Projectbeschrijving onderzoeksstage Diabetes mellitus bij katten

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The prevalence of underlying causes for type III diabetes mellitus in cats; Prevalence, risk factors and survival.

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Summary

This research is a substudy of 136 cats with diabetes from Dutch first line veterinary clinics with different glycaemic control, patients were selected for this research to determine the prevalence of underlying diseases that cause diabetes mellitus. Underlying causes for diabetes were found to be 31.1% for acromegaly, 15.7% for hyperadrenocortisism, 8.2% for hypothyroidism/SES and 5.7% for hyperthyroidism. Hypoglycaemia indicating Somogyi effect was found in 17.2% of the cases and pancreatitis was found to be present in 60.3% of our patients. Breed and gender predisposition was found in mixed breed and domestic short haired male cats which had a higher prevalence for acromegaly. The average life span of a cat after diagnosis is estimated to be about 5 years, hyperthyroidism increases life span and hypothyroidism decreases life span. Pancreatitis was significantly more present in patients with Acromegaly and pancreatitis decreases life span of the patients. Female cats were diagnosed at a later age and lived longer, domestic short haired cats also lived longer than breed cats with diabetes. The leading cause of death was found to be hypoglycaemia.

Samenvatting

Dit onderzoek is een deelonderzoek naar de prevalentie van onderliggende oorzaken voor suikerziekte bij 136 katten afkomstig uit Nederlandse eerstelijns dierenklinieken met verschillend gereguleerde suikerziekte. 31,1% van de katten had acromegalie, 15,7% had hyperadrenocortisisme, 8,2% had hypothyreoïdie/SES en 5,7% had hyperthyreoïdie. In 17,2% van de gevallen was er sprake van hypoglycaemie, dit werd waarschijnlijk veroorzaakt door het Somogyi effect, daarnaast werden er in 60,3% van de patiënten aanwijzingen gevonden voor pancreatitis. Een predispositie voor acromegalie werd gevonden in raskatten en in mannelijke dieren. De gemiddelde overlevingstijd na diagnose wordt geschat op 5 jaar, hyperthyreoïdie verhoogt en hypothyreoïdie verlaagt de levensduur van patiënten met diabetes. Pancreatitis werd meer aangetroffen in patiënten met acromegalie en kan de levensduur van katten met suikerziekte verkorten. Vrouwelijke dieren en Europese kortharen met suikerziekte leefden over het algemeen langer, daarnaast werd suikerziekte bij vrouwelijke dieren op latere leeftijd vastgesteld. De meest voorkomende doodsoorzaak was hypoglycaemie.

Introduction

Diabetes Mellitus (DM) has been identified as one of the leading endocrine disorders in cats since the dawn of feline veterinary medicine. DM is the condition in which there is an absolute or relative shortage of insulin, a condition that always leads to hyperglycaemia. Theoretically any chronic change in blood glucose level may eventually lead to diabetes. However only some forms of diabetes have been found and over the years there have been many classifications.

Prevalence values and risk factors have been found in the general population and in the population of diabetic cats. The prevalence of diabetes ranges about 0.5 – 2%[13] in the general cat population. Some predisposing factors for diabetes have been found, there is a breed predisposition in Burmese cats[19] and male neutered cats have a higher incidence to become diabetic. It is also shown that there is a higher prevalence of diabetes among older cats, especially older than 8 years with a peak incidence at 9-13 years.

Since veterinary medicine was based on human medicine, so was our first classification of this disease; type I and type II diabetes. This classification roughly tries to make a differentiation between insulin dependent or insulin resistant diabetes mellitus. However in veterinary science we came to the conclusion that in order to classify canine and feline diabetes we had to use a new classification system. Since a few years there has been a new Utrecht classification that involves therapy classification.

Type I Diabetes

In type I diabetes there is an autoimmune destruction of β-cells in the pancreas which leads to an absolute shortage of insulin. This is the most common type of diabetes in humans and dogs, but there is no evidence that this is one of the leading causes of diabetes in cats.

Type II diabetes

In type II diabetes there is a relative insulin shortage caused by insulin resistance. There is a multifactorial cause that is mainly attributed by a positive carbohydrate metabolism caused by lifestyle factors such as physical inactivity, high dietary carbohydrate intake, overweight or perhaps even a genetic background. In fact you could say this is idiopathic diabetes, the exact cause is unknown. Because there isn't any pancreatic destruction in the early stages it is possible to maintain this type of diabetes and even turn it into a remissive state as the insulin resistance is reversed. However type II diabetes can lead to the ultimate destruction of the pancreas if there has been a long period between the onset of the disease and diagnosis with concurrent treatment.

Therefore this time between onset and diagnosis is essential for the prognosis of diabetes in cats. Until now it has been assumed that this is the most likely cause of diabetes (DM) in cats and that the prevalence of this type is around 85% of all cats affected by DM, resulting in 0.4-1.7% of the entire population of cats.[20]

Type III diabetes

Type III diabetes is a group that includes all remaining types of diabetes and is mainly caused by other diseases that influence the carbohydrate mechanism leading to hyperglycaemia or that directly influence insulin production, insulin release or that influence the insulin signal transfer at the peripheral insulin receptors.

In most cases there is a questionable insulin resistance and it becomes harder to reach the right level of insulin.

A few examples of diseases that can influence this mechanism are acromegaly, hypercortisolism as part of Cushing's syndrome (iatrogenic, adrenal or pituitary dependant) or chronic stress, hyperthyroidism, hypothyroidism or chronic pancreatitis.
Until recently it was presumed that type III diabetes was rare among cats, although true prevalence was never established. However new information on this subject came when researchers were determining Insulin like growth factor-1 (IGF-1) levels in diabetic cats. In the research of Niessen et al. a prevalence of high IGF-1 was found, it was present in 59 out of 184 diabetic cats (32%), out of which eighteen cats were examined with CT/MRI scans and where seventeen of those eighteen cats were confirmed to have acromegaly. This would mean that about 30% of those cats would have had acromegaly as cause for their diabetes.[17]

Type IV diabetes

This type of diabetes is present in pregnant humans and animals. Progesterone causes growth hormone (GH) release in the mammary glands which is released into the circulation and causes hyperglycaemia.[9]
In dogs this is a cause for diabetes but in cats pregnancy can not lead to diabetes in the same way, because the growth hormone (GH) produced in the mammary glands in cats does not reach the circulatory system.

Proposed pathways of pathogenesis for different subtypes of DM type III

Acromegaly; the prevalence of this disease in the general population is uncommon to rare. Acromegaly in cats is mostly caused by a GH producing tumour in the pars distalis of the anterior pituitary gland. Acromegaly is found in older male domestic cats with a mean age of 10 years (range; 4-19 years).[9] Symptoms arise because of excess GH and IGF-1 production as well as space occupying cell-growth. Increased secretion of GH has anabolic as well as catabolic effects. The anabolic effects are most likely due to increased IGF-1 secretion causing proliferation of bone and cartilage as well as organomegaly. The catabolic effects are most likely due to antagonistic action of insulin caused by a GH induced post-receptor defect in the glucose transport leading to decreased insulin response and as result hyperinsulinism. Prolonged periods of hyperinsulinism lead to insulin resistance and DM type III.[7, 17]

The prevalence of diabetes among acromegaly patients is extremely high, as all will show signs of diabetes. The proposed prevalence of acromegaly among DM patients is also high (30%) according to research by Niessen et al.[17]

Specific symptoms for acromegaly are poorly controlled DM, increase in body size, enlargement of abdomen and head and weight gain. Later on the patient might experience prognatia inferior, degenerative arthropathies and organomegaly of heart, kidneys, liver and tongue. Also respiratory stridor has been found in large number of cats with Acromegaly.[17] Because of the organomegaly heart and kidney failure is a leading cause of death for acromegaly patients.[7, 9]
The space occupying aspect of acromegaly can also cause stupor, decreased appetite and anorexia, adipsia, loss of temperature regulation, circling, seizures and epilepsy and behavioural changes.[7]

True diagnosis can be made with a CT or MRI scan, however since this is a very invasive and costly procedure GH and IGF-1 levels can also be determined. GH tests are not readily available and IGF-1 is a solid method to identify possible acromegaly cases. However IGF-1 levels can be altered by other diseases like renal failure, some types of tumour and altered T_4 levels or other types of insulin resistance.[9] In addition humane research has shown a difference in GH-IGF-1 response between males and females.

Proposed therapies are cobalt radiotherapy, medical therapy with somatostatin analogues (octreotide), dopamine agonists and GH-receptor antagonists and hypofysectomy. [7,

[9] Hypofysectomy has shown to be successful in the treatment of cats presented with acromegaly, whereas other treatments have shown limited effectivity.[16] Additional therapy with ocreotide has also shown to effectively lower GH and ACTH in diabetic patients.[23] Permanent or temporary remission of DM has been found rarely due to spontaneous necrosis of the tumour due to an infarct.[9] Survival time of the untreated patient has been set on 4-42 months with an average survival time of 21 months.[7] Longer survival times have been found in treated patients.[9]

Hyperadrenocortisism; Hyperadrenocorisism is considered to be a rare disease in the general population of cats. 80% of the cases of hyperadrenocortisism in cats are caused by an ACTH producing adenoma in the pituitary gland. 20% of the cases are caused by a functioning adrenocortical tumour with a ratio of adenoma to carcinoma of 1:1. Hyperadrenocortisism is found in older cats with a mean age of 10-11 years (range 5-16 years), there seems to be no gender or breed predisposition.[9, 4, 7]

Cortisol antagonises Insulin and this causes DM in Cushing patients resulting in an extremely high prevalence of DM with possible insulin resistance among Cushing patients (>80%) [9, 7, 4]

Specific symptoms of Cushing patients are poorly controlled DM, polyphagia, dermatologic signs such as fragile, thin, easy to bruise and easily infected skin, patchy and asymmetrical alopecia, muscle wasting, increase in abdominal size, hepatomegaly, abnormally pigmented skin, recurrent upper respiratory and urinary tract infections.
The space occupying aspect of pituitary dependant Cushing can also cause stupor, decreased appetite and anorexia, adipsia, loss of temperature regulation, circling, seizures and epilepsy and behavioural changes.[7]

Screening for the presence of hyperadrenocortisism can be acquired by determining urine cortisol/creatinine ratios, however stress and increased T_4 can influence this test.[12] Low dose dexamethasone suppression test could also give an indication of Cushing's disease. If there is valid suspicion for Cushing's disease then differentiation tests can be performed to determine whether the tumour is located on the adrenal glands or in the pituitary. Tests that can help to determine this are a high dose dexamethasone suppression test, plasma ACTH concentration and ultrasonography of the abdomen, which are advised to be performed at specialised hospitals. Ultrasonography might show enlargement of the adrenals which can be an indication for Cushing's disease. Pituitary tumours can be seen on CT and/or MRI scans [7, 4]

Therapy of Cushing's disease is difficult and has a guarded prognosis, adrenalectomy has had better results than medical or radiation therapy. However bilateral adrenalectomy can't prevent space occupying symptoms in cats with pituitary dependant Cushing. Medicinal therapeutic options might be o'p'DDD (Mitotane) but this has shown little to no result, Trilostane (most promising), ketoconazole or metyrapone. Radiation therapy with cobalt-60 has also limited effectivity and is very expensive.[7, 9]

Prognosis of Cushing's disease before and after therapy is guarded especially if the cat presents with dermatologic signs before therapy. After therapy there is a risk the cat will develop Addisonian crisis which can result in death. However cats that undergo bilateral adrenalectomy with concurrent pituitary irradiation have the best prognosis, DM can go into remission and they have a normal life span.[7, 4]

Hyperthyroidism; Hyperthyroidism is the most common endocrine disorder in cats. The prevalence of hyperthyroidism in the normal feline population is 0.3%[9]. 99% of the cases involve a benign nodular hyperplasia, adenomatous hyperplasia or adenoma. Because of negative feedback there is atrophy of normal thyroid tissue. In 70-75% of the cases there is involvement of both thyroid glands and 1-3% of the cases are caused by a mild to moderate

malignant thyroid carcinoma. The mean age of onset of Hyperthyroidism is 13 years but ranges between 4 and 23 and hyperthyroidism has occasionally been found in animals younger than 1 year. No sex or breed predisposition has been found.[9]

Hyperthyroidism promotes hyperglycaemia by reducing the half-life of insulin because it increases the rate of degradation. In addition the excess of thyroid hormones increases the gut glucose absorption which also leads to hyperglycaemia. Endogenic glucose production is also enhanced and promoted by hyperthyroidism through influences on the GLUT-2 concentrations in hepatocyte plasma membranes. In addition hyperthyroidism increases other hormones (GH, glucagon and catecholamines) that can attribute to insulin resistance.[10]

Specific symptoms of hyperthyroid patients are weight loss, polyphagia, restlessness, greasy fur coat, vomiting, diarrhoea, bulky faeces, respiratory signs (dyspnoea, coughing and sneezing), aggression, seizures and muscle weakness. Concurrent cardiac hypertrophy might result in congestive heart failure which can cause dyspnoea, apathy, hind limb weakness or collapse.

A large proportion of animals with hyperthyroidism has hypertension and can develop retinopathies, cerebrovascular accidents, abnormal behaviour, dementia or renal failure.

10% of the hyperthyroid patients display apathetic hyperthyroidism.[9]

5% of the hyperthyroid patients display increased serum glucose levels, resulting in diabetes.

A definitive diagnosis of hyperthyroidism is based on elevated levels of T_4. However some cats with hyperthyroidism have a T_4 concentration within normal range or have another concurrent systemic illness that causes sick euthyroid syndrome (SES). Extra tests that can be performed are the T_3 suppression test, TRH stimulation test. Scintigraphy usually gives a good idea of the presence of hyperactive thyroid tissue, but is pretty expensive and can only be performed in specialised facilities.[9] In an extra notation hyperthyroid patients might display increased urinary cortisol/creatinine ratios because of an increase in ACTH release as well as an increase in metabolic clearance of cortisol.[12] Also it has been reported that Fructosamin levels are lower in patients with hyperthyroidism.[25]

Hyperthyroidism can be treated through surgical removal of the thyroid gland, with medication or with Radioiodine therapy (I^{131}).Some cats might develop renal failure after the treatment for hyperthyroidism and cats that already have renal failure might worsen.

Medical therapy consists of Medimazole or Carbimazole treatment.[9]

Thyroidectomy comes with risks during surgery and might eventually lead to hypothyroidism and hypoparathyroidism, Horner's syndrome and laryngeal paralysis.

The treatment of choice, if possible, is radioiodine therapy. 80% of treated patients have been treated successfully, 10% of cats become hypothyroid and 10% fail to respond to therapy. Radioiodine therapy is also the safest and most accurate method.[9]

The prognosis after treatment is extremely good to guarded, depending on additional disease; the life expectancy of treated cats is 2 years. Untreated disease might cause early death because of concurrent renal, heart or liver disease.

Hypothyroidism; Hypothyroidism is a rare disease in cats and in most cases it is caused by hyperthyroid treatment. Primary hypothyroidism in cats is extremely rare, however congenital hypothyroidism has been described more frequently. Also due to all-meat diets, kittens feeding on this might develop hypothyroidism due to Iodine shortage.

Congenital hypothyroidism has been found in domestic short hair cats and Abyssinian cats without sex predisposition.[9]

In humans and dogs auto-immune destruction of both thyroid gland and pancreas is a known cause for DM (DM type I in this case), this has not been reported in cats. However

hypothyroidism has been shown to lead to insulin resistance due to impaired insulin stimulated glucose uptake.[10]
Specific clinical signs are weight gain, lethargy, inappetence, hypothermia, bradycardia, dry skin, ill kept hair coat, alopecia of the pinnae, alopecia of pressure points, alopecia of tail base and caudal flanks, myxoedema.[9, 21]

Congenital hypothyroidism leads to slow kittens, disproportionate dwarfism (short stature), mental dullness, constipation, longer persistence of deciduous teeth, shaggy hair coat, goitre or death.[9]

Hypothyroid patients are shown to be more prone to hypoglycaemic insults and require less insulin than normal diabetic patients. Hypothyroid patients also show more severe retinopathies and renal problems when also suffering from diabetes.[10]
Circulating T_4 is low or in the low normal range. However Sick Euthyroid Syndrome could also cause low circulating T_4, confirmation is performed with TSH or TRH response tests or by determining basal TSH levels.[9]
Treatment consists of orally supplementing $_L$-Thyroxine.[9]
The response to this treatment is great and the prognosis after treatment is extremely good. Without treatment prognosis is usually guarded and in several congenital disorders leading to hypothyroidism death occurs at 16 weeks of age without treatment.[9]

Somogyi effect; the Somogyi effect is the effect which happens after an insulin overdose; iatrogenic hyperinsulinemia. Because of high levels of insulin, glucose drops rapidly and can drop below normal glucose levels.[1] In response the body releases glucocorticoids, catecholamines and increases glucagon output which causes rebound hyperglycaemia.[1] The Somogyi effect can severely interfere with the therapeutic control of cats with DM because elevated glucose concentrations can be found at 4-6 hours after insulin dose with Caninsulin. Because of this effect a dose that is too high can not be distinguished from a dose that is too low if glucose is only checked at low nadir. [1]

The Somogyi effect can occur in cases where insulin protocol is not followed by the veterinarian or the owner.[1] Remission of DM in cats has been reported and is also a possible cause for the Somogyi effect.

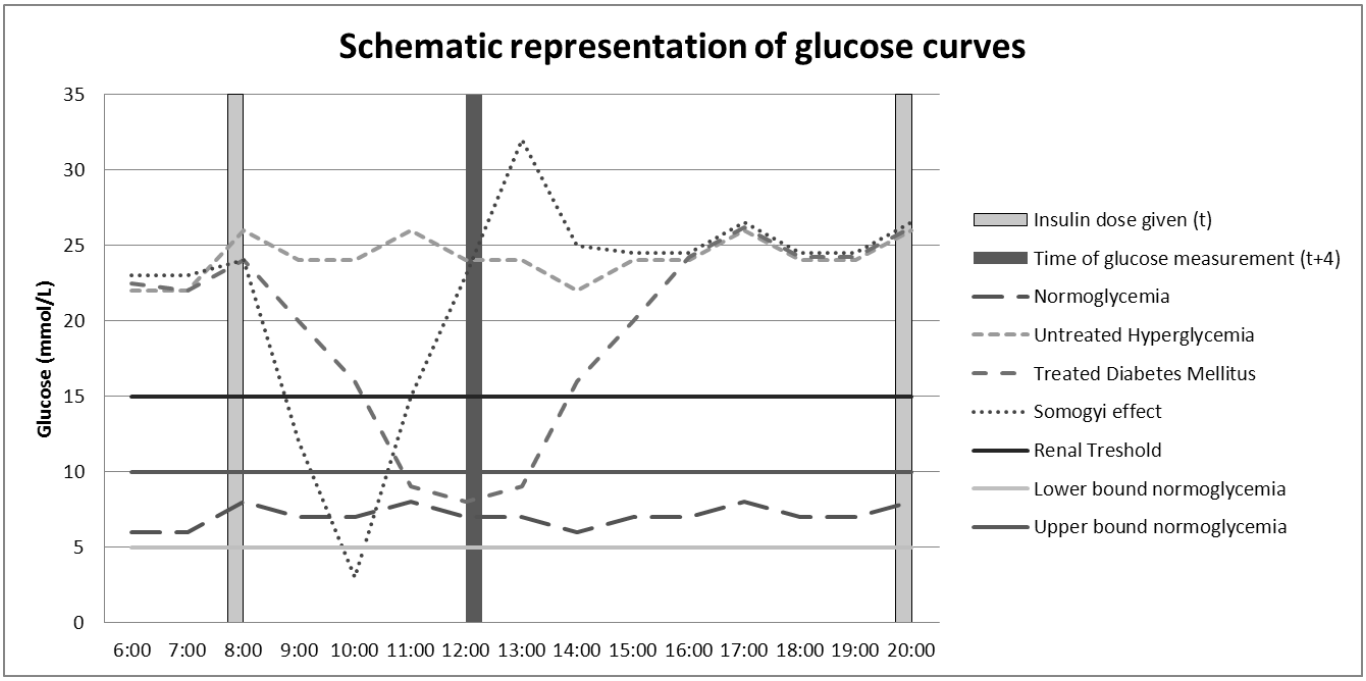
The Somogyi effect can occur in any patient – regardless of age, sex and breed – if diabetic control is insufficient. It is possible that underlying causes for diabetes, causing a difficult to control DM, might contribute to the occurrence of hypoglycaemic episodes. Specific signs of hypoglycaemia are depression, confusion, weakness, lethargy, ataxia, unresponsiveness, stupor, coma, seizures, and other neurological or behavioural signs.[1] Hypoglycaemia could be found with blood glucose tests, however hexokinase based glucose tests in the lower regions are not reliable, in these cases one should depend on clinical presentation of the patient. Glucose oxidase based glucose tests are more accurate. Also a delay in measurement or an error in sample storage could falsely lead to an indication of hypoglycaemia because of glucose use by enzymes and red blood cells.[5]

Evaluation of diabetic control can be performed by measuring a daily glucose curve, this measures the responsiveness of the animal to insulin and can aid in determining daily glucose control times. In addition fructosamines, GHb and urine glucose levels are valid to determine if long term glucose regulation is sufficient, however changes in insulin dosage should not be made on either of these parameters alone but always in combination with blood glucose.[1] The use of daily glucose curves should be limited because this also causes stress in the animal and might interfere with glycaemic control.[1]

To prevent the Somogyi effect from happening monitoring of the disease is essential, even if the disease appears to be stable. In case of the presence of Somogyi effect or hypoglycaemia a decrease in insulin dose of 25% is a necessity and is a safer option.[1]

Monitoring the patient closely and keeping track of insulin dose and signs of diabetes or hypoglycaemia might be essential in order to control the disease. Also always keep track that the patient is receiving the insulin in a correct manner, check if the insulin that is used is not older than 3 months and also check the integrity and storage of the insulin vial.[1]
In patients with poor control of diabetes long lasting insulin like Lantus might be considered, even though this insulin is not registered for use in cats.[1]

The effect always leads to hypoglycaemia which is potentially deadly. The Somogyi effect could be prevented if glucose and insulin levels are regularly monitored.[1]



This schematic representation shows that, during glycaemic control at 4 hours after insulin injection, patients presenting with Somogyi effect become indistinguishable of patients without therapy or with insufficient therapy resulting in a wrongfully increase in insulin dose.

Pancreatitis; although pancreatitis is pretty common in cats true prevalence is unknown. The clinical presentation of the disease ranges from subclinical cases to chronic cases or extreme hyperacute cases. There is no known cause for pancreatitis, but inflammatory bowel disease and cholangiohepatitis are known to occur simultaneously.[28] In addition pancreatitis might be caused by diabetes mellitus. Pancreatitis has no known prevalence for age of breed.

It is known that 65% of the diabetic cats show signs of amyloidosis of the pancreatic isles. Amyloidosis has also been found in non-diabetic cats. Amyloid is a pathologic deposit of polymerised proteins (feline Islet Amyloid PolyPeptide or fIAPP) most likely caused by the remnant of increased insulin release. It is suspected that this amyloidosis is caused by DM in cats and leads to chronic pancreatitis.[27]

In addition it is also suspected that any inflammation of the pancreas (both acute and chronic) may result in decreased function of the β-cells of the pancreas, which leads to an absolute insulin deficiency that causes DM. In some cases exocrine pancreas deficiencies can also develop causing EPI.

Specific clinical signs are anorexia, lethargy, dehydration, hypothermia, vomiting, abdominal pain, abdominal palpated mass, dyspnoea, diarrhoea, ataxia, icterus, shock. Symptoms of acute pancreatitis comprise of severe dehydration, tachycardia, tachypnea and pyrexia.

True diagnosis can be established by determining fPLI, but during episodes of pancreatitis other blood parameters should also be monitored. In addition an abdominal ultrasound might also show if there is pancreatitis present and the best non-invasive results were found in a combination of ultrasound and fPLI. fTLI is not a helpful parameter to determine the presence of pancreatitis in cats.[28]

Treatment of pancreatitis is very difficult and in most cases adjustments in treatment of DM should be halted when pancreatitis is present.
Severe acute pancreatitis should be treated aggressively by IV fluid replacement therapy, restriction of food and water uptake, controlling nausea and vomiting and providing pain relief. Restriction of food and water should not take longer than 2-3 days because this could lead to hepatic lipidosis, in these cases alternative nutritional supplementation should be provided.

Medicinal therapy consists of pain relief (Buprenorphine, Butorfenol, ketamine or Fentanyl), preventive broad spectrum antibacterial medicine (amoxicillin/clavulanic acid, enrofloxacin), anti-nausea medication (metoclopramide, ondansetron, dolasetron, chlorpromazine or maropitant citrate) and anti-thrombotic medicine (low molecular-weight heparin). The diet should consist of highly digestible food low on fat and high on carbohydrates. Force feeding should be avoided, if possible.[28]

Kidney failure; Diabetic nephropathy has been described to be present in many cases of different types of diabetes. Kidney damage is most likely due to macrovascular damage (atherosclerosis) leading to hypertension and destruction of renal tissue. In addition diabetes can cause ischemic damage because of concurrent cardiac disease, which may also damage the kidney. It is also known that kidney cells undergo cell-specific changes in phenotype in the presence of hyperglycaemia. All this causes damage to the renal glomerulus and influences the RAAS system with kidney failure as result.[3]

Kidney failure can also cause diabetes mellitus. Severe renal failure will result in a decline of glomerular filtration and will cause endogenous toxins to rise. These toxins can cause insulin resistance.

Other possible causes for DM type III; Multiple Adenomas have been described in feline and humane medicine and could cause both Cushing and Acromegaly in one patient with concurrent DM. [15]

Antemortem diagnosis of Pheochromocytomas in cats is rare.[9] DM is described in literature to be present in 35.6% of humane patients with pheochromocytomas and is most probably due to catecholamine induced insulin resistance or suppression. Humane patients with pheochromocytomas were significant younger and more likely to be female. The symptom both diseases have in common is hypertension.[2]
Next to endogenous hypercortisolism, exogenous hypercortisolism can also cause DM in cats. α-MSH producing tumours are also known to produce peripheral insulin resistance and by this route can play a role in the development of DM type III.

Tumours of the pancreas with destruction of ß-cells that lead to insulin deficiency could also lead to DM. In theory, glucagon and insulin secreting tumours could also lead to diabetes, however only a few documented cases of insulinoma's in cats are known and it is a very rare disease and animals are more likely to die from hypoglycaemia than that they have a chance of developing insulin resistance[11]

Diabetic complications

Urinary tract inflammation (UTF) and infection (UTI); Glucose crystals in the urine can damage the urinary tract and cause inflammation. Apart from that DM is a possible cause for stress and stress can result in cystitis.

UTI in cats is normally rare, but in several conditions prevalence is high. Glucosuria and Proteinuria are a perfect energy source for the development of certain types of bacteria. Urinary specific gravity has also shown to be related with the presence of bacteriuria. This is consistent with the finding that there is a high prevalence and high incidence of UTI in diabetic cats. 12% of the cats with DM were found to have an UTI and in addition 12% of the hyperthyroid cats and 22% of the cats with kidney disease had bacterial infestation of their urine tract. [14]

Cats with UTI tend to be older and female, however in diabetic patients – opposed to patients with hyperthyroidism or kidney disease – no relation could be found which may indicate that glycosuria may result in UTI indifferent of gender.[14] A shorter and wider urethra is the possible cause for the gender difference in patients presented with UTI.[14]
UTF and UTI can be found by routine urine examination (Urinary Specific Gravity, dip stick and microscopic examination) but urinary culture gives better results for UTI.[14]
Infections present were in most cases sensitive for amoxicillin/clavulanic acid treatment.[14]

Diabetic neuropathy; There is increasing evidence that diabetic neuropathy affects somatic, autonomous and several peripheral nervous systems. In addition suspicions rise on spinal cord and CNS involvement. Demyelination is present because of glucose toxicity and in cats this generally leads to paresis and plantigrade (bear-footed) stand of hind legs.[3]
In addition specific causes for diabetes are due to tumours of the pituitary that can also lead to damage to the surrounding tissue leading to neurological symptoms.

It is however also reported that neuropathy might be present due to insulin toxicosis[26] and this might explain the difference between the presence of diabetic neuropathies in feline and canine diabetes.

Also diabetic neuropathy can be present if there's concurrent disease like EPI. In these cases a Vitamin B12 deficiency might be the cause of neurological symptoms.

Diabetic ocular changes; Conjunctival infections, KCS, keratopathy, pupillary changes, uveitis, changes in lens refraction, cataract, vitreous changes and retinopathies have all been described as possible complications of humane DM. Most of these changes can also be found in feline patients.[22]

Diabetes is a known cause for blindness in human and feline medicine. Because of vascular damage and death or dysfunction of the retina and/or Nervus Opticus blindness can be a common complication in DM in cats. However retinopathies are mostly found in cats with additional disease leading to fast degeneration of the optic nerve, the retina or the retinal vessels.[3]

Impaired wound healing and dermatitis; Diabetes can cause impaired or delayed wound healing and can be a risk factor for infectious dermatitis such as *Malassezia* spp.[18] In addition poly endocrinologic disorders also impair wound healing and decrease dermatologic health. Dermatologic impairment might also be present in ears, eyes and food pads.

This research

In cats we assume they most likely have either DM type II or they are classified as type III DM, were the diabetes is a symptom of an underlying disease. Until recently there hasn't been much study on the prevalence of underlying causes for DM in cats in the general diabetic

population. As explained in the next session this research will try to give a complete overview of diabetes type III.

Research Goal

This study's research goal is to improve knowledge of diabetes mellitus by identifying the prevalence of underlying causes for diabetes mellitus in cats presented in first line clinics in the Netherlands. In this specific substudy the goal is to identify several risk factors and to determine possible therapeutic aspects as well as prognosis and survival time between different subtypes of diabetes type III.

Hypothesis

- 10% of all the cats presented with DM have Acromegaly
- 10% of all the cats presented with DM have Cushing's syndrome
- 10% of all the cats presented with DM have Hyperthyroidism
- 1% of all the cats presented with DM display signs of Somogyi effect
- Less than 1% of all the cats presented with DM have hypothyroidism
- 40% of all the cats presented with DM display signs of pancreatitis
- The total prevalence of type III diabetes is 30-35% in diabetic cats

- Male cats have a higher prevalence of Acromegaly than female cats
- The prevalence of Cushing, Pancreatitis and Somogyi effect doesn't differ significantly between males and females.

- Cats with type III diabetes need more insulin/$kg^{0.75}$ than cats with type II diabetes

- The median survival time after diagnosis of patients presenting with Type II diabetes is higher than those with Type III DM.

Materials and Methods

Identifying and selecting research group

In the Netherlands there are about 3.6 million cats and estimations of the prevalence of diabetes range from 0,44 to 2%. The number of cats with diabetes in the Netherlands can be estimated to be between 7200 and 36000 individuals.

Because we are interested in the prevalence of other underlying causes for diabetes in cats we could not simply take all the cats with diabetes from the faculty because this would result in a group of cats that had a difficult to treat DM resulting in an overrepresentation of DM type III patients. This is why we chose to try and find diabetic cats with the help of our colleagues through the country. In this way we can estimate the true prevalence through cluster sampling.

A sample size was estimated to 264-271 individuals with a 95% confidence level, a 5% confidence interval and a response rate of 90% in a population of 7200 – 36000 diabetic cats. Because of the technical difficulties concerning the collection of the results a target for 150 individuals was set.

Acquiring owner contact information

All four researchers contacted veterinary practices through the country to see if they were interested in participating in this research. All clinics were contacted with a standardized research folder (hard copy, via e-mail or both) and were contacted one week later by phone to find out if they would like to participate. Out of the approximately 80 clinics contacted, 60 veterinary clinics from all regions of the country were available for participation.

The veterinary clinics were asked to contact their clients with diabetic cats and ask them if they would like to participate. No actual data of the responsiveness has been acquired but is to be estimated at 95%.

Some of the owners were found by an appeal placed on a Dutch forum for diabetic cats which also provided several patients for our research group.

During the first contact with the owners the research was explained, the mode of action was explained and possible risks were evaluated. Here again there is no actual data collection, but about 5% of the owners did not want to participate mostly because their cats were of old age or incompliant during veterinarian examination and they were reluctant to put unnecessary stress on their animals.

The owners that did want to participate acquired an information folder about the research to inform them about all aspects concerning this research.

Patients

A total of 136 cats were selected for participation. The only selection criterion was that the cat should have some form of diabetes. The gender distribution was 93 castrated males, 40 castrated females and 3 intact females. At the time of research cats were 4-18 years old with a mean age of 12.3 years.

Data collection & sample storage

The owners were requested to collect morning urine from their cats. They were supplied with Katkor non-absorbing cat litter several days prior to the actual examination of the cat. In this way stress influences on the morning urine were to be minimal and gave the owners several opportunities to collect urine without having to punctate the bladder. The owners were asked to store the urine in the refrigerator at 4-8 ^{o}C until the appointment.

During the actual appointment there was an extensive anamnesis including a survey. In this period the cats were given the opportunity to come outside of their baskets to explore the room and settle down.

The anamnesis covered the following topics; demographics; name, breed, age, sex, body weight, date of diagnosis, current therapy and dosage, food and food additives, other treatments or history of treatments (especially corticosteroids), appetite, general condition, water intake, urine frequency and amount, weight development, change in body contour, presentation of hair/coat, vomiting, diarrhoea, locomotion problems, nervous problems, vision problems and any other problems at that time or relevant problems in the past.

After the anamnesis a clinical examination was performed that covered general impression, general examination, examination of the thyroid gland, abdomen, respiratory & circulatory system, nervous and locomotory system and eyesight.

After this the front side of the neck was shaved and 7-10 ml of blood was drawn from the V. Jugularis with a 22G x 16 mm needle tip connected to a 10 ml syringe. Because of the larger blood volume required a larger needle tip was required than normally to assure swift blood flow and to prevent coagulation before blood was stored in the EDTA collecting tube.

1 ml of blood was then transferred to a 1 ml EDTA collecting tube, while gently swirling the blood around the entire tube. The rest of the blood was equally divided between two 5 ml serum tubes. After the blood collection the client and patient were sent home.

The serum tubes were then transferred to a centrifuge system and were centrifuged for 10 minutes at 3000 rpm to separate the serum. After centrifugation the serum was transferred into 2-3 1.5 ml Sardstedt microtubes holding a total of 3-4.5 ml of serum.

The serum was stored and frozen as soon as possible (but always within 12 hours) at -18 ^{o}C, and the EDTA blood and urine were brought to the laboratory the same day or the next morning. In the meanwhile the EDTA blood and urine were stored at 4-8 ^{o}C.

Sample analysis & processing

The EDTA blood samples were analysed at the Utrecht University Veterinary Diagnostic Laboratory using the fully automated haematology analyser ADVIA 2120[a] with species-specific software adjustments made for veterinary samples. Leukocytes and erythrocytes were measured in different channels. Leukocytes were counted and differentiated using peroxidase, and in the basochannel cells were lysed. Haemoglobin was measured by two methods in the ADVIA 2120. In the first method cells were lysed and cell-free haemoglobin was measured by means of a colorimetric method. The second method was flow cytometry, in which isovolumetrically sphered erythrocytes and reticulocytes were passed through a laser beam. Light scattered by each individual cell was detected at two different angles: scattered laser light measured by low angle detection provided information on cell size, and high angle detection provided information on haemoglobin concentration.

The following analytes were assessed: total white cell count and differentiation (leucocytes, segmented, sticks, eosinophiles, basophiles, monocytes, lymphocytes, lymphoblasts), haematocrit (Ht; calculated), MCV, MCH, MCHC.

The urine sample was also analysed at the Utrecht University Veterinary Diagnostic Laboratory to determine the following analytes; Urine specific gravity was determined by refractometer. pH, glucose, ketones and the presence of blood were determined with a urine dipstick method (Menarini), protein was determined with the biuret method and measured on a Beckman DXC Unicel. Creatinine was determined with the Ratte Jaffe method and measured on a Beckman DXC Unicel. Cortisol was determined with a Radio Immuno Assay with specially UVDL developed antibodies. Macroscopic evaluation was also performed. Secondary parameters such as cortisol/creatinine coefficient and the protein/creatinine coefficient were derived from the primary parameters by calculation.

After a storage and collection period of 8-14 months the serum samples were sent to the university clinic in Zurich, Switzerland. Here the following parameters were determined; bilirubin, glucose, fructosamines, urea, creatinine, protein, albumin, cholesterol, triglycerides, alkaline phosphatase, amylase, lipase, ASAT(GOT), ALAT(GPT), Sodium, Potassium, Chloride, Calcium, Phosphorous, basal T_4 and IGF-1. During storage samples remained frozen.

The remaining samples were then sent to IDEXX in Kansas in the USA to determine fPLI, fTLI, Cobalamine (Vitamin B12) and Folate (Vitamin B9) levels. During storage samples remained frozen.

Between 22-11-2012 and 24-11-2012 a follow-up for the individual results were sent back to the concerned veterinary clinics and they were asked if they could give any information on the status of the diabetic patients. On 12-03-2013 there was another follow-up to see whether patients were still alive and if not to determine the cause of death. This data will be used to give an estimation of the prognosis and mean survival time of specific diabetic subtypes.

Determining diabetic subtypes

The following groups are subtypes of DM type III

- Patients with IGF-1 levels above 800 ng/mL (Utrecht reference) were identified as the acromegaly group.
- Patients with a urinary cortisol/creatinine ratio above 42 were classified as possible Cushing/stress group.
- Patients with T4 levels below 1 mcg/dL were classified as the possible hypothyroidism/Sick Euthyroid Syndrome group.
- Patients with T4 levels above 3.5 mcg/dL were classified as possible hyperthyroidism group.
- Patients with glucose below 4.0 mmol/L and high fructosamin or the presence of glucose in their urine were defined as the possible Somogyi/hypoglycaemia group.

Patients that did not present with DM type III were classified as DM type II. If patients had missing data they could not be classified as diabetes type II, however they would have been classified as diabetes type III if either of known values were out of range. If patients could not be classified as type II or III they would be classified as unknown.

The following patients were classified as pancreatitis group

- Patients with fPLI above 3,5 µg/L
- Patients with fTLI above 82.0 µg/L
- Patients with amylase above 1538 U/L
- Patients with lipase above 26 U/L
- Patients with amylase below 700 U/L
- Combinations of either deviation

Patients that did not present these signs of pancreatitis were classified as the group without pancreatitis.

Concurrent kidney disease, urine tract inflammation, urinary tract infection and presence of other complications will be discussed in a later article.

Statistics & interpretation

Data was collected with Microsoft Excel and statistics were performed with either Excel or with IBM SPSS

Reference samples were distributed by the concerning laboratories, the reference values that were used can be found in Appendix 1.

Results

As this is a substudy only a part of the chemical clinical results will be presented and discussed in this article, a later article will discuss all the results including clinical findings and anamnesis.

Prevalence

True prevalences were calculated from the observed prevalence with the Agresti-Coull method. Values shown are only prevalences to be used for populations of diabetic cats. The shown lower and upper bounds comprise a 95% confidence interval.

	Type II	Type III	Hypo Thyroidism	Hyper Thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Observed prevalence	41.4 %	58.6 %	8.2 %	5.7 %	17.2 %	15.7 %	31.1 %	60.3 %
Expected lower bound	32.8 %	49.5 %	4.4 %	2.6 %	11.5 %	10.0 %	23.6 %	52.4 %
Expected upper bound	50.5 %	67.2 %	14.6 %	11.6 %	25.0 %	23.9 %	39.9 %	69.6 %

Gender

The gender and status was known for 136 individual cats. There were 0 intact males, 93 castrated males, 3 intact females and 40 castrated females.

A significant relation between Gender and disease group (p=0.004) was found in a χ^2-test, and in specific disease groups only a significant likelihood ratio between Acromegaly and gender has been found (p=0.003). 34 out of 86 males (39.5%) were found to have increased IGF-1 levels opposed to 4 out of 32 females (12.5%). No significant difference in prevalence was found between diabetes type II and type III. No significant relation was found between gender and any other specific diabetes type. Because of the low prevalence of non-castrated animals in this research nothing significant can be said about a probable relation with castration.

	IGF-1 (ng/mL)	T4 (mcg/dL)	Cortisol/creatinine Ratio
Male castrated	798*	1.9	20.7
Female castrated & intact	524*	2.3	30.3

Mean IGF-1 levels between males and females also differed significantly (p=0.003) in an independent sampled t-test, with an IGF-1 level of 798 ng/mL in males and 524 ng/mL in females. No significant difference has been found in mean urinary cortisol/creatinine or mean serum T4 levels between males and females.

Breed

Breed was determined for 136 individuals, non-breed animals were classified as European short haired cats. Cross bred cats were classified for their main breed. The frequencies were; European shorthair (106), Persian (8), Norwegian forest cat (6), Maine coon (5), Siamese (2),

British Short hair (2), American short hair (1), Blue Russian (1), Burmese (1), Havana Brown (1), Ragdoll (1), Siberian (1), Somali (1).

Because of the low amount of cats with different breeds it was impossible to calculate statistics for specific breeds; however it was possible to calculate differences between mixed breed and non-breed cats that were classified as domestic short haired cats. Therefore 106 cats were classified as non-breed opposed to 30 breed cats.

χ^2-tests have been performed for disease group and every specific disease to determine if there was a significant difference between breed and non-breed cats. There was only a significant difference ($p=0.047$) in the χ^2-test between breed and non-breed cats and acromegaly. Out of the non-breed cats 25 out of 94 animals presented with a high IGF-1, whereas in cats with a specific breed this was 13 out of 28 animals.

	IGF-1 (ng/mL)	T4 (mcg/dL)	Cortisol/creatinine Ratio
Breed	797	1.9	20.7
ESH	693	2	25.0

Mean IGF-1, cortisol/creatinine and T_4 concentrations did not differ significantly between breed and European Short haired cats in an independent t-test.

Furthermore there was a significant likelihood ratio ($p=0.039$) in the χ^2-test between breed and non-breed in female castrated cats. 2 out of 4 female castrated cats belonging to a breed had increased IGF-1 levels (50%), opposed to 2 out of 29 female castrated domestic short haired cats. (6.9%)

In addition 11 out of 22 castrated males belonging to a breed had increased IGF-1 levels, (50%) opposed to 23 out of 64 domestic short haired castrated males. (35.9%) This difference however was not significant.

There was no significant difference between breed or non-breed found in any other disease type than acromegaly.

When breed groups and sex groups were combined there was a significant likelihood ratio ($p=0.001$) between groups for acromegaly in a χ^2-test, no other significant differences were found.

	All	Male ESH	Male Breed	Female ESH	Female Breed
IGF-1	717	794*	811*	471*	790
T4	2.0	1.9	1.9	2.3	2.1
Cortisol/creatinine Ratio	24.0	20.6	20.8	32.3	20.0

Mean IGF-1 concentrations differed significantly ($p=0.011$) in a one-way Anova between male breed and male ESH compared to female ESH cats. The mean IGF-1 level of male ESH cats was 794 ng/mL, of male breed was 811 ng/mL, of female ESH 471 ng/mL and of female breed 790 ng/mL. Mean T_4 and cortisol/creatinine ratios did not differ significantly.

<u>Insulin dose and therapy sufficiency</u>

	All	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Mean insulin dose (IE/dose)	3.4	3.1	3.7	1.9	3.3	3.3	4.3	4.5	3.7
Mean weighted insulin dose (IE/kg/dose)	1.3	1.2	1.4	0.8	1.1	1.1	1.8	1.6	1.4
Mean daily metabolic insulin dose (IE/kg^0.75/day)	1.9	1.8	2.1	1.1	1.7	1.7	2.6	2.4	2.1

There was a significant difference in mean insulin dose (p=0.011) and in metabolic insulin dose (p=0.032) in an independent t-test between patients with and without acromegaly. No other significant differences have been found in either insulin dose, mean weighted insulin dose or metabolic insulin dose with an independent t-test between groups.

Sufficiency of insulin therapy was based on normal glucose and fructosamine levels as well as the absence of glucose in the urine.

	All	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Sufficient therapy	15	3	9	1	0	0	4	7	7
Near sufficient therapy	20	11	8	2	2	0	0	5	8
Insufficient therapy	94	34	51	7	5	21	13	26	30

There was a significant difference in therapy sufficiency between Somogyi and non-Somogyi patients (p=0.006) in a χ^2-test with 21 patients having insufficient therapy and 0 near sufficient or sufficient therapy. There was also a significant difference (p=0.040) between patients with and without Cushing with 4 patients having sufficient therapy, 0 near sufficient therapy and 13 insufficient therapy. No other significant differences have been found.

Differences in glucose and fructosamine concentration

	All	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Glucose (mmol/L)	13.5	17.5	10.9	16.3	8.9	2.5	12.1	9.7	14.6
Fructosamine (µmol/L)	520	588	471	534	428	344	472	462	531

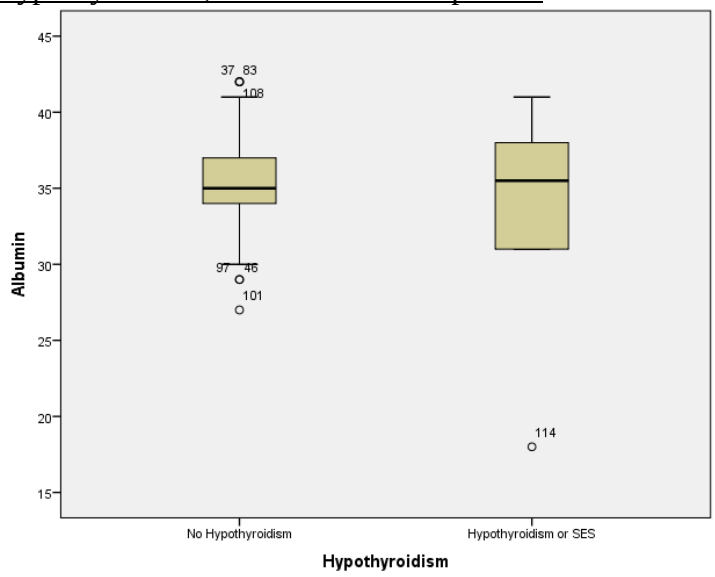
A significant difference was found between type II and type III diabetic patients in glucose (p<0.0005) and fructosamine (p<0.0005) levels. In addition significant differences in glucose were found in Somogyi patients (p<0.0005) and in Acromegaly patients (p=0.001). Significant differences were also found for fructosamin in Somogyi (p<0.0005) and Acromegaly (p=0.004) patients.

Presence of Pancreatitis

	All patients	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly
Pancreatitis	73	27	43	8	3	12	8	27
No pancreatitis	46	21	21	2	2	8	6	9

On the χ^2-tests a significant difference (p=0.044) was found between Acromegaly and non-Acromegaly patients in the presence of pancreatitis with patients with Acromegaly having more pancreatitis. No other significant differences were found.

Hypothyroidism, albumin and total protein



To differentiate between hypothyroidism and SES evaluation of albumin and total protein were performed between the hypothyroid group and the other samples.
No significant difference was found between the hypothyroid and non-hypothyroid group with a mean albumin of 35 g/L for the hypothyroid group and a mean albumin of 34 g/L for the non-hypothyroid group.
The mean protein levels were 79 g/L for hypothyroid patients, for non-hypothyroid patients the mean protein level was 73 g/L.

In the boxplot only 1 case was shown with severely decreased albumin for the hypothyroid patient group indicating SES.

Survival analysis

Multiple survival analysis were performed with Excel and SPSS to create Kaplan-Meier curves for all diabetes patients as well as specific curves for DM type II and DM type III and specific subsets of diabetes type III. Kaplan Meier survival statistics were run on the deceased patient group and on all living patients. The highest number of both would be considered to approach the true survival time in the best way.

All patients	All patients	DM type II	DM type III	Hypo-Thyroidism*	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Median survival	4.99	5.07	4.5	5.98	6.34	5.16	2.08	2.93	3.28
Lower bound	3.82	2.87	2.28	3.82	3.91	3.18	0	0.52	2.12
Upper bound	6.16	7.26	6.73	8.14	8.77	7.13	5.3	5.35	4.44

† Patient group	† patients	† DM type II	† DM type III	† hypo-thyroidism*	† Hyper-thyroidism	† somogyi	† Cushing	† acromegaly	† Pancreatitis
Median survival	2.62	3.28	2.02	1.05	5.16	2.93	1.99	2.08	2.08
Lower bound	1.96	2.4	1.9	0	0	2.14	1.82	1.67	1.67
Upper bound	3.27	4.16	2.15	2.43	10.38	3.73	2.16	2.5	2.5

* There were not enough hypothyroid patients to calculate median survival time, because of this mean survival time was used instead for this disease.

Patient group	Male castrated	Female intact	Female castrated	Breed*	ESH	Male Breed	Male ESH	Female Breed	Female ESH
Median survival	5.07	7.09*	3.64	4.50	4.99	6.27*	4.78	3.24	4.99
Lower bound	3.95	7.09*	3.95		3.76	4.37*	3.33	0.63	3.82
Upper bound	6.19	7.09*	6.19		6.22	8.17*	6.23	5.85	6.16

* Some of the data was limited and mean survival time was used instead of median survival time.

In this Kaplan-Meier curve patients still alive at the end of the research were counted as censored patients in Living Kaplan-Meier Curves (LKMC) and were counted as missing data in Deceased Kaplan-Meier Curves (DKMC). Truly deceased patients were regarded as deceased. Patients with missing data were excluded or counted as censored patients after contact was lost.

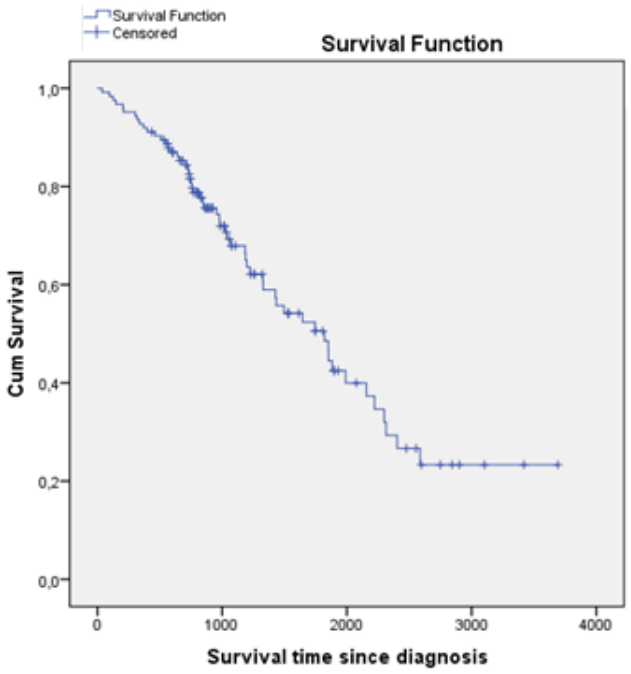
Survival of hypothyroid and non-hypothyroid differed significantly in the DKMC (Log rank; p=0.010, Breslow; p=0.015, Tarone-ware; p=0.012) but will not be shown here because of the low amount of patients (n=2) with hypothyroidism.

Survival of hyperthyroid and non-hyperthyroid differed significantly in the DKMC (Log rank; p=0.025) but will not be shown here because of the low amount of patients (n=4) with hyperthyroidism

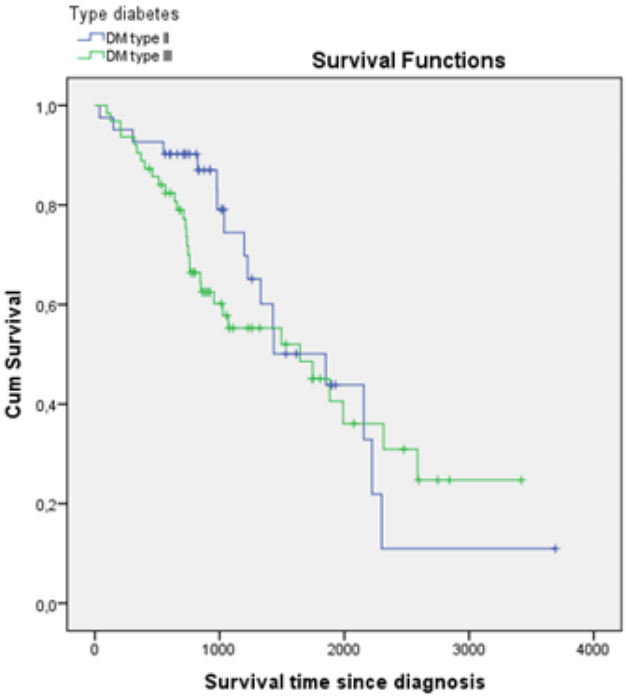
Significant differences were found between patients with and without pancreatitis with a shorter life span of patients with pancreatitis in LKMC with the following significances; Breslow; p=0.026, Tarone-ware; p=0.032. There was no significant difference between patients with or without pancreatitis in the DKMC.

Significant differences were found between ESH and breed with a shorter life span of patients belonging to a breed in DKMC with the following significances; Log rank; p=0.030, Breslow; p=0.039, Tarone-ware; p=0.035. There was no significant difference between ESH or breed in the LKMC.

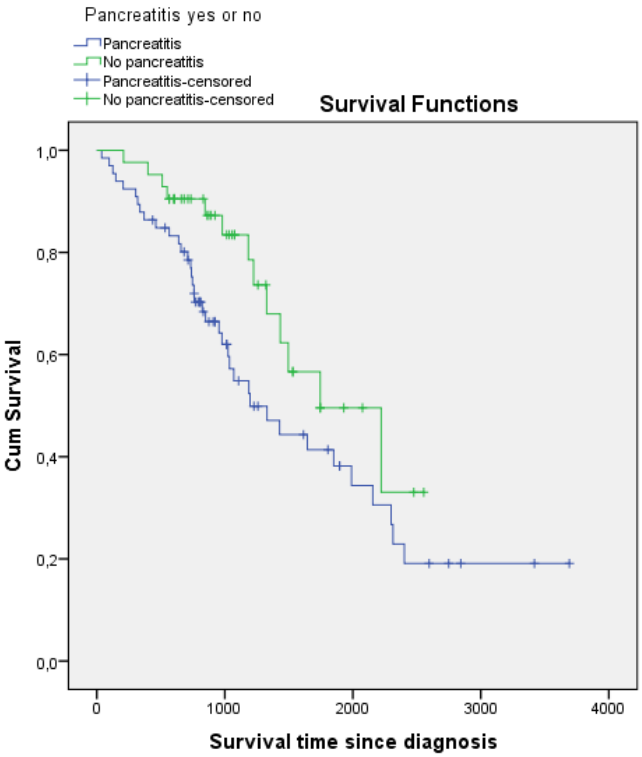
No significant differences were found between other groups.



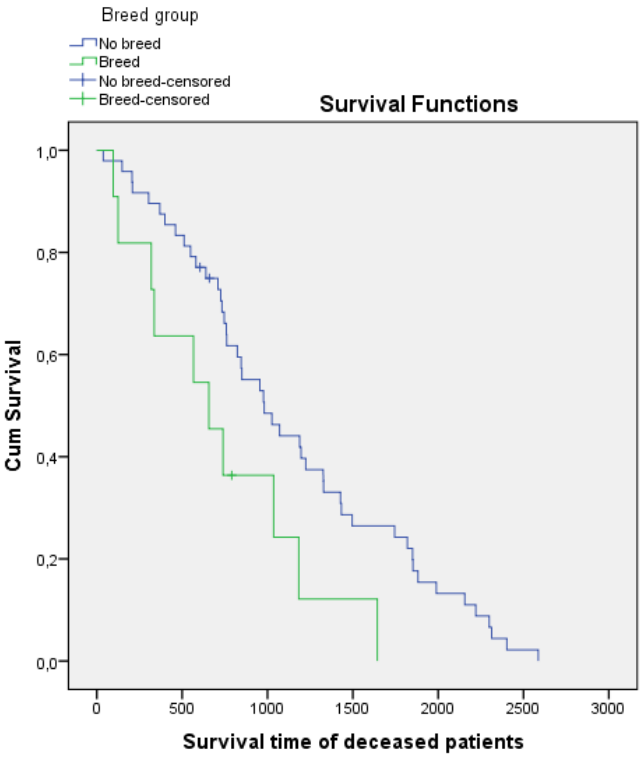
Survival of all patients



Survival of type II and type III diabetes



Survival time of patients with pancreatitis compared to patients without pancreatitis



Survival time of deceased patients for mixed breed and ESH cats

Survival time, Age at diagnosis, age at death and age at point of examination

	All	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
Age at onset	10.37	10.22	10.77	9.44	11.14	10.90	11.29	10.92	11.44
N	131	46	65	9	7	20	17	36	70
σ	3.22	3.12	3.34	3.91	2.61	3.85	2.80	3.29	3.32
Age at research	12.26	12.03	12.49	11.4	14.57	12.83	12.91	12.51	13.32
N	136	48	68	10	7	21	17	38	73
σ	3.23	3.23	3.14	3.82	1.13	3.78	2.59	2.96	2.88
Age at death	14.31	14.22	14.15	13.50	15.00	14.90	14.00	13.84	14.56
N	59	18	34	2	4	10	12	19	38
σ	2.79	3.14	2.56	3.54	1.41	2.56	2.73	2.71	3.32
Survival time since diagnosis	3.23	3.17	3.04	3.13	4.49	3.25	2.77	3.07	3.11
N	124	41	63	8	7	20	16	35	66
σ	2.01	1.87	2.04	2.32	2.45	1.73	1.74	2.00	2.17
Survival time of deceased patients	2.83	3.14	2.41	1.05	4.90	2.88	2.47	2.54	2.60
N	59	18	34	2	4	10	12	19	38
σ	1.81	1.87	1.72	0.99	2.71	1.82	1.85	1.45	1.80

	Male	Female	Breed	ESH	Male ESH	Male Breed	Female ESH	Female Breed
Age at onset	9.89	11.40	10.69	10.28	9.89	9.88	11.11	13.00
N	88	43	29	102	64	24	37	6
σ	3.20	3.10	3.05	3.28	3.21	3.23	3.22	0.89
Age at research	11.61	13.67	12.28	12.26	11.70	11.35	13.41	15.25
N	93	43	29	107	69	24	37	6
σ	3.26	2.70	3.28	3.23	3.29	3.24	2.78	1.04
Age at death	13.46	15.54	13.82	14.42	13.52	13.25	15.57	15.33
N	35	24	11	48	27	8	21	3
σ	2.68	2.52	1.83	2.97	2.93	1.75	2.68	1.16
Survival time since diagnosis	3.04	3.62	2.82	3.35	3.14	2.80	3.67	3.28
N	84	40	28	96	60	24	35	5
σ	1.83	2.32	2.07	1.99	1.74	2.07	2.36	2.24
Survival time of deceased patients	2.67	3.05	1.87	3.05	2.91	1.87	3.23	1.84
N	35	24	11	48	27	8	21	3
σ	1.69	1.99	1.30	1.85	1.73	1.33	2.02	1.50

Mean age at onset of diabetes was 10.37 years and range of onset of diabetes was 1-18 years. Maximum survival time reported was 10.12 years, however this patient was still alive at the end of this research. The shortest survival time was 38 days. Mean age of death was 14.31 years with a range of 8-20 years, however several patients were still alive at age 20.

A significant difference ($p=0.001$) between mean age at research for hyperthyroid patients in an independent t-test was found with hyperthyroid patients being older than patients without hyperthyroidism. Significant differences were also found in the independent t-test for survival time of deceased patients ($p=0.014$) with a longer survival time for hyperthyroid patients and another significant difference ($p=0.030$) was found in diagnosis – research interval which was longer in hyperthyroid patients.

There was also a significant difference ($p<0.00005$) in an independent t-test for age of onset between patients with and without pancreatitis with patients presenting with pancreatitis being older. In addition there was also a significant difference ($p<0.00005$) in the independent t-test for age at research between patients with and without pancreatitis, patients with pancreatitis were older.

There were significant differences in Age of onset ($p=0.013$), Age at research ($p<0.00005$) and age at death ($p=0.004$) in an independent t-test between males and females with females being older at all three points in life. There was a significant difference in survival time of deceased patients ($p=0.05$) in an independent t-test between breed and ESH cats with ESH cats living longer. No other significant differences were found between specific diseases, disease groups, gender groups and breed groups.

<u>Cause of death</u>

Cause of death or current status has been provided for 136 cats. 11 cats were untraceable in the system because they moved, ran away or simply could not be found in the veterinary database again. Out of the remaining 125 cats 66 were living at the end of the study and 59 were deceased. Of the 59 cases a specific cause of death could not be determined for 15 cats (listed as unknown cause) for 44 cats a cause of death has been established. Because of this wide variation direct statistics were impossible to perform, however the cause of death could be reclassified as following;

- Missing or Alive patients were excluded, as well as patients that died of unknown cause.
- Direct relation with diabetes; hypoglycemia (8), diabetes related causes (6), keto-acidosis (2), pancreatitis (1)
- Possible relation with diabetes or underlying causes; cachexia (5), kidney failure (4), epilepsy (3), collapse (2), coma (1), dyspnea (1), hematuria (1), paralysis posterior (1), urinary tract disease (1), non-healing wound (1)
- No possible relation; tumor (4), road kill accident (1), cripple (1), GI disease (1)

	All patients	Type II	Type III	Hypo-thyroidism	Hyper-thyroidism	Somogyi	Cushing	Acromegaly	Pancreatitis
COD established	44	14	25	1	4	7	8	16	29
Direct relation with DM	17	4	12	0	2	3	7	8	11
Possible relation with DM	20	9	8	1	2	2	1	4	12
No relation with DM	7	1	5	0	0	2	0	4	6

On the χ^2-tests only the difference in COD between Cushing and non-Cushing patients was significant ($P=0.011$) with 7 out of 8 Cushing patients dying of DM related causes

Discussion

Classification

Even though the classification of diabetes in cats in type II and type III can be adequate, classification on insulin dependence will not help in classifying DM in cats and renaming the diseases to avoid confusion might be a good idea. Both types might involve insulin resistance or pancreatic cell destruction and in the end many diabetes patients will remain insulin dependent for control of the disease. However it is possible to reclassify diabetes patients according to systems we have used in other diseases. If we would classify DM in primary/idiopathic DM (classic type II), secondary DM (classic type III, poly endocrinologic disorders) or complicated DM (presence of pancreatitis, kidney failure and perhaps the presence of Somogyi effect) diagnosis, treatment options and level of monitoring can be very clear. In this case the diagnosis Primary Diabetes Mellitus would be a diagnosis per exclusionem.

Primary DM (Classic type II) vs. secondary DM (Type III)

The true prevalence of DM type III was much higher than expected, and even though DM type II still remains the largest subtype of diabetes it is still possible that other causes that we have not looked for, like pheochromocytoma, were present in these animals.

In addition it is possible that the diabetes in the DM type II group was caused by pancreatitis or kidney failure in which case they should have been placed in the DM type III group. This however is impossible to determine unless patient charts are carefully checked on presence of either pancreatitis or kidney failure before the presence of DM.

One of the most surprising outcomes was that glucose and fructosamine concentrations are significantly higher in patients with DM type II. This may indicate that hypoglycemia does not play a role in patients with DM type II or that persistent hyperglycemia is present due to increased dietary intake.

It is presumable that no other significant data was found because of the extreme variability between specific subtypes of type III.

Acromegaly

In regard of acromegaly this research has shown that acromegaly might be a leading cause in the development in diabetes. Risk factors in the development of acromegaly have been found in this study and other studies and there is a higher prevalence of acromegaly in male cats and in breed cats.

A high prevalence of any disease based on male gender and breed might indicate that an X-chromosome related recessive trait might be the cause of the disease. However diagnosis of acromegaly was based on IGF-1 levels and it has been shown that GH is the hormone that causes insulin resistance and leads to diabetes. In cyclic humans it is known that 24 hour GH-secretion in women is 2-3 times higher than in men. On top of that it is known that GH-secretion is more irregular in women. The frequency of GH-bursts, the half-life of GH and the estimated GH-release have been reported to be the same in men and women. Estrogen has been suggested as causal factor for the difference in GH release.[6]

Because of this marked difference between GH and IGF-1 levels it is possible that female cats with GH-producing tumors have significantly lower IGF-1 levels while GH levels remain equal to men. This might indicate that we have to revise normal values of IGF-1 and we might have to differentiate between male and female IGF-1 reference levels.

If GH levels are influenced by estrogen it is possible that there is a distinct difference between intact and castrated females. However due to the small amount of intact animals in this study it was impossible to get significant results.

If we compare our findings with humane acromegaly we will find that IGF-1 levels in females in menopause (non-cyclic) are lower than their male counterparts even though the same levels of GH are present.[24] This relation could describe why IGF-1 levels are lower in castrated (non-cyclic) females.

If this is true then it is possible that we missed out on female cats with acromegaly causing DM because we have only checked IGF-1 levels. A follow-up research on basal IGF-1 levels between intact and castrated animals might be beneficial to determine if IGF-1 alone is a satisfactory parameter to determine the presence of acromegaly.

In this research we found that patients with acromegaly required more insulin. This might indicate that there is an increased insulin resistance when acromegaly is present. Another indication for this might be the fact that there was more pancreatitis present in patients with acromegaly. In this case the pancreatitis could have been induced by amyloid deposits because of increased insulin release caused by peripheral insulin resistance. However we did not perform pathology on any of the cases presented here so we cannot be certain if the proposed pathological pathway is correct.

It has always been presumed that acromegaly induced secondary diabetes is difficult to treat because of variable daily insulin requirements. Even though some acromegaly patients are treated adequately we cannot prove or disprove this statement because of lacking data. Glucose and fructosamine values were significantly lower in patients with acromegaly, which may actually indicate that these patients generally are more sufficiently treated than other patients. However this also indicates that cats with sufficient insulin therapy still may have acromegaly.

Life span between diabetic cats with and without acromegaly did not differ significantly on either survival analysis. The median age of onset of acromegaly induced secondary diabetes was found to be 10.92 years and the median age of death 13.84 years and a median life span of 2.93 years after diagnosis were not significantly lower than the average diabetic patient. However the difference that can be seen might be explained by the high prevalence of pancreatitis in patients with acromegaly. Pancreatitis is a known cause for decreased life span and monitoring the disease or treating acromegaly as a causal factor might increase life span and animal welfare.

Treating acromegaly has shown great results, but it is questionable if we need to treat the disease causally or rather continue treating the disease symptomatically e.g. treating the diabetes. In addition the treatment options are very expensive and these should be discussed with the owner. On the other hand diabetes can resolve after removal of the pituitary and the remaining hormonal deficit is easier to control.

The diagnosis of acromegaly in a diabetic patient might prepare the owner for a more difficult type of diabetes so even if treatment is not an option and considering the high prevalence of this disease, presumably especially among male cats, a diagnosis of acromegaly can support decisions in the future.

Acromegaly was possibly one of the most underdiagnosed causes for DM in cats. We have shown in this research that even in diabetic cats without difficult treatment, acromegaly can play a leading role and prevalence in diabetic cats may be as high as 50% in male mixed breed cats.

Cushing

Even though cortisol/creatinine ratios may be influenced by stress and possibly hyperthyroidism these ratios are still helpful in identifying possible Cushing patients. It is unknown how many true Cushing patients were presented in this study, however we have tried to minimalize the effects of stress on our research group.

15.7% of our patients presented increased cortisol/creatinine ratios and we can say that there must be cases of Cushing's disease or perhaps even double adenomas in this research because of the high prevalence of diabetes among Cushing's patients. In addition the prevalence of Cushing's in general population is thought to be rare, while these results indicate that increased cortisol/creatinine ratios in diabetic cats are very common. Cushing's and acromegaly status was known for 100 cats, with 5 cats presenting with both diseases and 43 patients presenting with either disease.

Patients with increased cortisol/creatinine ratios had a lower median survival time than patients with normalized cortisol/creatinine ratios; however this difference was not significant. It is possible that if we continue to follow these animals in the future we get better statistical results.

There was no significant difference in insulin requirement between patients with and without cortisol/creatinine ratios. However it is possible that this outcome is clouded by the presence of hyperthyroid patients and patients presenting chronic stress. Insulin requirements suggest a higher insulin requirement for patients with hyperadrenocortisism. Again just as in the acromegaly group, not much can be said about the possible difficulty in regulating Cushing's induced diabetic patients. There was a significant higher percentage of Cushing's patients that could be regulated, but still most of the cats presented with an insufficient regulated diabetes. As theory and previous research tells us Cushing's patients might be hard to regulate these patients might still benefit from early detection of Cushing's disease.

The therapy of Cushing's is expensive and results vary. Just as in acromegaly we have to wonder whether we want to treat this disease causally or symptomatically. In most cases of increased cortisol/creatinine ratios in this research cats presented without the presence of disease specific symptoms. Perhaps only patients that present with these symptoms should be treated causally as we have seen that there is no significant difference in treatment and prognosis of Cushing's induced DM compared to the other diseases. In the end it is for the owner to decide what should be done.

The diagnosis of Cushing's in a diabetic patient might prepare the owner for a more difficult type of diabetes so even if treatment is not an option and considering the high prevalence of this disease, a diagnosis of Cushing's can support decisions in the future.

Even though results remain unclear it is very plausible that the prevalence of Cushing's in diabetic patients is higher than earlier presumed. And we know that if Cushing's is present it is also presumable that this disease will influence and might lead to DM.

Hyperthyroidism

Even though hyperthyroidism is the most common endocrinologic disorder in cats affecting 1 in 300 cats we found that the prevalence among diabetic cats is higher affecting 17 in 300 cats (5.7%) supporting the idea that hyperthyroidism leads to DM.

Hyperthyroid patients were found to be older at examination suggesting a later onset of the disease and supporting the fact that diabetes was caused by hyperthyroidism in these cases. The thyroid gland was not palpable in all cases presented with hyperthyroidism. This however might have had something to do with the inexperience of the researchers at time of examination.

No gender differences or breed predisposition have been found, neither did mean T4 levels differ between male and female.

One interesting find is that in 3 out of 7 cases multiple endocrine abnormalities were found and only 3 out of 7 cases did not present with additional endocrine disorders, although one of those cases presented signs of Somogyi. In 1 case there was insufficient data to determine if any other endocrine abnormality was present. It is very plausible that the increase in all cell metabolisms can lead to metabolic and endocrine differences between hyperthyroid

diabetic and other diabetic patients. In addition previous research has shown that hyperthyroidism may lead to increased cortisol/creatinine release, but we could not support this with our findings because they were not significantly different.

Insulin requirement did not differ significantly between hyperthyroid and non-hyperthyroid patients neither did therapy sufficiency. No evidence for insulin resistance was found. This might be attributed to the fact that hyperthyroidism also leads to an increased breakdown of insulin and real insulin resistance is not the problem in most cases but rather an absolute insulin shortage. Lower glucose and fructosamin levels were found however they were not significant because of the low amount of hyperthyroid patients in this study.

There is some evidence that hyperthyroid diabetics have a significantly longer life span than non-hyperthyroid diabetics and this may also have something to do with increased general cell metabolism and perhaps even lower glucose and fructosamin values; A less severe type of diabetes.

Other findings supporting this suggestion are the longer diagnosis-examination interval. If patients with a specific disease causing DM would live longer their diagnosis-examination interval would also be considerably larger than other groups where patients diagnosed at the same time would already have been deceased.

The treatment for hyperthyroidism has had more success than the treatment for Acromegaly and Cushing. At this point it is questionable if hyperthyroidism increases the life span of diabetic cats and if life span in cats would increase or decrease after hyperthyroid treatment. As far as we can see now treating hyperthyroidism induced diabetes symptomatically has no influence on life span.

The diagnosis of hyperthyroidism in a diabetic patient might prepare the owner for a more difficult type of diabetes so even if treatment is not an option and considering the high prevalence of this disease, a diagnosis of hyperthyroidism can support decisions in the future.

The prevalence of hyperthyroidism is higher than previously expected and hyperthyroidism might be a leading cause for poly endocrinologic disorders.

Hypothyroidism/SES

Up to date hypothyroidism has only been reported in few cases in cats and most cases of low T_4 are caused by SES. By evaluating protein/albumin levels in the serum we can say that 9 out of 10 cases with hypothyroidism presented with normal albumin and protein levels indicating that the low T4 measured was not caused by an insufficiency in carrier molecules. Hypoproteinemia could be ruled out in these cases as a cause for low T4 values.

Even though not all causes of SES could be ruled out it is fair to say that there was a true low thyroxin level in nine out of ten cases. A low thyroxin level may point towards a low triiodothyronine level and a lowered metabolism which can definitely influence glucose and insulin metabolism, indifferent of the initial cause of the lowered thyroxin levels.

No significant differences were found in breed or gender suggesting that these parameters have no influence on the presence of this disease.

The mean insulin dose of the patients in the hypothyroid group was lower, but this difference was not significant. It is possible that mean insulin levels in hypothyroid patients are lower because of lowered metabolism and decreased glucose usage of cells, or that the decrease in insulin use is caused by the same causal origin of SES. Although no significant difference was found in glucose and fructosamines between hypothyroid patients and non-hypothyroid patients, the results of these parameters resemble those of patients with primary diabetes indicating a possible difference between this group and other types of secondary diabetes.

In the survival analysis a significant difference was found in the survival time of deceased patients between hypothyroid and non-hypothyroid patients indicating a clearly

decrease in survival. As the mean survival time was also decreased, although not significantly, it is possible that hypothyroid patients die sooner. However when we compared survival time including living patients we could see hypothyroid patients live a little longer. This may indicate that in the early phase of the disease patients may die sooner and in a later phase the disease stabilises.

If there is a hypothyroidism induced DM, treatment of hypothyroidism may be easier and may cost less than treating the DM on its own. Also treating hypothyroidism in the early phase may lead to an increased life span.

If we take the hypothyroid and hyperthyroid group together this may indicate that 13.9 % of diabetes in cats is caused by thyroid disturbances. In other words one in seven cats with diabetes might have a disturbance in thyroid hormone balance, rendering T_4 to be an important test in diabetic cats with and without insulin resistance.

Hypothyroidism may be another cause for diabetes and might not be as rare as we think it is in cats.

Somogyi/hypoglycaemia

The Somogyi effect in the cat may be a result of remission, but can also be caused by iatrogenic hyperinsulinemia. Iatrogenic hyperinsulinemia can be present because of known or unknown deviations and insufficient knowledge of the insulin protocols.

Evidence for the presence of the Somogyi effect was found in 17.2% of our patient group (one in six cats) and this high prevalence is worrying because most cases of Somogyi effect can be prevented by using the right insulin protocols and good monitoring of diabetic patients. In addition the true prevalence may exceed 17.2% because we only measured glucose at one point and we could have measured these patients glucose at any point in their glucose curve. However glucose concentrations could also have been lowered during the time between sample collection and sample storage leading to false positive Somogyi patients.

No breed or gender predisposition was found for the presence of the Somogyi effect. Insulin requirement was not found to be different from non-Somogyi patients indicating that the Somogyi effect can be present, even if low, normal or high amounts of insulin are given.

Significant lower values of glucose and fructosamin were found in our Somogyi group. Glucose was low because patients were selected on glucose, but mean fructosamin was also lower indicating that normalized fructosamin levels might also be indicatory for hyperinsulinemia.[8]

Hypoglycemia was found to be the leading cause of death in diabetic patients. It can be expected that hypoglycemia is always present in Somogyi patients and this would lead us to think that Somogyi patients would have a decreased life span. However if we compare life span between Somogyi and non-Somogyi patients, no significant difference can be found.

Although Somogyi might not lead directly to a decrease in life span, it should be said that many cases of Somogyi are preventable if adequate therapy and therapy monitoring is complied. Deviations of therapy and monitoring may still eventually lead to direct and unnecessary death of the patient.

Pancreatitis

The presence of pancreatitis based on clinical parameters was six in every ten cats, which is very high. Because of the lack of pathology reports true prevalence could not be determined, but earlier reports have also shown comparably high prevalence of this disease. Based on fPLI alone 69 out of 119 patients (58%) had increased fPLI levels.

No difference in the presence of pancreatitis in breed and gender could be found, neither was there a significant difference in insulin therapy and therapy sufficiency.

As stated before, an increased prevalence of pancreatitis was found in patients with acromegaly.

A significant decrease in life span was found when comparing patients with and without pancreatitis. Patients presented with pancreatitis tend to die at an earlier age. Median survival time did not differ significantly and patients with pancreatitis were diagnosed at a significantly older age and were significantly older at time of examination. These results may indicate that pancreatitis occurs at later age and may be another cause in the development of DM.

Pancreatitis may be the leading complication in patients with DM, it occurs at later age and may eventually influence life span. The treatment of pancreatitis might be challenging but treating the pancreatitis might eventually lead to improvement of quality and length of life.

Gender & breed

Significant differences have been found in gender & breed in patients with acromegaly. As reported above it remains questionable whether acromegaly really differs between male and female as the main parameter used to determine if a patient has acromegaly might differ between males and females.

Significant differences were found in lifespan between breed and ESH cats, with ESH cats living longer. No significant difference was found in lifespan after diagnosis between male and female, however females were diagnosed at a later age and also died at a later age.

Conclusion

The prevalence of underlying causes for DM in cats is very high, affecting six in every ten cats. Even though the treatment of these underlying causes can be challenging and might be very expensive it will be good to know whether these diseases are present or not. Underlying causes can affect the patients survival time.

Acromegaly is the most common underlying cause for DM affecting three in every ten cats and probably affects male cats more than female cats. Cats of mixed breed are also more affected. Cushing disease may affect one in every six cats, however results were not very clear in this research and additional research may be required. Thyroid disturbances were present in one in every seven cats. Thyroid disturbances are easier to track down and easier to treat than diseases involving the pituitary gland. 37% of our patients are expected to have acromegaly and/or Cushing and represent the population of diabetic cats with a possible tumor in the pituitary. 5% of our patients are suspected of a possible double pituitary adenoma for presenting with both acromegaly and Cushing.

At least one in every six cats presented with hypoglycemia, which was most likely due to the Somogyi effect as a result of lack of adequate therapy.

Pancreatitis decreases life expectancy in diabetic cats and was present in six in every ten cats. Pancreatitis however can be treated and early detection of this disease may result in better life expectancy of cats with diabetes.

In the end life expectancy of all diabetic cats does not differ much from other indoor and certainly outdoor cats. The disease is treatable and following protocol alongside with adequate monitoring will ensure a normal life span. Even though treatment ensures a normal lifespan, hypoglycemic insults are still the leading cause of death in diabetic cats.

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Appendix 1; Reference values for determined parameters

Temperature	Pulse frequency	Heart rate	Respiratory rate	Ht L/L	MCH fmol	MCHC g/dL	MCV fl
38.0 - 39.0	120-180	120-180	20-40	0,28-0,47	0,71 - 1,07	16,3 - 22,3	37,0 - 55,0

leucocyte 10^9/L	segmented 10^9/L	stick 10^9/L	eosinophil 10^9/L	basophil 10^9/L	monocyte 10^9/L	lymphocyte 10^9/L	Lymphoblast 10^9/L
6,3 - 19,6	3,0 - 13,4	0,0 - 0,1	0,3 - 1,7	0,0 - 0,1	0,0 - 1,0	2,0 - 7,2	< 0,0

Bilirubin umol/L	Glucose mmol/L	Fructosamine umol/L	urea mmol/L	Creatinine umol/L	Protein g/L	Albumin g/L	Cholesterol mmol/L
<0,3	4,0 - 9,0	202-299	7,4-12,6	98 - 163	64-80	30-40	2,6 - 6,8

Trigliceride mmol/L	Alkaline Phosphatase U/L	Amylase U/L	Lipase U/L	ASAT (GOT) U/L	ALAT (GPT) U/L
0,3 - 1,3	16 - 43	700 - 1538	8 - 26	19 - 44	34 - 98

Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Phosphorous mmol/L
158 - 165	3,8 - 5,4	121 - 131	2,4 - 2,8	0,9 - 1,8

urine specific gravity	pH (Urine)	protein (urine) g/L	glucose (urine)	ketone (urine)	cortisol/ creatinine quotient	Protein/ creatinine quotient
>1,05	6	< 0,56	-	-	< 42,0	< 1

T4 (total) basal mcg/dL	fPLI ug/L	fTLI ug/L	Folate (Vit B9) ug/L	Cobalamine (Vit B12) ng/L	IGF-1 ng/mL
1-3,5	<3,5 <5,3	12,0 - 82,0	8,9 - 19,9	276-1405	200-800

Appendix 2; Insulin therapy and therapy monitoring protocol

Food therapy;

- Cats without concurrent disease require a diet low on carbohydrates
    - Examples are Royal Canin diabetic or M/D from Hills.

Caninsulin therapy;

- Start dosage
    - Should not be higher than 2 IE/dose
    - If glucose is >20 mmol/L → 0.5 IE/kg/dose
    - If glucose is =< 20 mmol/L → 0.25 IE/kg/dose
    - Should be given twice daily
- Increments in dosage
    - Dosage should remain stable in the first 2-3 weeks unless hypoglycaemia presents
    - Should be increased weekly with a maximum of 0.5 IE/dose as long as symptoms of Pu/Pd are still present or as long as blood values indicate persistent hyperglycaemia.
    - Insulin dose should remain stable for 6-7 days before increasing insulin dosage each time.
- Therapy administration
    - Special insulin syringes have been produced for cats in which administering dosages of 0.5 IE is not a problem any more.

Diagnosis & Therapy monitoring;

- Diagnosis of diabetes mellitus should be based on high glucose and high fructosamin levels. Reference values vary among research labs but glucose levels above 15 mmol/L and in addition fructosamin levels above 350 µmol/L are indicative of the presence of DM.
- Daily glucose curve
    - Should have been performed at least once to determine the time of low nadir of the glucose curve
        - Should preferably be performed at home
    - Do not perform more than once a month
    - Should be performed every 3-6 months
- Blood glucose
    - Should be monitored at least twice a week
    - Can be checked daily at the time of low nadir by the owner
    - Pre-insulin glucose values may be required when using Lantus insulin
    - Should be checked around the suspected low nadir of the blood glucose level
- Fructosamin and urinary glucose
    - Can be checked up to bimonthly to determine if insulin therapy is satisfactory.
- Hypoglycaemia
    - In case of hypoglycaemia decrease insulin dosage with 50% next dose and continue from that level.
- Stable diabetic control
    - In case of stable diabetic control insulin therapy should be checked every 3 months by determining serum glucose and fructosamin levels.