinary glucose excretion and glycated haemoglobin concentrations were reduced after feeding the high fibre diets. The effects of these diets were not tested in dogs with naturally occurring diabetes mellitus, which differs from the experimentally induced disease in terms of pathophysiology and possibly digestive function if, in the former, there is co-existent chronic pancreatitis or gastrointestinal neuropathy. Blaxter *et al* (1990) studied the short term effects of dietary fibre by an acute change of diet in 4 dogs with naturally occurring diabetes mellitus but used home-made diets in which the fibre sources were sprinkled on to standard canned pet food. No statistically significant differences were observed in post-prandial glycaemia in these diabetic dogs following the change in diet although differences were observed in a control group of 6 normal beagles. Although these previously published preliminary studies have been directed at application in the naturally-occurring disease, there are no reports of the effects of canned high fibre diets in dogs with naturally occurring diabetes mellitus.

Diabetes mellitus is invariably associated with elevations in plasma concentrations of cholesterol, triglyceride, non-esterified fatty acids, alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase (Nelson, 1989a). These elevations have been reduced following improved management in diabetic dogs and/or humans (Nelson, 1989a; Nikkila, 1984), although Chapter 5 of this thesis suggests that alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase are of limited value in diabetic monitoring. Similarly, the concentrations of mean 24 hour plasma glucose, glycated haemoglobin (Wood and Smith, 1980; Bunn, 1981; Dolhofer *et al*, 1981; Mahaffrey and Cornelius, 1982;) and glycated plasma proteins (fructosamine) (Dolhofer and Weiland, 1979; Dolhofer *et al*, 1981; Mahaffrey *et al*, 1984; Reusch *et al*, 1993) have also been reduced. Lipoprotein lipase activity (Nikkila, 1984; Nelson, 1989a), lipoprotein profiles (Kissebah and Schectman, 1987) and VLDL triglyceride/apolipoprotein B ratios (Abbate and Brunzell, 1990) also tend to normalise following improved diabetic management. Any study assessing effectiveness of a diabetic management change designed to improve diabetic control should include monitoring of all the above parameters.

In addition to those parameters already mentioned, plasma glucose concentrations obtained at particular times in the day can be used as indicators of glycaemic control. In treated diabetic dogs, the plasma concentration of glucose in a sample taken just before the morning injection of insulin (fasting glucose) is often the highest that will occur in a 24 hour period. Fasting plasma glucose is often elevated well above normal ranges in treated diabetic dogs receiving a single daily injection of intermediate acting insulin. This is because intermediate duration insulins are seldom effective for as long as 24 hours in dogs. Fasting plasma glucose is usually the highest concentration of plasma glucose during the day, so it gives an indication of the worst degree of glycaemic control achieved by the treatment protocol. This information is generally used in conjunction with the afternoon (nadir) plasma glucose concentration which gives an indication of the best degree of glycaemic control achieved.

Plasma glucose concentration usually reaches its nadir just before the second meal of the day in diabetic dogs treated according to the protocol used in this study. Measuring plasma glucose concentration at this time gives an indication of the maximum degree of glycaemic control achieved by the treatment protocol and insulin dose administered. A well controlled, stable diabetic dog should have nadir plasma glucose concentrations of between 3.5 and 7.5mmol/l.

Materials and methods

Dogs

Thirteen privately-owned dogs with naturally occurring diabetes mellitus were recruited from diabetic dogs referred to the University of Glasgow Veterinary School over the period 1990 to 1992. To be included in the study each dog had to have been 'stable' for at least 4 months. With the exception of one dog which was diagnosed as hypothyroid after inclusion in the study, dogs with concomitant endocrinopathies were excluded. Two of the recruited dogs had to be excluded early in the study. Dog 7 was withdrawn by its owners and dog 12 died. Neither dog had received the CHF diet. The remaining 11 dogs were maintained on a once daily injection of an intermediate acting insulin (isophane, or IZS-P) and fed two evenly divided meals 6-8 hours apart, with the first meal fed at the time of the insulin injection. All dogs were insulin dependent. This did not mean, however, that they were all hypoinsulinaemic. To prove hypoinsulinaemia, an insulin response test by administration of an insulin secretagogue is required and this was not carried out routinely. Pre-treatment basal insulin concentrations in the 7 dogs in which it could be measured were at the low end of the reference range (SCL Bioscience 5-40μ IU/ml). There was wide variation in age and breed as would be expected in a study of this type and duration. Each dog was allocated a trial number in addition to their hospital

number for the puposes of this study. Details of dogs involved in the study are listed in Table 38.

Diet

Canned Pedigree Canine High Fibre Diet ® (CHF) was supplied by Waltham Centre for Pet Nutrition and stored indoors until use. This diet contains guar and pea fibre at a level which results in a total dietary fibre (DF) content of approximately 5.7g/100kcal. The study design required the dogs to be fed an amount of CHF diet which was iso-caloric with their previous diets. The caloric densities of common petfoods which were used to calculate the volume of CHF diet to be fed are given in Table 39.

No.	Case Number	Age (years)	Sex	Breed
1	115678	6.5	M	Rottweiler
2	119147	7.4	FN	Large cross-breed
3	115131	8.9	FN	Jack Russell terrier
4	115980	9.0	FN	Labrador
5	117222	11.1	FN	Whippet
6	113648	7.3	M	Scottish terrier
8	97218	8.5	FN	Labrador
9	117898	11.5	FN	Cairn terrier
10	121228	9.2	MN	Labrador
11	121252	3.2	FN	Rottweiler
13	120899	8.8	FN	Yorkshire terrier

Table 38. Details of diabetic dogs included in the dietary management study.

	Caloric density (kcal/100g)
Pedigree Canine High Fibre Diet®	95
Pedigree Chum®	77
Chappie®	82
Whiskas®	65
Bread/Biscuit	200

Table 39. Caloric densities of the foods used by the owners of 11 diabetic dogs prior to changing to CHF diet.

Study design

Because of the limited number of recruitable diabetic dogs, it was not possible to conduct this study with separate matched control and treatment groups. After a minimum period of 4 months of 'normal' diabetic management, each dog was monitored for a period of one month without change, then changed to CHF diet and monitored monthly for a further 4 months. Dogs started in the study in batches of up to three animals to reduce the influence of external time dependent factors, such as analytical methods, on the results of the study.

Hospitalisation

To facilitate conversion to CHF feeding, the dogs were hospitalised for approximately 10 days. During this time, afternoon blood glucose analyses were carried out so that any necessary adjustments in insulin dose could be made. The change to an iso-caloric quantity of CHF was made via an intermediate day when the dogs were fed meals made up of equal quantities of CHF and their previous diet. Clinical examination was performed on a daily basis and faecal quality was monitored.

Serial blood glucose analyses

At the beginning (-10 days) and end (0 days) of the hospitalisation period a 24 hour blood glucose curve was generated for each dog by taking blood samples for glucose estimation at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours post insulin injection. The second curve was made after 6-7 days of being fed CHF alone. Additionally, samples were taken at 6.16, 6.32, 6.5, 7, 7.5 and 9 hours post injection so that the effects of CHF on afternoon post-prandial glycaemia (APPG) could be more closely observed. The second meal of the day (fed at 6 hours post injection) was chosen for further study because the effects of exogenous insulin on blood glucose concentrations would be less dynamic at that time. In 5 of the dogs it was possible to generate a third 24 hour blood glucose curve after 4 months of being fed CHF diet.

Insulin dose and general health

Insulin dose (IU/kg) was recorded carefully so that any decrease in insulin requirement could be noted and increases in insulin dose could be considered in the explanation of any improvement in glycaemic control.

The following plasma biochemical and haematological parameters were measured, so that the effects of any unrelated illnesses could be accounted for and any adverse effects of feeding CHF identified: urea, creatinine, phosphate, sodium, potassium, chloride, calcium, bilirubin, gamma glutamyl transferase, amylase, total protein, albumin, white blood cell count, neutrophil count, lymphocyte count, eosinophil count, red blood cell count, packed cell volume, mean corpuscular volume and platelet count.

At clinical examination, bodyweight was recorded, and a visual analogue method was used to record coat condition, body condition, faecal volume, faecal consistency, activity and both owner and veterinary observations of demeanour and quality of vision.

Indicators of glycaemic control

The following biochemical parameters were monitored because of their known value as indicators of glycaemic control in diabetics: fructosamine (glycated plasma proteins), glycated haemoglobin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Direct measurements of plasma glucose concentration were also taken in the morning and afternoon when possible.

Although, daily water intake (ml/kg/day) is also a useful indicator of diabetic control it proved too difficult for owners to measure reliably at home and its monitoring was abandoned.

Lipid metabolism

To assess the effect of feeding CHF on lipid metabolism, plasma concentrations of cholesterol, triglyceride, non-esterified fatty acids and glycerol were measured. Changes in total plasma cholesterol concentration were studied further by quantitative analyses of the cholesterol concentration in each of the lipoprotein classes: VLDL, LDL and HDL. Post-heparin lipoprotein lipase activity and VLDL triglyceride:apolipoprotein B were also monitored. The analyses of these parameters require fasting blood samples and so they were only measured at -1 month, -10 days, 0 days, +2 months and +4 months.

Missing samples

Unfortunately, not all dogs were available for examination at every sampling time. Dog no. 2 was withdrawn by its owner after 2 months on CHF diet and dog no. 13 was not available at the +2 and +3 months sample times. Table 40 summarises the study design and the missing values.

Day	-1 mth	-10 days	0 days	+1 mth	+2 mth	+3 mth	+4 mth
Visit	Whole day	Hospitalisation		Afternoon sample	Whole day	Afternoon sample	Whole day
Diet	Original	Original	CHF	CHF	CHF	CHF	CHF
Dog 1	+	+	+	+	+	+	+
2	+	+	+	+	+	*	*
3	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+
13	+	+	+	+	*	*	+

Table 40. Study design and missing samples. * denotes data missing for all parameters

Clinical chemistry and haematology

Routine clinical chemistry and haematology analyses were performed as described in Chapter 2. Additional methods were employed for the quantative analysis of plasma lipoproteins, lipoprotein lipase (LPL), hepatic triglyceride lipase (H-TGL) and VLDL apolipoprotein B concentrations and these are outlined in Appendices 12 - 14.

Statistical analyses

Statistical analyses were performed using a proprietary statistical software package (MINITAB, Minitab Incorporated). Comparison between two times, e.g. before and one week after changing to CHF diet, was performed using a paired *t*-test. When more than one group was to be analysed, e.g., mean afternoon post-prandial plasma glucose concentrations in 5 dogs on three separate occasions, an analysis of variance (randomised block) method was used and the data blocked by dog. When there was missing data, e.g., plasma fructosamine concentrations in 11 dogs for the 7 sampling days over the 4 months of the study, a general linear model (GLM) analysis was used to determine the effects of dog and sample day (two-way analysis of variance). If GLM analysis revealed statistically significant differences then the data was grouped by sample day and re-interrogated using the Neuman-Keuls multiple range test[1]. A level of probability of 5% ($p<0.05$) was chosen as the cut-off for the presence of statistical significance in all of the above analyses.

Because of the wide inter-dog variation for certain parameters e.g. alkaline phosphatase, body condition etc., results were also analysed as a percentage change. The mean of the -1 month and -10 days results was taken as the baseline value.

Throughout this chapter a simple system of graphical representation of results has been employed. This was because of the number of animals in the study and the presence of marked inter-dog variation (resulting from: age, breed, sex, duration of diabetes, insulin type, etc.). In this system, the interquartile range (25th to 75th percentile) was plotted as a box, the median as a line, the mean as an asterisk and the data as individual dots. This system appeared to give an accurate and honest representation of the data. Wide discrepancies between the median and mean for any given set of data generally indicate that the data are not evenly distributed.

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[1]'Macro' for MINITAB written by D.R. Irvine, Department of Veterinary Medicine, University of Glasgow.

Results

Case summaries

Comments relating to the health and treatment of the dogs involved in the CHF diet study are contained in Appendix 15. An outline of the contents of the case reports is given below. More detailed information on certain aspects of their health and treatment (e.g. insulin dose, body weight, body condition, etc.) are included in relevant sections.

A variety of breeds and ages of dogs were included in the study and a variety of diets fed before the change to CHF diet. Two dogs were fed Chappie (Pedigree Petfoods) alone, two were fed Chappie and bread, two were fed Chum (Pedigree Petfoods) and bread, 4 were fed Chum and biscuit mixer and one was fed Whiskas (Pedigree Petfoods) and bread. Two different types of intermediate insulin preparations were used; 4 dogs received Caninsulin (porcine insulin zinc suspension (lente); Intervet) and 7 dogs received Hypurin Isophane (Fisons).

With the exception of dog no. 2, the dogs remained generally well throughout the study period. According to their owners, 6 of the dogs improved noticeably in terms of their general health and liveliness and with the exception of two dogs (no. 5 and no. 13) all maintained a good appetite throughout the period of the study. Three dogs suffered diarrhoea which required treatment following the change to CHF and in two this was attributed to the presence of small intestinal bacterial overgrowth. Two dogs (including one which had diarrhoea (no. 2)) suffered colitis after changing to CHF but in dog no. 9 this was only for a few days immediately following the change. One dog became constipated and was given vegetable oil supplements by its owner in an attempt to alleviate the problem. Three dogs (nos. 1, 2 and 8) had hypoglycaemic episodes during the period of the study and in two (nos. 2 and 8) this was whilst being fed CHF diet. The incidence of hypoglycaemic episodes in diabetic dogs fed standard diets managed under the regimen used is not known and so no comparison can be made. The visual ability of two dogs (nos. 8 and 10) deteriorated noticeably during the period of the study.

Post-prandial glycaemia

Twenty-four hour curves

Blood samples were taken at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours post injection from 11 dogs both before and one week after changing to CHF. The results are detailed in Appendix 16 and displayed graphically in Figures 51 and 52. These results were analysed both as absolute values and as incremental values by subtracting the nadir glucose concentration. The following parameters were analysed for statistical difference between original and CHF diets by the paired *t*-test method: mean absolute glucose

concentration (ABSMEAN), mean incremental glucose concentration (INCMEAN) and area under the incremental glucose concentration versus time curve (INCAUC).

The standard deviations of absolute glucose concentrations (ABSSD) were calculated as indicators of the fluctuation in plasma glucose over the sample period. Unfortunately, statistical analysis of the difference in fluctuation in plasma glucose between the two diets was not possible because of the complexity of the statistical method required and the means of these values are given for illustration only. The mean absolute and incremental glucose concentration were less ($p = 0.0087$ and $p = 0.034$ respectively) on the CHF day than on the original diet. There was a reduction in INCAUC which was close to statistically significant (Table 41).

	Original (mean ± sd)	CHF (mean ± sd)
ABSMEAN †	13.00 ± 5.68	6.09 ± 2.41
ABSSD	4.49	3.24
INCMEAN ††	6.08 ± 2.57	4.95 ± 1.17
INCAUC †††	8363 ± 3494	7220 ± 1673

Table 41. Means and standard deviations of values derived from 24 hour glucose concentrations in 11 dogs before and after changing to CHF (Statistically different between diets † (p=0.0087), †† (p=0.034)). ††† p = 0.056.

Afternoon post-prandial glycaemia

Blood samples were taken at 6, 6.16, 6.32, 6.5, 7, 7.5, 8, 9, 10, 12 and 14 hours post injection from 11 dogs both before and one week after changing to CHF. The results are included in Appendix 16 and displayed graphically in Figures 53 and 54. The results were standardised by subtracting the glucose concentration at 6 hours. The following parameters were derived from these results: mean afternoon post-prandial glucose concentration (APPGMEAN), standard deviation of afternoon post-prandial glucose concentrations (APPGSD) and area under the afternoon post-prandial glucose concentration versus time curve (APPGAUC). Adjusted means, standard deviations and areas under the curve were also calculated based on only the 6, 7, 8, 9, 10, 12 and 14 hour results (ADJMEAN, ADJSD and ADJAUC). Although there are striking trends towards reductions in APPGSD and ADJSD these are not mathematically sound measurements of plasma glucose fluctuation and appropriate statistical analysis could not be performed because of its complex nature. However, the means of APPGSD and ADJSD are given for illustration. The remaining derived values were analysed for statistically significant differences between the original and CHF diet using a paired *t*-test method. There were reductions in these 4 parameters which were not statistically significant (Table 42).

	Original (mean ± SD)	CHF (mean ± SD)
APPGMEAN	1.57 ± 2.76	0.88 ± 1.27
APPGSD	2.18	1.11
APPGAUC	1180 ± 1696	583 ± 723
ADJMEAN	2.08 ± 3.01	1.11 ± 1.33
ADJSD	1189	581
ADJAUC	2.21 ± 1.17	1.16 ± 0.60

Table 42. Means and standard deviations of values derived from afternoon post-prandial glucose concentrations in 11 dogs before and after changing to CHF.

Long-term effect on post-prandial glycaemia

In 5 dogs, 24 hour and afternoon post-prandial plasma glucose curves were generated after 4 months on CHF (Appendix 16) as well as before and one week after changing to CHF. These results are displayed graphically in Figures 55 - 60. The following parameters were statistically analysed using the an analysis of variance method: mean absolute glucose concentration (ABSMEAN), mean incremental glucose concentration (INCMEAN), area under the incremental glucose concentration versus time curve (INCAUC), mean afternoon post-prandial glucose concentration (APPGMEAN), and area under the afternoon post-prandial glucose concentration versus time curve (APPGAUC). Adjusted means and areas under the curve were also calculated based on only the 6, 7, 8, 9, 10, 12 and 14 hour results (ADJMEAN and ADJAUC). There were no statistically significant differences ($p<0.05$) in any of these parameters, although there were striking trends (Table 43). Previous comments relating to parameters derived from standard deviations apply to ABSSD, APPGSD and ADJSD (Table 43) and so statistical analyses were not performed.

	Original mean ± SD	CHF 1 week mean ± SD	CHF 4 months mean ± SD
ABSMEAN	13.00 ± 5.68	6.09 ± 2.41	7.71 ± 2.21
ABSSD	4.49	3.24	5.39
INCMEAN	5.24 ±2.49	5.13 ± 1.25	4.95 ± 1.16
INCAUC	7415 ± 3549	7580 ± 1691	7220 ± 1673
APPGMEAN	0.59 ± 1.35	0.42 ± 1.38	0.04 ± 0.57
APPGSD	1.85	1.26	0.61
APPGAUC	741 ± 933	309 ± 830	35 ± 320
ADJMEAN	1.34 ± 1.75	0.72 ± 1.55	0.12 ± 0.63
ADJAUC	786 ± 953	323 ± 796	45 ± 309
ADJSD	1.57	1.31	0.65

Table 43. Means and standard deviations of values derived from 24 hour and afternoon post-prandial glucose concentrations in 5 dogs before, 1 week and 4 months after changing to CHF diet.

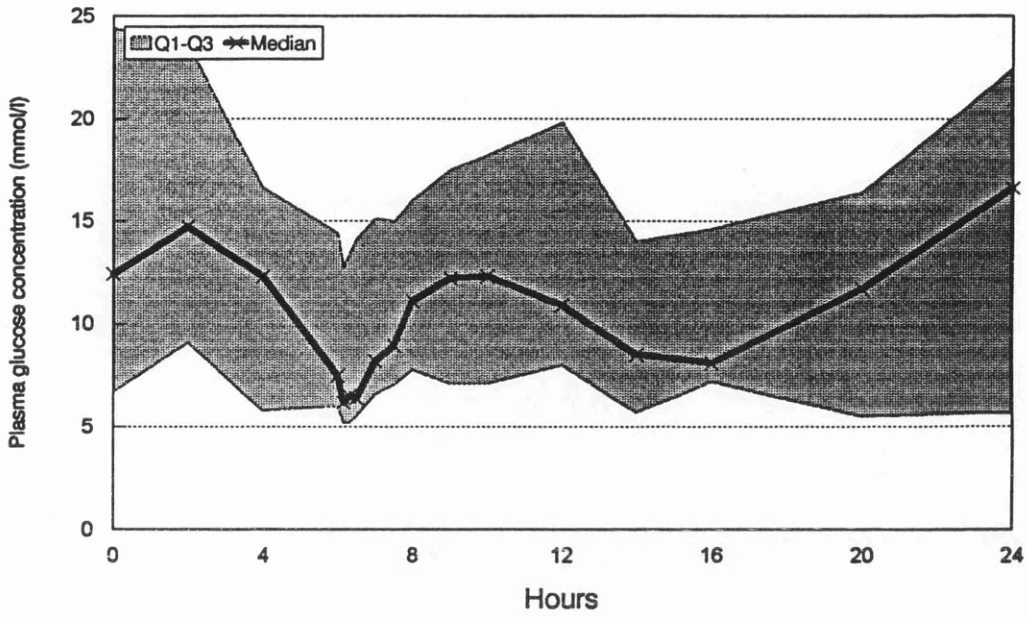

Figure 51. Absolute 24 hour glucose concentrations in 11 dogs on original diet fed in evenly divided meals at 0 and 6 hours (median and interquartile range).

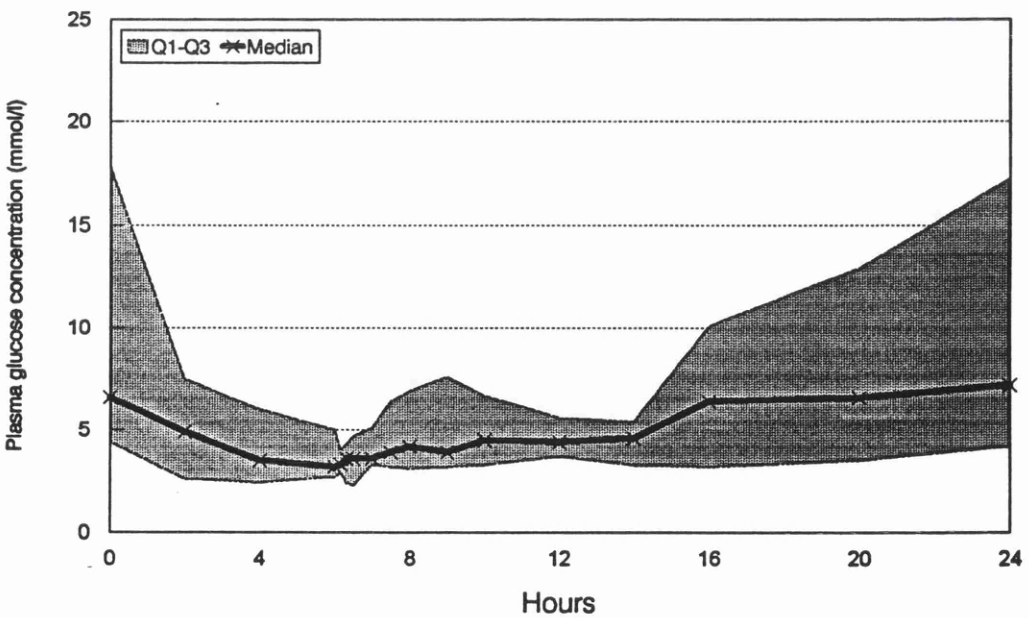

Figure 52. Absolute 24 hour glucose concentrations in 11 dogs on CHF diet fed in evenly divided meals at 0 and 6 hours (median and interquartile range).

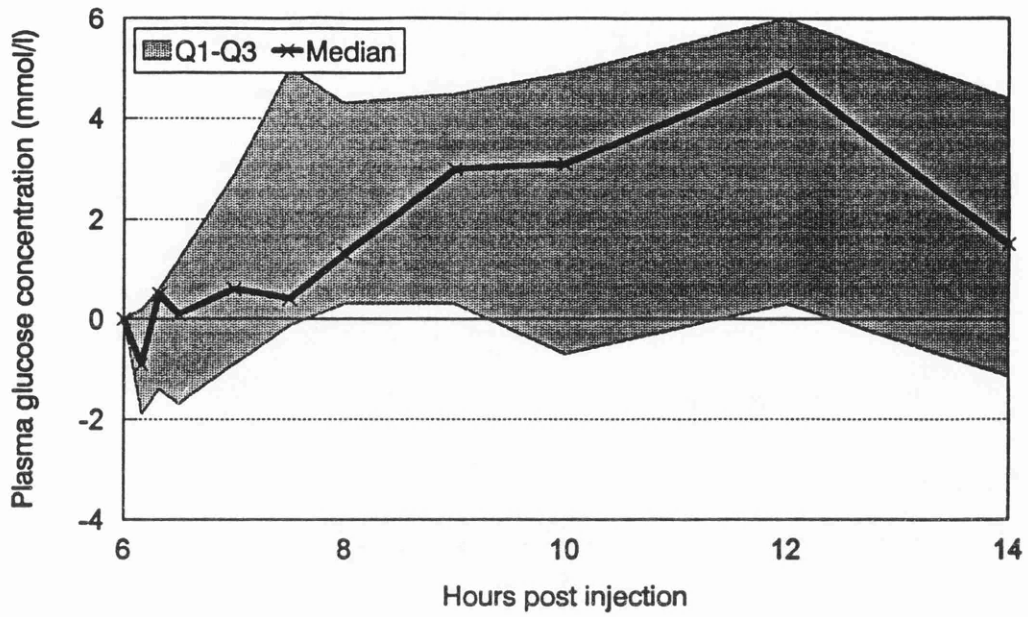

Figure 53. Afternoon post-prandial glycaemia in 11 dogs on original diet (median and interquartile range).

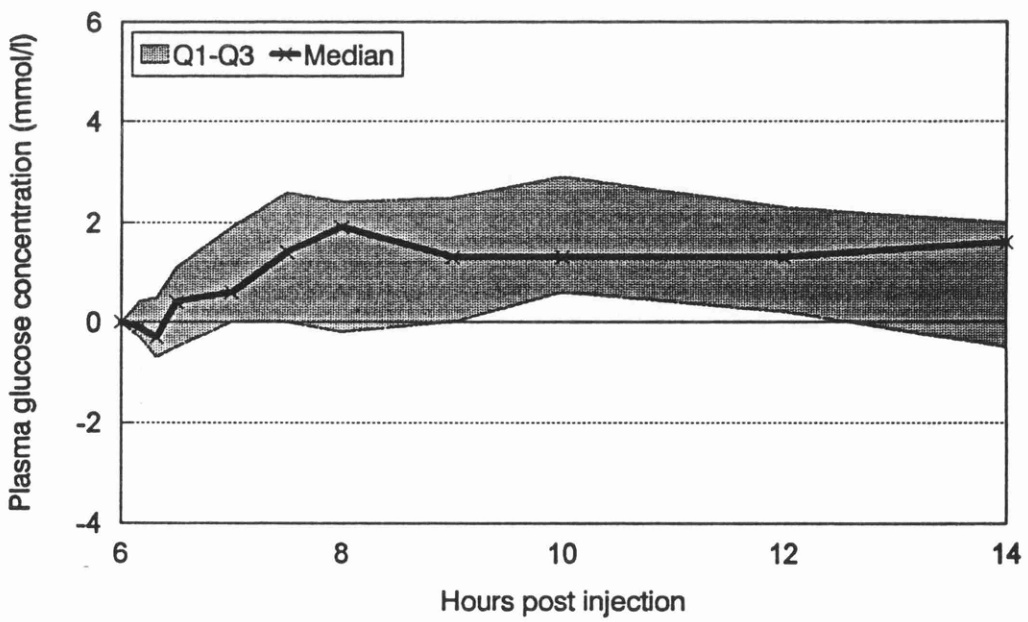

Figure 54. Afternoon post-prandial glycaemia in 11 dogs on CHF diet (median and interquartile range).

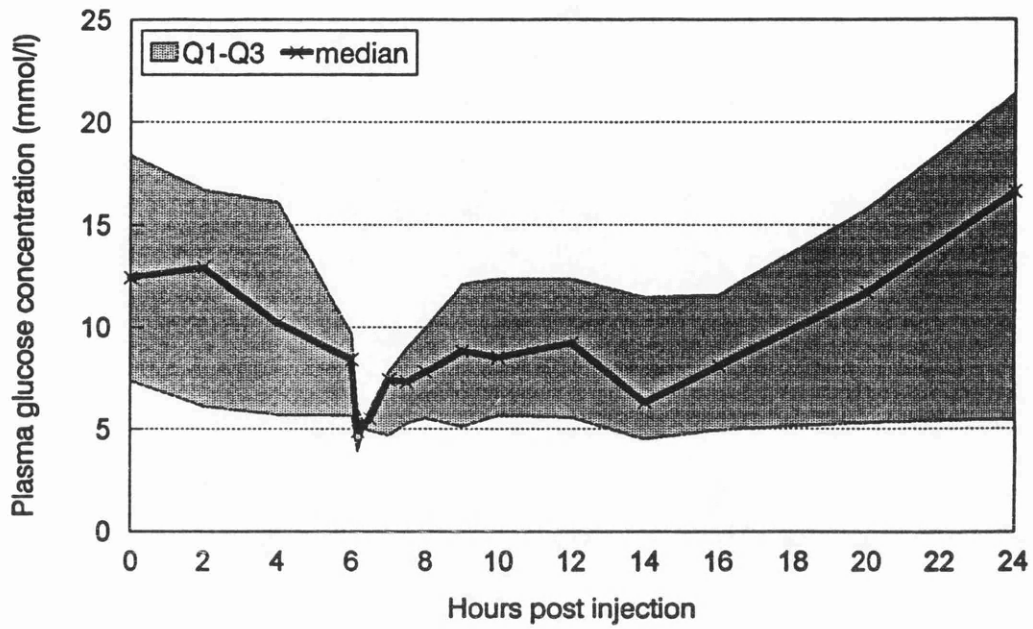

Figure 55. Absolute 24 hour glucose concentrations in 5 dogs on original diet fed in evenly divided meals at 0 and 6 hours (median and interquartile range).

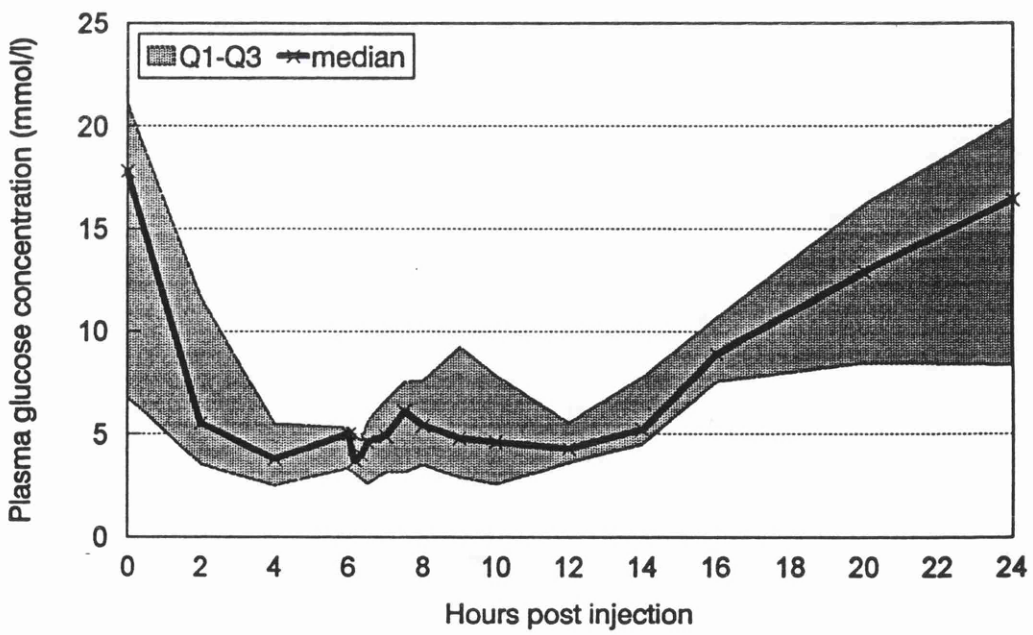

Figure 56. Absolute 24 hour glucose concentrations in 5 dogs after one week on CHF diet fed in evenly divided meals at 0 and 6 hours (median and interquartile range).

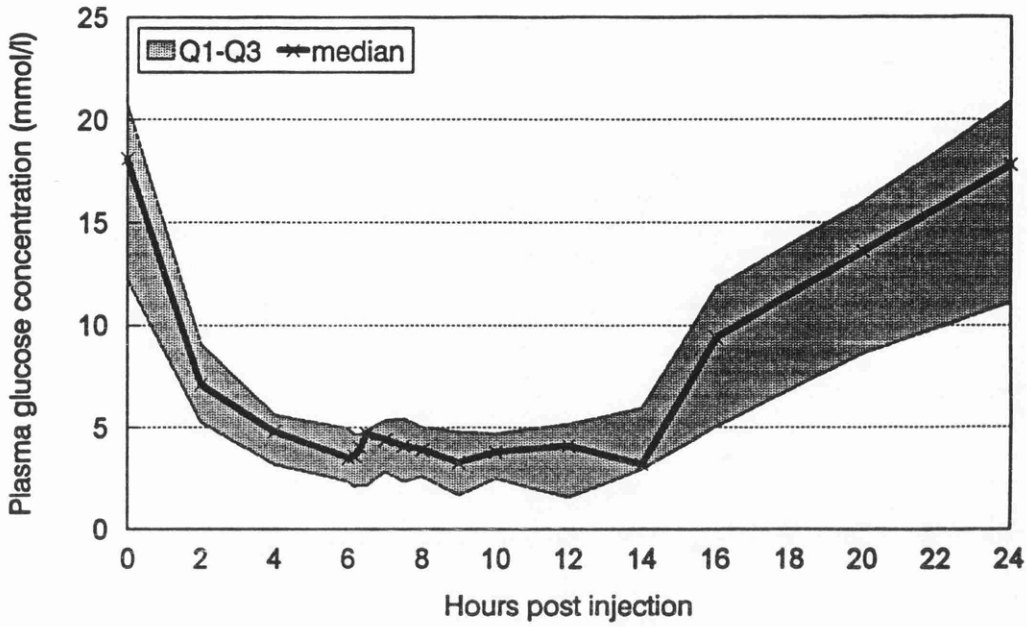

Figure 57. Absolute 24 hour glucose concentrations in 5 dogs after 4 months on CHF diet fed in evenly divided meals at 0 and 6 hours (median and interquartile range).

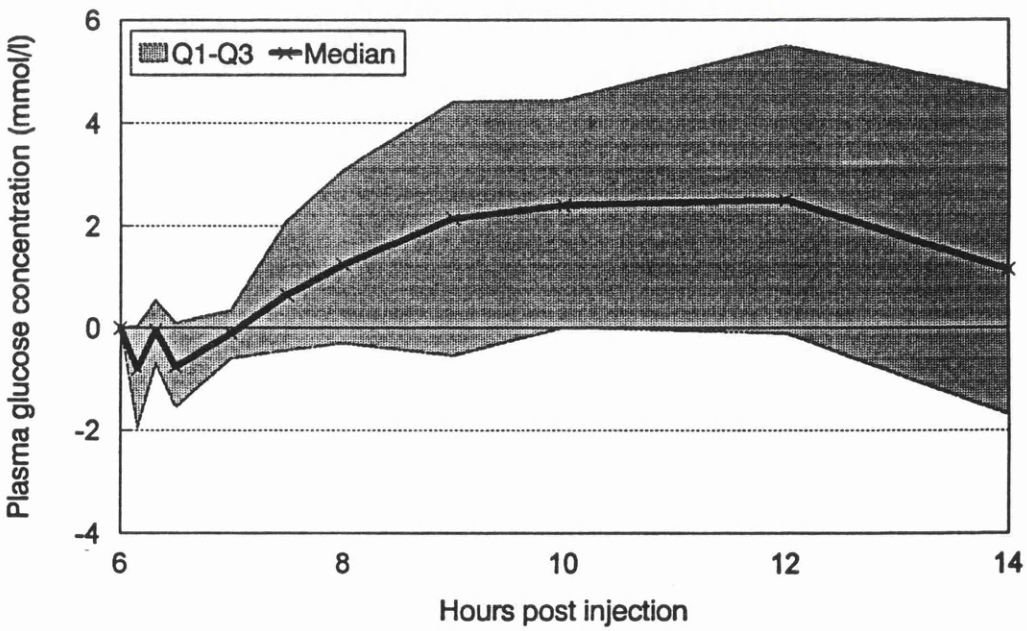

Figure 58. Afternoon post-prandial glycaemia in 5 dogs on original diet (median and interquartile range).

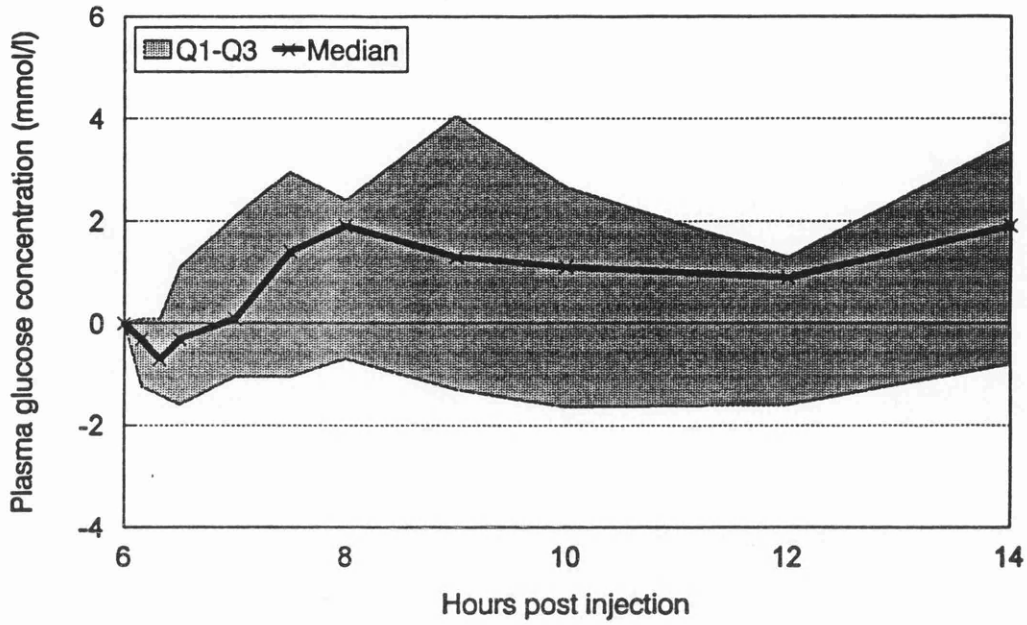

Figure 59. Afternoon post-prandial glycaemia in 5 dogs after one week on CHF diet (median and interquartile range).

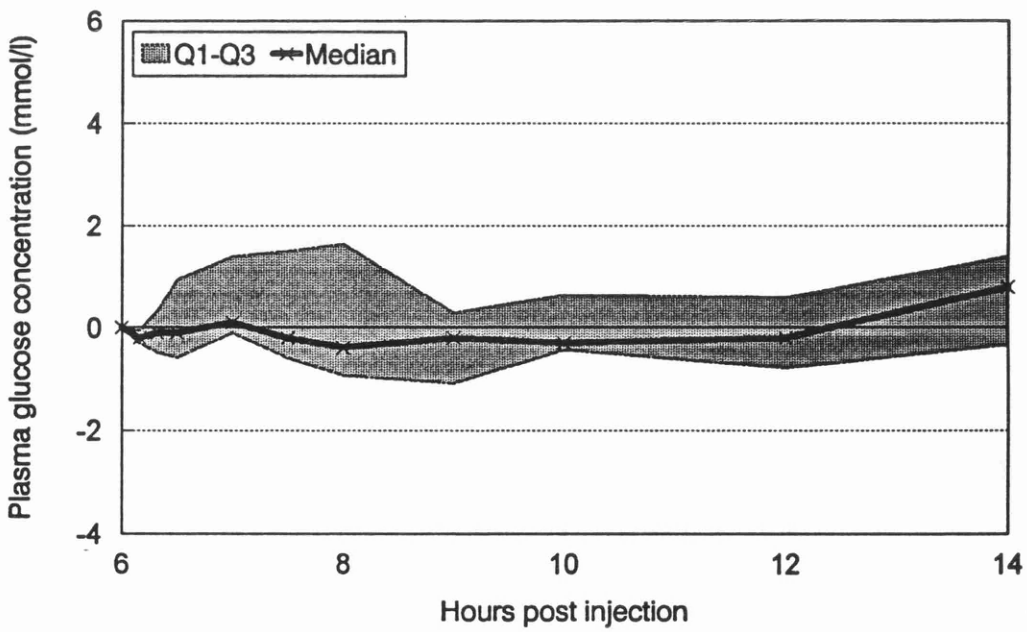

Figure 60. Afternoon post-prandial glycaemia in 5 dogs after 4 months on CHF diet (median and interquartile range).

Insulin dose and general health

Insulin dose

Insulin dose was recorded on an international units per kilogram basis because of the variation in size of the dogs included in the study. Absolute insulin doses for 11 dogs over the period of the study are given in Appendix 17, represented graphically in Figure 61 and as percentage change from basal values in Figure 62. There were no statistically significant changes in insulin dose over the period of the study and other than a slight increase in median dose at +4 months there were no apparent trends towards a change in insulin dose.

Bodyweight

Bodyweight was recorded at each visit by means of electronic scales. Absolute bodyweights (kg) are given in Appendix 17 and represented graphically in Figure 63 and as percentage change from basal values in Figure 64.

General linear model analysis revealed a statistically significant change in bodyweight ($p=0.0000$). Multiple range analysis indicated statistically significant differences between -1 month and +2 months; -1 month and +4 months; -10 days and +2 months and between -10 days and +4 months. There were also statistically significant differences between +2 months and all the other CHF diet days (bodyweight at +2 months was higher than on all the other days) and between +4 months and all the other CHF diet days (bodyweight at +4 months was lower than all the other days).

Analysis of bodyweight as a percentage of the mean of the two original diet days revealed a significant reduction over the period of the study ($p=0.0001$). Multiple range analysis indicated that there were statistically significant reductions in bodyweight from basal at +1 month, +2 months, +3 months and +4 months. The difference between basal and 0 days was close to statistically significant. There was also a statistically significant decrease in bodyweight between 0 days and +4 months.

Body condition

Body condition was assessed using a linear visual analogue scale 10 cm in length. A mark was made at a position which represented the dog's body condition and the distance of the mark from the zero end of the scale measured in millimetres. The zero end of the scale represented emaciation and the 10 cm end gross obesity. Dogs with a healthy, normal body condition generally received a body condition score of 45 - 55mm. Absolute body condition scores are given in Appendix 17, represented graphically in Figure 65 and as percentage change from the mean of the -1 month and -10 days results in Figure 66.

General linear model analysis revealed a statistically significant reduction in body condition (p=0.0000). Multiple range testing indicated that there were statistically significant differences between -1 month and both +3 months and +4 months and between -10 days and both +3 and +4 months. There were also statistically significant differences between +3 months and both 0 days and +1 month and between +4 months and all of the CHF days. There was also a statistically significant decrease in body condition when analysed on a percentage change basis (p=0.0003). On multiple range testing, there was a statistically significant difference between basal and +4 months. There were also statistically significant differences within the CHF days between 0 days and +3 months and between +4 months and all of 0 days, +1 month and +2 months.

Coat condition

Coat condition was given a score at each visit by means of a visual analogue scale. On this 10 cm scale the zero end represented very dull and the 10 cm end very glossy. Scores were converted to millimetres from the zero end for statistical analysis. The absolute coat condition scores are given in Appendix 17, represented graphically in Figure 67 and as percentage change from the mean of the original diet days values in Figure 68.

There were no statistically significant changes in coat condition nor any apparent trends.

Activity

An activity score was allocated to each dog on each visit based both on the owner's comments and veterinary observation by means of a visual analogue scale. The zero end of the 10 cm line represented lethargic and the 10 cm end active. These results were converted to millimetres for statistical analyses.

Absolute activity scores are given in Appendix 17, represented graphically in Figure 69 and as percentage change from the mean of the -1 month and -10 days results in Figure 70.

General linear model analysis revealed a statistically significant increase in activity score (p=0.0000). Multiple range testing indicated that there were statistically significant differences between -1 month and all of the CHF diet days and between -10 days and all of +1, +2, +3 and +4 months. There was also a statistically significant increase in activity score when considered on a percentage change basis (p=0.0022). Multiple range testing indicated statistically significant differences between basal (mean of -1 month and -10 days) and all of the CHF diet days.

Demeanour

Owner observed

Owner observations of demeanour were recorded on a 10 cm visual analogue scale. The zero end of this scale represented dull and the 10 cm end bright. Owners were asked to mark the scale 'blind' but then given an opportunity to amend their mark after seeing their judgement at the previous visit.

Owner observed demeanour scores are given in Appendix 17, represented graphically in Figure 71 and as a percentage change for the mean of the original diet days scores in Figure 72.

There were no statistically significant changes in owner observed demeanour over the course of the study. Graphically there was a slight upward trend.

Vet observed

Demeanour was also scored on a visual analogue scale by the veterinary surgeon. This score was based both on the owner's description of the dog's behaviour between visits and on direct observation.

Demeanour scores from veterinary observation are given in Appendix 17, represented graphically in Figure 73 and as percentage change from the mean of the original diet days scores in Figure 74.

There was a statistically significant improvement in veterinary observed demeanour by general linear model analysis ($p=0.0012$) and multiple range testing revealed statistically significant differences between -1 month and +3 and +4 months and between -10 days and +3 and +4 months. The differences between both -1 month and -10 days and +2 months were close to statistically significant. There were also statistically significant differences between 0 days and +3 and +4 months and a statistically significant improvement in veterinary observed demeanour when the results were considered on a percentage change basis ($p=0.0073$). Multiple range analysis revealed statistically significant differences between basal (mean of -1 month and -10 days) and +2, +3 and +4 months.

Vision

Owner and Vet observed

Vision was assessed both by the veterinary surgeon and the owner using a 10cm visual analogue scale which represented blind at the zero end and pre-diagnosis vision at the

10cm end. Owners marked their observation on the scale and were given an opportunity to amend their mark after seeing their assessment from their previous visit.

Owner and vet observed vision scores are given in Appendix 17, represented graphically in Figures 75 and 77 and as a percentage change from basal in Figures 76 and 78.

There were no statistically significant differences in owner observed or vet observed vision when considered as either absolute score or as percentage change in score. Positive percentage changes in vision score are more likely to be artefactual results of the scoring system, because genuine improvements in vision are not likely to be biologically feasible.

Faecal volume

Faecal volume score was recorded on a 10 cm visual analogue scale based on the veterinary surgeon's interpretation of owners comments.

Faecal volume scores are given in Appendix 17, represented graphically in Figure 79 and as a percentage change form the mean of the original diet days scores in Figure 80.

General linear model analysis revealed a statistically significant increase in faecal volume score ($p=0.0000$). Multiple range testing indicated that there were statistically significant differences between -1 month and all of the CHF diet days and between -10 days and all of the CHF diet days. There was also a statistically significant increase in faecal volume when considered on a percentage change basis ($p=0.0003$). Multiple range testing indicated statistically significant differences between basal (mean of -1 month and -10 days) and all of the CHF diet days.

Faecal consistency

Faecal consistency score was recorded on a 10 cm visual analogue scale. The zero end of the scale represented 'loose' which was interpreted as 'watery' and the 10 cm end of the scale represented 'hard'. Scores were allocated by veterinary interpretation of owner comments.

Faecal consistency scores are given in Appendix 17, represented graphically in Figure 81 and as a percentage of the mean of the original diet days scores in Figure 82.

There were no statistically significant changes in faecal consistency score when considered either as absolute scores or as percentage change in score from basal.

Routine biochemistry and haematology

Routine biochemical and haematological analyses were performed on blood samples taken at -1 month, -10 days, 0 days, +1 month, +2 months, +3 months and +4 months. The results of these analyses are given in Appendix 17. Very few of the results were outwith the reference ranges and there were no apparent trends in any of the parameters. Detailed statistical analyses were, therefore, not carried out.

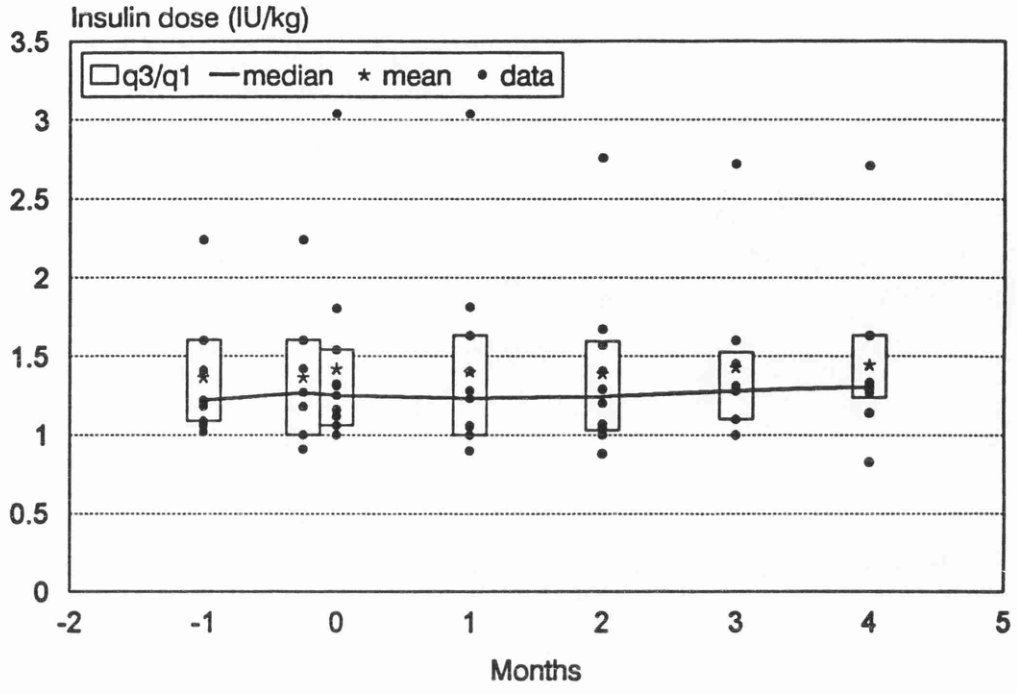

Figure 61. Absolute insulin dose (IU/kg) in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

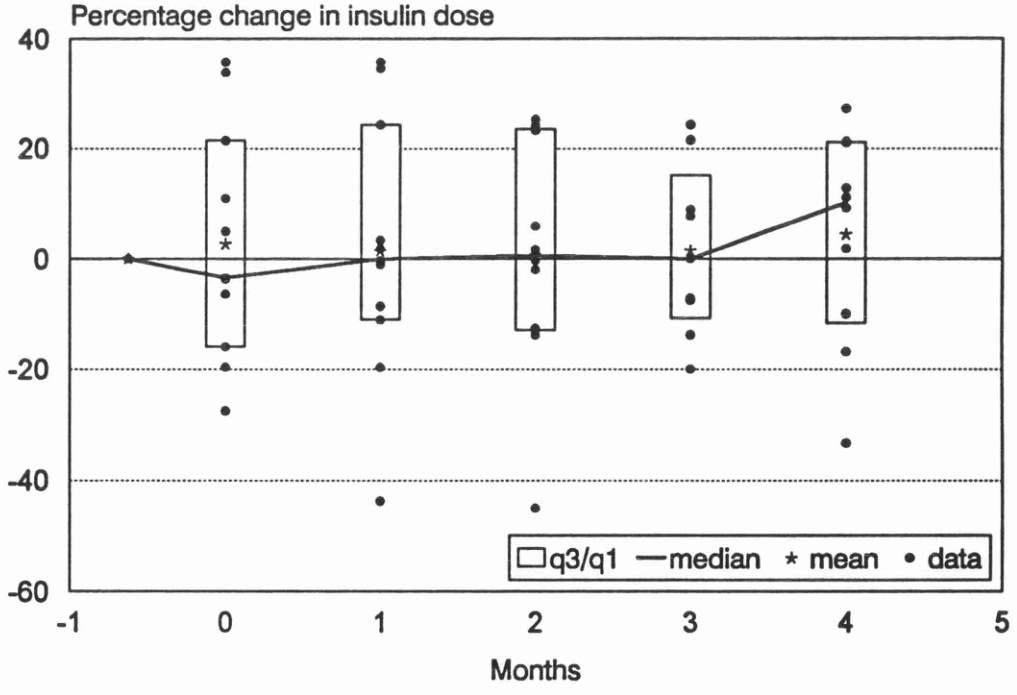

Figure 62. Percentage change in insulin dose in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

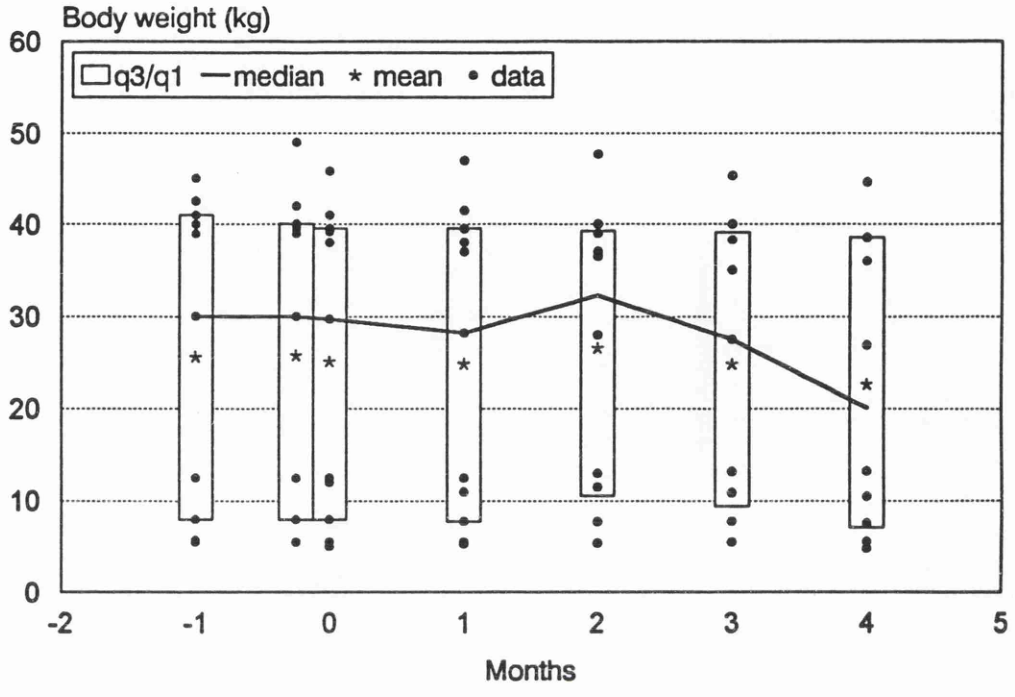

Figure 63. Absolute bodyweight (kg) in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

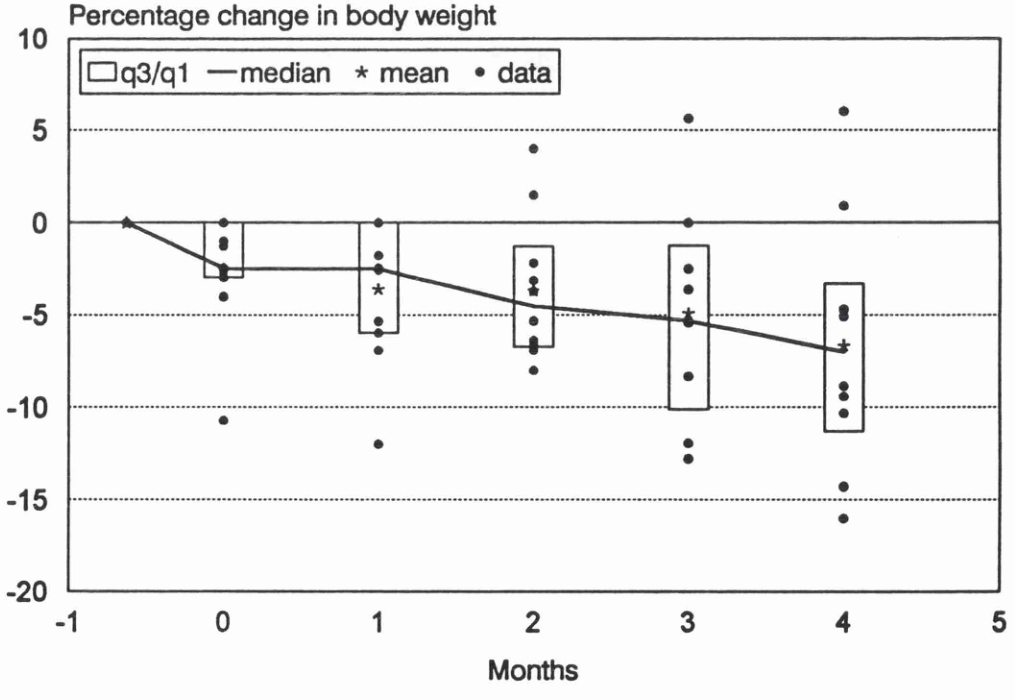

Figure 64. Percentage change in bodyweight in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

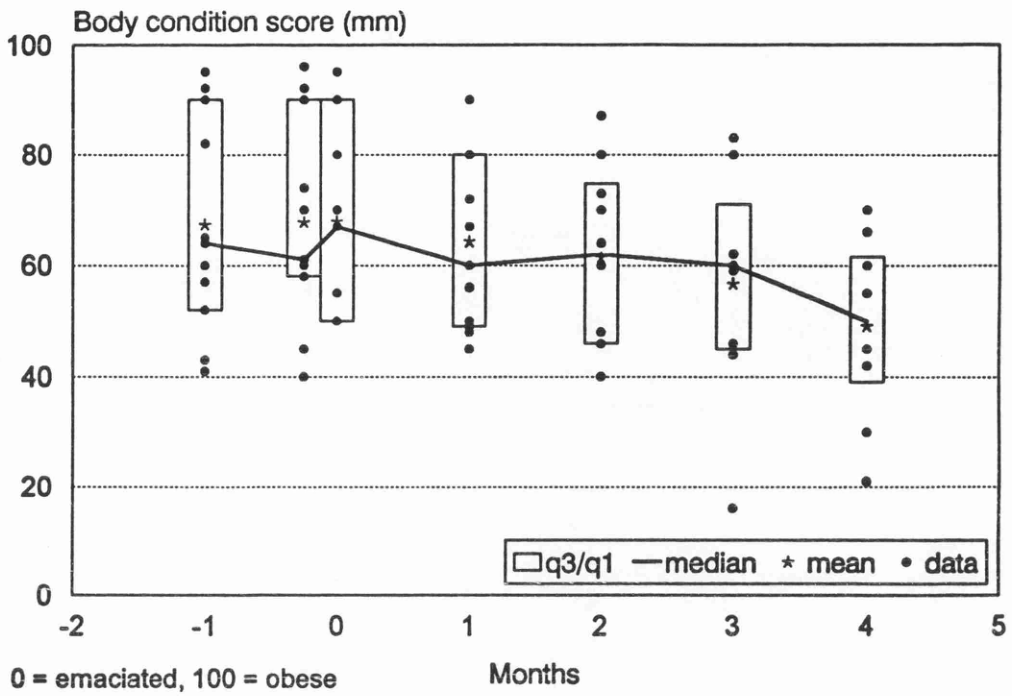

Figure 65. Absolute body condition score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

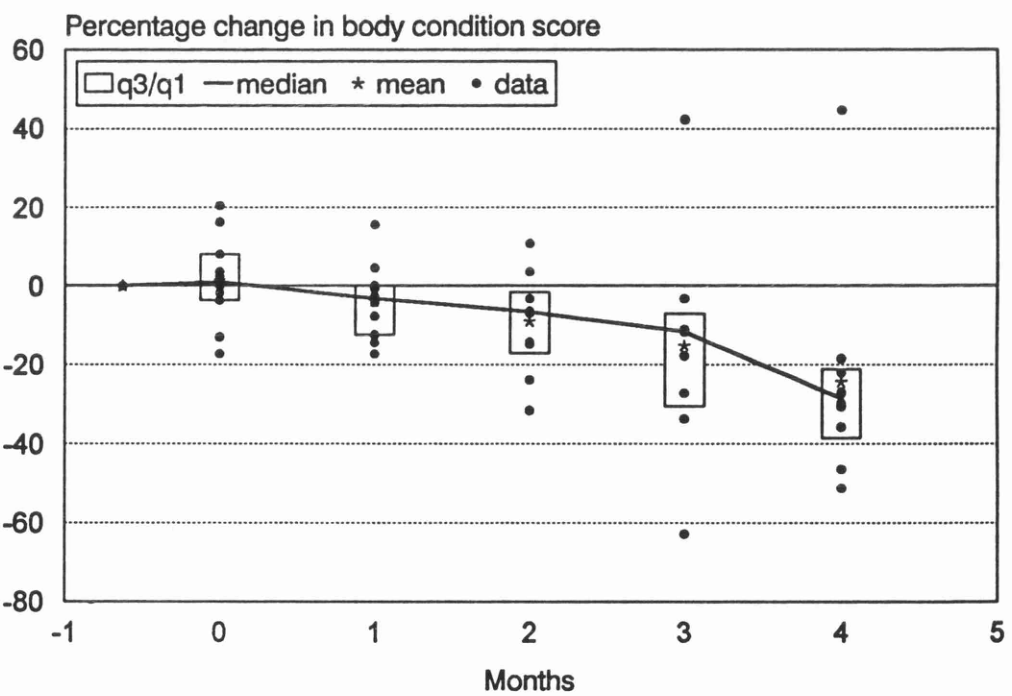

Figure 66. Percentage change in body condition score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

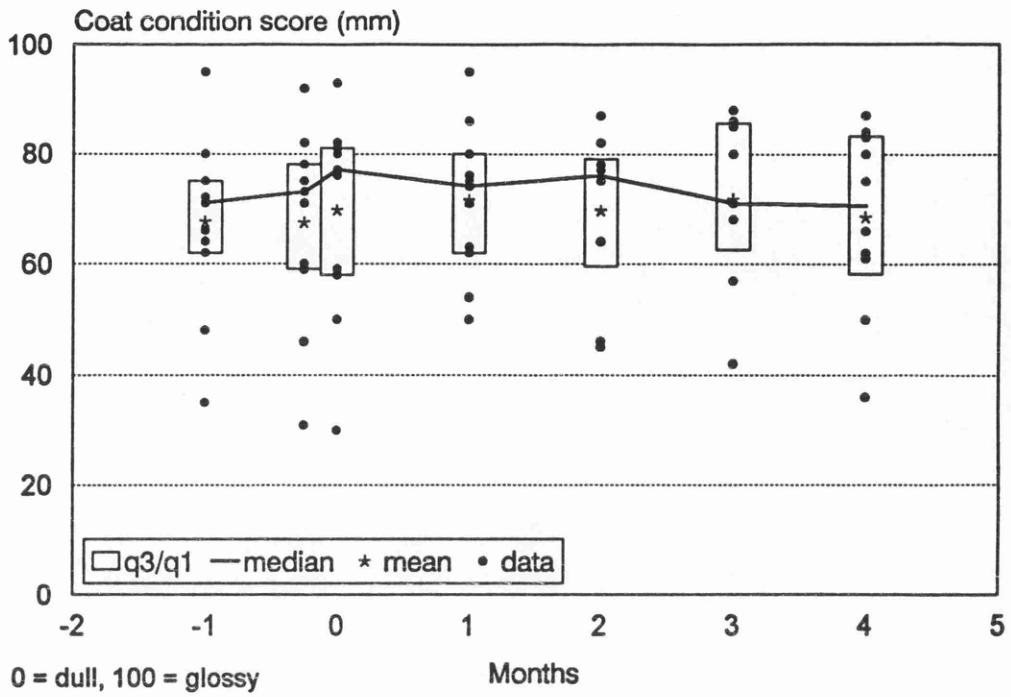

Figure 67. Absolute coat condition score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

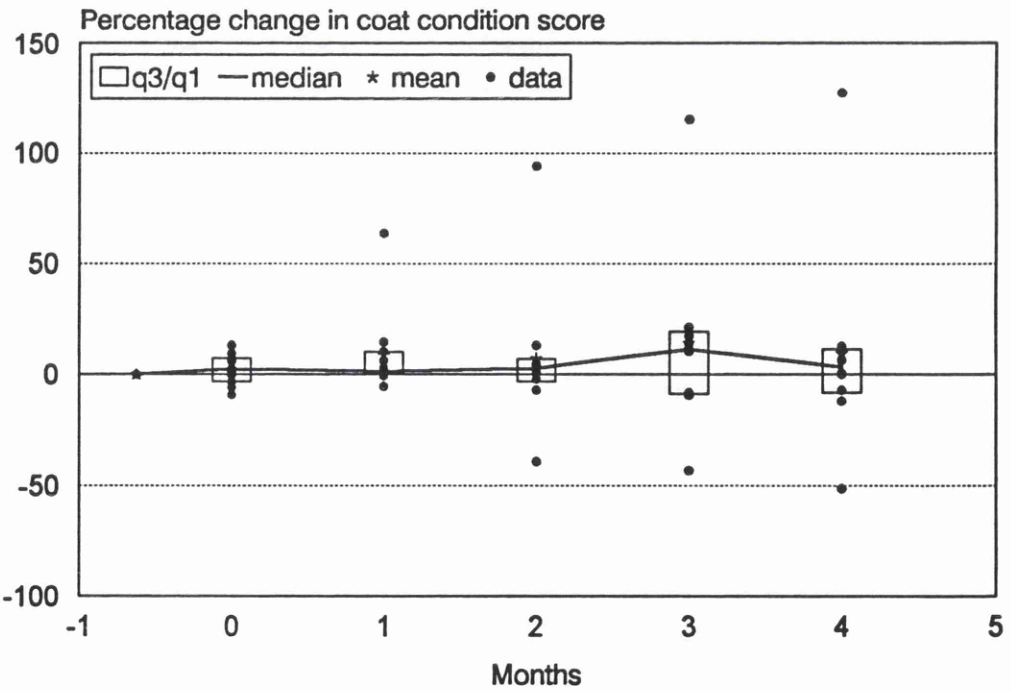

Figure 68. Percentage change in coat condition score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

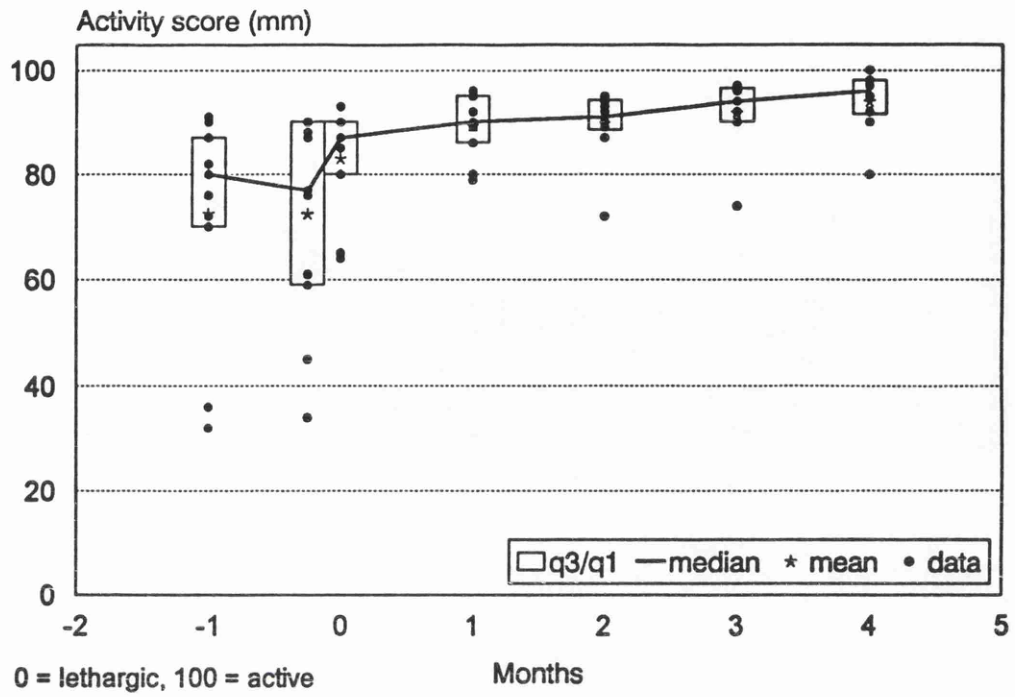

Figure 69. Absolute activity score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

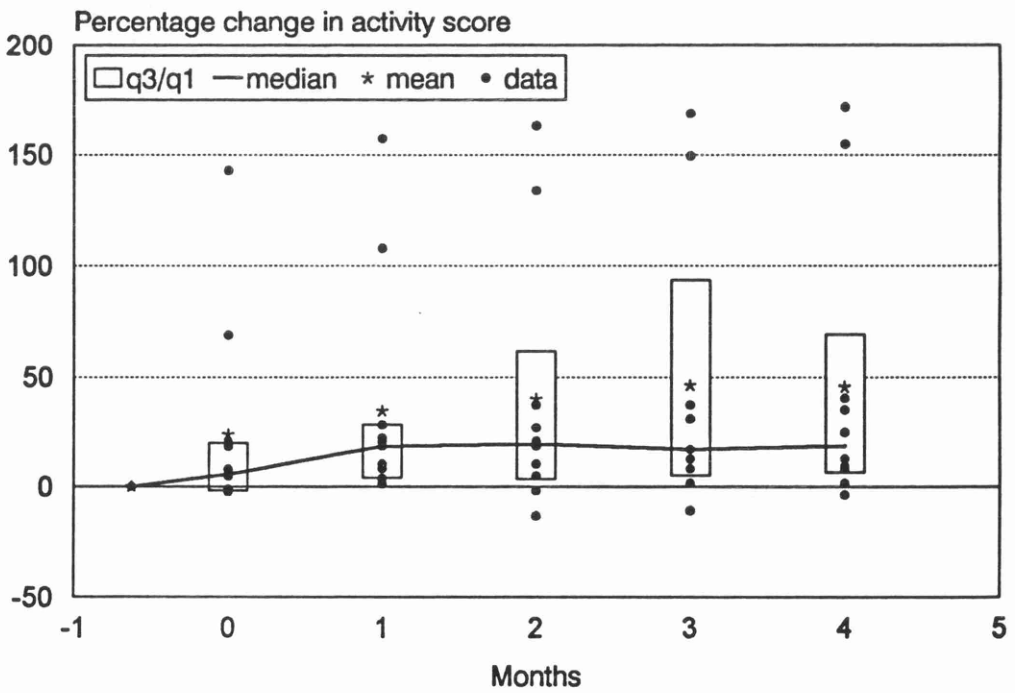

Figure 70. Percentage change in activity score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

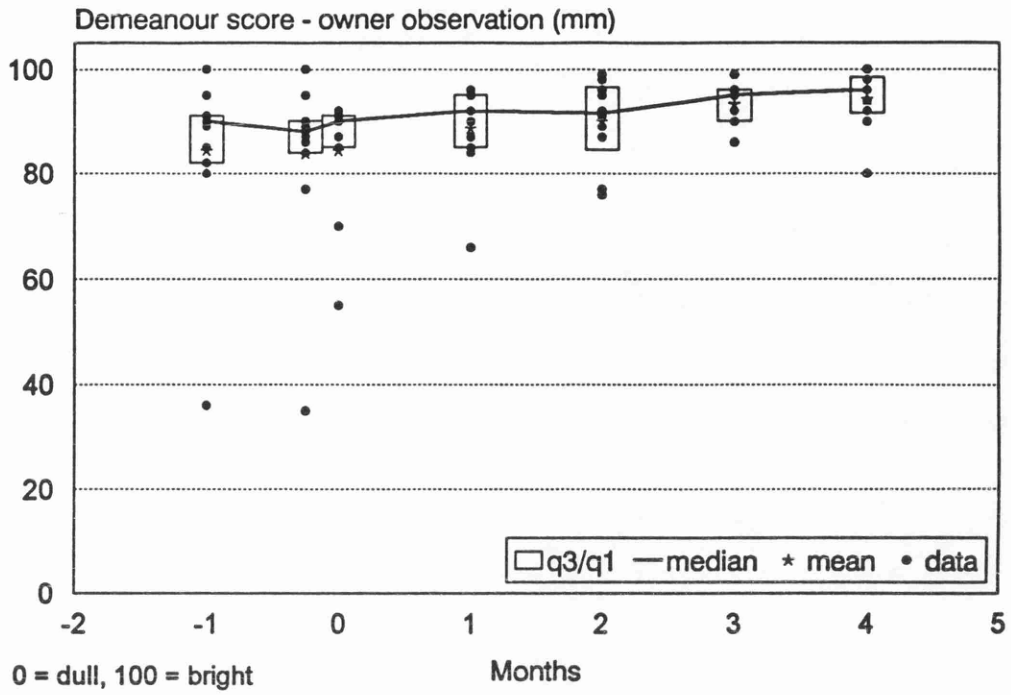

Figure 71. Absolute owner observed demeanour score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

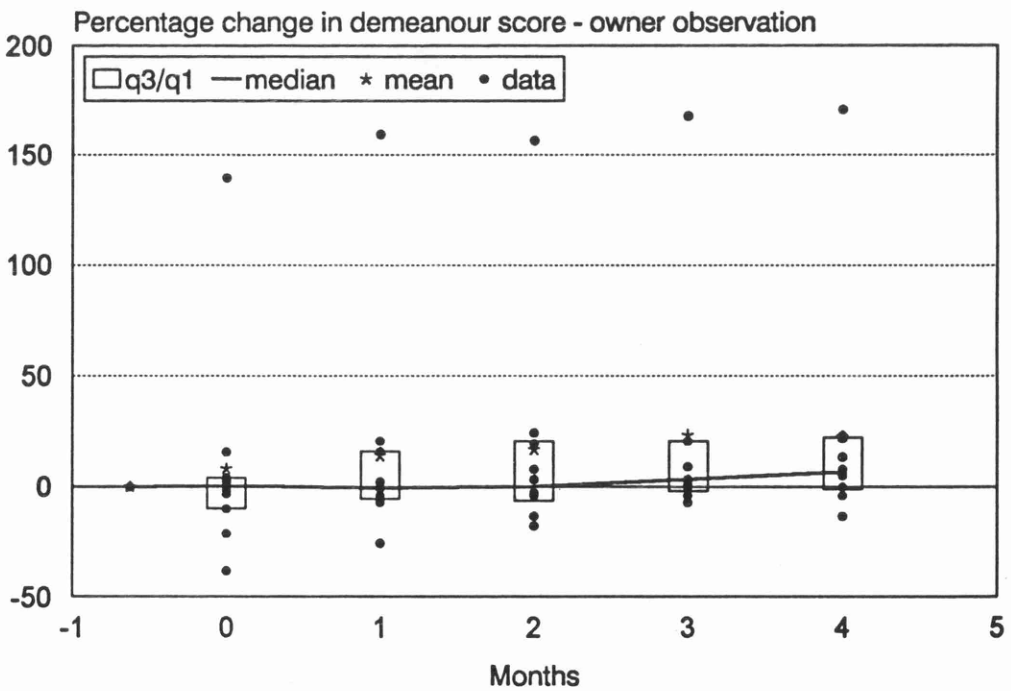

Figure 72. Percentage change in owner observed demeanour score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

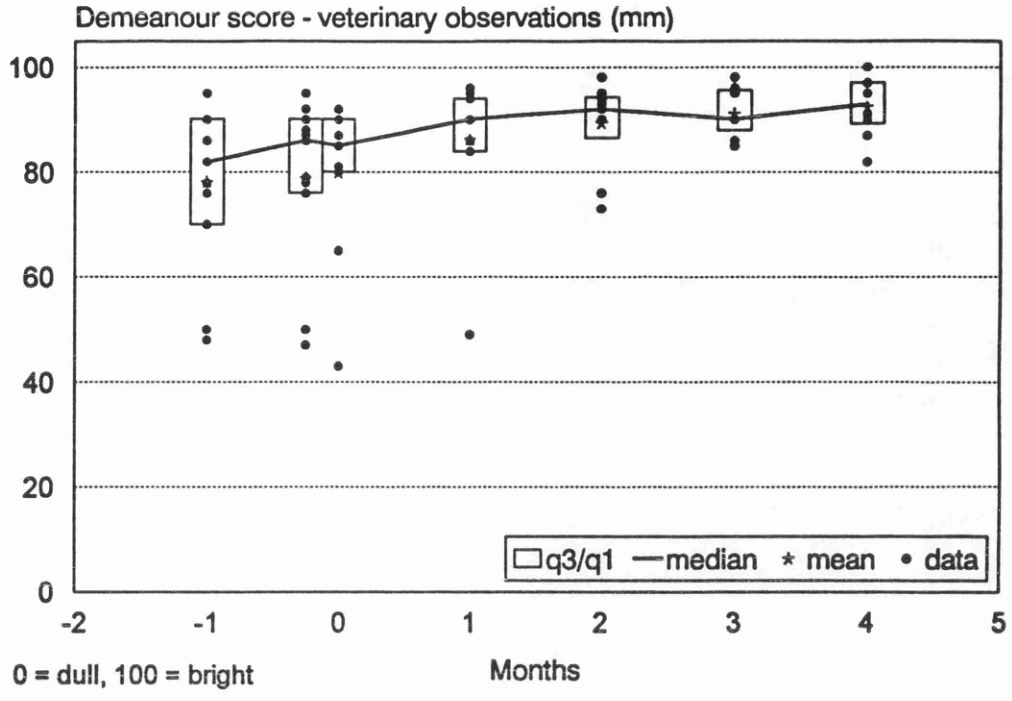

Figure 73. Absolute vet observed demeanour score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

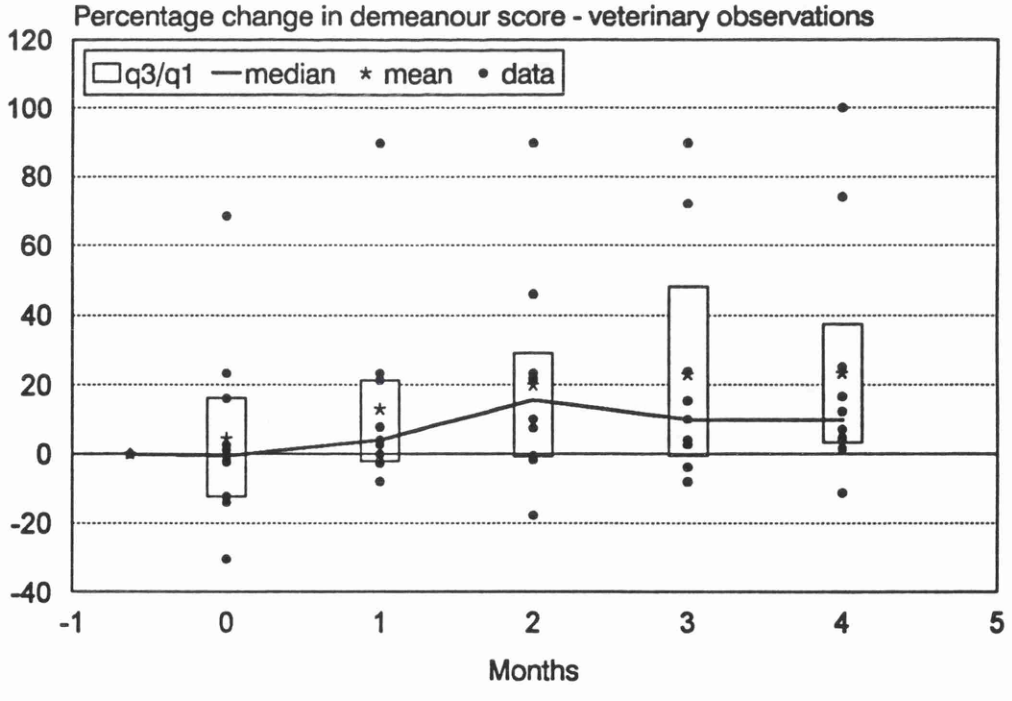

Figure 74. Percentage change in vet observed demeanour score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

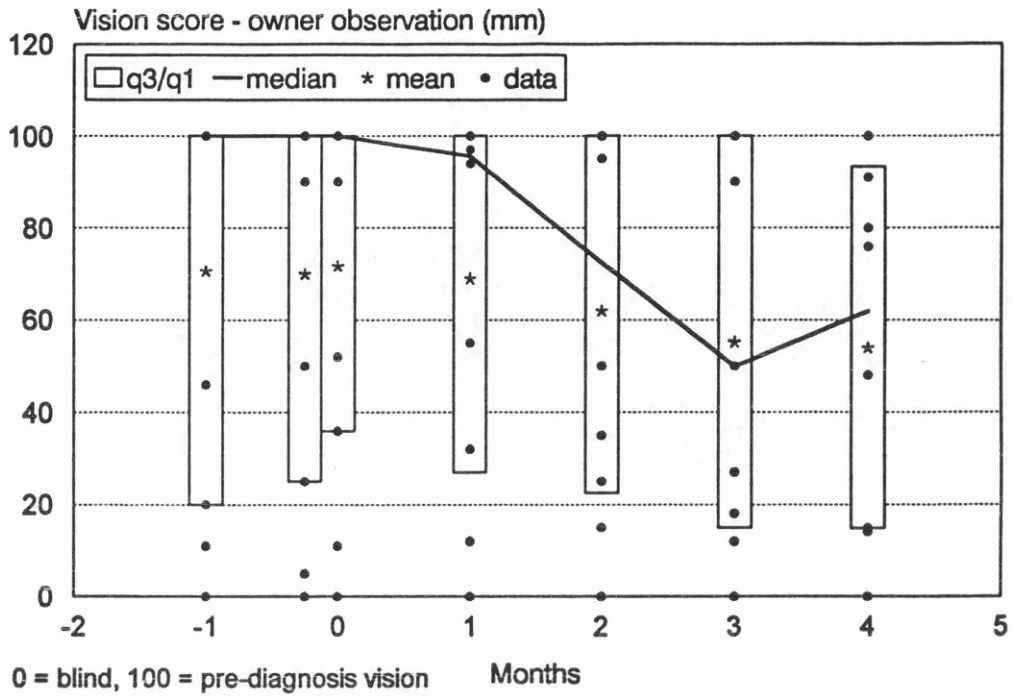

Figure 75. Absolute owner observed vision score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

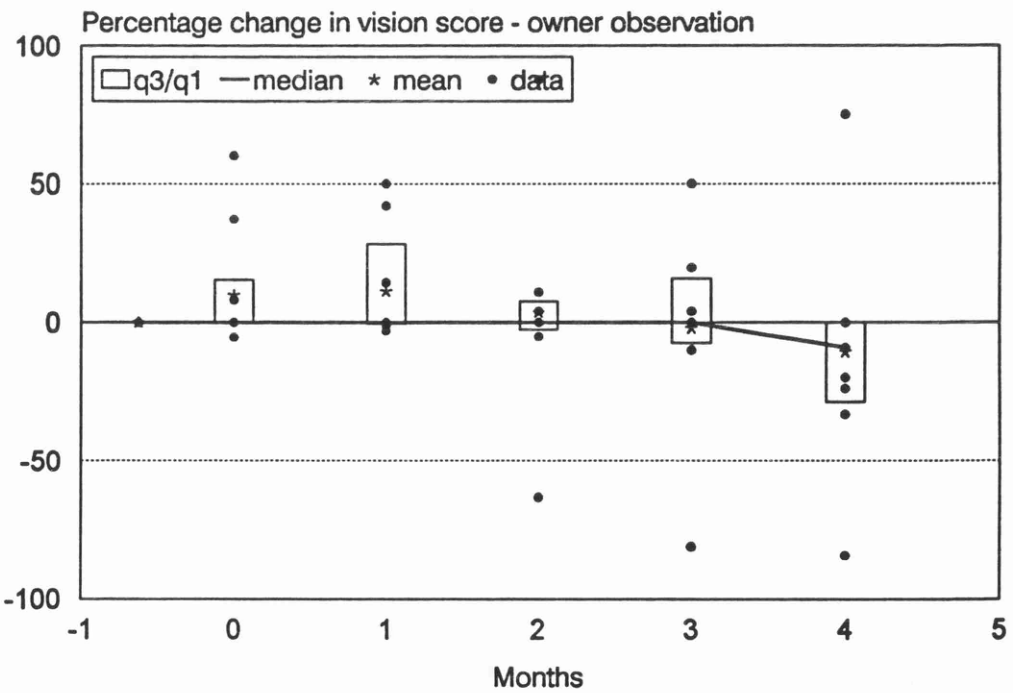

Figure 76. Percentage change in owner observed vision score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

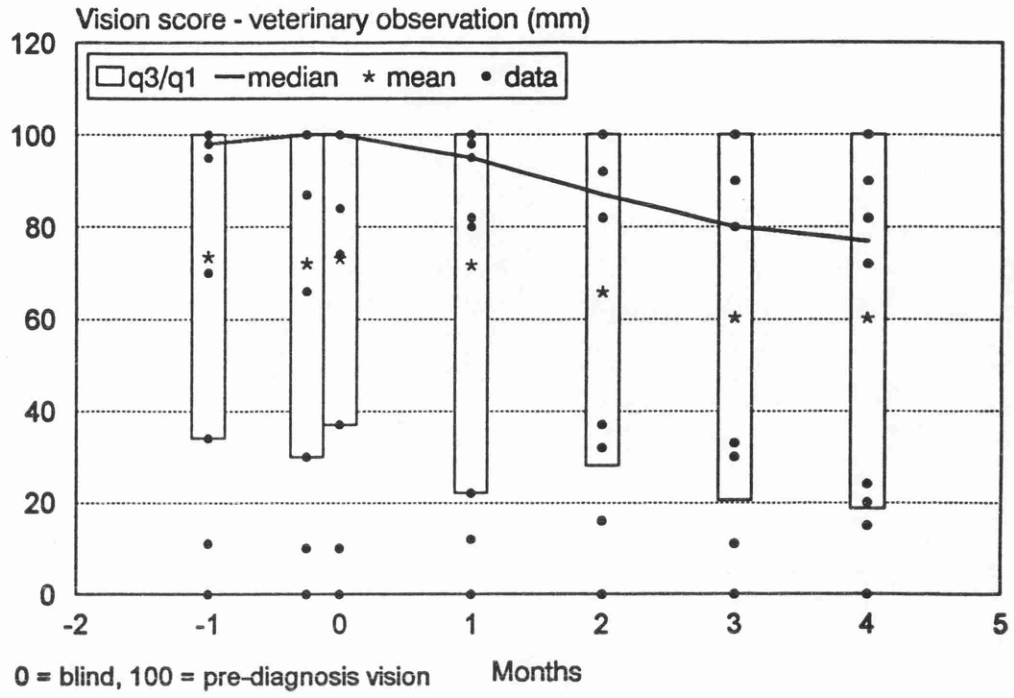

Figure 77. Absolute vet observed vision score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

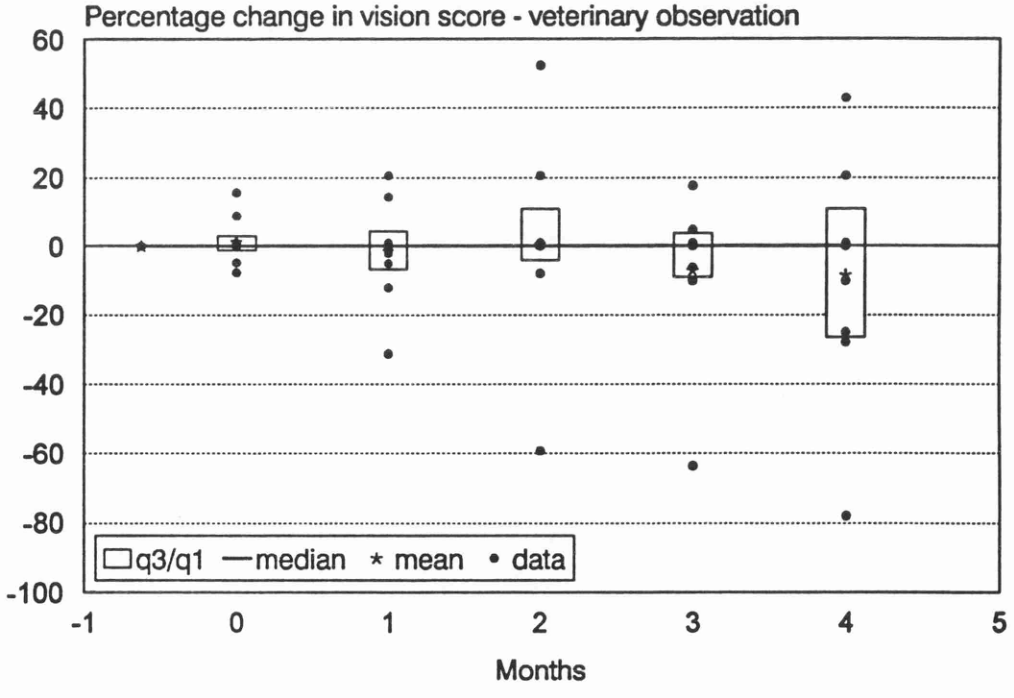

Figure 78. Percentage change in vet observed vision score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

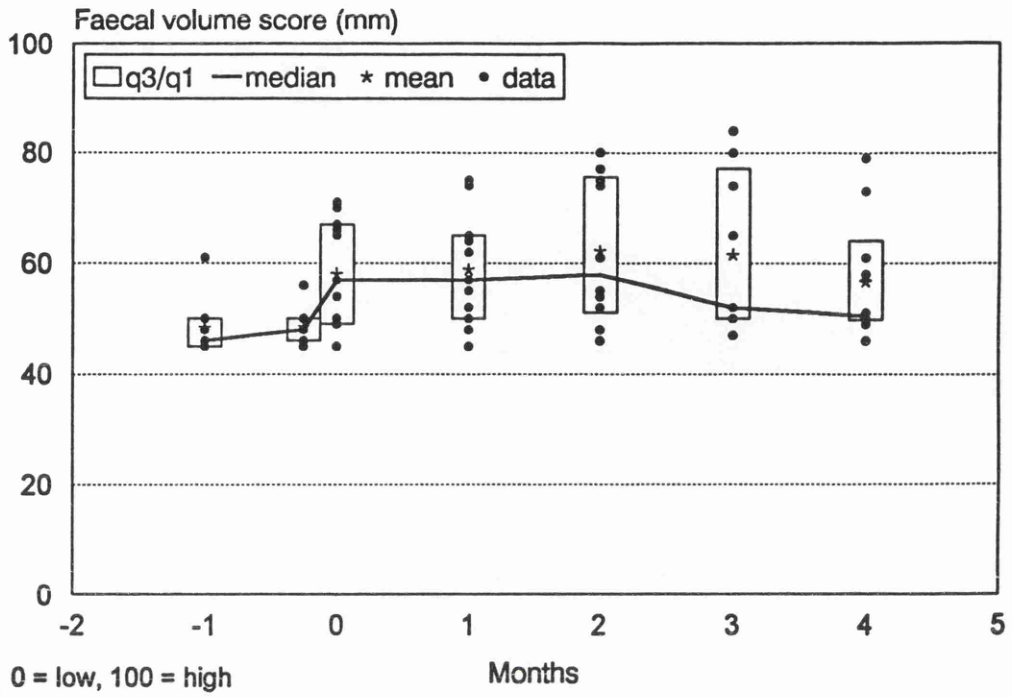

Figure 79. Absolute faecal volume score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

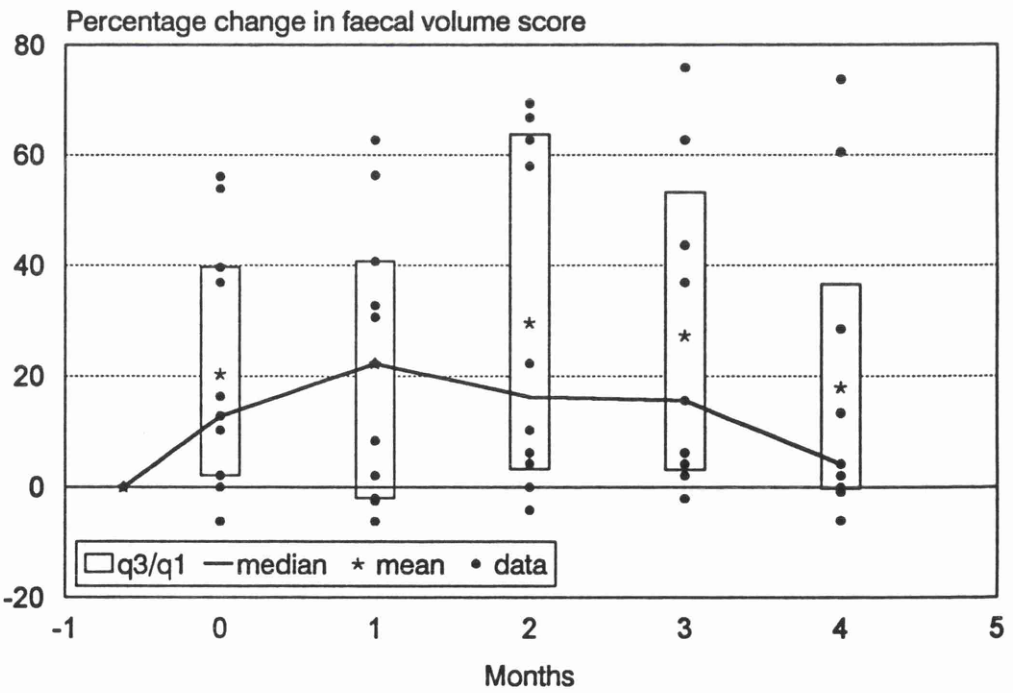

Figure 80. Percentage change in faecal volume score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

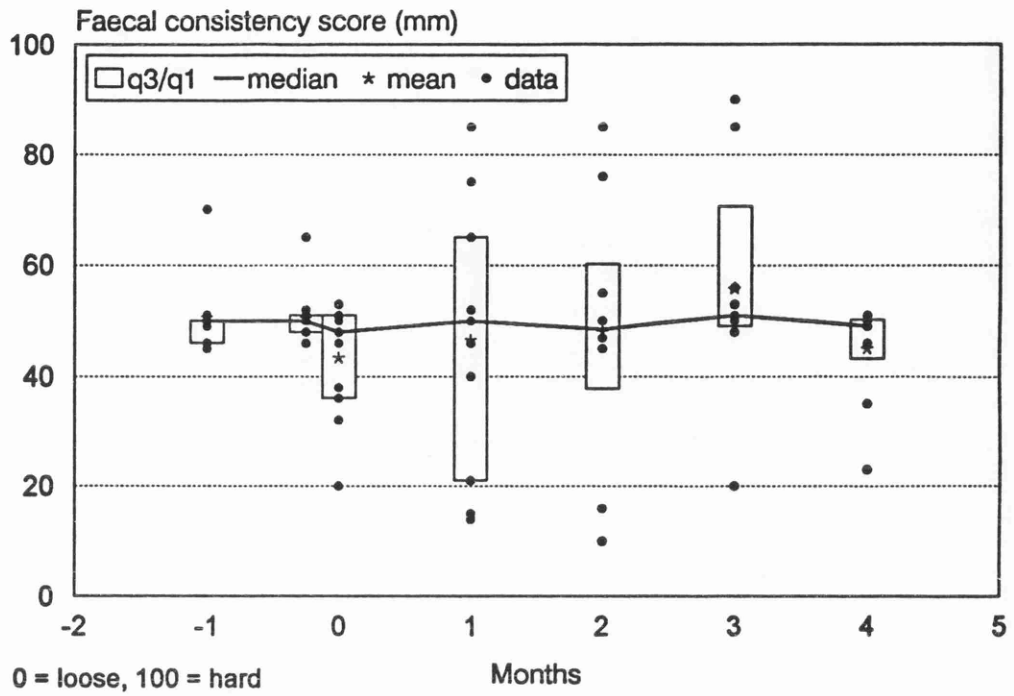

Figure 81. Absolute faecal consistency score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

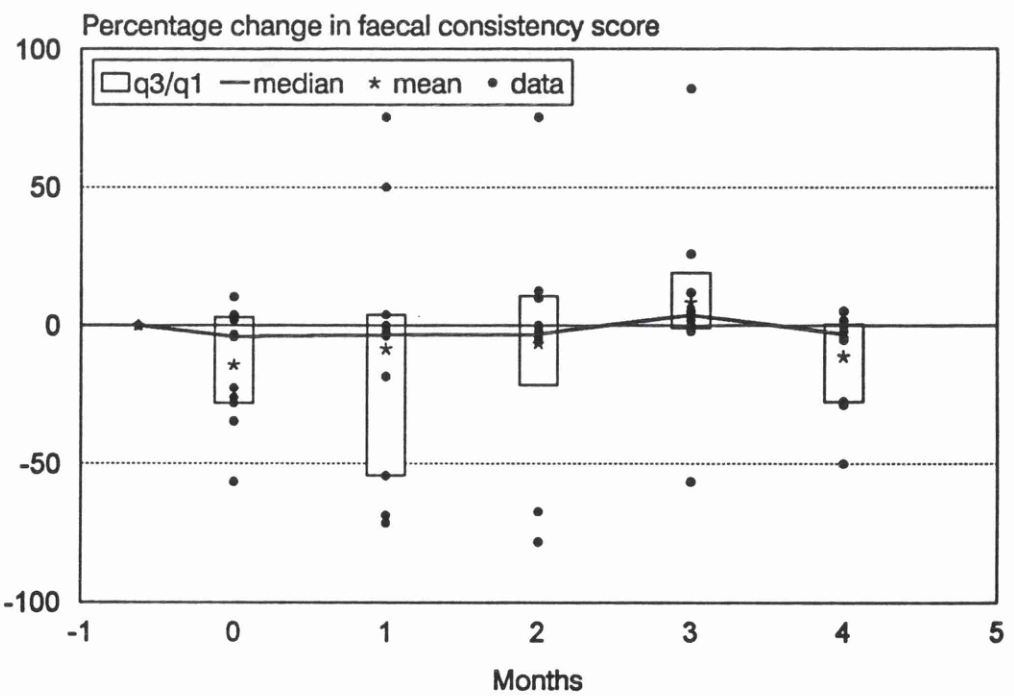

Figure 82. Percentage change in faecal consistency score in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

Indicators of glycaemic control

Fructosamine

The results of plasma fructosamine analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months +3 months and +4 months after changing to CHF are given in Appendix 18. Figure 83 displays these results graphically and Figure 84 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

General linear model analysis revealed a statistically significant reduction in absolute plasma fructosamine concentration ($p = 0.01$). Further analysis by Newman-Keuls multiple range test revealed the only significant difference to be -1 month versus +2 months. Differences between -10 days and +2 months and between -1 month and 0 days were close to statistically significant. General linear model analysis of percentage change in plasma fructosamine concentration revealed a statistically significant reduction ($p= 0.036$). Again, the only significant difference on multiple range analysis was between the basal (mean of -1 month and -10 days) and +2 months. Differences between basal and 0 days, basal and +3 months and basal and +4 months were close to statistically significant. Graphically, there is an apparent trend towards reduced plasma fructosamine on the CHF diet days.

Glycated haemoglobin

The results of glycated haemoglobin analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months +3 months and +4 months after changing to CHF are given in Appendix 18. Figure 85 displays these results graphically and Figure 86 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

General linear model analysis revealed a statistically significant reduction in absolute glycated haemoglobin concentration ($p < 0.002$). Further analysis by Newman-Keuls multiple range test revealed statistically significant differences between -10 days and +1 month, +2 months, +3 months and +4 months. Differences between -1 month and +1 month, +2 months, +3 months and +4 months were close to statistically significant. General linear model analysis of percentage change in glycated haemoglobin concentration revealed a statistically significant reduction ($p= 0.048$). Multiple range analysis failed to identify any significant differences. Differences between basal (mean of -1 month and -10 days) and +1 month, +2 months, +3 months and +4 months were very close to statistically significant. Graphically, there is a strong trend towards reduced glycated haemoglobin on the CHF diet days.

Alkaline phosphatase

The results of plasma alkaline phosphatase analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months +3 months and +4 months after changing to CHF diet are given in Appendix 18. Figure 87 displays these results graphically and Figure 88 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

General linear model analysis revealed a statistically significant decrease in plasma alkaline phosphatase concentrations ($p = 0.023$). Newman-Keuls multiple range test failed to identify any statistically significant differences but the differences between -10 days and 0 days, +1 month, +2 months and +4 months were close to statistically significant. There were no statistically significant differences in percentage change in plasma concentration of alkaline phosphatase by general linear model analysis. Following re-examination of the percentage change data it was apparent that dog 13's results may have been having a large influence on the statistical analyses.

Alanine aminotransferase

The results of plasma alanine aminotransferase analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months +3 months and +4 months after changing to CHF diet are given in Appendix 18. Figure 89 displays these results graphically and Figure 90 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

General linear model analysis found no statistically significant reductions in absolute or percentage change in plasma alanine aminotransferase concentrations. Figure 90 shows a moderate trend towards decreased alanine aminotransferase concentrations.

Aspartate aminotransferase

The results of plasma aspartate aminotransferase analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months +3 months and +4 months after changing to CHF diet are given in Appendix 18. Figure 91 displays these results graphically and Figure 92 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

There were no statistically significant differences in either the absolute or percentage change in plasma aspartate aminotransferase concentration but there is an apparent trend visible in Figure 92 in which most of the percentage change results are negative.

Fasting glucose

The results of fasting plasma glucose concentrations from 11 dogs sampled at -1 month, -10 days, 0 days, +2 months and +4 months after changing to CHF diet are given in Appendix 18. Figure 93 displays these results graphically and Figure 94 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

There were no statistically significant differences in the absolute or percentage change in fasting plasma glucose concentrations but there was a trend towards a reduction in absolute fasting plasma glucose concentration at the 0 days and +2 months sample points (Figure 93).

Afternoon (nadir) glucose

The results of afternoon plasma glucose concentrations from 11 dogs sampled at -1 month, -10 days, 0 days, +1 month, +2 months, +3 months and +4 months after changing to CHF diet are given in Appendix 18. Figure 95 displays these results graphically and Figure 96 shows these results expressed as a percentage change from the mean of the -1 month and -10 days values.

General linear model analysis revealed statistically significant reductions in afternoon plasma glucose concentration ($p<0.03$). Multiple range testing identified differences between -1 month and 0 days, -10 days and 0 days and between -1 month and +2 months. The difference between -10 days and +2 months was close to statistically significant. There was also a significant difference from basal in the percentage change data ($p<0.02$) and multiple range testing identified the differences between basal and 0 days and between basal and +2 months as statistically significant. The trend, apparent graphically, is towards a reduced afternoon plasma glucose concentration after changing to CHF diet (Figures 95 and 96).

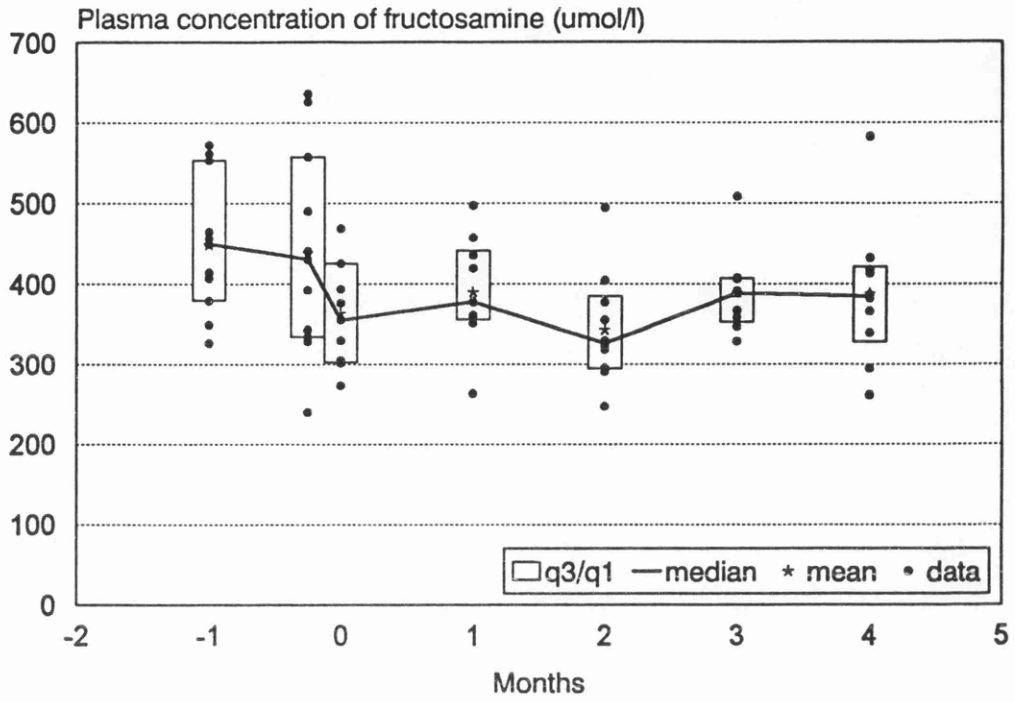

Figure 83. Absolute plasma fructosamine concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

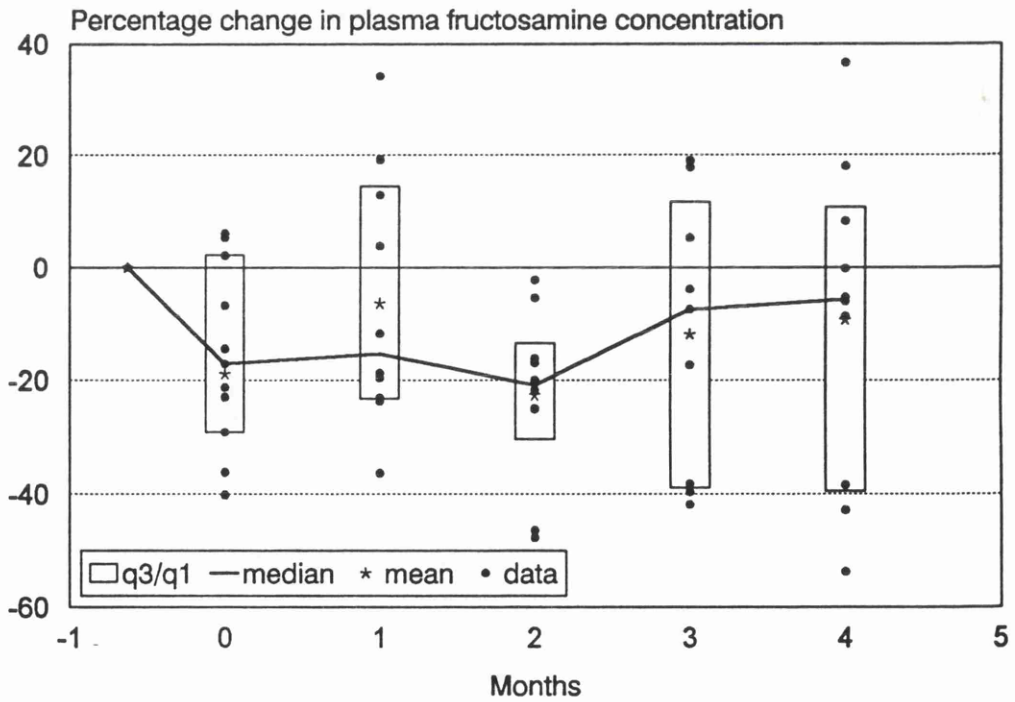

Figure 84. Percentage change in plasma fructosamine concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

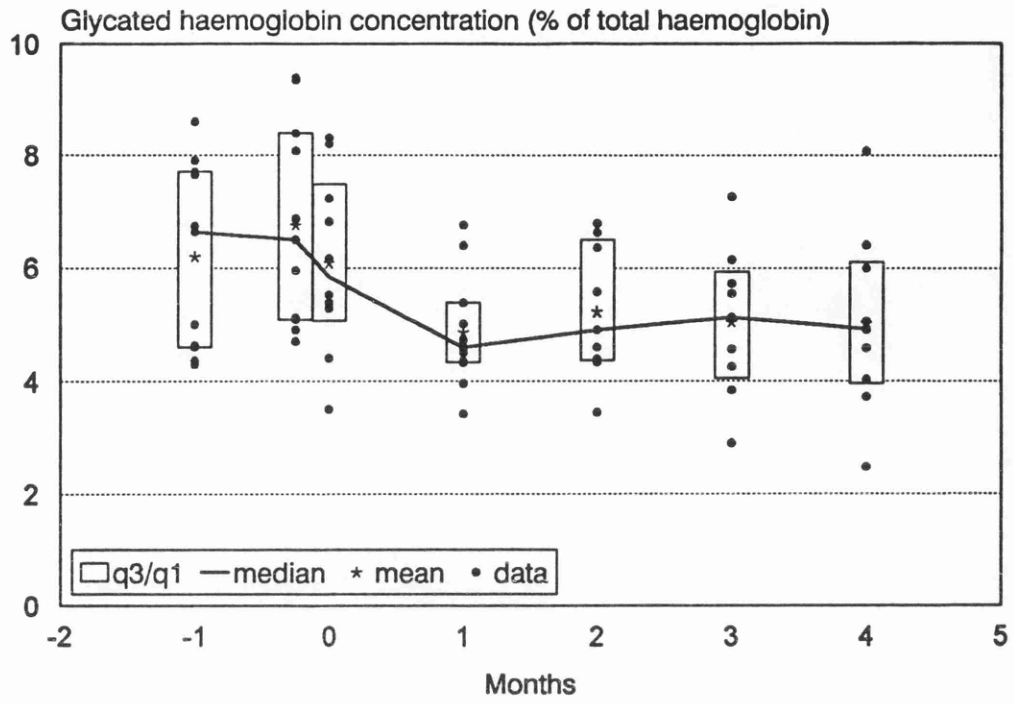

Figure 85. Absolute glycated haemoglobin concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

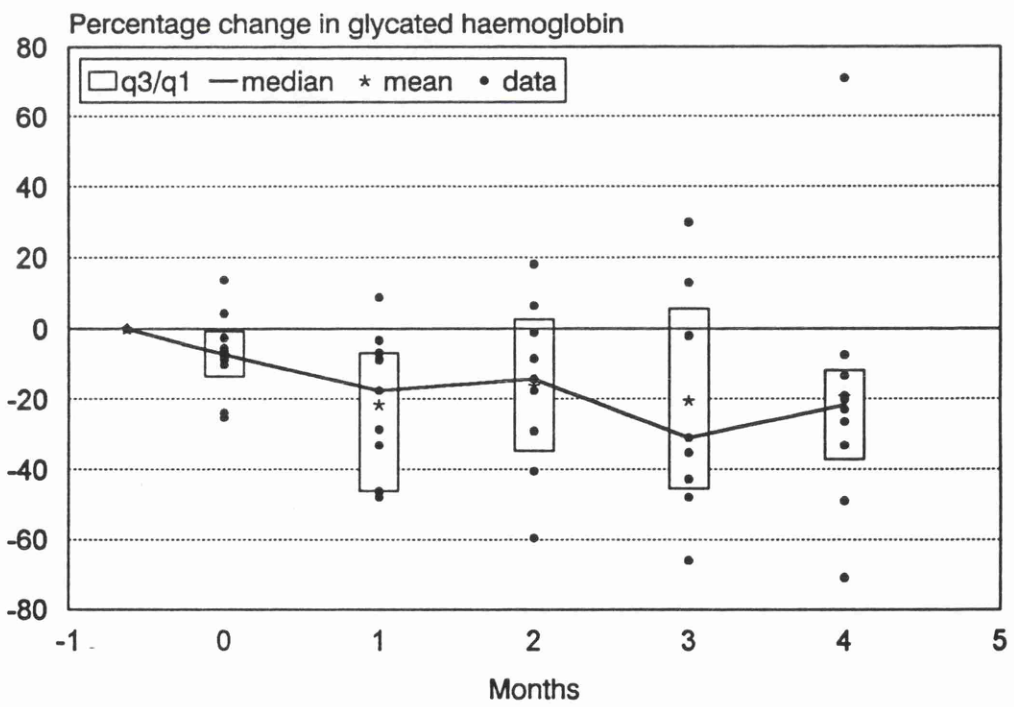

Figure 86. Percentage change in glycated haemoglobin concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

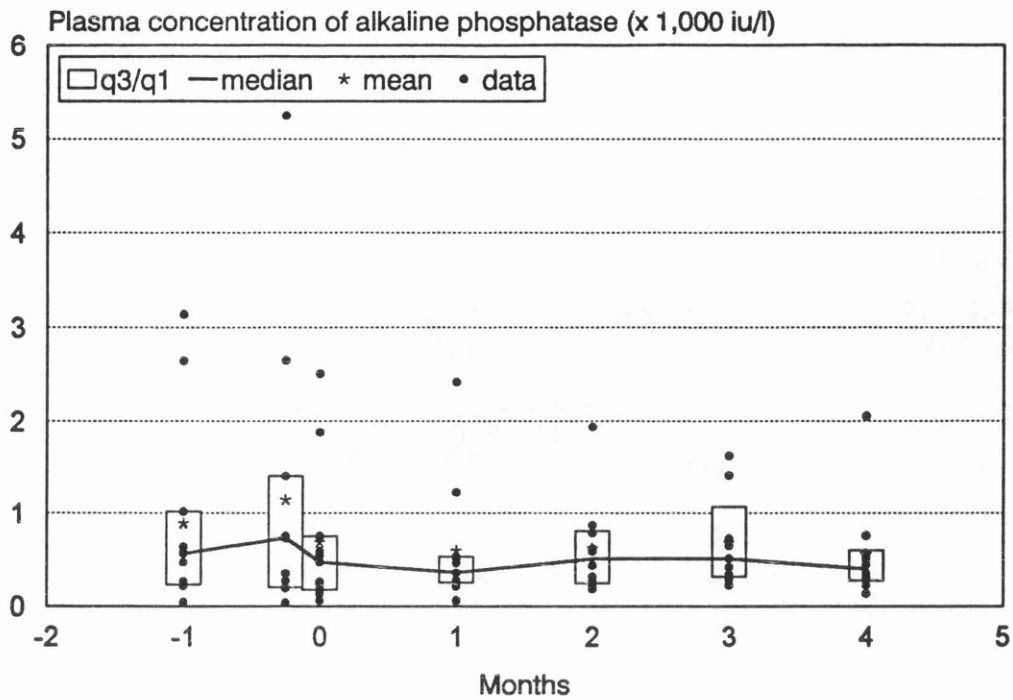

Figure 87. Absolute plasma alkaline phosphatase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

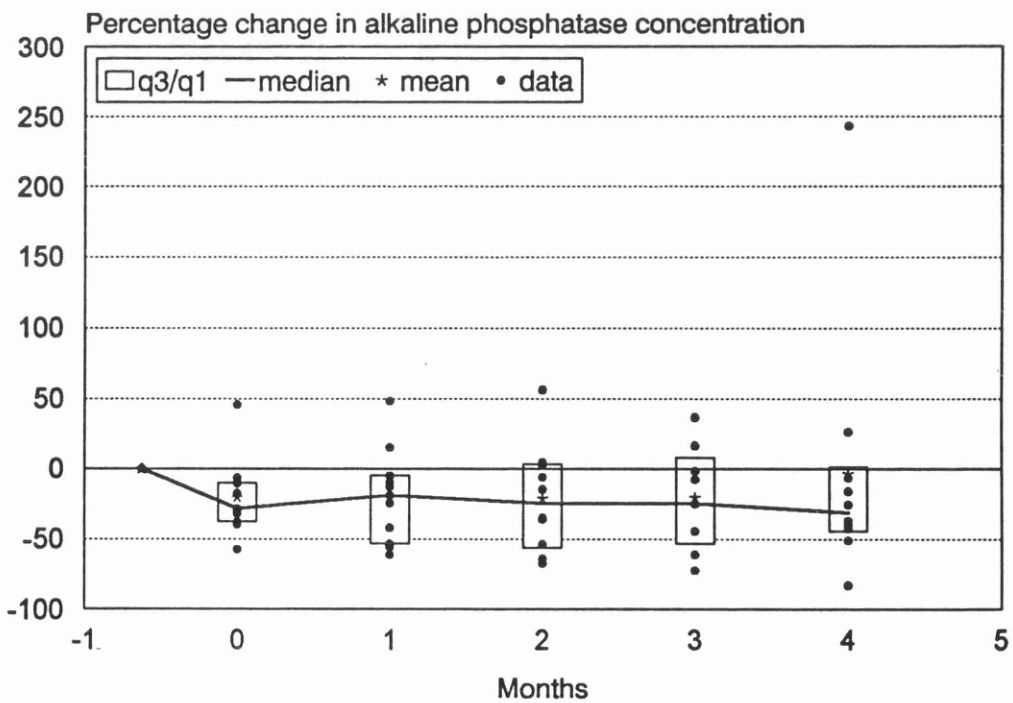

Figure 88. Percentage change in plasma alkaline phosphatase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

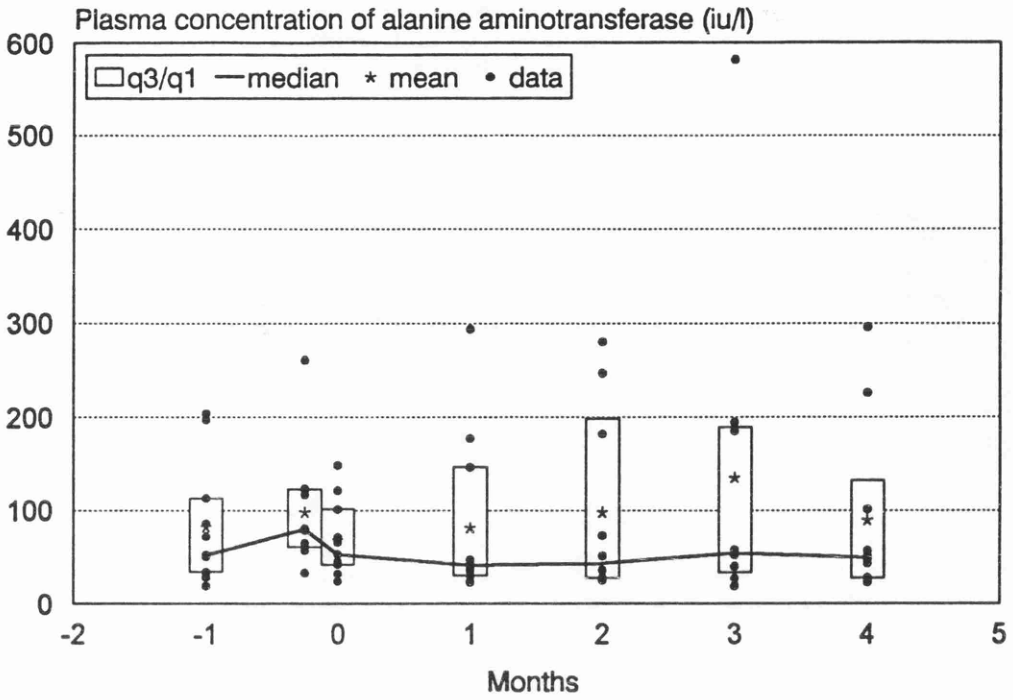

Figure 89. Absolute plasma alanine aminotransferase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

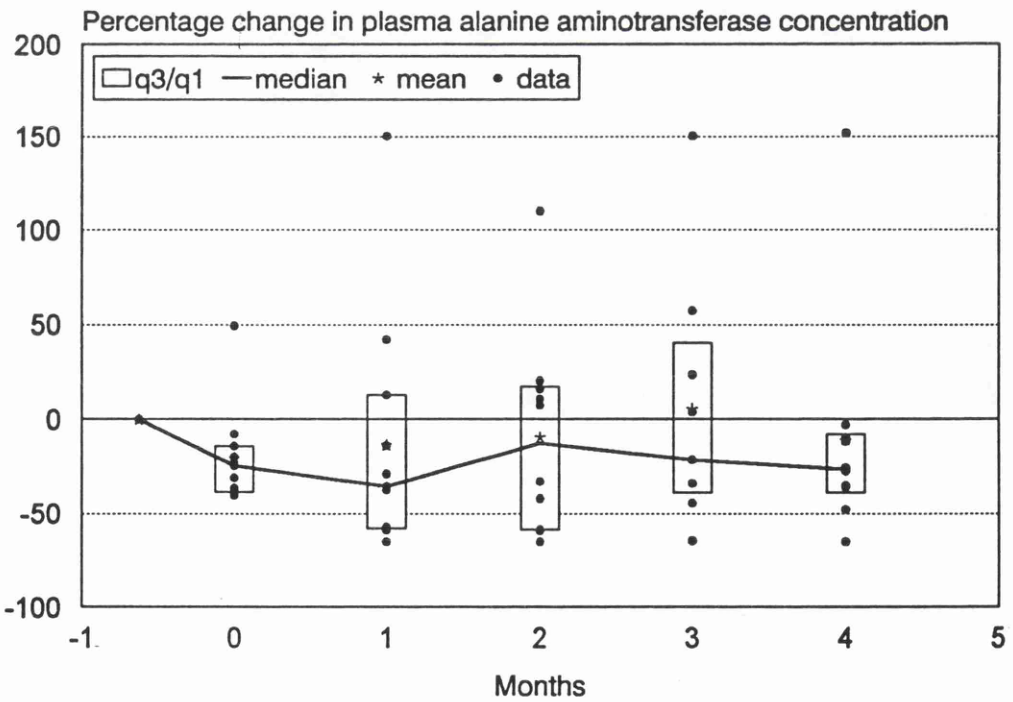

Figure 90. Percentage change in plasma alanine aminotransferase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

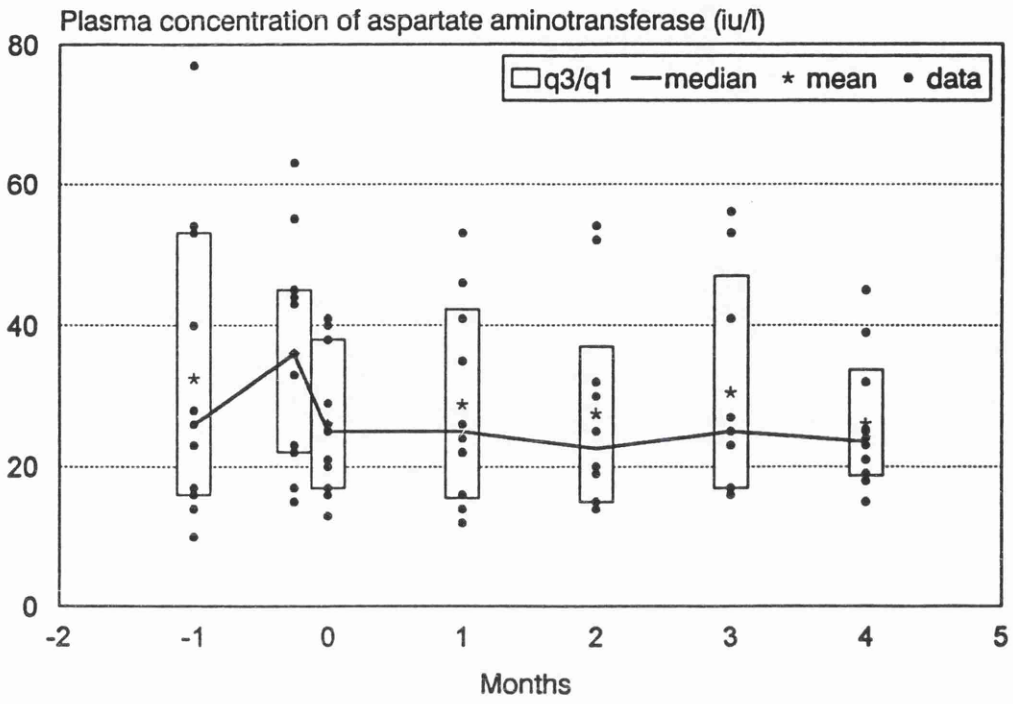

Figure 91. Absolute aspartate aminotransferase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

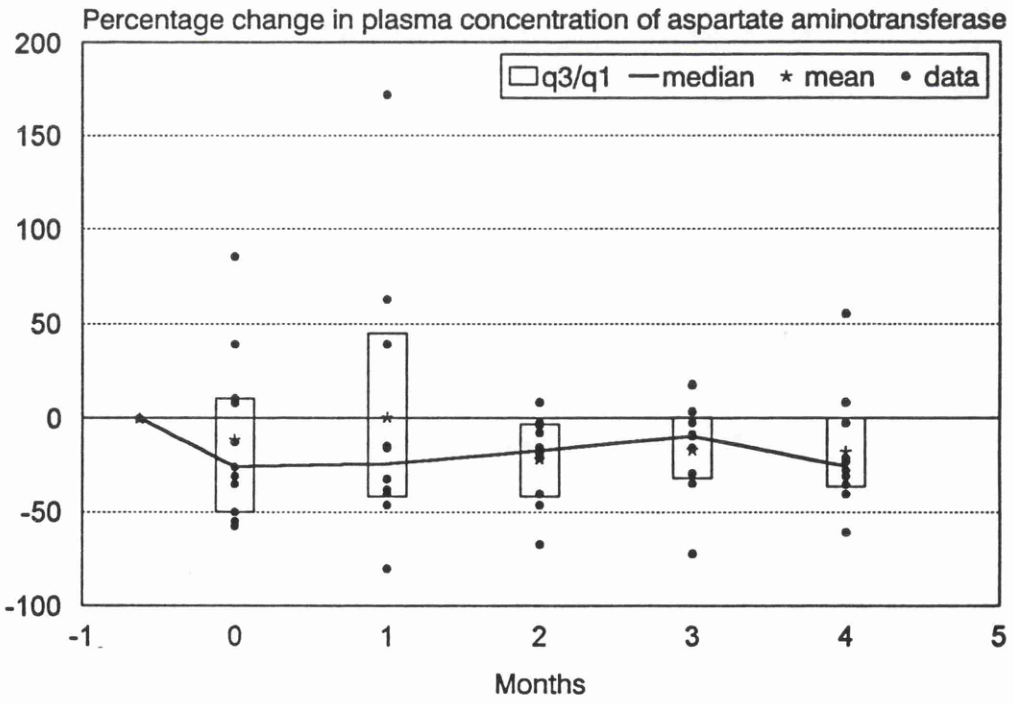

Figure 92. Percentage change in aspartate aminotransferase concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

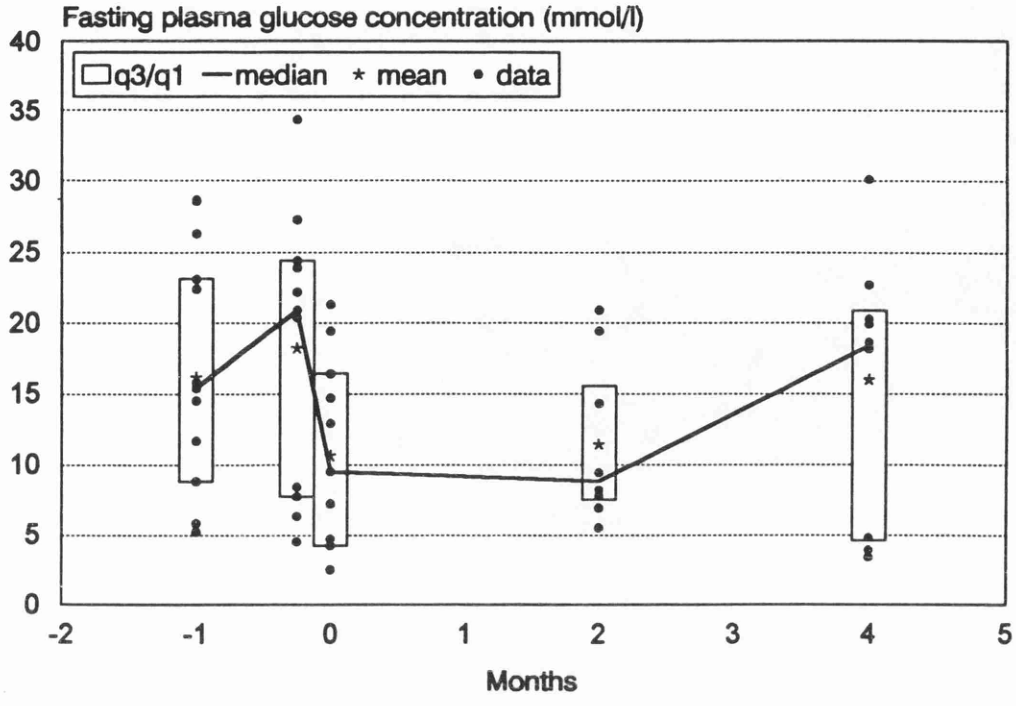

Figure 93. Absolute fasting plasma glucose concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

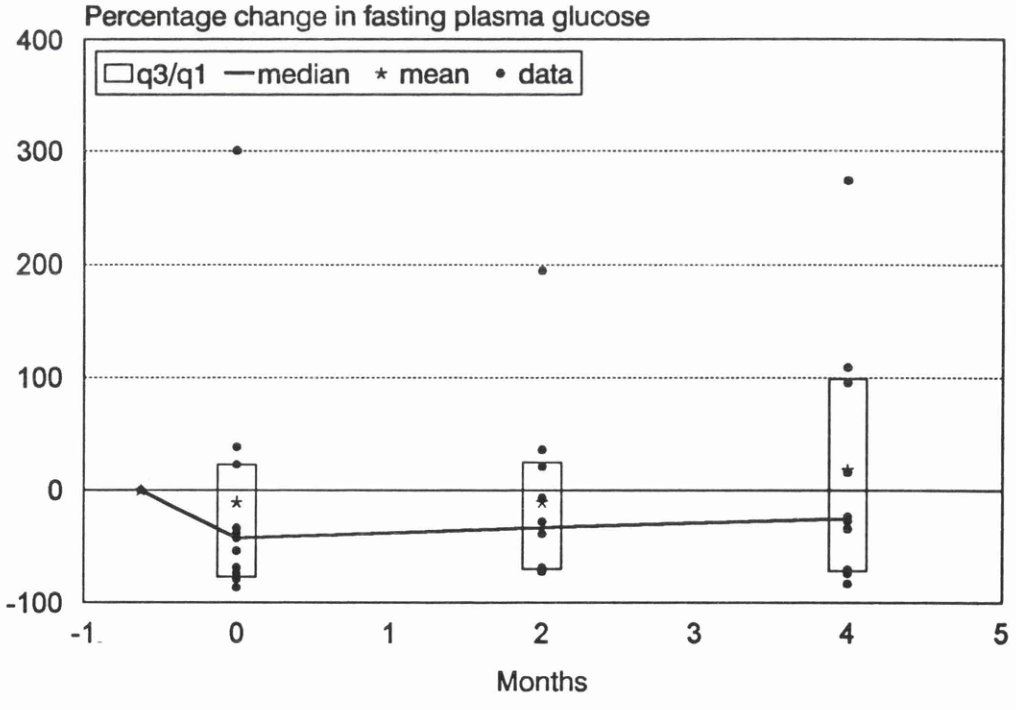

Figure 94. Percentage change in fasting plasma glucose concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

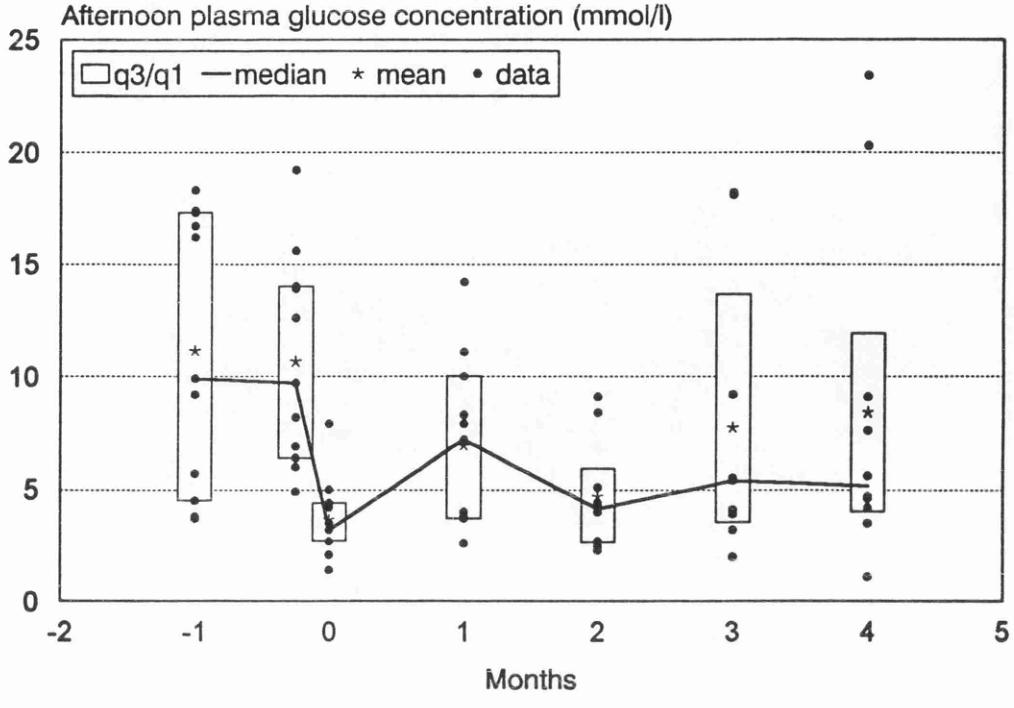

Figure 95. Absolute afternoon (nadir) plasma glucose concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

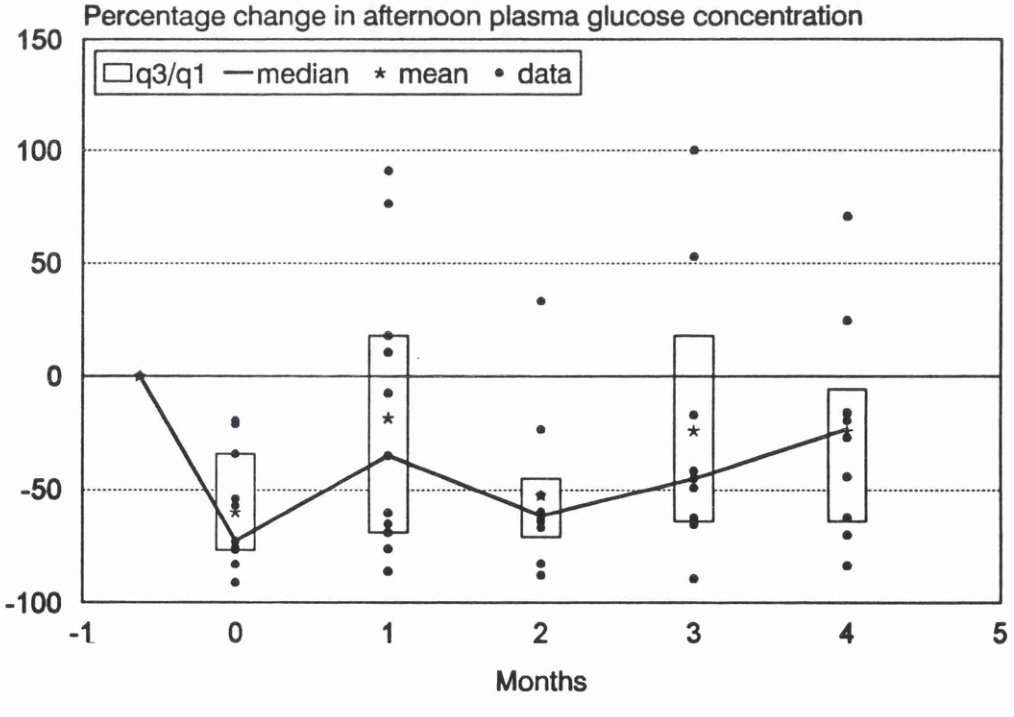

Figure 96. Percentage change in afternoon (nadir) plasma glucose concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

Lipid metabolism

Total cholesterol

Samples for cholesterol analyses were taken from fasted dogs to minimise the effects of post-prandial hypercholesterolaemia. The results of plasma cholesterol analyses from 11 dogs sampled at -1 month, -10 days, 0 days, +2 months and +4 months after changing to CHF are given in Appendix 19 and represented in Figure 97. Figure 98 represents the results expressed as a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed a statistically significant reduction in total fasting plasma cholesterol concentration ($p = 0.0023$). Multiple range testing indicated that there were statistically significant differences between -1 month and 0 days and between -10 days and 0 days. The differences between +2 months and both -1 month and -10 days were close to statistically significant. There was a highly statistically significant reduction in the percentage change data after changing to CHF diet ($p=0.0000$) and further analysis by multiple range testing indicated that there were statistically significant differences between all the CHF diet days and the mean of the two original diet days. There were also statistically significant differences between 0 days and both +2 months and +4 months.

Graphically, the reductions in absolute and percentage change data are striking. However, there is an apparent upward trend after 0 days.

VLDL cholesterol

The concentration of plasma cholesterol associated with very low density lipoproteins was measured after preparative ultracentrifugation. Technical difficulties were encountered in measuring this cholesterol fraction directly because of the very low concentrations of cholesterol involved and the difficulties in recovering the whole fraction intact after tube splitting. For the purposes of this study, a calculated VLDL cholesterol concentration was used (VLDL cholesterol = Total cholesterol - 'bottom fraction' cholesterol, i.e., VLDL cholesterol = Total cholesterol - (LDL cholesterol + HDL cholesterol)). VLDL cholesterol was measured in fasting plasma samples. The reference range for this method was 0.47 ± 0.70 (mean $\pm$ SD, n=33) (Barrie *et al*, 1993).

The results of VLDL cholesterol estimations are given in Appendix 19 and displayed graphically in Figure 99. Figure 100 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis did not reveal any statistically significant changes in VLDL cholesterol concentration. However, there is a slight trend towards reduced VLDL cholesterol evident graphically at the 0 days and +2 months sample points.

LDL cholesterol

The plasma concentration of cholesterol associated with low density lipoprotein was calculated after preparative ultracentrifugation from the difference in concentration of cholesterol in the infranatant before and after precipitation of the LDL by heparin-manganese chloride. The reference range for this method was 1.72 ± 1.88 (mean ± SD, n=33) (Barrie *et al*, 1993).

The results of LDL cholesterol estimations are given in Appendix 19 and displayed graphically in Figure 101. Figure 102 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed a statistically significant reduction in LDL cholesterol concentration (p= 0.0167) and further investigation by multiple range testing indicated that there was statistically significant difference between -10 days and 0 days. The difference between -1 month and 0 days was close to statistically significant. There was also a statistically significant reduction in LDL cholesterol concentration when expressed as percentage change form the mean of the -1 month and -10 days results (p = 0.0064) on general linear model analysis. Multiple range testing indicated that there were statistically significant differences between basal and all three CHF diet days (0 days, +2 months and +4 months).

Graphically, the reductions in LDL cholesterol concentration are striking (Figures 101 and 102).

HDL cholesterol

The plasma concentration of cholesterol associated with high density lipoprotein was measured after preparative ultracentrifugation and precipitation of LDL from the infranatant. The reference range for this method was 2.34 ± 0.70 (mean ± SD, n=33) (Barrie *et al*, 1993b).

The results of HDL cholesterol estimations are given in Appendix 19 and displayed graphically in Figure 103. Figure 104 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed no significant changes in HDL cholesterol concentration. Graphically it can be seen that there is a trend towards decreased HDL cholesterol at 0 days but that there is no change apparent at +2 months and +4 months.

Triglycerides

The results of plasma triglyceride estimations are given in Appendix 19 and displayed graphically in Figure 105. Figure 106 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed no statistically significant changes in plasma triglyceride concentrations and there are no trends apparent graphically.

Glycerol

The results of plasma glycerol estimations are given in Appendix 19 and displayed graphically in Figure 107. Figure 108 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed a statistically significant reduction in absolute free plasma glycerol concentrations ($p= 0.0072$). Multiple range testing indicated statistically significant differences between -1 month and +2 months and between -10 days and +2 months. There was also a statistically significant reduction in free plasma glycerol when expressed as a percentage change from basal ($p= 0.0397$). Again, multiple range testing indicated statistically significant differences between basal and +2 months and between 0 days and +2months. Differences between basal and +4 months and between 0 days and +4 months were close to statistically significant.

Non-esterified fatty acids

The results of plasma non-esterified fatty acids estimations are given in Appendix 19 and displayed graphically in Figure 109. Figure 110 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

General linear model analysis revealed a statistically significant reduction in plasma non-esterified fatty acid concentration ($p= 0.0302$). Multiple range testing indicated a statistically significant difference between -10 days and +2months. The difference between -1 month and + 2 months was close to statistically significant. There was no statistically significant change in plasma non-esterified fatty acid concentration when analysed on a percentage change basis although there was an apparent trend visible graphically (Figure 110).

VLDL triglyceride/apolipoprotein B

Plasma concentrations of very low density lipoprotein associated triglyceride were measured in the same supernatant obtained from preparative ultracentrifugation and tube slicing originally intended for the direct measurement of VLDL cholesterol. Some difficulty was experienced in recovering all of the supernatant (VLDL fraction) and, therefore, the resulting triglyceride results may not have been very reliable. This was principally the result of an imperfect seal in the upper section of the tube splitting apparatus which caused loss of supernatant during the process of flushing with saline.

Very low density lipoprotein associated apolipoprotein B concentrations were measured in the VLDL fraction which resulted from preparative ultracentrifugation of EDTA plasma samples for quantitative lipoprotein cholesterol analysis. In some cases the concentration of apolipoprotein B was undetectable making the creation of a VLDL triglyceride:apoliprotein B ratio impossible.

VLDL triglyceride/apolipoprotein B ratios are given in Appendix 19 and displayed graphically in Figure 111. Figure 112 represents these results expressed a percentage change from the mean of the -1 month and -10 days results. General linear model analyses revealed no statistically significant changes in VLDL triglyceride:apoliporotein B ratios and graphically there were no apparent trends.

Post heparin hepatic triglyceride lipase

The measurement of plasma concentrations of lipoprotein lipase was abandoned because of technical difficulties. This was believed to be mainly due to sample handling and storage during which the very labile lipoprotein lipase was destroyed. However, post-heparin hepatic triglyceride lipase is more stable and its measurement is a necessary part of lipoprotein lipase analysis. It was possible, therefore, to measure and record values for hepatic triglyceride lipase instead of lipoprotein lipase. The reference range for post heparin hepatic triglyceride lipase using this method is 6.92 ± 1.44 (mean $\pm$ SD, n=6) (Dr J. Barrie - personal communication).

The results of post-heparin plasma hepatic triglyceride lipase activities are given in Appendix 19 and displayed graphically in Figure 113. Figure 114 represents these results expressed a percentage change from the mean of the -1 month and -10 days results.

There were no statistically significant changes in post heparin plasma hepatic triglyceride lipase activity when analysed as absolute results nor when analysed as percentage change from basal results.

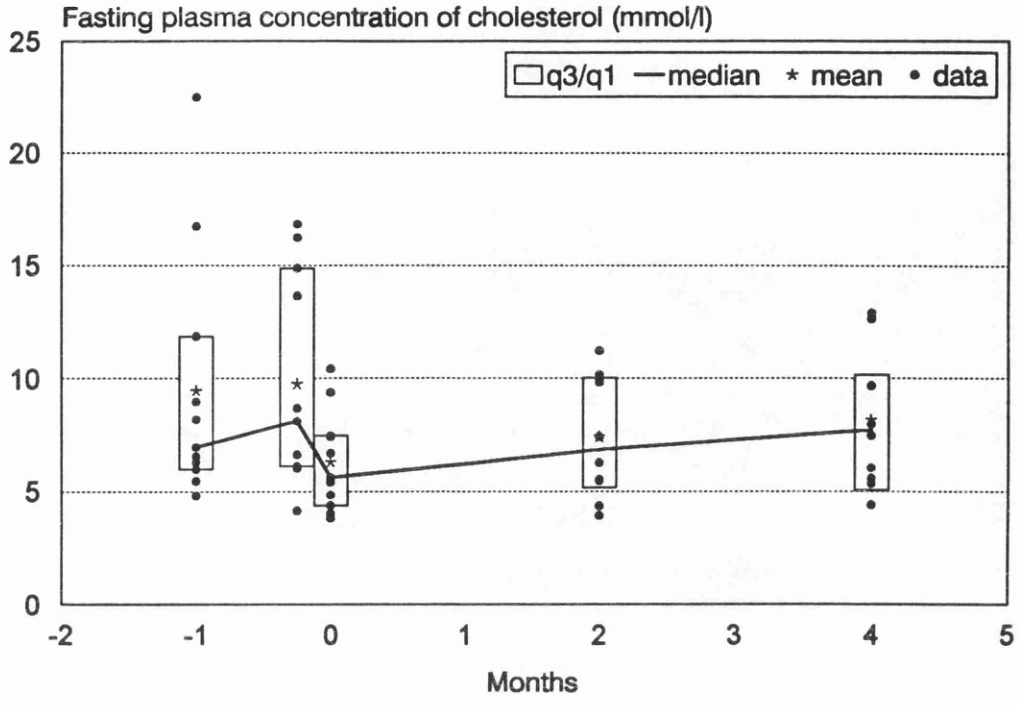

Figure 97. Absolute total plasma cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

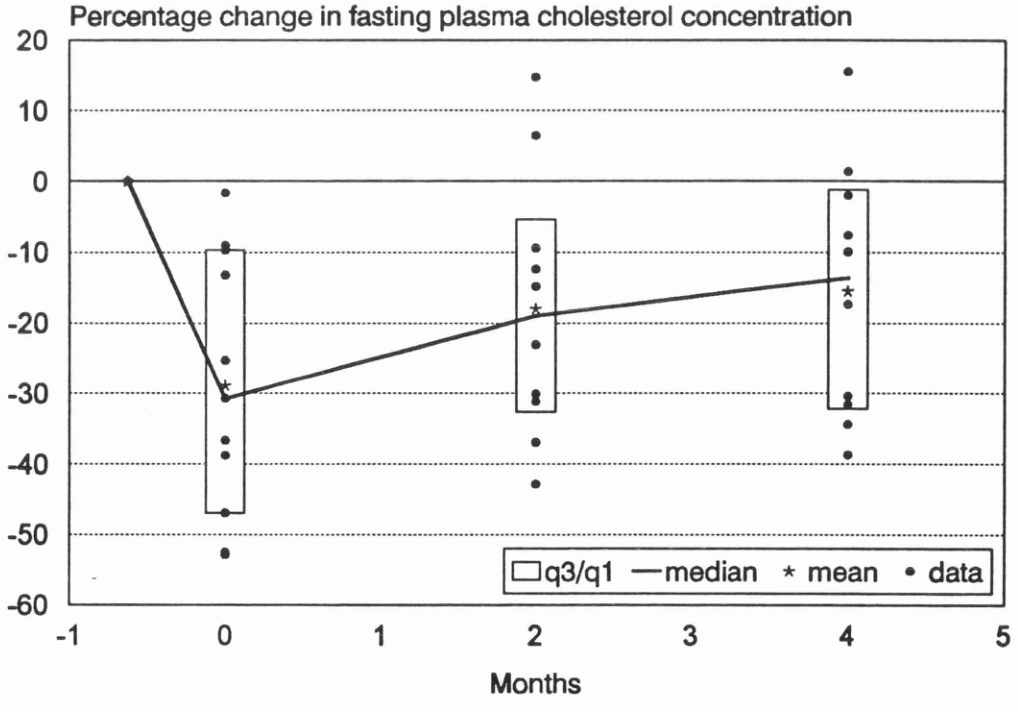

Figure 98. Percentage change in total plasma cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

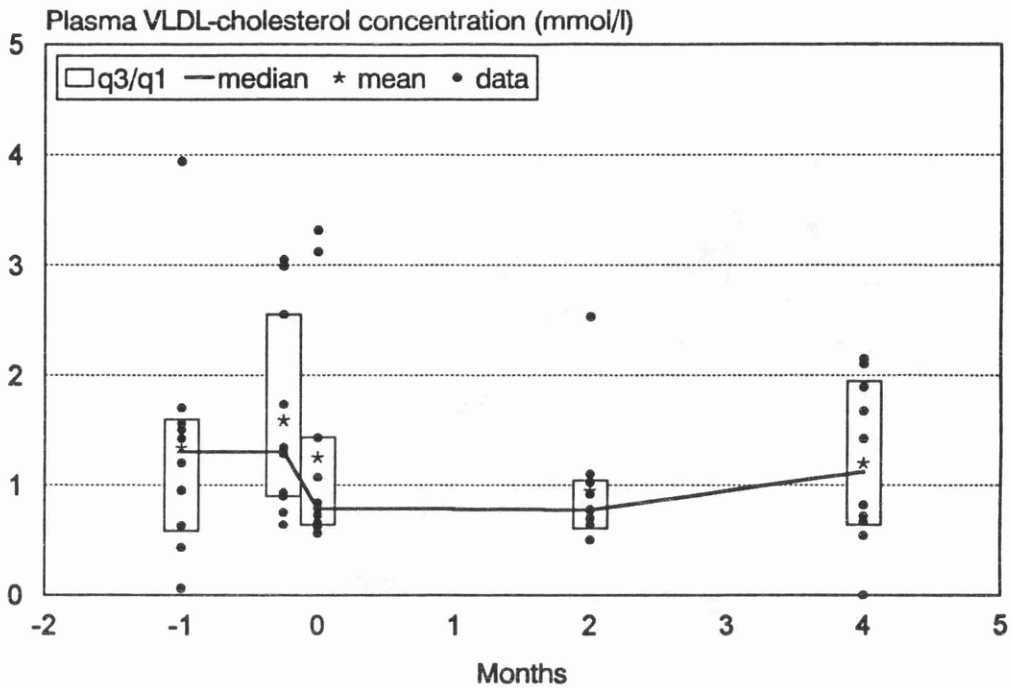

Figure 99. Absolute plasma VLDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

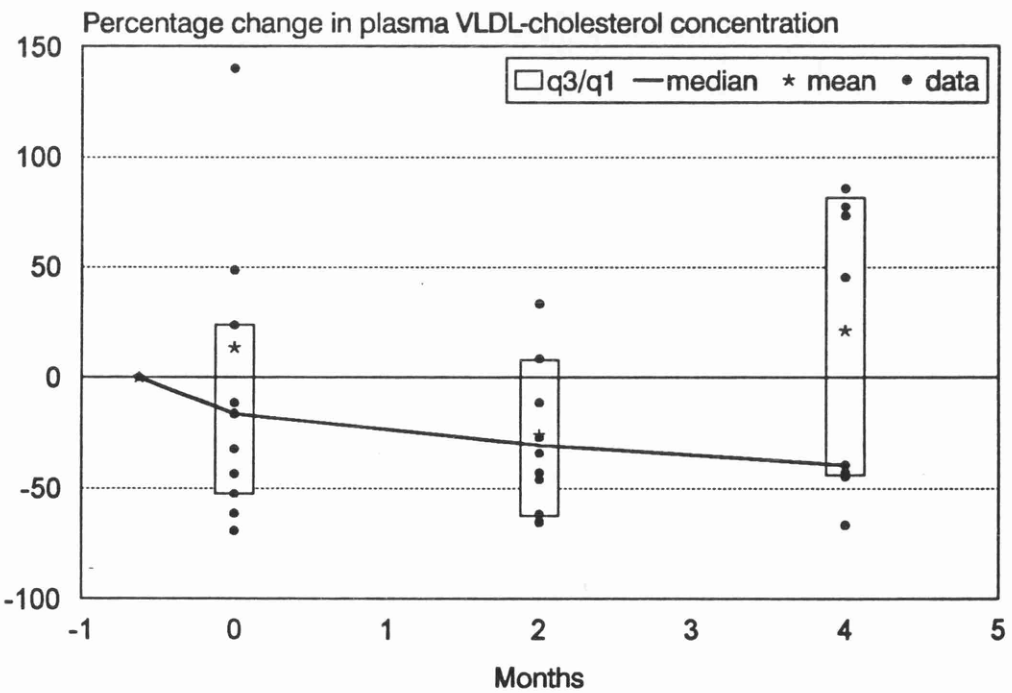

Figure 100. Percentage change in plasma VLDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

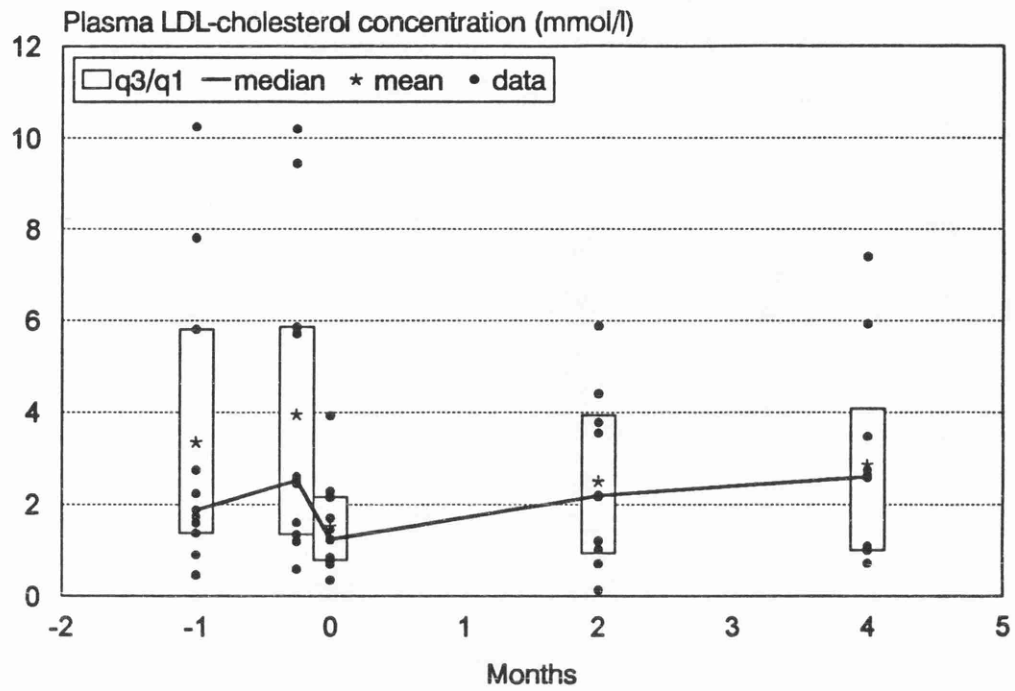

Figure 101. Absolute plasma LDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

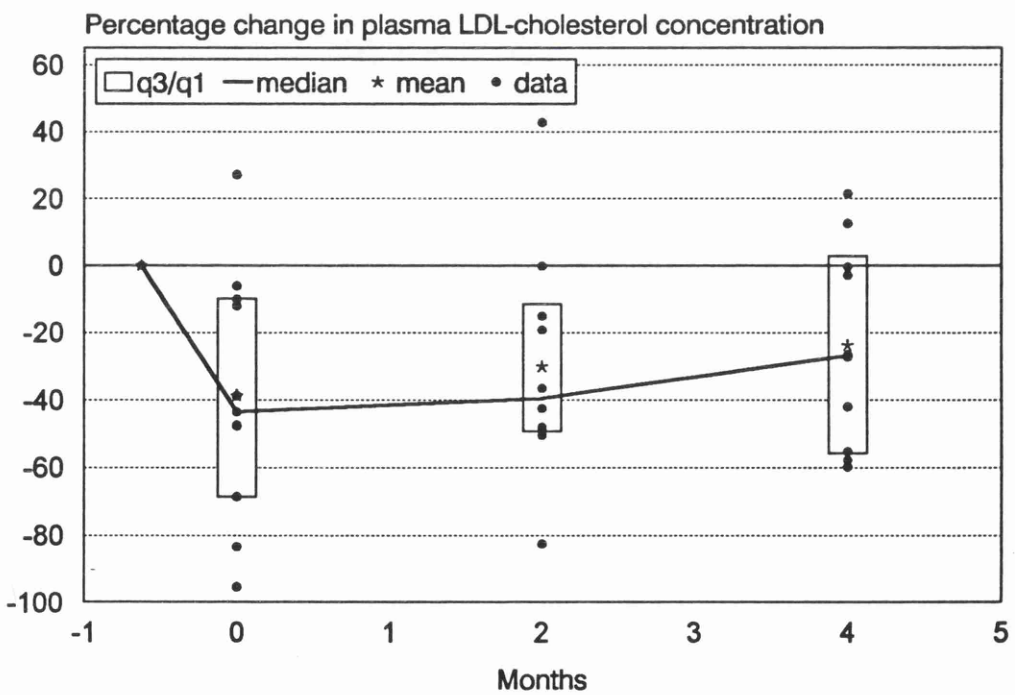

Figure 102. Percentage change in plasma LDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

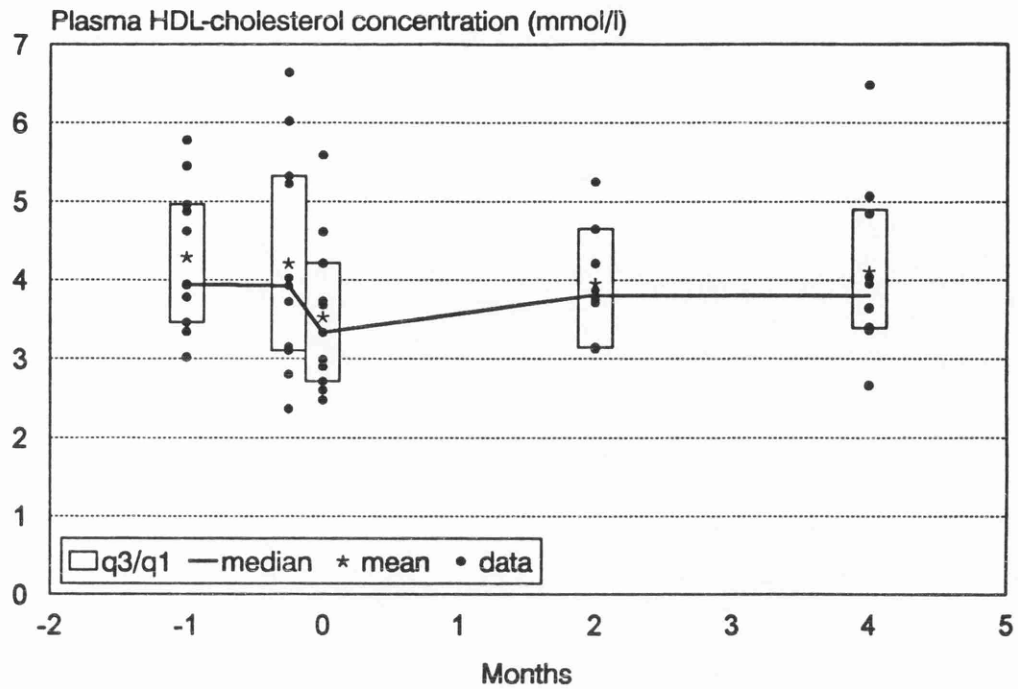

Figure 103. Absolute plasma HDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

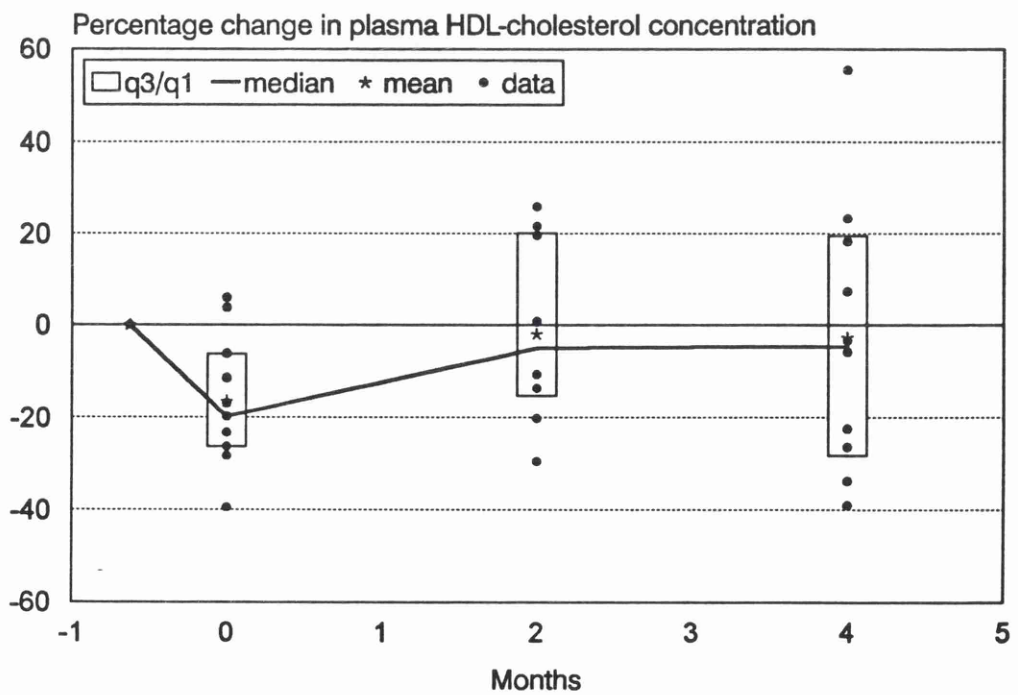

Figure 104. Percentage change in plasma HDL cholesterol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

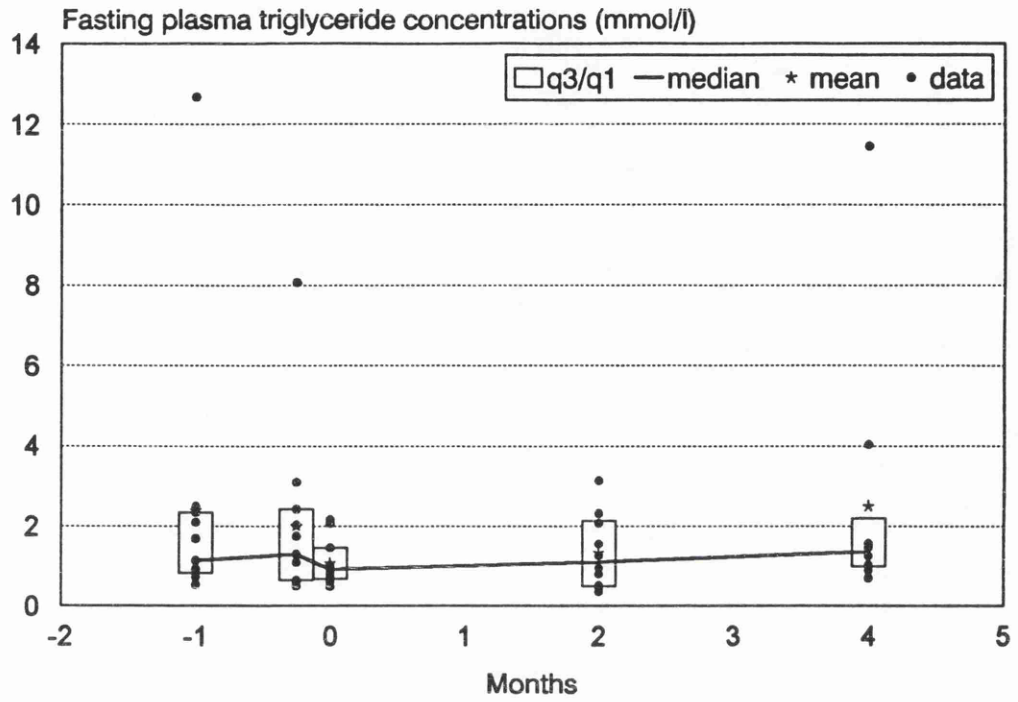

Figure 105. Absolute plasma triglyceride concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

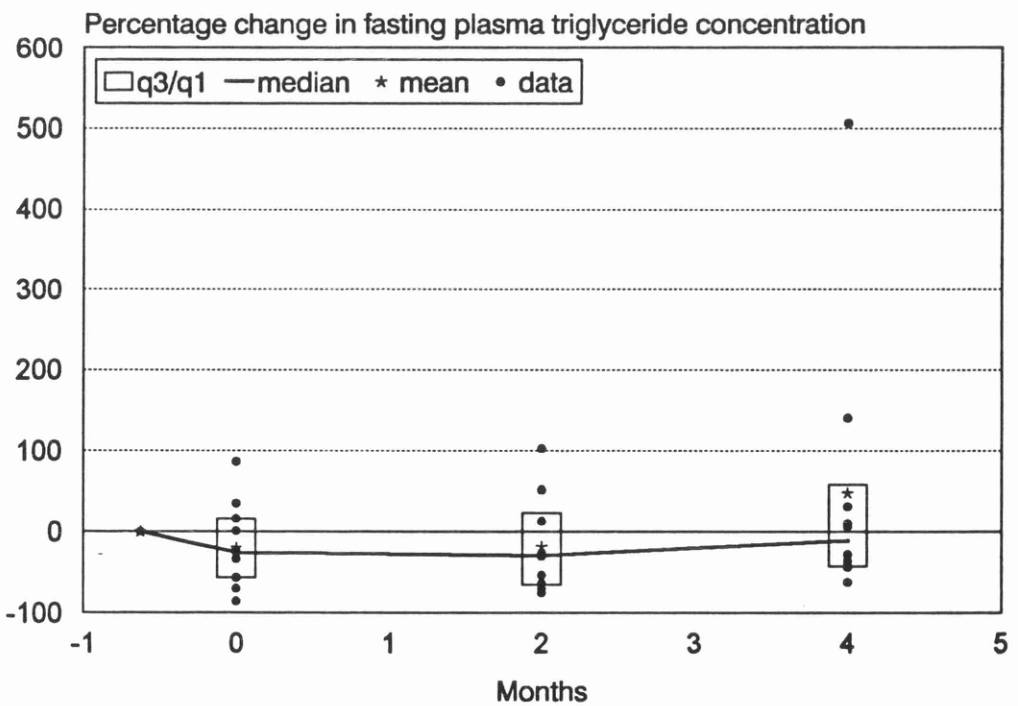

Figure 106. Percentage change in plasma triglyceride concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

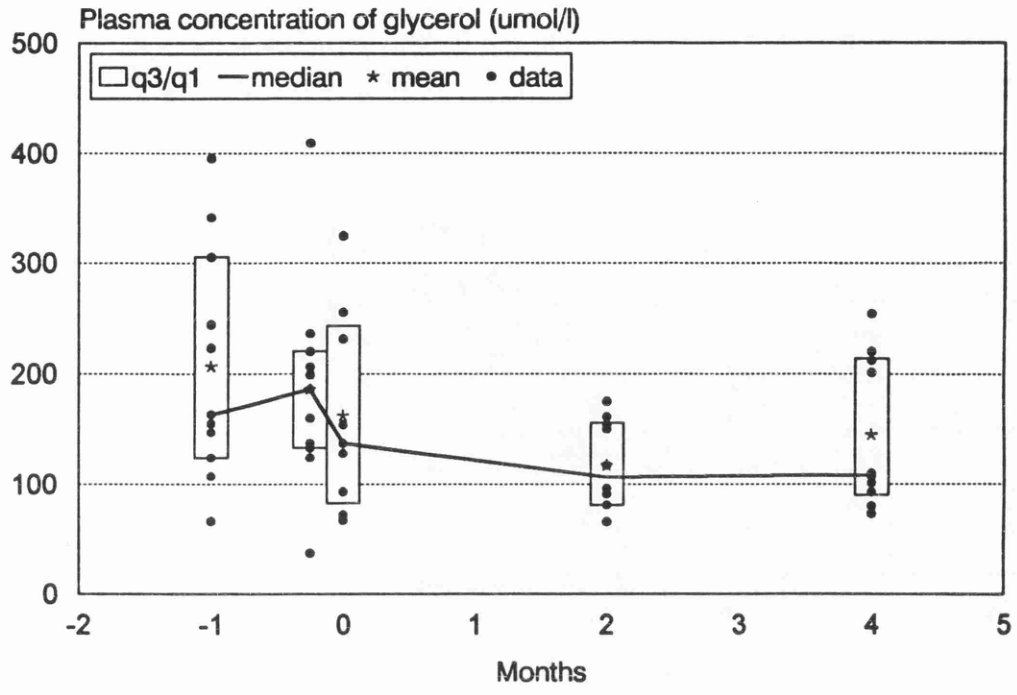

Figure 107. Absolute plasma free glycerol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

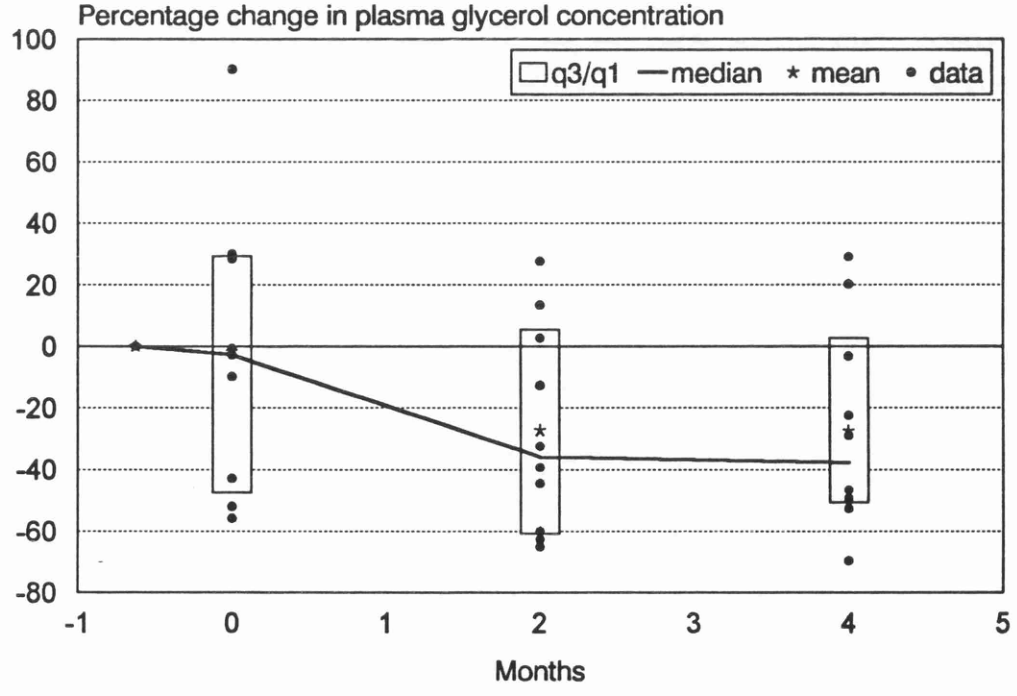

Figure 108. Percentage change in plasma free glycerol concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

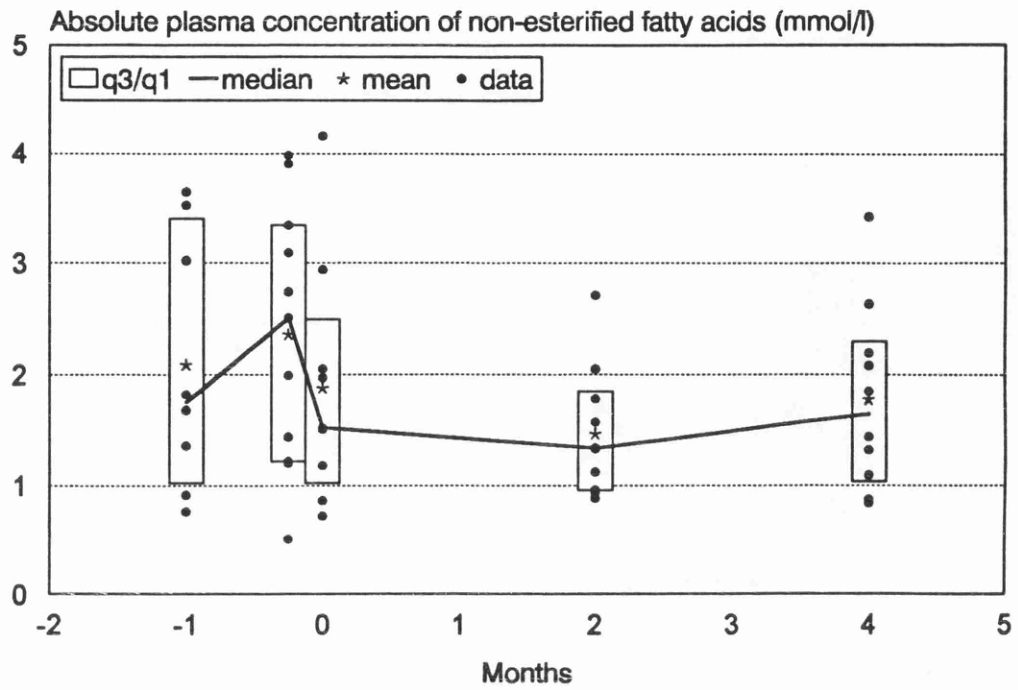

Figure 109. Absolute plasma non-esterified fatty acids concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

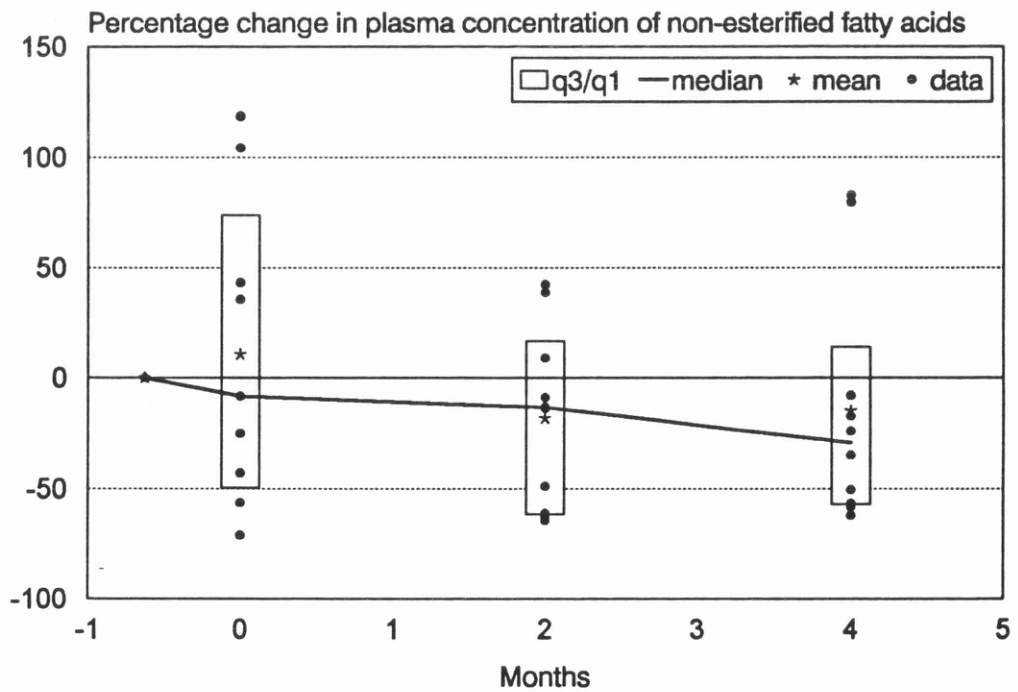

Figure 110. Percentage change in plasma non-esterified fatty acids concentrations in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

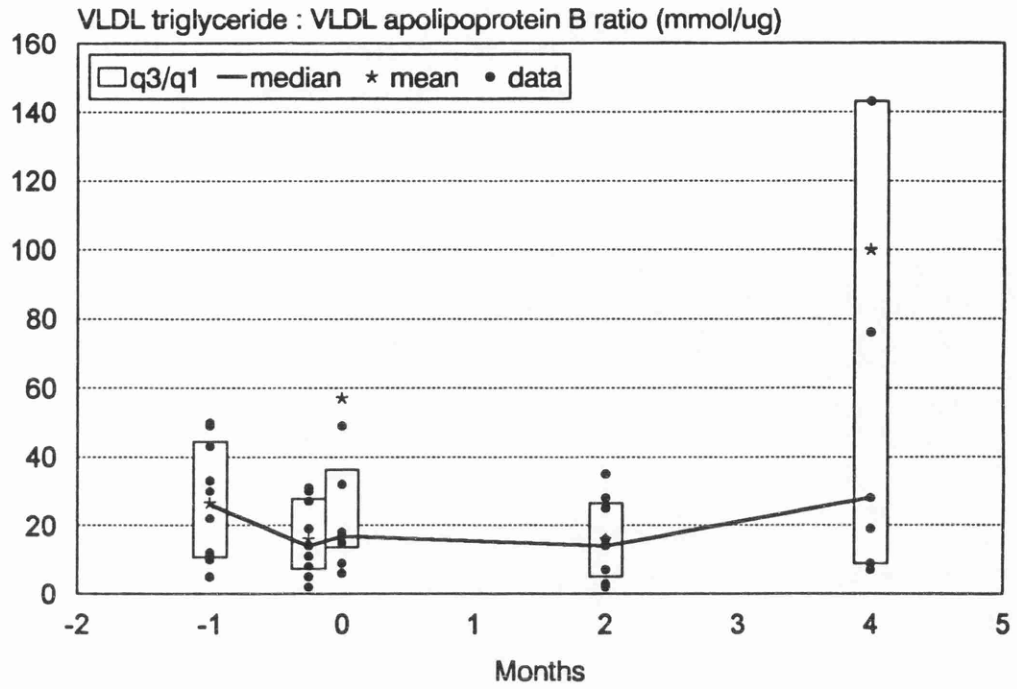

Figure 111. Absolute VLDL triglyceride/apolipoprotein B ratios in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges) - values greater than 200mmol/ug have been omitted.

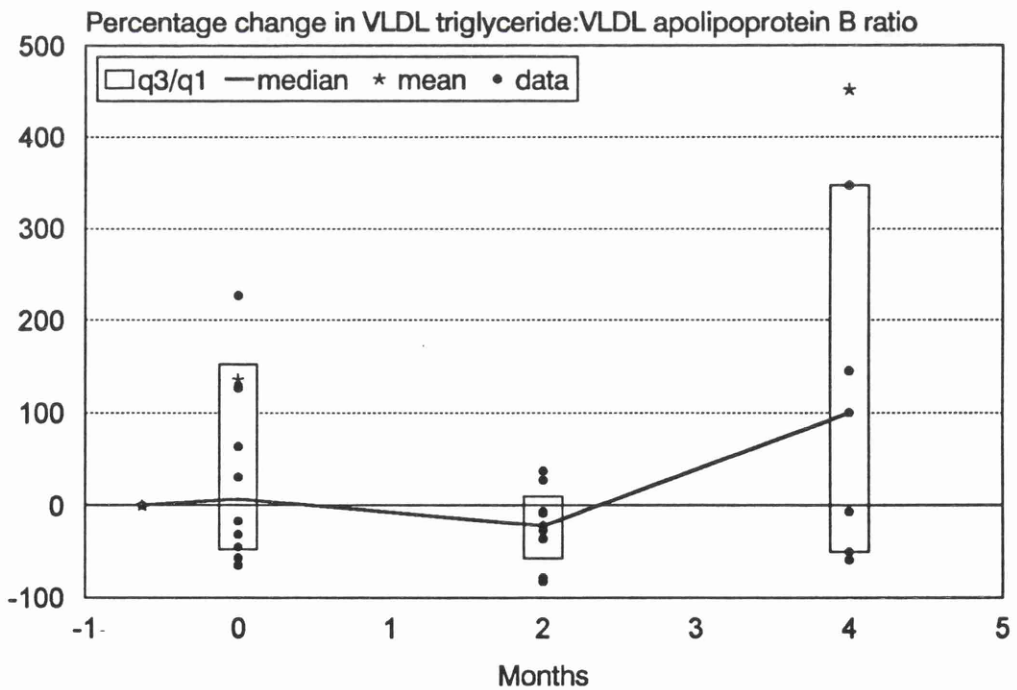

Figure 112. Percentage change in VLDL triglyceride/apolipoprotein B ratios in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges) - value greater than 500% have been omitted.

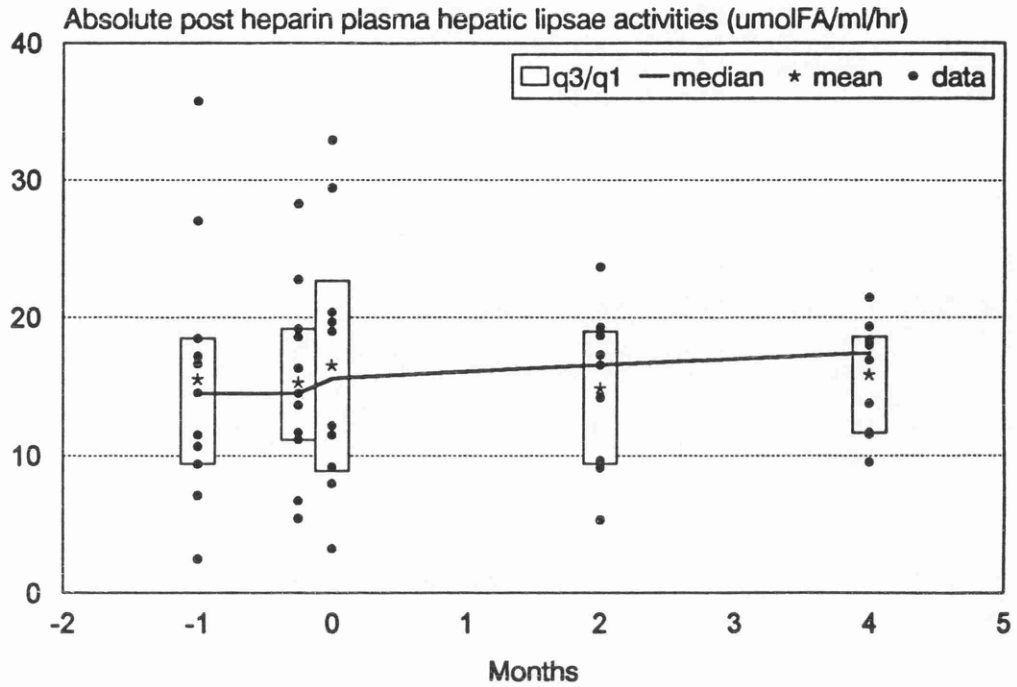

Figure 113. Absolute post heparin plasma hepatic triglyceride lipase activities (μmolFA/ml/hr) in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

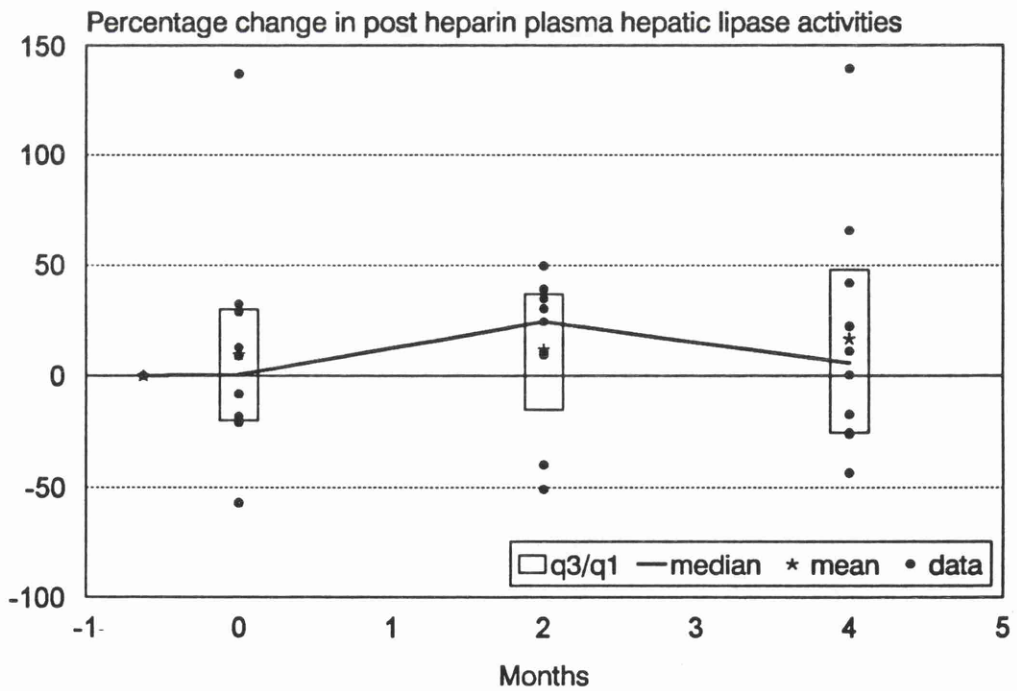

Figure 114. Percentage change in post heparin plasam hepatic triglyceride lipase activities in 11 dogs from one month before to 4 months after changing to CHF diet (median, mean and interquartile ranges).

Long-term follow-up (> 4 months)

All dogs which were still being fed CHF diet by the end of the 4 month study period continued to be fed CHF diet for variable periods of time thereafter. This section contains brief clinical descriptions of the effects of the long term (> 4 months) feeding of CHF diet to 10 dogs with naturally occurring diabetes mellitus.

Dog 1

Dog 1 required to be treated with antibiotics (metronidazole and oxytetracycline) for the last month of the 4 month study period to control small intestinal bacterial overgrowth (SIBO). Neither of these prolonged courses of antibiotic treatment nor a further one of tylosin successfully controlled the weight loss and intermittent diarrhoea associated with the SIBO and body and coat condition became very poor. By March 1993 (8 months of feeding CHF diet) the possibility of an underlying intestinal disease was considered as the cause of the refractory SIBO and small intestinal biopsies were taken at exploratory celiotomy. Histopathological examination of these biopsies yeilded a diagnosis of eosinophilic enteritis and as a result, treatment with a reducing course of corticosteroids and dietary management with Pedigree Canine Selected Protein (Pedigree Petfoods) was instituted. Following a little improvement the diet was changed to Eukanuba Lamb and Rice (Leander International) and a successful and uneventful recovery was achieved within 8 weeks. Lost weight was regained and body and coat condition became excellent.

Dog 3

Dog 3 continued to be fed CHF diet until October 1993 (15 months of CHF feeding) when body condition became unacceptably poor. At that time the dog weighed 6.86kg and had lost 12.5% of its pre-CHF bodyweight. This dog was in good bodily condition at the beginning of the study and weight loss had not been considered to be a necessary part of its management. No diarrhoea or signs of diabetic instability had been observed over the period that this dog was fed CHF diet. The dog was returned to its original diet of Chappie (Pedigree Petfoods), regained lost weight and continued to do very well.

Dog 4

Dog 4 continued uneventfully on CHF diet until April 1993 (8 months of feeding CHF diet) when it began to suffer occasional diarrhoea. By September 1993 (13 months) this had become a regular problem and was accompanied by signs of colitis. A course of sulphasalazine was instituted and B12 and folate estimations performed. A high plasma folate consistent with SIBO was discovered and treatment with metronidazole and dietary management with Prescription Diet i/d (Hills) was begun. Exploratory celiotomy was performed and intestinal biopsies obtained in an attempt to rule out underlying

intestinal disease. Histopathological examination of the biopsies revealed evidence of a mild non-specific enteropathy and this was thought to be a consequence of the SIBO rather than a contributor. Recovery was uneventful and the dog was returned to its original diet of Chappie and bread.

Dog 5

Dog 5 continued to be fed CHF diet along with small quantities of Pedigree Chum (Pedigree Petfoods) without any clinically obvious problems until the end of an agreed subsidised period of 12 months after the end of the 4 month study. Thereafter the dog was fed the original diet of Pedigree Chum (Pedigree Petfoods) and biscuit.

Dog 6

Dog 6 continued to be fed CHF diet until the end of a subsidised period of 12 months after the end of the 4 month study without any clinically obvious problems. The owners were pleased with the CHF diet and continued to obtained it from their own veterinary surgeon until changing the dog back to Chappie (Pedigree Petfoods) after 21 months of feeding CHF.

Dog 8

Dog 8 continued to be fed CHF diet without any problems until May 1993 (7 months of feeding CHF diet) when it was hospitalised for treatment of watery diarrhoea and signs of colitis. Therapy with sulphasalazine and metronidazole was begun and B12 and folate estimations revealed a low plasma B12 concentration consistent with SIBO. Weight loss and intermittent diarrhoea continued despite treatment and in August 1993 (10 months) an exploratory celiotomy was performed and intestinal biopsies obtained to investigate the refractory SIBO. After histopathological examination of the biopsies a diagnosis of immune-mediated lymphocytic/plasmacytic enteritis was made and as a result the feeding of CHF was discontinued and therapy with corticosteroids and metronidazole was begun in conjunction with dietary management with Pedigree Canine Selected Protein (Pedigree Petfoods). Within 6 weeks, recovery was complete and the dog was returned to the original diet of Chum (Pedigree Petfoods) and bread.

Dog 9

Dog 9 continued on CHF diet with only occasional bouts of sulphasalazine responsive colitis until July 1993 (9 months of feeding CHF diet) when intermittent diarrhoea became apparent. In November 1993 (15 months), body condition was unacceptably poor, with a loss of 9% of the pre-CHF bodyweight. Weight loss had not been a priority in this dog's management. The feeding of CHF diet was discontinued and the dog

returned to being fed Chappie. Weight loss continued and by January 1994 the dog had lost 12.7% of its pre-CHF weight and plasma folate concentration was low consistent with proximal small intestinal disease or dietary sensitivity. An exploratory celiotomy was performed and intestinal biopsies were obtained. Histopathological examination of the intestinal biopsies revealed evidence of an non-specific inflammatory enteropathy in which there was prominent intestinal infiltration with lymphocytes and eosinophils. Corticosteroid therapy was begun and dietary management with Pedigree Canine Selected Protein (Pedigree Petfoods) instituted. The dog made an impressive recovery and within 2 months had regained lost weight (to 98% of pre-CHF weight) and the diet was changed back to Chappie (Pedigree Petfoods).

Dog 10

Dog 10 was fed CHF diet until October 1994 (19 months) when his original diet was reinstituted without any significant problems. There has been a gradual loss of weight over this period but with acceptable body condition throughout. There was one brief bout of diarrhoea in September 1993 (6 months) which required no treatment in addition to the clavulanate potentiated amoxycillin which was being administered to control a bout of bacterial cystitis. Estimation of plasma B12 and folate concentration was performed at that time and found to be consistent with bacterial overgrowth (high folate). No additional treatment was administered and there was no further recurrence of diarrhoea.

Dog 11

Within a week of finishing the 4 month study period, dog 11 experienced a bout of vomiting and diarrhoea which was treated non-specifically. In September 1993 (6 months of feeding CHF diet), diarrhoea again became a problem. Plasma B12 was low, consistent with SIBO, and a 3 week course of metronidazole was begun. The diarrhoea was controlled by this treatment and this dog continued to be fed CHF until the end of a 12 months subsidised period. The dog remained in good body condition.

Dog 13

Dog 13 encountered no more problems after suffering diarrhoea during the 4 month study. However, by December 1993 (7 months of feeding CHF diet), this dog's body condition was unacceptably poor and 21% of its pre-CHF weight had been lost. The feeding of CHF diet was discontinued and the dog returned to its original diet. Over the months preceding the discontinuation of CHF feeding, polydipsia had been observed by the owners and so it is likely that some degree of diabetic instability contributed to this dog's dramatic weight loss.

In summary, 10 dogs continued to be fed CHF diet after the end of a 4 month study period. Of these, 4 remained on the diet until the end of a 12 month subsidised period and of these two continued on the diet thereafter. In six dogs, the feeding of CHF diet was discontinued after between 7 and 15 months. The reasons for discontinuation of CHF were unacceptable weight loss (three dogs) or recurrent or persistent diarrhoea (4 dogs). There was plasma biochemical evidence of SIBO in 5 dogs (one of which was unassociated with diarrhoea) and treatment with antibiotics was required in 4 of these dogs. Four dogs underwent exploratory celiotomy as part of the investigation of their weight loss or diarrhoea and in all of these dogs there was histopathological evidence of an enteropathy and in three dogs, this included the presence of an inflammatory cellular infiltrate. These three dogs required further treatment with corticosteroids and a change to an 'hypoallergenic diet' and made good recoveries. Table 57 summarises the duration of feeding CHF diet, the reasons for its discontinuation and any requirements for treatment in 10 dogs fed CHF diet for longer than 4 months.

Dog No.	Duration of feeding CHF (months)	Poor body condition	Diarrhoea	SIBO	Treatment for SIBO	Intestinal biopsy	Histopath. evidence of enteropathy	Further specific treatment
1	8	+	+	+	+	+	+	+
3	15	+						
4	13		+	+	+	+	+	
5	16							
6	21							
8	10		+	+	+	+	+	+
9	15	+				+	+	+
10	19			+				
11	16		+	+	+			
13	7	+						

Table 44. Further Observations: effects of very long term feeding (> 4 months) of CHF diet.

Discussion

Post-prandial glycaemia

The parameters ABSMEAN, ABSSD, INCMEAN, and INCAUC were measured in an attempt to describe the 24 hour plasma glucose curves as fully as possible. Standard deviations (ABSSD) were calculated for the plasma glucose concentrations as indicators of the degree of fluctuation in glucose concentrations over the sample period and areas under the glucose versus time curve (INCAUC) are indicators of the mean deviation from baseline adjusted for uneven sampling frequency. The incremental values were generated to try to compensate for any variation in the quality of glycaemic control among the dogs.

The reduction in absolute mean plasma glucose concentration (ABSMEAN) may not have been the result of the change of diet alone. It has to be remembered that these dogs were hospitalised between the generation of the two plasma glucose curves and their insulin doses were being adjusted. If the nadir plasma glucose concentrations for all the dogs on both original and CHF diets had been in the range 3.5-7.5mmol/l then the effects of hospitalisation and adjusted insulin dose could have been ruled out completely. However, nadir plasma glucose concentration for dogs nos. 2, 4, 6, and 8 were above this range on the original diet and well below this range in dogs nos. 4, 5, 8 and 9 on CHF diet. This complicates the interpretation of both the 24 hour and afternoon post-prandial glucose concentrations. However, of these 6 dogs, two (dog nos. 5 and 8) had had their insulin doses decreased between the original and CHF diet days; one dog (dog no. 4) had its insulin dose increased only very slightly and one dog (dog no. 9) whose insulin dose had been increased between diets was only below the 3.5-7.5mmol/l range for one sample point on the CHF diet day, leaving only two dogs (dog nos. 2 and 6) which had had an appreciable increase in insulin dose between the two diets. Taking these considerations into account along with the fact that the median nadir plasma glucose concentrations for the original and CHF diets fell into the acceptable range 3.5-7.5 mmol/l should have meant that valid conclusions could be drawn from the post-prandial glycaemia data.

There was no apparent or statistically significant absolute increase in insulin dose (IU/kg) over the hospitalisation period (Figures 115 and 116) and these dogs were clinically stable before hospitalisation. There may have been an effective increase in insulin dose if the CHF diet reduced insulin requirement and insulin doses were not decreased sufficiently to match this decreased requirement.

Despite the uncertainty over the main source of the reduced ABSMEAN, be it diet alone or absolute or effective insulin dose increase, there was a reduction in INCMEAN and apparent (but not statistically significant) decreases in ABSSD and INCAUC and Figures 51 and 52 highlight these reductions. The CHF plasma glucose curve is smoother and less erratic than the original diet curve and there is a striking reduction in afternoon post-prandial hyperglycaemia in the CHF curve.

The afternoon post-prandial period was chosen for further study because the potentially dramatic effects of exogenous insulin on plasma glucose concentrations were likely to be more stable at this time than after the morning meal. Plasma glucose concentrations were standardised by subtracting the 6 hour plasma glucose concentration in an attempt to compensate for variations in the degree of glycaemic control among the dogs. This should mean that unless dogs were hopelessly underdosed with insulin when on the original diet, or significantly overdosed when on the CHF diet, any changes due to hospitalisation or altered insulin dose were largely corrected for so that changes due to diet could be more readily identified.

Figures 53 and 54 show that in the immediate post-prandial period there is a greater decrease in plasma glucose concentration when on original diet than when on CHF. This was probably because it was the second meal in the day which was being studied. On the CHF diet, it is possible that substrate from the morning meal (6 hours earlier) was maintaining plasma glucose concentrations. Whereas, on the original diet, all the substrate from the morning meal may have already been absorbed by 6 hours allowing plasma glucose to fall. It was to reduce the bias created by the smaller sampling interval in this immediate post-prandial period that the adjusted values ADJMEAN, ADJAUC and ADJSD were created.

The standard deviations of the afternoon plasma glucose concentrations (APPGSD and ADJSD) were lower on the CHF diet than on the original diet, although this difference could not be tested by statistical analyses. This can be interpreted as a smoother, less erratic post-prandial plasma glucose curve. The mean concentrations of plasma glucose (APPGMEAN and ADJMEAN) and areas under the plasma glucose versus time curve (APPGAUC and ADJAUC) were reduced but not statistically different between the diets (Table 42).

In the longer term study of 5 dogs, there were no statistically significant differences between original diet, one week on CHF and 4 months on CHF for any of the derived values. Examination of the results Table 43 and the Figures 55 to 60 reveals striking

trends suggesting that the lack of statistical significance may have been a function of the small sample size.

Nelson *et al* (1991) successfully reduced mean 24 hour plasma glucose concentration and 24 hour plasma glucose fluctuation (SD) in 6 experimentally induced diabetic dogs using canned diets[1] which were high in soluble and insoluble fibre when compared to a low fibre canned diet. Similarly, Blaxter *et al* (1990) demonstrated a reduction in area under the plasma glucose versus time curve in normal dogs over a six hour morning post-prandial period following acute changes to home-made diets containing 20g of wheat bran and 20g of guar. In 4 dogs with naturally occurring diabetes mellitus there were similar trends but no statistically significant differences. Simpson *et al* (1981) studied the effects on 24 hour plasma glucose profiles of a high carbohydrate diet high in leguminous fibre versus a low carbohydrate diet in 9 insulin dependent diabetic humans. This high carbohydrate diet lowered and smoothed the mean plasma glucose curve and allowed the insulin dose to be reduced in 4 of the patients. Insulin dose was unchanged in the remaining 5 patients.

The present study is important and unique because there are no previous reports of the effects of canned high fibre diets on post-prandial glycaemia in dogs with naturally-occurring diabetes mellitus, none in which such a large group of dogs with naturally-occurring diabetes mellitus has been studied nor any in which a period of stable insulin dynamics has been chosen for further study.

The results of this study concur with previous findings of the effects of high-fibre diets on post-prandial glycaemia (Blaxter *et al*, 1990; Nelson *et al*, 1991) and confirm that a canned high fibre diet can reduce mean 24 hour glucose concentrations and may reduce post-prandial fluctuations in plasma glucose concentrations in dogs with naturally-occurring diabetes mellitus and that these effects may remain for at least 4 months.

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[1] Diets used by Nelson. *et al* (1991)

Fibre content (g/1000kcal ME)	High insoluble fibre	High soluble fibre	Low fibre
Total fibre	70	55	24
Insoluble fibre	60	21	17
Soluble fibre	10	34	7

Furda enzymatic digestion

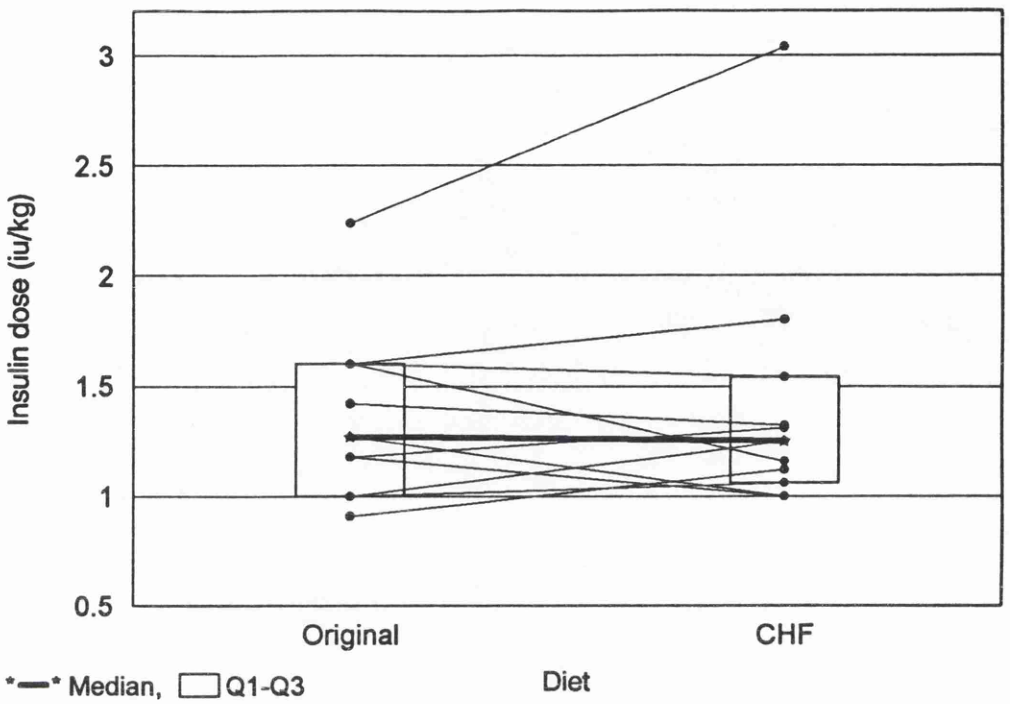

Figure 115. Insulin doses (IU/kg) in 11 dogs before and after one week on CHF diet.

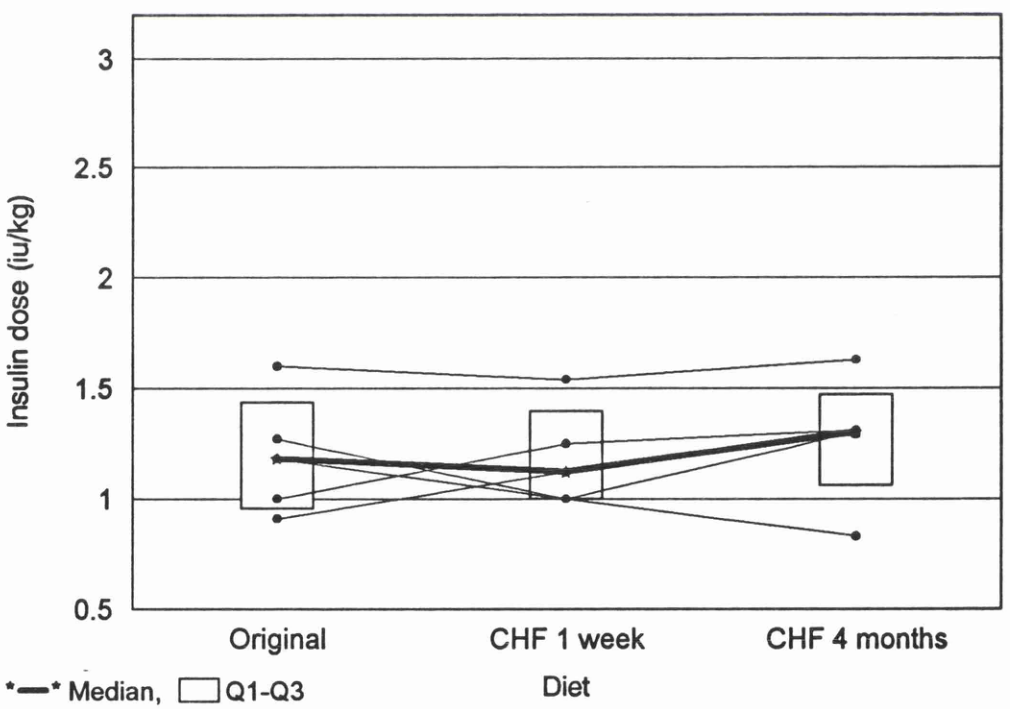

Figure 116. Insulin doses (IU/kg) in 5 dogs before, one week after and 4 months after changing to CHF diet.

Insulin dose and general health

Changing the diet of 11 dogs with naturally occurring diabetes mellitus to CHF diet resulted in statistically significant reductions in bodyweight and body condition score and increases in activity, demeanour and faecal volume scores. There were no statistically significant changes in insulin dose, coat condition score, vision score, faecal consistency score nor any apparent trends in routine haematological and biochemical parameters. There were no adverse changes in routine haematological or plasma biochemical parameters.

The change in bodyweight seen over the 4 month study period was generally a decrease with the exception of the absolute bodyweight for +2 months, the mean of which was higher than any of the other sample days. The reason for the weight loss is not instantly apparent since the quantity of CHF fed was isocaloric with each dog's previous weight maintaining diet. This may suggest that incorrect assumptions were made about the caloric density of the CHF diet or of the original diets. However, the volumes of food fed seemed, subjectively, to be about correct. Another possibility is that nutrient availability from the CHF diet was poorer than from the original diets and this may be reflected in the diarrhoea experienced by a number of the dogs (Appendix 15). In some of the dogs, a loss of bodyweight would have been beneficial because a number were overweight at the beginning of the study. This can be seen in the absolute body condition scores in which the majority of dogs have scores greater than 50 near the beginning of the study (Appendix 17). A score of around 50 was likely to be allocated to dogs in 'ideal' condition. If it were only the overweight dogs which had lost weight then a strong negative correlation between the mean body condition scores for the original diet days and the percentage of bodyweight lost at each of the CHF diet days would be expected. This was not the case. However, there was a statistically significant correlation between the percentage weight lost at 0 days and at both +3 months and +4 months (Pearson's correlation; $r > 0.60$, $p<0.05$) suggesting that, the dogs which started to lose weight within the first week of CHF were the ones which lost the most weight in the longer term.

Examination of the absolute body condition score results (Figure 65) revealed a trend towards the 'ideal' score of 50 but by the last month of the follow-up period it was apparent that a number of dogs had scores which suggested that body condition had in fact become poor. The reasons underlying this trend are, like those for the changes in bodyweight, unclear.

The increases in activity and veterinary observed demeanour scores most likely represent an improvement in glycaemic control and to some extent, in the late stages of the study, reduced bodyweight. The most likely explanation for the lack of statistically significant improvement in the owner observed demeanour scores in comparison with that in the vet observed scores is the owners' adaptation to any changes in demeanour because of continual exposure to their pets.

Increased faecal volume may be expected because of the inclusion of indigestible components in the CHF diet but also because of a contribution from the growth of colonic microflora (Cummings, 1983). Although there was no statistically significant trend in faecal consistency, clinically significant episodes of both constipation and diarrhoea were recorded in some dogs (Appendix 15).

There was no statistically significant change in insulin dose (IU/kg) over the period of the study, however, there was a trend towards a slight increase at +4 months. This is probably a reflection of the decrease in bodyweight seen especially at the end of the study rather than an active increase in insulin dose (IU) administered.

One previously published study of high fibre diets in experimentally induced diabetic dogs reported loose faeces and poor coat condition after feeding a high soluble fibre diet. In that study insulin dose was unchanged, as in the present study, and bodyweight was maintained by adjusting the volume of diet fed (the volume of high insoluble fibre diet fed was reduced compared to high soluble fibre and low fibre diets) (Nelson *et al* 1991). One other previously published study of home made high fibre diets in dogs with naturally occurring diabetes mellitus (Blaxter *et al* 1990) reported only the biochemical effects of an acute change in diet.

In human medicine, insulin doses have sometimes been decreased following a change to a high fibre diet (Simpson *et al*, 1981). Weight loss normally only occurs in human diabetics fed high fibre diets which are calorie restricted, however, concerns over the possibility of micro and macronutrient malabsorption associated with chronic feeding of high fibre diets have been voiced (Vinik and Jenkins, 1988). High levels of soluble (guar) and insoluble dietary fibre have been associated with gastrointestinal upset including increased faecal bulk and decreased faecal consistency (Fuessl *et al*, 1987; Vinik and Jenkins, 1988).

When fed to dogs with naturally occurring diabetes mellitus, CHF diet appeared to be safe and was capable of improving demeanour and activity without a change in insulin

dose over a 4 month study period but was associated with weight loss and increased faecal volume. It has no adverse effects on routine haematological or plasma biochemical parameters.

Indicators of glycaemic control

Changing the diets of 11 dogs to CHF diet during a period of hospitalisation resulted in statistically significant improvements in the concentrations of the indicators of glycaemic control: plasma fructosamine, glycated haemoglobin, plasma alkaline phosphatase and afternoon plasma glucose. There were trends towards improvement, which were not statistically significant, in the plasma concentrations of plasma alanine aminotransferase, aspartate aminotransferase and fasting glucose.

The reductions in fructosamine, glycated haemoglobin, alkaline phosphatase and afternoon glucose indicate that an improvement in glycaemic control was achieved. This improvement appeared to wane towards the end of the study in the cases of fructosamine and afternoon glucose but was consistently present in the case of glycated haemoglobin. This discrepancy can be explained by a lag-phase in the reflection of changes in glycaemic control due to the long half-life of haemoglobin compared to that of the plasma proteins.

The lack of statistically significant improvements in the concentrations of plasma alanine aminotransferase and plasma aspartate aminotransferase concurs with the finding in Chapter 5 that these are not sensitive tests for glycaemic control or may suggest that there was little room for improvements in these parameters, which is wholly feasible since these dogs were 'stable' diabetics at the beginning of the study.

Fasting plasma glucose concentrations are mostly dependent on the duration of the exogenous insulin preparation administered, so no particular improvement in this parameter was expected.

The improvements seen in glycaemic control (the reductions in fructosamine, glycated haemoglobin, alkaline phosphatase and afternoon glucose) may not have been the result of the CHF diet alone. The effects of the hospitalisation period and the changes in insulin dose have to be considered. As discussed under 'Insulin Dose and General Health', the insulin doses of this group did not change significantly over the period of the study and in some cases insulin dose decreased by 20 to 40%. There may have been an effective increase in insulin dose if the CHF diet reduced insulin requirement and this was not

followed by a decreased insulin dose. If this was the case, however, it could still only be described as a benefit of the diet.

The inclusion of previously stable diabetic dogs and the lack of an upward trend in insulin dose meant that any effect seen in the 4 month follow-up period was more likely to result from the CHF diet than the hospitalisation period.

With the exception of glycated haemoglobin, improvements in glycaemic control seemed to be more apparent in the first 2 months of the follow-up period and seem to wane towards the end of the study period. This may mean that the duration of the effects of the CHF diet are limited and that somehow there is gastrointestinal or metabolic adaptation to the diet. On the other hand, if any improvements in glycaemic control were the result of closer supervision in the hospitalisation period then these would be expected to diminish with time. One explanation for waning glycaemic control which could fit both a continuous effect of diet (unlimited duration) and the reduced level of supervision, is that, for endogenous reasons, these dogs may have had a steadily increasing requirement for insulin over the period of the study and that this increased requirement was not being followed. Yki-Jarvinen and Koivisto (1986) recorded a phenomenon of changing insulin requirement in treated Type I diabetic humans but whether this occurs in diabetic dogs is not yet known. Also, 7 of the dogs were receiving insulin of bovine origin. This is known to be antigenic in dogs and it is not inconceivable that its prolonged use leads to decreased potency and consequent increased requirement. Again, this has not yet been documented in dogs.

Previously published studies of the effects of dietary fibre in diabetic dogs have been limited to Blaxter *et al* (1990) and Nelson *et al* (1991). Blaxter *et al* (1990) studied the acute effects of two high fibre diets but did not monitor glycaemic control in a long term study. Nelson *et al* (1991) documented reduced glycated haemoglobin in a group of alloxan-induced diabetic dogs using a diet high in insoluble fibre over a 2 month study period. They, in concordance with the present CHF diet study, did not observe any statistical difference in fasting plasma glucose between low fibre and high soluble and insoluble fibre diets. They did not report the results of alkaline phosphatase, alanine aminotransferase or aspartate aminotransferase analyses and did not perform fructosamine measurements and so no direct comparison with their study can be made for these parameters.

In human diabetic medicine, Fuessl *et al* (1987) reduced fasting plasma glucose and glycated haemoglobin concentrations in 18 non-insulin dependent diabetic patients over a 4 week period using guar granules sprinkled onto food.

The protocol followed in this study improved glycaemic control during a 4 month follow-up period and, although the effects of hospitalisation on glycaemic control cannot be ruled out completely, this improvement was most likely to be the result of feeding CHF diet. These findings concur with previously published studies on 'high-fibre' diets in experimental diabetic dogs and non-insulin dependent diabetic humans, provide additional information by measurement of fructosamine and confirm that feeding a canned 'high-fibre' diet can improve long term glycaemic control in dogs with naturally-occurring diabetes mellitus.

Lipid metabolism

Feeding a high fibre diet to 11 dogs with naturally occurring diabetes mellitus over a period of 4 months following 10 days hospitalisation resulted in statistically significant reductions in the plasma concentrations of total cholesterol, low density lipoprotein associated cholesterol, free glycerol and non-esterified fatty acids. There were no statistically significant changes in plasma concentrations of triglyceride, VLDL cholesterol, HDL cholesterol or post-heparin hepatic lipase activity, although, there were trends towards reductions in VLDL and HDL cholesterol concentrations.

With the exception of percentage change in LDL cholesterol concentration from basal the effects of any change seemed to wane by the end of the study (+4 months). This may be a reflection of the importance of the effects of the hospitalisation period on lipid metabolism and suggests that the changes seen are not the result of the change of diet alone. However, insulin dose over the period of the study did not change (Insulin Dose and General Health; Indicators of Glycaemic Control) which suggests that any improvements in glycaemic control (which will contribute to any changes in lipid profiles) did not result from increased insulin dose. The CHF diet could also have directly affected lipid profiles without affecting glycaemic control.

Blaxter *et al* (1990) studied the acute effects of home-made high fibre diets on post prandial serum concentrations of total cholesterol and triglycerides in naturally occurring diabetic dogs and found none and Nelson *et al* (1991) found no effects on plasma total cholesterol or triglyceride after feeding diets high in soluble and insoluble fibre to dogs with experimentally induced diabetes mellitus for periods of two months. There are no previous reports of the effects of high fibre diets on the cholesterol content of the

lipoprotein classes in dogs with naturally occurring diabetes mellitus. High fibre diets have been shown to have effects on plasma concentrations of total (Vinik and Jenkins, 1988; Simpson *et al*, 1981; Riccardi *et al*, 1984), LDL (Riccardi *et al*, 1984; Vinik and Jenkins, 1988) and HDL cholesterol (Simpson *et al*, 1981; Riccardi *et al*, 1984) when fed to insulin dependent diabetic humans. VLDL cholesterol concentrations are seldom changed by high fibre diets (Miettinen, 1987; Simpson *et al*, 1981). A ratio calculated in human medicine to assess the atherogenic potential of a lipoprotein profile (VLDL cholesterol + LDL cholesterol/HDL cholesterol) has been reduced by high fibre diets in insulin dependent diabetic humans (Riccardi *et al*, 1984). This ratio was also reduced in the current study ($p=0.0426$) but the relevance of this to the health of diabetic dogs is unclear since it is known that the atherogenic lipid profile in dogs is different from that in humans (the most likely major canine atherogenic lipoprotein classes are an HDL and β-VLDL).

There was no statistically significant change in the plasma concentration of triglycerides in the present study and this is in agreement with the findings of previous reports studying the effects of high fibre diets in diabetic dogs (Nelson *et al*, 1991) and humans (Miettinen, 1987; Vinik and Jenkins, 1988; Riccardi, 1984; Simpson *et al*, 1981). High fibre diets have been associated with reduced total plasma triglyceride by others (Vinik and Jenkins, 1988) and with reduced VLDL triglyceride (Riccardi *et al*, 1984).

Plasma concentration of free glycerol and non-esterified fatty acids (NEFA) are qualitative markers of adipose tissue lipolysis but are generally not considered quantitative because the kinetics of adipose lipolysis and NEFA and glycerol re-esterification are complex and best studied by tracer isotope technology. Adipose tissue lipolysis is very sensitive to inhibition by insulin (via LPL and hormone sensitive lipase) and improved diabetic control has been associated with decreased lipolysis and plasma concentrations of NEFA and glycerol in diabetic humans (Coppack *et al*, 1994). A decrease in plasma concentrations of NEFA and glycerol in the present study is difficult to explain. The possibility that weight loss and reduced adipose depot has a role cannot be discounted but statistical analysis by multiple range testing did not identify differences in NEFA or glycerol concentrations at the times when weight loss was greatest. Since there was no increase in insulin dose this may mean that there is a mechanism by which a high fibre diet could contribute to the inhibition of lipolysis. Perhaps absorbed fermentation products can modulate insulin's inhibitory effect on lipolysis. Hjollund *et al* (1983) have demonstrated improved insulin sensitvity in non-insulin dependent diabetic humans fed diets high in complex carbohydrates including fibre.

Post heparin hepatic triglyceride lipase (H-TGL) activities in this study were generally much higher than the reference range and this is in agreement with Muller *et al* (1985) who found elevated activities of H-TGL in pancreatectomised diabetic dogs. In that study reductions in H-TGL activity towards normal were seen following improved glycaemic control by increased insulin dosage. Although feeding CHF diet to diabetic dogs in the present study does improve glycaemic control (see under Indicators of Glycaemic Control), this did not result from an increased insulin dose which probably explains why there was no change seen in H-TGL.

Feeding Pedigree Canine High Fibre Diet after a period of hospitalisation did appear to have beneficial effects on lipid metabolism in dogs with naturally occurring diabetes mellitus and these effects are similar to those produced by high fibre diets in diabetic humans.

Long-term follow-up (> 4 months)

The significance of these findings is difficult to ascertain. There was no comparative data available on the occurrence of diarrhoea or chronic weight loss when these dogs were fed their original diets because a double crossover study was not performed. Subjectively, the prevalence of weight loss, recurrent diarrhoea and small intestinal bacterial overgrowth (70% in total) in this group of dogs seems high even when considering that they are all diabetic. The incidence or prevalence of SIBO in dogs with naturally occurring diabetes mellitus has not been reported and is not currently known.

Those dogs in which the feeding of CHF diet was discontinued because of very poor body condition, had not been expected to lose weight because an amount of CHF was fed which was isocaloric with their previous weight maintaining diets (if the assumed caloric density of CHF was correct - see under Materials and Methods). The occurrence of unexpected weight loss, recurrent diarrhoea and SIBO in this group of dogs is difficult to explain but the possibility that the CHF diet could be a direct contributor to their occurrence has to be considered. It is likely that the induction of intestinal malabsorption is an underlying phenomenon common to unexpected weight loss, recurrent diarrhoea and SIBO. Mechanisms by which a high fibre diet could induce malabsorption, may include alteration of intestinal morphology (Wahlqvist, 1987), or the promotion of SIBO. Pre-existent SIBO might be exacerbated by a high fibre diet because of retention of nutrients within the intestinal lumen and promotion of bacterial growth (Cummings, 1983) but high fibre diets are unlikely to be able to initiate SIBO other than indirectly by inducing an intestinal malabsorption.

Very long term (>4 months) feeding of Pedigree Canine High Fibre diet to dogs with naturally occurring diabetes mellitus may be associated with unexpected weight loss, recurrent diarrhoea or SIBO. It may be prudent to recommend that CHF diet might be unsuitable for feeding to diabetic dogs which have a history of intestinal malabsorption or small intestinal bacterial overgrowth.

Conclusions

Pedigree Canine High Fibre Diet (CHF) was fed to 11 dogs with naturally occurring diabetes mellitus and its effects on post-prandial glycaemia, general health, biochemical indicators of glycaemic control and lipid metabolism were studied both in the short term (7 days) and in the longer term (4 months). In the short term, feeding CHF diet led to a statistically significant reduction in mean 24 hour plasma glucose concentration, and an apparent reduction in the fluctuation in afternoon post-prandial glycaemia. In the longer term, feeding CHF diet improved demeanour scores, activity scores, plasma concentrations of fructosamine, concentrations of glycated haemoglobin, plasma concentrations of alkaline phosphatase and afternoon plasma glucose concentrations. Reductions were also observed in the plasma concentrations of total cholesterol, low density lipoprotein cholesterol, free glycerol and non-esterified fatty acids. Thus, the effects of feeding CHF improved many aspects of diabetic control and this improvement was achieved without any statistically significant increase in insulin dose over the 4 month study period. There was no haematological or plasma biochemical evidence of any adverse effects of feeding CHF. However, there was an association between feeding CHF and weight loss, reduced body condition score and increased faecal volume score.

The change from each dog's original diet to CHF diet was made during a 7-10 day period of hospitalisation. The contribution of hospitalisation to the improvements in diabetic control seen is difficult to judge. Certainly, dogs were clinically stable when they entered the study and no apparent increase in insulin dose was observed over the 4 months of the study. This should mean that the effects seen were mostly due to the CHF diet. The only way to separate the effects of hospitalisation from those of diet would have been to perform a double crossover study. This was not possible because of the proposed study duration and cost.

The duration of effect of CHF diet on diabetic control is not clear from this study. The greatest improvements in diabetic control appear, in general, to be after feeding CHF diet for two months. However, the improvements in a number of parameters seem to wane after this time. Again the potential influence of changes in insulin dose within individual

animals and the effects of hospitalisation make it very difficult to be sure whether the beneficial effects of CHF diet did last for the 4 months of the study.

The opportunity was taken to follow the effects of feeding CHF diet to 10 dogs after the end of the 4 month study. The very long term (>4 months) feeding of CHF diet was associated with a subjectively high prevalence of unexpected weight loss, recurrent diarrhoea and small intestinal bacterial overgrowth.

The results of this study imply (assuming minimal effects of hospitalisation) that CHF diet has properties which enable it to significantly improve many aspects of diabetic control in dogs with naturally occurring diabetes mellitus when used in conjunction with an appropriate insulin replacement regimen. However, based on the observations made after the very long term feeding of CHF diet, this diet would be contraindicated in diabetic dogs with histories of intestinal malabsorption or small intestinal bacterial overgrowth.

Chapter 8:
Longevity in canine diabetes mellitus

Introduction

In the management of any chronic illness it is helpful to be able to predict longevity. One of the most common questions asked by owners of diabetic dogs at an initial consultation is: How long is my pet likely to survive on insulin treatment? In addition to the intrinsic value of such knowledge, a more accurate estimate of longevity would be helpful in calculating the size of the financial and psychological undertaking before owners commit themselves to have their dog treated. Moreover, comparative measures of longevity can be used to identify the most useful treatment strategies.

Two methods of survival analyses are commonly used by epidemiologists in order to ascertain likely survival following specific diseases in people and animals and to compare the relative benefit of different therapies. Of these, the product-limit (PL) method of estimating survirvorship function described by Kaplan and Meier (1958) is the more frequently used. This method facilitates the generation of step-shaped survivorship function curves, the calculation of median survival times and comparisons of subgroups or treatments.

Canine diabetes mellitus is an age-related disease and many diabetic dogs are aged when first diagnosed; consequently they can only be expected to have a relatively short life remaining, regardless of their diabetic state. The other common method of survival data analysis is the life table method for large sample estimates of survivorship function, which can assist in 'age-correcting' survival data. In the life table method, survival times are grouped into intervals (the PL method uses individual survival times) and because of the necessity for a reasonable number of observations in each interval, it is best suited to large sample sizes. Two types of life table can be created: a general life table which applies to the general population, in which the time intervals are age groups, and a clinical life table in which the intervals are months or years survived after diagnosis or start of therapy. By relating the survival of diseased patients to that expected from individuals of the same age, gender and ideally breed or race in the general life table a relative survival rate can be generated. For many years, general life tables have been

generated for humans by governmental agencies to monitor the health status of their populations and to make comparisons between nations, states and regions.

There are two techniques for creating general life tables: one is the cohort method in which a large group of people or animals are followed from birth to death and a table constructed of the number of deaths at each age. The other is the current life table, constructed from census data concerning the numbers of individuals in the population for each age interval combined with data from a register of deaths providing the number of deaths in each age interval, thereby creating age-specific death rates. Because of the very long follow-up period required by the cohort method, the current life table method is more commonly used.

In the UK there is neither a national dog census nor a register of canine deaths so a classical current life table for the study of canine survival cannot be generated. A modified current life table for dogs could be generated from a large random sample of dogs, followed for one year, so that age-specific death rates could be calculated. Such information is probably held by pet insurance companies because of the annual renewal of policies. However, during the period of this study, requests for data from a British pet insurance agency were refused. A modified cohort-based life table which forms the basis of the only previously published canine life table was reported by Hayashidani *et al* (1988). In this method, records of the age at death for 4915 dogs presented to a Tokyo pet cemetery between June 1981 and May 1982 were used to generate age-specific death rates.

The aims of the present study were: to generate values for longevity in diabetic dogs treated with single daily injections of an intermediate acting insulin preparation and to make comparisons of survival between males versus females, isophane-treated versus lente-treated and the presence of concurrent endocrinopathy versus no other identifiable endocrinopathy.

Materials and methods

Eighty six diabetic dogs seen at the University of Glasgow between 1989 and 1994 were used as the study population. Dogs in which only temporary insulin therapy was required (metoestrus-associated diabetes) were excluded. All but one dog received isophane or lente insulin by single daily injection. In two other dogs the nature of the intermediate insulin preparation used was not recorded. All three dogs were excluded from the insulin comparison.

Because dogs entered this study at different times; some were lost to follow-up and many were still alive at the end of the study, precise survival times were not known for all dogs. This is a common problem in survival data analysis and a technique known as censoring is employed (in this case Type III, random or progressive censoring). Censored survival times were calculated from the date at the end of the study or the date of last contact with the patient and denoted as censored by an asterisk or plus sign. All the statistical methods used were able to incorporate censored data.

Many of the following statistical methods are those recommended in *Statistical Methods for Survival Data Analysis* (Lee, 1992), *Principles of Biostatistics* (Pagano and Gauvreau, 1992) and *Stata Reference Manual: Release 3.1 6th ed* (Stata Corporation, 1993) but the originators of the methods are cited where appropriate.

Product-limit estimates

The method of Kaplan and Meier (1958) cited by Lee (1992) was used to estimate survivorship function. The following notation was used: n, the number of animals studied (in this case 86); t_1, t_2,.....t_n, the survival times ranked in ascending order; $\hat{S}_{(t)}$, the survivorship function or the proportion of sample alive at time t (the circumflex denotes an estimate of the function) and r, the ranked position of each observation. The survivorship function at time zero, $\hat{S}_{(t=0)} = 1$ because all dogs are alive at the beginning of the study. For each uncensored survival time the proportion of dogs surviving up to and through that time is given by $(n - r)/(n - r + 1)$. $\hat{S}_{(t)}$ is then calculated as the product of all preceding $(n - r)/(n - r + 1)$ since cumulative probability follows the multiplicative rule. Table 45 gives an example based on 18 dogs with concurrent hyperadrenocorticism and diabetes mellitus.

Separate survivorship plots were created to allow comparison between male and female, between those treated with isophane insulin and those treated with lente insulin and among those which had concurrent hypothyroidism (HypoT4), hyperadrenocorticism (HAC) or no other identifiable endocrinopathy (Norm). The log-rank test was performed to compare survival distributions using Stata Statistical Software (Stata Corporation) based on the methods described by Peto *et al* (1977). Probability values of < 0.05 were taken to indicate statistical significance.

t (years)	*r*	(n-r)/(n-r+1)	$\hat{S}(t)$
0.00	1	0.944	0.944
0.02	2	0.941	0.941 x 0.944 = 0.889
0.10	3	0.938	0.938 x 0.941 x 0.944 = 0.833
0.24	4	0.933	0.933 x 0.938 x 0.941 x 0.944 = 0.778
0.30	5	0.929	0.722
0.31 +			
0.31	7	0.917	0.662
0.36	8	0.909	0.602
0.39	9	0.900	0.542
0.84 +			
1.00 +			
1.60 +			
1.66	13	0.833	0.451
1.89	14	0.800	0.361
2.77	15	0.750	0.271
3.17	16	0.667	0.181
3.67 +			
3.86	18	0.000	0.000

Table 45. Calculation of survivorship function for 18 dogs with concurrent diabetes mellitus and hyperadrenocorticism using the product-limit method.

General life table

In the absence of survey data to generate a current life table, the method described by Hayashidani *et al* (1988) was used to create a modified cohort life table based on a sample of dead dogs. The sample of dead dogs used was generated from the hospital necropsy database at the University of Glasgow Veterinary School. An age at death was calculated for each canine necropsy between 1988 and 1994 from date received for post-mortem examination and date of birth. Following removal of those records where date of birth was incorrectly recorded or not recorded and where gender was not defined, a total of 926 necropsy records were used to generate two gender-specific (male and female) cohort life tables.

The general life tables were made up of the following columns:

Column 1. Age interval (years) 0 - 1, 1 - 2, 2 - 3, etc, denoted as $(x, x+t)$, where t is the length of the age interval. In this case $t = 1$ year.

Column 2. Number alive at time x, denoted as l_x. At $x_{(0)}$, $l = n$ (the number of observations in the sample). l_x is thereafter equal to n minus the sum of observed deaths preceding time x.

Column 3. Number dying in the age interval $(x, x+1)$, denoted as d_x.

Column 4. Conditional probability of dying in the age interval (x, x +1), denoted by q_x. $q_x = d_x/l_x$

Column 5. Conditional probability of surviving the age interval (x, x +1), denoted by p_x. $p_x = 1 - q_x$.

Column 6. Total number of years lived in age interval (x, x +1), denoted by L_x. Deaths within the age interval are assumed to be evenly distributed through the interval and are therefore each considered to contribute half an age interval. $L_x = t(l_{x+t} + (d_x/2))$.

Column 7. Total number of dog-years lived beyond age x by dogs alive at age x, denoted by T_x. T_x = the sum of L_x and all subsequent L's.

Column 8. Average number of years of life remaining at beginning of age interval (or age specific life expectancy), denoted by e_x. $e_x = T_x/l_x$.

Columns 5b and 5c were created to provide age specific probabilities of surviving 1 and 6 month intervals used in the age correction of clinical life table data. These contained the average probability of surviving 1 month = (1 - (q_x/12)) and of surviving 6 months = (1 - (q_x/2)) for each 1 year age interval.

Clinical life table

For 86 diabetic dogs, survival time (including censored observations) was grouped into intervals of 6 months. The first 6 month period was further subdivided into individual months.

A life table for all diabetics was constructed according to the following method (Lee, 1992):

Column 1. Time interval (t_i, t_{i+1}). In this case, 0 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 5, 5 - 6, 6 - 12 months, 1 - 1.5 years, 1.5 - 2 years etc. The final interval was >5 years.

Column 2. Midpoint of the time interval in years (t_{mi}). In this case, 0.041, 0.125, 0.208, 0.291, etc. The midpoint is used for plots of hazard and probability density below.

Column 3. Width of the time interval (t_i, t_{i+1}) in years (b_i).

Column 4. Number of cases lost to follow up (l_i)

Column 5. Number of cases withdrawn alive (w_i). Dogs still alive at the end of the study period.

Column 6. Number of cases dying (d_i).

Column 7. Number of cases entering the time interval (n'_i). For the first interval n'_i = the total sample size. Thereafter n'_i = the number entering the previous interval minus those lost to follow-up, withdrawn alive or dead in the previous interval i.e. $n'_i = n'_{i-1} - l_{i-1} - w_{i-1} - d_{i-1}$.

Column 8. Number of cases exposed to risk (n_i). Cases which are lost to follow-up or withdrawn alive are assumed to have been exposed to risk for half the interval. Consequently, $n_i = n'_i - 0.5(l_i + w_i)$.

Column 9. Conditional proportion dying ($\hat{q}_i$). $\hat{q}_i = d_i/n_i$.

Column 10. Conditional proportion surviving ($\hat{p}_i$). $\hat{p}_i = 1 - \hat{q}_i$.

Column 11. Cumulative proportion surviving ($\hat{S}(t_i)$). This is an estimate of survivorship function with time, i.e., the proportion of cases alive at time t. It is calculated from the probability of surviving to the beginning of, and then through, the previous interval, i.e. $\hat{S}(t_i) = \hat{p}_i\hat{S}(t_{i-1})$. At time zero $\hat{S}(t_i) = 1$.

Column 12. Estimated probability density function, $\hat{f}(t_{mi})$. This is the probability of dying in the ith interval per unit width (i.e., per year). It is estimated at the midpoint of the interval. $\hat{f}(t_{mi}) = \dfrac{\hat{S}(t_i)\hat{q}_i}{\hat{b}_i}$.

Column 13. Hazard function, $\hat{h}(t_{mi})$. This is the number of deaths per unit time (year) divided by the average number of survivors at the midpoint of the ith interval (or deaths per animal per year), i.e., $\hat{f}(t_{mi})/\hat{S}(t_{mi})$. $\hat{S}(t_{mi}) = 0.5[\hat{S}(t_{i+1})+\hat{S}(t_i)]$ since $\hat{S}(t_i)$ is the probability of surviving at the beginning and not the midpoint of the interval. This reduces to $\hat{h}(t_{mi}) = \dfrac{2\hat{q}_i}{b_i(1+\hat{p}_i)}$.

Column 14. Estimated median remaining lifetime at time t_i, denoted as $\hat{t}_{mr(i)}$. If $\hat{S}(t_i)$ is the proportion of cases surviving at time t_i, then the proportion of these cases which will

still be alive at the median survival time for interval i will be $\hat{S}(t_i)/2$. If, for each interval i, the interval containing $\hat{S}(t) = \hat{S}(t_i)/2$ is denoted (t_j, t_{j+1}), then:

$$\hat{t}_{mr(i)} = (t_j - t_i) + \frac{b_j[\hat{S}(t_j) - \frac{1}{2}\hat{S}(t_i)]}{\hat{S}(t_j) - \hat{S}(t_{j+1})}.$$

Columns 15-18. These are square roots of the variances of the estimated functions $\hat{S}(t_i)$, $\hat{f}(t_{mi})$, $\hat{h}(t_{mi})$ and $\hat{t}_{mr(i)}$ which give approximate confidence intervals. The variances are calculated using the following formulae (Lee, 1992):

$$\mathrm{Var}[\hat{S}(t_i)] \cong [\hat{S}(t_i)]^2 \sum \frac{\hat{q}_j}{n_j \hat{p}_j} \text{ (j refers to preceding intervals)}$$

$$\mathrm{Var}[\hat{f}(t_{mi})] \cong \frac{[\hat{S}(t_i)\hat{q}_i]^2}{b_i}\left[\sum \frac{\hat{q}_j}{n_j \hat{p}_j} + \frac{\hat{p}_i}{n_i \hat{q}_i}\right] \text{ (j refers to preceding intervals)}$$

$$\mathrm{Var}[\hat{h}(t_{mi})] \cong \frac{[\hat{h}(t_{mi})]^2}{n_i \hat{q}_i}\left\{1 - \left[\frac{1}{2}\hat{h}(t_{mi})b_i\right]^2\right\}$$

$$\mathrm{Var}[\hat{t}_{mr(i)}] \cong \frac{[\hat{S}(t_i)]^2}{4n_i[\hat{f}(t_{mj})]^2} \text{ (j refers to the start of the interval containing $\hat{S}(t) = \hat{S}(t_i)/2$).}$$

Following the generation of a clinical life table for all diabetic dogs separate tables were created for male and female dogs, isophane treated and lente treated dogs, and for all diabetic dogs minus those suffering from concurrent hyperadrenocorticism. It was not possible to make separate tables for concurrent hypothyroidism, concurrent hyperadrenocorticism and 'normal' diabetic dogs because the numbers of dogs in these individual groups were insufficient for the clinical life table method.

Relative survival rates

For each dog alive at the beginning of each interval an expected rate of survival for that dog for that interval, p^*, was obtained according to its age and gender from the general life table created above. For each additional year that a dog survived, its expected survival rate had to be taken from successive age groups within the general life table. The expected survival rate for each interval was calculated as the mean of all the individual expected survival rates of j dogs alive at the beginning of the interval, i.e. $p_i^* = \frac{1}{n'_i}\sum p_{ij}^*$.

A relative survival rate was then calculated from a ratio of the observed survival rate to the expected survival rate, i.e. $r_i = \hat{p}_i / p_i^*$. Relative survival rate curves were generated for each of the subgroups for which a clinical life table had been created.

Results

Product-limit estimates

Gender

The Kaplan-Meier survivorship function versus time plots for male (n=35) and female (n = 51) diabetics are given in Figure 117. Median survival time for males was 2.13 years and for females was 2.75 years. There was no statistically significant difference between the survival of these two groups. The overall median survival time was 2.71 years.

Insulin treatment

The Kaplan-Meier survivorship function versus time plots for isophane-treated (n=50) and lente-treated (n = 33) diabetics are given in Figure 118. Median suvival time for isophane-treated was 2.53 years and for lente-treated was 2.80 years. There was no statistically significant difference between the survival of these two groups.

Type

Survival plots for 'normal' diabetics (n = 61) and those with concurrent hypothyroidism (n = 7) or hyperadrenocorticism (n = 18) are represented in Figure 119. Median survival times were 2.93 years for the 'normal' group and 3.68 and 0.96 years for the hypothyroid and hyperadrenocorticism groups, respectively. There was a statistically significant difference among the survivals of these three groups (p = 0.02). Following exclusion of the hypothyroid group (because of the small number of cases), the dogs with hyperadrenocorticism had a significantly shorter survival than the 'normal' dogs (p = 0.03).

General life table

The general life tables generated for female and male dogs which underwent post-mortem examination at the University of Glasgow Veterinary School are given in Tables 46 and 47. Life expectancy at age zero was approximately 6.9 years for the two genders. There was no apparent difference between the male and female life tables. Figure 120 represents the age group specific death rates for male and females and also for the first 17 year intervals reported by Hayashidani *et al* (1988).

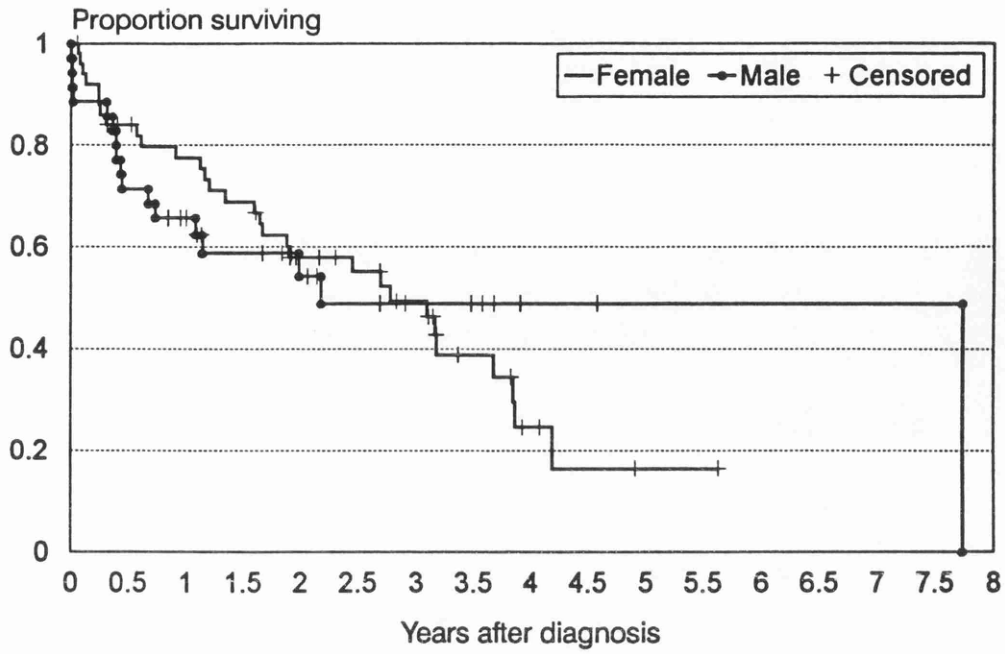

Figure 117. Survivorship function by product-limit method versus time for all 86 diabetic dogs according to gender (males n = 35, females n = 51).

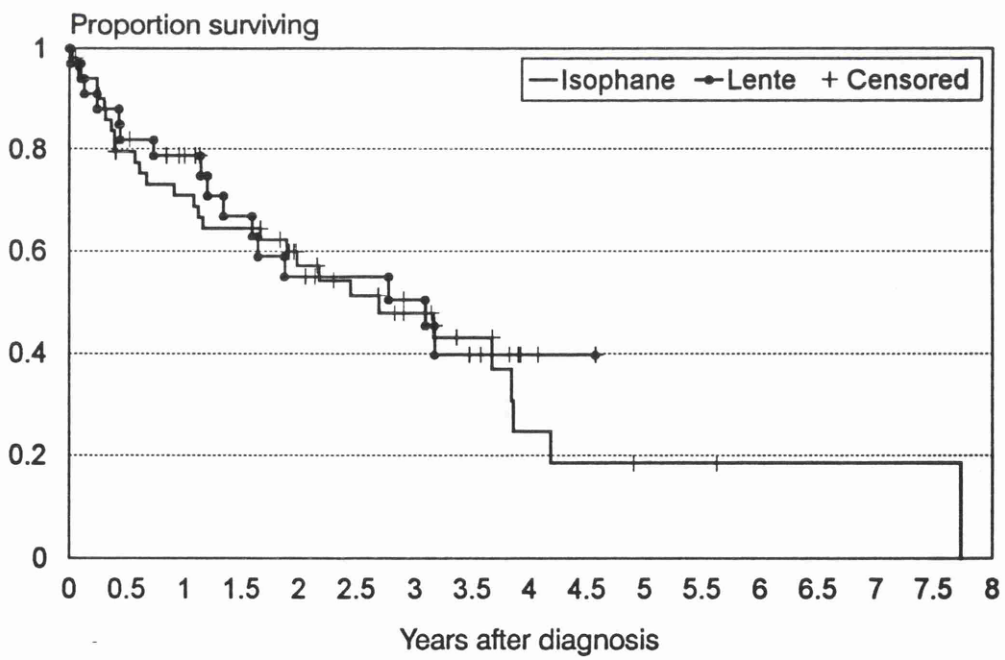

Figure 118. Survivorship function by product-limit method versus time according to type of intermediate acing insulin preparation (isophane n = 50, lente n = 33).

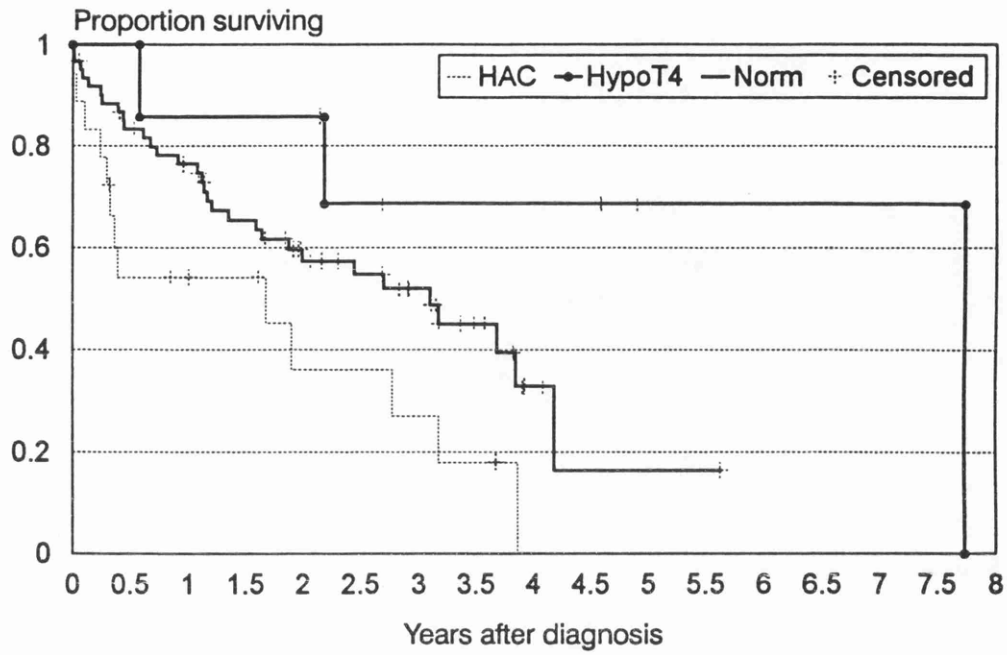

Figure 119. Survivorship function by product-limit method versus time according to type of diabetes mellitus. HAC (n = 18), HypoT4 (n = 7) and Norm (n = 61).

1	2	3	4	5a	5b	5c	6	7	8
$(x, x+t)$	l_x	d_x	q_x	p_x (/yr)	p_x (/mth)	p_x (/6mth)	L_x	T_x	e_x
0, -1	430	45	0.105	0.895	0.991	0.948	407.5	2941.0	6.84
1, -2	385	26	0.068	0.932	0.994	0.966	372.0	2533.5	6.58
2, -3	359	23	0.064	0.936	0.995	0.968	347.5	2161.5	6.02
3, -4	336	23	0.068	0.932	0.994	0.966	324.5	1814.0	5.40
4, -5	313	20	0.064	0.936	0.995	0.968	303.0	1489.5	4.76
5, -6	293	33	0.113	0.887	0.991	0.944	276.5	1186.5	4.05
6, -7	260	40	0.154	0.846	0.987	0.923	240.0	910.0	3.50
7, -8	220	34	0.155	0.845	0.987	0.923	203.0	670.0	3.05
8, -9	186	48	0.258	0.742	0.978	0.871	162.0	467.0	2.51
9, -10	138	35	0.254	0.746	0.979	0.873	120.5	305.0	2.21
10, -11	103	31	0.301	0.699	0.975	0.850	87.5	184.5	1.79
11, -12	72	35	0.486	0.514	0.959	0.757	54.5	97.0	1.35
12, -13	37	21	0.568	0.432	0.953	0.716	26.5	42.5	1.15
13, -14	16	9	0.563	0.438	0.953	0.719	11.5	16.0	1.00
14, -15	7	6	0.857	0.143	0.929	0.571	4.0	4.5	0.64
15, -16	1	1	1.000	0.000	0.917	0.500	0.5	0.5	0.50

Table 46. General life table for female dogs (n = 430) generated from the University of Glasgow Veterinary School necropsy database.

1	2	3	4	5a	5b	5c	6	7	8
$(x, x+t)$	l_x	d_x	q_x	p_x (/yr)	p_x (/mth)	p_x (/6mth)	L_x	T_x	e_x
0, -1	496	39	0.079	0.921	0.993	0.961	476.5	3458.0	6.97
1, -2	457	29	0.063	0.937	0.995	0.968	442.5	2981.5	6.52
2, -3	428	22	0.051	0.949	0.996	0.974	417.0	2539.0	5.93
3, -4	406	24	0.059	0.941	0.995	0.970	394.0	2122.0	5.23
4, -5	382	35	0.092	0.908	0.992	0.954	364.5	1728.0	4.52
5, -6	347	44	0.127	0.873	0.989	0.937	325.0	1363.5	3.93
6, -7	303	47	0.155	0.845	0.987	0.922	279.5	1038.5	3.43
7, -8	256	39	0.152	0.848	0.987	0.924	236.5	759.0	2.96
8, -9	217	57	0.263	0.737	0.978	0.869	188.5	522.5	2.41
9, -10	160	54	0.338	0.663	0.972	0.831	133.0	334.0	2.09
10, -11	106	33	0.311	0.689	0.974	0.844	89.5	201.0	1.90
11, -12	73	30	0.411	0.589	0.966	0.795	58.0	111.5	1.53
12, -13	43	21	0.488	0.512	0.959	0.756	32.5	53.5	1.24
13, -14	22	16	0.727	0.273	0.939	0.636	14.0	21.0	0.95
14, -15	6	3	0.500	0.500	0.958	0.750	4.5	7.0	1.17
15, -16	3	1	0.333	0.667	0.972	0.833	2.5	2.5	0.83
16, -17	2	2	1.000	0.000	0.917	0.500	1.0	0.0	0.00

Table 47. General life table for male dogs (n = 496) generated from the University of Glasgow Veterinary School necropsy database.

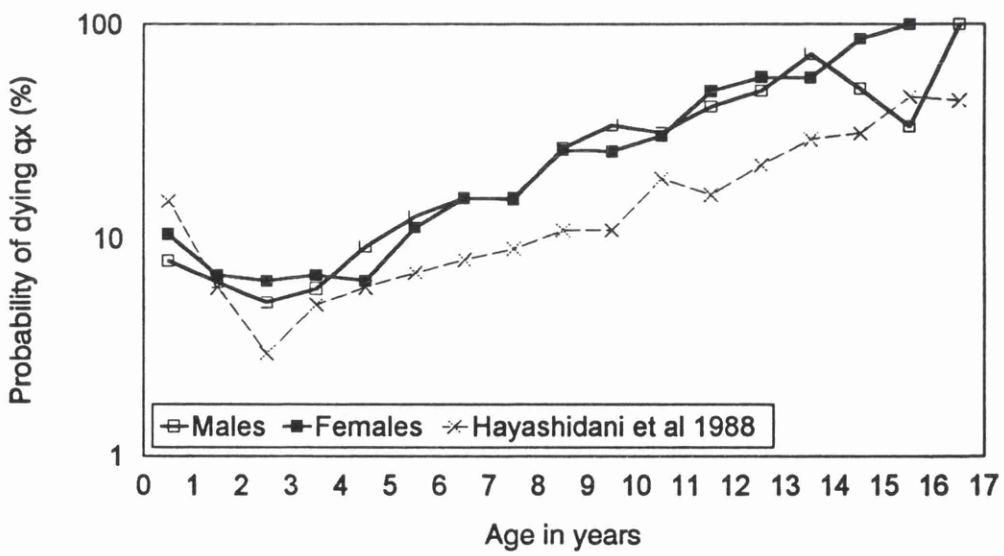

Figure 120. Probability of death versus age curve for male and female dogs from 926 canine post mortem records at the University of Glasgow Veterinary School (female n=430, male n=496) and for the first 17 years of data reported by Hayashidani *et al* (1988) from 4915 pet cemetery records.

Clinical life table

The life table for all 86 diabetic dogs is given in Table 48. Survivorship function, probability density and hazard function versus time are represented in Figures 121 - 123. Median survival time from the survivorship function graph was 2.82 years. Included in the survivorship function versus time graph there is another curve generated from a case series by Foster (1975) in which sufficient raw data on the survival times of 98 diabetic dogs was reported to create a clinical life table (median survival time 1.00 years). Unfortunately, these data were not presented in a form from which product-limit estimates or statistical comparisons could be made. In the present study, the hazard function was greatest during the first month of treatment, remains relatively low from 6 months to 2.5 years and then begins to increase again. The reasons for failure (death) in the uncensored observations during this initial 'hazardous' 6 month period (n= 17) were: owners unwilling to continue therapy (6), no response to initial treatment (3), unknown (2), hypoglycaemia (1), renal failure (1), cirrhosis (1), failure to recover following ovariohysterectomy (1), septicaemia (1) and aggression (1).

Because of the difference between survival of 'normal' diabetics and those with concurrent hyperadrenocorticism within the first 6 months of treatment, highlighted in Figure 119, an expanded plot of hazard function for all diabetics and those without HAC was generated to compare these two groups within the early part of treatment (Figure 124). There is an apparent difference in hazard between all dogs and those without HAC especially between 2 and 5 months after start of therapy.

Median remaining lifetime is represented in histogram form in Figure 125 and increases from 2.82 years at the start of treatment to 3.11 years for those dogs which have survived the first 5 months of therapy. Median remaining lifetime could not be calculated for dogs surviving longer than 2.50 years because of an insufficient follow-up period. Median remaining lifetimes based on data from Foster (1975) are also represented in Figure 125, where they increase from 1.00 year at diagnosis to a maximum of 2.84 years for dogs which survived the first 5 months.

1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(t_i, t_{i+1})	t_i	t_{mi}	b_i	l_i	w_i	d_i	n'_i	n_i	$\hat{q}_i$	$\hat{p}_i$	$\hat{S}(t_i)$	$\hat{f}(t_{mi})$	$\hat{h}(t_{mi})$	$\hat{t}_{mr(i)}$	$\sqrt{\text{Var}[\hat{S}(t)]}$	$\sqrt{\text{Var}[\ (\ _i)]}$	$\sqrt{\text{Var}[\hat{h}(t_i)]}$	$\sqrt{\text{Var}[\hat{t}_{mr(i)}]}$
A (1)	0.00	0.042	0.083	1	0	5	86	85.5	0.058	0.942	1.000	0.702	0.723	2.82	*	0.09	0.323	0.103
B (2)	0.08	0.125	0.083	0	0	3	80	80.0	0.038	0.963	0.942	0.424	0.459	3.02	0.025	0.07	0.265	0.108
C (3)	0.17	0.208	0.083	0	0	2	77	77.0	0.026	0.974	0.906	0.282	0.316	3.05	0.032	0.06	0.223	0.106
D (4)	0.25	0.292	0.083	1	0	3	75	74.5	0.040	0.960	0.883	0.427	0.493	3.05	0.035	0.07	0.285	0.105
E (5)	0.33	0.375	0.083	1	0	3	71	70.5	0.043	0.957	0.847	0.433	0.522	3.08	0.039	0.07	0.301	0.104
F (6)	0.42	0.458	0.083	0	0	2	67	67.0	0.030	0.970	0.811	0.291	0.364	3.11	0.043	0.06	0.257	0.121
G (6-12)	0.50	0.750	0.500	1	2	5	65	63.5	0.079	0.921	0.787	0.124	0.164	3.08	0.045	0.04	0.073	0.120
H (12-18)	1.00	1.250	0.500	0	3	6	57	55.5	0.108	0.892	0.725	0.157	0.229	2.73	0.049	0.04	0.093	0.118
I (18-24)	1.50	1.750	0.500	0	7	6	48	44.5	0.135	0.865	0.647	0.174	0.289	2.41	0.053	0.05	0.118	0.118
J (24-30)	2.00	2.250	0.500	1	3	2	35	33.0	0.061	0.939	0.559	0.068	0.125	2.22	0.057	0.03	0.088	0.160
K (30-36)	2.50	2.750	0.500	1	4	2	29	26.5	0.075	0.925	0.525	0.079	0.157	1.87	0.058	0.04	0.111	0.168
L (36-42)	3.00	3.250	0.500	0	5	3	22	19.5	0.154	0.846	0.486	0.149	0.333	2.00 +	0.060	0.06	0.192	*
M (42-48)	3.50	3.750	0.500	0	5	3	14	11.5	0.261	0.739	0.411	0.214	0.600	1.50 +	0.064	0.08	0.342	*
N (48-54)	4.00	4.250	0.500	0	1	1	6	5.5	0.182	0.818	0.304	0.110	0.400	1.00 +	0.071	0.07	0.398	*
O (54-60)	4.50	4.750	0.500	0	2	0	4	3.0	0.000	1.000	0.249	0.000	0.000	0.50 +	0.077	*	*	*
P (60+)	5.00	*	*	0	1	1	2	1.5	1.000	0.000	0.249	*	*	0.00 +	0.077	*	*	

Table 48. Clinical life table for 86 diabetic dogs (see text for key to column headings).

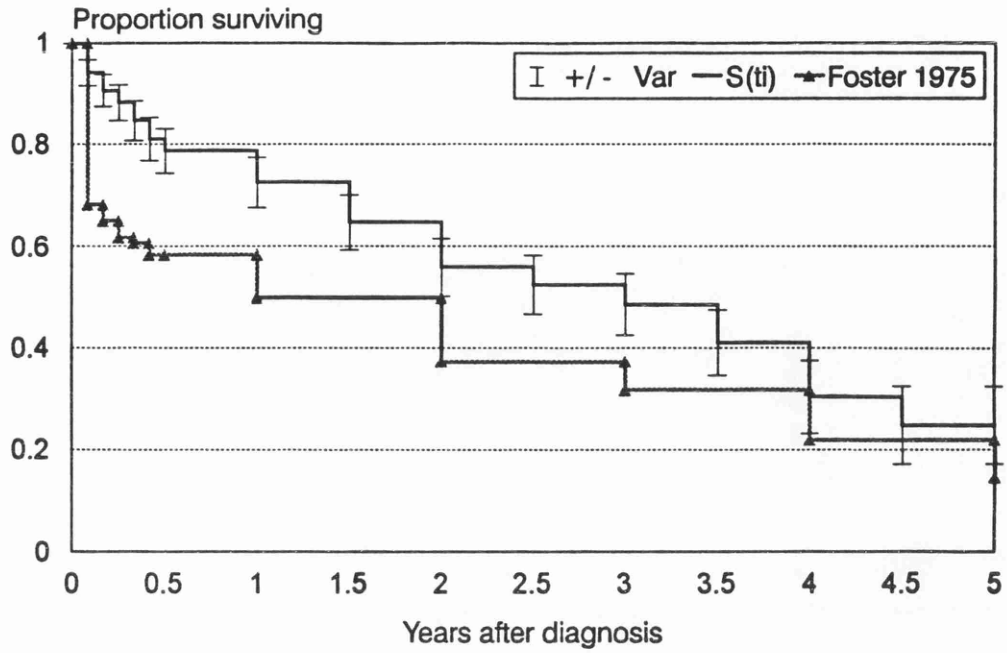

Figure 121. Survivorship function of 86 diabetic dogs seen at the University of Glasgow Veterinary School and also for data derived from 98 diabetic dogs reported by Foster (1975).

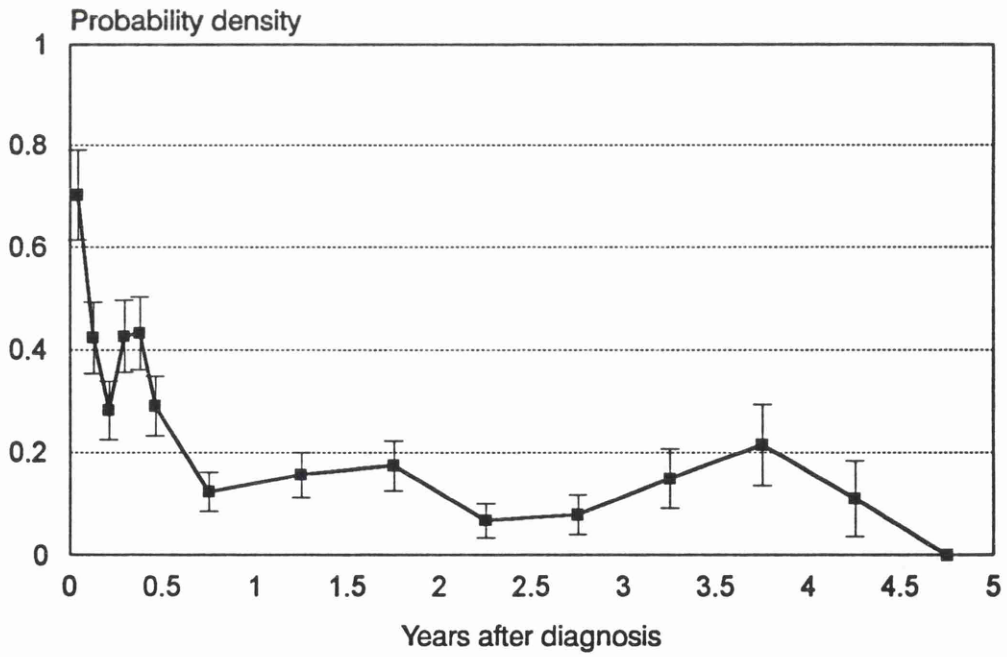

Figure 122. Probability density function for death in 86 diabetic dogs seen at the University of Glasgow Veterinary School.

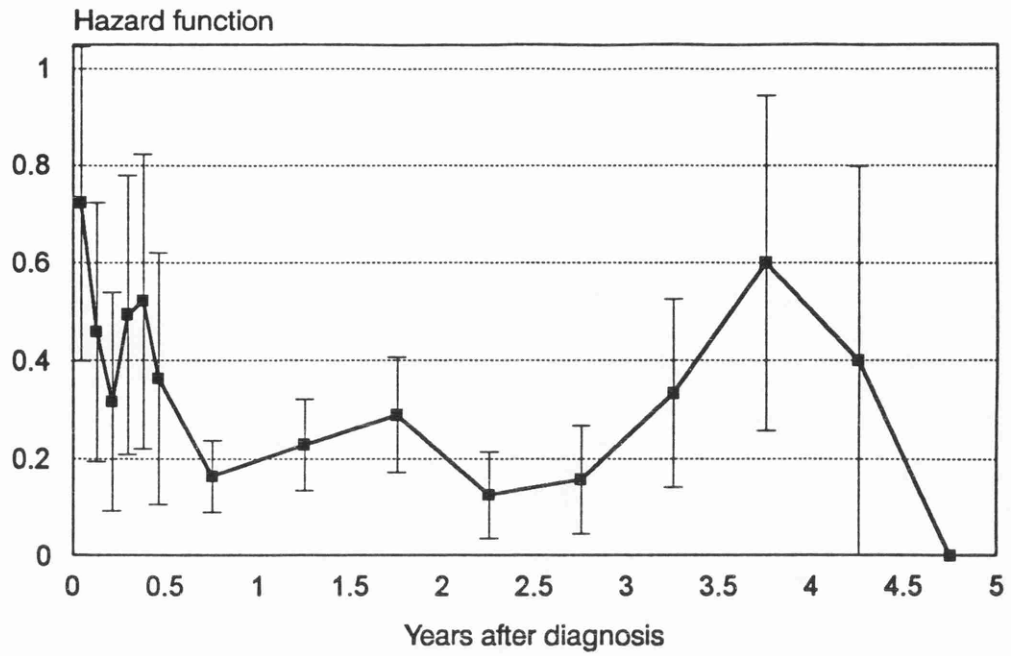

Figure 123. Hazard function for 86 diabetic dogs seen at the University of Glasgow Veterinary School.

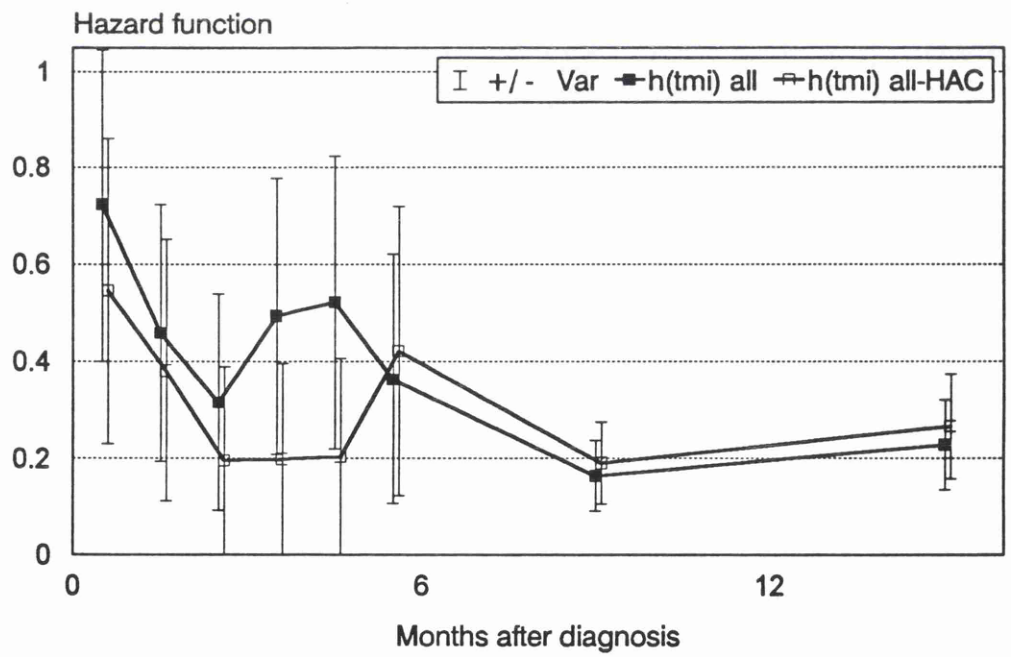

Figure 124. Hazard function for all diabetic dogs (n = 86) and for all diabetic dogs without hyperadrenocorticism (all - HAC, n = 68).

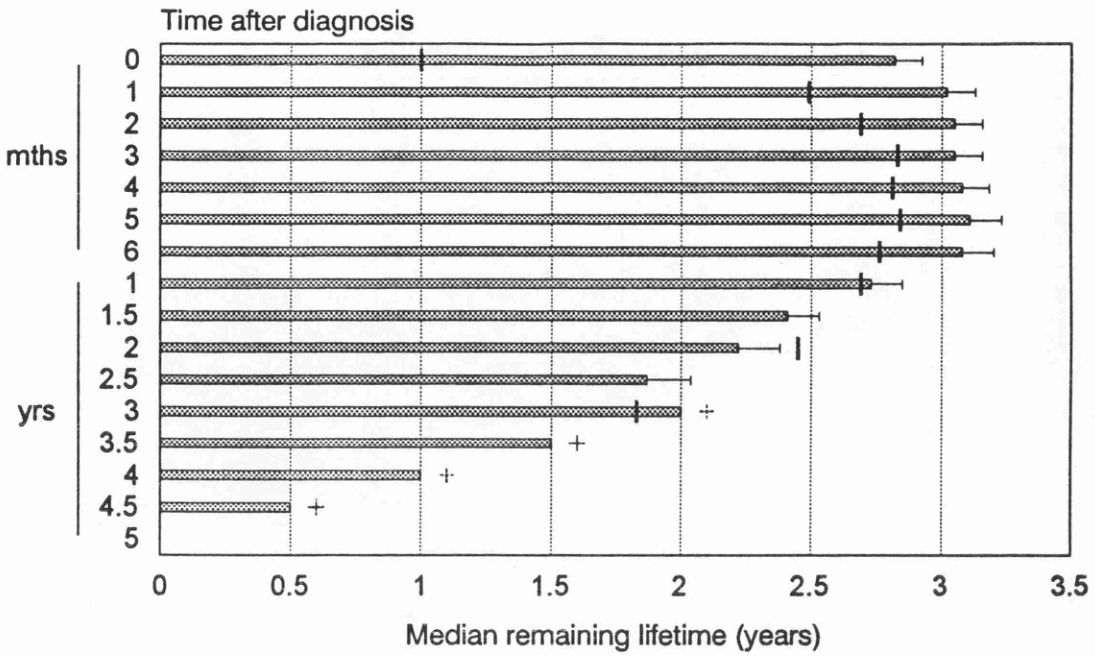

Figure 125. Median remaining lifetime for 86 diabetic dogs seen at the University of Glasgow Veterinary School (shaded bars) and also for data derived from 98 diabetic dogs reported by Foster (1975) (vertical bars).

Relative survival rates

Relative survival rates for all 86 dogs were close to or greater than 100% for up to 4 to 4.5 years of treatment. After that time there were insufficient data to make a useful estimate of relative survival rate. This means that, for the first 4.5 years, treated diabetic dogs have a near equal or greater chance of survival than that expected from a general population (generated from canine post-mortem data).

The relative survival rate curves for the gender and insulin groups are presented in Figures 126 and 127. Relative survival rate appeared to be higher for males during the period 3 to 4.5 years after diagnosis and for lente-treated dogs during the preriod 3.5 to 4.5 years. The numbers of dogs involved in these survival intervals and the erratic nature of the curves made the significance of these 'differences' in estimates of relative survival rate difficult to interpret.

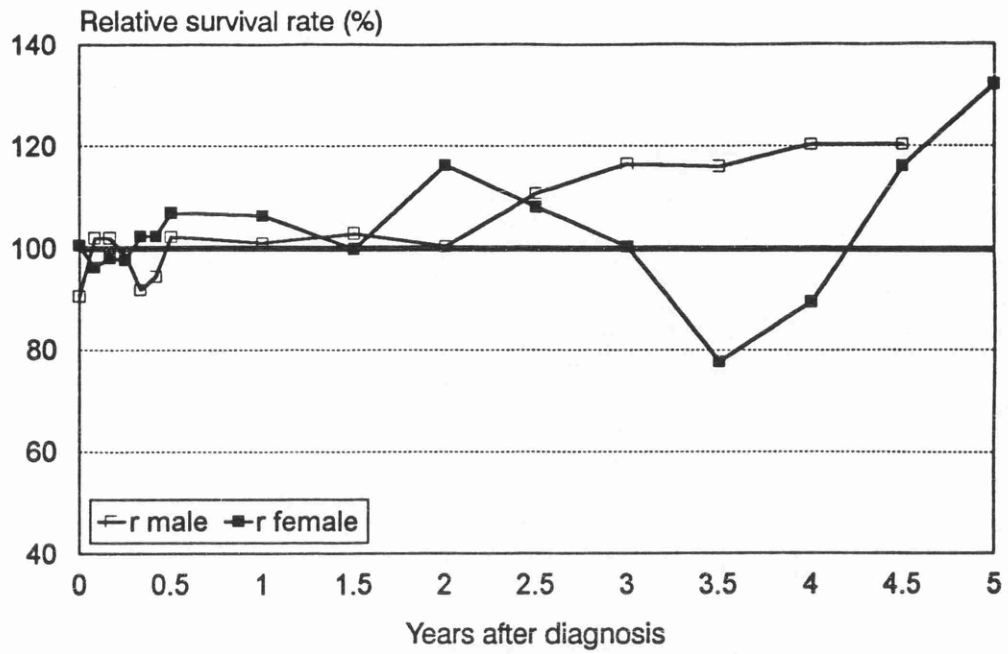

Figure 126. Relative survival rates by gender (female n = 51; male n = 35). NB y - axis extends from 40% to 140%.

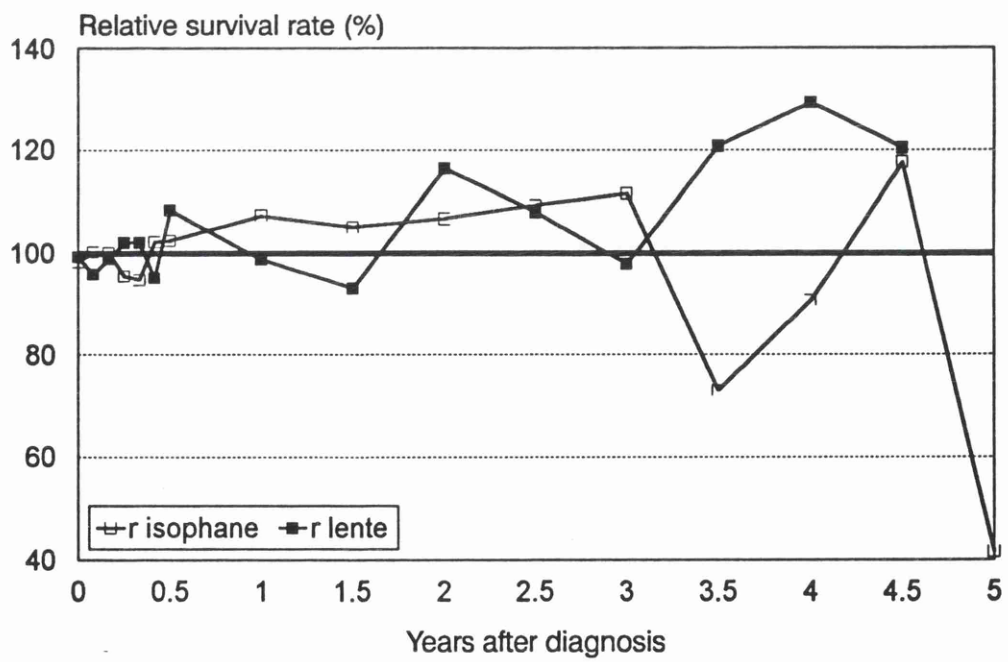

Figure 127. Relative survival rates by insulin type (isophane n = 50; lente n = 33). NB y - axis extends from 40% to 140%.

Because of the difference in survival (product-limit estimates) and hazard between the 'normal' diabetics and those with concurrent hyperadrenocorticism, a relative survival rate for those dogs without HAC was plotted alongside that for all diabetics (Figure 128). It appears from the relative survival rate graph that following age-correction there is very little difference in survival related to the presence of concurrent HAC.

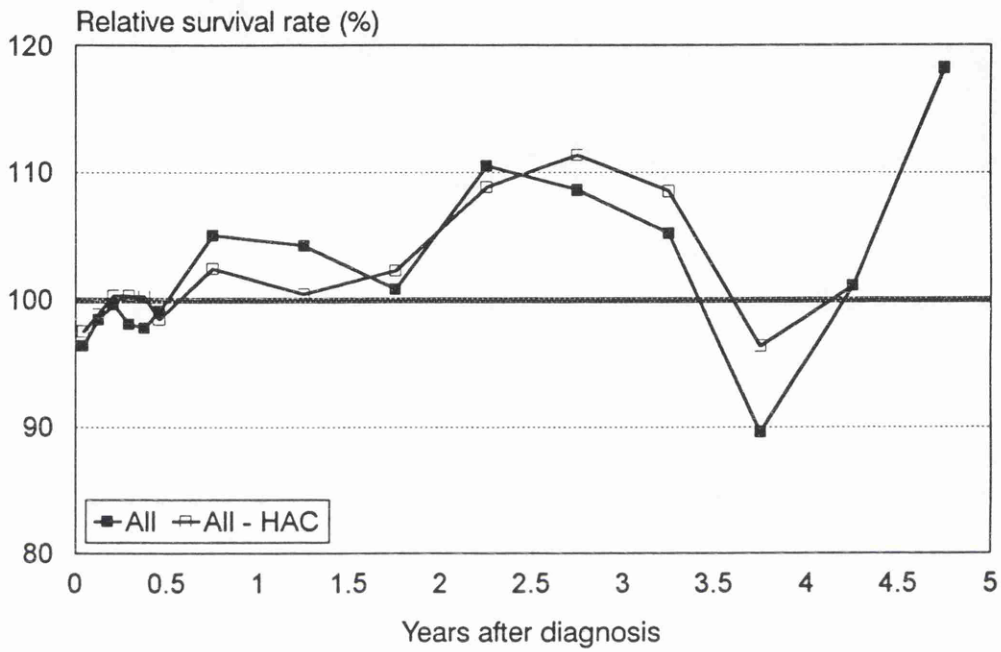

Figure 128. Relative survival rates for all diabetic dogs (n = 86) and for those without hyperadrenocorticism (All - HAC, n = 68). NB y - axis extends from 80% to 120%.

Discussion

The duration of survival in diabetic dogs treated with single daily injections of intermediate acting insulin was not dependent on gender or the type of insulin preparation used. The product limit and clinical life table methods gave very similar overall median survival times of 2.71 and 2.80 years, respectively. The presence of concurrent hyperadrenocorticism significantly affected survival time and median survival time for these diabetic dogs was only 0.96 years. The hazard (of death) to diabetic dogs is greatest within the first 6 months of treatment especially for those with concurrent HAC and this is reflected in the median remaining lifetime which reaches a peak in dogs which have survived the first 5 months of treatment. The greatest single hazard to diabetic dogs within the first 6 months of treatment is their owner's unwillingness to continue treatment. Following correction (principally for age) of survival data using a general population based on veterinary school necropsy data, it appears that treated

diabetic dogs have a near equivalent or greater chance of survival than expected and that the increased 'hazard' experienced by dogs with concurrent HAC may be age related.

There are few previous reports of survival of treated canine diabetics and none in which statistical methods for survival data analysis have been employed. Some reports contain very vague estimates of survival such as 3 to 12 months and sometimes 1.5 to 2 years (Joshua, 1963) or 2-5 years (Feldman and Nelson, 1987).

Some previously published reports contain sufficient information for some inferences about survival to be derived. In 1960, Wilkinson reported the first large British case series of canine diabetes mellitus at which time insulin treatment in dogs was still relatively new. Of 58 cases from a veterinary hospital based population in London, 30 had died very early in the course of treatment and so a median survival time would probably have only been one or two months at the most. Unwillingness by owners to continue treatment was responsible for about 14% of deaths (7% in the present study) and renal and hepatic failure were believed to be responsible for many of the other treatment failures. In 1972, Lauder reported a review of 97 veterinary hospital based cases at the University of Glasgow which provided some survival data. At the end of the first month of treatment only 60% remained alive (94% in the present study), after 6 months 40% were alive (79% in the present study) and only 15% were alive after one year (72% in the present study). No explanation was given as to the causes of treatment failure. Ling *et al* (1977) reported on a veterinary hospital based series of 59 diabetic dogs from the United States. In that study, at least 33 were dead within two months of starting treatment, i.e., only 44% survived longer than two months (90% in the present study). Again the unwillingness of owners to continue treatment was a major cause of death (11%) followed by ketoacidosis, necrotising pancreatitis and renal failure. Doxey *et al* (1985) reported a veterinary hospital based group of 43 diabetic dogs from Edinburgh in which 29 survived the initial stabilisation period. Of these 29 dogs, 64% survived to one year, i.e. an overall one year survivorship function of 43% (72% in the present study).

Foster (1975) reported a large veterinary practice survey-based case series of 98 dogs in such a way that the survival data could be manipulated and incorporated into the present study for comparison (Figures 121 and 125). In that series there was a much higher death rate in the first few months of treatment than in the present study, resulting in a median survival time of only one year. However, after the first three months, median remaining life was reasonably comparable with that in the present study. Possible reasons for the very large difference between the studies in survival during the first month are that Foster's survey was practice based (first opinion) and not hospital based (second

opinion) and that early management of canine diabetes mellitus has improved considerably over the last 20 years. It is reassuring that the median remaining lifetimes are generally greater in a group of referred diabetic dogs than in those derived from a practice based population nearly 20 years previously. However, the difference between the two groups is not large after the first three months, suggesting that there has only been a small improvement in the long-term management of canine diabetes mellitus in 20 years. This may be a reflection of the age at which many dogs become diabetic and the limits that the natural canine life-span places on improvements in survival time.

Based on the above reports, it seems that in the present study dramatic improvements in the longevity of dogs with diabetes mellitus have been achieved and that the principal improvements are in early management and during the first year after diagnosis.

None of the previous reports indicated whether the dogs they studied were diabetic only or whether they were complicated by the presence of other endocrinopathies. Peterson *et al* (1981) reported on 30 dogs presented with concurrent diabetes mellitus and hyperadrenocorticism from the United States. Approximate survivorship functions derived from survival data in that report are (present study in brackets): 1 month 76% (84%), 6 months 63% (53%) and at one year 53% (50%). Peterson *et al* (1981) reported that ketoacidosis, renal failure and pancreatitis were responsible for most deaths in this group and that owner unwillingness to continue was responsible for around 13% of deaths. In the United Kingdom, Blaxter and Gruffydd-Jones (1990) reported on a much smaller series of concurrent diabetes mellitus and hyperadrenocorticism in 8 dogs from Bristol in which median survival time was approximately 0.66 years (0.96 years in the present study). The results of the present study concur with those of Peterson *et al* (1981) and Blaxter and Gruffydd-Jones (1990) and suggest that survival time in dogs with concurrent diabetes mellitus and hyperadrenocorticism is short compared with diabetic dogs which do not have hyperadrenocorticism.

There are no previous reports of relative survival rates for canine diabetes mellitus but it is very encouraging that, based on the general population used for comparison in the present study, it appears that 'normal' life expectancy can be restored or even exceeded in diabetic dogs treated with once daily insulin therapy. The possibilty of a relative survival rate greater than 100% may be explained by the unusually high degree of committed care and attention that owners will provide for diabetic dogs, manifested by their decision to opt for treatment of their pet's diabetes in the first place and also the amount of disciplined contact necessitated by the insulin injection and dietary regimen.

The use of a general life table generated from veterinary hospital necropsy data may be considered inappropriate by some but there are a number of points in its favour. It may be suggested that hospital necropsies are performed on animals which are both terminally ill (including those which are indirectly terminal, i.e., the presence of an illness causes owners to elect euthanasia for their pet) and referred to a specialist centre. However, if these dogs had not been referred, their survival times would have been equivalent or shorter and they consequently represent a cross-section of survival experience by the general canine population. Such a cross-section may be biased by factors which affect the decision to refer to the hospital such as age, gender, breed and nature of disease but the hospital canine diabetic population is similarly biased because the effects of these factors will also apply to their selection for referral. A comparison between the two groups on this basis is therefore valid.

Further observations suggest that the general life table created in the present study is likely to be a reasonable reflection of the general UK canine population. The similarity in shape between the age specific probability of death curves created in the present study and those of Hayashidani *et al* (1988) is striking (Figure 120). However, the probability of death curve of Hayashidani *et al* (1988) is generally lower than that generated in the present study. This is not surprising after consideration of the following information. Hayashidani *et al* (1988) reported on a group of Tokyo dogs which were putatively up to 26 years of age at death and of which 42% lived longer than 10 years. This seems unlikely to be the case in the British canine population and this is confirmed in two publications on the demography of British dogs. Thrusfield (1989) reported that only 15% of British dogs were greater than 10 years of age based on a very large owner survey, which supported results of a veterinary practice survey which showed that 18% were greater than or equal to 10 years old (Edney and Smith, 1986).

The percentages of a population dying and the percentage alive at a certain age are not directly interchangeable, but of the necropsy data in the present study 22% of dogs were alive at equal to or greater than 10 years old and only 2.4% were alive after 14 years of age (2.45% of British dogs were greater than 14 years old in 1986 (Thrusfield, 1989)). This suggests that, at least in terms of aged dogs, the necropsy data used in the present study reflects the general UK canine population reasonably well and that a higher probability of death curve generated from this data than from Hayashidani *et al* (1988) is to be expected given the considerably greater longevity of dogs in Tokyo than in the UK.

In conclusion, the median survival time for diabetic dogs treated with single daily injections of an intermediate acting insulin was 2.71 years. Survival is not affected by gender or choice of insulin type. Median remaining lifetime was 3.11 years for those

diabetic dogs which survived the first 5 months of treatment. Dogs with concurrent hyperadrenocorticism had a much poorer prognosis but this may have been a reflection of their age at diagnosis. Survival times for diabetic dogs have improved considerably in the last 20 - 30 years but improvements in long-term survival may be limited by a natural canine lifespan. Survival of treated diabetic dogs is near equivalent to or greater than that of an age and gender matched general canine population using a necropsy based modified cohort general life table.

During the conduct of this study areas for further research have been highlighted. More vigorous attempts to create a current life table for UK dogs need to be made, preferably with a large enough data set to allow breed specific tables to be created. This would allow validation of the relative survival rate results presented in this study. Furthermore, in order to increase the usefulness of the results of this study, logistic and linear regression modelling could help identify prognostic or risk factors associated with longevity such as: preliminary concentrations of certain plasma biochemical analytes, including basal insulin, initial response to treatment, mean or integrated values for plasma biochemical analyses during treatment, in addition to age at diagnosis and the presence of concurrent illness.

Chapter 9:
Conclusions and future study

The results of each section of this study have been discussed in detail at the end of each chapter. The aim of this chapter is to summarise the findings of the work and to highlight potential areas for future study.

Epidemiological investigation of a large group of diabetic dogs concluded that age, gender and breed were all important factors associated with the presence of diabetes mellitus. Similar breed predispositions to those in previous reports (Wilkinson, 1960; Krook *et al*, 1960; Foster, 1975; Ling *et al*, 1977; Marmor *et al*, 1982; Doxey *et al*, 1985) were found. In particular, the over-representation of Poodles and Cairn terriers and the under-representation of German shepherd dogs have once again been confirmed. The findings of this study also served to emphasise the large proportion of diabetic dogs which were young (20% were < 7 years) and the complex relationship between age and gender associated with the condition.

No previous reports have compared the epidemiological characteristics of the condition in dogs from high and low or normal-risk breeds. The work presented in this thesis suggests that the disease in these breed groups may be different and highlights another area for future study. Candidate approaches for such an investigation include genetic and immunological techniques to attempt the identification of markers for disease sub-groups and to confirm or reject the hypothesis that an autoimmune process (similar to human Type I disease) may be involved in the 'high risk' breeds. In humans and experimental mouse models, genetic markers based in the major histocompatability complex (MHC) have been identified for susceptibility to diabetes mellitus (Vadheim and Rotter, 1992). Considerable work on MHC typing in the dog has been undertaken and it would now be possible to investigate MHC based genetic markers in spontaneous canine diabetes mellitus.

In Chapter 4, an attempt was made to identify characteristics of the clinically recognisable different forms of canine diabetes mellitus. This work showed that diabetic dogs with hypothyroidism were generally younger and those with hyperadrenocorticism were generally older than 'normal' diabetic dogs. In addition half of the dogs with

concurrent hypothyroidism and diabetes mellitus were of only two (uncommon) breeds. The pathophysiology of diabetes mellitus in these dogs may be different from 'normal' diabetic dogs and warrants further investigation again by genetic and immunological techniques to discover if this condition is a canine manifestation of an autoimmune based polyendocrine failure similar to those found in man.

Based on a small number of observations, basal plasma insulin analysis prior to ovariohysterectomy was a useful prognostic tool in metoestrus associated diabetes mellitus. However, more examples of the use of this technique need to be gathered in order for a more definite conclusion of its value to be reached.

Two tests new to the veterinary clinical laboratory, fructosamine and glycated haemoglobin, were validated for use with canine samples. Both tests performed well in the laboratory and were useful for diabetic monitoring. This was in contrast to alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase which were not useful for this purpose. The assessment of these tests was performed using relative operating characteristic curve analysis, a powerful technique for the comparison of laboratory tests which is likely to become increasingly popular in the realm of veterinary laboratory medicine.

Future work on the use of fructosamine and glycated haemoglobin in diabetic dogs must include an attempt to define algorithms which can relate fructosamine or glycated haemoglobin concentrations to mean plasma glucose concentrations. Such work would involve repeated plasma glucose measurements on a daily basis for a number of weeks or months and, therefore, would be better suited to an experimental environment than a clinical setting.

The pharmacokinetic analysis of a highly purified porcine mixed insulin zinc suspension (IZS-P) revealed that it is well absorbed following subcutaneous injection and that it has two reasonably predictable times of peak activity at around 4 and 11 hours and an overall duration of activity of between 14 and 24 hours. This is the first report of the pharmacokinetics of a lente insulin preparation in dogs. Opportunities for further study in this area include: a comparison of the pharmacokinetics of lente preparations of bovine and porcine origin and an investigation of the pharmacokinetics of protamine zinc insulin preparations which have not been reported so far, despite the availability of a veterinary licensed formulation (Insuvet PZI; Schering-Plough).

An efficacy study of IZS-P showed it to be effective in both the short and long term management of canine diabetes mellitus when used on a single daily basis in combination with a twice daily feeding schedule. Rates of bacterial infections and blindness resulting from diabetic cataracts were calculated during its long term use and were found to be 1 per 1.11 and 1 per 2.23 diabetic-dog-years respectively. Unfortunately, no comparison with other insulin preparations could be made at this stage but future work on the long term complications of canine diabetes mellitus (including cataracts, bacterial infections and small intestinal bacterial overgrowth) will be performed and will consider risk factors such as: age at diagnosis, gender, insulin type and dietary management using statistical methods for survival data analysis, including logistic regression modelling.

The use of a commercially produced canned 'high fibre' diet as an adjunct to the management of canine diabetes mellitus was studied. Feeding this diet reduced mean 24 hour plasma glucose concentration and the fluctuation in afternoon post-prandial glycaemia. Demeanour and activity scores and plasma concentrations of afternoon glucose, fructosamine and alkaline phosphatase were also improved. In addition, reductions were observed in plasma concentrations of total cholesterol, LDL cholesterol, free glycerol and non-esterified fatty acids. There was an association between feeding this diet and weight loss, reduced body condition scores and increased faecal volume score and in the very long term (> 4 months) there was a subjectively high prevalence of unexpected weight loss, recurrent diarrhoea and SIBO.

Two broad areas of future work were highlighted by the 'high fibre' diet study. The first is the need to characterise the absorbable fermentation products produced by this kind of diet and to investigate the association with decreased lipolysis evidenced by the reductions in plasma free glycerol and non-esterified fatty acid concentrations, perhaps utilising stable isotope tracer technology. Secondly, the association between this diet and colitis, unexpected weight loss, recurrent diarrhoea and SIBO needs to be investigated further. Relatively non-invasive technology such as proctoscopy and breath hydrogen analysis could be of use in such an investigation. Recently, encouraging work supported by Leander International Pet Foods Ltd., has highlighted the effects of short chain fatty acids resulting from intestinal bacterial fermentation of different fibre sources on colonic structure and the presence of colonic cryptitis (Reinhart, 1994).

Median survival time for diabetic dogs treated at the University of Glasgow Veterinary School by single daily injection of intermediate duration insulin was 2.71 years by product limit analysis. Survival was not affected by gender or the choice of insulin preparation but the presence of concurrent hyperadrenocorticism was important and

these dogs had a much poorer prognosis. A clinical life table method was also used to analyse the survival data and this method gave a very similar median survival time of 2.82 years confirming its validity in this situation. The clinical life table method also facilitated the generation of other survival indices such as hazard function and median remaining life time. Median remaining lifetime increased to a maximum of 3.11 for those dogs which survived the first 5 months of therapy and hazard function was greatest during the first 6 months. Comparisons with previously published series of dogs with diabetes mellitus confirmed that improvements in survival times have been achieved in the last 20 - 30 years but that improvements in very long term survival have been limited, perhaps by the combination of mean age at diagnosis and the natural life span of dogs.

The use of the clinical life table method in combination with necropsy based modified cohort general life tables created for dogs, facilitated the creation of relative survival rates. This proved to be a useful technique for the study of longevity in canine diabetes mellitus because of the wide age range of dogs affected. Survival rates for diabetic dogs were shown by this method to be close to or greater than that expected from an age and gender matched general canine population.

Both the longevity and epidemiology studies have highlighted the need for a national canine census and disease survey as the ultimate method of validation for their findings. The first and most readily achievable future study in this area would be the creation of gender and breed specific general life tables for dogs based, perhaps, on pet insurance company data. In this way, the survival data of many chronic, and particularly neoplastic, canine diseases can be more accurately studied and compared.

During this study, a number of aspects of the presentation and management of canine diabetes mellitus were investigated and many original observations were made such that significant progress in our understanding of the condition and its management was achieved. The practical application of this new information in the veterinary clinic has the potential to substantially improve the health, well-being and life-expectancy of many diabetic dogs.

As might be expected in a study of this kind, almost as many new questions arose as were answered, thus confirming the existence of further scope for the continued investigation of canine diabetes mellitus.

Appendices

Appendix 1:
List of manufacturers

Laboratory reagents and equipment

Amersham International plc	Amersham Place, Little Chalfont, Bucks. HP7 9BR
Ames	see Bayer
Bayer	Diagnostics Manufacturing, B-7501 Orcq-Tournai, Belgium
Beckman	Progress Road, Sands Industrial Estate, High Wycombe, Buckinghamshire. HP12 4JL
Bio-Stat Ltd	40 Osborne St, Bredbury, Stockport. SK6 2BT
Boehringer Mannheim (Diagnostics and Biochemicals) UK Ltd	Bell Lane, Lewes, East Sussex. BN7 1LG
Canberra Packard	Brook House, 14 Station Road, Pangbourne, Berkshire. RG8 7DT
Ciba-Corning	Colchester Road, Halstead, Essex. CO9 2DX
Diagnostic Products Corporation	5700 West 96th Street, Los Angeles, CA 90045 USA
Dynatech Laboratories Ltd	Daux Road, Billingshurst, West Sussex. RH14 9SJ
Greiner Labortechnik Ltd	Station Road, Cam, Dursley, Gloucestershire. GL11 5NS
Helena Laboratories UK	Team Valley Trading Estate, Gateshead, Tyne and Wear. NE11 0LH
IDDEX Laboratories Ltd	Milton Court, Churchfield Road, Chalfont St Peter, Buckinghamshire SL9 9EW
Instrumentation Laboratories Ltd	Kelvin Close, Warrington, Cheshire. WA3 7BP
Randox Laboratories	Ardmore, Diamond Road, Crumlin, Co Antrim, Northern Ireland. BT29 4QY
Roche Products Ltd	PO Box 8, Welwyn Garden City, Hertfordshire. AL7 3AY
Sigma Chemical Company Ltd	Fancy Road, Poole, Dorset. BH17 7NH
Sarstedt Ltd	66 Boston Road, Beaumont Leys, Leicester. LE14 1AW
Terumo Europe BV	3001 Leuven, Belgium
Wako Chemical GmBH	Nissanstr. 2, W-4040 Neuss 1, Germany.

Pharmaceuticals and foodstuffs

The following products were used in the management of the dogs included in this study:

Pharmaceuticals

Isopto-plain	artificial tears	Alcon Laboratories Ltd, Imperial Way, Watford, Herts. WD2 4YR
Lacrilube	liquid paraffin	Allergan Ltd, Crown Centre, Coronation Road, Cressex Industrial Estate, High Wycome, Bucks, HP12 3SH
Metronidazole Tablets	metronidazole	Approved Prescription Service (APS) Ltd, Water Street, Towngate, Bradford, West Yorks. BD12 9AF
Co-trimoxazole Tablets	co-trimoxazole	
Synacthen	tetracosactrin	Ciba Laboratories, Wimblehurst Road, Horsham, West Sussex. RH12 4AB
Evan's Isophane	isophane insulin (bovine) 100iu/ml	Evans Medical Ltd, Langhurst, Horsham, Sussex. RH12 4QD
Hypurin Isophane	isophane insulin (bovine) 100iu/ml	Fisons, Pharmaceutical Division, 12 Derby Road, Loughborough, Leicestershire. LE11 0BB
Eltroxin	l-thyroxine	Goldshield Pharmaceuticals, Bensham House, 324 Bensham Lane, Thornton Heath, Surrey. CR7 7EQ
Caninsulin	mixed insulin zinc suspension (porcine) 40iu/ml	Intervet UK Ltd, Science Park, Milton Road, Cambridge. CB4 4FP
Covinan	proligestone	
Salazopyrin Tablets	sulphasalazine	Kabi Pharmacia Ltd, Davy Ave, Knowlhill, Milton Keynes. MK5 8PH
Intralipid	soya bean oil and glycerol emulsion	
Lysodren	mitotane (o,p'-DDD)	Kingston Animal Health Ltd, Canbury 2000 Business Centre, Elm Crescent, Kingston-upon-Thames, Surrey, KT2 6HJ
Heparin (Mucous) Injection BP		Leo Laboratories Ltd, Longwick Road, Princes Risborough, Aylesbury, Bucks. HP17 9RR
Oxytetracycline Tablets	oxytetracycline	Millpledge Pharmaceuticals Ltd, Whinleys Estate, Church Lane, Clarborough, Retford, Notts. DN22 9NA
Phenylbutazone Tablets	phenylbutazone	
Amfipen Capsules	ampicillin	Mycofarm UK Ltd, Science Park, Milton Road, Cambridge. CB4 4FP
Delvosterone	proligestone	

Pharmaceuticals (cont.)

Lentard MC	mixed insulin zinc suspension (30% porcine, 70% bovine) 100iu/ml	Novo-Nordisk Pharmaceutical Ltd, Novo-Nordisk House, Broadfield Park, Brighton Road, Pease Pottage, Crawley, West Sussex. RH11 9RT
Chloromycetin Ophthalmic Ointment	chloramphenicol 1%	Parke-Davis Veterinary, Usk Road, Pontypool, Gwent. NP4 0YH
Zaquilan 12%	baquiloprim and sulphadimethoxine	Pitman-Moore Ltd, Crewe Hall, Crewe, Cheshire. CW1 1YR
Insuvet Lente	mixed insulin zinc suspension (bovine) 100iu/ml	Schering-Plough Animal Health, Schering-Plough House, Shire Park, Welwyn Garden City, Hertfordshire. AL7 1TW
Insuvet Neutral	soluble insulin (bovine) 100iu/ml	
Bovine TSH		Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset. BH17 7NH
Synulox Tablets	clavulanic acid and amoxycillin	SmithKline Beecham Animal Health Ltd, Hunter's Chase, Walton Oaks, Dorking Road, Tadworth,Surrey. KT20 7NT
Emequell Tablets	metoclopramide	
Kaobiotic	kaolin and neomycin	Upjohn Ltd, Animal Health Division, Fleming Way, Crawley, West Sussex. RH10 2NJ
Soloxine	l-thyroxine	Vet-2-Vet Marketing, PO Box 98, Bury St Edmunds, Suffolk. IP33 2QN

Feedstuffs

Prescription Diets r/d, i/d	Hill's Pet Products Ltd, 1 The Beacons, Beaconsfield Road, Hatfield, Herts. AL10 8EQ
Eukanuba Lamb and Rice	Leander International Petfoods Ltd, Arden Grange, London Road, Albourne, Hassocks, Wets Sussex. BN6 9BJ
Pedigree Canine High Fibre Diet	Pedigree Petfoods, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire. LE14 4RS
Pedigree Canine Selected Protein Diet	
Pedigree Chum	
Chappie	
Whiskas	

Appendix 2:
Details of 89 referred diabetic dogs used for epidemiological investigation

Case Number	Breed	Date of Birth	Date First Seen	Age (years)	Gender
109826	West Highland White terrier	10/01/76	08/01/89	13.00	F
109899	German shepherd dog	17/01/81	17/01/89	8.00	F
110113	Whippet	08/02/79	08/02/89	10.00	F
101600	Rottweiler	06/11/81	08/05/89	7.50	FN
111353	Labrador retriever	08/06/78	08/06/89	11.00	F
111513	Labrador retriever	03/07/81	03/07/89	8.00	F
111876	Cross breed	20/08/80	21/08/89	9.00	FN
109126	West Highland White terrier	04/10/78	20/09/89	10.96	MN
112324	Labrador retriever	04/10/78	04/10/89	11.00	F
112527	West Highland White terrier	23/10/80	24/10/89	9.00	M
112598	Samoyed	31/10/80	31/10/89	9.00	F
112714	Tibetan terrier	08/11/85	08/11/89	4.00	FN
113105	Cross breed	04/01/78	04/01/90	12.00	M
113430	Cross breed	07/02/82	07/02/90	8.00	M
113498	Cross breed	13/02/80	13/02/90	10.00	FN
113648	Scottish terrier	04/03/85	05/03/90	5.00	M
113936	Cairn terrier	08/04/76	09/04/90	14.00	FN
114460	Dobermann	05/12/82	06/06/90	7.50	F
114511	Miniature smooth-haired dachshund	12/06/81	13/06/90	9.00	M
114653	Cross breed	02/07/79	02/07/90	11.00	F
114695	Terrier cross	05/07/81	06/07/90	9.00	FN
114895	Bull terrier	07/08/83	07/08/90	7.00	M
115009	Miniature smooth-haired dachshund	15/08/80	16/08/90	10.00	FN
115088	Labrador retriever	29/08/80	30/08/90	10.00	MN
115131	Jack Russell terrier	05/09/82	05/09/90	8.00	F
115164	Cairn terrier	11/09/86	11/09/90	4.00	FN
115258	Cocker spaniel	24/09/80	25/09/90	10.00	FN
115678	Rottweiler	05/11/85	06/11/90	5.00	M
107674	Beagle	22/11/83	22/11/90	7.00	F
115980	Labrador retriever	07/12/82	07/12/90	8.00	FN
116146	English setter	11/01/84	11/01/91	7.00	M
116315	West Highland White terrier	30/07/76	30/01/91	14.50	FN
116318	Labrador retriever	29/01/85	30/01/91	6.00	M
116334	Cross breed	04/02/80	04/02/91	11.00	M
116594	Cairn terrier	05/09/85	07/03/91	5.50	M
116724	German shepherd dog	23/09/84	26/03/91	6.50	M
116744	Miniature poodle	01/04/82	02/04/91	9.00	M
116825	Australian terrier	23/06/83	11/04/91	7.80	M
117056	Jack Russell terrier	06/05/80	07/05/91	11.00	F
117162	Border terrier	16/05/78	17/05/91	13.00	M

Case Number	Breed	Date of Birth	Date First Seen	Age (years)	Gender
117164	Cross breed	19/05/80	20/05/91	11.00	M
117222	Whippet	22/05/81	25/05/91	10.01	F
117369	Cross breed	05/06/82	06/06/91	9.00	M
117705	Cross breed	10/07/77	11/07/91	14.00	FN
117783	Rottweiler	28/07/84	29/07/91	7.00	F
117898	Cairn terrier	12/03/91	12/08/91	0.42	F
103348	Border collie	26/08/81	27/08/91	10.00	F
118388	Labrador retriever	14/10/81	15/10/91	10.00	M
118568	Greyhound	03/11/82	04/11/91	9.00	F
118601	King Charles spaniel	05/11/88	06/11/91	3.00	M
118626	Jack Russell terrier	10/11/80	11/11/91	11.00	M
118680	Cocker spaniel	18/11/83	18/11/91	8.00	F
118839	Border terrier	04/12/84	05/12/91	7.00	FN
118939	Cross breed	23/12/83	23/12/91	8.00	F
119136	English setter	02/09/86	27/01/92	5.40	M
119147	Retriever cross	20/12/84	27/01/92	7.10	F
119173	Labrador retriever	28/01/83	29/01/92	9.00	M
119446	Cairn terrier	03/03/87	03/03/92	5.00	FN
119447	Wire haired fox terrier	03/03/84	03/03/92	8.00	M
97218	Labrador retriever	10/03/84	10/03/92	8.00	F
119821	Miniature poodle	05/05/80	05/05/92	12.00	F
120173	Collie cross	17/12/78	17/06/92	13.50	FN
120177	Collie cross	18/06/82	18/06/92	10.00	M
120811	Tibetan terrier	07/10/85	07/10/92	7.00	FN
120827	Rottweiler	07/03/87	12/10/92	5.60	M
120899	Yorkshire terrier	19/04/84	19/10/92	8.50	FN
120931	Cairn terrier	16/03/88	22/10/92	4.60	MN
121031	Cocker spaniel	02/11/83	02/11/92	9.00	FN
121062	Cairn terrier	09/11/85	09/11/92	7.00	FN
121143	Border collie	17/11/82	17/11/92	10.00	F
121192	Cairn terrier	24/11/83	24/11/92	9.00	MN
121228	Collie cross	01/12/83	01/12/92	9.00	MN
121252	Rottweiler	03/12/89	03/12/92	3.00	F
121320	Toy poodle	05/12/83	15/12/92	9.03	F
109434	Miniature poodle	07/11/80	09/02/93	12.26	FN
121626	Jack Russell terrier	16/02/81	16/02/93	12.00	FN
122026	Bearded collie	26/04/82	26/04/93	11.00	FN
122125	Border collie	11/05/86	11/05/93	7.00	M
122131	German shepherd dog	11/05/81	11/05/93	12.00	M
122150	Yorkshire terrier	17/05/81	17/05/93	12.00	FN
122200	Collie cross	18/06/81	24/05/93	11.93	F
122551	Labrador retriever	13/07/86	13/07/93	7.00	F
122686	Labrador retriever	13/08/87	13/08/93	6.00	M
122739	Shetland sheepdog	22/08/88	23/08/93	5.00	M
122768	Jack Russell terrier	30/08/87	30/08/93	6.00	M
122970	Jack Russell terrier	23/02/82	30/09/93	11.60	M
122973	Labrador retriever	01/10/83	01/10/93	10.00	FN
123086	Labrador retriever	18/10/86	18/10/93	7.00	M
124186	King Charles spaniel	24/03/83	24/03/94	11.00	FN

Appendix 3:
Numbers of dogs in the hospital control population categorised by age, gender and referral group

Age (years)	Gender	Referral group						
		General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
0	F	20	7	4	3	23	0	57
	FN	0	0	0	0	1	0	1
	M	13	16	6	5	41	0	81
0 Total		33	23	10	8	65	0	139
1	F	6	7	2	8	16	1	40
	FN	3	5	0	1	1	0	10
	M	15	6	3	4	14	1	43
	MN	0	1	0	0	3	0	4
1 Total		24	19	5	13	34	2	97
2	F	5	6	2	0	7	1	21
	FN	2	1	2	0	1	1	7
	M	8	6	3	1	8	0	26
	MN	3	0	1	0	1	0	5
2 Total		18	13	8	1	17	2	59
3	F	5	8	0	1	6	3	23
	FN	3	3	3	0	1	1	11
	M	6	4	3	2	4	0	19
	MN	1	0	0	1	0	0	2
3 Total		15	15	6	4	11	4	55
4	F	7	11	2	2	5	2	29
	FN	6	5	0	0	6	0	17
	M	5	8	6	1	7	0	27
	MN	1	0	0	0	1	0	2
4 Total		19	24	8	3	19	2	75
5	F	8	6	3	0	4	0	21
	FN	3	4	0	0	4	0	11
	M	10	7	4	4	9	0	34
	MN	0	1	1	0	0	0	2
5 Total		21	18	8	4	17	0	68
6	F	6	3	0	2	5	0	16
	FN	3	7	1	6	2	0	19
	M	12	15	3	0	4	0	34
	MN	0	1	0	0	0	0	1
6 Total		21	26	4	8	11	0	70
7	F	2	5	2	1	4	0	14
	FN	7	6	2	4	5	0	24
	M	12	7	6	4	5	0	34
	MN	0	2	0	0	0	0	2
7 Total		21	20	10	9	14	0	74

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
8	F	3	8	0	0	0	0	11
	FN	8	7	3	1	1	0	20
	M	10	17	1	4	7	0	39
	MN	0	1	2	0	0	0	3
8 Total		21	33	6	5	8	0	73
9	F	5	4	1	0	0	0	10
	FN	6	5	2	5	4	0	22
	M	9	9	6	1	4	0	29
	MN	1	1	1	0	0	0	3
9 Total		21	19	10	6	8	0	64
10	F	2	0	1	2	3	0	8
	FN	2	1	2	0	0	0	5
	M	9	11	2	0	2	1	25
	MN	0	1	2	0	0	0	3
10 Total		13	13	7	2	5	1	41
11	F	5	0	0	0	0	0	5
	FN	3	3	1	1	1	0	9
	M	9	7	0	1	2	0	19
	MN	0	2	0	0	1	0	3
11 Total		17	12	1	2	4	0	36
12	F	0	3	0	1	0	0	4
	FN	2	3	0	0	0	0	5
	M	2	3	0	4	1	0	10
12 Total		4	9	0	5	1	0	19
13	F	1	1	0	0	0	0	2
	FN	1	1	0	2	1	0	5
	M	2	2	0	1	0	0	5
13 Total		4	4	0	3	1	0	12
14	F	0	0	0	1	0	0	1
	FN	0	1	0	0	0	0	1
	M	0	2	0	0	0	0	2
14 Total		0	3	0	1	0	0	4
15	F	1	0	0	0	0	0	1
	FN	1	1	0	0	0	0	2
	M	0	1	0	0	0	0	1
15 Total		2	2	0	0	0	0	4
Grand total		254	253	83	74	215	11	890

Appendix 4:
Numbers of dogs in a time matched hospital control population categorised by breed, gender and referral group

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Akita								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Beagle								
	F	0	0	0	0	0	1	1
	M	0	1	0	1	0	0	2
Total		0	1	0	1	0	1	3
Belgian shepherd dog								
	M	0	2	1	0	0	0	3
Total		0	2	1	0	0	0	3
Bernese mountain dog								
	F	1	1	1	0	2	0	5
	M	0	0	0	0	2	0	2
Total		1	1	1	0	4	0	7
Bichon frise								
	F	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Bloodhound								
	M	0	0	0	0	2	0	2
Total		0	0	0	0	2	0	2
Bouvier de Flandres								
	M	0	2	0	0	0	0	2
Total		0	2	0	0	0	0	2
Boxer								
	F	3	1	1	1	2	0	8
	FN	0	1	0	1	0	0	2
	M	4	6	2	1	1	0	14
	MN	0	1	0	0	0	0	1
Total		7	9	3	3	3	0	25
Briard								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Bulldog								
	FN	0	1	0	0	0	0	1
	M	1	1	0	0	0	0	2
Total		1	2	0	0	0	0	3

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Bullmastiff								
	F	0	0	0	0	1	0	1
	FN	1	0	0	0	0	0	1
	M	0	2	2	0	2	0	6
Total		1	2	2	0	3	0	8
Chihuahua								
	F	0	0	1	1	0	0	2
Total		0	0	1	1	0	0	2
Chow Chow								
	M	0	0	0	1	0	0	1
Total		0	0	0	1	0	0	1
Bearded collie								
	F	0	0	0	0	3	1	4
	FN	0	1	0	0	0	0	1
	M	3	1	0	0	2	0	6
	MN	1	0	0	0	0	0	1
Total		4	2	0	0	5	1	12
Border collie								
	F	5	4	0	0	3	0	12
	FN	1	3	0	0	0	1	5
	M	3	6	0	1	5	0	15
	MN	1	0	1	0	1	0	3
Total		10	13	1	1	9	1	35
Rough collie								
	F	0	1	0	0	0	0	1
	M	2	2	0	0	0	0	4
Total		2	3	0	0	0	0	5
Smooth collie								
	F	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Huntaway collie								
	F	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Collie cross								
	FN	2	2	0	0	0	0	4
	M	2	7	2	0	2	0	13
Total		4	9	2	0	2	0	17
Corgi								
	F	0	0	0	0	1	0	1
	FN	0	0	0	1	0	0	1
Total		0	0	0	1	1	0	2
Dachshund								
	FN	0	1	0	0	1	0	2
	M	0	1	0	0	0	0	1
Total		0	2	0	0	1	0	3

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Long haired dachshund								
	M	0	0	0	0	0	1	1
Total		0	0	0	0	0	1	1
Miniature long-haired dachshund								
	F	1	0	0	0	0	0	1
	M	1	0	0	0	0	0	1
Total		2	0	0	0	0	0	2
Miniature smooth-haired dachshund								
	F	1	1	0	0	0	0	2
	M	0	0	0	1	0	0	1
Total		1	1	0	1	0	0	3
Miniature wire-haired dachshund								
	F	0	0	1	0	0	0	1
Total		0	0	1	0	0	0	1
Smooth-haired dachshund								
	F	0	0	1	0	0	0	1
	M	0	0	1	0	0	0	1
Total		0	0	2	0	0	0	2
Dalmatian								
	F	1	0	0	0	0	0	1
	FN	1	0	0	0	0	0	1
	M	1	1	0	0	1	0	3
Total		3	1	0	0	1	0	5
Deerhound								
	M	1	0	0	0	1	1	3
Total		1	0	0	0	1	1	3
Dobermann								
	F	0	4	1	0	1	0	6
	FN	1	2	1	1	1	0	6
	M	4	0	0	1	2	0	8
Total		5	6	3	2	4	0	20
Elkhound								
	M	0	1	0	0	0	0	1
Total		0	1	0	0	0	0	1
Foxhound								
	F	1	0	0	0	0	0	1
	M	1	0	0	0	0	0	1
Total		2	0	0	0	0	0	2
German shepherd dog								
	F	10	11	1	1	9	1	33
	FN	5	3	1	1	3	0	13
	M	7	25	9	0	16	0	57
	MN	0	1	0	0	1	0	2
Total		22	40	11	2	29	1	105

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalmology	Orthopaedics	Reproduction	Total
German shepherd dog cross								
	FN	0	1	0	0	1	0	2
	M	3	0	0	0	0	0	3
	MN	0	0	0	0	1	0	1
Total		3	1	0	0	2	0	6
Great Dane								
	F	0	0	0	0	0	2	2
	FN	0	1	0	0	1	0	2
	M	4	1	0	0	3	0	8
Total		4	2	0	0	4	2	12
Greyhound								
	F	2	0	1	0	2	0	5
	FN	0	0	0	0	1	0	1
	M	0	1	0	0	2	0	3
Total		2	1	1	0	5	0	9
Afghan hound								
	M	0	0	1	0	0	0	1
Total		0	0	1	0	0	0	1
Basset hound								
	F	0	1	0	0	0	0	1
	M	1	0	0	0	1	0	2
Total		1	1	0	0	1	0	3
Irish wolfhound								
	F	2	0	0	0	0	0	2
	M	0	0	1	0	0	0	1
Total		2	0	1	0	0	0	3
Husky								
	M	1	0	0	0	0	0	1
	MN	0	1	0	0	0	0	1
Total		1	1	0	0	0	0	2
Japenese chin								
	FN	0	1	0	0	0	0	1
Total		0	1	0	0	0	0	1
Keeshond								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Lhasa apso								
	FN	1	0	1	0	0	0	2
Total		1	0	1	0	0	0	2
Lurcher								
	F	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Alaskan malamute								
	F	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Maltese								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Tibetan mastiff								
	F	0	0	0	0	0	1	1
Total		0	0	0	0	0	1	1
Newfoundland								
	F	0	1	0	0	1	0	2
	FN	0	1	0	0	1	0	2
Total		0	2	0	0	2	0	4
Old English sheepdog								
	FN	1	1	0	1	0	0	3
	M	3	2	1	1	1	0	8
	MN	0	0	1	0	1	0	2
Total		4	3	2	2	2	0	13
Papillon								
	F	0	0	1	0	0	0	1
	FN	1	0	0	0	0	0	1
Total		1	0	1	0	0	0	2
Pekingese								
	F	0	0	1	1	0	0	2
Total		0	0	1	1	0	0	2
Pinscher								
	F	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Miniature pinscher								
	M	0	1	0	0	0	0	1
Total		0	1	0	0	0	0	1
Standard pinscher								
	F	0	0	1	0	0	0	1
Total		0	0	1	0	0	0	1
English pointer								
	F	0	0	0	0	1	0	1
	FN	0	0	1	0	0	0	1
	MN	0	0	1	0	0	0	1
Total		0	0	2	0	1	0	3
German short-haired pointer								
	F	0	0	0	1	0	0	1
	FN	0	0	0	1	0	0	1
	M	1	1	0	0	0	0	2
Total		1	1	0	2	0	0	4
Pomeranian								
	M	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Miniature poodle								
	F	0	1	0	0	0	0	1
	FN	1	0	0	3	0	0	4
Total		1	1	0	3	0	0	5
Standard poodle								
	F	0	2	0	0	0	0	2
	FN	0	1	1	0	0	1	3
	M	1	0	0	0	0	0	1
Total		1	3	1	0	0	1	6
Toy poodle								
	FN	0	1	0	0	0	0	1
	M	0	1	0	0	0	0	1
	MN	0	0	1	0	0	0	1
Total		0	2	1	0	0	0	3
Pug								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Curly coated retriever								
	M	0	0	1	0	0	0	1
Total		0	0	1	0	0	0	1
Flat coated retriever								
	F	1	0	0	1	0	0	2
	FN	1	0	0	0	0	0	1
	M	1	0	1	0	0	0	2
	MN	0	1	0	0	0	0	1
Total		3	1	1	1	0	0	6
Golden retriever								
	F	7	1	1	0	4	0	13
	FN	5	2	2	0	0	0	9
	M	11	3	0	2	6	0	22
	MN	1	0	1	1	0	0	3
Total		24	6	4	3	10	0	47
Labrador retriever								
	F	4	8	0	3	9	1	25
	FN	4	6	2	4	3	0	19
	M	11	14	3	0	24	0	52
	MN	2	0	1	0	0	0	3
Total		21	28	6	7	36	1	99
Retriever cross								
	F	1	0	0	0	0	0	1
	FN	0	2	0	0	1	0	3
	M	1	1	0	0	1	0	3
	MN	1	0	0	0	0	0	1
Total		3	3	0	0	2	0	8

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Rhodesian ridgeback								
	F	0	0	0	0	2	0	2
	M	1	0	0	0	0	0	1
Total		1	0	0	0	2	0	3
Rottweiler								
	F	2	1	1	0	8	0	12
	FN	0	0	1	0	1	0	2
	M	1	2	1	1	5	0	10
	MN	0	0	0	0	1	0	1
Total		3	3	3	1	15	0	25
Saint Bernard								
	F	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Samoyed								
	FN	0	3	0	0	0	0	3
Total		0	3	0	0	0	0	3
Giant schnauzer								
	FN	1	0	0	0	0	0	1
	M	1	0	0	0	0	0	1
Total		2	0	0	0	0	0	2
Miniature schnauzer								
	F	0	0	0	0	1	0	1
	M	0	1	0	0	1	0	2
Total		0	1	0	0	2	0	3
Standard schnauzer								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
English setter								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Gordon setter								
	F	1	0	0	0	1	0	2
	FN	1	1	0	0	0	0	2
	M	1	2	1	0	1	0	5
Total		3	3	1	0	2	0	9
Irish setter								
	F	2	1	1	0	1	0	5
	M	3	0	0	0	1	0	4
	MN	0	1	0	0	0	0	1
Total		5	2	1	0	2	0	10
Setter cross								
	M	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Shetland sheepdog								
	F	1	1	0	0	1	0	3
	FN	1	0	0	0	1	0	2
	M	2	2	0	0	1	0	5
Total		4	3	0	0	3	0	10
Shih tzu								
	FN	0	0	1	1	0	0	2
	M	0	1	0	0	0	0	1
Total		0	1	1	1	0	0	3
Cavalier King Charles spaniel								
	F	1	4	0	0	2	0	7
	FN	3	1	0	0	2	0	6
	M	0	1	1	2	1	0	5
	MN	0	1	0	0	0	0	1
Total		4	7	1	2	5	0	19
Cocker spaniel								
	F	3	1	1	2	2	0	9
	FN	1	0	0	0	0	0	1
	M	6	4	0	3	1	0	14
Total		10	5	1	5	3	0	24
English Springer spaniel								
	F	4	5	0	2	1	0	12
	FN	1	2	1	1	0	0	5
	M	3	4	3	2	6	0	18
Total		8	11	4	5	7	0	35
King Charles spaniel								
	F	1	0	0	0	2	0	3
	FN	0	1	0	0	0	0	1
	MN	0	1	0	0	0	0	1
Total		1	2	0	0	2	0	5
Tibetan spaniel								
	F	0	0	0	2	0	0	2
	M	0	1	0	0	0	0	1
Total		0	1	0	2	0	0	3
Welsh Springer spaniel								
	F	0	1	0	1	0	0	2
Total		0	1	0	1	0	0	2
Blue roan spaniel								
	FN	0	0	0	1	0	0	1
Total		0	0	0	1	0	0	1
Spaniel cross								
	FN	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Spinone								
	M	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Australian terrier								
	FN	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Bedlington terrier								
	F	0	2	0	0	0	0	2
	M	1	0	0	0	0	0	1
Total		1	2	0	0	0	0	3
Border terrier								
	F	0	1	0	0	0	0	1
	M	0	0	1	0	1	0	2
Total		0	1	1	0	1	0	3
Bull terrier								
	F	3	0	0	1	0	0	4
	M	6	0	0	0	0	0	6
Total		9	0	0	1	0	0	10
Cairn terrier								
	F	1	3	0	0	0	0	4
	FN	0	2	0	0	0	0	2
	M	0	1	0	1	0	0	2
Total		1	6	0	1	0	0	8
Dandie Dinmont terrier								
	M	0	1	0	0	0	0	1
Total		0	1	0	0	0	0	1
Wire-haired fox terrier								
	F	0	0	0	1	1	0	2
	FN	1	0	1	0	0	0	2
	MN	0	1	0	0	0	0	1
Total		1	1	1	1	1	0	5
Jack Russell terrier								
	F	2	2	0	2	0	0	6
	FN	0	2	0	1	0	0	3
	M	0	2	3	1	2	0	8
Total		2	6	3	4	2	0	17
Lakeland terrier								
	FN	0	0	0	0	1	0	1
Total		0	0	0	0	1	0	1
Scottish terrier								
	F	1	0	0	0	0	0	1
	FN	1	0	0	0	0	0	1
	M	0	2	0	0	0	0	2
Total		2	2	0	0	0	0	4
Skye terrier								
	FN	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1

Age (years)	Gender	Referral group: General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Soft Coated Wheaten terrier								
	F	6	0	0	0	0	0	6
	M	4	0	0	0	0	0	4
Total		10	0	0	0	0	0	10
Staffordshire bull terrier								
	F	1	0	0	0	0	0	1
	FN	1	0	0	0	1	0	2
	M	0	0	1	0	2	0	3
Total		2	0	1	0	3	0	6
Tibetan terrier								
	M	1	0	0	0	0	0	1
Total		1	0	0	0	0	0	1
Welsh terrier								
	M	0	0	0	1	0	0	1
Total		0	0	0	1	0	0	1
West Highland White terrier								
	F	2	3	0	0	0	0	5
	FN	1	3	1	1	1	0	7
	M	3	2	0	2	1	0	8
	MN	0	1	0	0	0	0	1
Total		6	9	1	3	2	0	21
Yorkshire terrier								
	F	2	1	2	0	4	0	9
	FN	0	1	0	0	0	0	1
	M	4	3	3	0	2	0	12
Total		6	5	5	0	6	0	22
Terrier cross								
	FN	0	1	0	0	1	0	2
	M	0	0	1	0	0	0	1
Total		0	1	1	0	1	0	3
Hungarian vizsla								
	F	0	1	0	0	0	0	1
	FN	0	0	0	0	1	0	1
	M	0	0	1	0	0	0	1
Total		0	1	1	0	1	0	3
Weimaraner								
	F	0	1	0	1	0	0	2
	M	1	0	0	2	0	0	3
	MN	0	1	0	0	0	0	1
Total		1	2	0	3	0	0	6
Whippet								
	F	0	1	0	0	0	0	1
	M	1	0	0	1	0	0	2
Total		1	1	0	1	0	0	3
Sharpei								
	M	0	1	0	0	0	0	1
Total		0	1	0	0	0	0	1

Age (years)	Gender	Referral group						
		General Medicine	General Surgery	Neurology	Ophthalm-ology	Ortho-paedics	Repro-duction	Total
Cross bred								
	F	1	3	0	0	3	0	7
	FN	11	5	2	2	5	0	25
	M	8	8	1	7	6	0	30
	MN	0	0	1	0	1	0	2
Total		20	16	4	9	15	0	64
Grand total		254	253	83	74	215	11	890

Appendix 5:
Synacthen stimulation test results in 7 dogs with concurrent hypothyroidism and diabetes mellitus

Hospital number	Date	Basal cortisol (nmol/l)	Post-ACTH cortisol at 45 or 60 minutes	Post-ACTH cortisol at 90 minutes
107303	18/02/88	57	240	*
112714	08/02/90	38	556	*
113648	18/02/91	57	373	575
116146	11/01/91	35	178	250
120811	07/10/92	33	201	239
120931	22/10/92	228	287	*
124684	07/06/94	56	306	*

Appendix 6:
Initial biochemistry, body condition score and basal insulin concentrations in dogs with straightforward diabetes mellitus and dogs with associated syndromes

Hospital number	Gender	Age years	Days	Glucose mmol/l	Choles mmol/l	ALKP U/l	ALT U/l	AST U/l	Insulin µIU/ml	Condition score
Hyperadrenocorticism										
109434	FN	12.3	0	27.2	12.3	668	66	25	9.2	0
109826	F	13.0	7	8.9	10.0	861	218	51	*	4
113105	M	12.0	0	30.1	9.7	3114	397	77	*	5
113936	FN	14.0	0	31.0	5.8	*	161	72	*	4
116315	FN	14.5	0	24.8	4.7	1602	79	43	*	4
116318	M	6.0	0	19.3	10.0	117	81	14	*	4
116744	M	9.0	0	26.0	6.0	627	317	330	4.1	4
117369	M	9.8	0	19.6	7.6	495	179	54	5.8	3
117705	FN	13.8	77	*	*	*	*	*	27.0	*
118388	M	10.0	3	27.3	6.0	1431	100	66	3.6	4
118626	M	11.0	0	71.5	12.2	15873	114	74	17.2	4
122970	M	11.6	0	23.0	16.1	1948	255	49	41.0	3
122973	FN	10.0	0	16.2	8.2	3850	182	21	41.0	4
Hypothroidism										
112714	FN	4.0	0	24.6	7.8	1202	180	163	*	4
113648	M	5.0	0	19.9	14.0	8259	78	43	5.0	4
119136	M	5.4	0	25.0	20.2	100	162	64	4.8	3
124684	FN	8.0	0	29.7	8.1	891	86	82	11.5	5
Metoestrus										
103348	F	10.0	0	19.6	7.1	237	173	47	3.2	3
109899	F	8.0	0	32.4	9.8	119	29	18	*	4
110113	F	10.0	0	30.4	11.9	164	138	55	*	3
111353	F	11.0	0	24.5	8.4	822	67	27	*	2
114460	F	7.5	0	21.5	12.2	1465	85	53	6.0	3
114653	F	11.0	0	20.5	8.3	1479	51	23	*	4
117056	F	11.0	0	16.4	12.8	929	113	23	93.0	3
118568	F	9.0	0	20.1	7.8	145	82	35	189.0	4
119821	F	12.0	6	28.2	9.5	288	67	20	16.2	4
120899	F	8.5	0	13.1	6.4	168	80	38	6.2	2
121143	F	9.9	36	*	*	*	*	*	10.7	4
122200	F	11.9	4	24.6	8.1	1129	254	60	6.5	3

Hospital number	Gender	Age years	Days	Glucose mmol/l	Choles mmol/l	ALKP U/l	ALT U/l	AST U/l	Insulin µIU/ml	Condition score
Progestagen associated										
112598	F	8.0	2	21.0	9.3	571	76	28	*	4
115131	F	8.0	0	22.7	7.0	97	135	99	*	3
Islet cell hypolasia										
117898	F	0.4	0	23.6	3.7	748	55	56	3.0	3
'Normal'										
97218	FN	8.0	6	27.3	4.1	2984	133	82	8.0	6
101600	FN	7.5	0	32.5	7.7	245	62	25	*	4
105869	F	8.6	0	27.9	*	2046	31	18	*	1
107674	F	7.0	0	25.4	8.9	152	199	43	*	4
109126	M	11.0	0	21.7	7.8	211	84	26	*	4
111513	F	8.0	0	31.8	6.4	925	44	43	*	2
111876	FN	9.0	0	23.6	5.7	3372	399	274	*	4
114511	M	9.0	0	21.8	11.1	1596	269	53	*	4
114695	FN	8.7	141	*	*	*	*	*	13.0	*
115009	FN	10.0	16	18.1	13.2	1164	444	85	7.0	3
115164	FN	4.0	0	21.8	8.4	1085	61	38	*	4
115678	M	5.0	0	22.8	8.7	472	185	144	*	3
115980	FN	8.0	1	27.5	10.0	3858	61	34	*	5
116334	M	11.0	0	31.1	6.2	372	97	24	*	4
116594	M	5.5	0	22.4	6.9	224	50	14	9.0	4
116724	M	6.7	0	20.3	9.2	89	134	119	*	0
116825	M	7.8	0	26.6	5.0	140	172	36	*	4
117164	M	11.0	0	25.7	13.5	1835	965	143	*	3
117222	F	10.0	1	19.5	14.6	274	146	25	6.2	4
117783	F	7.0	3	14.2	10.9	3102	97	270	*	4
118601	M	3.0	1	13.1	8.3	6930	64	43	5.7	4
118680	F	8.0	0	18.5	10.6	320	41	35	9.6	5
118839	FN	7.0	1	19.0	6.1	505	37	43	3.0	4
119147	FN	7.1	0	20.0	6.7	322	252	92	4.3	4
119173	M	11.0	4	35.1	13.5	3746	468	204	9.6	4
119447	M	8.0	0	17.0	7.1	1774	61	35	*	3
120173	FN	13.5	0	27.3	12.2	125	94	74	*	4
120177	M	10.0	0	16.9	20.4	1267	172	75	*	0
120827	M	5.5	31	*	*	*	*	*	10.8	*
121062	FN	7.0	2	16.9	6.2	7568	192	56	9.0	3
121192	M	9.1	0	19.0	7.9	5786	307	109	14.9	4
121228	M	9.0	0	21.1	6.0	492	178	109	7.1	5
121626	FN	12.0	0	24.7	6.1	526	105	92	*	4
122125	M	7.0	0	25.7	8.8	2585	151	86	5.9	3
122131	M	12.0	0	16.9	7.9	535	115	44	8.7	4
122686	M	6.0	0	16.8	7.7	1039	84	21	9.3	5
122768	M	5.7	0	18.4	5.6	368	94	38	10.1	4
123086	M	7.0	0	18.3	6.3	192	57	27	12.0	5
124186	FN	11.0	0	35.7	12.7	4128	229	58	10.5	4
124748	FN	10.0	0	22.2	6.2	522	17	20	*	4
124785	FN	9.0	0	19.8	5.2	398	62	17	15.6	2
125113	FN	7.6	0	20.4	6.8	1338	267	222	*	4

Appendix 7:
Endocrine tests in dogs with straightforward diabetes mellitus

Hospital number	Date	Cortisol pre-ACTH	Cortisol at 45 or 60 min	Cortisol at 90 min	Thyroxin pre-TSH	Thyroxin at 6 hrs post-TSH
97218	17/06/92	*	*	*	18.1	64.3
101600	23/02/93	*	*	*	20.7	53.7
107674	21/01/91	*	*	*	19.4	*
109126	29/10/90	122	320	*	*	*
114695	14/08/91	*	*	*	16.3	40.8
115009	21/08/90	27	524	527	*	*
115678	20/08/93	*	*	*	28.0	63.0
116594	21/03/91	78	289	325	*	*
116825	11/04/91	53	194	222	*	*
117164	25/07/91	112	301	380	*	*
118601	29/09/93	*	*	*	32.0	75.0
118680	06/12/91	*	*	*	15.3	*
118839	08/08/94	66	308	*	*	*
119173	11/02/92	29	245	378	*	*
119446	03/03/92	40	301	*	*	*
119447	03/03/92	69	524	*	*	*
121062	28/09/93	89	475	520	24.0	87.0
121192	22/09/93	151	435	551	38.0	88.0
121228	01/12/92	68	340	354	*	*
122686	13/08/93	*	*	*	37.0	54.0
122739	23/08/93	149	285	*	52.9	*
122768	09/09/93	*	*	*	24.0	94.0
123086	18/10/93	*	*	*	16.3	47.0
124186	24/03/94	44	302	*	*	*
124748	14/06/94	129	296	*	*	*

Appendix 8:
Post-stabilisation Insulin doses for dogs with straightforward diabetes mellitus and those with associated syndromes

Hospital number	Mean insulin dose IU/kg	Minimum insulin dose	Maximum insulin dose	Number of visits
Hyperadrenocorticism				
109434	2.08	1.73	2.40	4
109826	2.70	1.25	3.56	7
113105	1.21	1.21	1.21	1
113498	2.34	2.34	2.34	2
113936	2.33	2.33	2.33	1
114895	1.25	1.25	1.25	1
116315	1.32	1.15	2.08	6
116318	2.42	2.42	2.42	1
116744	2.34	2.00	2.67	9
117705	2.51	2.02	3.00	2
118388	1.78	1.72	1.83	2
119010	4.33	4.33	4.33	1
122026	2.24	2.24	2.24	1
122150	5.24	5.21	5.26	2
122970	3.79	3.79	3.79	2
Hypothyroidism				
107303	1.72	1.72	1.72	1
112714	2.51	1.60	3.09	5
113648	2.32	1.45	2.80	9
116146	0.76	0.40	0.76	1
120811	2.73	2.73	2.73	1
120931	3.24	3.24	3.24	1
Metoestrus				
103348	0.82	0.47	1.20	4
109899	0.53	0.00	1.06	2
110113	0.18	0.00	0.31	6
110364	1.19	0.68	1.52	3
111353	1.07	0.44	1.51	10
112324	0.92	0.92	0.92	1
114460	1.02	0.53	1.66	17
114653	1.14	1.14	1.14	1
120899	1.26	0.83	1.94	10
121143	0.60	0.54	0.66	2
122200	0.75	0.00	1.46	8
Islet cell hypoplasia				
117898	1.42	0.89	2.08	18

Hospital number	Mean insulin dose IU/kg	Minimum insulin dose	Maximum insulin dose	Number of visits
Progestagen associated				
112598	0.78	0.70	0.83	3
115131	1.20	0.93	1.49	26
122551	2.67	1.58	3.45	6
'Normal'				
97218	1.29	1.02	1.72	18
101600	1.07	0.00	1.78	12
107674	0.92	0.51	1.16	8
109126	1.83	1.57	2.20	9
111513	3.00	3.00	3.00	1
111876	1.01	0.95	1.10	6
114695	1.50	1.33	1.90	14
115009	2.31	1.79	2.64	5
115164	2.18	1.70	2.35	5
115258	1.80	1.60	2.00	2
115678	1.24	0.79	2.31	23
115980	1.03	0.78	1.68	29
116334	1.31	1.08	1.47	6
116594	2.09	1.67	2.73	17
116825	1.31	0.92	1.56	26
117162	1.77	0.92	2.62	2
117164	0.60	0.38	0.93	13
117222	1.45	0.52	8.85	18
117783	1.62	1.00	1.88	5
118601	0.66	0.00	1.15	16
118680	0.55	0.55	0.55	2
118839	1.13	0.35	1.52	6
118939	1.59	1.59	1.59	1
119147	1.28	0.72	1.93	18
119173	1.25	0.93	1.70	10
119446	3.42	3.30	3.60	5
119447	1.74	1.46	2.15	4
120173	1.00	0.53	1.39	18
120827	1.27	1.02	1.50	15
121031	1.90	1.90	1.90	1
121062	2.97	1.95	3.89	4
121192	1.81	1.42	2.60	8
121228	1.93	1.55	2.47	23
121252	1.20	1.00	1.45	11
121320	3.17	3.17	3.17	1
121626	1.66	1.47	1.82	4
122131	1.10	1.00	1.22	4
122686	1.55	1.32	1.88	5
122739	0.59	0.36	0.83	8
122768	1.64	1.58	1.74	8
123086	1.49	1.25	1.64	5
124186	1.13	0.95	1.29	4
124748	3.14	2.13	3.93	5
124785	1.43	1.19	1.82	3
125113	1.02	0.77	1.28	4

N.B. excludes insulin doses for dogs on Lysodren or thyroxin therapy

Appendix 9:
Caiculation of the area under a relative operating characteristic curve

The calculation of area under the ROC curve was calculated according to the method of Jensen and Poulsen (1992) based on that of Hanley and McNeil (1982). The following example presents data from 10 cut-off values for plasma fructosamine concentration used to differentiate between samples form dogs under poor or very poor glycaemic control (glycaemic control score 2 and 3) and those from dogs with fair to excellent diabetic control (glycaemic control groups 4, 5 and 6).

	fructosamine cut-off value µmol/l									
	430	431	432	434	435	439	440	442	443	444
Nx	3	2	2	1	0	0	2	1	2	1
Nbx	174	177	179	181	182	182	182	184	185	187
Ax	0	0	0	0	2	1	1	1	1	0
Aax	66	66	66	66	64	63	62	61	60	60
WPartial	198	132	132	66	0	0	125	62	121	60
Q1Partial	13068	8712	8712	4356	0	0	7813	3782	7321	3600
Q2Partial	0	0	0	0	66248	33124	33489	34040	34596	0

Nx The number of results from dogs with glycaemic control score 4, 5 or 6 at the cut-off value
Nbx The number of results from dogs with glycaemic control score 4, 5 or 6 below the cut-off value
Ax The number of results from dogs with glycaemic control score 2 or 3 at the cut-off value
Aax The number of results from dogs with glycaemic control score 2 or 3 above the cut-off value

At each cut-off value the partial terms of the are under the ROC curve (WPartial) and of the intermediate probabilities Q1 Partial and Q2 Partial are calculated using the formulae:

$$W_{Partial} = (Nx * Aax) + (0.5 * Nx * Ax)$$
$$Q1_{Partial} = Nx * [(Aax * Aax) + (Aax * Ax) + (0.33 * (Ax * Ax))]$$
$$Q2_{Partial} = Ax * [(Nbx * Nbx) + (Nbx * Nx) + (0.33 * (Nx * Nx))]$$

When W Partial, Q1 Partial and Q2 Partial have been calculated for each cut-off value, the final value of the area under the ROC curve (W) and of the intermediate probabilities Q1 and Q2 are calculated using the following formulae in which Nd represents the number of results from dogs in glycaemic control groups 2 and 3 and Nn represents the number from dogs in glycaemic control groups 4, 5 and 6.

$W = (\Sigma\ W_{Partial})/(Nd * Nn)$
$Q_1 = (\Sigma\ Q_{1\ Partial})/(Nd * Nd * Nn)$
$Q_2 = (\Sigma\ Q_{2\ Partial})/(Nn * Nn * Nd)$

The standard error of the area under the ROC curve (SE_W) is then calculated as:

$$SE_W = \sqrt{\frac{(W*[1-W])+([Nd-1]*[Q_1-W^2])+([Nn-1]*[Q_2-W^2])}{Nd*Nn}}$$

To statistically test whether the area under the ROC curve was different from 0.5, a *z*-score was calculated as:

$$z = (W - 0.5)/SE_W$$

and the resulting score was compared to *z*-scores from the normal distribution to obtain a probability (p) value.

Appendix 10:
24 hour profiles of plasma concentrations of alkaline phosphatase, alanine aminotransferase, fructosamine and glucose in three diabetic dogs

Case No.	Hours post - injection	Alkaline phosphatase IU/l	Alanine amino-transferase IU/l	Fructosamine µmol/l	Glucose mmol/l
117222	0.00	209	53	356	6.2
	2.00	221	51	322	3.6
	4.00	242	50	316	4.6
	6.00	239	51	329	6.0
	6.16	232	49	*	6.2
	6.32	248	48	*	6.5
	6.50	200	50	*	5.6
	7.00	219	46	*	6.6
	7.50	216	44	*	7.6
	8.00	241	43	316	8.0
	9.00	227	44	*	7.1
	10.00	219	50	320	6.4
	12.00	205	44	302	10.9
	14.00	213	45	310	7.7
	16.00	216	43	313	7.8
	20.00	199	44	301	5.3
	24.00	216	48	317	5.4
113648	0.00	4109	109	526	11.1
	2.00	4179	109	520	14.7
	4.00	4630	111	533	16.6
	6.00	3934	103	517	15.4
	6.16	4019	114	*	18.9
	6.32	4266	112	*	20.2
	6.50	3985	114	*	18.7
	7.00	4195	109	*	21.1
	7.50	4395	106	*	23.1
	8.00	3669	103	538	21.8
	9.00	4306	107	*	24.1
	10.00	4389	102	521	25.1
	12.00	4130	100	529	25.5
	14.00	3737	93	520	23.1
	16.00	3656	92	521	20.6
	20.00	3727	96	525	16.4
	24.00	3537	93	518	14.8

Case No.	Hours post - injection	Alkaline phosphatase IU/l	Alanine amino-transferase IU/l	Fructosamine µmol/l	Glucose mmol/l
115980	0.00	1194	194	634	27.1
	2.00	1170	187	647	29.8
	4.00	1187	180	610	20.9
	6.00	1138	168	605	25.6
	6.16	1166	182	*	24.7
	6.32	1171	183	*	26.1
	6.50	1121	183	*	26.4
	7.00	1125	178	*	27.1
	7.50	*	*	*	*
	8.00	1140	191	630	26.5
	9.00	1073	183	*	26.7
	10.00	1176	183	663	24.2
	12.00	1215	170	612	29.5
	14.00	*	*	*	*
	16.00	*	*	613	19.9
	20.00	1275	170	593	21.4
	24.00	1300	115	644	22.7

Appendix 11:
Case reports of 19 dogs treated with mixed insulin zinc suspension

The following abbreviations and acronyms have been used throughout this appendix (units of measurement indicated where appropriate):

WBCC	Total white cell count	$x10^9/l$
NEUTROPHILS	Circulating neutrophils	$x10^9/l$
LYMPHOCYTES	Circulating lymphocytes	$x10^9/l$
MONOCYTES	Circulating monocytes	$x10^9/l$
EOSINOPHILS	Circulating eosinophils	$x10^9/l$
RBCC	Total red cell count	$x10^{12}/l$
HAEMOGLOBIN (HB)	Haemoglobin concentration	g/dl
HAEMATOCRIT		l/l
MEAN CELL VOLUME	Mean red cell volume	fl
MEAN CELL HB	Mean cell haemoglobin	pg
MEAN CELL HB CONC.	Mean cell haemoglobin concentration	g/dl
PLATELETS		$x10^9/l$
UREA	Plasma urea concentration	mmol/l
SODIUM	Plasma sodium concentration	mmol/l
POTASSIUM	Plasma potassium concentration	mmol/l
CHLORIDE	Plasma chloride concentration	mmol/l
CALCIUM	Plasma calcium concentration	mmol/l
PHOSPHATE	Plasma phosphate concentration	mmol/l
GLUCOSE	Plasma glucose concentration	mmol/l
CHOLESTEROL	Plasma cholesterol concentration	mmol/l
CREATININE	Plasma creatinine concentration	umol/l
TOTAL BILIRUBIN	Plasma bilirubin concentration	umol/l
ALKALINE PHOS	Plasma alkaline phosphatase concentration	U/l
ALT	Plasma alanine aminotransferase concentration	U/l
AST	Plasma aspartate aminotransaminase concentration	U/l
TOTAL PROTEIN	Plasma total protein concentration	g/l
ALBUMIN	Plasma albumin concentration	g/l
GLOBULIN	Plasma globulin concentration	g/l
GAMMA GT	Gamma-glutamyl transferase	U/l
CORTISOL	Plasma cortisol concentration	nmol/l
POST ACTH STIM	Plasma cortisol concentration after injection of ACTH analogue	nmol/l
CRT	Capillary refill time	seconds
NEG	Negative test result	
TRACE	Slightly positive test result	
MOD or SMALL	Positive test result	
LARGE or (L)	Strongly positive test result	
NM	Not measured	
NS	No sample	

CASE REPORT DOG C3

BREED: Scottish terrier

AGE: 5 years

SEX: Male

HISTORY:

This animal was referred for diabetic stabilisation with the following history. Two months earlier the dog had begun to lose weight and had become polydipsic, weaker and exercise intolerant. The dog had also been affected by ocular, nasal and pedal infections over this two month period. Initially polyphagia had been marked but a few days before admission to the University of Glasgow Veterinary School (G.U.V.S.) appetite had become severely depressed. There was no history of previous major medical or surgical illness, prolonged steroid therapy nor any history consistent with pancreatitis.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was noticeably dull and smelt strongly of ketones. The coat was sparse for a dog of this type. Mucous membranes were pink, CRT was less than 2 seconds and pulse volume was good. Heart rate was 112/minute. On auscultation, there were no cardiac murmurs nor adventitous lung sounds. Abdominal palpation revealed a 'full' feeling abdomen and hepatic enlargement. There was bilateral thick, tacky, mucopurulent ocular discharge but no evidence of cataract formation.

Schirmer tear test gave results of 7mm/min. and 12mm/min. (normal > 10 mm/min) in the left and right eyes respectively, confirming keratoconjuntivitis sicca of the left eye.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	1.3	WBCC	38.9
SODIUM	143	NEUTROPHILS	35.70
POTASSIUM	3.7	LYMPHOCYTES	0.58
CHLORIDE	104	MONOCYTES	1.56
CALCIUM	2.41	EOSINOPHILS	0
PHOSPHATE	0.80	RBCC	5.47
GLUCOSE	19.9	HAEMOGLOBIN	11.9
CHOLESTEROL	13.96	HAEMATOCRIT	33.7
CREATININE	50	MEAN CELL VOLUME	62
TOTAL BILIRUBIN	3	PLATELETS	661
ALKALINE PHOS	8,259		
ALT	78		

BIOCHEMISTRY (CONT):	
AST	43
TOTAL PROTEIN	64
ALBUMIN	28
GLOBULIN	36
CORTISOL	34
POST SYN 45MIN	38
GAMMA GT	12

Bacteriological examination of urine failed to demonstrate the presence of any significant urinary infection.

In view of the thin hair coat and elevated plasma alkaline phosphatase concentration, the presence of concurrent Cushing's disease was suspected. Plasma cortisol concentrations before and after stimulation with ACTH analogue (Synacthen) indicated that this was not the case.

Radiography of thorax and abdomen revealed only hepatomegaly and prostatomegaly. The day following admission, stabilisation with Caninsulin was begun. The starting dose was 5IU. Diet consisted of 285g of canned dog food (Chum: Pedigree Petfoods) and 70g of bread fed twice daily (75kcal/kg/day).

HOSPITALISATION:

For the first 3 days of hospitalisation this dog remained moderately dull, after which he was bright, alert and playful. The ocular discharge resolved following treatment with artificial tears (Isopto-plain: Allergan Ltd), ocular lubricant (Lacri Lube: Alcon Labs. UK, Ltd) and Chloromycetin Ophthalamic Ointment (Parke-Davis). A course of clavulanate potentiated amoxycillin (Synulox: SK Beecham) was instituted in view of the elevated total white blood cell count.

Rectal temperature during the hospitalisation period did not exceed 100.6°F and water intake reduced from a maximum of 900ml/day to 350ml/day. Heart rate remained below 120/minute at all examination times. Bodyweight remained constant.

The dog was discharged on day 12. The owners were instructed to increase insulin dose only if polydipsia returned.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	5	LARGE	2.0	17.1	700	11
5pm				22.8		
9.30pm		MOD./L	1.5	25.5		
DAY NO. 2						
9.30am	7	LARGE	2.0	22.3	800	
5pm				20.5		
9.30pm		MOD.	2.0	17.7		
DAY NO. 3						
9.30am	12	MOD	2.0	17.6	475	
5pm				17.3		
9.30pm		SMALL	1.5	26.7		
DAY NO. 4						
9.30am	16	SMALL	1.5	17.8	250	
5pm				12.5		
9.30pm		NEG	2.0	19.1		
DAY NO. 5						
9.30am	20	TRACE	2.0	23.5	500	
5pm				13.9		
9.30pm		NEG	1.5	17.4		
DAY NO. 6						
9.30am	20	NEG	2.0	17.3	400	
5pm				11.6		
9.30pm		NEG	2.0	11.7		
DAY NO. 7						
9.30am	24	NEG	2.0	25.4	900	
5pm				11.7		
9.30pm		NEG	1.5	29.4		
DAY NO. 8						
9.30am	26	NEG	1.5	16.8	550	
5pm				8.5		
9.30pm		NEG	2.0	12.8		
DAY NO. 9						
9.30am	26	NEG	1.0	12.6	150	
5pm				5.1		
9.30pm		NEG	1.5	17.3		
DAY NO. 10						
9.30am	26	NEG	1.5	22.1	550	
5pm				7.8		
9.30pm		NEG	2.0	20.3		

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 11						
9.30am	26	NEG	1.5	17.8	NM	11
5pm				4.3		
9.30pm		NEG	0.5	10.7		
DAY NO. 12						
9.30am	26	NEG	1.5	15.6		

CASE REPORT DOG C4

BREED: West Highland White terrier

AGB: 11 years

SEX: Castrated male

HISTORY:

This animal was first seen for diabetic stabilisation 9 months previously, when he was stabilised on 26IU Isophane insulin (Evans). Previous illnesses included cystitis and bladder haemangioma.

Prior to re-admission this dog had been stable and polydipsia had been well controlled. He was transferred to Caninsulin therapy whilst boarding for a period of 2 weeks.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright and alert. Mucous membranes were pink and CRT was less than 2 seconds. Heart rate was 120/minute. Respiratory rate was 30/minute. On auscultation there were no cardiac murmurs or pulmonary adventitious sounds. Rectal temperature was 101°F. There was marked cataract formation in the left eye and to a lesser degree in the right. Vision was limited but the dog was not blind.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	4.8	WBCC	7.5
SODIUM	147	NEUTROPHILS	5.96
POTASSIUM	4.7	LYMPHOCYTES	1.20
CHLORIDE	108	MONOCYTES	0.26
CALCIUM	2.70	EOSINOPHILS	0.04
PHOSPHATE	1.05	RBCC	7.65
GLUCOSE	8.8	HAEMOGLOBIN	17.4
CHOLESTEROL	6.92	HAEMATOCRIT	49.0
CREATININE	61	MEAN CELL VOLUME	64
TOTAL BILIRUBIN	0	MEAN CELL HB	22.7
ALKALINE PHOS	209	MEAN CELL HB CONC.	35.5
ALT	29	PLATELETS	438
AST	20		
TOTAL PROTEIN	70		
ALBUMIN	34		
GLOBULIN	36		

Radiography was not performed

This dog was included in the Caninsulin trial from the day following admission for boarding. Starting dose of insulin was 26IU and diet comprised 250g canned dog food (Chum; Pedigree Petfoods) and 100g bread fed twice daily.

HOSPITALISATION:

The dog remained bright and alert throughout the 2 weeks. Insulin dose was decreased by decrements of 2IU from the initial 26IU to a final dose of 18IU. Water intake over this period varied between 200 and 1500ml/day. There was a 0.3kg loss of bodyweight.

The dog was discharged on 18IU Caninsulin and owners were advised on the use of urine test strips for glucose.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	26	NEG	0.0	9.8	NM	11.8
5pm				4.5		
9.30pm		NEG	0.0	9.7		
DAY NO. 2						
9.30am	26	NEG	0.0	8.5	NM	
5pm				2.6		
9.30pm		NEG	0.0	12.7		
DAY NO. 3						
9.30am	26	NEG	0.0	5.7	750	
5pm				3.0		
9.30pm		NEG	0.0	9.7		
DAY NO. 4						
9.30am	26	NEG	0.0	7.5	NM	11.5
5pm				3.0		
9.30pm		NEG	0.0	5.7		
DAY NO. 5						
9.30am	26	NEG	0.0	9.3	500	
5pm				2.6		
9.30pm		NEG	2.0	14.1		
DAY NO. 6						
9.30am	26	NEG	0.25	6.5	NM	
5pm				3.0		
9.30pm		NEG	0.0	3.6		
DAY NO. 7						
9.30am	26	NEG	0.0	4.4	NM	
5pm				1.7		
9.30pm		NEG	0.0	4.0		

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 8						
9.30am	24	NEG	0.0	2.2	NM	
5pm				1.9		
9.30pm		NEG	0.0	5.1		
DAY NO. 9						
9.30am	22	NEG	0.0	7.0	NM	
5pm				3.1		
9.30pm		NEG	0.0	5.0		
DAY NO. 10						
9.30am	22	NEG	0.25	10.1	NM	
5pm				2.3		
9.30pm		NEG	0.0	9.0		
DAY NO. 11						
9.30am	20	NEG	0.0	4.6	1500	11.5
5pm				3.3		
9.30pm		NEG	0.25	12.3		
DAY NO. 12						
9.30am	18	NEG	0.0	8.1	1500	
5pm				2.5		
9.30pm		NEG	0.0	7.8		
DAY NO. 13						
9.30am	18	NEG	0.0	15.4	1500	
5pm				7.7		
9.30pm		NEG	0.25	15.1		
DAY NO. 14						
9.30am	18	NEG	0.25	11.0	1500	
5pm				6.8		
9.30pm		NEG	1.0	17.3		
DAY NO. 15						
9.30am	18	NEG	0.0	8.2		

CASE REPORT DOG C5

BREED: Labrador-X

AGE: 12 years

SEX: Speyed female

HISTORY:

This dog was first seen 10 months prior to entry to Caninsulin trial with a history of polyuria, polydipsia and weight loss. Clinical examination at that time revealed bilateral cataracts and a grade III, holosystolic cardiac murmur. Faeces were also noted to be pale, suggesting an exocrine pancreatic component to this dog's condition. The dog was initially stabilised on 10IU of Isophane insulin (Evans). Four months later ovariohysterectomy was performed following increasing diabetic instability.

The dog was converted from Isophane (Evans) to Caninsulin when re-admitted to G.U.V.S. for boarding for a three week period.

CLINICAL EXAMINATION AND INVESTIGATION:

On clinical examination this dog was thin, bright and alert, but was obviously blind. There were bilateral mature cataracts. Mucous membranes were pink and CRT was less than 3 seconds. Heart rate was 112/minute. Auscultation revealed a grade III holosystolic murmur but no pulmonary adventitious sounds. Abdominal palpation revealed hepatomegaly.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	2.7	WBCC	7.0
SODIUM	142	NEUTROPHILS	4.69
POTASSIUM	5.2	LYMPHOCYTES	1.47
CHLORIDE	107	MONOCYTES	0.63
CALCIUM	2.40	EOSINOPHILS	0.21
PHOSPHATE	0.69	RBCC	6.39
GLUCOSE	21.8	HAEMOGLOBIN	15.1
CHOLESTEROL	4.42	HAEMATOCRIT	43.4
CREATININE	69	MEAN CELL VOLUME	68
TOTAL BILIRUBIN	2	MEAN CELL HB	23.6
ALKALINE PHOS	2,686	MEAN CELL HB CONC.	34.7
ALT	120	PLATELETS	509
AST	28		
TOTAL PROTEIN	61		
ALBUMIN	30		
GLOBULIN	31		

Radiography was not performed.

Diet was maintained as before at 500g canned dog food (Chum: Pedigree Petfoods) and 100g of bread twice daily. Starting dose of Caninsulin was 17 IU.

HOSPITALISATION:

During the 3 week hospitalisation period the rectal temperature did not exceed 101.8°F. Daily water intake varied between 600ml and 2900ml. On day 6, two-hourly blood samples and four-hourly urine samples were collected for pharmacokinetic study. On day 12 a purulent ocular discharge was noted. This responded to a five day course of Chloromycetin Ophthalamic Ointment (Parke-Davis).

From day 5 to day 14, 5.00pm plasma glucose concentrations were consistently subnormal despite decreasing insulin dosages. On day 12, an episode of clinical hypoglycaemia occurred which required treatment with 1 IU of glucagon intramuscularly (im), 100ml of 40% dextrose saline per os and 1500ml of 4% dextrose saline intravenously (iv). Faeces from this dog were pale and steatorrhoeic between days 5 and 16. However, during this period the presence of exocrine pancreatic insufficiency was not demonstated by faecal trypsin activity analyses. Between admission and discharge the dog lost 2 kg bodyweight (14 kg to 12 kg) and became observably thin.

The dog was discharged on 15 IU of Caninsulin daily and the owner was instructed to increase the dose only if the dog remained polydipsic.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	17	NEG	2.0	23.2	NM	14
5pm				8.4		
9.30pm		NEG	2.0	24.5		
DAY NO. 2						
9.30am	17	NS	NS	36.1	NM	
5pm				6.3		
9.3Opm		TRACE	2.0	24.6		
DAY NO. 3						
9.30am	17	TRACE	2.0	19.0	1000	
5pm				31.4		
9.3Opm		NS	NS	37.4		

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	17	MOD	2.0	29.0	1250	14
5pm				16.5		
9.30pm		TRACE	1.0	18.6		
DAY NO. 5						
9.30am	17	NS	NS	7.4	1200	
5pm				2.2		
9.30pm		NEG	0.0	3.2		
DAY NO. 6						
9.30am	19	NS	NS	17.5	1200	
5pm				2.0		
9.30pm		NEG	0.0	1.5		
DAY NO. 7						
9.30am	17	NEG	0.0	18.9	1100	
5pm				1.0		
9.30pm		NEG	0.0	2.0		
DAY NO. 8						
9.30am	15	NEG	0.0	22.1	1250	
5pm				3.9		
9.30pm		NS	NS	NS		
DAY NO. 9						
9.30am	15	NS	NS	NS	1200	
5pm				1.8		
9.30pm		NS	NS	NS		
DAY NO. 10						
9.30am	15	NS	NS	21.2	850	
5pm				1.7		
9.30pm		NS	NS	NS		
DAY NO. 11						
9.30am	15	NEG	0.0	16.8	1050	13.5
5pm				2.1		
9.30pm		NEG	0.0	3.1		
DAY NO. 12						
9.30am	11	NEG	0.0	4.3	1200	
5pm				1.1		
9.3Opm		NS	NS	3.5		
DAY NO. 13						
9.30am	8	NS	NS	6.9	900	
5pm				1.8		
9.30pm		NEG	0.0	6.4		

	Insulin dose (IU)	Urine ketones	Urine glucose	Plasma glucose	Water intake (mls)	Body-weight (kg)
DAY NO. 14						
9.30am	6	NEG	0.75	11.7	600	
5pm				12.7		
9.30pm		NEG	0.75	20.4		
DAY NO. 15						
9.30am	6	TRACE	2.0	27.4	750	
5pm				15.7		
9.30pm		NEG	2.0	21.0		
DAY NO. 16						
9.30am	8	NEG	0.0	20.7	1500	
5pm				21.2		
9.30pm		NEG	1.5	31.0		
DAY NO. 17						
9.30am	10	TRACE	1.5	30.5	2900	
5pm				20.1		
9.30pm		NS	NS	38.5		
DAY NO. 18						
9.30am	12	SMALL	1.5	29.3	2000	
5pm				10.8		
9.30pm		NEG	2.0	33.3		
DAY NO. 19						
9.30am	13	SMALL	1.0	28.8	1900	
5pm				18.2		
9.30pm		NEG	2.0	18.9		
DAY NO. 20						
9.30am	15	NEG	2.0	32.2		12
5pm						
9.3Opm						

CASE REPORT DOG C6

BREED: Dobermann

AGE: 7 years

SEX: Female

HISTORY:

This dog was referred for investigation of polydipsia of 2 months duration (water intake was > 8 litres/day). There was no history consistent with pancreatitis nor prolonged steroid therapy but she had been in season 2.5 months previously.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was alert and bright. The coat was dry, dull and scurfy. Ketotic breath was obvious and enlarged prescapular lymph nodes were palpated. Heart rate was 120/minute. Mucous membranes were pink and CRT was less than 2 seconds. Rectal temperature was 101°F. Moderate hind limb ataxia was noticed at walk. The bitch's mammary glands were developed and milk could be expressed from them. There were no vulval discharges. There were no cardiac murmurs or pulmonary adventitious sounds on auscultation. Abdominal palpation revealed no abnormalities.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	5.9	WBCC	11.8
SODIUM	138	NEUTROPHILS	9.20
POTASSIUM	4.2	LYMPHOCYTES	1.77
CHLORIDE	99	MONOCYTES	0.53
CALCIUM	2.65	EOSINOPHILS	0.30
PHOSPHATE	1.80	RBCC	4.96
GLUCOSE	21.5	HAEMOGLOBIN	11.9
CHOLESTEROL	12.16	HAEMATOCRIT	33.0
CREATININE	61	MEAN CELL VOLUME	67
TOTAL BILIRUBIN	0	MEAN CELL HB	23.9
ALKALINE PHOS	1,465	MEAN CELL HB CONC.	35.8
ALT	85	PLATELETS	216
AST	53		
TOTAL PROTEIN	63		
ALBUMIN	32		
GLOBULIN	31		
CORTISOL	60		
CORTISOL POST SYNACTHEN	497		

Examination of lateral thoracic and abdominal radiographs revealed hepatomegaly but no evidence of uterine enlargement.

A lymph node aspirate was taken from a prescapular node which showed that the lymphadenopathy was non-neoplastic.

This dog was entered into the Caninsulin study 5 days after admission. The starting dose of insulin was 18IU and diet comprised 495g canned dog food (Chappie; Pedigree Petfoods) an 110g bread fed twice daily (40kcal/kg/day).

HOSPITALISATION:

The dog was hospitalised for a further 23 days for stabilisation. During this period daily dose was increased steadily until day 22. Much weight (8.7kg) was lost during the hospitalisation period (body weight 27.3kg at discharge). Water intake reduced from approximately 2000ml/day to 200-800ml/day. Ketotic breath could not be detected after day 4. After day 10, milk could no longer be expressed from mammary glands and all evidence of lymphadenopathy had disappeared.

The dog remained bright and lively throughout.

The dog was discharged at a dose of 46IU and the owners were advised that the bitch was probably suffering from progesterone related insulin resistance, that she would require less insulin in time and that ovariohysterectomy was stongly recommended. Urine glucose testing strips were supplied and the owners were instructed in adjusting insulin dose according to morning urine glucose concentrations.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	18	SMALL	2.0	26.5	2000	34
5pm				25.3		
9.30pm		SMALL	2.0	29.3		
DAY NO. 2						
9.30am	20	LARGE	1.0	22.9	NM	
5pm				21.9		
9.30pm		MOD	2.0	27.2		
DAY NO. 3						
9.30am	22	SMALL	1.0	25.1	1900	
5pm				23.5		
9.30pm		NEG	2.0	37.7		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	24	MOD	1.0	29.3	2400	
5pm				27.0		
9.30pm		NEG	2.0	28.5		
DAY NO. 5						
9.30am	26	SMALL	2.0	28.8	6500	
5pm				21.0		
9.30pm		NEG	2.0	38.5		
DAY NO. 6						
9.30am	28	SMALL	2.0	30.1	2500	
5pm				21.9		
9.30pm		NEG	2.0	35.6		
DAY NO. 7						
9.30am	28	SMALL	1.0	34.6	1500	
5pm				27.3		
9.30pm		TRACE	2.0	38.0		
DAY NO. 8						
9.30am	30	NEG	2.0	29.0	3750	
5pm				22.5		
9.30pm		NEG	2.0	33.0		
DAY NO. 9						
9.30am	36	NEG	2.0	26.3	NM	32
5pm				21.0		
9.30pm		NEG	2.0	33.7		
DAY NO. 10						
9.30am	40	TRACE	2.0	29.9	4000	
5pm				17.4		
9.30pm		NEG	2.0	28.0		
DAY NO. 11						
9.30am	40	SMALL	2.0	29.6	3000	
5pm				16.8		
9.30pm		NEG	2.0	28.0		
DAY NO. 12						
9.30am	40	NEG	1.0	32.0	4500	
5pm				15.1		
9.30pm		NEG	2.0	27.0		
DAY NO. 13						
9.30am	42	NEG	1.0	30.1	3000	
5pm				14.0		
9.30pm		NEG	2.0	28.8		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 14						
9.30am	42	NEG	2.0	32.0	1000	
5pm				17.5		
9.30pm		NEG	2.0	32.0		
DAY NO. 15						
9.30am	42	NEG	2.0	26.2	550	29.5
5pm				12.9		
9.30pm		NEG	2.0	23.7		
DAY NO. 16						
9.30am	44	NEG	2.0	30.1	1500	
5pm				12.1		
9.30pm		NEG	2.0	25.2		
DAY NO. 17						
9.30am	46	NEG	2.0	30.2	800	
5pm				10.2		
9.30pm		NEG	1.0	18.2		
DAY NO. 18						
9.30am	48	NEG	2.0	21.5	200	
5pm				7.5		
9.30pm		NEG	1.5	21.1		
DAY NO. 19						
9.30am	48	NEG	1.0	25.2	250	
5pm				7.5		
9.30pm		NEG	0.75	17.1		
DAY NO. 20						
9.30am	48	NEG	0.75	18.4	800	
5pm				6.1		
9.30pm		NEG	1.0	7.8		
DAY NO. 21						
9.30am	48	NEG	0.25	13.7	200	
5pm				8.5		
9.30pm		NEG	1.0	15.8		
DAY NO. 22						
9.30am	48	NEG	0.25	15.9	NM	
5pm				4.0		
9.30pm		NEG	0.5	7.8		
DAY NO. 23						
9.30am	46	NEG	0.25	14.3		27.3

CASE REPORT DOG C7

BREED: Spaniel X

AGE: 11 years

SEX: Female

HISTORY:

This dog had been polydipsic, to some extent, for 1 year before being referred for diabetic stabilisation. There had been a further increase in thirst two weeks prior to referral and this had reduced, in part, following oral hypoglycaemic therapy instituted by the referring veterinary surgeon. This bitch had been in season 3 weeks prior to referral and had commonly suffered from pseudopregnancy. There was no history of prolonged steroid or reproductive hormone therapy.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright and alert. Mucous membranes were pink and CRT was less than 2 seconds. Heart rate was 108/minute. Rectal temperature was 100°F. On auscultation there was a left sided, systolic cardiac murmur but no adventitious pulmonary sounds. The left submandibular lymph node was enlarged. There was marked periodontal disease.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	5.5	WBCC	12.7
SODIUM	139	NEUTROPHILS	10.92
POTASSIUM	3.8	LYMPHOCYTES	1.40
CHLORIDE	96	EOSINOPHILS	0.39
CALCIUM	2.83	RBCC	5.89
PHOSPHATE	1.47	HAEMOGLOBIN (HB)	13.3
GLUCOSE	20.5	HAEMATOCRIT	38.1
CHOLESTEROL	8.32	MEAN CELL VOLUME	65
CREATININE	62	MEAN CELL HB	22.5
TOTAL BILIRUBIN	1	MEAN CELL HB CONC.	34.9
ALKALINE PHOS	1,479	PLATELETS	567
ALT	51		
AST	23		
TOTAL PROTEIN	66		
ALBUMIN	32		
GLOBULIN	34		

Radiography of thorax and abdomen did not demonstrate any abnormalities.

The day following admission this dog was included in the Caninsulin study. Starting dose of insulin was 28IU and diet comprised 300g canned dog food (Chum; Pedigree Petfoods) and 60g bread fed twice daily (60kcal/kg/day).

HOSPITALISATION:

Whilst hospitalised the insulin dose was increased in 2-4IU increments until reaching 62IU on day 10. This dose was continued until day 16 when the dose was reduced by increments of 4IU until day 24 when the dog was obviously no longer diabetic and insulin therapy was stopped. During the hospitalisation period water intake was reduced from a maximum of 4950 to less than 400ml/day and the dog lost 2kg bodyweight. Rectal temperature never exceeded 101.6°F. Clinical condition and bright demeanour did not vary whilst hospitalised. This dog was considered to have been suffering from a progesterone induced diabetes mellitus.

The dog was boarded after discontinuing insulin therapy until ovariohysterectomy and mammary tumour removal was performed on day 28.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	28	NEG	2.0	20.5	3000	24.5
5pm				18.5		
9.30pm				26.8		
DAY NO. 2						
9.30am	32	NEG	2.0	22.9	3000	
5pm				19.9		
9.30pm		NEG	2.0	29.3		
DAY NO. 3						
9.30am	34	NEG	2.0	23.7	4500	
5pm				20.3		
9.30pm		NEG	2.0	26.8		
DAY NO. 4						
9.30am	38	NEG	2.0	22.0	NM	
5pm				NS		
9.30pm		NEG	2.0	25.5		
DAY NO. 5						
9.30am	42	NEG	2.0	24.8	NM	
5pm				26.3		
9.30pm		NEG	2.0	28.0		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 6						
9.30am	46	TRACE	2.0	22.3	NM	
5pm				23.6		
9.30pm		NEG	2.0	25.0		
DAY NO. 7						
9.30am	50	SMALL	2.0	NS	3000	
5pm				15.3		
9.30pm		NEG	2.0	26.6		
DAY NO. 8						
9.30am	54	NEG	2.0	23.5	4950	
5pm				2.0		
9.30pm		NEG	1.0	24.4		
DAY NO. 9						
9.30am	58	NEG	2.0	20.3	2750	
5pm				15.2		
9.30pm		NEG	0.0	17.5		
DAY NO. 10						
9.30am	62	NEG	0.0	16.8	2200	
5pm				5.0		
9.30pm		NEG	0.0	13.8		
DAY NO. 11						
9.30am	62	NEG	0.0	4.2	2100	23.5
5pm				5.0		
9.30pm		NEG	0.0	8.2		
DAY NO. 12						
9.30am	62	NEG	0.0	10.8	NM	
5pm				4.7		
9.30pm		NEG	0.0	14.2		
DAY NO. 13						
9.30am	62	NEG	0.0	6.0	NM	
5pm				4.1		
9.30pm		NEG	o.o	4.5		
DAY NO. 14						
9.30am	62	NEG	0.0	5.0	NM	
5pm				3.5		
9.30pm		NEG	0.0	4.4		
DAY NO. 15						
9.30am	62	NEG	0.0	NS	300	
5pm				2.3		
9.30pm		NEG	0.0	4.4		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 16						
9.30am	58	NEG	0.0	5.0	200	
5pm				2.3		
9.30pm		NEG	0.0	5.1		
DAY NO. 17						
9.30am	54	NEG	0.0	4.2	300	22.75
5pm				2.0		
9.30pm		NEG	0.0	3.9		
DAY NO. 18						
9.30am	50	NEG	0.0	4.0	NM	
5pm				2.4		
9.30pm		NEG	0.0	3.7		
DAY NO. 19						
9.30am	46	NEG	0.0	5.1	NM	
5pm				2.4		
9.30pm		NEG	0.0	5.3		
DAY NO. 20						
9.30am	40	NEG	0.0	4.8	400	
5pm				2.2		
9.30pm		NEG	0.0	5.2		
DAY NO. 21						
9.30am	38	NEG	0.0	5.3	650	
5pm				4.4		
9.30pm		NEG	0.0	4.7		
DAY NO. 22						
9.30am	36	NEG	0.0	5.0	100	
5pm				3.0		
9.30pm		NEG	0.0	3.3		
DAY NO. 23						
9.30am	32	NEG	0.0	4.0	200	
5pm				1.8		
9.30pm		NEG	0.0	4.1		
DAY NO. 24						
9.30am	0	NEG	0.0	4.6	350	
5pm				5.5		
9.30pm		NEG	0.0	5.3		
DAY NO. 25						
9.30am	0	NEG	0.0	5.5	100	
5pm				5.4		

CASE REPORT DOG C8

BREED: Terrier X

AGE: 9 years

SEX: Speyed female

HISTORY:

This dog had been in the owners' possession for 6 years and had been neutered prior to their ownership. A diagnosis of diabetes mellitus had been made by the referring veterinary surgeon 5 months prior to admission to G.U.V.S., after a one-week-period of polydipsia and nocturia. There had been no history of prolonged therapy with steroid hormones or consistent with pancreatitis. The dog was initially stabilised at home by the owners using isophane insulin of bovine origin and urine glucose testing strips. The dose had been increased from 10 to 20IU. No blood samples were taken from this dog prior to her admission to G.U.V.S. Weight loss was noted around the time of original diagnosis but latterly the dog had maintained weight well. The eyes were dry and mattery initially but this had improved after a few weeks of insulin therapy. She was admitted to G.U.V.S. for thorough assessment and conversion to Caninsulin.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright and alert and appeared moderately overweight. Heart rate was 110/min. Respiratory rate was 30/min. Mucous membranes were pink and capillary refill time was less than 2 seconds. There were no pulmonary adventitious sounds or cardiac murmurs detected on thoracic auscultation. Abdominal palpation revealed no abnormalities.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.9	WBCC	9.9
SODIUM	142	NEUTROPHILS	7.62
POTASSIUM	4.2	LYMPHOCYTES	1.88
CHLORIDE	108	MONOCYTES	0.30
CALCIUM	2.54	EOSINOPHILS	0.10
PHOSPHATE	0.82	RBCC	6.33
GLUCOSE	5.0	HAEMOGLOBIN (HB)	14.9
CHOLESTEROL	17.87	HAEMATOCRIT	41.1
CREATININE	42	MEAN CELL VOLUME	65
TOTAL BILIRUBIN	2	MEAN CELL HB	23.5
ALKALINE PHOS	909	MEAN CELL HB CONC.	36.2
ALT	53	PLATELETS	443

BIOCHEMISTRY (cont):	
TOTAL PROTEIN	72
ALBUMIN	40
GLOBULIN	32

Lateral thoracic and abdominal radiographs demonstrated enlargement of the hepatic shadow.

In view of the plasma glucose concentration of 5mmol/1 at the time of blood sampling insulin therapy was withheld for one day. The fasting plasma glucose concentration the following day was 22mmol/1. Therapy with Caninsulin was then started at a dose of 15IU. Diet comprised 9oz canned dog food (Pedigree Chum; Pedigree Petfoods) and 2oz of bread fed twice daily (75kcal/kg/day).

HOSPITALISATION:

This dog was hospitalised for a further 12 days, during which time it remained very bright, active and ate well. Rectal temperature never exceeded 101.8°F. Insulin dose was initially increased to 19IU but reduced to a final dose of 14IU. Water intake remained below 250ml/day. Bodyweight remained constant.

The dog was discharged on day 13 and the owners were advised to continue monitoring morning urine glucose concentrations but to contact G.U.V.S. before adjusting the insulin dose.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	15	TRACE	2.0	25.4	NM	10.5
5pm				15.4		
9.30pm		TRACE	2.0	19.9		
DAY NO. 2						
9.30am	19	NEG	2.0	19.7	50	
5pm				4.6		
9.3Opm		TRACE	2.0	8.1		
DAY NO. 3						
9.30am	18	TRACE	0.25	11.2	100	
5pm				3.3		
9.30pm		TRACE	0.25	9.8		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	17	TRACE	0.25	NS	NM	
5pm				4.3		
9.3Opm		TRACE	0.25	NS		
DAY NO. 5						
9.30am	17	NEG	0.0	NS	200	
5pm				4.9		
9.3Opm		NEG	0.0	15.3		
DAY NO. 6						
9.30am	17	NEG	2.0	12.0	250	10.5
5pm				4.6		
9.30pm		NEG	0.0	4.9		
DAY NO. 7						
9.30am	17	NEG	0.0	7.1	NM	
5pm				4.9		
9.30pm		NEG	0.0	7.6		
DAY NO. 8						
9.3Oam	17	NEG	0.0	NS	NM	
5pm				5.4		
9.30pm		NEG	0.0	5.5		
DAY NO. 9						
9.30am	17	NEG	0.0	4.9	NM	
5pm				7.3		
9.30pm		NEG	0.0	7.0		
DAY NO. 10						
9.30am	17	NEG	0.0	7.4	150	
5pm				3.9		
9.30pm		NEG	0.0	3.1		
DAY NO. 11						
9.30am	15	NEG	0.0	9.9	150	
5pm				4.0		
9.30pm		NEG	0.0	3.9		
DAY NO. 12						
9.30am	15	NEG	0.0	6.5	150	
5pm				3.4		
9.3Opm		NEG	0.0	3.9		
DAY NO. 13						
9.30am	14	NEG	0.0	8.6	NM	10.5

CASE REPORT DOG C9

BREED: Miniature Smooth-haired Dachshund

AGE: 10 years

SEX: Speyed female

HISTORY:

This dog was referred to G.U.V.S. for thorough investigation and re-stabilisation. A progressively increasing thirst had been noted for three months prior to referral. Weight loss and exercise intolerance became obvious in the 4 weeks before admission. The referring veterinary surgeon diagnosed diabetes mellitus and ketoacidosis when weight loss became apparent and attempted stabilisation using single daily evening injections of Lentard MC (Novo, Nordisk). Doses of insulin were high (20-28IU for a 6kg dog), hence referral for further investigation.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was bright and alert. Rectal temperature was 100.5°F. There was obvious cataract opacification of the left lens. There were no ocular discharges. Lymph nodes were normal. Heart rate was 120/min. Colour, CRT and pulse volume were good. On auscultation, there was a grade III/V left sided systolic cardiac murmur but no adventitious pulmonary sounds. On abdominal palpation, hepatomegaly was obvious. There was also erythema and tacky purulent discharge around the vulva. The skin of the axillae, abdominal folds and ear pinnae was also erythematous.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.0	WBCC	8.5
SODIUM	146	NEUTROPHILS	6.72
POTASSIUM	4.6	LYMPHOCYTES	1.11
CHLORIDE	107	MONOCYTES	0.43
CALCIUM	2.33	EOSINOPHILS	0.26
PHOSPHATE	1.15	RBCC	6.67
GLUCOSE	18.1	HAEMOGLOBIN	14.6
CHOLESTEROL	13.24	HAEMATOCRIT	42.4
CREATININE	64	PLATELETS	321
TOTAL BILIRUBIN	1		
ALKALINE PHOS	1,164		
ALT	444		
AST	85		
TOTAL PROTEIN	65		
ALBUMIN	33		
GLOBULIN	32		

Radiography of thorax and abdomen revealed only hepatomegaly.

This dog was included in Caninsulin trial on the day of admission. Starting dose of Canisulin was 15IU because of the recent history of high insulin requirement. Diet comprised 125g canned dog food (Chum; Pedigree Petfoods) and 25g of bread fed twice daily (80kcal/kg)

HOSPITALISATION:

Over the first 5 days of hospitalisation the clinical condition varied very little. Insulin dose was increased to 20IU and then decreased to 10IU. Water intake varied between 150 and 300ml/day. The dog remained bright and alert throughout this initial period. On the sixth day, however, she dog passed soft faeces and a course of kaolin and neomycin (Kaobiotic; Upjohn) was instituted. The next day (day 7), watery diarrhoea was passed but she remained bright. On days 8 and 9, the dog became dull, anorexic and passed fresh blood with diarrhoea. Insulin dose for these and subsequent 2 days was halved and a light, easily digested diet was offered. Bacterial culture of faeces subsequently yielded significant growth of β-haemolytic *E. coli.* Diet and appetite returned to normal and insulin dose returned to 10IU on day 12. The dog was discharged on day 14 on an insulin dose of 12 IU .

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	15	NEG	2.0	24.4	150	6
5pm				18.1		
9.30pm		NEG	2.0	15.9		
DAY NO. 2						
9.30am	20	NEG	1.0	18.7	200	
5pm				4.7		
9.30pm		NEG	1.0	12.5		
DAY NO. 3						
9.30am	20	NEG	0.0	10.5	NM	
5pm				3.6		
9.30pm		NEG	0.0	13.9		
DAY NO. 4						
9.30am	18	NEG	0.0	3.5	NM	
5pm				3.3		
9.30pm		NEG	0.0	3.2		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 5						
9.30am	14	NEG	0.0	13.4	250	5.8
5pm				3.2		
9.30pm		NEG	1.5	21.9		
DAY NO. 6						
9.30am	10	NEG	1.5	16.7	300	
5pm				7.2		
9.30pm		NEG	1.5	26.0		
DAY NO. 7						
9.30am	10	NEG	2.0	20.8	200	5.5
5pm				3.4		
9.30pm		NEG	2.0	3.2		
DAY NO. 8						
9.30am	8	NEG	0.25	15.1	NM	
5pm				4.0		
9.30pm		NEG	0.0	6.4		
DAY NO. 9						
9.30am	6	NEG	0.75	18.0	400	5.25
5pm				3.0		
9.30pm		NEG	0.75	14.4		
DAY NO. 10						
9.30am	5	NEG	0.75	18.8	400	
5pm				5.8		
9.3Opm		TRACE	1.0	22.2		
DAY NO. 11						
9.30am	5	NEG	0.75	22.7	400	
5pm				17.9		
9.30pm		NEG	1.0	29.1		
DAY NO. 12						
9.30am	10	TRACE	1.0	27.5	450	5.5
5pm				10.6		
9.30pm		NEG	1.0	27.3		
DAY NO. 13						
9.30am	14	NEG	1.0	22.4	175	
5pm				3.5		
9.3Opm		TRACE	0.5	21.0		
DAY NO. 14						
9.30am	12	NEG	0.5	18.9	300	5.75
5pm				4.7		

CASE REPORT DOG C10

BREED: Samoyed

AGE: 8 years

SEX: Entire female

HISTORY:

This dog was first referred for diabetic stabilisation 5 months prior to admission to the Caninsulin study. This was achieved using an isophane insulin (Isophane; Evans) and the dog had been 'stable' on 20IU since that time. The owners were advised to have the dog speyed but refused. Conversion to Caninsulin was carried out whilst the dog was boarding.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was bright and lively. Heart rate was 108/minute. Mucous membrane colour, CRT and pulse volume were all good. Rectal temperature was 101.8°F. Vision was good but there mild increases in density of the ventral parts of both lenses. No abnormalities were detected on thoracic auscultation nor on abdominal palpation. Lymph nodes were normal.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.3	WBCC	8.6
SODIUM	141	NEUTROPHILS	6.19
POTASSIUM	4.6	LYMPHOCYTES	1.98
CHLORIDE	107	MONOCYTES	0.34
CALCIUM	2.46	EOSINOPHILS	0.04
PHOSPHATE	1.07	RBCC	7.14
GLUCOSE	24.3	HAEMAGLOBIN (HB)	17.0
CHOLESTEROL	9.12	HAEMATOCRIT	45.0
CREATININE	19	MEAN CELL VOLUME	63
TOTAL BILIRUBIN	2	MEAN CELL HB	23.8
ALKALINE PHOS	380	MEAN CELL HB CONC.	37.7
ALT	30	PLATELETS	224
AST	13		
TOTAL PROTEIN	66		
ALBUMIN	32		
GLOBULIN	34		

Radiography was not performed.

This dog was included in the Caninsulin study from the day of re-admission. Starting dose of Caninsulin was 24IU and diet was as before at 500g canned dog food (Pedigree Chum; Pedigree Petfoods) and 100g bread fed twice daily (45kcal/kg/day).

HOSPITALISATION:

This dog was hospitalised for 6 days for conversion to Caninsulin, during which time, there was no change in clinical condition. The dog remained bright and lively. Rectal temperature remained below 102.0°F at all examination times. Insulin dose was increased to 26IU but then lowered again to 24IU. Water intake varied between 750 and 1400ml/day. Bodyweight remained constant at 29kg.

The dog was discharged on 24IU and the owners were advised to monitor morning urine glucose concentrations using test strips but not to alter the insulin dose unless directed by G.U.V.S. hospital staff.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	24	NEG	2.0	24.3	NM	29
5pm				8.1		
9.30pm		NEG	2.0	9.2		
DAY NO. 2						
9.30am	25	NEG	2.0	23.4	900	
5pm				9.7		
9.30pm		NEG	1.0	20.4		
DAY NO. 3						
9.30am	26	NEG	0.25	14.3	750	
5pm				3.8		
9.30pm		NEG	0.0	6.5		
DAY NO. 4						
9.30am	26	NS	NS	19.4	1400	
5pm				7.4		
9.30pm		NEG	0.0	3.8		
DAY NO. 5						
9.30am	24	NEG	0.0	13.0	NM	
5pm				3.7		
9.30pm		NEG	0.0	9.9		
DAY NO. 6						
9.30am	24	NEG	0.0	18.0		

CASE REPORT DOG C11

BREED: Jack Russell terrier

AGE: 8 years

SEX: Entire female

HISTORY:

This dog was referred to G.U.V.S. for diabetic stabilisation, having been polydipsic (water intake approximately 1 litre/day), polyphagic and losing weight for the preceeding two months. These signs become more marked in the fortnight prior to referral.

The referring veterinary surgeon prescribed a low calorie/high fibre diet (r/d: Hill's), 6 weeks prior to referral, and this seemed to reduce the polydipsia. There was no history of prolonged steroid therapy or consistent with pancreatitis. An injection to suppress oestrus had been administered one month before admission to G.U.V.S.. The preceeding oestrus had been 7 months earlier.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was noticeably thin but bright and alert. Mucous membranes were dry but pink and capillary refill time was less than 2 seconds. Heart rate was 84/minute. There were no cardiac murmurs or adventitious pulmonary sounds to be heard on auscultation. Abdominal palpation revealed a 'full' feeling abdomen, especially cranially, suggesting hepatic enlargement. There was no vaginal discharge and no signs of cataract formation but bilateral nuclear sclerosis of both lenses was present.

Lateral radiographs of thorax and abdomen revealed only hepatomegaly. There was no obvious uterine shadow.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	5.9	WBCC	6.5
SODIUM	142	NEUTROPHILS	3.90
POTASSIUM	4.2	LYMPHOCYTES	1.98
CHLORIDE	107	MONOCYTES	0.49
CALCIUM	2.25	EOSINOPHILS	0.13
PHOSPHATE	0.64	RBCC	6.78
GLUCOSE	22.7	HAEMOGLOBIN (HB)	14.9
CHOLESTEROL	6.95	HAEMATOCRIT	44.1
CREATININE	62	MEAN CELL VOLUME	65
TOTAL BILIRUBIN	2	MEAN CELL HB	21.9

BIOCHEMISTRY (CONT):		HAEMATOLOGY (CONT):	
ALKALINE PHOS	97	MEAN CELL HB CONC.	33.7
ALT	135	PLATELETS	432
AST	99		
TOTAL PROTEIN	57		
ALBUMIN	32		
GLOBULIN	25		

The day following admission this dog was included in the Caninsulin trial. Starting dose of Caninsulin was 7IU and diet comprised 172g of canned dog food (Chappie:Pedigree Petfoods) and 42g of bread fed twice daily (65kcal/kg/day).

HOSPITALISATION:

The dog was hospitalised for a total of 16 further days, during which time the insulin dose rose to 10IU but settled down at 8IU. After a series of very low afternoon plasma glucose concentrations, it was decided to reduce the insulin dose drastically and no insulin was given on day 7. Water intake, whilst hospitalised, varied between 150ml and 600ml. Body weight increased from 5.8kg on admission to 6.5 kg at discharge. The clinical condition of this dog did not vary throughout the hospitalisation period. The dog remained bright, alert and playful. Rectal temperature varied between 100.4°F and 101.6°F. Heart rate remained below 120/minute.

This dog was discharged on day 16 and instructions were given to the owner to monitor morning urine glucose concentrations using Keto-diastix (Ames). The owners were advised to increase Caninsulin dose by 1IU if morning urine glucose concentrations were 2% or greater for more than two consecutive days.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	7	NS	NS	21.7	200	5.8
5pm				8.8		
9.30pm		NS	NS	15.3		
DAY NO. 2						
9.30am	9	NEG	1.5	18.7	400	
5pm				10.6		
9.3Opm		NS	NS	NS		
DAY NO. 3						
9.30am	11	NEG	0.0	4.5	600	
5pm				20.5		
9.3Opm		NS	NS	NS		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	9	NS	NS	7.7	200	
5pm				4.1		
9.30pm		TRACE	0.0	3.8		
DAY NO. 5						
9.30am	8	NEG	0.0	11.1	400	6.5
5pm				2.9		
9.30pm		NEG	2.0	24.5		
DAY NO. 6						
9.30am	6	NS	NS	17.0	600	
5pm				2.6		
9.30pm		NEG	0.0	8.0		
DAY NO. 7						
9.30am	0	NEG	0.0	17.9	500	
5pm				18.0		
9.30pm		NEG	2.0	21.5		
DAY NO. 8						
9.30am	4	NEG	2.0	28.5	500	
5pm				7.9		
9.30pm		NEG	2.0	19.6		
DAY NO. 9						
9.30am	4	NEG	2.0	17.3	650	6.0
5pm				20.6		
9.30pm		NEG	2.0	22.1		
DAY NO. 10						
9.30am	6	NEG	2.0	26.3	500	
5pm				11.1		
9.30pm		NEG	2.0	18.5		
DAY NO. 11						
9.30am	8	NEG	2.0	17.2	275	
5pm				7.3		
9.30pm		NEG	2.0	13.4		
DAY NO. 12						
9.30am	10	NEG	0.25	18.9	350	
5pm				4.0		
9.30pm		NEG	0.25	9.4		
DAY NO. 13						
9.30am	10	NEG	2.0	19.7	150	
5pm				3.2		
9.30pm		NEG	2.0	4.5		
DAY NO. 14						
9.30am	9	NEG	0.5	20.5	200	
5pm				3.8		
9.30pm		NEG	0.25	6.4		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 15						
9.30am	8	MEG	0.0	7.3	400	
5pm				4.0		
9.30pm		NEG	0.0	20.1		
DAY NO. 16						
9.30am	8	NEG	0.0	14.2	NM	6.5
5pm				4.1		
9.3Opm						

CASE REPORT DOG C12

BREED: Cairn terrier

AGE: 4 years

SEX: Speyed female

HISTORY:

This bitch had been speyed prior to her first oestrus and had no history of previous major medical or surgical illness. Over a 6 week period, prior to admission to G.U.V.S., she became noticeably polydipsic, polyuric, polyphagic and was presented to the referring veterinary surgeon 4 days prior to admission. Ampicillin was prescribed pending referral. There was no history of previous therapy with corticosteroids or reproductive hormones nor of any pancreatic disease.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was bright and alert. Mucous membranes were pink and moist and capillary refill time was less than 2 seconds. Heart rate was 120/minute. There were no cardiac murmurs or adventitious pulmonary sounds on auscultation and pulses were good. The dog's eyes were clear and bright with no obvious discharges or cataract formation. Abdominal palpation revealed a 'fullness' and possible hepatomegaly. Lymph nodes were not enlarged.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.4	WBCC	9.3
SODIUM	144	NEUTROPHILS	7.30
POTASSIUM	3.9	LYMPHOCYTES	1.81
CHLORIDE	105	MONOCYTES	0.09
CALCIUM	2.46	EOSINOPHILS	0.09
PHOSPHATE	1.00	RBCC	6.93
GLUCOSE	21.8	HAEMOGLOBIN (HB)	16.4
CHOLESTEROL	8.38	HAEMATOCRIT	47.1
CREATININE	55	MEAN CELL VOLUME	68
TOTAL BILIRUBIN	2	MEAN CELL HB	23.6
ALKALINE PHOS	1,085	MEAN CELL HB CONC.	34.8
ALT	61	PLATELETS	529
AST	38		
TOTAL PROTEIN	66		
ALBUMIN	40		
GLOBULIN	26		

Examination of lateral abdominal and thoracic radiographs confirmed only the presence of hepatomegaly.

The dog was included in the Caninsulin trial from the day of admission. The starting dose of Caninsulin was 11IU and diet comprised 195g canned commercial dog food (Chappie:Pedigree Petfoods) and 45g bread fed twice daily (70kcal/kg/day).

HOSPITALISATION:

The dog was hospitalised for a further 10 days, during which time the dose of Caninsulin rose to 16IU but was reduced to 15IU. At this point the dog was discharged. Whilst hospitalised water intake dropped from 600-800ml/day to 150ml/day. The dog's general condition improved marginally although she was bright and lively throughout. Rectal temperature remained normal whilst hospitalised.

On discharge (day 10), the owners were instructed in the use of urine glucose test strips and were advised to adjust insulin dose according to morning urine glucose concentrations.

Two days following discharge the dog was re-admitted as she had become dull and the owner was unhappy with her condition. Morning urine glucose concentrations had been 2% or greater. On examination no abnormalities were detected. Re-stabilisation was undertaken over the following 10 days. A rectal temperature of 103°F was recorded the day after the second admission and a course of ampicillin was instituted. Water intake again fell to 150ml/day. Doses varied up to 22IU during this second period but the dog was discharged again on a dose of 18IU.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	11	LARGE	1.0	22.3	600	8.5
5pm				7.5		
9.30pm		LARGE	2.0	16.9		
DAY NO. 2						
9.30am	12	SMALL	1.0	20.3	NM	
5pm				13.2		
9.30pm		NEG	2.0	15.0		
DAY NO. 3						
9.30am	14	NEG	2.0	27.4	800	
5pm				16.5		
9.30pm		NEG	2.0	14.6		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	16	NEG	2.0	27.3	750	8.5
5pm				6.4		
9.30pm		NEG	2.0	16.4		
DAY NO. 5						
9.30am	16	NEG	2.0	24.0	600	
5pm				5.8		
9.30pm		NEG	2.0	17.8		
DAY NO. 6						
9.30am	16	NEG	1.0	9.6	250	
5pm				4.5		
9.30pm		NEG	2.0	20.0		
DAY NO. 7						
9.30am	16	NEG	2.0	19.3	150	
5pm				5.5		
9.30pm		NEG	2.0	17.9		
DAY NO. 8						
9.30am	16	NEG	2.0	15.1	150	
5pm				3.9		
9.30pm		NEG	2.0	12.6		
DAY NO. 9						
9.30am	15	NEG	2.0	15.0	400	
5pm				4.4		
9.30pm		NEG	2.0	14.8		
DAY NO. 10						
9.30am	15	NEG	2.0	10.9		8.8
5pm				7.1		

CASE REPORT DOG C13

BREED: Beagle.

AGE: 7 years

SEX: Entire female

HISTORY:

This dog was referred for diabetic stabilisation with a history of weight loss, lethargy and polydipsia of 2 months duration. She had been in season 3 to 4 months earlier. There was no history of reproductive hormone therapy, prolonged corticosteroid therapy nor of generalised pancreatic disease. Two years prior to referral thyroxin (Eltroxin) therapy had been prescribed.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright but quiet. Mucous membranes were pink and moist. Heart rate was 92/minute. CRT and pulse volume were both good. There were no ocular discharges nor evidence of cataract formation. On auscultation, there were no cardiac murmurs or pulmonary adventitous sounds detected. Abdominal palpation revealed a 'full' feeling abdomen and possible hepatomegaly. Hypothyroidism was not suspected.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	3.5	WBCC	11.2
SODIUM	140	NEUTROPHILS	9.52
POTASSIUM	4.7	LYMPHOCYTES	0.90
CHLORIDE	97	MONOCYTES	0.67
CALCIUM	2.48	EOSINOPHILS	0.11
PHOSPHATE	1.12	RBCC	6.85
GLUCOSE	25.4	HAEMOGLOBIN HB	16.7
CHOLESTEROL	8.89	HAEMATOCRIT	47.8
CREATININE	79	MEAN CELL VOLUME	70
TOTAL BILIRUBIN	0	MEAN CELL HB	24.3
ALKALINE PHOS	152	MEAN CELL HB CONC.	34.9
ALT	199	PLATELETS	277
AST	43		
TOTAL PROTEIN	66		
ALBUMIN	35		
GLOBULIN	31		

Radiography revealed no abnormalities.

Bacteriological culture of urine yielded β-haemolytic *Streptococcus* and non-haemolytic *E. coli*. Ampicillin therapy was instituted.

The day following admission this dog was included in the Caninsulin trial. The starting dose of Caninsulin was 13IU. Diet comprised 370g of canned dog food (Chum:Pedigree Petfoods) and 50g bread fed twice daily.

HOSPITALISATION:

The dog remained bright until day 10 when she began to pass bloody diarrhoea, which continued until day 13. During this three day period her appetite was considerably reduced. The diarrhoea was treated with clavulanate potentiated amoxycillin (Synulox: SK Beecham). Bacterial culture of faeces yielded β-haemolytic *E. coli* and *Campylobacter spp.*. Rectal temperature never exceeded 101.8°F at all examination times. Whilst hospitalised bodyweight initially increased and then fell again to 12kg. Water intake remained below 450mls. Insulin dose was increased to 18IU then lowered again to a final dose of 13IU.

The dog was boarded after this stabilisation period until ovariohysterectomy, which was performed on day 18. Recovery was uneventful.

The dog was discharged on 13 IU of Caninsulin and owners were advised to adjust insulin dose according to morning urine glucose concentrations i.e., to increase the insulin dose by one unit if morning glucose concentrations were 2% or greater for more than two consecutive days and to lower it if urine glucose concentrations were negative for more than two days.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	13	NEG	2.0	20.7	50	11.5
5pm				23.1		
9.30pm		NEG	2.0	23.2	50	
DAY NO. 2						
9.30am	15	NS	NS	22.3	50	
5pm				21.7		
9.30pm		NEG	2.0	20.7		
DAY NO. 3						
9.30am	15	NEG	0.0	11.2	200	
5pm				14.0		
9.30pm		NEG	0.0	14.7		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 4						
9.30am	15	NEG	0.0	12.1	200	
5pm				10.5		
9.30pm		NEG	2.0	10.1		
DAY NO. 5						
9.30am	17	NEG	2.0	8.0	450	12.75
5pm				7.7		
9.30pm		NEG	2.0	15.3		
DAY NO. 6						
9.30am	18	NEG	0.0	6.1	150	
5pm				4.5		
9.30pm		NEG	0.25	10.4		
DAY NO. 7						
9.30am	17	NEG	0.25	5.1	100	
5pm				5.6		
9.30pm		NEG	0.25	8.9		
DAY NO. 8						
9.30am	17	NEG	0.0	5.4	100	12.5
5pm				6.2		
9.30pm		NEG	2.0	13.7		
DAY NO. 9						
9.3Oam						
5pm	17	NEG	0.0	4.1	150	
9.30pm				2.8		
		NEG	0.75	8.1		
DAY NO. 10						
9.30am	13	NEG	0.0	8.6	200	12.5
5pm				8.2		
9.30pm		NEG	0.0	8.8		
DAY NO. 11						
9.30am	13	NEG	0.0	7.4	25	
5pm				5.1		
9.30pm		NEG	0.0	9.8		
DAY NO. 12						
9.30am	13	NEG	0.0	11.1	100	
5pm				11.6		
9.30pm		NEG	0.0	16.5		
DAY NO. 13						
9.30am	13	NEG	0.0	14.6	100	
5pm				8.1		

CASE REPORT DOG C14

BREED: Rottweiler

AGE: 5 years

SEX: Male

HISTORY:

This dog was referred for diabetic stabilisation with a history of weight loss, polyphagia, polydipsia and polyuria of 4 weeks duration. Diarrhoea, vomiting, dullness and exercise intolerance had been noted over the latter 2 weeks. There was no history of chronic steroid therapy or pancreatitis.

CLINICAL EXAMINATION AND INVESTIGATION:

This dog was alert but dull in demeanour. His breath smelt strongly of ketones. Heart rate was 80/min. Rectal temperature was 101°F. Thoracic auscultation and abdominal palpation revealed no abnormalities. Hair coat was in poor condition. There was bilateral, tacky, mucopurulent ocular discharge.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	4.7	WBCC	12.9
SODIUM	137	NEUTROPHILS	10.06
POTASSIUM	3.4	LYMPHOCYTES	2.06
CHLORIDE	102	MONOCYTES	0.36
PHOSPHATE	1.19	EOSINOPHILS	0.32
GLUCOSE	22.8	RBCC	7.33
CHOLESTEROL	8.71	HAEMOGLOBIN (HB)	16.7
CREATININE	68	HAEMATOCRIT	47.8
TOTAL BILIRUBIN	4	MEAN CELL VOLUME	65
ALKALINE PHOS	472	MEAN CELL HB	22.7
ALT	185	MEAN CELL HB CONC.	34.9
AST	144	PLATELETS	239
TOTAL PROTEIN	68		
ALBUMIN	27		
GLOBULIN	49		

Lateral thoracic and abdominal radiographs revealed moderate hepatomegaly.

Schirmer tear test on both eyes showed tear production to be normal and trypsin-like-immunoreactivity test showed exocrine pancreatic function to be normal.

The day following admission this dog was entered into the Caninsulin study. Starting dose was 40IU and diet comprised 750g canned dog food (Chappie; Pedigree Petfoods) and 175g bread fed twice daily (60kcal/kg/day).

HOSPITALISATION:

This dog was hospitalised for a period of 14 days. During this time rectal temperature never exceeded 102°F. Therapy with Chloromycetin Ophthalmic ointment (Parke Davis) was begun on day 1. Water intake fell from 3000 - 3500ml/day to 1600 - 1900ml/day. Insulin dose increased to 49IU by day 8 and remained at that level until discharge. Bodyweight increased by lkg whilst hospitalised. The dog became noticeably bright by day 7 and coat condition had improved markedly by this time. The ocular discharge resolved by day 4.

The dog was discharged on day 14 and the owners were advised to monitor morning urine glucose concentrations.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	40	LARGE	2.0	21.4	NM	40
5pm				17.4		
9.30pm		MOD.	1.0	33.1		
DAY NO. 2						
9.30am	44	NEG	0.0	22.1	NM	
5pm				3.8		
9.30pm		NEG	0.0	3.5		
DAY NO. 3						
9.30am	42	NS	NS	30.1	3000	
5pm				12.4		
9.30pm		NEG	2.0	19.6		
DAY NO. 4						
9.30am	44	NEG	2.0	22.2	3500	
5pm				16.2		
9.30pm		NEG	2.0	19.8		
DAY NO. 5						
9.30am	46	NEG	2.0	21.3	3000	
5pm				11.7		
9.30pm		NEG	0.0	9.9		
DAY NO. 6						
9.30am	46	NEG	2.0	25.2	3500	
5pm				10.7		
9.30pm		NEG	2.0	19.6		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 7						
9.30am	47	NEG	2.0	23.9	2000	
5pm				19.6		
9.30pm		NEG	2.0	27.1		
DAY NO. 8						
9.30am	49	NEG	2.0	15.1	1800	
5pm				5.5		
9.30pm		NEG	2.0	17.9		
DAY NO. 9						
9.30am	49	NEG	0.25	9.3	1600	41
5pm				5.2		
9.30pm		NEG	0.0	4.5		
DAY NO. 10						
9.30am	49	NEG	0.25	24.5	1800	
5pm				8.1		
9.30pm		NEG	0.25	16.7		
DAY NO. 11						
9.30am	49	NEG	0.0	17.5	NM	
5pm				5.2		
9.30pm		NEG	0.0	9.6		
DAY NO. 12						
9.30am	49	NEG	0.0	16.0	1900	
5pm				8.1		
9.30pm		NEG	0.0	8.6		
DAY NO. 13						
9.30am	49	NEG	0.25	22.3	1600	
5pm				10.5		
9.30pm		NEG	0.0	6.9		
DAY NO. 14						
9.30am	49	NEG	0.75	17.5		41
5pm				7.4		

CASE REPORT DOG C15

BREED: Labrador

AGE 8 years

SEX: Speyed female

HISTORY:

One year before referral the dog developed a painful neck and was placed on continuing corticosteroid therapy (5mg eod). Three months later an ovariohysterectomy was performed as treatment for pyometritis. Extreme polydipsia and marked weight loss developed during the 3 weeks prior to referral. The dog had formerly been obese. The dog's condition progressively deteriorated until she was referred to G.U.V.S. in a collapsed state.

CLINICAL EXAMINATION AND INVESTIGATION:

On admission this dog was collapsed. Heart rate was 124/minute. Mucous membranes were tacky but pink. Abdominal palpation revealed no abnormalities and was not resented. No cardiac murmurs or adventitious pulmonary sounds were detected on auscultation.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	18.2	WBCC	18.3
SODIUM	141	NEUTROPHILS	17.39
POTASSIUM	2.7	LYMPHOCYTES	0.73
CHLORIDE	111	MONOCYTES	0.18
CALCIUM	2.35	RBCC	6.29
PHOSPHATE	1.62	HAEMOGLOBIN (HB)	14.4
GLUCOSE	27.5	HAEMATOCRIT	43.1
CHOLESTEROL	9.95	MEAN CELL VOLUME	69
CREATININE	112	MEAN CELL HB	22.8
TOTAL BILIRUBIN	2	MEAN CELL HB CONC.	33.4
ALKALINE PHOS	3,858	PLATELETS	584
ALT	61		
AST	34		
TOTAL PROTEIN	64		
ALBUMIN	30		
GLOBULIN	34		
AMYLASE	16,120		

Radiographs of thorax and abdomen revealed an area of localised peritonitis consistent with pancreatitis.

Urine was very dark and red/brown in colour and on bacteriological culture was found to contain β-haemolytic *E. coli*.

Diagnoses of diabetes mellitus, ketoacidosis, pancreatitis, hypokalaemia and bacterial cystitis were made. The dog was denied access to oral food or fluids for 8 days and treated with intravenous fluids, intravenous potassium supplementation, neutral insulin (Hypurin Neutral, Fisons), Caninsulin and clavulanate potentiated amoxycillin (Synulox: SK Beecham).

At the end of this initial 8 day period this dog was included in the Caninsulin trial. Starting dose of Caninsulin was 25IU and diet comprised 560g of canned dog food (Chappie: Pedigree Petfoods) and 135g of bread twice daily.

HOSPITALISATION:

The dog was hospitalised for a further 11 days for stabilisation with Caninsulin. During this period insulin dose increased to 48IU. Unfortunately because of owner pressure this dog was discharged before reaching 'ideal' glycaemic stability. During stabilisation the dog's clinical condition varied very little, remaining bright and lively throughout. Rectal temperature never exceeded 101.8°F at any examination time. By day 4, urine was clear and culture negative for bacteria. Polydipsia reduced as a result of treatment with Caninsulin but this was impossible to record because the dog regularly tipped its water bowl.

The dog was discharged before good glycaemic control was achieved and the owners were instructed to continue to adjust insulin dosage according to morning urine glucose concentrations.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	25	NEG	0.0	3.5	NM	30
5pm				5.3		
9.30pm		NEG	0.0	6.3		
DAY NO. 2						
9.30am	21	NEG	0.0	12.7	NM	
5pm				3.7		
9.30pm		NEG	0.0	7.7		
DAY NO. 3						
9.30am	21	NEG	1.0	31.1	NM	
5pm				17.5		
9.30pm		NEG	2.0	17.1		
DAY NO. 4						
9.3Oam	23	NEG	1.0	21.0	NM	
5pm				20.2		
9.30pm		NEG	2.0	24.8		
DAY NO. 5						
9.30am	27	NEG	2.0	22.9	NM	
5pm				24.8		
9.30pm		NEG	2.0	31.1		
DAY NO. 6						
9.30am	30	NEG	2.0	18.7	NM	
5pm				22.7		
9.30pm		NEG	2.0	25.3		
DAY NO. 7						
9.30am	32	NEG	2.0	20.7	NM	
5pm				21.0		
9.3Opm		NS	NS	23.6		
DAY NO. 8						
9.30am	36	NEG	2.0	23.4	NM	33
5pm				22.8		
9.30pm		NEG	2.0	24.2		
DAY NO. 9						
9.30am	40	NEG	0.0	NS	NM	
5pm				22.0		
9.3Opm		NEG	2.0	NS		
DAY NO. 10						
9.30am	44	NEG	2.0	NS	NM	
5pm				24.0		
9.3Opm		NEG	2.0	NS		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 11						
9.30am	48	NEG	1.0	7.5	NM	
5pm				15.4		
9.3Opm		NS	NS	NS		
DAY NO. 12						
9.30am	48	NEG	1.0	NS	NM	
5pm				7.2		

CASE REPORT DOG C16

BREED: West Highland White terrier

AGE: 14.5 years

SEX: Speyed female

HISTORY:

This dog was referred to G.U.V.S. for diabetic stabilisation following sudden onset blindness. There was no history of previous major medical or surgical illness. Polydipsia and polyuria had been apparent for approximately 14 days. There was no history of pancreatic disease or therapy with reproductive or corticosteroid hormones. Ovariohysterectomy had been performed when the dog was less than one year of age.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was dull and disinterested. There was a tacky mucopurulent discharge from the right eye. Much dental calculus and gingivitis were present. Heart rate was 130/minute and colour, CRT and pulse volume were good. There were no cardiac murmurs. Rectal temperature was 103.4°F. Peripheral lymph nodes, i.e., submandibular, prescapular and popliteal were all significantly enlarged. The dog smelt stongly of ketones. There were bilateral, diffuse, mature cataracts. The hair coat was thin for this breed of dog. Abdominal palpation revealed a pendulous abdomen and hepatomegaly.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	4.7	WBCC	16.0
SODIUM	142	NEUTROPHILS	11.12
POTASSIUM	5.7	LYMPHOCYTES	3.68
CHLORIDE	96	MONOCYTES	1.12
CALCIUM	2.85	EOSINOPHILS	0.08
PHOSPHATE	0.94	RBCC	6.96
GLUCOSE	24.8	HAEMOGLOBIN (HB)	15.6
CHOLESTEROL	4.74	HAEMATOCRIT	44.7
CREATININE	74	MEAN CELL VOLUME	64
TOTAL BILIRUBIN	0	MEAN CELL HB	22.4
ALKALINE PHOS	1,602	MEAN CELL HB CONC.	34.8
ALT	79	PLATELETS	507
AST	43		
TOTAL PROTEIN	68		
ALBUMIN	39		
GLOBULIN	29		
AMYLASE	1891		

A Schirmer tear test was performed and tear production was normal. Aspirates of enlarged lymph nodes were examined and found to contain a mixed population of cells consistent with reactive hyperplasia. Radiographic examination of the thorax and abdomen revealed hepatomegaly, osteoporosis, bronchial calcification and a pendulous, fat-filled abdomen.

This dog was entered into the Caninsulin trial on the day after admission to G.U.V.S.. The starting dose of Caninsulin for this dog was 12IU. Diet comprised 325g canned dog food (Chum; Pedigree Petfoods) and 65g bread fed twice daily. Therapy with potentiated sulphonamide (co-trimoxazole) was instituted on day 1 because of the lymphadenopathy.

HOSPITALISATION:

The dog was hospitalised for 14 days and for the first 3 days she remained dull, although her appetite remained good. After day 3, her rectal temperature was below 102.6°F at all examination times, she became bright and alert and the ocular discharge improved. Insulin dose was increased to 25IU over the first week but this was then reduced to a final dose of 14IU. On day 4 the previous day's insulin results were not available when the decision to increase insulin dose to 24IU was made. This was an incorrect decision, as Somogyi overswing phenomen would appear to have occurred on day 3 and perhaps also on day 4. During the 14 day period of hospitalisation water intake reduced from 1500ml/day to 600ml/day.

On day 15 the dog was discharged on 14IU. Lymphadenopathy was still present and co-trimoxazole therapy was continued for another 7 days. The owners were not provided with urine testing equipment.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	12	NS	NS	20.7	1500	12
5pm				22.5		
9.3Opm		SMALL	2.0	20.7		
DAY NO. 2						
9.30am	16	NEG	2.0	23.7	1500	
5pm				19.7		
9.30pm		SMALL	2.0	21.2		
DAY NO. 3						
9.30am	20	TRACE	2.0	22.0	1450	
5pm				2.0		
9.30pm		NEG	2.0	32.4		
DAY NO. 4						
9.30am	24	TRACE	2.0	28.7	1350	
5pm				5.5		
9.30pm		NEG	2.0	31.7		
DAY NO. 5						
9.30am	24	TRACE	2.0	22.8	NM	
5pm				3.8		
9.3Opm		SMALL	2.0	23.0		
DAY NO. 6						
9.30am	25	MOD	2.0	25.7	650	
5pm				2.8		
9.30pm		NEG	0.0	7.1		
DAY NO. 7						
9.30am	22	NEG	1.0	13.5	850	12.25
5pm				2.8		
9.30pm		NEG	2.0	23.4		
DAY NO. 8						
9.30am	18	NEG	0.0	16.8	650	
5pm				2.4		
9.30pm		NEG	0.5	15.8		
DAY NO. 9						
9.30am	14	NEG	1.0	17.3	NM	
5pm				3.1		
9.30pm		NEG	0.25	12.3		
DAY NO. 10						
9.30am	14	NEG	0.25	9.3	1000	
5pm				3.1		
9.30pm		NEG	0.0	12.8		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 11						
9.30am						
5pm	14	NEG	1.0	21.1	950	
9.30pm				3.4		
		NEG	2.0	23.8		
DAY NO. 12						
9.30am	14	TRACE	2.0	20.7	900	
5pm				5.2		
9.30pm		NEG	2.0	13.2		
DAY NO. 13						
9.30am	14	TRACE	2.0	22.2	600	
5pm				5.9		
9.30pm		NEG	0.0	6.2		
DAY NO. 14						
9.30am	14	NEG	0.25	20.5		
5pm				6.3		
9.30pm		NEG	0.0	11.8		
DAY NO. 15						
9.30am	14	NEG	1.0	20.4		12

CASE REPORT DOG C17

BREED: Collie-X

AGE: 11 years

SEX: Male

HISTORY:

This dog was referred for diabetic stabilisation following polydipsia and polyuria over the preceeding 2 weeks. Weight loss and exercise intolerance had become apparent during the previous week. There was no history of prolonged steroid therapy nor of generalised pancreatic disease.

CLINICAL EXAMINATION AND INVESTIGATION:

On examination this dog was bright and alert. Colour, CRT and pulse volume were all good. Heart rate was 108/minute. There were no ocular discharges. Auscultation revealed no cardiac murmurs and no pulmonary adventitious sounds. Lymph nodes were normal in size. Abdominal palpation revealed no significant abnormalities.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.1	WBCC	14.6
SODIUM	141	NEUTROPHILS	12.56
POTASSIUM	4.0	LYMPHOCYTES	1.31
CHLORIDE	102	MONOCYTES	0.37
PHOSPHATE	0.94	EOSINOPHILS	0.79
GLUCOSE	31.1	RBCC	6.62
CHOLESTEROL	6.16	HAEMOGLOBIN (HB)	15.2
CREATININE	91	HAEMATOCRIT	42.9
TOTAL BILIRUBIN	0	MEAN CELL VOLUME	65
ALKALINE PHOS	372	MEAN CELL HB	22.9
ALT	97	MEAN CELL HB CONC.	35.4
AST	24	PLATELETS	274
TOTAL PROTEIN	73		
ALBUMIN	37		
GLOBULIN	36		

Radiography of thorax and abdomen revealed only slight hepatomegaly.

Bacteriological culture of urine yielded significant growth of a β-haemolytic *Streptococcus* sensitive to ampicillin. A 7 day course of ampicillin was commenced.

On the day of admission this dog was included in the Caninsulin trial. Starting dose of Caninsulin was 19IU and diet comprised 450g of canned dog food (Chum; Pedigree Petfoods) and 100g of bread fed twice daily (65kcal/kg/day).

HOSPITALISATION:

During the subsequent 11 days of hospitalisation the dog remained bright and active. Rectal temperature remained below 101.6°F at all examination times. Water intake varied between 300 and 1400ml/day. Insulin dose was increased to 28IU by day 4 and maintained at that level until the dog was discharged. Body weight remained constant.

Upon discharge the owners were advised to maintain the diet and insulin dose at the hospitalisation levels unless instructed to change them at at follow up check. They were not supplied with urine glucose testing equipment.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	19	NEG	2.0	31.1	NM	19
5pm				19.5		
9.30pm		NEG	2.0	21.7		
DAY NO. 2						
9.30am	20	NEG	2.0	28.5	800	
5pm				16.1		
9.30pm		NEG	2.0	19.6		
DAY NO. 3						
9.30am	24	NS	NS	16.6	300	
5pm				12.2		
9.30pm		NEG	1.0	20.6		
DAY NO. 4						
9.30am	28	NEG	0.5	14.3	550	
5pm				5.2		
9.30pm		NEG	1.0	14.7		
DAY NO. 5						
9.30am	28	NEG	0.0	5.0	NM	
5pm				4.5		
9.30pm		NEG	0.0	12.1		
DAY NO. 6						
9.30am	28	NEG	0.0	10.1	450	
5pm				3.5		
9.30pm		NEG	0.0	14.0		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 7						
9.30am	28	NEG	0.0	7.9	NM	
5pm				4.2		
9.30pm		NEG	0.0	11.9		
DAY NO. 8						
9.30am	28	NEG	1.5	16.6	1400	
5pm				4.7		
9.3Opm		NEG	2.0	5.5		
DAY NO. 9						
9.30am	28	NEG	0.0	15.1	NM	
5pm				4.2		
9.30pm		NEG	1.0	17.1		
DAY NO. 10						
9.30am	28	NEG	1.5	7.2	450	
5pm				3.3		
9.30pm		NEG	0.0	6.3		
DAY NO. 11						
9.30am	28	NEG	0.0	9.1	NM	19
5pm				3.5		

CASE REPORT DOG C18

BREED: Cairn terrier

AGE: 5.5 years

SEX: Male

HISTORY:

This dog was referred for diabetic stabilisation having had a mature diffuse cataract of the right eye for 5 months and polyuria, polydipsia and weight loss during the few weeks prior to referral. The polydipsia was noted to be greatest in the evenings following meals. Exercise intolerance became apparent one week before referral. There was no history of prolonged steroid or reproductive hormone therapy nor of generalised pancreatic disease.

CLINICAL EXAMINATION AND INVESTIGATION:

On clinical examination this dog was bright and alert and apparently in good bodily condition. There was a dense cataract obvious in the right eye. The dog smelt strongly of ketones. Mucous membrane colour, CRT and pulses were good. Heart rate was 108/min. There were no cardiac murmurs nor adventitious lung sounds detected on thoracic auscultation. Rectal temperature was 102.4°F. Abdominal palpation revealed a tense fat abdomen and possible hepatomegaly.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	4.5	WBCC	10.1
SODIUM	137	NEUTROPHILS	7.58
POTASSIUM	4.0	LYMPHOCYTES	1.11
CHLORIDE	99	MONOCYTES	0.86
CALCIUM	2.58	EOSINOPHILS	0.56
PHOSPHATE	0.73	RBCC	6.56
GLUCOSE	22.4	HAEMOGLOBIN (HB)	15.1
CHOLESTEROL	6.89	HAEMATOCRIT	44.1
CREATININE	68	MEAN CELL VOLUME	67
TOTAL BILIRUBIN	1	MEAN CELL HB	23.0
ALKALINE PHOS	224	MEAN CELL HB CONC.	34.2
ALT	50	PLATELETS	475
AST	14		
TOTAL PROTEIN	74		
ALBUMIN	40		
GLOBULIN	34		

Radiography of thorax and abdomen demonstrated only hepatomegaly.

The day following admission, the dog was entered to the Caninsulin trial. Starting dose of Caninsulin was 12IU and diet comprised 260g canned dog food (Chappie: Pedigree Petfoods) and 60g bread fed twice daily (60kcal/kg/day).

HOSPITALISATION:

This dog was hospitalised for 14 days during which time the dose of insulin was increased to 21IU over the first 5 days and then reduced to 19IU. The dog remained bright and alert throughout the hospitalisation period. Rectal temperature remained below 101.4°F at all examination times. Water intake varied between 200 and 1500ml/day. On day 14, the dog was discharged and the owners were advised to continue the new diet and 19IU of insulin unless directed otherwise at follow up examinations.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	12	LARGE	2.0	19.2	1500	12.0
5pm				21.7		
9.30pm		TRACE	1.0	22.4		
DAY NO. 2						
9.30am	16	SMALL	1.0	28.4	NM	
5pm				7.5		
9.30pm		NEG	0.25	28.5		
DAY NO. 3						
9.30am	17	NEG	0.25	9.4	100	
5pm				6.4		
9.30pm		NEG	0.0	5.9		
DAY NO. 4						
9.30am	17	NEG	0.0	5.3	950	
5pm				4.2		
9.30pm		NEG	0.0	4.6		
DAY NO. 5						
9.30am	21	NEG	0.75	12.0	900	
5pm				3.7		
9.30pm		NEG	0.0	6.9		
DAY NO. 6						
9.30am	20	NEG	0.0	3.5	NM	12.5
5pm				3.4		
9.30pm		NEG	0.0	5.0		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 7						
9.3Oam	19	NEG	0.0	11.9	NM	
5pm				4.3		
9.30pm		NEG	0.0	8.7		
DAY NO. 8						
9.30am	19	NEG	0.25	11.7	200	
5pm				4.9		
9.30pm		NEG	0.0	6.6		
DAY NO. 9						
9.30am	19	NEG	0.5	12.6	450	
5pm				4.6		
9.30pm		NEG	0.0	5.1		
DAY NO. 10						
9.30am	20	NEG	0.0	13.0	NM	
5pm				3.6		
9.30pm		NEG	0.0	6.6		
DAY NO. 11						
9.30am	19	NEG	0.0	8.0	NM	12.0
5pm				4.3		
9.30pm		NEG	0.0	7.1		
DAY NO. 12						
9.30am	19	NEG	0.0	8.4	345	
5pm				4.2		
9.30pm		NEG	0.0	10.1		
DAY NO. 13						
9.30am	19	NEG	0.5	14.6	400	12.0
5pm				3.6		
9.30pm		NEG	0.0	2.5		
DAY NO. 14						
9.30am	19	NEG	0.0	11.8		

CASE REPORT DOG C19

BREED: Miniature Poodle

AGE: 9 years

SEX: Male

HISTORY:

For one month prior to referral for diabetic stabilisation, this dog had become significantly polydipsic and polyphagic and had lost weight in the two weeks before referral. There was no history of prolonged steroid therapy or pancreatitis. There was no history of any previous major medical or sugical illness.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright and alert. Hair coat appeared thin over caudal dorsum but the dog had recently been clipped. The dog also smelt strongly of ketones. Rectal temperature was 101°F. Colour and CRT were good. Auscultation revealed no cardiac murmurs nor adventitious pulmonary sounds. There was bilateral purulent ocular discharge and bilateral enlargement of submandibular and prescapular lymph nodes. Abdominal palpation revealed hepatomegaly.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	7.4	WBCC	14.1
SODIUM	137	NEUTROPHILS	9.73
POTASSIUM	4.6	LYMPHOCYTES	2.68
CHLORIDE	97	MONOCYTES	1.62
CALCIUM	2.24	RBCC	6.93
PHOSPHATE	1.35	HAEMATOCRIT	44.4
GLUCOSE	26.0		
CHOLESTEROL	5.95		
CREATININE	44		
ALKALINE PHOS	627		
ALT	317		
AST	330		
TOTAL PROTEIN	70		
ALBUMIN	33		
GLOBULIN	37		
CORTISOL	40		
POST SYN 45MIN	195		
POST SYN 90MIN	231		
BASAL INSULIN	4.1		

Radiography of thorax and abdomen revealed changes consistent with hypovolaemia in addition to hepatomegaly and obesity.

This dog was included in the Caninsulin study from the day after admission on a starting dose of 6IU and a diet comprising 190g canned dog food (Chum;Pedigree Petfoods) and 40g bread fed twice daily.

HOSPITALISATION:

Diabetic stabilisation of this dog was undertaken during 11 days of hospitalisation. Ampicillin therapy was prescribed on admission because of the enlarged lymph nodes. Clinical condition did not vary much over this period and the dog remained bright, alert and appetite improved after 3 days of hospitalisation. Submandibular and prescapular lymph nodes decreased in size only slightly. The purulent ocular discharge resolved after 7 days. Rectal temperature remained below 101°F. Water intake reduced from 1300 to 150-200ml/day. Insulin dose was increased up to 18IU but was reduced to a final dose of 16IU.

The dog was discharged on 16IU on day 10 and the owners were advised to increase the insulin dose if polydipsia became marked.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	6	MOD	2.0	21.5	1300	6
5pm				9.4		
9.30pm		MOD	2.0	30.6		
DAY NO. 2						
9.30am	6	SMALL	2.0	31.6	850	
5pm				27.2		
9.30pm		NEG	2.0	30.6		
DAY NO. 3						
9.30am	10	MOD	2.0	25.6	720	
5pm				25.1		
9.30pm		NEG	2.0	22.1		
DAY NO. 4						
9.30am	14	TRACE	2.0	27.0	800	
5pm				21.0		
9.30pm		NEG	2.0	20.1		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 5						
9.30am	16	NEG	2.0	24.9	650	
5pm				19.4		
9.30pm		NEG	2.0	19.1		
DAY NO. 6						
9.30am	18	NEG	2.0	30.7	150	
5pm				6.7		
9.30pm		NEG	2.0	18.7		
DAY NO. 7						
9.30am	18	NEG	2.0	4.6	250	
5pm				7.7		
9.30pm		NEG	1.5	17.0		
DAY NO. 8						
9.30am	18	NEG	0.25	5.9	150	6
5pm				4.8		
9.30pm		NEG	0.5	5.1		
DAY NO. 9						
9.30am	18	NEG	1.0	3.5	200	
5pm				2.5		
9.30pm		NEG	0.0	5.9		
DAY NO. 10						
9.30am	16	NEG	0.25	3.4		6
5pm				4.8		

CASE REPORT DOG C20

BREED: Australian terrier

AGE: 8 years

SEX: Male

HISTORY:

This dog was referred to G.U.V.S. for diabetic stabilisation after being polydipsic for 2 months. In the 2 weeks prior to referral, polyphagia, weight loss and exercise intolerance had also become apparent. There was no history of prolonged corticosteroid therapy nor of generalised pancreatic disease.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was quiet and dull but interested in its surroundings. His coat was sparse and he smelt strongly of ketones. On auscultation there were no cardiac murmurs or pulmonary adventitious sounds. Heart rate was 130/minute. Pulse volume, colour and CRT were all good. There was no cataract formation nor lymphadenopathy.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	6.6	WBCC	16.6
SODIUM	142	NEUTROPHILS	12.62
POTASSIUM	4.2	LYMPHOCYTES	1.91
CHLORIDE	103	MONOCYTES	1.25
CALCIUM	2.22	EOSINOPHILS	0.83
PHOSPHATE	0.71	RBCC	7.34
GLUCOSE	26.6	HAEMOGLOBIN (HB)	17.2
CHOLESTEROL	5.02	HAEMATOCRIT	46.8
CREATININE	131	MEAN CELL VOLUME	64
TOTAL BILIRUBIN	0	MEAN CELL HB	23.4
ALKALINE PHOS	14	MEAN CELL HB CONC.	36.7
ALT	172	PLATELETS	192
AST	36		
TOTAL PROTEIN	69		
ALBUMIN	38		
GLOBULIN	31		

Because of the thin hair coat and a tendency to bruise on blood sampling, an ACTH stimulation test was performed but Cushing's disease was not present (plasma cortisol; pre-ACTH 53, post-ACTH 45min 94, post-ACTH 90min 222nmol/1).

Radiography of thorax and abdomen revealed hepatomegaly, prostatomegaly, right sided cardiomegaly and a pendulous abdomen containing much fat.

The day following admission this dog was included in the Caninsulin study. The starting dose of insulin was 10IU and diet comprised 150g canned dog food (Chappie; Pedigree Petfoods) and 50g of bread fed twice daily (75kcal/kg/day).

HOSPITALISATION:

In the first 3 days of hospitalisation the dog's clinical condition improved significantly and he became brighter and more active. Rectal temperature never exceeded 101.6°F at all examination times. Water intake reduced from 1000-1050 to 200-300ml/day. Insulin dose was increased initially to 13IU but was reduced to 8IU. Body weight remained constant.

The dog was discharged on day 12 with instructions to the owners to maintain the dog on this dose and diet until instructed to do otherwise. They were not supplied with urine testing equipment.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	10	TRACE	1.75	26.5	800	8.7
5pm				15.3		
9.30pm		NEG	2.0	18.7		
DAY NO. 2						
9.30am	12	NEG	2.0	19.8	600	
5pm				6.2		
9.30pm		NEG	2.0	18.2		
DAY NO. 3						
9.30am	13	NEG	2.0	16.5	1000	
5pm				5.2		
9.30pm		NEG	2.0	23.5		
DAY NO. 4						
9.30am	13	NEG	1.0	14.7	750	8.7
5pm				2.9		
9.30pm		NEG	0.0	3.8		
DAY NO. 5						
9.30am	11	NEG	0.25	8.8	750	
5pm				3.4		
9.30pm		NEG	0.0	5.1		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 6						
9.30am	10	NEG	0.1	11.8	1050	
5pm				4.1		
9.30pm		NEG	0.0	6.0		
DAY NO. 7						
9.30am	10	NEG	2.0	4.0	800	
5pm				3.9		
9.30pm		NEG	2.0	20.1		
DAY NO. 8						
9.30am	8	NEG	1.5	14.0	NM	
5pm				4.8		
9.30pm		NEG	2.0	28.7		
DAY NO. 9						
9.30am	8	NEG	1.5	13.9	450	
5pm				6.3		
9.30pm		NEG	1.5	25.4		
DAY NO. 10						
9.30am	8	NEG	1.0	19.7	200	
5pm				14.9		
9.30pm		NEG	2.0	21.4		
DAY NO. 11						
9.30am	8	NEG	1.0	14.7	300	8.7
5pm				5.3		
9.30pm		NEG	0.25	7.0		
DAY NO. 12						
9.30am	8	NS	NS	NS		

CASE REPORT DOG C21

BREED: Jack Russell terrier

AGE: 11 years

SEX: Female

HISTORY:

This dog was referred for diabetic stabilisation with a two week history of polydipsia and progressive exercise intolerance. She had suffered a similiar condition for a few weeks 7 months previously. There was no history of previous therapy with long acting corticosteroids nor progestagens and she had been in oestrus 4-6 weeks prior to referral.

CLINICAL EXAMINATION AND INVESTIGATION:

The dog was bright and alert. Mucous membranes were congested in colour and there was much dental calculus. Heart rate was 130/minute. Capillary refill time and pulse volume were good. Auscultation of the thorax revealed no cardiac murmurs nor adventitious pulmonary sounds. There were small cataracts in the ventral borders of both lenses and, in addition, a number of mammary tumours were present.

LABORATORY FINDINGS:

BIOCHEMISTRY:		HAEMATOLOGY:	
UREA	4.7	WBCC	25.3
SODIUM	145	NEUTROPHILS	20.24
POTASSIUM	3.6	LYMPHOCYTES	3.29
CHLORIDE	97	MONOCYTES	1.01
CALCIUM	2.94	EOSINOPHILS	0.13
PHOSPHATE	1.08	RBCC	5.04
GLUCOSE	16.4	HAEMOGLOBIN (HB)	11.0
CHOLESTEROL	12.75	HAEMATOCRIT	33.9
CREATININE	65	MEAN CELL VOLUME	67
TOTAL BILIRUBIN	0	MEAN CELL HB	21.8
ALKALINE PHOS	929	MEAN CELL HB CONC.	32.4
ALT	113	PLATELETS	396
AST	23		
TOTAL PROTEIN	71		
ALBUMIN	37		
GLOBULIN	34		

Lateral radiographs of thorax and abdomen revealed no significant abnormalities.

The day following admission to G.U.V.S. this dog was entered into the Caninsulin trial. The starting dose of insulin was 6IU and diet comprised 170g canned dog food (Chappie; Pedigree Petfoods) and 40g of bread fed twice daily (70kcal/kg).

HOSPITALISATION:

The clinical condition of this dog remained unchanged during hospitalisation. Rectal temperature remained normal. Because of the initial neutrophilia, ampicillin therapy was instituted on day 1. Insulin dose increased up to a maximum of 20IU by day 6. Water intake varied between 50 and 650ml/day. Collection of urine samples was seldom possible. By day 9, it was apparent that plasma glucose concentrations were never significantly greater than normal values, suggesting that the insulin requirement had become minimal. Insulin dosages from this time were, therefore, reduced dramatically until only very low doses of insulin were required to control plasma glucose concentrations. Ovariohysterectomy was performed on day 13 as it was felt that the dog had diabetes mellitus secondary to raised circulating levels of progesterone following the recent oestrus. She made an uneventful recovery during which insulin replacement therapy was discontinued.

When discharged on day 17 the owner was instructed to treat the dog normally. A follow up check was arranged for one week post discharge with the intention of re-instituting insulin therapy if the plasma glucose concentration was markedly elevated at that time but this was not necessary.

HOSPITAL SUMMARY:

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 1						
9.30am	6	NS	NS	17.2	300	6.3
5pm				31.1		
9.30pm		NS	NS	32.2		
DAY NO. 2						
9.30am	10	NS	NS	20.9	600	
5pm				25.7		
9.30pm		NS	NS	35.8		
DAY NO. 3						
9.30am	14	NS	NS	19.5	400	6.5
5pm				19.5		
9.30pm		NS	NS	30.9		
DAY NO. 4						
9.30am	16	NS	NS	20.6	300	
5pm				21.6		
9.30pm		NS	NS	30.1		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 5						
9.30am	18	NS	NS	18.9	600	
5pm				22.6		
9.3Opm		NS	NS	20.2		
DAY NO. 6						
9.30am	20	NS	NS	18.4	400	6.6
5pm				6.6		
9.30pm		NS	NS	8.8		
DAY NO. 7						
9.30am	20	NS	NS	15.2	200	
5pm				5.4		
9.30pm				7.2		
DAY NO. 8						
9.30am	20	NS	NS	10.1	200	
5pm				3.1		
9.3Opm		NS	NS	11.6		
DAY NO. 9						
9.30am	18	NS	NS	8.2	400	
5pm				3.0		
9.3Opm		NS	NS	6.8		
DAY NO. 10						
9.30am	14	NS	NS	8.1	100	
5pm				3.1		
9.3Opm		NS	NS	5.3		
DAY NO. 11						
9.30am	10	NS	NS	6.8	650	
5pm				3.0		
9.30pm		NEG	0.0	6.7		
DAY NO. 12						
9.30am	10	NEG	0.0	7.2	650	
5pm				2.9		
9.30pm		NEG	0.0	6.3		
DAY NO. 13						
9.30am	0	NS	NS	7.3	NM	
5pm				9.1		
9.30pm		NS	NS	9.6		
DAY NO. 14						
9.30am	4	NS	NS	8.1	50	
5pm				7.9		
9.3Opm		NS	NS	9.1		

	Insulin dose (IU)	Urine ketones	Urine glucose (g/dl)	Plasma glucose (mmol/l)	Water intake (mls)	Body-weight (kg)
DAY NO. 15						
9.30am	4	NS	NS	6.9	NM	
5pm				5.4		
9.30pm		NS	NS	7.1		
DAY NO. 16						
9.30am	3	NS	NS	6.4	50	
5pm				6.1		
9.30pm		NS	NS	6.8		
DAY NO. 17						
9.30am	3	NS	NS	6.3		6.6
5pm				6.1		

Appendix 12:
Quantitative analysis of canine plasma lipoproteins

The concentration of cholesterol in each lipoprotein fraction (very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)) were measured following separation by a combined ultracentrifugation/precipitation technique (Lipids Research Clinics, 1982; Barrie *et al*, 1993b).

In this method, 4ml of plasma was placed in a thermoplastic ultracentifuge tube (Ultraclear, 13x64mm; Beckman) and overlayered with 2.5ml normal saline (density 1.006g/ml). The tubes were capped and centrifuged at 164,000g at 4°C for 18 hours in a fixed angle rotor (type 50.4 Ti, Optima L-70 preparative ultracentrifuge; Beckman). The lipoproteins with density less than 1.006g/ml (VLDL) were removed in the top fraction by tube slicing and made up to a total volume of 3ml with normal saline. The cholesterol concentration of the plasma and infranatant were measured using a Cobas Mira clinical chemistry analyser and enzymatic colorimetric method (Chapter 2). The VLDL cholesterol was calculated as the difference between the cholesterol concentration of the plasma and that of the infranatant.

The apolipoprotein B containing lipoproteins (LDL) were precipitated from 1ml of the infranatant by the addition of 50µl of 92nM heparin-manganese chloride followed by incubation at 4°C for 30 minutes and separated by centrifugation at 10,800g at 4°C for 30 minutes. The cholesterol concentration of the resulting supernatant (HDL cholesterol) was measured and the LDL cholesterol concentration was calculated as the difference in cholesterol concentration between the infranatant and HDL.

Appendix 13:
Method for the analyses of LPL and H-TGL

Post-heparin plasma lipase activities were measured in heparinsed plasma from fasted dogs according to a method validated in horses (Watson *et al,* 1992) under the supervision of Dr TDG Watson in The Department of Pathological Biochemistry, Glasgow Royal Infirmary. Blood was collected 10 minutes after the intravenous adminstration of sodium heparin (70IU/kg) (Heparin (Mucous) Injection BP; Leo Laboratories Ltd) into lithium heparin containers and placed on ice before separation by centrifugation at 4°C for 15 minutes. Plasma was divided into 1 ml aliquots and stored at -20°C until analysis.

Radioactive triolein was formed by the addition of 50μCi glycerol (1-^{14}C) oleate (CFA 258; Amersham International plc) to 24.5 toluene, and then divided into 3.5ml aliquots and placed in round bottom flasks. To each flask was then added 3.5ml cold triolein (500mg triolein (T7140; Sigma) in 25ml toluene). The substrate was dried down under nitrogen in a water bath at 55°C, washed three times with 3ml heptane and stored under nitrogen at -20°C. The substrate emulsion was prepared 30 minutes prior to use by the addition of 5.5ml 5% gum arabic in 0.2M Tris-HCl pH8.4, followed by sonication at 18 microns for 4 minutes. Then 5.5ml 10 % bovine serum albumin (Fraction V (A-4503); Sigma) in 0.2M Tris HCl, pH8.4 was added.

For the measurement of total post-heparin plasma (PHP) lipase activity, 10μl of post-heparin plasma was diluted in 30μl 0.15M NaCl and incubated in duplicate with 200μl substrate, 250μl low salt buffer (0.2M Tris, 0.2M NaCl, pH 8.4) and 50μl pooled, heat-inactivated serum at 28°C for 60 minutes. Heparin triglyceride lipase (H-TGL) activity was assayed by the dilution of 10μl post-heparin plasma in 30μl 0.15M NaCl and incubated in duplicate with 200μl substrate, 250μl high salt buffer (0.2M Tris, 2.0M NaCl, pH 8:4) under the same conditions. Fatty acids were extracted by adding 3.25ml methanol:chloroform:heptane (1.41:1.25:1 parts) and 0.75ml 0.14M K_2CO_3, 0.14M H_3BO_3, pH 10.5. The tubes were vortexed and centrifuged at 3 000 rpm at 4°C for 30 minutes. One ml of the upper fraction was removed and counted in 10ml scintillation fluid (Ultima Gold; Canberra Packard) and 200μl acetic acid. the radioactivity in 1 ml of the upper fraction of blank incubations (containing 10μl 0.15M sodium chloride instead

of post-heparin plasma) was subtracted from the sample counts and the total radioactivity taken as the counts in 1ml of the lower fraction of the blank incubations. The lipolytic activity was then calculated according to the following equation:

$$\text{Activity } (\mu\text{molFA/ml/hr}) = ((\text{Sample cpm} - \text{Blank cpm}) * 755.1)/ (\text{Total cpm} - \text{Background cpm})$$

Lipoprotein lipase was calculated as the difference between total post-heparin lipolytic activity and hepatic triglyceride lipase activity

Appendix 14:
Method for the analysis of VLDL apolipoprotein B concentration

A colorimetric microplate protein method was designed using bicinchonic acid/copper sulphate microprotein reagent (B9643; Sigma) read against bovine serum albumin (BSA) standards (Sigma procedure No. TPRO-562). Apolipoprotein B concentrations were calculated from the differences between the total protein concentrations of the supernatants before and after precipitation of apolipoprotein B by isopropanol (Egusa, *et al* 1983).

To 2ml of VLDL containing top fraction resulting from quantitative analysis of plasma lipoproteins (Appendix 12) was added 2ml of 100% isopropanol. The mixture was vortexed for 1 minute, incubated overnight at room temperature in sealed conical plastic centrifuge tubes and then centrifuged (1000g for 30 minutes). If the precipitate was incompletely sedimented an aliquot was passed through a syringe filter (0.22μm pore).

Protein estimations were performed using a microplate modification of Sigma procedure number TPRO-562. 5μl of sample was added to 200μl of bicinchonic acid/copper sulphate solution containing 1% sodium dodecyl sulphate (to reduce light dispersal by the lipid content of the samples) in quadruplicate in a 96 well flat bottomed microplate (Cat no. 655101; Greiner Labortechnik Ltd). Bovine serum albumin standards were also included in quadruplicate in each plate at concentrations of 12.5, 25, 50, 100, 150, 200 and 400 μg/l. Each plate was read in a microplate reader (MR 5000, Dynatech Laboratories Ltd) at 560 nm. Integral curve fitting software calculated the protein concentration of each sample from a linear standard curve based on the the BSA standards.

Apolipoprotein B concentration was calculated from the difference in protein concentration between the original VLDL fraction and the post-precipitation supernatant (x2).

Appendix 15:
Case reports of 11 diabetic dogs from one month before to 4 months after changing to a canine high fibre diet

Dog 1

Case No.	115678
Dog name (+ owner surname)	Schultz Mulligan
Breed	Rottweiler
Age (at initial visit)	6yrs 6 mths
Sex	Male
Diagnosis, visit dates and treatment	
Date at -1 mth	8/6/92
Diagnosis	Diabetes mellitus
Time since diagnosis	November 1990
Treatment	Insulin - Caninsulin Diet - Chappie
Dietary and insulin history	
Previous diet	Chappie 1238g twice daily
Insulin type and dose	Caninsulin dose varied (38 - 60IU) once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 2030kcal/day) Insulin dose varied (38 - 56IU) because of 4 hypoglycaemic episodes.
Date at -10 days	6/7/92
Treatment	Insulin dose 46IU at the moment
New diet	1143g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	14/7/92
Treatment	46IU Caninsulin. No hypoglycaemic crises Soft faeces and mild diarrhoea on days 1,2 and 5
Weeks since last visit	1 week
Date at 1 mth	10/8/92
Treatment	Dose constant at 44IU. No hypoglycaemic episodes. One bout of bacterial cystitis - treated with Zaquilan 12% injection. Faeces soft
Weeks since last visit	4 weeks
Date at 2 mths	7/9/92
Treatment	38IU Caninsulin Faeces diarrhoeic. Some weight loss. Body condition good.
Weeks since last visit	4 weeks
Date at 3 mths	20/10/92
Treatment	40IU Caninsulin. Metronidazole 400mg BID for suspected SIBO. Faeces diarrhoeic.
Weeks since last visit	6 weeks
Date at 4 mths	3/12/92
Treatment	40IU Caninsulin. SIBO confirmed by plasma B12 and folate analyses (2/11/92) Oxytetracycline 500mg BID
Weeks since last visit	5 weeks

Dog 2

Case No.	119147
Dog name (+ owner surname)	Mitzi Macaldowie
Breed	Large cross-bred
Age (at initial visit)	7yrs
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	8/6/92
Diagnosis	Diabetes mellitus
Time since diagnosis	January 1992
Treatment	Insulin - Hypurin Isophane Diet - Chappie
Dietary and insulin history	
Previous diet	Chappie 825g and 2 slices of bread twice daily
Insulin type and dose	Isophane insulin 38-46IU once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 1785kcal/day) Insulin dose constant 46IU
Date at -10 days	6/7/92
Treatment	Insulin dose still 46IU. No other treatment.
New diet	950g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	14/7/92
Treatment	50IU Isophane insulin. No other treatment. Soft faeces and mild diarrhoea on days 1 and 2.
Weeks since last visit	1 week
Date at 1 mth	10/8/92
Treatment	Insulin dose down to 44IU. One hypoglycaemic episode in first week home. 4 diarrhoeic 'accidents' in house.
Weeks since last visit	4 weeks
Date at 2 mths	7/9/92
Treatment	Dog withdrawn from trial at owners' request. Seen at hospital 4 times for vomiting, colitis and diarrhoea. Treated with sulphasalazine, ampicillin and metaclopramide to little effect. Occasionally defecating in the house. Investigated for haematemesis - NAD. SIBO diagnosed (high plasma folate).
Weeks since last visit	4 weeks

Dog 3

Case No.	115131
Dog name (+ owner surname)	Shira Robertson
Breed	Jack Russell terrier
Age (at initial visit)	8.9yrs
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	8/6/92
Diagnosis	Diabetes mellitus
Time since diagnosis	September 1990
Treatment	Insulin - Caninsulin Diet - Chappie and bread
Dietary and insulin history	
Previous diet	176g Chappie and 40g bread twice daily
Insulin type and dose	Caninsulin 8-9IU once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 450kcal/day) Caninsulin 8IU
Date at -10 days	6/7/92
Treatment	8IU Caninsulin. No other treatment.
New diet	250g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	14/7/92
Treatment	10IU Caninsulin. No other treatment. No diarrhoea
Weeks since last visit	1 week
Date at 1 mth	11/8/92
Treatment	10IU Caninsulin Doing very well. Owners believe dog is much livelier
Weeks since last visit	4 weeks
Date at 2 mths	7/9/92
Treatment	10IU Caninsulin. Very bright and lively.
Weeks since last visit	4 weeks
Date at 3 mths	20/10/92
Treatment	10IU Caninsulin. Still doing very well.
Weeks since last visit	6 weeks
Date at 4 mths	3/12/92
Treatment	10IU Caninsulin Still very bright and active. Some weight loss
Weeks since last visit	5 weeks

Dog 4

Case No.	115980
Dog Reference No.	4
Dog name (+ owner surname)	Wendy Rowan
Breed	Labrador retriever
Age (at initial visit)	9yrs
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	8/7/92
Diagnosis	Diabetes mellitus, cervical disc disease.
Time since diagnosis	December 1990
Treatment	Insulin - Caninsulin Diet - Chappie and bread
Dietary and insulin history	
Previous diet	324g Chappie and 108g bread twice daily
Insulin type and dose	38-42IU Caninsulin
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 963kcal/day) 40IU Caninsulin
Date at -10 days	10/8/92
Treatment	40IU Caninsulin
New diet	500g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	20/8/92
Treatment	42IU Caninsulin No diarrhoea
Weeks since last visit	1 week
Date at 1 mth	19/9/92
Treatment	42IU Caninsulin Brighter and livelier.
Weeks since last visit	4 weeks
Date at 2 mths	20/10/92
Treatment	42IU Caninsulin Still very bright and happy.
Weeks since last visit	4 weeks
Date at 3 mths	24/11/92
Treatment	42IU Caninsulin One bout vomiting and diarrhoea but generally very much more active.
Weeks since last visit	4 weeks
Date at 4 mths	6/1/93
Treatment	44IU Caninsulin Still doing well.
Weeks since last visit	6 weeks

Dog 5

Case No.	117222
Dog name (+ owner surname)	Lina Tannoch
Breed	Whippet
Age (at initial visit)	11.1yrs
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	13/7/92
Diagnosis	Diabetes mellitus, anal sacculitis/sinus.
Time since diagnosis	May 1991
Treatment	Insulin - Hypurin Isophane Diet - Pedigree Chum and biscuit.
Dietary and insulin history	
Previous diet	400g Chum and 80g biscuit twice daily
Insulin type and dose	20IU isophane once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 936kcal/day) Insulin dose constant at 20IU
Date at -10 days	10/8/92
Treatment	20IU isophane
New diet	500g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	20/8/92
Treatment	14IU isophane. Appetite poor. No diarrhoea.
Weeks since last visit	10 days
Date at 1 mth	24/9/92
Treatment	10IU isophane Appetite poor. Constipation complicated by anal surgery (24/8). Vegetable oil fed with CHF
Weeks since last visit	4 weeks
Date at 2 mths	20/10/92
Treatment	10IU isophane. Very bright and happy. Appetite returned.
Weeks since last visit	
Date at 3 mths	24/11/92
Treatment	10IU isophane. Appetite variable. Still getting some vegetable oil in food. Faeces firm.
Weeks since last visit	4 weeks
Date at 4 mths	4/2/93
Treatment	14IU isophane Thin; otherwise doing well.
Weeks since last visit	8 weeks

Dog 6

Case No.	113648
Dog name (+ owner surname)	Bertie Elliot
Breed	Scottish terrier
Age (at initial visit)	7.3yrs
Sex	Male
Diagnosis, visit dates and treatment	
Date at -1 mth	13/7/92
Diagnosis	Diabetes mellitus and pyoderma.
Time since diagnosis	March 1990
Treatment	Insulin - Caninsulin Diet - Pedigree Chum and biscuit
Dietary and insulin history	
Previous diet	284g Chum and 60g biscuit twice daily
Insulin type and dose	28IU Caninsulin once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 680 kcal/day) Insulin dose constant at 28IU Co-trimoxazole ½ x 480 twice daily for pyoderma.
Date at -10 days	11/8/92
Treatment	28IU Caninsulin. Skin still poor. TSH stimulation test suggests hypothyroidism. Thyroxin replacement therapy begun.
New diet	370g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	20/8/92
Treatment	38IU Caninsulin Appetite good. Bright and lively. No diarrhoea.
Weeks since last visit	9 days
Date at 1 mth	18/9/93
Treatment	38IU Caninsulin Bright and alert. Skin improved and antibiotics stopped.
Weeks since last visit	4 weeks
Date at 2 mths	26/10/92
Treatment	36IU Caninsulin Still very bright and active.
Weeks since last visit	5 weeks
Date at 3 mths	7/12/92
Treatment	36IU Caninsulin Still doing well.
Weeks since last visit	5 weeks
Date at 4 mths	6/1/93
Treatment	36IU Caninsulin Doing well. Some weight gain.
Weeks since last visit	4 weeks

Dog 8

Case No.	97218
Dog name (+ owner surname)	Mandy Leitch
Breed	Labrador
Age (at initial visit)	8yrs 6mths
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	15/9/92
Diagnosis	Diabetes mellitus, osteoarthritis.
Time since diagnosis	March 1992
Treatment	Insulin - Hypurin Isophane Diet - Pedigree Chum and bread
Dietary and insulin history	
Previous diet	405g Chum and 95g bread twice daily
Insulin type and dose	56IU isophane once daily
Treatment	Phenylbutazone 200mg twice daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 1000kcal/day) 56IU isophane
Date at -10 days	20/10/92
Treatment	56IU isophane
New diet	500g pedigree CHF twice daily
Weeks since last visit	4weeks
Date at time zero	28/10/92
Treatment	52IU isophane Uneventful changeover. No diarrhoea.
Weeks since last visit	8 days
Date at 1 mth	1/12/92
Treatment	52IU isophane Bright and lively. 1 bout hypoglycaemia.
Weeks since last visit	4 weeks
Date at 2 mths	5/1/93
Treatment	52IU isophane Bright and active at home but vision worsening
Weeks since last visit	4 weeks
Date at 3 mths	9/2/93
Treatment	46IU isophane Still doing well but vision deteriorating further
Weeks since last visit	4 weeks
Date at 4 mths	16/3/93
Treatment	46IU isophane Still doing well.
Weeks since last visit	4 weeks

Dog 9

Case No.	117898
Dog name (+ owner surname)	Jodie Downs
Breed	Cairn terrier
Age (at initial visit)	1yr 6mths
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	17/9/92
Diagnosis	Islet cell hypoplasia
Time since diagnosis	August 1991
Treatment	Insulin - Hypurin Isophane Diet - Whiskas and bread
Dietary and insulin history	
Previous diet	200g Whiskas and 14g bread twice daily
Insulin type and dose	Isophane 4-6IU once daily
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 360kcal/day) 6-9IU isophane
Date at -10 days	20/10/92
Treatment	9IU isophane. No other treatment.
New diet	200g CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	28/10/92
Treatment	10IU isophane. No other treatment. Colitis days 1 and 2.
Weeks since last visit	8 days
Date at 1 mth	1/12/92
Treatment	10IU isophane. No other treatment.
Weeks since last visit	4 weeks
Date at 2 mths	7/1/93
Treatment	9IU isophane Lively and active. Slimmer appearance.
Weeks since last visit	4 weeks
Date at 3 mths	11/2/93
Treatment	8IU isophane. No other treatment.
Weeks since last visit	4 weeks
Date at 4 mths	10/3/93
Treatment	9IU isophane. No other treatment. Still doing well.
Weeks since last visit	4 weeks

Dog 10

Case No.	121228
Dog Reference No.	10
Dog name (+ owner surname)	Flip Bissett
Breed	Labrador X
Age (at initial visit)	9.2yrs
Sex	Male neutered
<u>Diagnosis, visit dates and treatment</u>	
Date at -1 mth	17/2/93
Diagnosis	Diabetes mellitus
Time since diagnosis	December 1992
Treatment	Insulin - Hypurin Isophane Diet - Pedigree Chum and biscuit
<u>Dietary and insulin history</u>	
Previous diet	500g Chum and 100g biscuit twice daily
Insulin type and dose	46-50IU isophane
Details of diet and insulin dose during month pre-trial	Diet as above (approx. 1200kcal/day) 50IU isophane
Date at -10 days	15/3/93
Treatment	50IU isophane. No other treatment
New diet	600g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	24/3/93
Treatment	46IU isophane. No other treatment No diarrhoea
Weeks since last visit	9 days
Date at 1 mth	20/4/93
Treatment	46IU isophane More lively and playful. Some weight loss. Owner very pleased.
Weeks since last visit	4 weeks
Date at 2 mths	18/5/93
Treatment	44IU isophane Still very bright and lively. Further weight loss.
Weeks since last visit	4 weeks
Date at 3 mths	15/6/93
Treatment	44IU isophane Vision deteriorated.
Weeks since last visit	4 weeks
Date at 4 mths	22/7/93
Treatment	44IU isophane Doing well but very little vision.
Weeks since last visit	4 weeks

Dog 11

Case No.	121252
Dog name (+ owner surname)	Roxanne McNally
Breed	Rottweiler
Age (at initial visit)	3.2yrs
Sex	Female neutered
<u>Diagnosis, visit dates and treatment</u>	
Date at -1 mth	25/2/93
Diagnosis	Diabetes mellitus
Time since diagnosis	December 1992
Treatment	Insulin - Hypurin Isophane Diet - Pedigree Chum and biscuit
<u>Dietary and insulin history</u>	
Previous diet	600g Chum and 225g biscuit twice daily
Insulin type and dose	54-58IU isophane once daily
Details of diet and insulin dose during month pre-trial	Diet as before (approx. 1870kcal/day) 58IU isophane
Date at -10 days	16/3/93
Treatment	58IU isophane
New diet	1200g Pedigree CHF twice daily
Weeks since last visit	3 weeks
Date at time zero	24/3/93
Treatment	46IU isophane No diarrhoea.
Weeks since last visit	8 days
Date at 1 mth	4/5/93
Treatment	58IU isophane for last two weeks. Doing well. Insulin dose reduced to 50IU based on blood glucose concentration.
Weeks since last visit	5 weeks
Date at 2 mths	1/6/93
Treatment	50IU isophane. Still doing very well
Weeks since last visit	4 weeks
Date at 3 mths	29/6/93
Treatment	50IU isophane Healthy, bright and active.
Weeks since last visit	4 weeks
Date at 4 mths	21/7/93
Treatment	54IU isophane Polydipsic and blood glucose high; insulin dose increased to 60IU
Weeks since last visit	4 weeks

Dog 13

Case No.	120899
Dog name (+ owner surname)	Jenna Michie
Breed	Yorkshire terrier
Age (at initial visit)	8.8yrs
Sex	Female neutered
Diagnosis, visit dates and treatment	
Date at -1 mth	16/3/93
Diagnosis	Diabetes mellitus
Time since diagnosis	November 1992
Treatment	Insulin - Hypurin Isophane Diet - Pedigree Chum and bread
Dietary and insulin history	
Previous diet	125g Chum and 30g bread twice daily
Insulin type and dose	7IU isophane once daily
Details of diet and insulin dose during month pre-trial	Diet as before (approx. 312kcal/day) 7IU isophane
Date at -10 days	27/4/93
Treatment	7IU isophane
New diet	200g Pedigree CHF twice daily
Weeks since last visit	4 weeks
Date at time zero	5/5/93
Treatment	5IU isophane Appetite very poor over week. No diarrhoea
Weeks since last visit	8 days
Date at 1 mth	4/6/93
Treatment	5IU isophane Normal faeces when first went home. Very watery diarrhoea for the last few days. Diarrhoea continued (7-8/6/93). Started 100mg metronidazole bid. Plasma B12 and folate concentrations normal.
Weeks since last visit	4 weeks
Date at 2 mths	
Treatment	Unable to contact owners
Weeks since last visit	
Date at 3 mths	
Treatment	Unable to contact owners
Weeks since last visit	
Date at 4 mths	13/9/93
Treatment	4IU isophane No more diarrhoea since last visit
Weeks since last visit	13 weeks

Appendix 16:
24 hour plasma glucose concentrations and derived values in 11 dogs before and 10 days after changing to a canine high fibre diet and in 5 dogs after 4 months.

ABSMEAN	Mean of plasma glucose concentrations at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after insulin injection
ABSSD	Standard deviation of plasma glucose concentrations at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after insulin injection
INCMEAN	Mean of plasma glucose concentrations at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after insulin injection following subtractionof the nadir concentration
INCAUC	Area under the plasma glucose versus time curve generated from plasma glucose concentrations at 0, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after insulin injection
APPGMEAN	Mean of plasma glucose concentrations at 6, 6.16, 6.32, 6.5, 7, 7.5, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours
APPGSD	Standard deviation of plasma glucose concentrations at 6, 6.16, 6.32, 6.5, 7, 7.5, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours
APPGAUC	Area under the plasma glucose versus time curve generated from plasma glucose concentrations at 6, 6.16, 6.32, 6.5, 7, 7.5, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours
ADJMEAN	Mean of plasma glucose concentrations at 6, 7, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours
ADJAUC	Standard deviation of plasma glucose concentrations at 6, 7, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours
ADJSD	Area under the plasma glucose versus time curve generated from plasma glucose concentrations at 6, 7, 8, 9, 10, 12 and 14 hours post insulin injection following subtraction of the plasma glucose concentration at 6 hours

Time/Dog	1	2	3	4	5	6	8	9	10	11	13
0.00	12.4	7.3	19.0	27.1	6.2	11.1	27.3	24.4	17.8	8.4	6.3
2.00	18.7	16.8	12.9	29.8	3.6	14.7	24.1	23.6	14.4	9.1	3.1
4.00	12.5	15.7	12.3	20.9	4.6	16.6	16.8	7.1	5.6	10.2	5.8
6.00	7.5	14.4	9.2	25.6	6.0	15.4	13.9	6.0	4.5	6.4	4.9
6.16	5.2	12.5	6.2	24.7	6.2	18.9	12.7	9.7	4.6	5.5	4.7
6.32	5.2	13.4	5.2	26.1	6.5	20.2	13.3	11.6	5.1	5.0	5.4
6.50	5.5	14.1	6.4	26.4	5.6	18.7	14.0	13.4	5.7	4.7	5.1
7.00	7.4	14.6	8.2	27.1	6.6	21.1	14.7	15.1	7.4	5.5	3.9
7.50	7.3	14.7	9.3	*	7.6	23.1	14.4	15.7	8.6	6.3	4.2
8.00	7.8	15.7	11.1	26.5	8.0	21.8	14.1	16.0	8.8	7.0	4.0
9.00	12.0	17.5	12.2	26.7	7.1	24.1	12.9	15.7	8.8	6.7	3.5
10.00	12.4	18.2	12.3	24.2	6.4	25.1	9.3	16.1	8.5	7.1	4.2
12.00	9.2	19.8	15.2	29.5	10.9	25.5	7.3	15.3	9.5	6.7	4.4
14.00	4.7	15.7	13.4	*	7.7	23.1	6.0	9.3	9.5	6.3	4.3
16.00	5.4	13.4	14.6	19.9	7.8	20.6	7.2	8.0	8.1	8.6	4.5
20.00	5.1	8.3	11.7	21.4	5.3	16.4	18.6	8.1	15.8	15.8	5.5
24.00	5.2	7.5	16.6	22.7	5.4	14.8	36.6	22.4	21.4	20.8	5.7
ABSMEAN	9.17	13.89	13.48	24.76	6.54	18.65	16.47	14.21	11.26	9.73	4.79
ABSSD	4.40	4.33	2.73	3.52	1.99	4.79	9.64	6.96	5.33	4.72	0.95
INCMEAN	4.47	6.59	8.28	4.86	2.94	7.55	10.47	8.21	6.76	5.03	1.69
INCAUC	5532	9186	11448	6156	4176	11166	14130	10104	9702	7980	2412
APPGMEAN	0.15	1.11	0.68	0.71	1.15	6.15	-1.85	7.08	2.86	-0.29	-0.48
APPGSD	2.64	2.19	3.23	1.52	1.46	3.08	3.06	3.44	2.00	0.81	0.56
APPGAUC	665	1293	1364	776	947	3908	-1708	4060	1953	51	-328
ADJMEAN	1.21	2.16	2.46	1.00	1.53	6.90	-2.73	7.36	3.64	0.13	-0.73
ADJAUC	759	1344	1485	786	948	3795	-1680	3951	1950	93	-357
ADJSD	2.73	2.00	2.40	1.76	1.65	3.45	3.58	4.03	1.76	0.54	0.44

Absolute 24 hour glucose concentrations and derived values in 11 dogs on original diet.

Time\Dog	1	2	3	4	5	6	8	9	10	11	13
0.00	17.8	6.6	23.9	4.4	4.8	3.1	4.3	9.3	7.0	18.3	6.6
2.00	15.8	10.1	7.5	6.1	1.6	4.2	2.4	4.9	5.5	4.5	2.6
4.00	5.0	6.5	6.0	2.9	2.9	2.4	1.7	6.0	3.5	3.8	1.5
6.00	5.2	3.9	5.4	2.7	2.1	3.2	1.4	2.7	5.0	3.2	3.5
6.16	3.3	3.8	4.8	2.5	3.0	2.9	1.8	4.0	5.0	2.9	3.7
6.32	3.2	3.6	5.1	2.4	3.7	3.2	1.7	4.8	4.3	2.4	4.0
6.50	2.9	4.2	6.5	2.2	3.4	3.6	2.1	6.6	4.7	2.3	4.6
7.00	3.6	4.5	8.2	2.7	3.4	3.6	3.3	7.9	5.1	2.7	4.9
7.50	3.1	4.6	8.7	3.9	3.8	3.1	3.7	7.7	6.4	3.2	6.1
8.00	4.4	4.2	7.9	2.5	4.0	3.1	3.8	6.9	7.3	2.6	5.4
9.00	2.6	6.0	7.6	3.2	3.3	3.0	3.9	7.7	10.9	3.2	4.8
10.00	2.6	5.6	6.7	3.3	4.5	3.8	4.3	6.8	9.0	2.5	4.6
12.00	3.1	5.6	4.3	4.4	6.3	3.4	3.7	5.4	6.3	4.1	4.8
14.00	4.1	5.5	4.9	3.3	4.6	2.3	3.2	3.7	10.1	5.2	5.4
16.00	8.9	4.7	8.7	2.1	10.5	1.7	3.2	4.4	10.1	11.2	6.4
20.00	12.9	3.5	18.4	2.1	8.0	2.1	5.8	6.4	10.3	14.0	6.6
24.00	16.4	4.7	19.4	4.2	4.2	2.5	6.9	17.2	9.5	21.3	7.2
ABSMEAN	8.75	5.54	10.28	3.45	4.86	2.89	3.70	6.70	7.60	8.25	4.96
ABSSD	5.87	1.81	6.86	1.22	2.59	0.76	1.64	3.91	2.36	6.81	1.81
INCMEAN	6.15	2.04	5.98	1.35	3.26	1.19	2.30	4.00	4.10	5.95	3.46
INCAUC	8814	2574	8876	1650	5418	1536	3498	5430	6414	8826	5172
APPGMEAN	-1.74	0.78	0.97	0.31	1.73	0.00	1.59	3.14	1.74	-0.08	1.21
APPGSD	0.81	0.85	1.56	0.68	1.07	0.41	1.04	1.81	2.31	0.86	0.77
APPGAUC	-940	634	396	336	1169	22	1050	1660	1312	94	682
ADJMEAN	-1.54	1.14	1.03	0.43	1.93	0.00	1.97	3.17	2.67	0.16	1.27
ADJAUC	-852	633	405	312	1134	30	1053	1617	1320	99	645
ADJSD	0.97	0.82	1.56	0.64	1.31	0.49	0.94	2.02	2.38	0.98	0.64

Absolute 24 hour glucose concentrations and derived values in 11 dogs on CHF diet.

Time/dog	1	3	10	11	13
0.00	7.5	18.1	16.9	18.9	22.7
2.00	5.4	5.1	8.3	7.1	9.9
4.00	2.5	6.0	3.9	4.8	5.3
6.00	2.2	5.6	4.3	2.5	3.5
6.16	2.0	5.3	4.1	2.2	3.6
6.32	2.0	4.8	4.5	2.4	4.0
6.50	1.9	4.7	4.7	2.4	5.0
7.00	3.2	5.4	4.4	2.5	5.3
7.50	2.8	5.0	4.1	1.9	5.9
8.00	3.5	4.5	3.9	1.7	5.5
9.00	2.1	5.4	3.3	1.3	4.2
10.00	2.8	5.2	3.8	2.2	4.2
12.00	1.3	5.8	4.1	1.8	4.5
14.00	3.0	7.0	3.2	2.9	4.9
16.00	3.9	11.3	9.4	6.2	12.4
20.00	6.5	16.2	15.8	13.6	10.7
24.00	7.7	20.9	20.9	14.5	17.8
ABSMEAN	4.21	9.61	8.59	6.93	9.22
ABSSD	2.22	6.02	6.39	6.02	6.29
INCMEAN	2.91	5.11	5.39	5.63	5.72
INCAUC	4248	7740	8178	8142	7794
APPGMEAN	0.16	-0.39	-0.16	-0.37	0.97
APPGSD	0.67	0.68	0.47	0.45	0.78
APPGAUC	84	-42	-208	-219	561
ADJMEAN	0.39	-0.04	-0.44	-0.37	1.09
ADJAUC	126	-18	-213	-207	537
ADJSD	0.76	0.76	0.46	0.56	0.70

Absolute 24 hour glucose concentrations and derived values in 5 dogs after 4 months on CHF.

Appendix 17:
Insulin dose and indicators of general health in 11 dogs from one month before to 4 months after changing to canine high fibre diet

Shaded area represents hospitalisation period

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	1.41	0.91	1.12	1.06	1.00	1.00	1.29
2	119147	1.18	1.18	1.31	1.05	1.20	*	*
3	115131	1.06	1.00	1.25	1.28	1.29	1.28	1.31
4	115980	1.02	1.00	1.06	1.00	1.07	1.10	1.14
5	117222	1.60	1.60	1.16	0.90	0.88	1.28	1.33
6	113648	2.24	2.24	3.04	3.04	2.76	2.72	2.71
8	97218	1.40	1.42	1.32	1.40	1.40	1.31	1.27
9	117898	1.09	1.60	1.80	1.81	1.67	1.45	1.63
10	121228	1.60	1.60	1.54	1.63	1.57	1.60	1.63
11	121252	1.20	1.18	1.00	1.23	1.04	1.10	1.30
13	120899	1.22	1.27	1.00	1.00	*	*	0.83

Absolute insulin dose (IU/kg).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	42.5	42.0	41.0	41.5	40.0	40.0	38.5
2	119147	39.0	39.0	38.0	38.0	36.5	*	*
3	115131	8.00	8.00	8.00	7.80	7.75	7.80	7.62
4	115980	41.0	40.0	39.5	39.5	39.0	38.3	38.6
5	117222	12.5	12.5	12.0	11.0	11.5	10.9	10.5
6	113648	12.5	12.5	12.5	12.5	13.0	13.2	13.2
8	97218	40.0	39.5	39.2	37.0	37.0	35.0	36.0
9	117898	5.50	5.50	5.50	5.50	5.38	5.50	5.55
10	121228	30.0	30.0	29.7	28.2	28.0	27.5	26.9
11	121252	45.0	49.0	45.8	47.0	47.7	45.3	44.6
13	120899	5.7	5.5	5.0	5.3	*	*	4.8

Absolute bodyweight (kg).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	41	45	50	45	40	16	21
2	119147	82	74	80	72	73	*	*
3	115131	52	60	55	49	48	46	30
4	115980	92	96	95	90	80	83	66
5	117222	64	60	67	60	60	60	45
6	113648	43	40	50	48	46	59	60
8	97218	95	92	90	80	64	62	60
9	117898	60	61	50	50	46	44	42
10	121228	90	90	90	90	87	80	70
11	121252	65	70	70	67	70	60	55
13	120899	57	58	50	56	*	*	42

Absolute body condition score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	66	82	81	75	45	42	36
2	119147	80	71	80	76	77	*	*
3	115131	72	73	82	80	82	80	80
4	115980	75	75	77	86	78	88	83
5	117222	71	78	80	74	75	85	84
6	113648	35	31	30	54	64	71	75
8	97218	95	92	93	95	87	86	87
9	117898	48	46	50	50	46	57	50
10	121228	62	60	59	63	64	68	61
11	121252	75	75	76	71	78	68	66
13	120899	64	59	58	62	*	*	62

Absolute coat condition score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	76	90	87	90	72	74	80
2	119147	91	59	90	89	89	*	*
3	115131	82	90	85	95	95	97	97
4	115980	32	45	65	80	90	96	98
5	117222	70	61	64	79	90	90	92
6	113648	36	34	85	90	92	94	95
8	97218	90	87	87	90	93	96	97
9	117898	80	77	93	96	95	92	98
10	121228	72	76	80	95	94	97	100
11	121252	87	90	87	92	87	90	90
13	120899	82	88	90	86	*	*	92

Absolute activity score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	90	95	91	92	76	86	80
2	119147	95	88	92	85	89	*	*
3	115131	100	100	90	96	96	96	96
4	115980	91	87	70	84	92	92	96
5	117222	89	89	55	66	77	90	94
6	113648	36	35	85	92	91	95	96
8	97218	90	86	90	90	95	96	100
9	117898	82	77	92	96	99	96	98
10	121228	80	84	85	95	98	99	100
11	121252	90	90	87	92	87	90	90
13	120899	85	90	91	87	*	*	92

Absolute owner observed demeanour score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	90	95	92	95	76	85	82
2	119147	70	76	90	90	90	*	*
3	115131	78	78	80	84	95	90	91
4	115980	95	92	65	86	92	90	95
5	117222	50	50	43	49	73	86	87
6	113648	48	47	80	90	90	90	95
8	97218	95	90	81	90	92	95	97
9	117898	76	79	90	94	94	96	97
10	121228	90	88	87	96	98	98	100
11	121252	86	87	85	90	93	90	90
13	120899	82	86	85	84	*	*	90

Absolute vet observed demeanour score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	100	100	100	97	95	90	91
2	119147	100	100	100	100	100	*	*
3	115131	46	50	52	55	50	50	48
4	115980	20	25	36	32	25	27	15
5	117222	0	0	0	0	0	0	0
6	113648	11	5	11	12	15	12	14
8	97218	100	90	90	94	35	18	15
9	117898	100	100	100	100	100	100	100
10	121228	100	100	100	100	100	100	76
11	121252	100	100	100	100	100	100	100
13	120899	100	100	100	100	*	*	80

Absolute owner observed vision score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	100	100	100	95	92	90	90
2	119147	100	100	100	100	100	*	*
3	115131	70	66	74	82	82	80	82
4	115980	34	30	37	22	32	30	24
5	117222	0	0	0	0	0	0	0
6	113648	11	10	10	12	16	11	15
8	97218	95	87	84	80	37	33	20
9	117898	100	100	100	98	100	100	100
10	121228	100	100	100	100	100	100	72
11	121252	98	100	100	100	100	100	100
13	120899	100	100	100	100	*	*	100

Absolute vet observed vision score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	45	46	71	74	77	74	73
2	119147	50	46	67	75	80	*	*
3	115131	46	50	49	52	46	50	50
4	115980	46	45	70	64	74	80	79
5	117222	61	56	66	57	61	84	58
6	113648	45	50	65	62	75	65	61
8	97218	46	50	45	45	48	47	50
9	117898	50	48	50	48	52	52	50
10	121228	45	45	45	55	55	52	51
11	121252	50	48	57	50	54	50	49
13	120899	48	50	54	65	*	*	46

Absolute faecal volume score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	46	46	20	21	10	20	23
2	119147	50	48	32	14	16	*	*
3	115131	49	51	48	52	47	50	50
4	115980	70	65	50	65	76	85	49
5	117222	51	46	50	85	85	90	46
6	113648	45	50	46	46	45	50	50
8	97218	50	50	51	75	50	56	51
9	117898	50	48	38	40	47	48	35
10	121228	50	52	53	50	50	53	51
11	121252	50	50	36	50	55	51	49
13	120899	46	50	53	15	*	*	46

Absolute faecal consistency score.

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	4.5	4.1	5.7	8.1	5.3	7.9	4.4
2	119147	2.4	2.5	2.9	5.7	2.1	*	*
3	115131	2.3	2.4	3.7	6.3	2.1	4.5	4.1
4	115980	2.7	2.9	3.8	5.4	3.2	3.8	3.3
5	117222	2.7	6.0	2.8	6.7	5.5	6.5	4.3
6	113648	3.6	5.6	2.8	5.0	2.1	5.2	2.8
8	97218	5.0	8.5	2.3	6.3	4.9	4.0	5.4
9	117898	5.6	4.5	4.1	8.0	3.8	6.9	3.4
10	121228	4.6	5.0	3.0	4.8	2.9	3.8	3.3
11	121252	3.5	4.0	4.6	3.9	3.3	5.9	3.9
13	120899	3.7	3.2	2.8	4.2	*	*	5.2

Plasma urea concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	99	100	107	84	90	94	92
2	119147	79	86	80	61	70	*	*
3	115131	66	66	56	70	64	64	76
4	115980	74	88	69	67	92	69	79
5	117222	57	66	54	60	72	64	71
6	113648	71	73	56	64	60	57	59
8	97218	64	104	73	76	83	73	94
9	117898	75	78	76	66	63	59	66
10	121228	86	117	76	73	84	70	82
11	121252	97	90	87	79	91	81	105
13	120899	75	71	67	74	*	*	81

Plasma creatinine concentrations (μmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	1.44	1.32	1.62	1.69	1.57	1.36	1.23
2	119147	1.13	1.11	1.02	2.20	1.18	*	*
3	115131	0.85	0.80	1.01	1.60	0.81	1.22	0.98
4	115980	0.83	0.79	1.01	1.79	0.83	1.00	0.81
5	117222	1.28	1.16	1.37	1.19	1.08	0.54	1.06
6	113648	1.23	1.34	1.33	1.14	1.02	1.41	0.96
8	97218	1.44	1.56	1.11	0.77	0.41	0.73	0.72
9	117898	1.57	1.50	1.40	2.05	1.28	1.92	1.19
10	121228	1.04	0.88	0.92	0.87	0.81	0.56	0.83
11	121252	1.43	1.15	1.32	1.62	1.59	1.96	1.42
13	120899	1.19	0.98	1.24	0.79	*	*	1.17

Plasma phosphate concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	150	144	147	145	150	149	150
2	119147	146	150	147	147	146	*	*
3	115131	145	146	138	147	142	148	142
4	115980	142	143	145	146	146	148	151
5	117222	142	141	145	*	144	146	140
6	113648	140	140	143	144	1448	149	150
8	97218	138	140	150	148	145	150	144
9	117898	137	142	143	145	148	144	141
10	121228	138	133	144	146	146	148	140
11	121252	140	148	140	147	142	138	136
13	120899	146	148	151	146	*	*	141

Plasma sodium concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	3.9	4.4	4.1	4.7	3.8	4.0	3.9
2	119147	4.1	3.9	3.8	3.5	4.2	*	*
3	115131	3.7	3.9	3.9	3.8	4.0	3.9	4.3
4	115980	4.8	4.9	4.1	4.6	4.6	4.3	4.1
5	117222	4.1	4.6	4.3	*	4.5	5.0	3.8
6	113648	4.3	4.6	3.8	3.8	4.5	4.2	4.3
8	97218	4.8	4.9	4.8	4.0	3.6	3.9	4.8
9	117898	4.3	4.6	4.1	4.3	4.1	4.4	4.1
10	121228	5.0	5	4.2	3.7	4.3	3.6	4.6
11	121252	4.6	3.9	4.6	4.2	4.5	4.0	4.6
13	120899	4.6	4.0	4.0	4.0	*	*	4.5

Plasma potassium concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	116	107	117	113	119	115	117
2	119147	111	112	117	113	115	*	*
3	115131	113	108	110	115	109	114	106
4	115980	113	109	110	114	107	113	112
5	117222	115	104	111	109	105	101	108
6	113648	111	103	108	110	115	110	109
8	97218	106	104	116	115	109	114	109
9	117898	104	102	110	111	108	113	104
10	121228	96	99	119	116	103	110	103
11	121252	101	105	111	107	106	105	96
13	120899	100	110	108	110	*	*	101

Plasma chloride concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	2.55	2.47	2.96	2.62	2.92	2.32	2.3
2	119147	2.66	2.81	2.94	2.78	2.81	*	*
3	115131	2.56	2.64	2.70	2.53	2.72	2.37	2.47
4	115980	2.53	2.8	2.89	2.66	2.66	2.64	2.63
5	117222	2.96	2.72	2.78	1.88	2.62	*	2.5
6	113648	3.20	2.69	2.94	2.86	2.72	2.77	2.66
8	97218	2.79	2.54	2.70	2.73	2.81	2.69	2.54
9	117898	2.77	2.84	2.90	2.85	2.83	2.68	2.70
10	121228	2.80	2.56	2.80	2.84	2.72	2.90	2.75
11	121252	2.70	2.71	2.69	2.70	2.60	2.79	2.63
13	120899	2.73	2.92	2.54	2.32	*	*	2.52

Plasma calcium concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	0	2	0	0	0	0	1
2	119147	1	2	0	*	0	*	*
3	115131	2	1	0	1	0	0	1
4	115980	0	0	0	*	0	0	0
5	117222	0	0	0	*	1	*	2
6	113648	0	1	0	1	0	0	1
8	97218	1	1	1	0	0	0	0
9	117898	0	1	0	0	0	0	0
10	121228	4	*	0	*	0	*	*
11	121252	1	*	1	1	0	1	1
13	120899	1	1	1	1	*	*	1

Plasma bilirubin concentrations (μmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	8	9	10	6	17	18	6
2	119147	4	9	7	12	19	*	*
3	115131	9	13	10	12	10	22	6
4	115980	8	9	5	2	30	*	26
5	117222	8	9	6	1	31	*	26
6	113648	13	6	4	5	24	23	18
8	97218	5	20	14	14	45	5	1
9	117898	19	11	*	15	2	11	21
10	121228	*	1	23	*	43	4	*
11	121252	10	9	14	27	28	*	10
13	120899	19	25	18	*	*	*	37

Plasma gamma glutamyl transferase concentrations (IU/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	2710	2881	2991	2095	2771	2606	2771
2	119147	1833	1963	1770	1250	1100	*	*
3	115131	1675	1888	1376	1398	1270	1180	1116
4	115980	2888	2667	2994	2766	2950	2617	2786
5	117222	1237	781	1038	900	863	891	654
6	113648	1560	933	1000	950	1043	1611	1262
8	97218	1511	1332	1491	1379	1469	*	1344
9	117898	694	1042	1057	995	1137	1195	1069
10	121228	1946	1781	2352	*	1774	1879	1701
11	121252	3164	5054	2878	2583	2551	*	1701
13	120899	1010	1340	1860	2240	*	*	1317

Plasma amylase concentrations (IU/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	72	65	68	69	65	59	63
2	119147	73	67	66	71	66	*	*
3	115131	70	67	62	72	64	61	63
4	115980	64	69	66	70	65	68	65
5	117222	69	73	62	69	62	71	63
6	113648	74	66	68	63	66	64	63
8	97218	66	65	60	70	63	64	57
9	117898	67	63	58	66	64	61	62
10	121228	71	84	66	76	68	72	68
11	121252	66	69	65	70	62	67	63
13	120899	70	74	68	64	*	*	68

Plasma total protein concentrations (g/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	27	29	30	28	27	23	25
2	119147	31	32	33	35	29	*	*
3	115131	30	32	32	31	29	27	27
4	115980	32	34	35	35	29	33	32
5	117222	31	36	32	40	28	34	31
6	113648	36	33	35	34	28	32	31
8	97218	35	30	26	31	30	32	26
9	117898	38	31	30	33	32	34	32
10	121228	33	33	30	34	36	37	35
11	121252	30	33	29	33	30	32	30
13	120899	37	39	36	34	*	*	32

Plasma albumin concentrations (g/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	10.6	10.9	14.1	11.6	10.9	12.0	8.9
2	119147	12.0	16.6	*	13.4	10.4	*	*
3	115131	6.9	6.4	6.6	7.9	6.1	6.4	6.6
4	115980	12.1	9.6	10.6	11.0	*	10.5	8.4
5	117222	6.6	11.1	8.4	15.5	7.2	7.6	6.6
6	113648	9.8	8.0	8.3	8.9	8.5	16.8	10.4
8	97218	6.7	9.2	9.3	6.4	6.7	7.6	5.1
9	117898	6.9	6.2	*	6.5	5.6	7.7	5.9
10	121228	12.1	9.6	12.8	11.0	9.6	12.3	11.6
11	121252	8.3	9.7	8.8	9.6	10.0	13.8	8.8
13	120899	9.7	4.1	11.3	6.0	*	*	9.6

Total white blood cell counts (10^9/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	6.70	6.20	9.58	6.14	7.14	8.70	5.11
2	119147	7.70	10.30	*	8.10	6.34	*	*
3	115131	4.70	4.07	5.21	5.92	5.00	4.86	4.55
4	115980	8.30	6.00	6.94	7.04	*	5.56	6.76
5	117222	4.02	9.87	5.79	12.80	5.18	5.20	5.10
6	113648	6.37	5.72	5.76	6.00	5.57	14.11	7.64
8	97218	4.99	7.90	6.83	3.58	4.79	5.16	3.13
9	117898	6.90	4.46	*	6.50	5.60	7.70	5.90
10	121228	7.74	6.80	9.10	7.37	6.24	7.81	7.94
11	121252	3.81	4.90	4.80	3.84	5.20	8.10	3.96
13	120899	7.76	2.00	8.70	4.17	*	*	4.56

Absolute neutrophil counts (10^9/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	2.80	2.90	2.89	3.53	1.96	2.16	2.14
2	119147	2.90	3.00	*	3.01	2.55	*	*
3	115131	1.60	1.20	1.12	1.50	0.52	1.12	1.12
4	115980	2.84	2.59	3.12	3.08	*	4.41	1.10
5	117222	2.17	0.77	2.05	2.24	1.87	2.09	1.22
6	113648	2.94	1.84	1.53	2.44	2.34	1.01	2.13
8	97218	1.17	0.64	2.18	2.49	1.67	1.98	1.71
9	117898	1.41	0.93	*	1.40	1.34	1.69	1.06
10	121228	2.66	1.92	2.43	1.98	2.59	2.89	2.14
11	121252	3.36	2.57	2.72	3.93	3.05	4.20	3.16
13	120899	1.45	1.43	1.41	1.17	*	*	3.55

Absolute lymphocyte counts (10^9/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	0.90	0.71	0.14	0.58	0.76	0.54	0.80
2	119147	0.84	2.98	*	1.00	2.55	*	*
3	115131	0.31	0.22	0.00	0.19	0.12	0.16	0.23
4	115980	0.61	0.62	0.21	0.38	*	0.16	0.25
5	117222	0.10	0.00	0.04	0.16	0.04	0.00	0.01
6	113648	0.10	0.32	0.29	0.31	0.21	0.34	0.36
8	97218	0.03	0.05	0.00	0.06	0.00	0.23	0.13
9	117898	0.10	0.09	*	0.07	0.17	0.23	0.09
10	121228	0.97	0.45	0.64	0.88	0.38	1.17	1.22
11	121252	0.78	0.82	0.70	0.91	1.40	1.30	1.40
13	120899	0.05	0.23	0.17	0.30	*	*	1.30

Absolute eosinophil counts (10^9/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	7.10	7.56	6.43	7.38	6.58	6.64	6.49
2	119147	6.64	8.87	*	7.07	6.55	*	*
3	115131	7.10	6.95	6.37	7.38	6.95	7.29	6.96
4	115980	6.77	6.95	5.77	6.49	*	6.99	6.55
5	117222	7.44	8.27	7.20	7.87	7.81	8.11	7.67
6	113648	6.49	6.28	5.62	6.28	7.27	7.49	6.99
8	97218	6.86	6.77	5.91	7.70	7.45	7.98	7.32
9	117898	7.13	7.04	*	6.96	7.05	6.86	5.69
10	121228	6.99	7.01	6.14	7.05	6.93	6.93	6.96
11	121252	6.71	7.26	6.02	7.45	6.12	6.88	6.27
13	120899	8.49	8.93	7.64	8.20	*	*	7.48

Total red blood cell counts (10^{12}/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	45.5	49.5	41.7	49.5	43.2	42.9	42.2
2	119147	44.5	60.3	*	50.4	43.8	*	*
3	115131	48.8	48.8	44.3	51.1	48.1	49.5	47.8
4	115980	45.2	46.2	38.0	45.2	*	46.5	47.8
5	117222	51.8	57.4	49.6	59.7	54.6	57.4	53.4
6	113648	44.0	40.5	36.0	44.2	46.3	50.1	45.8
8	97218	44.0	44.3	40.2	49.8	48.1	50.8	48.1
9	117898	48.1	48.3	*	46.8	46.8	48.1	38.2
10	121228	46.5	47.8	41.2	46.5	46.2	45.7	44.8
11	121252	43.8	48.1	40.5	48.8	39.6	46.8	40.9
13	120899	54.7	64.1	49.5	52.1	*	*	49.8

Packed cell volumes (%).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	64	65	65	67	66	65	65
2	119147	67	68	*	71	67	*	*
3	115131	69	70	70	69	69	68	69
4	115980	67	66	66	70	*	67	67
5	117222	70	69	69	76	70	71	70
6	113648	68	64	64	70	64	67	66
8	97218	64	65	68	65	65	64	66
9	117898	67	69	*	67	66	70	67
10	121228	67	67	67	66	67	66	67
11	121252	65	66	67	66	65	68	65
13	120899	64	61	65	64	*	*	67

Mean corpuscular volumes (fl).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	352	264	317	252	295	353	363
2	119147	168	114	*	*	228	*	*
3	115131	249	267	329	330	216	254	324
4	115980	304	342	295	427	*	300	308
5	117222	195	248	270	207	273	258	285
6	113648	242	252	340	179	195	163	189
8	97218	416	440	398	358	428	372	375
9	117898	518	516	*	492	392	427	502
10	121228	310	412	343	319	307	327	338
11	121252	375	308	255	345	310	199	333
13	120899	289	321	235	318	*	*	234

Absolute platelet counts (10^9/l).

Appendix 18:
Concentrations of indicators of glycaemic control in 11 dogs from one month before to 4 months after changing to a canine high fibre diet

Shaded area represents hospitalisation period

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	349	240	301	351	247	347	294
2	119147	407	334	393	497	290	*	*
3	115131	464	343	425	419	323	388	382
4	115980	553	636	468	457	494	367	366
5	117222	379	392	273	435	377	406	417
6	113648	572	557	468	*	295	328	261
8	97218	561	626	355	378	318	358	339
9	117898	449	430	376	357	329	407	413
10	121228	456	490	302	361	355	391	432
11	121252	414	440	329	377	404	508	583
13	120899	326	328	305	263	*	*	386

Absolute plasma fructosamine concentrations (μmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	5.00	5.12	5.28	4.72	4.34	5.72	3.72
2	119147	4.30	4.90	3.50	5.01	4.90	*	*
3	115131	4.60	4.70	4.40	4.50	4.60	4.56	4.03
4	115980	8.60	9.36	8.20	6.40	6.36	5.13	4.58
5	117222	6.74	8.08	6.82	6.76	6.79	7.26	6.00
6	113648	7.65	9.39	8.30	4.59	3.45	2.90	2.48
8	97218	7.90	6.87	5.52	3.96	4.39	3.84	4.93
9	117898	6.64	6.50	6.16	3.42	*	4.25	5.06
10	121228	7.70	8.39	7.23	5.38	6.63	5.55	6.41
11	121252	4.36	5.09	5.38	4.33	5.58	6.14	8.08
13	120899	4.63	5.95	*	4.36	*	*	4.90

Absolute glycated haemoglobin concentrations (% of total haemoglobin).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	475	750	547	269	219	338	350
2	119147	227	204	177	248	183	*	*
3	115131	639	737	477	516	439	514	509
4	115980	1016	1400	752	467	783	1406	760
5	117222	263	351	130	276	315	419	286
6	113648	3134	5247	2506	2416	1936	1619	2050
8	97218	2642	2645	1877	1227	865	725	451
9	117898	219	270	228	212	255	225	308
10	121228	565	191	256	358	590	282	226
11	121252	582	734	587	532	617	647	550
13	120899	42	35	56	57	*	*	132

Absolute plasma alkaline phosphatase concentrations (IU/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	52	123	66	36	51	58	57
2	119147	50	80	45	42	27	*	*
3	115131	72	65	42	24	28	54	51
4	115980	204	261	148	146	280	582	226
5	117222	28	79	32	23	36	19	28
6	113648	113	122	101	294	247	185	296
8	97218	86	57	44	30	25	40	25
9	117898	19	33	24	37	28	27	23
10	121228	197	117	121	177	182	194	101
11	121252	51	81	53	47	73	52	48
13	120899	34	61	71	41	*	*	43

Absolute plasma alanine aminotransferase concentrations (IU/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	28	43	16	24	30	25	23
2	119147	14	23	20	*	15	*	*
3	115131	40	44	29	26	25	41	25
4	115980	54	63	38	35	54	53	45
5	117222	17	22	17	53	19	23	19
6	113648	26	33	41	41	32	27	32
8	97218	77	45	26	12	20	17	24
9	117898	23	15	21	16	15	16	15
10	121228	53	55	40	46	52	56	39
11	121252	16	36	13	14	14	17	18
13	120899	10	17	25	22	*	*	21

Absolute aspartate aminotransferase concentrations (IU/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	5.8	20.9	16.4	*	8.2	*	3.9
2	119147	8.8	7.7	4.7	*	7.7	*	*
3	115131	5.2	4.5	19.4	*	14.3	*	18.1
4	115980	15.8	23.9	4.2	*	14.3	*	3.4
5	117222	11.7	20.4	4.2	*	19.4	*	18.6
6	113648	14.5	22.2	2.5	*	5.5	*	4.8
8	97218	28.6	27.3	12.9	*	7.8	*	20.3
9	117898	23.1	24.4	14.7	*	6.9	*	18.2
10	121228	26.3	34.3	9.5	*	9.4	*	19.9
11	121252	22.4	8.4	21.3	*	20.9	*	30.1
13	120899	15.4	6.3	7.2	*	*	*	22.7

Absolute fasting plasma glucose concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	3.7	9.7	4.4	7.9	2.5	3.9	1.1
2	119147	16.7	14.0	4.2	14.2	5.1	*	*
3	115131	4.5	15.6	7.9	4.0	4.0	5.5	5.6
4	115980	5.7	6.9	2.7	11.1	8.4	3.2	4.6
5	117222	9.9	8.2	2.1	10	4.3	18.1	7.6
6	113648	18.3	19.2	3.2	2.6	2.3	2.0	23.4
8	97218	17.3	13.9	1.4	3.7	2.7	5.4	4.7
9	117898	16.2	6.0	2.7	7.2	4.0	9.2	4.2
10	121228	9.2	12.6	5.0	3.8	4.4	4.1	9.1
11	121252	17.4	6.4	3.2	3.7	9.1	18.2	20.3
13	120899	3.8	4.9	3.5	8.3	*	*	3.5

Absolute afternoon (nadir) plasma glucose concentrations (mmol/l).

Appendix 19:
Plasma concentrations of indicators of lipid metabolism in 11 dogs from one month before to 4 months after changing to a canine high fibre diet

Shaded area represents hospitalisation period

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	8.17	8.09	7.39	*	6.25	*	7.96
2	119147	4.79	4.14	4.03	*	3.91	*	*
3	115131	6.92	6.00	4.82	*	5.50	*	5.34
4	115980	5.95	6.09	3.81	*	5.45	*	5.56
5	117222	11.84	16.21	6.66	*	9.80	*	12.63
6	113648	22.46	16.81	10.41	*	11.21	*	12.88
8	97218	6.29	6.60	5.59	*	7.39	*	7.44
9	117898	6.52	6.10	4.37	*	4.34	*	4.39
10	121228	16.71	14.86	7.43	*	9.95	*	9.67
11	121252	5.44	13.63	9.37	*	10.15	*	9.66
13	120899	8.95	8.67	5.39	*	*	*	6.03

Absolute total plasma cholesterol concentrations (mmol/l).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	1.50	1.73	1.43	*	0.92	*	0.54
2	119147	0.43	0.75	0.73	*	0.64	*	*
3	115131	1.70	0.64	0.66	*	0.77	*	0.67
4	115980	1.56	1.34	0.56	*	0.50	*	0.82
5	117222	1.42	3.05	3.32	*	0.78	*	0.00
6	113648	*	1.30	3.12	*	0.70	*	1.89
8	97218	0.63	0.90	0.64	*	1.02	*	1.42
9	117898	0.06	2.55	0.62	*	0.50	*	0.72
10	121228	3.94	2.99	1.07	*	2.53	*	2.10
11	121252	1.20	1.28	0.84	*	1.10	*	2.15
13	120899	0.95	0.93	0.79	*	*	*	1.67

Absolute plasma VLDL cholesterol concentrations (mmol/l)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	2.74	2.44	2.28	*	2.20	*	2.58
2	119147	0.90	0.59	0.70	*	0.13	*	*
3	115131	1.88	1.34	1.45	*	1.02	*	0.72
4	115980	1.37	1.60	0.78	*	1.20	*	1.09
5	117222	5.80	9.44	0.35	*	3.77	*	7.40
6	113648	10.24	10.19	1.70	*	5.87	*	5.92
8	97218	1.73	2.60	1.22	*	2.16	*	2.63
9	117898	1.59	1.19	0.85	*	0.71	*	1.01
10	121228	7.81	5.85	2.15	*	3.55	*	2.74
11	121252	0.46	5.71	3.92	*	4.40	*	3.47
13	120899	2.23	2.52	1.24	*	*	*	1.00

Absolute plasma LDL cholesterol concentrations (mmol/l)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	3.93	3.92	3.68	*	3.13	*	4.84
2	119147	3.46	2.80	2.60	*	3.15	*	*
3	115131	3.34	4.02	2.71	*	3.71	*	3.95
4	115980	3.02	3.15	2.47	*	3.75	*	3.65
5	117222	4.62	3.72	2.99	*	5.25	*	6.48
6	113648	5.45	5.32	5.59	*	4.65	*	5.07
8	97218	3.94	3.10	3.73	*	4.21	*	3.40
9	117898	4.87	2.36	2.90	*	3.12	*	2.66
10	121228	4.96	6.02	4.21	*	3.87	*	3.64
11	121252	3.78	6.64	4.61	*	4.65	*	4.04
13	120899	5.78	5.22	3.33	*	*	*	3.36

Absolute plasma HDL cholesterol concentrations (mmol/l)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	1.14	2.41	2.06	*	1.26	*	1.04
2	119147	0.89	0.64	1.03	*	1.55	*	*
3	115131	0.54	0.50	0.97	*	0.79	*	1.25
4	115980	1.69	2.02	0.80	*	0.46	*	0.70
5	117222	0.82	1.09	0.71	*	0.35	*	1.02
6	113648	0.93	1.30	0.49	*	0.52	*	1.46
8	97218	2.34	1.75	0.61	*	2.31	*	1.48
9	117898	2.09	0.64	0.91	*	0.95	*	0.88
10	121228	12.67	8.07	1.46	*	3.12	*	11.44
11	121252	2.48	3.08	2.16	*	2.06	*	1.57
13	120899	0.72	0.61	0.67	*	*	*	4.03

Absolute plasma triglyceride concentrations (mmol/l)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	107	133	154	*	81	*	93
2	119147	66	37	67	*	66	*	*
3	115131	155	186	324	*	175	*	220
4	115980	124	160	128	*	161	*	101
5	117222	147	206	*	*	154	*	212
6	113648	223	199	93	*	117	*	107
8	97218	395	409	*	*	150	*	201
9	117898	163	137	72	*	91	*	80
10	121228	244	236	137	*	96	*	73
11	121252	341	124	231	*	81	*	110
13	120899	305	220	255	*	*	*	254

Absolute plasma free glycerol concentrations (μmol/l) in 11 dogs from one month before to 4 months after changing to CHF diet).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	0.91	1.20	1.51	*	0.96	*	0.87
2	119147	0.76	0.51	0.86	*	0.88	*	*
3	115131	1.82	1.99	4.16	*	2.71	*	3.42
4	115980	1.36	1.22	1.18	*	1.12	*	0.84
5	117222	1.68	3.09	*	*	2.05	*	2.19
6	113648	3.02	3.90	1.97	*	1.34	*	1.44
8	97218	3.52	3.98	*	*	1.33	*	1.85
9	117898	*	2.51	0.72	*	0.93	*	1.09
10	121228	3.64	3.34	1.52	*	1.78	*	1.32
11	121252	*	1.44	2.94	*	1.57	*	2.63
13	120899	*	2.74	2.05	*	*	*	2.08

Absolute plasma non-esterified fatty acids concentrations (mmol/l)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	30	14	15	*	14	*	9
2	119147	11	NR	18	*	14	*	*
3	115131	10	5	17	*	7	*	7
4	115980	12	11	15	*	2	*	NR
5	117222	NR	14	6	*	3	*	28
6	113648	5	30	NR	*	16	*	NR
8	97218	43	19	17	*	NR	*	76
9	117898	49	2	9	*	35	*	NR
10	121228	50	27	32	*	28	*	19
11	121252	33	31	393	*	25	*	143
13	120899	22	8	49	*	*	*	418

NR denotes samples in which the concentration of apolipoprotein B was undetectable

Absolute VLDL triglyceride/apolipoprotein B ratios (mmol/ug equiv. bovine serum albumin)).

No	DOG REFERENCE	MONTHS						
		-1	-10 days	0	1	2	3	4
1	115678	2.48	5.45	3.25	*	5.34	*	9.49
2	119147	7.11	6.73	9.16	*	9.63	*	*
3	115131	9.35	11.64	11.44	*	*	*	11.66
4	115980	14.49	19.15	18.97	*	18.64	*	16.89
5	117222	17.17	14.44	20.37	*	23.65	*	19.32
6	113648	11.46	16.29	32.89	*	17.27	*	11.49
8	97218	35.73	28.23	29.40	*	19.25	*	18.05
9	117898	18.44	18.54	7.96	*	9.09	*	13.76
10	121228	10.63	11.10	*	*	14.15	*	17.97
11	121252	16.60	13.61	12.13	*	16.53	*	21.43
13	120899	27.01	22.74	19.63	*	*	*	18.36

Absolute post heparin hepatic lipase activities (μmolFA/ml/hr)).

Glossary

β	beta
α	alpha
Ci	Curie
cm	centimetre
cpm	counts per minute
CV	coefficient of variation
EDTA	ethylenediaminetetra-acetate
FA	fatty acid
HDL	high density lipoprotein
hr	hour
IU	international unit
kg	kilogram
l	litre
LDL	low density lipoprotein
M	molar
m	milli
mmol	millimole
n	number of observations
OR	odds ratio
p	probability
r	correlation coefficient
rpm	revolutions per minute
SD	standard deviation of a sample
SE	standard error
SEM	standard error of the mean
μ or u	micro
VLDL	very low density lipoprotein

Laboratory reference ranges

Biochemistry

Alanine aminotransferase	0 - 65	U/l
Albumin	22 - 35	g/l
Alkaline phosphatase	< 230	U/l
Ammonia	< 100	µmol/l
Amylase	400 - 3500	U/l
Aspartate aminotransferase	0 - 35	U/l
Bilirubin	< 10	µmol/l
Calcium	2.34 - 3.03	mmol/l
Chloride	95 - 115	mmol/l
Cholesterol	1.80 - 6.96	mmol/l
Creatinine	44 - 132	µmol/l
Gamma-glutamyl transferase	0 - 6	U/l
Globulin	22 - 45	g/l
Glucose	2.5 - 4.5	mmol/l
Inorganic phosphorus	1.29 - 2.90	mmol/l
Lipase	< 1000	U/l
Potassium	3.4 - 5.8	mmol/l
Sodium	136 - 160	mmol/l
Total protein	50 - 78	g/l
Urea	0 - 7.47	mmol/l

Haematology

Red blood cell count	4.25 - 8.50	$x10^{12}/l$
Haemoglobin	10.5 - 21.0	g/dl
Haematocrit	0.34 - 0.58	l/l
Mean cell volume	60 - 77	fl
Mean cell haemoglobin	19.5 - 24.5	pg
Mean cell haemoglobin conc.	32 - 36	g/dl
Reticulocytes	0.0 - 1.5	%
White blood cell count	6.0 - 17.5	$x10^{9}/l$
Neutrophils	3.0 - 11.5	$x10^{9}/l$
Lymphocytes	1.0- 4.8	$x10^{9}/l$
Monocytes	0.15 - 1.35	$x10^{9}/l$
Eosinophils	0.1 - 1.2	$x10^{9}/l$
Platelets	200 - 500	$x10^{9}/l$

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