Feline diabetes mellitus

Aspects on epidemiology and pathogenesis

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Feline diabetes mellitus. Aspects on epidemiology and pathogenesis

Abstract

Feline diabetes mellitus (DM) is strikingly similar to human type 2 diabetes. Cats and humans share many risk factors for the disease, including an association with insulin resistance coupled to obesity and a sedentary lifestyle. There are also pathophysiological resemblances, with β-cell loss and amyloid deposition in the islets of Langerhans in the pancreas. In people, ethnicity has been associated with an increased risk of DM, and in cats, a breed predisposition has been identified, with the Burmese breed at increased risk. The aims of this thesis were to identify risk factors for DM in cats and to increase understanding of disease pathogenesis.

We used insurance data from Agria Pet Insurance to determine the general incidence of DM in Swedish cats, and the incidence in relation to age, sex and breed. We found that incidence rates peaked when cats were 13 years old. Male cats developed DM twice as often as female cats. The Burmese breed, along with the Russian Blue, Abyssinian, and Norwegian Forest cat breeds, showed an increased risk of DM, while several breeds showed a lower risk. Owners of diabetic and non-diabetic cats from the same cohort were invited to participate in a web survey, with questions on *e.g.* type of diet, feeding regime, and activity levels. We found associations between overweight and an increased risk of DM. Access to the outdoors was protective. There was an increased risk of DM in cats eating predominantly dry food, compared to wet food, in the group of cats considered normal-weight by their owners. Since overweight was shown to be a strong risk factor for DM, we studied if factors associated with DM were also associated with risk of overweight. Several shared risk factors for DM and overweight were found, such as eating predominantly dry food, being male, and considered being a greedy eater. Cats from Birman and Persian breeds, with a decreased risk of DM, were less often overweight. Finally, we studied the metabolism in the Burmese, Birman, and Maine coon breeds, using nuclear magnetic resonance (NMR) spectroscopy and hormone immunoassays. There were differences in the metabolic profiles between breeds, with patterns of metabolites in the Burmese cats resembling patterns seen with insulin resistance in people.

In conclusion, both new and previously reported factors associated with DM and overweight in cats were identified in this thesis. Knowledge of predisposing factors can help owners and veterinarians to identify cats at risk, and enables prevention of disease.

Keywords: Burmese, cat, dry food, epidemiology, *Felis catus*, lifestyle, metabolomics, overweight, obesity, type 2 diabetes.

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Diabetes mellitus hos katt. Aspekter på epidemiologi och patogenes

Sammanfattning

Diabetes mellitus (DM) är en endokrin sjukdom hos katt, som är slående lik typ 2 diabetes hos människa. Katter och människor delar många riskfaktorer för sjukdomen, inklusive ett samband med insulinresistens kopplat till övervikt och en stillasittande livsstil. Det finns också patofysiologiska likheter, med förlust av β-celler och inlagring av amyloid i Langerhans öar i bukspottkörteln. En raspredisposition har setts hos katt, med en förhöjd risk för rasen burma, vilket liknar situationen hos människa där etnicitet har visat sig ha ett samband med en ökad risk för DM. Övergripande syften med avhandlingen var att identifiera riskfaktorer för DM hos katt och öka förståelsen för sjukdomens uppkomst.

Vi använde försäkringsdata från Agria djurförsäkring för att fastställa den generella incidensen av DM hos svenska katter, samt incidensen i relation till ålder, kön och ras. Vi fann att incidensen var som högst för katter vid 13 års ålder. Hankatter drabbades av DM dubbelt så ofta som honkatter. Burma, tillsammans med raserna russian blue, abessinier och norsk skogkatt, visade en ökad risk för DM, medan flera andra raser istället visade en minskad risk. Vi bjöd in ägare till katter med och utan DM att delta i en enkät-undersökning, med frågor om bland annat fodertyp, utfodring och aktivitetsnivå. Vi fann ett samband mellan övervikt och en ökad risk för DM. Tillgång till utevistelse skyddade däremot mot DM. I gruppen katter som bedömts som normalviktiga av sina ägare påvisades en ökad risk för DM hos katter som åt mestadels torrfoder jämfört med katter som åt mestadels blötmat. Eftersom övervikt visade sig vara en stark riskfaktor för DM undersökte vi om faktorer som visat samband med DM också hade ett samband med övervikt. Vi fann flera gemensamma riskfaktorer för DM och övervikt, som att äta mestadels torrfoder, vara av hankön, och att vara glupsk. Raserna birma och perser, med en nedsatt risk för DM, var mindre ofta överviktiga. Slutligen studerade vi metabolismen hos katter av raserna burma, birma och maine coon med NMR-spektroskopi och hormonanalyser. Den metabola profilen skilde mellan raserna, med ett mönster av metaboliter hos burmorna som liknar det som ses hos människor med insulinresistens.

Sammanfattningsvis har både nya och tidigare kända riskfaktorer för DM och övervikt hos katt identifierats i avhandlingen. Kunskap om predisponerande faktorer kan hjälpa ägare och veterinärer att identifiera katter med en förhöjd risk, och ge möjlighet att förebygga sjukdom.

Nyckelord: Burma, epidemiologi, *Felis catus*, fetma, livsstil, metabolomik, torrfoder, typ 2-diabetes, övervikt.

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Dedication

To my boys

Jag vet inte vad som var värst. Att alla alltid hade fel eller att jag alltid hade rätt.

Villfarelser

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Öhlund M*, Fall T, Holst BS, Hansson-Hamlin H, Bonnett B and Egenvall A (2015). Incidence of Diabetes Mellitus in Insured Swedish Cats in relation to Age, Breed and Sex. *Journal of Veterinary Internal Medicine*, 29 (5), pp. 1342–1347.

II Öhlund M*, Egenvall A, Fall T, Hansson-Hamlin H, Röcklinsberg H and Holst BS (2017). Environmental Risk Factors for Diabetes Mellitus in Cats. *Journal of Veterinary Internal Medicine*, 31 (1), pp. 29–35.

III Öhlund M, Palmgren M and Holst BS. Overweight in Adult Cats: A Cross-Sectional Study (submitted).

IV Öhlund M, Müllner E, Moazzami A, Hermansson U, Pettersson A, Häggström J, Hansson-Hamlin H and Holst BS. Characterization of the feline metabolic profile reveals differences between breeds (manuscript).

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The contribution of Malin Öhlund to the papers included in this thesis was as follows:

I Malin Öhlund and Agneta Egenvall performed the statistical analyses. Malin Öhlund drafted the manuscript. All authors contributed to the design of the study, interpretation of the results, and revision of the final manuscript.

II Malin Öhlund created the questionnaire, compiled the results, performed the statistical analyses, and drafted the manuscript. All authors contributed to the design of the study, interpretation of the results, and revision of the final manuscript.

III Malin Öhlund and Malin Palmgren collected the data. Malin Öhlund conducted the analyses and drafted the manuscript. All authors contributed to the design of the study, interpretation of the results, and revision of the final manuscript.

IV Malin Öhlund and Ulrika Hermansson collected the data. Malin Öhlund, Elisabeth Müllner and Ali Moazzami performed the statistical analyses of the data. Malin Öhlund drafted the manuscript. All authors contributed to the design of the study, interpretation of results, and revision of the final manuscript.

Related publication not included in the thesis

- Öhlund M, Franzén P, Andersson G, Holst BS and Lau J (2014). Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats. *Journal of Gastroenterology, Pancreatology & Liver Disorders*, 1 (4), pp. 1-9.

Abbreviations

AIC	Akaike information criterion
ALAT	Alanine aminotransferase
BCS	Body condition score
BMI	Body mass index
CI	Confidence interval
CYAR	Cat-years at risk
DAG	Directed acyclic graph
DEXA	Dual energy X-ray absorptiometry
DM	Diabetes mellitus
DPP-4	Dipeptidyl peptidase-4
ELISA	Enzyme-linked immunosorbent assay
FFA	Free fatty acids
GLP-1	Glucagon-like peptide-1
HDL	High-density lipoprotein
IAPP	Islet amyloid polypeptide
IDDM	Insulin-dependent diabetes mellitus
IGF-I	Insulin-like growth factor-I
IR	Incidence rate
IRR	Incidence rate ratio
LC-MS	Liquid chromatography mass spectrometry
MC4R	Melanocortin-4 receptor
MCO	Maine coon
NIDDM	Noninsulin-dependent diabetes mellitus
NMR	Nuclear magnetic resonance
OR	Odds ratio
PLS-DA	Partial least squares discriminant analysis
SLU	Sveriges lantbruksuniversitet, Swedish University of Agricultural Sciences

SVA	Statens veterinärmedicinska anstalt, National Veterinary Institute
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
UDS	Universitetsdjursjukhuset, University Animal Hospital
VLDL	Very low-density lipoprotein
WHO	World Health Organization

1 Introduction

Diabetes mellitus (DM) is a disease with a worldwide increasing prevalence in people, as a consequence of the obesity epidemic encountered in many parts of the world (Chan *et al.*, 2009; Chen *et al.*, 2012). In 2015, the International Diabetes Federation estimated that more than 452 million people suffered from DM, and it was postulated that by 2040, one in ten adults will have developed DM. Diabetes mellitus is considered to be a disease with an increasing prevalence also in domestic cats (Prahl *et al.*, 2007).

There are several types of DM in people, of which type 1 and type 2 are the most prevalent. Type 1 DM (T1DM) is generally thought to be caused by an immune-associated destruction of insulin-producing pancreatic β-cells, leading to absolute insulin deficiency (Atkinson *et al.*, 2014). Type 2 DM (T2DM) on the other hand, is characterised by a relative insulin deficiency caused by pancreatic β-cell dysfunction coupled with insulin resistance in target organs such as muscle and adipose tissue (Chatterjee *et al.*, 2017). Type 2 DM is by far the most common type of DM in people, accounting for approximately 90% of all cases (Chen *et al.*, 2012).

Diabetic cats most frequently suffer from a type of DM strikingly similar to T2DM in people (Rand, 1999; O'Brien, 2002). In fact, cats are the only known non-primate species to develop spontaneous DM that is similar to human T2DM (Verkest & Bjornvad, 2012). Cats and people also share risk factors for DM, such as obesity and a sedentary lifestyle, and have similar pathophysiological changes within the pancreas, with β-cell loss and amyloid deposition (Rand *et al.*, 2004; Osto *et al.*, 2013). Naturally occurring amyloid deposition is a feature so far only encountered in cats and primates, and not in other animals (Osto & Lutz, 2015).

Obesity is a major risk factor for DM in cats and people, and the risk factors for DM are similar to the risk factors for obesity. Therefore, risk factors for both DM and obesity will be acknowledged and discussed in this thesis.

1.1 History

1.1.1 Diabetes mellitus in people

Diabetes mellitus was described as a disease in humans with "too great emptying of the urine" already in 1500 BC in Egypt. The first described cases were most likely to have suffered from T1DM. Diabetes as a term has been recognised from around 250 BC in Greece. *Diabetes* means "to pass through", and refers to the increased urination encountered in diabetic patients. The term *mellitus* means "from honey" and was added by Thomas Willis in the 17th century to describe the sweetness of the urine, and to distinguish DM from *diabetes insipidus*, another disease with similar clinical signs, but related to the hormone vasopressin (antidiuretic hormone) and unrelated to DM (Zajac *et al.*, 2010; Karamanou *et al.*, 2016). However, the observation that urine from diabetic patients attracts flies was noted by physicians in India, already at around 1500 BC. The separation of the diabetic patients into two groups; today's type 1 and 2 DM, was observed by Indian physicians Sushruta and Charaka in the fifth century AD, who associated the disease with youth and obesity, respectively (Zajac *et al.*, 2010).

The prognosis for the disease was grave for over three millennia, with an invariably fatal outcome. Aretaeus of Cappadocia, a Greek physicist, wrote in the second century AD (Zajac *et al.*, 2010):

> Diabetes is a dreadful affliction, not very frequent among men, being a melting down of the flesh and limbs into urine. The patients never stop making water and the flow is incessant, like the opening of the aqueducts. Life is short, unpleasant and painful, thirst unquenchable, drinking excessive and disproportionate to the large quantity of urine, for yet more urine is passed. . . . If for a while they abstain from drinking, their mouths become parched and their bodies dry; the viscera seem scorched up, the patients are affected by nausea, restlessness and a burning thirst, and within a short time they expire.

Before the discovery of insulin by Banting and colleagues in the 20th century (Banting & Best, 1922; Banting *et al.*, 1923; Banting *et al.*, 1991), DM was mainly treated with different sorts of diets. John Rollo, a surgeon general in the British army, developed a low-carbohydrate diet for patients with DM (Anderson, 1965). Others, like the leading American diabetologist of the time Frederick Allen, suggested that limiting of food supply would improve the disease.

Other significant scientific advances preceded the discovery of insulin. In 1869, the German pathologist Paul Langerhans described the tiny islets of pancreatic tissue that were later suggested by the French scientist Gustave-

Edouard Laguesse to be the source of a substance involved in blood glucose control. The Belgian physician Jean de Mayer named this substance "insulin" in 1909 (Zajac *et al.*, 2010).

In 1922, Frederick Grant Banting and his student Charles Best (Figure 1), treated the first human DM patient, a 14-year old boy, with a pancreatic extract, after first successfully treating a pancreatectomised diabetic dog with the same substance. The pancreatic duct in dogs was ligated, causing the pancreas to atrophy, after which the degenerated pancreas with the remaining islets was harvested, in order to produce the extract (Banting *et al.*, 1922). As a result of this treatment, the boy survived an additional 13 years. Another boy, one of the first to receive insulin treatment in 1922, survived for more than 70 years with insulin therapy. Banting was awarded the Nobel Prize in October 1923, together with John J.R. MacLeod, a professor at the University of Toronto where Banting performed his experiments.

Figure 1. Sir Frederick Banting (right) and Dr Charles Best, co-discoverers of insulin, with the dog Marjory (depancreatised and treated with insulin), 1921. Wellcome Library, London.

Throughout the 20th century, DM was classified based on clinical manifestations as juvenile or adult. In 1979, diabetic patients were classified mainly into two groups by the International Diabetes Data Group. The insulin-dependent type of DM (IDDM), was considered a distinct subclass, as was the noninsulin-dependent type (NIDDM). At the end of the century, this terminology was slowly replaced by type 1 and 2 DM, and the terms IDDM and NIDDM were abandoned (Gilor *et al.*, 2016a).

1.1.2 Diabetes mellitus in cats

When searching for "diabetes AND cat" in biomedical databases such as PubMed, Scopus, and Web of Science; only reports of experimentally induced DM in cats are encountered prior to the 1950's (Homans, 1914; Long & Lukens, 1936; Dohan & Lukens, 1947). A suspected case of DM in a laboratory cat is briefly mentioned in a footnote in a paper from 1916 (Mettam & Craig, 1916). In a report on nine cases of DM (eight dogs and one cat) from 1951, the authors describe a spontaneous case of DM in a Persian cat (Schlotthauer & Millar, 1951).

It appears that the earliest reports of feline DM concerned cases with concurrent pancreatic disease, such as a large adenoma replacing the pancreatic tissue, or chronic pancreatitis (Bloom 1937; Holzworth & Coffin, 1953), and not the obesity-related disease most often encountered today.

Joan Joshua described the disease in cats in a review from 1963, stating that most of the cases seen were aged neutered male cats, and that according to her experience, DM was not necessarily associated with obesity (Joshua, 1963).

Contrary to today's situation, in 1986, DM in cats was still considered to be less common than in dogs (Stogdale, 1986; Rios & Ward, 2008a). The association of DM in cats with advancing age and obesity was starting to be noticed, but in the 1990's, cats were still mainly classified as having IDDM, although scientists commented on the fact that the cats' insulin secretion in response to glucose tolerance testing rather suggested resemblance with NIDDM (Nelson & Lewis, 1990; Wallace & Kirk, 1990). Others however, started using the T2DM classification we use today at this time point (Wolfsheimer, 1990; Lutz & Rand, 1993; Lutz & Rand, 1995).

1.1.3 Overweight and obesity in people and cats

Chronic food shortage and malnutrition have been a curse for mankind from the dawn of history. During prehistoric times, natural selection rewarded a "thrifty" genotype, where an ability to store fat was beneficial for survival. In times of nutrient shortage; excess weight has also been a symbol of wealth, represented here by the figurine Venus of Willendorf (Figure 2), an icon of fertility from 25,000 BC.

Figure 2. The Venus of Willendorf, by user Matthias Kabel, CC-BY-SA-3.0, via Wikimedia Commons.

The current worldwide epidemic of obesity, now recognised as a public health crisis is however just a few decades old. Only after the technological advances of the 19th century did food supply gradually increase in the industrialised world. The initial effects on public health were beneficial, with increased longevity and body size (Eknoyan, 2006).

However, the overabundance of food coupled with reduced physical activity, has resulted in the dramatic increase in overweight and obesity seen in the last decades. Overweight and obesity are today important health problems and considered a growing global

epidemic, in both people and animals (Kopelman, 2000; Flegal *et al.*, 2002; Eknoyan, 2006; German, 2006).

The prevalence of obesity among US adults increased from 13% in the early 1960's, to 31% at the turn of the millennium. Most investigators agree that the prevalence of obesity is also increasing in cats. In a report from 1974, the prevalence of obesity in cats was only 6–12 % (Anderson, 1973). Later reports have shown higher prevalences, up to 63% in some cat populations (Cave *et al.*, 2012).

In people, one way to classify overweight or obesity in adults is by calculating the body mass index (BMI). The BMI is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). Overweight is defined as having a BMI greater than or equal to 25 kg/m^2 and below 30 kg/m^2, and obesity occurs when the BMI is greater than or equal to 30 kg/m^2 (Guh *et al.*, 2009). The BMI measurement however, does not discriminate between excess body fat and increased muscle mass. Other techniques, all with their own shortcomings, such as dual energy X-ray absorptiometry (DEXA) (Prentice & Jebb, 2001), bioelectrical impedance (Meeuwsen *et al.*, 2010), and skin-fold thickness (Freedman *et al.*, 2009) measurements can also be used.

In cats, obesity can be assessed subjectively, for example using a body condition score (BCS) system, or objectively using DEXA or magnetic resonance imaging (Laflamme, 1997; Hoenig, 2012). In clinical practice, the objective ways to evaluate body condition in cats are not usually performed for practical reasons. The BMI system has also been used in cats and can be calculated according to the following formula, but it has not gained wide acceptance in general veterinary practice or research (Bjornvad *et al.*, 2011; Nelson *et al.*, 1990; Hoenig, 2012):

$$\text{BMI} = \text{body weight (kg)} / [\text{body length (m)} \times \text{height (m)}]$$

The 5- or 9-grade scale BCS systems provide a semi-quantitative assessment of the cat's body condition that is easy to use in a clinical setting, and the systems have been validated and shown to be repeatable both within and between scorers (Laflamme, 1997; German *et al.*, 2006; Baldwin *et al.*, 2010).

1.2 Obesity and diabetes mellitus in cats and people today

In 2014, more than 1.9 billion adults were overweight, and more than half a billion were obese, according to the World Health Organization (WHO) (Figure 3).

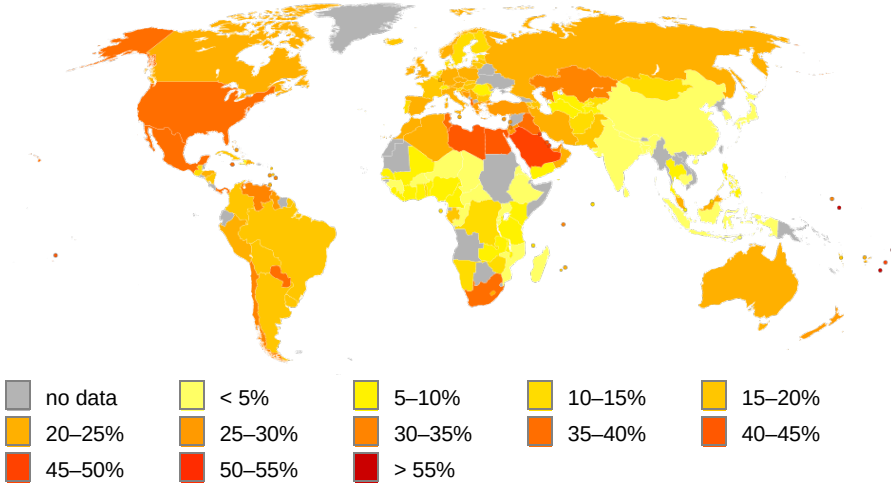

Figure 3. Map of obesity prevalence in adult females (% with a BMI ≥ 30) per country (2016). Data from WHO, map by user Lukepryke (own work), CC BY-SA 4.0, via Wikimedia Commons.

The prevalence of obesity has more than doubled since 1980, and at least 2.8 million people each year die as a result of being overweight or obese. About 422 million people today suffer from DM, and the prevalence has been steadily increasing during the last decades, mirroring the increasing prevalence of overweight and obesity. In 2012, DM was the direct cause of 1.5 million deaths.

Today, similar to the situation in humans, 80–95% of all diabetic cats are believed to suffer from T2DM associated with insulin resistance, with obesity as the most prominent risk factor (Rand *et al.*, 2004; Chen *et al.*, 2012). The prevalence of overweight and obesity among cats today differs between populations, but recent reports from countries like the United States, United Kingdom, the Netherlands, and New Zeeland show that between one and two thirds of included cats were either overweight or obese (Lund *et al.*, 2005; Courcier *et al.*, 2010; Cave *et al.*, 2012; Corbee, 2014). The prevalence and incidence of feline DM is more difficult to measure. The prevalence will differ depending on the population examined. For example, the prevalence of DM in a cohort of insured cats in the United Kingdom was found to be 1 in 230 cats, or 0.43% (McCann *et al.*, 2007). Others have reported animal hospital prevalence or clinical laboratory prevalence from 1 in 179 (0.6%) to 1 in 98 cats (1.0%) (Rand *et al.*, 1997; Baral *et al.*, 2003). The current prevalence and incidence of feline DM are unknown.

1.3 Measuring disease frequency

1.3.1 Prevalence and incidence

When describing how often a disease or another trait occurs in a population, different measures can be used. *Prevalence* describes the number of existing cases, and is affected by a number of mechanisms, for example length of disease time (Jager *et al.*, 2007). If the disease investigated is lethal and untreatable, like DM before the discovery of insulin, few prevalent cases will exist because of the short lifespan. On the other hand, if an effective treatment is discovered and used, patients will survive, and the number of prevalent cases will therefore increase. The incidence, another measure of disease occurrence, can however remain unaffected. *Incidence*, in contrast to prevalence, measures the number of new cases arising in a population over a given period of time. The difference between prevalence and incidence can thus be summarised as "how many individuals have this disease right now?" and "how many individuals per year acquire this disease?", respectively (Rothman, 2012). A schematic drawing of the concepts incidence and prevalence is shown in Figure 4.

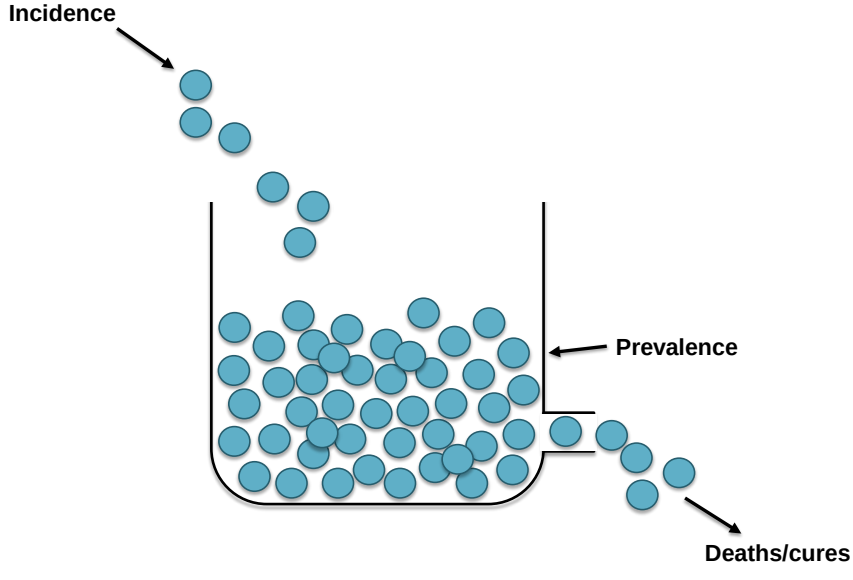

Figure 4. Schematic drawing of the concepts incidence and prevalence, with incidence as "all new cases" and prevalence as "all existing cases", where prevalence is affected by both disease length and survival.

To assess incidence, the population at risk needs to be known. Incidence is often expressed as the incidence rate (IR), which is the number of new cases divided by the person-time at risk. When calculating the IR, each individual at risk of contracting the disease contributes with person-time at risk. Incidence is usually more useful than prevalence in understanding the disease aetiology. If the incidence increases in a population, there is probably an emerging new risk factor that promotes the disease. Prevalence is more important as a measure of the burden of the disease on society, with no regard to time at risk or when individuals may have been exposed to a possible risk factor (Rothman, 2012).

Studies on DM and obesity frequency in cats have mainly been reporting prevalence (Panciera *et al.*, 1990; Sloth, 1992; Scarlett *et al.*, 1994; Rand *et al.*, 1997; McCann *et al.*, 2007; Prahl *et al.*, 2007; Colliard *et al.*, 2009; Lederer *et al.*, 2009; Courcier *et al.*, 2010; Cave *et al.*, 2012; Sallander *et al.*, 2012). The reason for this is probably a lack of information on the population at risk which is necessary to estimate the incidence. It has been generally accepted that the prevalence of overweight, obesity, and DM have increased during the last decades, even though there are relatively few studies supporting this allegation. Therefore, more studies are needed, and especially studies on incidence would be beneficial.

1.4 Risk factors for overweight, obesity, and diabetes mellitus

The exact aetiology of T2DM is unknown in both people and cats. The disease is characterised by a combination of an insulin secretory defect and insulin resistance (Kahn, 2003; Scott-Moncrieff, 2010). Insulin resistance is a condition where cells in the body fail to respond normally to the hormone insulin. Insulin resistance is an important component of the pathogenesis of T2DM in both people and cats. With insulin resistance, if the pancreatic β-cells are unable to produce sufficient amounts of insulin to maintain normal blood sugar levels; DM develops (Shulman, 2000; Kahn, 2003; Scott-Moncrieff, 2010). A number of factors associated with an increased risk of overweight, obesity and DM have been identified in both people and cats.

In epidemiology, a risk factor is defined as a variable associated with an increased risk of disease. Risk factors are not necessarily causal, because association or correlation does not prove causation which is important to bear in mind when interpreting results from such studies. The concepts of bias and confounding are also important to recognise when carrying out epidemiological studies, as they can influence the results. Bias in an epidemiological study may be defined as any systematic error that results in an incorrect estimate of the

association between exposure and risk of disease. Bias can for example result from a non-representative sampling of a population, or relate to facts such as a tendency to more easily remember events preceding a disease occurrence, *e.g.* a recall bias. For example, owners of diabetic cats may search their memories more thoroughly than owners of unaffected control cats to try to recall exposure to factors that they believe might be associated with the disease. Confounding on the other hand, occurs when the effects of two associated exposures or factors have not been identified and separated, and can result in the interpretation that the effect is due to one variable rather than the other. A classic example is the strong association seen between ice cream sales and the number of shark attacks. The confounding variable here is temperature. Warmer temperatures cause ice cream sales to go up, and also bring more people to the beach, increasing the risk of shark attacks.

Risk factors for a disease can be categorised as either modifiable or non-modifiable. Type of diet and activity level represent modifiable, or environmental, factors. Non-modifiable factors cannot be changed in the individual, and include factors such as age, ethnicity, and genetic susceptibility. They are also referred to as demographic factors. The word demography originates from the Greek words *dêmos*, "the people", and *graphô*, meaning "description of". Both modifiable and non-modifiable risk factors contribute to an increased risk for DM in people, as well as in cats, although the genetic predisposition to DM in cats is still unexplored. In people, most of the identified genetic risk loci are related to β-cell dysfunction rather than to insulin resistance or obesity (Florez, 2008; Dupuis *et al.*, 2010; Herder & Roden, 2011). However, in both cats and people, overweight and obesity are the most important predictors of T2DM (Panciera *et al.*, 1990; Chen *et al.*, 2012).

Another aspect to consider is the relationship between time points of the investigated factors with the outcome, *i.e.* the disease of interest. To use the word risk factor, the event must precede disease onset. In an observational cross-sectional study design, data on both explanatory factors and outcome are measured simultaneously at a specific point in time, and it is not possible to make a distinction between which variable is the cause and which is the effect. Only associations between the investigated variables and the effect can thus be measured.

Another type of observational study is the case-control study. Two groups differing in outcome are compared to identify factors that may contribute to disease development. In case-control studies, subjects who have the disease ("cases") are compared with those who do not have the disease ("controls"). Even though the identified factors precede disease development, causal inference cannot be claimed (Rothman, 2012). Following an association study

using a case-control population, the identified genetic risk factors for complex diseases such as diabetes can be defined as associated with an increased risk of developing the disease. The particular risk for any given genetic risk factor needs to be defined specifically. Typically, for complex diseases there are multiple genetic and environmental risk factors that interact, and these factors combined will determine the risk for the individual.

In the following sections risk factors for overweight, obesity and DM will be discussed simultaneously, since the same or similar factors appear to be associated with all the conditions, making a distinction between them difficult.

1.4.1 Non-modifiable factors

Age

Increasing age has been associated with an increased risk of DM in both people and cats. Most diabetic cats are > 8 years of age, with a peak incidence between 10 and 13 years of age (Panciera *et al.*, 1990; Baral *et al.*, 2003). Mature age is also associated with an increased risk of overweight and obesity in cats (Russell *et al.*, 2000; Lund *et al.*, 2005; Courcier *et al.*, 2012). In people, it has been shown that β-cell function deteriorates with age. The age-associated decline of β-cell function likely plays a role in the increased prevalence of abnormal glucose tolerance encountered with age (Chiu *et al.*, 2000).

Sex

In cats, it has been shown that males are more prone to develop DM (Panciera *et al.*, 1990; McCann *et al.*, 2007; Prahl *et al.*, 2007; Lederer *et al.*, 2009). However, male cats are also more prone to develop obesity, which will contribute to an increased DM risk (Scarlett *et al.*, 1994; Appleton *et al.*, 2001; Lund *et al.*, 2005; Colliard *et al.*, 2009; Courcier *et al.*, 2010). In people on the other hand, sex has not been associated with the risk of developing T2DM, with the exception of gestational DM, a condition where women develop DM during pregnancy (Chu *et al.*, 2007; Chen *et al.*, 2012).

Neutering is routinely performed in many domestic cats worldwide, to prevent from over-population, and is here considered a non-modifiable risk factor. Neutering has been recognised as a risk factor for DM in both male and female cats (Panciera *et al.*, 1990; McCann *et al.*, 2007; Prahl *et al.*, 2007), although it did not remain as a risk factor in the multivariable models in these studies (McCann *et al.*, 2007; Prahl *et al.*, 2007). It is possible that the effect of

neutering on DM risk is a reflection of an increase in both age and obesity, rather than an effect of the gonadectomy itself (Prahl *et al.*, 2007).

Breed and genetic susceptibility

Breed as a potential risk factor for feline DM has been investigated, but most studies have failed to identify other breeds than the Burmese as high risk breeds for DM. The Burmese cat (Figure 5) had an increased risk for DM in studies from Europe, Australia, and New Zeeland, but not from the US (Panciera *et al.*, 1990; Rand *et al.*, 1997; Wade *et al.*, 1999; McCann *et al.*, 2007; Prahl *et al.*, 2007; Lederer *et al.*, 2009).

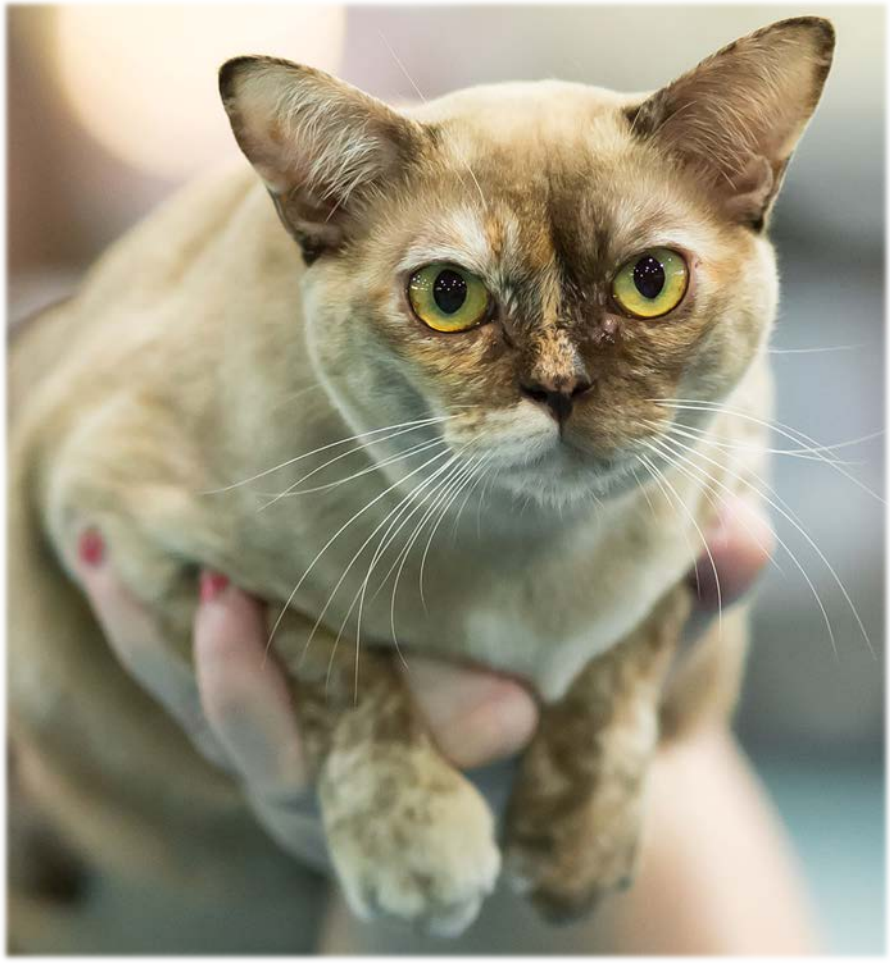

Figure 5. A Burmese cat. By user: Heikki Siltala (URK cat show Kirkkonummi 2013-10-13), CC BY 3.0, via Wikimedia Commons.

However, the European/Australian and the American Burmese breed lines have been kept separate since the 1970s, and can thus be considered to represent two different subpopulations (Alhaddad *et al.*, 2013).

The cause of the predisposition to DM in the Burmese cats is unknown, but likely genetic (O'Leary *et al.*, 2013). A dyslipidaemia is present in the breed, and is suspected to be associated with insulin resistance and thereby an increased risk of DM (Kluger *et al.*, 2009; Kluger *et al.*, 2010; Lee *et al.*, 2013). Lean Burmese cats had aberrant gene expression patterns and cholesterol lipoprotein fraction profiles that mimicked the profile of obese domestic cats, suggesting a propensity for obesity and insulin resistance, and supporting the hypothesis that these Burmese cats were in a prodromal obese metabolic state (Lee *et al.*, 2013).

Ethnicity in people has also been associated with differences in T2DM risk (Ravussin *et al.*, 1994; Cowie *et al.*, 2010; Chatterjee *et al.*, 2017), with the Pima Indians as a well-known example of an indigenous population with a very high prevalence of T2DM, with more than half of the adults affected (Barceló & Rajpathak, 2001). The "westernisation" process has been proposed to play a major role in the DM epidemic in people. Several populations previously free of DM, have recently been shown to have a high prevalence of DM, and it is possible that the progression to DM in different ethnic groups may be determined by a prevalent insulin resistance in some, and by β-cell dysfunction in others (Abate & Chandalia, 2003).

Type 2 DM depends on multiple genetic loci as well as environmental factors as previously discussed, and is known as a complex polygenic disorder. In people, more than 100 susceptibility loci have so far been detected by genome-wide association studies (Gaulton, 2017). Most of these genes seem to be related to β-cell function rather than to obesity or insulin resistance, which was anticipated earlier. Beta cell dysfunction is more severe than insulin resistance, since insulin secretion is impaired, whereas with insulin resistance, insulin may still be secreted. As β-cell function deteriorates, hyperglycaemia develops initiating the onset of T2DM (Cerf, 2013). Likely, there are also differences in the locations of risk alleles and frequencies of occurrence of particular risk alleles across different ethnic groups (Chen *et al.*, 2012).

In cats, very little is known about the genetic background in DM pathogenesis. Insulin sensitivity varies between individual cats, and it is speculated that an underlying low insulin sensitivity in some cats is genetically determined (Appleton *et al.*, 2001). Others have investigated heritability for DM within the Burmese cat breed (Wade *et al.*, 1999; O'Leary *et al.*, 2013). Heritability was estimated to be around 9%, assuming that all included animals with unknown status were unaffected from DM. Authors suggested a dominant expression with the risk allele frequency at 15% with 60% penetrance to be the best fitting

genetic model in this breed (O'Leary *et al.*, 2013). Others, however, stated that the condition was not sex linked, and unlikely to be dominant (Wade *et al.*, 1999). Apart from these two pedigree analyses on Burmese cats, a recent study found that a mutation in the melanocortin 4 receptor (MC4R) gene was associated with DM in overweight domestic shorthaired cats. Mutations in this gene are the most common single genetic cause of human obesity, but an association between presence of the mutation and overweight has not been demonstrated in cats (Xi *et al.*, 2012; Forcada *et al.*, 2014).

1.4.2 Modifiable factors

Overweight and obesity

Overweight and obesity are important factors contributing to insulin resistance in both cats and people, and also highly important risk factors for DM (Biourge *et al.*, 1997; Scarlett & Donoghue, 1998; German, 2006; Scott-Moncrieff, 2010; Chen *et al.*, 2012; Hoenig, 2012; Bjornvad *et al.*, 2014; Hoenig, 2014; Osto & Lutz, 2015; Clark & Hoenig, 2016). Scarlett and Donoghue found an almost fourfold increased risk of DM in obese cats (Scarlett & Donoghue, 1998).

In people, overweight can be defined as having a BMI greater than 25, and obesity if above 30, as mentioned previously (Guh *et al.*, 2009). Cats have an optimal body condition when scored 5/9, are considered overweight when scored 6–7/9, and obese when scored 8–9/9, when using the 9-grade scale (Brooks *et al.*, 2014). When using a 5-grade scale, 3 is considered an optimal BCS, while scores below or above 3 are considered as being underweight or overweight, respectively (Baldwin *et al.*, 2010).

In people, obesity, especially so called central obesity, increases insulin resistance by a number of mechanisms. Enhanced lipolysis will occur, since the normal anti-lipolytic effects of insulin will be diminished when adipose tissue has become resistant to the effects of insulin. This will contribute to increased release of free fatty acids (FFA) from the adipocytes. Free fatty acids negatively affect the liver by impairing insulin uptake, thereby contributing to an increased gluconeogenesis and glucose release, creating a vicious circle (Kopelman, 2000). Moreover, adiponectin concentrations are lower in obesity, leading to a decreased stimulation of fatty acid oxidation further contributing to insulin resistance (Kahn *et al.*, 2006).

Several other adipocyte-derived factors are also believed to play a role in the development of insulin resistance, for example tumour necrosis factor-α and interleukin-6 (Kahn *et al.*, 2006). In cats, obesity has also been shown to be

associated with insulin resistance, as showed by Hoenig *et al*. using a euglycaemic hyperinsulinaemic clamp, the gold standard method for evaluating insulin resistance. Glucose sensitivity in obese cats was only 40% of that of lean cats (Hoenig *et al.*, 2006).

Physical inactivity

In humans, it is well known that a sedentary lifestyle is associated with an increased risk for both obesity and T2DM (Chatterjee *et al.*, 2017). Inactivity has also been shown to induce changes consistent with insulin resistance in people (Hamburg *et al.*, 2007). In cats, indoor confinement and physical inactivity have been associated with an increased risk of both obesity and DM (Scarlett *et al.*, 1994; Robertson, 1999; Slingerland *et al.*, 2009).

Dietary factors

Several studies have investigated associations between different types of food and obesity or DM in cats, but results have been inconclusive. It has been hypothesised that the change in diet, from the feral cats' low-carbohydrate, high-protein diet to the high-carbohydrate diet given to many domesticated cats today, is partially responsible for the increase in overweight and DM seen in cats (Rand *et al.*, 2004). Cats are strict carnivores, and a cat's natural diet consists of small prey with a low carbohydrate content (Dierenfeld *et al.*, 2002), but many commercial diets today are moderate to high in carbohydrate content. In one study, it was shown that overweight cats were more likely to be fed dry foods purchased from veterinarians or other non-grocery retailers, or to receive specialty or prescribed diets (Scarlett *et al.*, 1994). In younger cats, an association between eating predominantly dry food and overweight has been detected (Rowe *et al.*, 2015; Rowe *et al.*, 2017). Others did not detect associations between type of diet and the cats' weight (Robertson, 1999). Slingerland *et al*. investigated associations between diet and DM, but their results indicated that the proportion of dry food in a cat's diet was not an independent risk factor for DM (Slingerland *et al.*, 2009).

Type of feeding regime has also been investigated as a risk factor for both obesity and DM. Again, studies have shown different results. *Ad libitum* feeding has been associated with a higher body condition compared to meal-feeding for cats fed a canned diet, but not for cats on a dry-food diet (Russell *et al.*, 2000). Another study found owners feeding their cats twice or three times a day to be more likely to have overweight or obese cats than those who fed *ad libitum* (Courcier *et al.*, 2010). Yet others found no associations between feeding management and risk of obesity (Allan *et al.*, 2000).

Eating behaviour has been analysed as a potential risk factor for obesity and DM in both cats and humans. In people, eating slowly was associated with a lower caloric intake and an enhanced satiety (Andrade *et al.*, 2008). Diabetic Burmese cats tended to more often be described by their owners as "greedy eaters" compared to age- and gender matched nondiabetic Burmese cats, although the difference was not statistically significant (Lederer *et al.*, 2003).

Drugs

Corticosteroid treatment, and administration of progestins such as megestrol acetate, have been reported as risk factors for feline DM (Rios & Ward, 2008b). However, most of these studies are intervention studies, where cats experimentally have been given high doses of one or both of these types of drugs, in some cases leading to DM or glucose intolerance (Buse *et al.*, 1957; Middleton & Watson, 1985; Peterson, 1987; Hoenig *et al.*, 2000). Authors concluded that if cats are treated with a glucocorticoid or megestrol acetate, overt DM is most likely to occur in animals with a subclinical DM present (Peterson, 1995). In Sweden, medroxyprogesterone acetate is used in cats and megestrol acetate is not available. Risk factor analysis has shown corticosteroid treatment to be a risk for DM development in the univariate analysis, but not in the following multivariate model (McCann *et al.*, 2007).

1.5 Clinical features of diabetes mellitus in cats

1.5.1 Pathophysiology

There are striking pathophysiological similarities between diabetic cats and people. In people, insulin resistance in tissues, will lead to an increased production of insulin from pancreatic β-cells in order to maintain normal glucose homeostasis. If β-cells are incapable of this task, glucose concentrations will rise and T2DM will evolve. Reduced β-cell function is present in certain groups of people at increased risk for DM, and it has been shown that β-cell function is heritable (Kahn *et al.*, 2014). In the diabetic human pancreas, the number of β-cells is reduced. In diabetic cats, there is also a mean loss of β-cells of approximately 50% (O'Brien *et al.*, 1986). The reason for this β-cell loss is multifactorial, and includes glucotoxicity and deposition of amyloid. Chronic hyperglycaemia impairs insulin secretion by increased apoptosis of the β-cells, mediated by activation of oxidative stress as a result of increased generation of reactive oxygen species (Del Prato, 2009). Amyloid is a proteinaceous deposit with a β-sheet structure. Amylin, or islet amyloid polypeptide (IAPP), is a

polypeptide hormone co-secreted with insulin from the pancreatic β-cells, that forms the amyloid deposits present in the pancreas of most diabetic people and cats (Johnson *et al.*, 1989a; O'Brien, 2002; Westermark *et al.*, 2011; Herndon *et al.*, 2014). Islet amyloidosis (Figure 6) has been shown to occur in only a limited number of species (*e.g.* people, macaques and cats), and not in rodents or dogs; animal species in which a diabetic syndrome similar to T2DM in people does not seem to develop spontaneously (Johnson *et al.*, 1989b; O'Brien *et al.*, 1993; Höppener *et al.*, 2002; O'Brien, 2002).

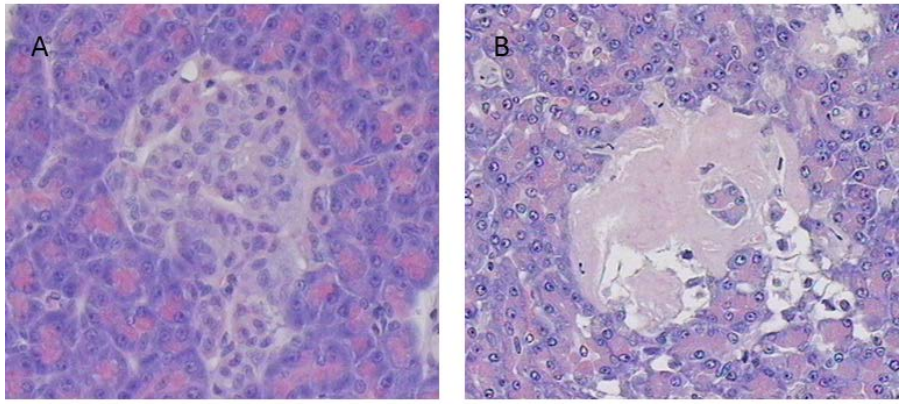

Figure 6. An islet of Langerhans from a healthy cat pancreas (A), and an islet with severe amyloidosis from a diabetic cat (B). Photo: Erika Karlstam.

It has been debated whether the islet amyloid present in T2DM is a cause or merely a consequence of the disease. It is possible that islet amyloidosis can be both a cause and a consequence, as it is induced by insulin resistance (stimulating both insulin and IAPP secretion), and subsequently contributes to the development of insulin insufficiency by promoting β-cell failure, since the location of the amyloid can impair the supply of glucose and other nutrients to the islet cells (Höppener *et al.*, 2002).

Other types of DM in cats can occur, for example as a consequence of overproduction of hormones with an insulin-antagonistic effect, such as cortisol (hyperadrenocorticism) and growth hormone (hypersomatotropism or acromegaly) (Niessen *et al.*, 2015). Pancreatitis and other pancreatic diseases are disorders that can lead to widespread damage to the pancreas and the islets of Langerhans, with subsequent β-cell failure and DM development (Rand, 2013).

1.5.2 Diagnosis

Diabetes mellitus in cats is usually diagnosed based on the presence of clinical signs and persistent hyperglycaemia. Classic clinical signs of feline DM include polyuria, polydipsia, lethargy, weight loss, and polyphagia. Stress hyperglycaemia and glycosuria must be excluded, as stress in cats can cause a marked transient hyperglycaemia. Measurement of serum fructosamine, a glycated plasma protein, is helpful to confirm a diagnosis, as it is indicative of the average blood glucose concentration during approximately the preceding week (Link & Rand, 2008; Sparkes *et al.*, 2015). In people, HbA1c, or glycated haemoglobin, is used as an index of mean plasma glucose over the preceding weeks to months. HbA1c is a "weighted" average of the blood glucose levels during the preceding 120 days, which is the lifespan of erythrocytes. The glucose concentration from the last month contributes to approximately 50% of the final result (Rohlfing *et al.*, 2002).

In people, the concentration of C-peptide can be measured as a marker for endogenous insulin production. C-peptide is removed from proinsulin when insulin is formed, and it is produced in equal amounts to insulin. It can be used to distinguish patients with T1DM from T2DM, because with time, C-peptide becomes undetectable in T1DM patients. C-peptide can be measured even if the patient receives insulin injections (Clark, 1999; Jones & Hattersley, 2013). C-peptide assays are currently not available for cats.

1.5.3 Treatment

Treatment of diabetic cats aims at controlling or eliminating clinical signs by achieving good glycaemic control. This usually involves insulin treatment together with lifestyle adjustments, such as diet and weight control (Sparkes *et al.*, 2015).

In human T2DM patients, lifestyle adjustments with focus on diet and obesity treatment, alone or with the addition of oral drugs, are generally appropriate as initial treatment, depending on severity of the disease. Metformin remains the therapy of choice for people with T2DM (Chatterjee *et al.*, 2017). Metformin reduces glucose output from the liver, enhances insulin sensitivity, stimulates glucagon-like peptide (GLP)-1 secretion, and lowers HbA1c concentrations without causing hypoglycaemia. Targeting the incretin system, of which GLP-1 is a member, has become an important novel therapeutic approach for treating T2DM. Incretins are a group of metabolic hormones that are produced in the intestines and released in response to food intake. They augment the secretion of insulin from the β-cells, thereby stimulating a decrease in blood glucose levels. The incretin effect explains the difference between responses to oral and

intravenous glucose administration, where glucose given orally produces a greater insulin response than that of an intravenous infusion. In people, the incretin effect is reduced in T2DM patients and in obesity (Muscelli *et al.*, 2008; Meier & Nauck, 2010; Nauck, 2011). Concentrations of the incretin glucagon-like peptide GLP-1 were lower in obese than in lean cats. The low concentrations might indicate a contribution of GLP-1 to the lower insulin sensitivity of obese cats, but this hypothesis needs to be further investigated (Hoenig *et al.*, 2010). Two drug classes targeting the incretin system have been developed; GLP-1 receptor agonists and dipeptidyl peptidase (DPP)-4 inhibitors (Højberg *et al.*, 2009). The DPP-4 inhibitors act by blocking the enzyme DPP-4, responsible for the degradation of incretins such as GLP-1.

In contrast to cats, people are often diagnosed with T2DM or prediabetes when their blood glucose is only mildly elevated, and most often years before the hyperglycaemia would cause clinical signs. Since the DM diagnosis in cats is usually made when blood glucose concentrations are much higher, this presumably occurs at a later stage when the pancreatic β-cell mass is already significantly decreased. The window of opportunity to treat by targeting the incretin system might therefore be smaller for diabetic cats (Gilor *et al.*, 2016b). Exenatide, a GLP-1 mimetic, was well tolerated when given to obese cats for 12 weeks, and tended to decrease body weight even without dietary changes (Hoelmkjaer *et al.*, 2016). It potentiates insulin secretion in healthy cats (Gilor *et al.*, 2011), and has also been used in a pilot study on diabetic cats, where it was not associated with local or systemic adverse effects (Riederer *et al.*, 2016). In healthy cats, a DPP-4 inhibitor significantly reduced glucagon output, and increased insulin output, after an intravenous glucose tolerance test (Furrer *et al.*, 2010). The potential usefulness of incretin therapy in diabetic cats remains to be established.

In cats, in contrast to most human T2DM patients, insulin treatment is usually necessary at diagnosis. Insulin is often administered subcutaneously twice daily. Insulin-induced hypoglycaemia should be avoided, sometimes at the expense of allowing periods of hyperglycaemia. The owner's concerns and expectations will influence the level of glycaemic control to be aimed for (Sparkes *et al.*, 2015). As insulin treatment is preferable in cats, oral hypoglycaemic drugs are infrequently used (Sparkes *et al.*, 2015). Metformin is of limited value in diabetic cats (Nelson *et al.*, 2004), and an alternative oral agent, glipizide, is often ineffective (Sparkes *et al.*, 2015).

Secondary or concurrent diseases such as diabetic neuropathy, infections, chronic pancreatitis, acromegaly, and dental problems, as well as other factors which might contribute to insulin resistance, such as corticosteroid treatments, should be addressed (Rand, 1999; Rios & Ward, 2008a; Stanko & Izakovicova

Holla, 2014; Sparkes *et al.*, 2015). Suspected acromegaly can be diagnosed with a combination of typical clinical findings, hormonal tests (insulin-like growth factor (IGF)-I and feline growth hormone) and intracranial imaging (computed tomography or magnetic resonance imaging) (Niessen, 2010). In cats, IGF-I concentrations > 1,000 ng/mL have been suggested as a tentative diagnosis of acromegaly (Niessen *et al.*, 2007).

In people, the bidirectional association between DM and inflammatory periodontal disease is well-known (Taylor *et al.*, 1996; Stanko & Izakovicova Holla, 2014). In cats, studies in this field are lacking. However, anecdotal reports indicate that diabetic cats commonly have dental disease and chronic gingivitis (Diehl, 1995). There are also anecdotal reports where insulin-treated diabetic cats had markedly improved glycaemic control when dental disease was treated (Martin & Rand, 2000).

Lifestyle interventions for a diabetic cat typically includes weight loss management and dietary interventions. Increased exercise is encouraged as a useful adjunct to the weight management (German, 2006). Wet foods can be beneficial, as they tend to lower the caloric consumption compared to dry food, which often is more calorie-dense due to the lower water content (Wei *et al.*, 2011). A low-carbohydrate diet is recommended to achieve better glycaemic control (Frank *et al.*, 2001; Mazzaferro *et al.*, 2003; Bennett *et al.*, 2006). The low-carbohydrate dietary recommendations have replaced the earlier recommendations of a high-fibre diet to diabetic cats (Nelson *et al.*, 2000). Interestingly, regarding treatment of diabetic cats, already in the 1960's Joan Joshua stated the following:

> "The cat. A much more rewarding species to treat. Diet is not effective since most cats are already on a very low carbohydrate intake anyway." (Joshua, 1963)

This context has changed dramatically since the 1960's, and today, commercial dry food is a typical diet for domestic cats. As the carbohydrate content in commercial dry cat food constitutes approximately 20–40% of the metabolisable energy, it cannot be considered to be a low-carbohydrate feed (Villaverde & Fascetti, 2014).

1.5.4 Remission

Resolution of peripheral insulin resistance in cats with T2DM together with good glycaemic control may result in diabetic remission (Scott-Moncrieff, 2010). Remission can be defined as the ability to maintain euglycaemia without insulin therapy for at least 4 consecutive weeks, without the reappearance of clinical signs (Zini *et al.*, 2010; Gottlieb *et al.*, 2015). Cats in remission should remain

on a low-carbohydrate diet and kept in optimal body condition (Sparkes *et al.*, 2015). Remission is more likely to occur with good glycaemic control and early institution of treatment (Zini *et al.*, 2010; Gottlieb & Rand, 2013; Nack & DeClue, 2014).

2 Aims

The hypothesis underlying this thesis was that the lifestyle change in domestic cats during the last century would be associated with both overweight and DM. The main aims were to identify new potential risk factors for DM in cats, to confirm previously identified risk factors, and to increase understanding of disease pathogenesis.

The specific aims were to:

- Determine the general incidence of DM in Swedish cats.
- Determine incidence rates (IRs) for demographic risk factors for DM, such as age, breed and sex.
- Investigate associations between environmental risk factors and DM in cats.
- Determine the prevalence of overweight in cats.
- Investigate associations between overweight in cats and demographic and environmental factors.
- Explore the predisposition of the Burmese cat breed to DM by studying the metabolic profile in the Burmese, Birman and Maine coon breeds.

3 Comments on materials and methods

The methods used are described in detail in each respective paper. Study I focused on demographic (non-modifiable) risk factors for feline DM and used data from the Agria Pet Insurance company database. Owners were recruited from the database for participation in a questionnaire study where environmental (modifiable) risk factors for DM were assessed (study II). Study III investigated factors associated with overweight in a cross-sectional study, and included data from hospital records obtained for cats visiting the University Animal Hospital, Swedish University of Agricultural Sciences (SLU), and questionnaire data from the non-diabetic control group used in study II. Study IV included clinical data and blood samples from healthy cats, visiting the University Animal Hospital, SLU, and the Anicura Bagarmossen Animal Referral Hospital (Figure 7).

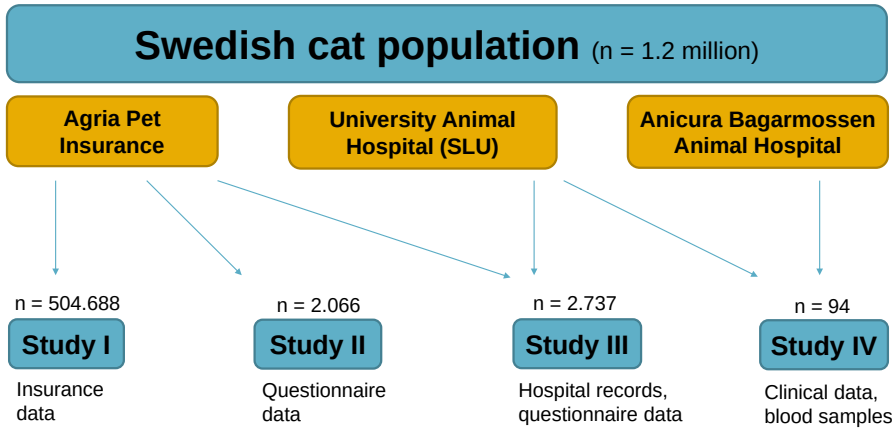

Figure 7. Overview of number of included cats in each study, and types of data and sample material used for the studies included in this thesis.

3.1 Measuring disease frequency

3.1.1 Incidence of diabetes mellitus in insured cats (paper I)

As mentioned previously, determining IRs require knowledge about the population at risk of developing the disease. We used data on all cats insured in the Agria Pet Insurance Company's database during a 5-year period (2009–2013) to investigate the incidence of DM in Swedish cats. It is estimated that more than one third of all cats in Sweden are insured, and Agria Pet Insurance Company is the largest pet insurance company in Sweden. The database includes records of both healthy and sick cats, and is believed to fairly represent the Swedish cat population, although there is a larger proportion of purebred than domestic cats that is covered by an insurance (Egenvall *et al.*, 2010). The exact time each cat was covered by the insurance was calculated and summarised. Cats were at risk from start of year 2009 or start of the insurance if cats entered the system at a later date, to the end of 2013, or until the date of a DM diagnosis, death, or withdrawal from the insurance. The time at risk is expressed as cat-years at risk (CYAR) (Figure 8). All cases of DM were counted, and IRs were thereafter calculated as number of cases with DM divided by the total time at risk.

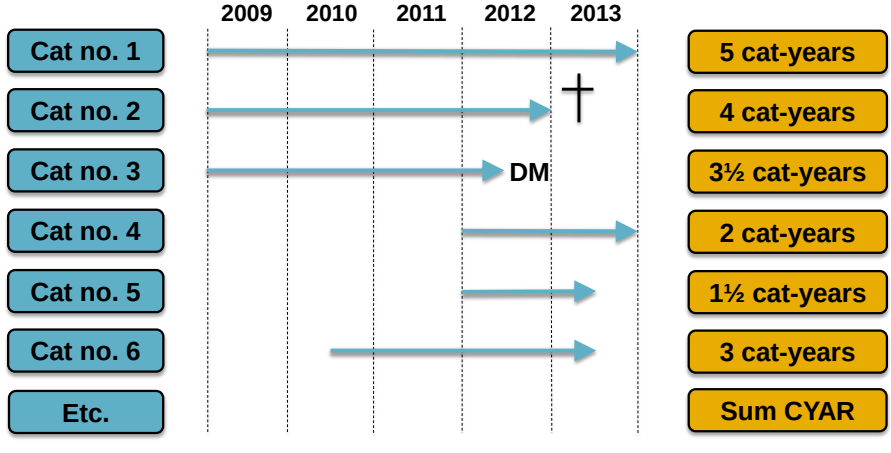

Figure 8. Each cat in the insurance database contributes with time at risk for developing DM. Before entry into the insurance program, or in case of death (†), diabetes mellitus (DM) diagnosis, or withdrawal from the insurance, the cat does not contribute to time-at-risk as illustrated in the figure. The exact time-at-risk for each individual cat is calculated as cat-years at risk (CYAR) and thereafter summarised.

A strength with using entries in an insurance database as a study population is the considerable size of the registry, which allows stratifications by for example age, sex and breed. We also stratified on study year, since we wanted to examine if the incidence of DM in Swedish cats is increasing or not.

3.1.2 Prevalence of overweight in two cohorts of cats (paper III)

In many circumstances, calculating the incidence of a condition is unfeasible, and researchers therefore choose to report disease occurrence as prevalence. The prevalence is often used to measure chronic diseases such as obesity which have a long duration, and time of onset can be insidious and difficult for the individual to pinpoint. Many veterinary studies report hospital prevalence. This can be misleading, as the prevalence of a disease is likely higher when measured at a hospital where fewer healthy individuals are normally encountered, compared to the general population.

Since obesity has been reported as an important risk factor for feline DM (Scarlett & Donoghue, 1998), we decided to examine if cat breeds associated with an increased risk of DM would also be associated with overweight. We did this by investigating the prevalence of overweight in two cohorts of cats. The first cohort consisted of cats visiting the University Animal Hospital, SLU, Uppsala, Sweden, during years 2013 to 2015. The second cohort consisted of the non-diabetic control cats used in study II, whose owners answered a web questionnaire. Medical records and questionnaire data were used from each cohort of cats. Only cats older than one year of age at the time of visit to the hospital were included. All medical records where the cat's body condition were scored using a 9-grade scale (Laflamme, 1997) were used for further analysis. For the questionnaire cohort where owners scored their cat, a 5-grade scale was used (Baldwin *et al.*, 2010). The 5-grade was selected because of its simplicity and user-friendliness.

If the body condition was scored from 6 to 9 in the medical record, the cat was considered overweight, and a score of 5 or lower was considered as not being overweight. The overweight group included both overweight and obese cats. Cats in the questionnaire cohort were considered overweight if scored by owners as grade 4 or 5, and not overweight if scored 3 or lower. Prevalence of overweight was calculated as the percentage of overweight cats in each cohort. A major difference between the two cohorts was the examiner of the cats' body condition; in the first cohort the cats were examined using a 9-grade scale by either a veterinarian or a veterinary student, and in the second cohort owners judged their cats' body condition on a 5-grade scale with the help of illustrations and accompanying descriptions (Baldwin *et al.*, 2010).

3.2 Factors associated with disease

3.2.1 Demographic risk factors for diabetes mellitus (paper I)

In this case-control study, we investigated if non-modifiable demographic factors such as age, breed, and sex were associated with risk of DM in cats. Information on neutering status was unavailable in the database, and sex was defined as male or female. Data used consisted of all records in the Agria Pet Insurance' database during 2009 to 2013. Incidence rates were calculated as number of diabetic cats divided by CYAR. Dividing two IRs, *e.g.* the IR for male versus female cats, renders the incidence rate ratio (IRR), or rate ratio. We calculated IRRs to compare the IRs between male and female cats, age groups, and between different cat breeds and all other purebred cats or all other cats. A 95% confidence interval (CI) was calculated for each IRR. If the CIs for the IRR did not include 1, it was considered evidence that the groups were significantly different from each other.

3.2.2 Environmental risk factors for diabetes mellitus (paper II)

To complement data from study I, where non-modifiable demographic risk factors for DM in Swedish cats were assessed, we decided to extend our knowledge by including assessment of modifiable environmental risk factors, using the same cohort of cats from the Agria Pet Insurance database. An invitation to participate in this questionnaire study was sent out to owners of diabetic cats with a diagnosis received during 2009–2013 (*i.e.* the same cats identified as DM cases in study I). A non-diabetic control group, four times larger in size than the diabetic group as suggested by Linden and Samuels (Linden & Samuels, 2013), was recruited from the same database and time period, with cats matched on birth year with the diabetic cats. No other selection was made for the control group.

Owners received a web address to the questionnaire, where a maximum of 48 questions were provided. The questionnaire included questions on the cat's birth year, breed, sex, and neutering status, as well as questions related to activity level, access to outdoors, type of diet, feeding regime, eating behaviour, body condition etc. Sex was defined as male or female, and neutering status as intact or neutered. Data were analysed with multiple logistic regression, with DM as the dependent variable, after excluding clearly non-significant variables ($P > 0.15$) in a univariable logistic regression. A backwards stepwise elimination approach was used to exclude variables based on a lowered Akaike information criterion (AIC), as a measure of best goodness of fit of the final statistical model.

Odds ratios (OR) were calculated with 95% CIs. The significance level was set as $P < 0.05$.

There were interactions present between some of the variables assessed, and these interactions were also included in the multiple logistic regression model if $P < 0.15$. An interaction can be interpreted as a combination of two variables where the combination itself leads to a different effect on the outcome. This occurs when the simultaneous influence of two variables on the outcome is not additive. If two variables interact, the relationship between each of the interacting variables and the outcome depends on the value of the other interacting variable. Therefore, the two interacting variables need to be interpreted in context to correctly asses their effect on the outcome.

3.2.3 Factors associated with overweight (paper III)

In this cross-sectional retrospective study, we investigated factors associated with overweight in cats. We were interested in whether the risk factors previously identified for DM would be similar to the factors associated with overweight. In particular, we wanted to know if the breed predisposition to DM was similar to the breed predisposition to overweight, and if breeds with a low risk of DM would be similar to the breeds with a low prevalence of overweight.

As mentioned previously, data from two cohorts of cats was used in this study. The first cohort included cats visiting the University Animal Hospital during 2013 to 2015, and medical records were used. From these records, information on the cats' age, breed, sex, neutering status, diagnosis assigned when visiting the hospital, and information on BCS were collected. The diagnosis can also include clinical signs, such as fever or anorexia. Sex was defined as male or female, and neutering status as intact or neutered. If BCS was 6 or higher on the 9-grade scale used, cats were grouped as overweight, and if BCS was 5 or lower into the group not overweight.

The second cohort comprised cats insured by the Agria Pet Insurance Company during 2009 to 2013. Data from the questionnaire from the non-diabetic control group in study II was used. Data included information about the cats' age, breed, and sex, type of diet, eating behaviour, and activity level. Cats were considered overweight if scored 4–5 (on a 5-grade scale), and not overweight if scored 3 or lower. A multiple logistic regression analysis with backwards stepwise removal of non-significant variables was applied to each cohort based on a lowered AIC, with overweight as the response variable of interest. Odds ratios were calculated with 95% CIs. The significance level was set as $P < 0.05$.

3.3 Burmese cats predisposed to diabetes mellitus (paper IV)

The Burmese cat breed has been identified as a high-risk breed for developing DM in previous studies (Rand *et al.*, 1997; McCann *et al.*, 2007; Lederer *et al.*, 2009). A dyslipidaemia is present in the breed, possibly causing this predisposition to DM (Kluger *et al.*, 2009; Kluger *et al.*, 2010). Therefore, we decided to study the metabolism in the Burmese cat breed. Healthy Burmese cats were recruited to the study in collaboration with the Swedish Burmese cat club. All cats were examined at the University Animal Hospital, Uppsala, or at the Bagarmossen Anicura Referral Hospital, Stockholm, during two sampling periods, the first in 2013, and the second in 2015 to 2016. Healthy cats from a low-risk breed for DM (the Birman breed) and a medium-risk breed (the Maine coon (MCO)) were used as controls, and sampled in 2015 to 2016. Samples from 94 cats (46 Burmese cats, 23 Birman cats, and 25 MCO cats) were analysed for serum biochemistry parameters (alanine aminotransferase (ALAT), creatinine, fructosamine, FFA, and lipoprotein fractions), and hormones (insulin, adiponectin, and IGF-I analyses performed with enzyme-linked immunosorbent assay (ELISA)). Analysis of the serum metabolome with nuclear magnetic resonance (NMR) spectroscopy was performed in 63 of the cat samples, since 31 of the Burmese samples had to be excluded due to differences in sample handling.

Nuclear magnetic resonance spectroscopy is an analytical technique that has emerged as a promising approach to identify new biomarkers, and has been applied to research on many different diseases in people, *e.g.* T2DM, and other metabolic traits. The metabolic aetiology of T2DM makes it a suitable condition to be characterised with metabolomics techniques such as NMR or liquid chromatography mass spectrometry (LC-MS) (Orešič, 2009; Connor *et al.*, 2010). Another advantage with metabolomics is the possibility to identify biomarkers that can predict disease before overt symptoms have manifested, enabling interventions that may delay or even prevent a progression to T2DM. The metabolomics techniques have identified several metabolites associated with T2DM, insulin resistance and prediabetes in people (Gall *et al.*, 2010; Wang *et al.*, 2011; Floegel *et al.*, 2013). We wanted to investigate if Burmese cats might have a metabolic profile compatible with insulin resistance patterns in people, which might anticipate a progression into DM at a later stage.

A multivariate statistical model was used to investigate the effects of breed, weight, and BCS on the concentrations of the serum biochemistry variables creatinine, ALAT, fructosamine, FFA, very low-density lipoprotein-triglycerides (VLDL-TG), high-density lipoprotein (HDL)-cholesterol and concentrations of the hormones insulin, adiponectin, and IGF-I. The model was not adjusted for age, sex or neutering status as these parameters did not differ between groups.

For NMR metabolites, a principal components analysis was used, followed by a one-way ANOVA with a post-hoc Tukey test, or a Kruskal-Wallis test for not normally distributed data, applied on each metabolite with breed as the explanatory variable. Thereafter correction for multiple testing was done (Benjamini & Hochberg, 1995), and a multivariate partial least squares discriminant analysis (PLS-DA) was performed. A univariate regression model was finally applied to all metabolites differing significantly between breeds from the multivariate approach, to adjust for weight and BCS, which differed between breeds. For normally distributed data, concentrations were reported as least square means with 95% CI. Non-normally distributed data were logged for analysis, and least square means were back-transformed and reported as geometric means, with 95% CI.

4 Results

4.1 Measuring disease frequency

4.1.1 Incidence of diabetes mellitus in cats (paper I)

In total, 504,688 individual cats contributed to the study. The number of CYAR was 1,229,669, which was used to calculate the IR of DM. 1,432 cases of DM was registered, giving a general IR of 11.6 cases per 10,000 CYAR (95% CI, 11.0–12.2). The IRs were similar across years 2009 to 2013.

4.1.2 Prevalence of overweight in cats (paper III)

The prevalence of overweight differed between the two cohorts ($P < 0.0001$). In the cohort of cats visiting an animal hospital, the prevalence of overweight was 45%. In the questionnaire cohort, the prevalence of overweight was 22%.

4.2 Factors associated with disease

Risk factors associated with DM in cats were assessed in two studies. In study I, we focused on non-modifiable demographic risk factors such as age, breed and sex. In study II, a follow-up study was performed with investigation on modifiable environmental risk factors for DM in the same cohort of cats. Factors associated with overweight were assessed in study III.

4.2.1 Demographic risk factors for diabetes mellitus (paper I)

Age

The IR of DM was close to zero in young cats, and started to increase when cats were about 5 to 6 years of age, with a peak IR seen in cats at 13 years of age. In cats older than this, the IRs declined (Figure 9).

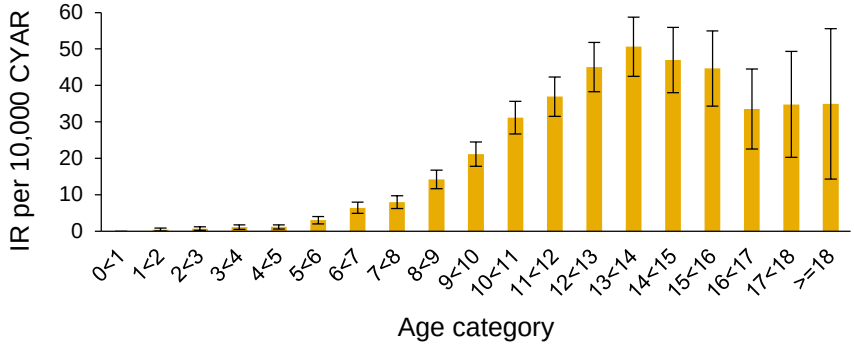

Figure 9. Incidence rates (IRs) of diabetes mellitus per age category. IRs start to increase at around 6 years of age, peak at 13 years of age, and decline in the geriatric cats. Error bars represent 95% confidence intervals.

Breed

Certain cat breeds had a higher IR of DM compared to all other cats, and also when compared to other purebreds. The most prominent was the Burmese breed, with an IR of 48.8 cases per 10,000 CYAR (95% CI, 31.0–65.7), compared to the IR of domestic cats of 12.1 (95% CI, 11.4–12.8). Other breeds with an increased risk of DM were the Russian Blue, Norwegian Forest cat, and Abyssinian. The IRR for Burmese cats versus all other purebred cats was 5.2 (95% CI, 3.6–7.6). The Bengal, Siberian, Birman, Ragdoll, British shorthair, Persian, and MCO breeds had a decreased risk of DM, with lower IRs compared to other cats. The IRR of DM for domestic cats compared to all purebreds was 1.2 (95% CI, 1.0–1.3).

Sex

The IR was higher for male cats (15.4 cases per 10,000 CYAR; 95% CI, 14.4–16.4) than for females (7.6 cases per 10,000 CYAR; 95% CI, 6.9–8.3), with an IRR for males compared to females of 2.0 (95% CI, 1.8–2.3). The increased risk of DM in male cats was seen in most breeds and was significant in several breeds, however not evident in the Burmese breed (Figure 10).

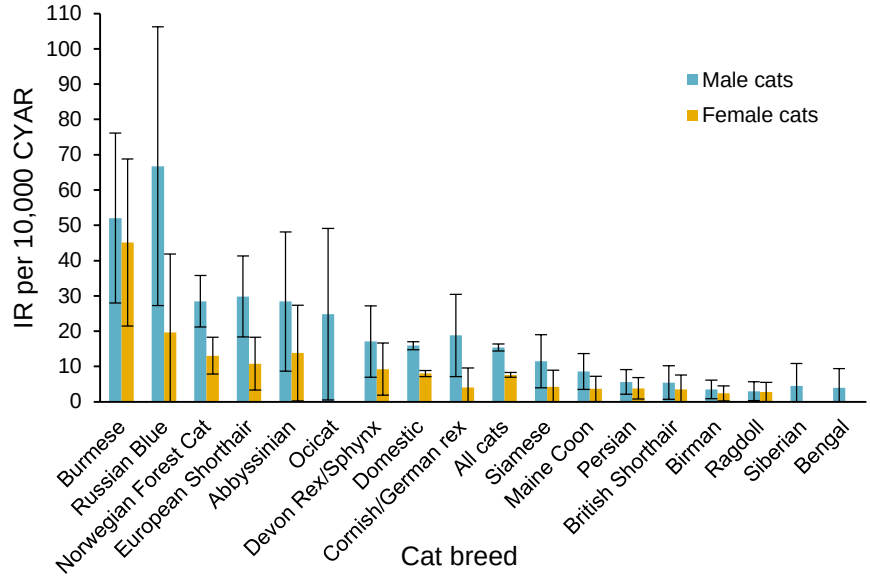

Figure 10. Incidence rates (IRs) of diabetes mellitus stratified on breed and sex. Most breeds have more male (blue bars) than female (orange bars) cats affected with diabetes mellitus. No discrimination was made based on neutering status. Only breeds with more than five cases of DM are shown in this graph. Error bars represent 95% confidence intervals.

4.2.2 Environmental risk factors for diabetes mellitus (paper II)

From this questionnaire study, answers from 2,066 cats were used for statistical analysis. Of these cats, 396 were from owners of diabetic cats and 1,670 from owners of non-diabetic control cats. In the multiple logistic regression, the variables breed, sex, vaccination status, corticosteroid injections, eating behaviour, and presence of other pets in the household remained associated with an increased risk of DM after stepwise removal of all non-significant variables. As expected, the Burmese and Norwegian Forest cat breeds showed an increased risk of DM, as did being male. Fully vaccinated cats were more often diagnosed with DM, as were cats receiving corticosteroid injections the year preceding a DM diagnosis. Living in a household with no other pets was associated with an increased risk of DM. Being considered a greedy eater was associated with increased odds of DM, compared to cats that had a nibbling eating behaviour. Two interactions remained significant in the final model. First, an interaction between BCS and type of diet, and secondly, an interaction between activity level and outdoor access versus indoor confinement. The variables included in an interaction always have to be interpreted in context. Being an indoor cat was associated with an increased risk of DM for the inactive and moderately active

cats, but such an association was not evident for the group of active cats. For the interaction between BCS and type of diet (Figure 11); the OR for DM was significantly higher for cats eating predominantly dry food in the normal-weight group (OR 3.8; 95% CI, 1.3–11.2), but in the overweight or underweight cats no associations between type of diet and DM risk were seen. On the other hand, regardless of type of diet, being overweight was always associated with an increased risk of DM. For cats on a diet consisting predominantly of dry food, the OR for developing DM was 2.3 (95% CI, 1.5–3.4) when comparing overweight cats with normal weight cats. For cats on a mixed diet the OR for DM was 2.6 (95% CI, 1.8–3.9), and for cats on a wet food diet 15.7 (95% CI, 4.5–55.1), for overweight cats compared to normal weight cats.

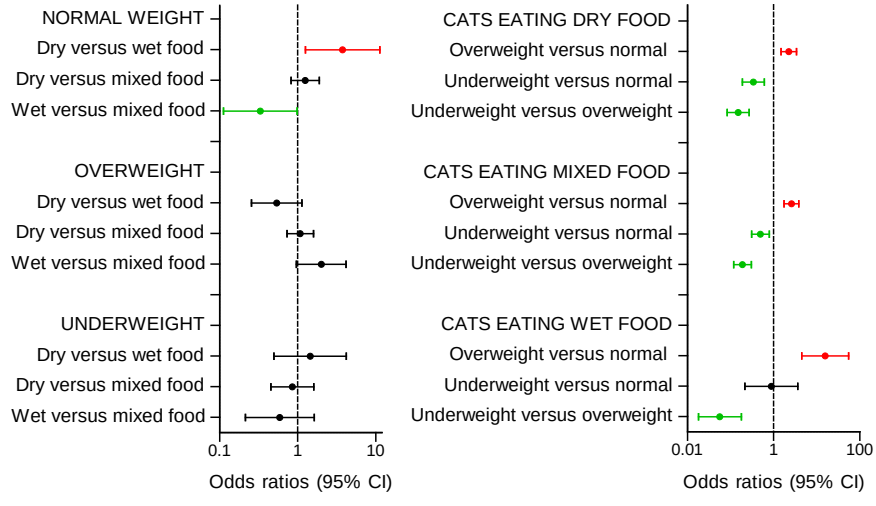

Figure 11. Odds ratios for diabetes mellitus for the interaction between body condition and type of diet. The interaction is shown within each body condition group (left), and diet group (right), respectively. Note that the log scale for the graph on the right reaches 100, in contrast to the left graph, which ends at 10, indicating a far greater effect of being overweight, regardless of diet type, compared to the effect of diet, where significance is present only within the normal weight group. Red data points and error bars show an increased risk of diabetes mellitus, whereas green indicates a decreased risk. Black points and error bars represent non-significant results. Error bars represent 95% confidence intervals (CI).

4.2.3 Factors associated with overweight in cats (paper III)

The non-modifiable variables age, breed, and sex were significantly associated with overweight in the final multiple logistic regression model for both cohorts of cats. For the medical records cohort (n = 1,072), neutering status and type of diagnosis was associated with overweight. In the questionnaire cohort

(n = 1,665), type of food, eating behaviour, and activity level were factors associated with an increased, or decreased risk of overweight. In both cohorts, geriatric cats were less often overweight than mature cats. No individual cat breed was identified as being at an increased risk of overweight in this study, but in both cohorts, the Birman and Persian cat breeds showed a decreased risk of being overweight as compared with the domestic cats. When comparing domestic cats with all purebred cats, the domestic cats were more often overweight (OR 1.3; 95% CI, 1.0–1.7 in the medical records cohort, and OR 1.8; 95% CI, 1.3–2.5 in the questionnaire cohort). Male cats were at increased risk of being overweight compared to females in both cohorts (OR 1.6; 95% CI, 1.3–2.1, and OR 1.4; 95% CI, 1.1–1.7, respectively).

Several different diagnoses were associated with overweight in the group of cats visiting the animal hospital. We compared different diagnostic groups with diagnoses referring to the whole animal, including diagnoses such as “lethargy” or “fever”. Cats were more often overweight if they had a diagnosis related to the respiratory tract (OR 2.6; 95% CI, 1.4–4.8), skin (OR 2.4; 95% CI, 1.3–4.2), lower urinary tract (OR 2.2; 95% CI, 1.3–3.8), locomotor system (OR 2.1; 95% CI, 1.1–3.9), or trauma (OR 1.6; 95% CI, 1.0–2.4).

Eating predominantly dry food was associated with being overweight when compared with eating predominantly wet food (OR 2.4; 95% CI, 1.4–4.0). Being a greedy eater was also associated with overweight, compared with preferring to nibble (OR 2.9; 95% CI, 1.4–2.7). Inactive cats were more often overweight than cats with a normal activity level (OR 1.9; 95% CI, 1.5–2.5).

4.3 The metabolic profile of Burmese cats (paper IV)

There were several differences discovered in the metabolic profiles between the three cat breeds included in the study; the Burmese, Birman, and MCO. Data from the multivariate model revealed that 22 of the 58 quantified NMR metabolites were discriminant between breeds. Two branch-chained amino acids, leucine and valine, and one aromatic amino acid, tyrosine, were found to differ between breeds. Additionally, the amino acids arginine, asparagine, histidine, lysine, and methionine, and the amino acid metabolites 2-oxoisocaproic acid, 3-methyl-2-oxovaleric acid, and dimethylglycine were found discriminative. The metabolites 2-propanol, acetic acid, acetylcarnitine, carnitine, creatine, creatinine, O-phosphocholine, succinic acid, taurine, 2-hydrobutyric acid, and glucose also differed between breeds. After adjusting for potential effects of weight and BCS, which differed between the breed groups, breed remained significant for 15 metabolites (Figure 12).

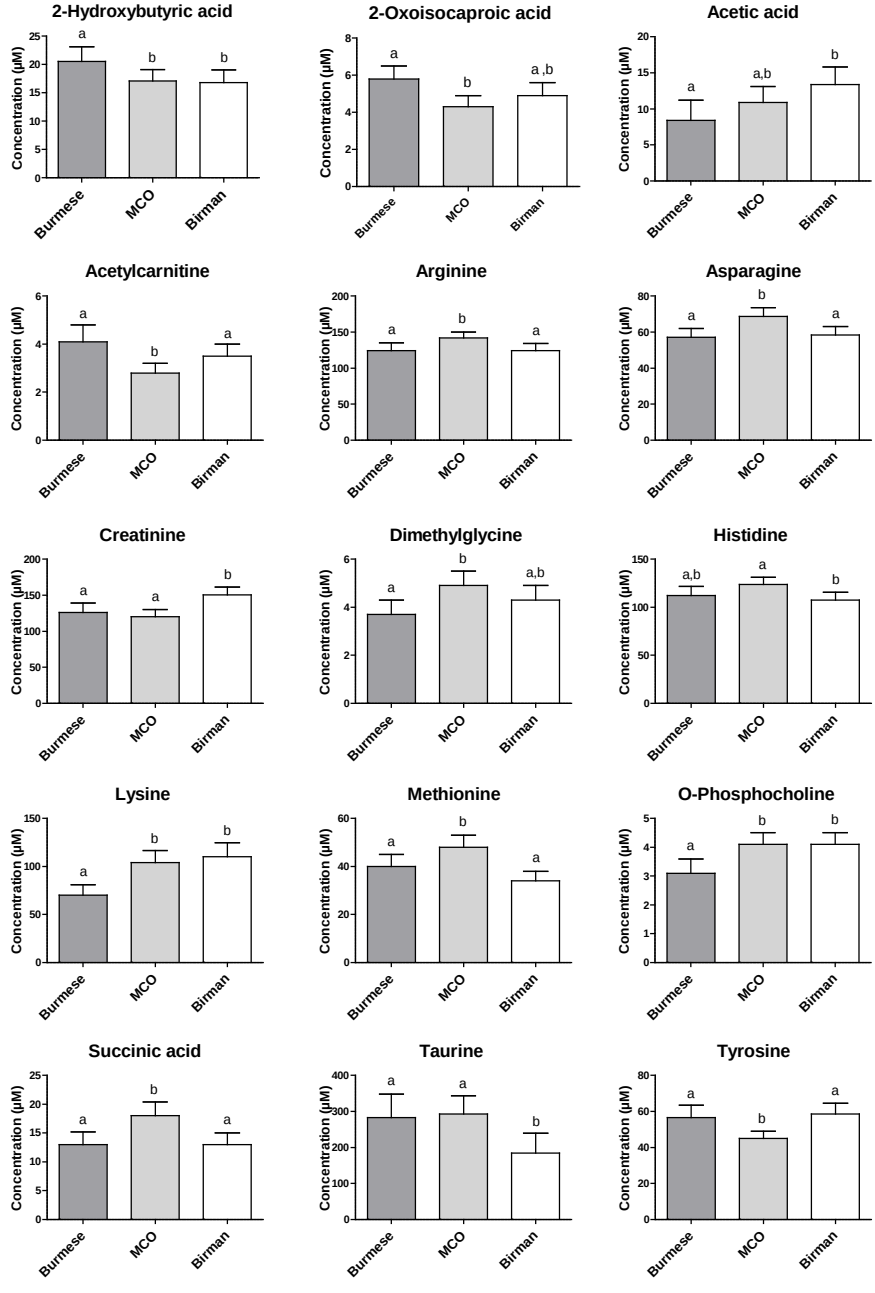

Figure 12. Metabolites that were discriminant between breeds after adjusting for weight and body condition. Different letters (a, b) indicate breeds that differ from other breeds at $P < 0.05$. Concentrations are shown as least square means, or geometric means, with 95% confidence intervals. MCO, Maine coon.

Breed differences were found also in the clinical biochemistry and hormonal immunoassay results. Breed ($P < 0.0001$) and weight ($P < 0.0001$) had significant overall effects in the multivariate model, but not BCS ($P = 0.14$). A significant model could be established for creatinine ($P < 0.0001$), fructosamine ($P = 0.004$), VLDL-TG ($P < 0.0001$), HDL-cholesterol ($P < 0.0001$), insulin ($P = 0.036$), adiponectin ($P < 0.0001$), and IGF-I ($P < 0.0012$), but not for ALAT ($P = 0.11$) and FFA ($P = 0.17$). The breed effects are shown in Figure 13. Apart from breed, some parameters were also affected by weight, or BCS. For example, overweight cats had 20.3 µmol/L higher average creatinine concentrations than normal weight cats. Fructosamine concentrations were affected only by weight, with an increase in concentration of 8.1 µmol/L per kg of weight ($P = 0.0015$). Concentrations of VLDL-TG increased with 27% per kg of body weight ($P < 0.0001$). Adiponectin concentrations were lower in Burmese cats than in MCO cats ($P < 0.0001$), and were also lowered by an increasing body weight, with –40.3 ng/mL ($P = 0.0003$) per kg. Weight influenced the IGF-I concentrations with a 33% increase per kg of body weight ($P < 0.0001$).

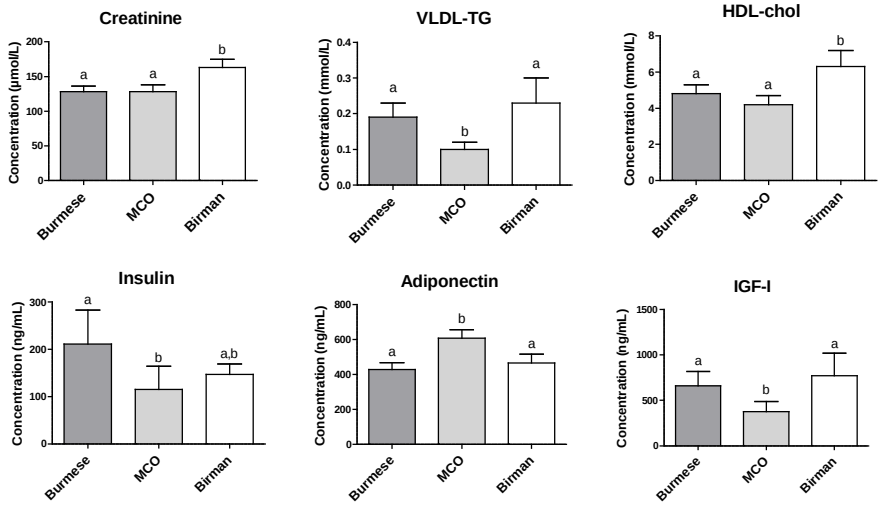

Figure 13. Serum biochemistry and hormonal concentrations that were discriminant between breeds after adjusting for weight and body condition. Different letters (a, b) indicate breeds that differ from other breeds at $P < 0.05$. Concentrations are shown as least square means, or geometric means, with 95% confidence intervals. MCO, Maine coon; VLDL, very low-density lipoprotein; TG, triglycerides; HDL, high-density lipoprotein; chol, cholesterol; IGF, insulin-like growth factor.

5 Discussion

The main aims of this thesis were to investigate risk factors, such as demographic and environmental factors, for DM in cats, and to increase understanding of disease pathogenesis. Better knowledge of predisposing factors can help cat owners and veterinarians to identify cats at risk of developing DM at an earlier stage, thereby increasing the possibilities to apply preventive measures to avoid disease occurrence, or delay its progression.

Although DM in cats was uncommon only a few decades ago, it is now recognised as one of the most common feline endocrinopathies. The incidence is suspected to increase, similar to what is observed among people. Our results indicate that overweight, as has been observed in people, is one of the major risk factors for developing feline DM.

The cat was probably domesticated around 10,000 years ago, at the time of the origin of agriculture and permanent human settlements, which created a new environment where both mice (*Mus musculus domesticus*), and subsequently wild cats, could thrive (Driscoll *et al.*, 2009a). It is likely that the domestication of cats led to fewer adaptations than in dogs, where several genes involved in starch digestion and fat metabolism have shown signals of selection, giving dogs a better ability to cope with an increasingly starch-rich diet (Axelsson *et al.*, 2013). Domestication of dogs was likely driven by artificial selection, with deliberate breeding by human intervention, while the domestic cat was a product of natural selection, without human intervention. Artificial selection to produce modern cat breeds has only occurred during the last 200 years (Driscoll *et al.*, 2009b).

With the urbanisation process in the last century, the cat has transformed from being an active, outdoor hunter to a sedentary, indoor pet. Domestic cats are often fed commercially produced diets, often in excessive amounts and consisting of less protein and more carbohydrates than is found in their natural diet. Altogether, these changes are hypothesised to play a role in the development of feline DM (Slingerland *et al.*, 2009; Zoran & Buffington, 2011).

5.1 Incidence of diabetes mellitus in Swedish cats (paper I)

We started by investigating the general incidence of DM in a large cohort of Swedish cats, to be able to make comparisons between breeds, age groups, and sex. We did not identify any increase in incidence over time during the years 2009 to 2013, but the study period might have been too short to enable us to document a potential increase. It is also possible that the suspected increase in incidence might have already taken place, and that a plateau now has been reached. However, we were able to identify several cat breeds at either an increased, or a decreased, risk for DM. The top risk breed identified in this study was the Burmese cat, a breed that has been identified previously as a high-risk breed for DM (Rand *et al.*, 1997; McCann *et al.*, 2007; Lederer *et al.*, 2009).

We also identified three previously unreported high-risk breeds for DM (the Russian Blue, Norwegian Forest cat, and Abyssinian), as well as breeds with a decreased risk of DM (the Bengal, Birman, Persian, Ragdoll, and British Shorthair breeds).

In all breeds analysed except the Burmese, there were more male than female cats with DM, similar to previous reports (Panciera *et al.*, 1990; McCann *et al.*, 2007; Prahl *et al.*, 2007; Lederer *et al.*, 2009). The male predilection for DM can be explained in several ways. First, male cats are prone to develop obesity (Lund *et al.*, 2005). Secondly, male cats tend to have lower insulin sensitivity than female cats (Appleton *et al.*, 2001). The lack of sex predilection in the Burmese breed indicates that the pathogenesis of DM might differ from that in other breeds, and we therefore decided to study the metabolism in the Burmese breed in study IV.

Neutering is another factor that has been associated with an increased risk of both obesity and DM. We were not able to investigate the effect of neutering in study I, since there was no reliable information on neutering status available in the database. If the tendency to neuter male cats was higher than for female cats, this could influence the male predilection found. Therefore, we decided to investigate more potential risk factors for DM, such as neutering, in study II.

Age was associated with DM risk, with a peak incidence in middle-aged cats. We speculated if this could be due to a simultaneous increase in the incidence of obesity, most often seen in middle-aged cats (Lund *et al.*, 2005). The hypothesis that some risk factors identified for DM are also risk factors for overweight and obesity, was investigated further in study III. The demographic risk factors age, sex and breed investigated in study I are all examples of non-modifiable risk factors, *i.e.* factors that cannot be changed in the individual animal. We also examined modifiable risk factors, where owners can make adjustments to decrease the risk of future development of DM (study II).

5.2 Environmental risk factors for diabetes mellitus (paper II)

Diabetes mellitus develops when the insulin-producing β-cells cannot keep pace with the cells' demand for insulin. For feline DM and human T2DM, this is associated with exhaustion of the β-cells in conjunction with insulin resistance (Rand, 1999; Chatterjee *et al.*, 2017). Most often, there is more than one factor contributing to the insulin resistance. To decrease insulin resistance, one or more of these contributors need to be identified and addressed. Knowledge of the modifiable risk factors in the individual cat is important. In study II, we examined modifiable risk factors for DM in cats, by inviting owners of all diabetic cats that participated in study I to complete a web survey. As a control group, a larger cohort of non-diabetic cats were selected from the same database, matched only on birth year with the diabetic cats to avoid introducing unnecessary bias due to matching (Rothman, 2012).

Our results highlight the importance of overweight as a risk factor for DM, in concordance with other studies on both cats and people (Scarlett & Donoghue, 1998; German, 2006; McCann *et al.*, 2007; Chen *et al.*, 2012; Chatterjee *et al.*, 2017). Since there was an interaction present between BCS and type of diet fed to the cats, these two variables needed to be interpreted together. Overweight was associated with an increased risk of DM, regardless of type of diet. The effect of diet on DM risk was significant only within the group of normal weight cats, where cats on a diet consisting predominantly of dry food had a fourfold increased risk of DM compared to cats on a wet food diet. There was no significant effect of type of diet for the group of overweight cats, possibly reflecting the higher impact of the overweight itself rather than the effects of diet. This study is, to our knowledge, the first study to report dry food as a potential risk factor for DM in cats. In people, availability of sugar is a significant statistical determinant of DM prevalence rates worldwide (Basu *et al.*, 2013).

Cats are obligate carnivores, and their original diet consists of small prey, with a naturally low carbohydrate content (Plantinga *et al.*, 2011). Even though cats were domesticated around 10,000 years ago, they have not been intentionally bred for form or function, instead selection was often based on simple traits such as coat colour or pattern. Unlike the dog, where some breeds are thousands of years old, most cat breeds were developed within the past 150 years, mainly in Europe and in the United States (Lipinski *et al.*, 2008). It is unlikely that a selection for breed-specific exterior traits would have resulted in major changes in the physiology (Plantinga *et al.*, 2011). Likely, cats have not yet adapted metabolically to the modern commercial dry food diets, with its often high carbohydrate content, served to many of our cats today. For example, cats lack the gene for sweet taste, and subsequently also sweet taste receptors.

They also lack salivary amylase, the enzyme that initiates digestion of starch, and have low concentrations of intestinal amylase and disaccharidases. In the liver, the functioning levels of hepatic glucokinase are minimal. Glucokinase is the enzyme that phosphorylates glucose to glucose-6-phosphate for further metabolic processing such as glycogen formation. There are also minimal functioning concentrations of hepatic glycogen synthetase, the enzyme that converts glucose into glycogen, in the feline liver. Finally, cats lack fructokinase and thus the ability to metabolise fructose (Zoran, 2010). Altogether, this indicates that cats are not adapted to handle carbohydrates to the same extent as omnivores, such as dog and man. Cats have a rather limited capacity to metabolise sugars, resulting in a slower glucose clearance from blood than dogs after ingesting a high-starch or glucose-loaded meal (Kienzle, 1994; Hewson-Hughes *et al.*, 2011b). It has been suggested that high-carbohydrate diets fed to cats might contribute to the development of DM by a gradual exhaustion of the β-cells due to the increased insulin production in response to the elevated plasma glucose concentrations, and by a direct glucotoxic effect on the pancreas with apoptosis of the β-cells (Hewson-Hughes *et al.*, 2011a).

It is unclear from study II if the association between dry food and DM can be separated from dry food as a potential risk factor for obesity, even if the cats in which the association was seen were subjectively judged as being normal weight by the owners. We decided to analyse data from the non-diabetic cats from this study in a new study (study III), where we investigated factors associated with overweight, to add information on the relationships between exposure (*e.g.* diet, overweight) and outcome (*e.g.* overweight, DM). These relationships are illustrated with a simple directed acyclic graph (DAG) in Figure 14.

Figure 14. A schematic directed acyclic graph (DAG), used in epidemiology to help adjusting for and identifying potential confounders and minimise bias. For feline diabetes mellitus (DM), it is not known if type of diet has a direct effect on DM risk, or if it is mediated by an increased risk of obesity, or both.

The DAG approach can be used to help identify covariates which should be included in the statistical approach to adjust for confounders and minimise bias (Shrier & Platt, 2008).

Being considered a greedy eater was associated with an increased risk of DM. This is another example of a variable that also could be affecting DM risk via an increased risk for overweight, or by affecting both DM and overweight risks. The potential effect from eating behaviour on body condition is addressed in study III.

Finally, we found that indoor cats more often developed DM than outdoor cats. Again, there was an interaction present between indoor confinement and access to the outdoors with the cats' reported activity level. Indoor confinement was a risk factor for DM compared to being allowed outdoor access for all cats except those reported to be very active by their owners, where no differences could be seen. The protective effect from being an outdoor cat is likely mediated via a higher amount of exercise compared to indoor cats, but other factors, such as an opportunity to hunt, are also plausible as explanations for this finding. A lack of activity and exercise have been associated with an increased risk of feline DM and T2DM in people (McCann *et al.*, 2007; Slingerland *et al.*, 2009; Chatterjee *et al.*, 2017), while physical activity of moderate intensity, such as regular walking, has been shown to reduce the risk of T2DM in people (Jeon *et al.*, 2007).

5.3 Prevalence and factors associated with overweight in cats (paper III)

Since overweight is an important risk factor for feline DM, we wanted to investigate if the risk factors we identified for DM in study I and II would also be associated with overweight. For example, we wanted to investigate if the breed predisposition found to DM could be due to a propensity for overweight in these breeds. We used cats from two cohorts for study III, the first set of data consisted of medical records obtained from cats visiting the University Animal Hospital, and the second data set consisted of questionnaire data from the non-diabetic control group used in study II. The two groups are not directly comparable due to differences in the study populations. In the medical records group, the BCS was assessed by a veterinarian or veterinary student, whereas in the questionnaire group, the owners themselves judged their pet's BCS. Owners are known to underestimate their pet's body condition, and our belief is that this was reflected in the lower prevalence of overweight found in this group (22%), as compared to the medical records group (45%). A high prevalence of overweight among domestic cats, as in the medical records cohorts in the present study, has been reported previously (Sloth, 1992; Scarlett *et al.*, 1994; Colliard *et al.*, 2009; Courcier *et al.*, 2010; Cave *et al.*, 2012; Vandendriessche *et al.*, 2017).

Another complicating factor is the possibility that the increasing occurrence of overweight and obesity in both animals and people, can affect and alter our opinion of what is in fact a normal body condition. An association between overweight and a diagnosis of DM was observed in cats from the medical records cohort. This finding is similar to results presented in previous reports (Scarlett & Donoghue, 1998; German, 2006), and results from study II.

In cross-sectional studies, like the present, it is not possible to determine causality, as all variables are estimated at the same point of time. Therefore, only associations can be evaluated. Another drawback is the potential problems with recall bias, for data drawn from the questionnaire, and selection bias, for the medical records data. Finally, estimating a pet's body condition is a subjective measurement, with a potential for inter-observer variability, and also a risk that uneducated owners are unable to correctly asses their pets' BCS.

We were unable to distinguish any cat breed as predisposed to overweight. However, the Birman and Persian cat breeds had a decreased risk of being overweight. Based on analyses presented in paper I, these two breeds were also identified as low-risk breeds for DM. When comparing all domestic cats with all purebreds, the domestic cats were more prone to overweight than the purebreds, similar to previous findings (Lund *et al.*, 2005; Colliard *et al.*, 2009; Teng *et al.*, 2017). Interestingly, the domestic cats were also at a higher risk for DM when compared with all purebred cats, as shown in study I.

Type of diet was associated with risk of overweight, with cats eating predominantly dry food more often being overweight than cats eating predominantly wet food. The study population in the questionnaire cohort consisted of mainly adult, neutered cats, and conclusions should not be drawn for cats outside these criteria. However, studies on younger cats have shown similar results, with feeding a dry food diet as a risk factor for overweight (Rowe *et al.*, 2015; Rowe *et al.*, 2017). Dry food is typically more energy dense than wet food, which can help explain these findings (Zoran, 2002). The nutritional content in the food given to cats in this study is unknown, but in general, there is a higher carbohydrate content in dry food than in wet food (Villaverde & Fascetti, 2014). The higher water content in wet food has also been shown to decrease energy intake and body weight in cats (Wei *et al.*, 2011). Cats eat approximately the same amount of food (g/kg body weight) irrespective of which diet they are given (Morris *et al.*, 2006), which will result in a higher energy intake if the diet is more energy dense, as most often is the case for dry food.

Moreover, feeding cats one of two diets with the same amount of calories, but different protein and carbohydrate content, led to weight loss for cats fed the high-protein, low-carbohydrate diet, as compared to the low-protein, high-carbohydrate version (Hill *et al.*, 2015). High-carbohydrate diets resulted in

higher postprandial glucose and insulin concentrations, and the duration of postprandial glycaemia in cats was markedly longer compared with durations observed in dogs and humans (Coradini *et al.*, 2011; Farrow *et al.*, 2012). This is likely a reflection of the cat's strict carnivorous nature, and cats do not appear to have any dietary requirements for carbohydrates (Macdonald *et al.*, 1984). Cats have as previously discussed very low hepatic glucokinase activity, which partly explains the cat's lower ability to handle glucose. Glycaemia increases pancreatic β-cell insulin production. Insulin is an anabolic hormone that promotes uptake of glucose from the blood into fat, liver and skeletal muscle tissues, where the glucose is converted into either glycogen in skeletal muscle, or stored as fat in adipose tissue, or both in the liver. In people, consumption of sugar (as in sugar-sweetened beverages) can lead to weight gain and increase the risk of obesity, and also increase the risk of developing T2DM (Malik *et al.*, 2006; Hu & Malik, 2010; Malik *et al.*, 2010; Hu, 2013).

It is estimated in people that approximately 30% to 40% of the variance in BMI can be attributed to genetics, and 60% to 70% to the environment (Pi-Sunyer, 2002). If the situation for cats is similar is unknown, but modifiable factors such as diet and exercise are likely of high importance also for cats.

5.4 The metabolic profile of Burmese cats (paper IV)

The metabolic profile of the Burmese breed was examined, and compared to that of the Birman and MCO breeds, using NMR spectroscopy, routine serum biochemistry, and hormone immunoassays. Metabolomics is a powerful tool for investigating metabolites in biological samples. From obtained data it is possible to draw conclusions regarding the individual's metabolism status and potential alterations from normal levels. Significant breed differences were observed from the NMR data, serum biochemistry and hormonal assays.

The pattern of metabolites in the Burmese breed resembled that encountered in people with insulin resistance. For example, Burmese cats had higher concentrations of 2-hydrobutyric acid and tyrosine, and lower concentrations of dimethylglycine (Gall *et al.*, 2010; Stančáková *et al.*, 2012; Magnusson *et al.*, 2015). 2-Hydroxybutyric acid has been suggested useful as an early indicator of insulin resistance in non-diabetic people, and elevated serum concentrations also predicted a worsening of glucose tolerance (Gall *et al.*, 2010; Ferrannini *et al.*, 2013). Increased tyrosine concentrations predicted future T2DM in people in another study (Stančáková *et al.*, 2012). It is unknown if the Burmese cats in our study were in fact insulin resistant or not, since we did not include any interventions to assess insulin sensitivity in the study protocol.

Concentrations of 2-oxoisocaproic acid were also higher in Burmese cats compared to MCO cats. 2-Oxoisocaproic acid, or alpha-ketoisocaproic acid, is an intermediate in the metabolism of leucine, a branch-chained amino acid associated with insulin resistance and T2DM in people according to several studies (Shaham *et al.*, 2008; Wang *et al.*, 2011; Menni *et al.*, 2013; Guasch-Ferré *et al.*, 2016). High concentrations of 2-oxoisocaproic acid have been shown to induce insulin secretion in rat islets, and stimulation of insulin secretion could with time promote DM development via exhaustion of the β-cells (Gao *et al.*, 2003; Wang *et al.*, 2011). The Burmese cats also had higher concentrations of insulin than the other breeds, although the difference was significant only compared with the MCO. Moreover, Burmese cats had lower adiponectin concentrations than the MCO breed. Adiponectin is an adipocyte-derived hormone, an adipokine, with an inverse correlation with obesity in both cats and people (Hoenig *et al.*, 2007; Ishioka *et al.*, 2009; Muranaka *et al.*, 2011; Tvarijonaviciute *et al.*, 2012; Okada *et al.*, 2017), although conflicting results exist in cats (Bjornvad *et al.*, 2014; Witzel *et al.*, 2015). In people, adiponectin regulates glucose metabolism by stimulating FFA oxidation, glucose uptake and reducing gluconeogenesis, thereby increasing insulin sensitivity (Yamauchi *et al.*, 2002; Kadowaki *et al.*, 2006; Gao *et al.*, 2013). Adiponectin has been suggested to play a role in explaining why some species, like cats and people, develop T2DM in association with obesity, whereas others, such as dogs and horses, do not (Verkest & Bjornvad, 2012).

The Burmese cats included in our study had metabolic patterns compatible with insulin resistance patterns in people, however, we did not assess insulin sensitivity directly in these cats. Further studies investigating the predisposition of Burmese cats to DM is warranted. We did not detect any breed differences in the concentrations of FFA, which was somewhat unexpected, since it is the increased availability of FFA in people with hypertriglyceridemia due to obesity or T2DM that leads to the deleterious effects on insulin uptake by the liver. The increased hepatic gluconeogenesis and glucose release that occurs creates a vicious circle of hyperglycaemia, further augmenting the insulin resistance (Kopelman, 2000; Subramanian & Chait, 2012). Hypertriglyceridemia alone has been suggested, in some studies, to cause insulin resistance even without concomitant obesity or T2DM (Steiner & Vranic, 1982). Beta-oxidation of FFA causes increased intracellular content of metabolites which may interfere with insulin-receptor signalling, thereby contributing to the insulin resistance found in tissue (Kahn *et al.*, 2006). There is a theory that the dyslipidaemia in Burmese cats could be caused by a deficient lipoprotein lipase (LPL), the enzyme responsible for hydrolysing TG into FFA for subsequent adipose and skeletal muscle tissue utilisation. A deficient LPL would likely have led to a decreased,

rather than increased, β-oxidation. Abnormalities in LPL function have also been associated with insulin resistance and T2DM in people (Mingrone *et al.*, 1999; Mead *et al.*, 2002). To measure LPL concentrations it is necessary to administer heparin intravenously to release the enzyme into the circulation (Jeppesen *et al.*, 1995), and therefore assessment of LPL activity was not performed in our study.

6 Conclusions and future remarks

The hypothesis underlying this thesis was that the change in lifestyle of domestic cats, which has occurred during the last century, would be associated with both obesity and DM.

In this thesis, we have shown that:

- The general incidence of DM in insured, Swedish cats was 11.6 cases per 10,000 CYAR.
- Several cat breeds displayed an increased risk of developing DM, with the Burmese breed at a fivefold risk (IRR 5.2; 95% CI, 3.6–7.6) compared to other purebred cats.
- Middle-aged, male cats were at increased risk of both overweight and DM.
- Indoor confinement and inactivity were associated with an increased risk of DM, and overweight, respectively.
- Being fed predominantly dry food was associated with an increased risk of being overweight. In normal-weight cats, there was an association between eating predominantly dry food and an increased risk of DM.
- Cats visiting an animal hospital had a high prevalence of overweight (45%).
- For cats scored by their owners, one out of five cats (22%) was considered overweight.
- The metabolic profile of Burmese cats resembled that of people with insulin resistance.

Since obesity is an important risk factor for DM in cats and T2DM in people, we studied factors associated with both overweight and DM in this thesis. Cats from several breeds displayed an increased risk of developing DM, with the Burmese breed as the most predisposed with a fivefold increased risk compared to other purebred cats. We were not able to detect any breed associated with overweight, but on the other hand, the Birman and Persian cat breeds had lower risk for overweight than other breeds, and also a decreased risk for DM.

Age, breed and sex are examples of non-modifiable risk factors, important to recognise, but obviously unfeasible to adjust in the individual animal. Modifiable risk factors, on the other hand, are possible for the veterinarian and cat owner to address. We confirmed that overweight is an important risk factor for feline DM. The prevalence of overweight varied between groups of cats. Cats visiting an animal hospital were scored by either a veterinarian or a veterinary student, and showed a high prevalence of overweight with almost every other cat considered overweight. In contrast, for cats scored by their owners, one out of five cats was considered overweight. We believe that this discrepancy is mainly due to an inability of cat owners to truly apprehend a problem with overweight in their pets. When increasing numbers of pets and people become overweight or obese, there is a risk that our perception of what is actually normal is altered. Therefore, it is important for veterinarians to assess the BCS in all pets to make owners aware of their pet's current and ideal body condition, and to address deviances properly.

A new finding was the association between feeding predominantly dry food, and an increased risk of DM in middle-aged, neutered cats, judged as being normal weight by their owners. We also detected an association between eating mainly dry food and being overweight. Cats are obligate carnivores, adapted for hunting, and eating, small prey. During the last decades, there has been a change in the cats' diet, with the introduction of commercial pet foods, often with a higher carbohydrate content in comparison with the cats' natural diet. Simultaneously, there has been a shift from a predominantly outdoor environment for domesticated cats to an indoor lifestyle, with a decreased physical activity, because cats no longer need to hunt to obtain food (Verbrugghe *et al.*, 2012). The possible association between dry food and both overweight and DM as indicated by our studies, warrants further attention, since dry food is fed to a vast number of cats all over the world.

In people, despite the fact that genetic association studies so far have identified more than 100 gene loci influencing the risk of T2DM, these only have small to moderate effects on the individual's susceptibility to the disease (Gaulton, 2017). In cats, the genetic predisposition to DM is mainly unknown, however, the breed predisposition seen is an indicator of a genetic influence. Therefore, other factors influencing the DM risk are important to acknowledge. Demographic factors such as age, breed, and sex, are non-modifiable, but knowledge of both modifiable and non-modifiable risk factors is important. It is the sum of all factors contributing to insulin resistance that in susceptible individuals, will contribute to the development of disease. In people, most of the identified genetic risk loci are associated with β-cell function. It is not unlikely that the same is true for cats. Further studies are needed to investigate the β-cell

function in cats with or without DM, for example using gene expression studies. For individuals with an increased susceptibility to DM and T2DM, it is important to recognise all potential risk factors, and to address the modifiable factors to possibly avoid disease development. We have shown that overweight, a lack of exercise, and possibly dry food, are examples of modifiable risk factors for DM in cats.

Finally, we investigated the predisposition of Burmese cats to develop DM by characterising their metabolic profile and compare it with that of two other cat breeds, of low or medium risk of DM. Results from NMR spectroscopy, serum biochemistry and hormone immunoassays showed a metabolic profile of the Burmese cats resembling that of people with insulin resistance. Differing concentrations of several NMR metabolites, as well as increased concentrations of insulin, and lower concentrations of adiponectin, were found in the Burmese cats compared to other breeds. Further investigations are necessary to elucidate the factors and mechanisms underlying the predisposition of Burmese cats to develop DM.

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Popular science summary

Diabetes mellitus (DM) in cats is similar to type 2 diabetes in people. Cats and humans share many risk factors for the disease, such as obesity and a sedentary lifestyle. In people, ethnicity has been associated with an increased risk of DM. In cats, a breed predisposition has been described, with the Burmese breed at increased risk. The main aim of this thesis was to investigate risk factors for DM in cats.

We used records from a pet insurance company to investigate how common the disease is in Swedish cats. We also investigated if the risk of DM varies with age, sex and breed of the cats. The highest risk of DM was seen in 13-year old cats. Male cats developed DM twice as often as female cats. The Burmese breed, along with the Russian Blue, Abyssinian, and Norwegian Forest cat breeds, had an increased risk of DM, while cats from several other breeds had a lower risk.

We invited owners of diabetic and non-diabetic cats, insured by the same insurance company, to participate in a web survey. The survey contained questions on *e.g.* body condition, eating behaviour, activity levels, type of diet, and if the cat was an indoor or outdoor cat. We found that overweight cats had an increased risk of developing DM. Cats described as being greedy eaters by their owners also had a higher risk of developing disease. On the other hand, access to the outdoors was protective against DM. In normal weight cats, there was an increased risk of DM in cats eating mostly dry food, compared to wet food.

Since overweight turned out to be a strong risk factor for DM, we decided to study factors associated with overweight. We found several risk factors that were similar for both DM and overweight, such as eating mainly dry food, being male, middle-aged, and considered a greedy eater. Birman and Persian breeds, with a decreased risk of DM, were less often overweight.

Finally, we studied the metabolism in cats of the Burmese breed. There were differences in the metabolic profiles between the Burmese, Birman, and Maine coon cat breeds, with a pattern in the Burmese cats resembling people with

insulin resistance. Insulin resistance is a condition usually seen in people before type 2 diabetes develops.

In conclusion, both new and previously reported factors associated with DM in cats were identified. The Burmese breed predisposition was verified, and three new breeds with an increased risk of DM were identified. We could confirm that overweight is an important risk factor for DM in cats. Being a middle-aged, male, inactive cat increased the risk of both overweight and DM. Knowledge of predisposing factors can help owners and veterinarians to identify cats at risk, enabling a possibility to prevent disease or delay its development.

Populärvetenskaplig sammanfattning

Diabetes mellitus (DM) hos katt liknar människans typ 2-diabetes. Katter och människor delar flera riskfaktorer för sjukdomen, såsom övervikt och en stillasittande livsstil. Hos människor har etnicitet visat sig ha ett samband med risken för DM. Hos katt har vissa raser, som t.ex. burma, en ökad risk för DM. Det huvudsakliga syftet med denna avhandling har varit att undersöka riskfaktorer för DM hos katter.

Vi använde statistik från ett djurförsäkringsbolag för att undersöka hur vanlig sjukdomen är hos våra svenska katter. Vi undersökte också om risken för DM varierade med katternas ålder, kön och ras. Störst risk för DM sågs hos katter vid 13 års ålder. Hankatter utvecklade DM dubbelt så ofta som honkatter. Katter av raserna burma, russian blue, abessinier och norsk skogkatt hade en ökad risk för DM, medan flera andra raser hade en minskad risk.

Vi bjöd in ägare till katter med och utan DM, försäkrade i samma försäkringsbolag, att delta i en enkätundersökning. Enkäten innehöll frågor om t.ex. kattens hull, ätbeteende, aktivitetsnivå, om katten var inne- eller utekatt, och typ av foder som katten åt. Vi fann att överviktiga katter hade en klart förhöjd risk att drabbas av DM. Katter som beskrevs av sina ägare som glupska hade också en förhöjd risk för sjukdomen. Att vara utekatt var däremot skyddande mot DM. I gruppen normalviktiga katter hade katter som åt mestadels torrfoder en ökad risk för DM jämfört med katter som åt mest blötmat.

Eftersom övervikt visade sig vara en så stark riskfaktor för DM, studerade vi vilka faktorer som hade ett samband med övervikt. Vi fann flera faktorer som var desamma för både DM och övervikt, som t.ex. att vara hankatt, medelålders, glupsk, och att äta mestadels torrfoder. Katter av raserna birma och perser hade en lägre risk för DM, och var också mer sällan överviktiga.

Slutligen undersökte vi ämnesomsättningen hos katter av rasen burma jämfört med katter av raserna birma och maine coon. Vi såg skillnader i ämnesomsättningen mellan raserna, med ett mönster hos burmorna som liknar det som

ses hos människor med insulinresistens. Insulinresistens är ett tillstånd som vanligtvis ses hos människor innan typ 2-diabetes utvecklas.

Sammanfattningsvis har vi identifierat både nya och tidigare rapporterade faktorer som har ett samband med DM hos katt. Vi kunde bekräfta att rasen burma är predisponerad för DM, och vi identifierade tre nya raser med en ökad risk för DM. Att vara en medelålders, inaktiv hankatt ökade risken för både övervikt och DM. Kunskap om vilka faktorer som bidrar till att öka risken för DM är viktiga att känna till, för att tidigare kunna hitta de katter som har en förhöjd risk, och därmed ge veterinärer och kattägare en möjlighet att förebygga sjukdomen eller fördröja sjukdomsdebuten.

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