# Risk factors

# for

# feline diabetes mellitus

**Lars Slingerland**

**2008**

# Risk factors for feline diabetes mellitus

**Risicofactoren voor diabetes mellitus bij de kat**
(met een samenvatting in het Nederlands)

**Proefschrift**

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door
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# 1

# Aims and scope of the thesis

The general aim of the studies presented in this thesis was to increase our understanding of certain risk factors for the development of feline diabetes mellitus (DM).

Several aspects of (feline) DM are discussed in the general introduction (**Chapter 2**). First, an introduction to β-cell function and insulin action is discussed together with some methods for their assessment. Then the classification and the pathophysiology of DM are discussed and finally the *metabolic syndrome*, as defined in human medicine, is introduced and the dietary strategies to prevent and treat this syndrome and DM are addressed.

New techniques for assessing measures of glucose homeostasis and arterial blood pressure in cats are described in **Part I of the thesis**. The hyperglycemic glucose clamp (HGC) is the gold standard test to assess β-cell secretion *in vivo* in humans, but its application in cats has not previously been described. **Chapter 3** reports the use of the HGC in conscious, healthy cats. The results of the HGC are compared with those of intravenous glucose tolerance tests in the same cats.

Correct performance of the HGC requires frequent arterial blood sampling and arterial catheterisation is necessary to accomplish this in cats without causing stress. Repeated catheterisation is necessary for follow-up studies and thus the method of catheterisation should not sacrifice the catheterised artery. In **Chapter 4** such a catheterisation technique is described using a catheter that also allows direct measurement of arterial blood pressure (ABP) in conscious cats. ABP was measured continuously in resting, inactive but attentive, and active cats. In addition, the effect on the ABP of short-term interaction with a familiar and an unfamiliar person was evaluated.

Although the HGC is the gold standard test for measurement of β-cell secretion, it is time-consuming and costly and hence its use is mainly restricted to research. Ideally, tests used to assess β-cell function in a clinical setting should cause minimal discomfort and should be quick, easy to perform, and inexpensive. The universal secretogogue $Ca^{2+}$ has been demonstrated to cause a transient increase in β-cell insulin secretion *in vitro* and *in vivo*. This has raised the question whether intravenous calcium administration could serve as a reliable stimulus to evaluate insulin secretion. In **Chapter 5** the results of intravenous calcium stimulation tests are compared with the results of the HGC.

Risk factors for the development of diabetes mellitus are studied in **Part II of the thesis**. When cats with DM are classified according to the scheme for classifying DM in humans, most are found to have type 2 DM. However, in a significant minority of them the disease is classified as an "other specific type" of DM. A well-known disorder causing DM in cats is acromegaly. This pituitary disorder causes DM through the development of insulin resistance. Sixteen diabetic cats with varying insulin requirements were studied for concurrent (pituitary) disease (**Chapter 6**).

There are only anecdotal reports of the treatment of acromegalic cats with analogues of the hypophysiotrophic hormone somatostatin. The effect of administering the short-acting somatostatin analogue octreotide on plasma concentrations of several pituitary hormones, insulin-like growth factor-1, and cortisol was studied in 16 diabetic cats (**Chapter 6**).

Cats are true carnivores and their original diet of prey animals contained only small amounts of carbohydrate. The high-carbohydrate intake that is associated with consumption of most of the industrially manufactured dry cat foods has been postulated to be a risk factor for

the development of feline DM. This assumes that the increased demand for insulin secretion leads to the exhaustion and loss of β-cells. In addition, domestication and urbanisation have reduced physical exercise considerably, which is known to be a risk factor for DM in humans. In a questionnaire-based retrospective case-control study these two risk factors were studied in cats (**Chapter 7**).

To further investigate the role of dietary macronutrients in the development of feline DM, the effects of feeding 3 different diets (high-fat, high-carbohydrate, and high-protein) for 9 months on glucose-induced insulin secretion, glucose disposal, insulin sensitivity, blood pressure, and body composition were studied in a prospective, longitudinal study, using the techniques described in chapters 3 and 4 (**Chapter 8**).

In **Chapter 9** the results are summarised and discussed.

# 2

# General introduction

Diabetes mellitus (DM) is a common endocrine disorder in cats, with a reported incidence of 2.45 cases per 1,000 cat-years-of-risk.[1] As in humans, its incidence in cats is believed to be increasing.[2] An expert committee on the diagnosis and classification of human DM has defined DM as a group of metabolic diseases characterized by persistent hyperglycemia (high blood glucose concentrations) caused by defects in insulin secretion, insulin action, or both.[3]

*Insulin secretion*

Insulin is synthesized and secreted by the β-cells of the islets of Langerhans, which are small 'islands' of endocrine tissue scattered in a 'sea' of exocrine pancreas tissue. In addition to β-cells, the islets of Langerhans contain α-cells, δ-cells, and PP-cells, which secrete glucagon, somatostatin, and pancreatic polypeptide, respectively. It is the prevailing insulin:glucagon ratio that influences carbohydrate metabolism. However, defects and loss of β-cells are important factors in the pathophysiology of DM and therefore research has focused mainly on insulin and the β-cell as opposed to glucagon and the α-cell.

Insulin is synthesized by transcription and translation of the insulin gene. This process produces preproinsulin, which is subsequently converted into proinsulin. Proinsulin is stored in secretory granules and cleaved into insulin and C-peptide. Insulin and C-peptide are released by exocytosis. Also present in these granules is islet amyloid polypeptide (IAPP), also known as amylin. This hormone is thus co-secreted with insulin and C-peptide. At least three pools of secretory granules can be distinguished in the β-cell. There is a pool docked to the plasma membrane and a large non-docked reserve pool (Figure 1). The docked pool can be subdivided into a pool that is not yet ready for release and one that is readily releasable.[4]

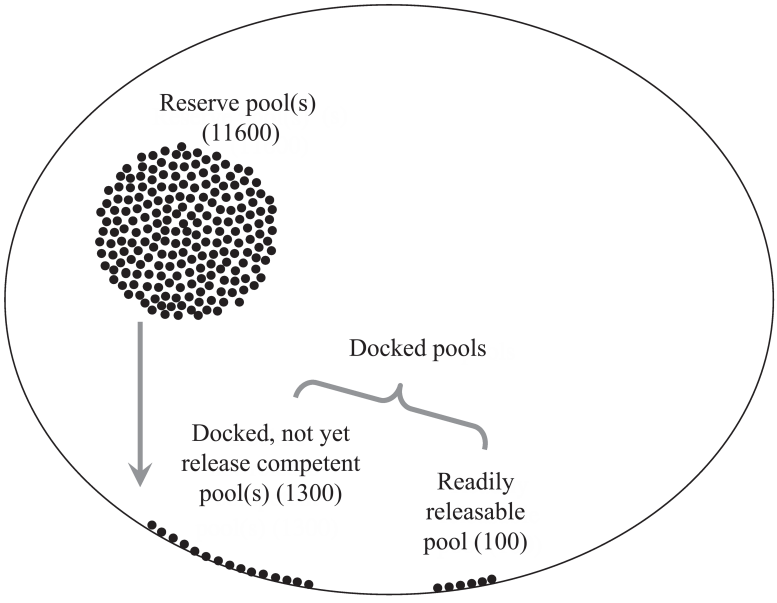


**Figure 1** Schematic overview of the pools of secretory insulin granules. The numbers in brackets are the numbers of granules in β-cells of mice. Adapted from Bratanova-Tochkova et al.[4]

Secretion of insulin is a tightly regulated process triggered by a variety of stimuli, the most important being glucose (Figure 2). Contrary to what might be expected, there is no 'glucose-receptor' on the cell membrane of the β-cell. Instead, glucose is transported into the β-cell by glucose transporter 2 (GLUT2) and subsequently metabolised, mainly through glycolysis and subsequently the tricarboxylic acid cycle. This leads to an increase in the ATP:ADP ratio, resulting in closure of ATP-sensitive potassium channels and consequently depolarization of the β-cell. The depolarisation of the β-cell allows $Ca^{2+}$ influx through type L voltage-dependent $Ca^{2+}$ channels. The thereby increased cytosolic $Ca^{2+}$ concentration is the trigger for the docked, readily-releasable pool of secretory granules to fuse with the β-cell plasma membrane, which completes the release of insulin into the extracellular space.[5]

Aside from glucose, other nutrients, hormones, neurotransmitters, ions, and drugs can stimulate insulin secretion, sometimes in a glucose-dependent way. For example, fatty acids stimulate insulin secretion through an incompletely understood process that requires their oxidation.[6] The amino acid leucine stimulates insulin release via the same pathway as glucose, whereas arginine directly depolarises the β-cell. Sulfonylurea drugs such as glipizide and glibenclamide depolarise the β-cell through activation of a protein termed the sulfonylurea-receptor, which is directly attached to the ATP-sensitive potassium channels.[7] The neurotransmitter acetylcholine and the gastrointestinal hormone cholecystokinin increase intracellular $Ca^{2+}$ concentration in the β-cell by inducing the release of $Ca^{2+}$ from the endoplasmic reticulum, via generation of the second messengers inositol triphosphate and diacylglycerol.[5] Although the exact mechanism is not understood, an abrupt increase in the extracellular $Ca^{2+}$ level leads to a brief elevation of cytosolic $Ca^{2+}$ concentration and a transient increase in insulin secretion.[8] Other gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) increase *glucose-induced* insulin secretion by various mechanisms.[9]

As mentioned above, a cascade of processes leads to the exocytosis of insulin-containing granules by raising cytosolic $Ca^{2+}$ concentration in the β-cell. The exocytosis process itself can be modulated by many of the stimuli mentioned in the previous paragraph. In addition, some of these stimuli influence insulin synthesis by affecting insulin gene transcription, preproinsulin mRNA stability, translation of preproinsulin mRNA, and the 'maturation' of secretory granules.[10]

β-cells are equipped with insulin receptors, and insulin receptor function is of great importance to foetal (and presumably adult) beta cell growth and function.[11] The β-cell response to glucose bears a close relation to the sensitivity of various tissues to insulin's actions. Whether this is related to an effect of the β-cell insulin receptor signalling pathways is unknown. Insulin secretion should therefore be assessed in the context of the prevailing insulin sensitivity.[12]

*Insulin action*

After release from the β-cell by exocytosis, insulin reaches the liver via the portal vein. In the hepatocytes a high insulin:glucagon ratio stimulates glycolysis, glycogenesis, and lipogenesis, while it suppresses gluconeogenesis and glycogenolysis. Through these mechanisms, insulin *indirectly* stimulates glucose uptake by the hepatocytes. Insulin does not *directly* stimulate

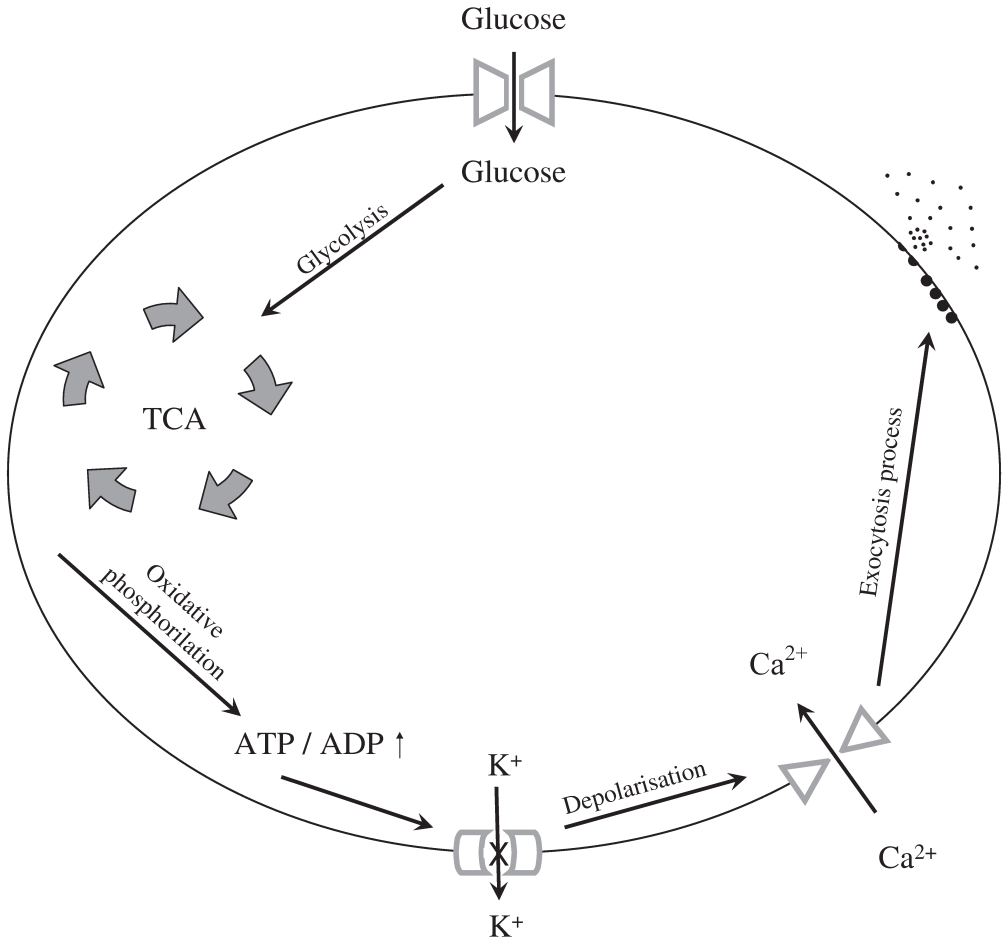


**Figure 2** Schematic overview of glucose-stimulated insulin secretion.

glucose uptake by the hepatocytes: the hepatocyte glucose transporter (GLUT2) is not regulated by insulin.

Part of the insulin is extracted from the circulation by the liver. In addition, after passing through the liver the blood from the hepatic vein is mixed with venous blood returning from other parts of the body. This leads to an approximately 1:3 ratio between peripheral insulin concentration and portal vein insulin concentration in anesthetised, fasted, non-obese, non-diabetic humans.[13] In fasted, anesthetised cats the ratio of peripheral to portal insulin concentrations was reported to be 1:8, although the design of the study may not have been adequate for determination of the ratio.[14]

Insulin has a multitude of effects in peripheral tissues. With respect to energy metabolism, the main target tissues for insulin are cardiac and skeletal muscle and adipose tissue. In these tissues insulin stimulates cellular uptake of glucose from the blood. This is

accomplished by stimulating translocation (and activity) of glucose transporter 4 (GLUT4) from within the cell to the cell membrane. Consequently, the blood glucose concentration decreases and the intracellular glucose concentration increases.

In adipose tissue, insulin also stimulates ❶ the use of intracellular glucose for the production of triacylglycerol by increasing glycolysis and lipogenesis, ❷ increased adipocyte uptake of fatty acids from very-low-density lipoproteins and chylomicrons by stimulation of the synthesis of lipoprotein lipase (LPL), and ❸ up-regulation of the acylation of glycerol-3-phosphate into triacylglycerol. Insulin suppresses adipocyte hormone-sensitive lipase, thereby slowing down hydrolysis of existing triacylglycerol. As a result, the concentration of the gluconeogenic precursor glycerol in blood decreases. Thus, the main action of insulin in adipose tissue is anabolic, in that it promotes energy storage in the form of triacylglycerol.[15]

In muscle, insulin stimulates the storage of intracellular glucose as glycogen by stimulating glycogen synthase. Glucose can also be transformed into triacylglycerol and stored as such. Glycogen stored in muscle can be converted back to glucose-6-phosphate when the insulin:glucagon ratio falls. The glucose-6-phosphate can then be shunted into the glycolytic pathway. In contrast to liver, muscle expresses very little glucose-6-phosphatase activity, which means that glucose-6-phosphate cannot be converted to glucose. Thus, muscle glycogen stores cannot be used to increase blood glucose concentration.

Insulin also affects protein metabolism, by decreasing hydrolysis of proteins and by increasing amino acid uptake by cells, resulting in a net synthesis of protein and decreased availability of amino acids to serve as gluconeogenic precursors.

Insulin resistance is the state in which higher concentrations of insulin are needed to obtain a given biological effect. It may be present in all insulin-sensitive tissues, or be restricted to one, as in the case of normal insulin action in the liver but reduced action in muscle.[16] Insulin resistance may even selectively affect carbohydrate, lipid, and protein metabolism within the same tissue.[16]

*Measurement of insulin secretion and insulin action*

Insulin and its secretion play a pivotal role in metabolism. Various tests of the response of the β-cell to different stimuli are used to assess insulin secretion. The hyperglycemic glucose clamp (HGC) is the gold standard test to assess glucose-stimulated insulin secretion.[17-22] By means of a variable glucose infusion, the arterial glucose concentration is set (‘clamped’) at a desired level. After approximately two hours the glucose infusion reaches a constant level, allowing multiple steady state measurements of the glucose infusion rate and insulin concentration.

Glucose tolerance tests (GTT) can also be used to study glucose-induced insulin secretion: a standardised dose of glucose is administered orally (oGTT) or intravenously (ivGTT) and the resulting glucose and insulin concentrations in plasma are measured. The area under the insulin curve represents the β-cell response to glucose.

Other approaches to assess β-cell function include the use of other stimulating agents, such as amino acids or tolbutamide. Because of its simplicity, measurement of fasting insulin concentration is also used as an indicator of β-cell function. Calculations based on fasting concentrations of glucose and insulin, such as the homeostasis model assessment B (HOMA-

B) may also be used, although there are several differences between the fasted and stimulated state.

There are several tests of *insulin action*, but the euglycemic, hyperinsulinemic glucose clamp (EGC) is considered the gold standard.[22,23] A continuous insulin infusion is combined with a glucose infusion that is adjusted to maintain arterial euglycemia. After approximately two hours the glucose infusion reaches a constant level, which allows multiple steady state measurements of the glucose infusion rate ($M_{EGC}$). $M_{EGC}$ equals whole body glucose disposal and is the gold standard measure of insulin action.

The area under the glucose curve divided by the area under the insulin curve after an oGTT or an ivGTT also provides a measure of insulin action. The frequently sampled ivGTT is a somewhat more sophisticated variant. The results of more frequently sampled glucose and insulin concentrations are modelled (minimal model assessment) and result in variables that reflect the ability of glucose to stimulate insulin secretion, the ability of insulin to stimulate glucose uptake, and the ability of glucose to stimulate its own uptake. Similar to the HOMA-B, the homeostasis model assessment R (HOMA-R) can be calculated from fasting glucose and insulin concentrations and provides an easy, though less accurate, measure of insulin action.

The clamping technique has several advantages. ❶ Multiple measurements of a steady state result in a more reproducible measure of insulin action (EGC) or β-cell response to glucose (HGC). ❷ Any preset insulin and glucose concentration can be achieved, allowing reproduction of these stimuli between individuals, which minimises interindividual differences. ❸ Other techniques can be combined with the clamping technique (e.g., tracer glucose infusion for modelling of hepatic glucose output) and interference by other hormones can be assessed by co-infusing them during the clamp. On the other hand, the glucose clamping technique requires frequent blood sampling and costly equipment, such as an accurate glucose analyser and infusion pumps.

For glucose clamps, arterial (or arterialised) blood samples should be collected in conscious subjects, for determination of glucose and insulin concentrations. By measuring the glucose and insulin concentrations in arterial blood during a glucose clamp, stimulation of the β-cell (HGC) or insulin-sensitive tissues (EGC) is known exactly. Unwanted variation is introduced when venous blood is used.[24-26] Also, anesthesia is known to influence glucose metabolism in cats.[27]

It is clear that the EGC is the gold standard measure of insulin action and the HGC is the gold standard measure of insulin secretion. In situations in which one is equally interested in both measures, ideally both tests should be performed, but if only one can be performed, preference should be given to the HGC. Not only is the HGC a measure of insulin secretion, but it can also provide a measure of insulin action. The latter measure correlates well with the measure provided by the EGC, over a wide range of insulin sensitivities.[22,28,29] In contrast, the EGC cannot provide a measure of insulin secretion because it employs exogenous insulin infusion. On the other hand, the EGC-technique facilitates study of responses to controlled hypoglycemia.

Although somewhat simpler to perform than the glucose clamp, the GTT has several drawbacks. ❶ The glucose clamp technique breaks the feedback loop between glucose and

insulin concentrations, whereas the (frequently sampled) ivGTT and oGTT leave this feedback loop intact. Hence there is no steady state in a GTT, which consequently leads to a less reproducible outcome and confuses the interpretation of results. ❷ The administration of a standardised intravenous dose of glucose (e.g., 1 g/kg body weight) results in a wide range of peak glucose concentrations in different subjects and therefore different stimuli to these individuals. The magnitude of the stimulus has been demonstrated to influence glucose tolerance in cats.[30] ❸ Renal loss of glucose is not considered in GTT-derived calculations and hepatic glucose output varies during the test. With regard to the minimal model assessment of the frequently sampled glucose tolerance test, Ferrannini and Defronzo wrote: "Not minimal is the problem that the data analysis requires a computer program that, like all package deals, deprives users of critical evaluation."[31]

The choice of test largely depends on the number of subjects. Glucose clamping is time consuming and costly and thus usually unsuitable if large numbers of individuals are to be studied. For smaller numbers of subjects, however, it is the method of choice, because it is accurate and reproducible, and will therefore increase the power of the study. When possible, the glucose clamping technique is the method of choice when assessing glucose metabolism.

*Classification of DM*

Due to the heterogeneous etiology of DM, classification enables better insight into the pathogenesis and thereby aids formulation of strategies for prevention and treatment. In former times classification of human DM was based on treatment, but currently it is classified according to etiology. Thus, the categories "non-insulin-dependent diabetes mellitus" and "insulin-dependent diabetes mellitus" have been replaced by those in Table 1.

| | |
|---|---|
| I. | **Type 1 diabetes mellitus** (β-cell destruction, usually leading to absolute insulin deficiency) |
| | A. Immune-mediated |
| | B. Idiopathic |
| **II.** | **Type 2 diabetes mellitus** (may range from predominantly insulin resistance with relative insulin deficiency to predominantly a secretory defect with insulin resistance) |
| **III.** | **Other specific types** |
| | A. Genetic defects in β-cell function |
| | B. Genetic defects in insulin action |
| | C. Diseases of the exocrine pancreas |
| | D. Endocrinopathies |
| | E. Drug- or chemical-induced |
| | F. Infections |
| | G. Uncommon forms of immune-mediated diabetes mellitus |
| | H. Other genetic syndromes associated with diabetes mellitus |
| **IV.** | **Gestational diabetes mellitus** |

**Table 1** Classification of DM.[3]

Although exact figures are not available, it is thought that 80-95% of cases of feline DM resemble type 2 DM of the human classification system.[32] As in humans, obesity and older age are risk factors for the development of DM in domestic cats.[1,33] Other similarities include insulin resistance,[34] absence or diminution of the first phase of insulin secretion in glucose tolerance tests together with exaggeration or absence of the second phase,[35] and formation of amyloid in the pancreatic islets of Langerhans.[36,37]

There are but few case reports of (suspected) type 1 DM in cats,[38-40] whereas a substantial minority can be classified as 'other specific types' of DM. For example, feline DM can be induced by administering progestagens and by glucocorticoid treatment.[41,42] Growth hormone excess and hypercortisolism are also known to cause it,[43-46] and a melanotroph pituitary adenoma secreting large amounts of α-melanocyte stimulating hormone (α-MSH) has been reported in a cat with concurrent DM.[47] This cat's DM went into remission after surgical removal of the adenoma by transsphenoidal hypophysectomy, suggesting that α-MSH had a role in the pathogenesis of the DM. Double (somatotroph and corticotroph) pituitary adenomas and pancreatic adenocarcinomas have also been reported in diabetic cats.[48,49]

*Pathogenesis of type 2 DM*

Even with the subdivision of DM into different types, type 2 DM may still include a variety of different causes and pathogeneses.[3] Typically, type 2 DM is characterized by both decreased insulin action and a relative or absolute insulin deficiency. In humans, genetic background is an important factor in the pathogenesis of DM,[50] and persons with a low birth weight are at increased risk of developing DM.[51] Risk factors such as obesity and physical inactivity lead to decreased insulin action, initially compensated by increased insulin secretion by the ß-cells. In some individuals the increased demand for insulin cannot be sustained long term, as shown by a subsequent decline in the ß-cell response to glucose and a decrease in the ß-cell mass.[52,53] At some point insulin secretion becomes insufficient for maintenance of fasting normoglycemia, and/or normal glucose tolerance. After an intermediate phase of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), DM ensues.

The cause(s) of the changes in the ß-cell response to glucose and the ß-cell mass are not fully understood. The decrease in ß-cell mass is generally accepted to be mainly caused by increased apoptosis of ß-cells (Figure 3).[54] Several mechanisms have been proposed, including glucose toxicity,[55] lipotoxicity,[56] and islet amyloidosis.[57] The role of these mechanisms in the progression of ß-cell failure is well established, although there is still considerable debate whether these mechanisms should be considered as primary factors causing ß-cell failure. Nevertheless, it is intriguing that the species that naturally develop type 2 DM (humans, non-human primates, and cats) also naturally develop islet amyloidosis, whereas other species do not.

It is also interesting to note that almost all type 2 diabetic subjects have insulin resistance, but only about half of obese (and thus insulin-resistant) persons develop impaired glucose tolerance or DM.[58] Also, in normal, glucose-tolerant, first-degree relatives of Caucasian DM patients, defects in ß-cell function have been demonstrated prior to the development of insulin resistance.[59,60] Thus, ß-cell dysfunction may be the primary pathogenetic mechanism that, accelerated by insulin resistance, leads to the development of

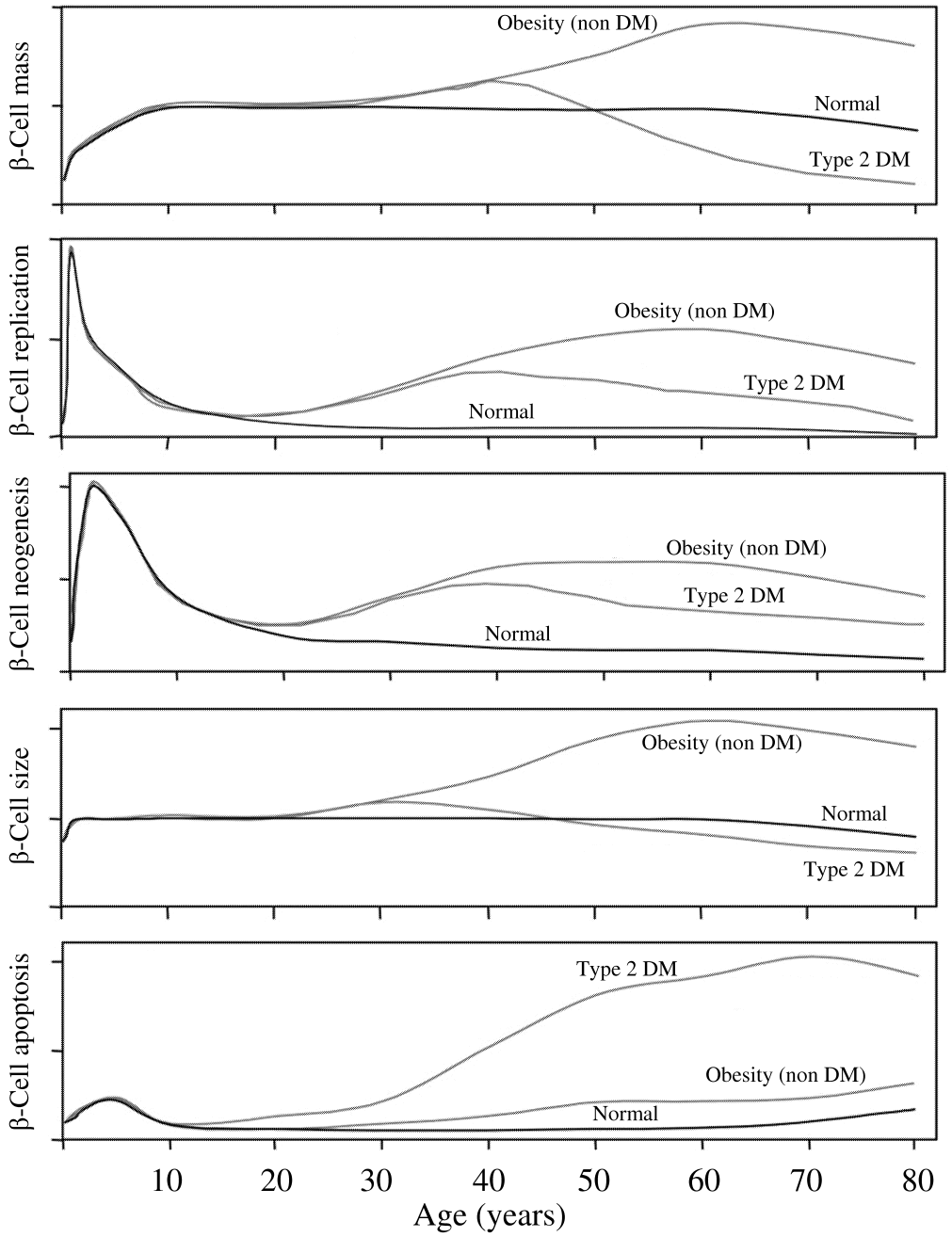


**Figure 3** Hypothetical model for β-cell growth in type 2 diabetics, obese non-diabetics, and normal humans throughout life. Units on the y-axis are arbitrary. Adapted from Rhodes.[54]

type 2 DM in Caucasian individuals. In Mexican Americans, however, the primary pathogenetic mechanism is insulin resistance,[61] which illustrates the putative diversity of pathogeneses that are grouped together in the type 2 DM category.

Much less is known about the pathogenesis of DM in cats. One study found that DM occurred 2.45 times per 1,000 cat-years-of-risk, and obesity and old age were shown to be risk factors.[1] In Australia, Burmese cats are at higher risk of developing DM,[62] which may indicate a genetic background. As obesity is increasingly a problem in domestic cats, it is thought that the incidence of DM in cats is also increasing, but studies of this widely accepted hypothesis are lacking. O'Brien et al. compared ivGTT results in three groups of cats: normal glucose tolerant (NGT), IGT, and DM.[35] First phase insulin secretion was lower and second phase insulin secretion higher in IGT cats than in NGT cats, whereas both first and second phase insulin secretion were decreased in diabetic cats. Obesity induces insulin resistance in healthy cats and normalisation of body weight reverses it, and diabetic cats have been reported to be six times less insulin sensitive than healthy cats.[34,63,64] Obesity decreases both GLUT4 expression in muscle and adipose tissue, and post-heparin plasma LPL activity (mainly through a decrease in fat tissue LPL activity) in cats.[65,66] Islet amyloidosis occurs in diabetic cats in approximately the same frequency as in humans and is associated with comparable reductions in ß-cell mass. It is not considered to be a primary diabetogenic event but a contributor to the progression of ß-cell failure.[67]

*The metabolic syndrome*

In humans, disturbances in carbohydrate metabolism (insulin resistance, IFG, IGT, or type 2 DM) are criteria used for the diagnosis of the metabolic syndrome (also called "Syndrome X" or "Insulin Resistance Syndrome"). The metabolic syndrome has been proposed as a clinical tool to aid in the identification of individuals at high risk of developing macrovascular atherosclerotic disease. There are several definitions of the metabolic syndrome with different cut-off points, but the definitions always include ❶ a measure of body composition (elevated waist circumference, waist:hip ratio, and/or body mass index), ❷ a measure of insulin resistance and/or disturbed glucose homeostasis (IGT, IFG, type 2 DM), ❸ arterial hypertension, and ❹ a measure of dyslipidemia (elevated triglycerides, low HDL-cholesterol).[68]

The individual components of the metabolic syndrome are risk factors for macrovascular atherosclerotic disease. The clustering of these risk factors exceeds coincidental clustering and the risk factors coexist more than they occur in isolated form.[69,70] The risk factors have therefore been proposed to share a "common soil".[71] Presumably obesity and the insulin resistance that accompanies it underlie these disturbances. Improper nutrition and lack of physical exercise are considered to be the root causes of the metabolic syndrome, as they stimulate the development of obesity.[72]

It is interesting to note that Dr. Reaven, who laid the basis for the concept of the metabolic syndrome,[73] has recently proposed to bury this entity.[74] However, his criticism is directed at the clinical utility of the metabolic syndrome as a diagnostic tool and not so much at the pathophysiological mechanism that links many risk factors for macrovascular atherosclerotic disease.

It is generally accepted that cats do not develop the metabolic syndrome. Although atherosclerotic macrovascular disease may occur in cats under specific conditions,[75-77] this is quite uncommon. Also, the relationship between type 2 DM and arterial hypertension in cats is unclear. The prevalence of arterial hypertension does not seem to be higher in diabetic cats.[78-80] Others have reported increased systolic (but not diastolic or mean) blood pressure in diabetic cats,[81] but this was not consistently demonstrable with different techniques (oscillometry versus Doppler technique) or different sites of measurement (base of the tail versus front leg). Studies using sufficient numbers of cats and designed to investigate a possible relation between DM and arterial hypertension are lacking. In addition, handling of cats during measurement of blood pressure can induce stress-related variation of the arterial blood pressure.[82] It may therefore be difficult to detect subtle changes in arterial blood pressure using indirect measurement techniques.

*Diet and exercise in the prevention and treatment of DM*

Recommendations with regard to physical exercise and nutrition have been made for the prevention and treatment of type 2 DM and the metabolic syndrome in humans.[83,84] An active lifestyle is advocated: at least 30 minutes daily of physical activity of at least moderate intensity. With regard to diet, it is recommended that 45-60 % of energy be provided by carbohydrate, 10-20 % by protein, and less than 35% by fat. Naturally-occurring food containing saturated fatty acids, trans-unsaturated fatty acids, and cholesterol should be minimized. Naturally-occurring food containing cis-monounsaturated fatty acids (MUFA), moderate amounts of cis-polyunsaturated fatty acid (PUFA) and high amounts of dietary fibre (~50% soluble) are recommended. There is disagreement between American and European expert committees concerning the glycemic index or glycemic load. However, many foods that are high in soluble fibre also have a low glycemic index.[85] Thus, selecting naturally-occurring foods that contain high amounts of soluble fibre automatically results in lowering of the overall glycemic index of the diet. Dietary sodium intake should be less than 6 grams per day. These guidelines can be met with a diet containing wholegrain products, vegetables, legumes, fruit, MUFA-containing plant oils, low-fat diary products, low-fat meat, and some fish.

Dietary recommendations for the prevention and treatment of DM in cats may be different from those for humans. Cats do not appear to develop the metabolic syndrome spontaneously and therefore dietary recommendations need not be influenced by possible development of atherosclerotic macrovascular disease. Also, unlike humans, cats are obligate carnivores whose natural diet consists of small prey animals, which contain little carbohydrate.[86,87]

It has been hypothesised that evolutionary pressures arising from this low-carbohydrate natural diet have led to several adaptations in the cat.[88] For example, feline saliva lacks the enzyme amylase [89] and feline hepatocytes do not contain glucokinase [90-92] and also synthesise glycogen relatively slowly.[90] Conversely, hexokinase is more active in feline hepatocytes than in canine hepatocytes.[91,92] The ability of cats to digest carbohydrates is a matter of debate; some researchers have reported it to be low [93-96] and others have reported it to be high.[97]

It has also been suggested that the metabolic adaptations of the carnivorous cat compromise its ability to metabolise large amounts of carbohydrate.[88] Others have postulated that the "carnivore connection theory"[98,99] could also apply to cats.[32,100] According to this theory carnivores (human hunter/gatherers during Ice Ages) may have evolved to be insulin resistant as an adaptation to their low carbohydrate diet: glucose is utilized less by insulin-sensitive tissues and remains available for glucose-dependent tissues such as the brain. However, humans now consume high-carbohydrate diets having a high glycemic index and thus insulin resistance is no longer an advantage. These diets may instead lead to DM by increasing the demand for insulin and thereby possibly resulting in exhaustion of the β-cells. For the same reason, high-carbohydrate diets might be thought to contribute to the development of DM in cats, but the effects of dietary macronutrient differences on glucose disposal and β-cell function have not been investigated in this species.

Dry cat food is at present the major food source for domestic cats. On average, dry cat food provides approximately 50% of metabolisable energy as carbohydrates, compared with approximately 10% for canned cat foods (Figure 4).[101,102] If the amount of dietary carbohydrate were found to play an important role in the development of feline DM, it would

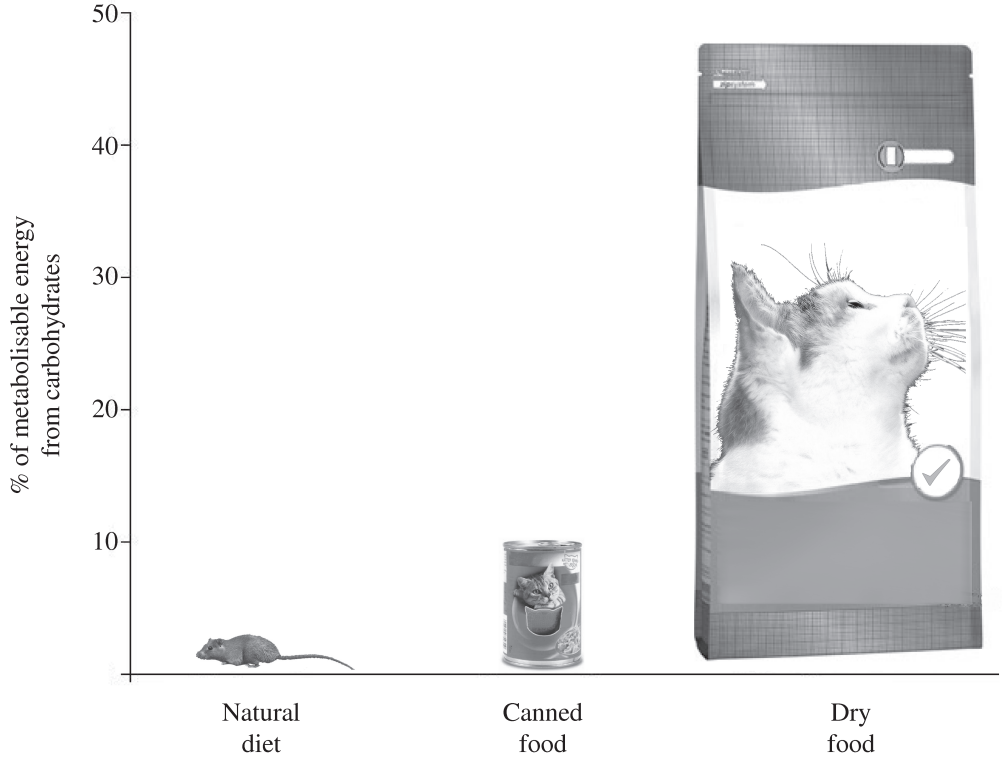


**Figure 4** Percent metabolisable energy from carbohydrate in the cat's natural diet, canned cat food, and dry cat food.

imply that dry cat food is a risk factor. An alternative mechanism could be that the higher energy content of dry food promotes obesity, which subsequently increases the risk of developing DM. Two studies have addressed nutritional support in the treatment of feline DM. In the first study, a diet high in insoluble fibre (HFi) was compared with a low-fibre (LFi) diet in a crossover design.[103] The HFi diet provided 29% of metabolisable energy as carbohydrate, whereas the LFi diet provided 37%. Diabetic cats receiving the HFi diet had lower fasting blood glucose concentrations, tended to require lower doses of insulin, and tended to have lower glycated hemoglobin concentrations. This led the authors to advocate the HFi diet for nutritional support of DM therapy. A more recent study found that a low-carbohydrate, low-fibre (LCLFi) diet may be advantageous as part of the treatment of type 2 DM in cats.[104] In this study a medium-carbohydrate, high-fibre (MCHFi) diet was compared with the LCLFi diet. More cats became independent of exogenous insulin on the LCLFi diet than on MCHFi diet and thus the former is now advocated as part of the treatment of type 2 DM in cats.

One possible mechanism which might explain these results is that lower (via low-carbohydrate diets) or slower (via high-fibre diets) uptake of dietary carbohydrates leads to decreased or a more even demand for insulin. As a result, endogenous insulin secretion may be able to cover this demand better, leading to decreased exogenous insulin demand and possibly even independence from exogenous insulin. However, this hypothesized interpretation of available data has yet to be tested.

It remains unknown whether treatment of DM and prevention of DM require the same dietary approach, since the possibility of preventing feline type 2 DM by dietary measures have yet to be explored.

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# I

# Measurement techniques

# Insulin sensitivity and ß-cell function in healthy cats: Assessment with the use of the hyperglycemic glucose clamp

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## Abstract

A hyperglycemic clamp (HGC) was developed for use in conscious cats. In 21 healthy normal glucose tolerant cats we measured: glucose disposal rate (M), insulin sensitivity ($ISI_{HGC}$), and β-cell response (I) at arterial plasma glucose of 9 mmol•$L^{-1}$.

The HGC was tolerated well and steady state glucose infusion was achieved. Compared to values reported for humans, M values for the cats were low, which appeared to relate to both a low $ISI_{HGC}$ and a low I. HGC measures correlated with fasting plasma glucose and insulin concentrations as well as with their HOMA (homeostasis model assessment) and (quantitative insulin sensitivity check index) counterparts. Also, I and $ISI_{HGC}$ correlated with their counterparts derived from intravenous glucose tolerance tests.

In conclusion, this is the first report of hyperglycemic glucose clamping in cats. The procedure (HGC) allows for measurements of glucose disposal, β-cell response and insulin sensitivity. Compared to human data, both insulin sensitivity and insulin secretion appeared to be low in cats. This is compatible with the carnivorous nature of this species, for which insulin resistance would be advantageous during periods of restricted food availability.

# Introduction

In cats diabetes mellitus is a common disease with a reported incidence of 2.45 cases per 1,000 cat-years-of-risk.[1] The main risk factors are age and obesity.[1] The disease is characterised by disorders of insulin action and insulin secretion, both of which are present by the time the disease is clinically manifested.[2,3] Diabetic cats have been reported to be six times less insulin sensitive than healthy cats.[3] The (relative) impairment of insulin secretion is associated with islet amyloidosis.[4] These characteristics bear close resemblance to (human) type 2 diabetes mellitus.[5]

Insulin resistance and insulin secretory dysfunction can be identified in prediabetic humans years before they acquire type 2 diabetes mellitus.[6] The euglycemic hyperinsulinemic glucose clamp is the gold standard measurement of insulin sensitivity in man,[7] while the hyperglycemic glucose clamp (HGC) allows for the gold standard assessment of the ß-cells' response to glucose and also provides a measure of insulin sensitivity.[8-12] There are four reports on methods of assessing insulin sensitivity in cats using euglycemic hyperinsulinemic clamp techniques. However, these studies were performed under anesthesia and/or venous blood was used for measurement of plasma glucose concentration,[13-16] both of which are known to influence results.[17-19]

We describe (to our knowledge) the first report on a new hyperglycemic glucose clamp technique in conscious cats, using arterial blood for glucose sampling. The data are compared with fasting glucose and insulin concentrations and indices such as the quantitative insulin sensitivity check index (QUICKI) and the homeostasis model assessment (HOMA-R and HOMA-B), as well as with values derived from intravenous glucose tolerance tests (ivGTT).

# Materials and methods

*Cats*

Ten castrated male and 11 spayed female cats were studied. The ages had been estimated to range from 5 to 9 years. One male was a mixed breed (Persian x domestic shorthair) cat, all others were domestic shorthairs. Findings at physical examination and routine biochemical and hematological examination were unremarkable. All cats were accustomed to daily handling. The cats were housed with natural daylight in three equal sized groups in enclosures enriched with shelves, rotation of toys and other structures. In addition, cats were provided with human socialisation by dedicated, non-research staff. Also, each group of cats had free access to a playground for at least two hours per day. All cats were fed a standardised mix of commercially available dry and canned cat food, resulting in a macronutrient composition of 38% of metabolisable energy from protein, 29% from fat and 33% from carbohydrates. Cats were fed for weight stasis and had been fed this diet for at least 10 weeks prior to testing.

*DXA scanning*

Five weeks after performance of the HGC, body composition was measured using a pencil beam DXA machine (QDR-1000/W, Hologic Europe NV, Zaventem, Belgium), which has

been validated for use in cats.[20] Anesthetised cats were scanned in sternal recumbence with front legs stretched forward, hind legs stretched backwards and a phantom calibration block next to them. The means (standard deviation (SD)) of the fat mass percentage and lean body mass were 24.3% (4.8%) and 72.5% (4.9%), respectively. The infant whole body scan-option was used [20] with software version 6.20D. The variation coefficient for the quality control measurements (performed before each scanning session) was 0.34%.

*Hyperglycemic glucose clamp*

Forty-eight to 72 hours prior to the HGC, in all cats double lumen catheters (Certofix Duo Paed S420, B. Braun Medical BV, Oss, The Netherlands, Seldinger technique) were placed in a common carotid artery, under general anesthesia and sterile conditions. The catheter was placed guided by ultrasonography, rather than a cut down technique, and the catheter tip was manoeuvred into either the descending aorta or the brachiocephalic trunk under fluoroscopic guidance. An identical catheter was inserted 8 cm caudally into the contralateral external jugular vein. After full recovery from anesthesia, cats were housed in individual cages and fed according to their usual feeding regime. All catheter lumens were flushed at 8 hour intervals with 1.5 mL 0.9% saline solution with added heparin (Leo Pharma BV, Breda, The Netherlands) in a final concentration of 50 IU•mL$^{-1}$.

After withholding food for 18 h and then obtaining 4 fasting glucose and insulin samples each (at t= -15, -10, -5 and 0 minutes), the hyperglycemic clamp was started (9 mmol•L$^{-1}$ arterial plasma glucose concentration for 3 h) with a glucose bolus (of 0.075 ml • body weight (kg) • desired increment in plasma glucose concentration (mmol•L$^{-1}$)), followed by a continuous glucose (20%) infusion (with KCl 20 mmol•L$^{-1}$) using an infusion pump (Perfusor fm, B. Braun Medical BV, Oss, The Netherlands). For both the glucose bolus and the continuous glucose infusion the same glucose solution (with added KCl) was used. Arterial blood for glucose and insulin measurements was collected at 3 to 5 minute intervals; Glucose Infusion Rates were adjusted accordingly in order to maintain glucose levels at 9 mmol•L$^{-1}$ and recorded at 15 minute intervals. The infused glucose was delivered through the proximal lumen of the venous catheter and the distal venous lumen was used to return arterial blood drawn prior to sample collection.

For removal of the catheters, cats were anesthetised and digital pressure was applied for at least 10 minutes to prevent hemorrhage.

*Intravenous glucose tolerance test*

On average 3 months before the HGC, 5 castrated male and 6 spayed female cats from the 21 cats that underwent HGC, also underwent ivGTTs. The cats' average (and SD) weight difference between both tests was 0.03 kg (0.17 kg). Forty-eight to 72 hours prior to the ivGTTs, triple lumen catheters (Multicath 3, 4 French, length 20 cm, Vygon Nederland BV, Veenendaal, The Netherlands, Seldinger technique) were inserted 8 cm caudally into an external jugular vein, under anesthesia and sterile conditions. Catheter lumens were capped with a safety connector (Safsite®, B.Braun Medical BV, Oss, The Netherlands). Flushing of catheters and feeding of cats was as for the HGC.

Food was withheld 18 hours prior to the ivGTT and the cats remained in their group cages during the ivGTT. Over a period of 30 seconds 1 g glucose per kg bodyweight [21] (glucose 50%, B. Braun Medical BV, Oss, The Netherlands) was administered through one lumen of the jugular catheter. Blood was collected through another catheter lumen at –15, -2, 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes for glucose and insulin measurements.

*Sample analysis*

Plasma glucose concentration was measured in 0.2 mL of blood, collected into a dry heparin containing syringe (ivGTT: PICO 30 and HGC: PICO 50, Radiometer Nederland BV, Zoetermeer, The Netherlands) with an automated glucose analyser (EML 105, Radiometer Nederland BV, Zoetermeer, The Netherlands). Blood for insulin measurement (0.4 mL) was transferred immediately into prechilled EDTA tubes (Vacuette, Greiner Bio-One BV, Alphen aan den Rijn, The Netherlands). After sample collection and the venous return of the blood drawn prior to sample collection, the catheter lumen was flushed with 1.5 mL (ivGTT) or 0.6 mL (HGC) of a 0.9% saline solution containing 2 IU•mL$^{-1}$ heparin. Blood for insulin determination was centrifuged for 12 minutes at 4$^{o}$ C at 3500 rpm. Plasma was stored at -20$^{o}$ C. Insulin was determined in a heterologous immunoradiometric assay (INS-Irma, Biosource Nederland BV, Etten-Leur, The Netherlands) in three runs of the same batch of assays. Results of measurements from serial dilutions of a feline sample were parallel to the standard curve. Also, a linear relationship ($y = 1.00x - 0.60$) with an $R^2$ of 0.99 existed between measured dilution concentrations (y) and calculated dilution concentrations (x) of a feline sample. The assay's sensitivity was 1 mU•L$^{-1}$. Interassay variation for the insulin measurements between the three runs on separate days was 5.7% at 12 mU•L$^{-1}$ (3 samples per run), and 5.1% at 100 mU•L$^{-1}$ (3 samples per run). Insulin unit conversion from mU•L$^{-1}$ to pmol•L$^{-1}$ was performed by multiplying by 6.71, according to the manufacturer's instructions.

*Anesthesia*

For inserting catheters for the HGC, anesthesia comprised of premedication with 80 µg•kg$^{-1}$ medetomidine (Domitor®, Pfizer BV, Capelle aan den IJssel, The Netherlands) intramuscularly (IM), followed by induction of anesthesia with 2 to 4 mg•kg$^{-1}$ propofol (Fresenius Kabi Nederland BV, Schelle, Belgium) intravenously (IV) through a canula in a cephalic vein. After induction of anesthesia, cats were intubated and anesthesia was maintained with a mix of isoflurane, oxygen and air. After inserting the catheters, anesthesia was reversed with 200 µg•kg$^{-1}$ atipamezol IM (Antisedan®, Pfizer BV, Capelle aan den IJssel, The Netherlands) and the canula was removed from the cephalic vein. For the ivGTTs, the above procedures were followed except that maintenance of anesthesia was not necessary. Cats were allowed to recover from anesthesia 48 to 72 hours before HGCs or ivGTTs were performed.

For the DXA scan, cats were anesthetised with 5 mg•kg$^{-1}$ ketamine IM (Narketan 10®, Vétoquinol BV, 's Hertogenbosch, The Netherlands) and 0.3 mg•kg$^{-1}$ midazolam IM (Hameln Pharmaceuticals GMBH, Hameln, Germany) as premedication, followed by induction (1-2 mg•kg$^{-1}$ IV) and maintenance (3-7 mg•kg$^{-1}$•h$^{-1}$ IV) of anesthesia with propofol.

*Calculations: HGC*

HCG variables were calculated according to Defronzo et al [22] (Table 1), with an extracellular glucose space of 0.19 $L \bullet kg^{-1}$ body weight (the average extracellular water space of cats)[23] for the calculation of space correction.

$ISI_{HGC}$ was calculated as M divided by average insulin concentration from 120 through 165 minutes. The plasma insulin concentration at 180 minutes was not included in this average because the 180 minute plasma insulin concentration could not yet have influenced M. The first phase of insulin secretion during HGC (AUC-Ins1) was defined as the AUC of the insulin concentration ($nmol \bullet L^{-1} \bullet min$) during the first 12 minutes of the hyperglycemic period. AUC-Ins2, a measure of the steady state of the second phase of insulin secretion during HGC, was calculated as the AUC of the insulin concentration during the last 45 minutes of the hyperglycemic period.

*Calculations: ivGTT*

Calculations of the K value (% glucose disappearance per minute), $T_{1/2}$ (glucose half-life), first phase insulin secretion (Phi-1), second phase insulin secretion (Phi-2) and the total area under the curve for glucose and insulin ($AUC_{glucose}$, Phi-1+2) were performed as previously described for cats.[21] The total area under the curve (AUC) was estimated using the trapezoidal rule. The sensitivity index ($SI_{ivGTT}$) for insulin was calculated by dividing the $AUC_{glucose}$ by Phi-1+2. Plasma glucose concentration was expressed in $mmol \bullet L^{-1}$, plasma insulin concentration was expressed in $pmol \bullet L^{-1}$.

| Variable | Measure of | Calculation | Units |
|---|---|---|---|
| I | B | average insulin concentration (120' - 180') | $pmol \cdot L^{-1}$ |
| M | G | INF - UC - SC | $\mu mol \cdot (kg \cdot min)^{-1}$ |
| | | INF = Infused glucose | $\mu mol \cdot (kg \cdot min)^{-1}$ |
| | | UC = Correction for urinary loss of glucose | $\mu mol \cdot (kg \cdot min)^{-1}$ |
| | | SC = Correction for changes in glucose concentration in the extracellular glucose space (0.19 L/kg) | $\mu mol \cdot (kg \cdot min)^{-1}$ |
| $ISI_{HGC}$ | S | M / average insulin concentration (120' - 165') | $\mu mol$ glucose $\cdot L \cdot (kg \cdot min \cdot pmol$ insulin$)^{-1}$ |
| AUC-Ins1 | B | AUC for insulin (0' - 12') -- First phase secretion | $nmol \cdot L^{-1} \cdot min$ |
| AUC-Ins2 | B | AUC for insulin (135' - 180') -- Second phase secretion | $nmol \cdot L^{-1} \cdot min$ |

**Table 1** Summary of the calculations of measures of glucose disposal (G), β-cell response to glucose (B) and insulin sensitivity (S) from the HGC.

*Calculations from fasting values*

HOMA-R, QUICKI and HOMA-B were calculated using the fasting glucose and fasting insulin concentrations.[24,25]

*Statistical analysis*

Statistical analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, USA) and Microsoft Excel 2000 (Microsoft NL BV, Amsterdam, The Netherlands). Log transformed glucose infusion rate data were analysed with repeated measures ANOVA with orthogonal polynomial contrasts to determine whether or not a steady state glucose infusion was achieved during the last hour of the HGC.

For the calculation of correlation coefficients, statistical outliers were identified using Grubb's T statistic ($P<0.01$; two tailed) first.[26] The datasets from each variable with outliers were log transformed and retested for outliers. Then, data were tested for normality using the Kolmogorov-Smirnov test with Lilliefors significance correction ($P<0.10$).[27] If log transformation had not already been applied due to outliers, non-parametric data were log transformed and retested for normality. Pearson's correlation coefficients were calculated for parametric data and Spearman's rank correlation test was used for non-parametric data. Values of $P\leq0.05$ (two tailed) were considered statistically significant.

*Ethics*

The research protocols and care for the cats were evaluated and approved by the Committee for the ethical treatment of animals of Utrecht University (The Netherlands) according to Dutch and European law.

# Results

Arterial catheterisations were uneventful in 16 out of 21 cats. In 5 cats, catheterisation was stopped because good entrance into the artery could initially not be achieved. In all cats catheterisation was repeated successfully several weeks later. In one cat, catheter patency was lost; the catheters were removed and the clamp was repeated several weeks later.

There were no striking adverse reactions to the procedures. During the HGC the behaviour of the cats did not change. Within approximately 30 minutes into the hyperglycemic period most cats went to sleep.

The mean plasma glucose concentration during the whole hyperglycemic period of the HGC was 9.0 mmol•$L^{-1}$ with a mean SD of 0.3 mmol•$L^{-1}$ (variation coefficient (VC) of 3.2%) (Figure 1). During the third hour, glucose infusion rates were at steady state since there were no significant linear ($P=0.47$), quadratic ($P=0.63$) or cubic ($P=0.29$) trends in the glucose infusion rate curve. (Figure 2).

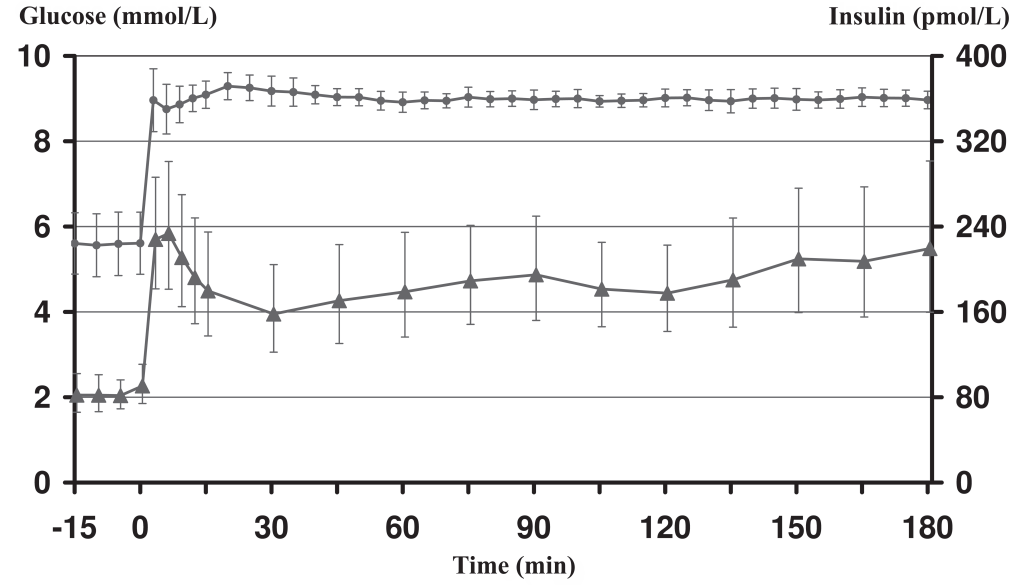


**Figure 1** Plasma glucose (●) and insulin (▲) concentrations during the hyperglycemic glucose clamp in 21 cats. The glucose values are expressed as means ± standard deviations and the insulin concentrations as geometrical means with 95% confidence intervals.

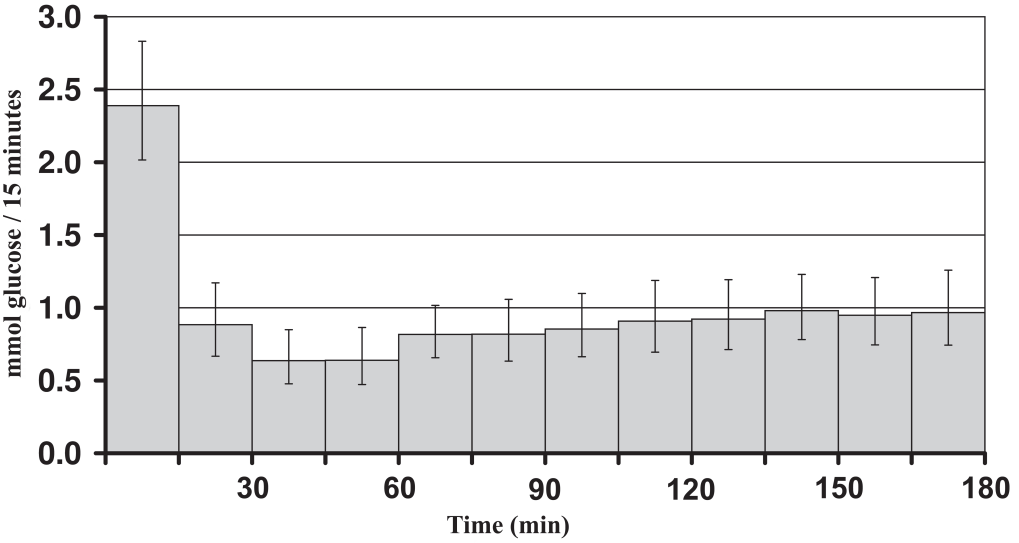


**Figure 2** Infused glucose recorded at 15 minute intervals during the hyperglycemic glucose clamp in 21 cats expressed as geometrical means with 95% confidence intervals. The bar of the first 15 minutes includes the initial glucose bolus.

| Variable | Measure of | Mean | Median | Range | Units |
|---|---|---|---|---|---|
| fasting glucose | G | 5.6 | 5.8 | 4.4 - 6.6 | mmol • $L^{-1}$ |
| fasting insulin | S | 85[§] | 84 | 33 - 225 | pmol • $L^{-1}$ |
| I | B | | 246 | 51 - 772 | pmol • $L^{-1}$ |
| M | G | 15.9[§] | 13.9 | 8.8 - 36.0 | µmol • (kg • min)$^{-1}$ |
| $ISI_{HGC}$ | S | 0.081[§] | 0.081 | 0.035 - 0.249 | µmol glucose • L • (kg • min • pmol insulin)$^{-1}$ |
| AUC-Ins1 | B | 2.77 | 2.73 | 0.89 - 5.08 | nmol • $L^{-1}$ • min |
| AUC-Ins2 | B | | 11.37 | 2.31 - 37.65 | nmol • $L^{-1}$ • min |
| K | G | 1.61 | 1.41 | 0.93 - 2.68 | % • $min^{-1}$ |
| $T_{1/2}$ | G | 49.2 | 49.3 | 25.9 - 74.4 | min |
| $AUC_{glucose}$ | G | 1.47 | 1.53 | 0.99 - 2.17 | mol • $L^{-1}$ • min |
| phi-1 | B | 9.42[§] | 10.09 | 4.16 - 23.39 | nmol • $L^{-1}$ • min |
| phi-2 | B | 34.00[§] | 28.93 | 12.60 - 119.25 | nmol • $L^{-1}$ • min |
| phi-1+2 | B | 44.90[§] | 35.20 | 19.56 - 142.64 | nmol • $L^{-1}$ • min |
| $SI_{ivGTT}$ | S | 0.037 | 0.044 | 0.008 - 0.062 | mmol glucose • min • (pmol insulin • min)$^{-1}$ |
| HOMA-R | S | 3.14[§] | 3.04 | 1.33 - 9.81 | |
| QUICKI | S | 0.323 | 0.324 | 0.278 - 0.366 | |
| HOMA-B | B | 130.3[§] | 141.8 | 37.2 - 333.7 | |

**Table 2** Measures of glucose disposal (G), β-cell response to glucose (B) and insulin sensitivity (S) calculated from the HGC (upper panel), the ivGTT (middle panel) and fasting plasma glucose and insulin concentrations (lower panel). No corrections for outliers were made for calculation of these measures. [§]: Geometrical mean is reported because of the log-normal distribution of these measures.

During ivGTTs, peak plasma glucose concentrations ranged from 33.3 to 50.1 mmol•$L^{-1}$ (mean 42.8 mmol•$L^{-1}$ and SD 4.2 mmol•$L^{-1}$) and plasma glucose concentrations at time point 15' ranged from 22.2 to 32.3 mmol•$L^{-1}$ (mean 28.1 mmol•$L^{-1}$ and SD 2.5 mmol•$L^{-1}$).

Variables and indices derived from HGCs, ivGTTs and fasting values are presented in Table 2. Correlations between variables calculated from HGC data are presented in Table 3. For the calculation of correlation coefficients, one outlier was detected in the data sets of the $ISI_{HGC}$, I, HOMA-R, AUC-Ins2 and fasting insulin concentrations. After log transformation of these data sets, no outliers were detected anymore. Data for M, Phi-1, Phi-2, Phi-1+2, and HOMA-B were log transformed in order to obtain parametric distributions. For I and AUC-Ins2, log transformation did not result in parametric distributions. Therefore, the non transformed data of these two variables were analyzed in a non-parametric fashion.

Comparison for corresponding variables of the HGC and the ivGTT indicated good correlations for insulin sensitivity ($ISI_{HGC}$ and $SI_{ivGTT}$ : Pearson's r=0.63, *P*=0.036), first phase insulin secretion (AUC-Ins1 and Phi-1; Pearson's r=0.84, *P*=0.001), second phase insulin secretion (AUC-Ins2 and Phi-2; Spearman's r=0.63, *P*=0.039) and for the measures of "total" ß-cells' response to glucose (I and Phi-1+2; Spearman's r=0.67, *P*=0.023).

| | Insulin action | | ß-cell function | | |
|---|---|---|---|---|---|
| | Glucose disposal rate (M) | Insulin Sensitivity Index ($ISI_{HGC}$) | Mean insulin concentration (120' - 180') (I) | First phase insulin secretion (AUC-Ins1) | Second phase insulin secretion (AUC-Ins2) |
| | | | Θ | | Θ |
| Fasting glucose concentration | -0.70*** | | -0.68*** | -0.69*** | -0.60** |
| Fasting insulin concentration | | -0.61** | 0.61** | 0.70*** | 0.72*** |
| HOMA-R | | -0.60** | 0.44* | 0.46* | 0.56** |
| QUICKI | | 0.63** | -0.44* | -0.48* | 0.56** |
| HOMA-B | 0.52* | | 0.81*** | 0.94*** | 0.83*** |

**Table 3** Pearson's correlation coefficients between variables of the hyperglycemic glucose clamp, except for the columns marked with a Θ, where Spearman's correlation coefficient is given. Non-significant correlations have been omitted. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

# Discussion

To the authors' knowledge, this is the first report on the use of a hyperglycemic glucose clamping technique in conscious cats using arterial blood measurements. All cats were middle aged, with normal glucose tolerance.[28]

In earlier reports on the use in cats of the hyperinsulinemic euglycemic glucose clamp, cats were either anesthetised and/or venous blood was used for measurement of the plasma glucose concentration.[13-16] As anesthesia is known to influence glucose metabolism in cats [17] we conducted tests in conscious cats. Since ß-cell insulin secretion depends on arterial glucose concentrations we used arterial rather than venous plasma levels to prevent unwanted variation.[18,19] In the present study, a stable arterial plasma glucose concentration of 9.0 mmol•$L^{-1}$ was achieved.

The techniques of arterial catheterisation, maintenance of patency and catheter removal require great care.[29] Catheterisation of the aorta through a common carotid artery has been described for dogs, cats and rats [30] and is advocated for long-term (>48 h) use in dogs and cats.[29]

The glucose clamp was set well below the maximum level of the renal tubular resorption capacity for glucose in healthy cats.[31,32] Therefore, no corrections for urinary loss of glucose were necessary (UC = 0) for the calculation of M.

The HGC technique is the gold standard method for the measurement of the ß-cells' response to glucose (I),[9] while the euglycemic hyperinsulinemic glucose clamp (EGC) is considered to be the gold standard for the measurement of insulin sensitivity;[7] both M and M/I provided by the EGC ($M_{EGC}$ and $M/I_{EGC}$) are considered to be measures of insulin sensitivity. We used the HGC not only for the measurement of M and I, but also for calculation of the insulin sensitivity index ($ISI_{HGC}$). The $ISI_{HGC}$ has been demonstrated to correlate strongly with both $M_{EGC}$ and $M/I_{EGC}$ through a wide range of insulin sensitivities.[22,33,34]

Hyperglycemic glucose clamps have been performed in several other species, including humans and dogs. In our cats the mean M was 39% of that reported in a study in 11 humans, clamped at 11.8 mmol•$L^{-1}$.[22] In a second study, 184 normal glucose tolerant humans were clamped at 10 mmol•$L^{-1}$ plasma glucose.[35] Under the assumption of an average lean body mass of 0.75 kg per kg bodyweight, in our cats the mean M was 32% of that reported for humans. In another study, HGCs were performed on 10 healthy mongrel dogs, raising plasma glucose to 9.6 mmol•$L^{-1}$. The mean M in our cats was 21% of these dogs.[36]

The differences between the glucose levels in aforementioned studies and this study are not big enough to explain the observed differences in M when comparing our cats to humans and dogs. Thus, cats have a lower glucose disposal rate than dogs and normal glucose tolerant humans. In addition, even when our data are compared with those obtained in humans with impaired glucose tolerance M in our cats is still low.[35]

Interspecies comparisons of both I and $ISI_{HGC}$ can be hampered by methodological aspects of insulin measurements.[37] However, the glucose disposal rate at a certain level of stimulation results from the interaction of insulin release (I) and insulin action ($ISI_{HGC}$).[38] The apparently lower M is therefore caused by a lower I or a lower $ISI_{HGC}$ or a combination of both. When all possible confounding factors are disregarded in the comparison of our results

to those of humans, the low M of cats is related to both low insulin sensitivity and low β-cell response to stimulation by glucose.[22,35]

Cats are obligate carnivores (low-carbohydrate ingestion) and their life style may be associated with relatively long periods of starvation. To meet the specific glucose requirements of the brain, foetus and mammary gland, hepatic and peripheral insulin resistance with respect to blood glucose lowering effects are advantageous. Glucose uptake by brain, foetus and mammary gland do not depend on insulin and are therefore preserved under conditions of insulin resistance. The relatively low insulin sensitivity and/or β-cell response to glucose observed in this study thus seem to fit the carnivorous nature of cats.

Similarly to what has been reported in man,[24,25] in our cats the HOMA-R, HOMA-B and QUICKI correlated well with their respective counterparts measured by the HGC. The HOMA model is used in epidemiologic studies in humans.[39]

Our observations favor the use of the HGC over the ivGTT in cats when possible, for the following reasons. ❶ The cats tolerated the HGC procedure well. ❷ The magnitude of the glucose stimulus during an ivGTT varies strongly between cats, as illustrated by the range of peak plasma glucose concentrations (between 33 and 50 mmol•$L^{-1}$) and by the variation in glucose levels measured at 15 minutes. Indeed, variation in glucose exposure is known to influence results of ivGTTs (K, $T_{1/2}$ and $AUC_{glucose}$) in cats.[21] Conversely, during the HGC in each cat the stimulus could be fixed at 9 mmol•$L^{-1}$ plasma glucose concentration. Thus, compared to the ivGTT, the variation in results introduced by the measuring technique is reduced when using the HGC. ❸ In contrast to the ivGTT, in the HGC multiple measurements of the variables M and I are made at steady state. These measurements can be averaged leading to reproducible determinations of M, I and $ISI_{HGC}$. Thus, the HGC is well suited for the determination of M, I and $ISI_{HGC}$ in cats, for instance for studies on the effects of diets or dietary supplements.[40]

In conclusion the HGC, newly developed for use in conscious cats, allowed for measurements of glucose disposal rate, ß-cells' response to glucose and insulin sensitivity. Compared to humans these variables are low in cats, which is compatible with the obligate carnivorous nature of this species.

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# Response of cats to familiar and unfamiliar human contact using continuous direct arterial blood pressure measurement

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## Abstract

Continuous direct measurement of feline arterial blood pressure (ABP) was carried out via a modified method with percutaneous, ultrasound guided catheterisation of the common carotid artery. In 21 healthy, conscious cats the ABP was measured during rest, alertness and activity. Furthermore, the ABP response to being petted by familiar and unfamiliar persons was assessed.

Linear mixed-effects modelling revealed that the mean blood pressure (MBP) in resting cats (114.6 mmHg) was lower ($P<0.001$) than in alert cats (122.7 mmHg), which was lower ($P<0.001$) than that of active cats (136.8 mmHg). The MBP during petting by a familiar person (144.7 mmHg) tended to be higher ($P=0.065$) than that during petting by an unfamiliar person (139.4 mmHg). The MBP of active cats was lower ($P=0.003$) than MBP of cats petted by a familiar person, but not different from MBP of cats petted by an unfamiliar person. The MBP returned to resting values between 16 and 20 minutes after the familiar person had left, whereas resting values were reached between 11 and 15 minutes after the unfamiliar person had left. The complications of the described method were limited considering the potential risks of continuous direct ABP measurement.

In conclusion, the described technique enables accurate measurement of feline ABP, which is influenced by the cat's activity level and the familiarity of persons.

# Introduction

In cats, arterial hypertension is found in renal disease,[1,2] endocrine diseases, such as hyperthyroidism [3] and primary hyperaldosteronism,[1] left ventricular hypertrophy,[4] and ocular lesions.[5] In clinical practice arterial blood pressure (ABP) is usually measured by indirect non-invasive methods, relying on physical principles such as the Doppler effect and oscillometry. These indirect measurements are easy to perform and safe.[6] With indirect methods reference ranges for systolic and diastolic blood pressure in cats have been established, but they vary between and within techniques. An important factor for these variations with non-invasive techniques is the experience of the operator.[7] Furthermore, due to the innate temperament of cats, the outcome of the measurement can be seriously influenced by the measurement itself, the use of restraint, and (unfamiliar) surroundings.[8-11]

Direct (or invasive) methods to measure ABP are generally accepted as more accurate. In studies in which the variable under investigation is expected to provoke only small ABP changes, direct measurements of ABP are therefore preferred.[12] Apart from its reliability, the direct measurement also allows for long-term continuous monitoring and the elimination of factors that influence the cat's innate temperament. Two methods for long-term continuous monitoring of ABP in cats have been described. One method uses radiotelemetry catheters implanted into a femoral artery.[13] With another technique an arterial catheter is introduced into the descending aorta via the common carotid artery, permitting ABP measurement as well as collection of blood samples.[14] Both techniques use a traumatic surgical approach and involve ligation of the catheterised vessel. Therefore these techniques are not suited for repeated catheterisations, which may be required in a follow-up study.

We have modified the latter technique by omitting the cut-down approach of the artery. Instead, the artery was catheterised percutaneously under ultrasound guidance, making the procedure less traumatic and repetition of the procedure in the same cat at a later date possible. ABP was measured in healthy cats without the physical presence of a person. Measurements were divided into categories according to the behaviour of the cats. Additionally, the ABP was measured while the cats were petted by a person familiar to the cats and by an unfamiliar person.

# Materials and methods

*Cats*

Ten castrated male and 11 spayed female cats were studied. Estimated ages ranged from 5 to 9 years. All cats were domestic shorthairs except for one male mixed breed (Persian x domestic shorthair) cat. Findings at physical examination and routine hematological and biochemical examination were unremarkable. Cats were housed with natural daylight in three equal sized groups in 2.10 by 2.75 by 2.90 metre enclosures, allowing for inter-cat socialisation. Environment was enriched with shelves, rotation of toys and other structures. In addition, cats were provided with human socialisation (brushing, playing, petting, etc.) by dedicated, non-research staff for at least 30 minutes per day per group, for 5 days a week. Also, each group of

cats had free access to a playground (6.30 by 1.80 by 2.90 metres, enriched with shelves and toys) for at least two hours per day. By rotation, each group had access to this playground during the night.

*Protocol*

Catheters were placed on mornings between 08.00 and 12.00 h (24-hour clock) with a maximum of 2 cats per session. After full recovery from anesthesia, cats were relocated to a quiet room with natural daylight, each cat in a separate cage and with the possibility of visual contact between cats. A basket to lie in, a litter box and ad libitum fresh water were supplied. Cats were fed twice daily. Video camera recordings of the cats and of the screen of the patient monitor were mixed into one image, which was recorded by a video recorder. At approximately 17.00 h the continuous recording of the ABP was started. After thirty minutes a person who had never been in contact with the cats before entered the room, opened one of the cages and petted a cat inside its cage for 2 minutes. This person then proceeded to the other cat, petted it for 2 minutes and left the room. The recording of ABP was stopped 30 minutes after the individual had left the room. Starting at 23.00 h, the procedure was repeated by a person familiar to the cats. Both petting sessions were recorded onto the same videotape. In situations that there was only one cat under study, petting of the second cat was imitated. The familiar person petted the cats in the same order as the unfamiliar person. An additional recording was started on a second videotape at 24.00 h. This 3-hour recording was used to obtain ABP values from cats, without a person present in the room.

*Insertion and removal of catheters*

Anesthesia for inserting catheters comprised premedication with 80 $\mu g \bullet kg^{-1}$ medetomidine (Domitor®, Pfizer BV, Capelle aan den IJssel, the Netherlands) intramuscularly (IM), followed by induction of anesthesia with 2 to 4 $mg \bullet kg^{-1}$ propofol (Fresenius Kabi Nederland BV, 's Hertogenbosch, the Netherlands) intravenously (IV) through a canula in a cephalic vein. Following induction, the trachea was intubated and anesthesia was maintained with a mix of isoflurane, oxygen and air. The anesthetised cat was placed in dorsal recumbency, with extended neck.

Double lumen polyurethane catheters (Certofix Duo Paed S420 (20 cm, 4 French)), B. Braun Medical BV, Oss, the Netherlands) were inserted under sterile conditions using the Seldinger technique.[15] The catheters were inserted percutaneously under ultrasound guidance by an experienced ultrasonographer using a high definition ultrasound machine (ATL 3000, Philips Medical Systems Nederland B.V., Eindhoven, The Netherlands) equipped with a 10-5 MHz broad band compact linear array transducer. The carotid artery was located by placing the transducer in the jugular furrow with the scan plane directed perpendicular to the long axis of the neck and medially at a 20-45 degree angle to the parasagittal plane. Pulsatile blood flow was detected by color flow Doppler analysis. After localisation of the carotid artery the transducer was rotated 90 degrees around its axis to image the carotid artery in the longitudinal plane. A stab incision of 2-3 mm was made in the skin. With ultrasound guidance and using the freehand technique, the introduction needle was inserted in the direction of the thoracic inlet at a 30-60 degree angle with the carotid artery. Careful attention was paid to

avoid the external and internal jugular veins. The bevel of the needle was positioned "on top of" the carotid artery, after which the artery was punctured. Blood loss via the needle indicated successful penetration of the arterial wall. While maintaining the position of the needle, a second individual inserted the guide wire through the needle. The needle was then removed while pressure was applied to the insertion point to prevent further hemorrhage. The wire was used to guide the catheter into the lumen of the artery. Under fluoroscopic guidance, the J-tip of the guide wire was positioned in the tip of the catheter, giving it a slight bend. This enabled manoeuvring the catheter into either the descending aorta or the most proximal part of the brachiocephalic trunk, after which the guide wire was removed. Another, identical catheter was inserted into the contra-lateral jugular vein. Both catheters were then partly taped to the lateral side of the cat's neck and covered by a collar bandage.

After placement of the catheters, anesthesia was reversed with 200 $\mu g \cdot kg^{-1}$ atipamezol IM (Antisedan®, Pfizer BV, Capelle aan den IJssel, the Netherlands). and the canula was removed from the cephalic vein. Total duration of the procedure, including anesthesia, was approximately 90 minutes.

After completion of the studies the arterial catheters were removed under anesthesia with digital pressure at the insertion site for at least 10 minutes to prevent hemorrhage. The anesthetic protocol for removal of the catheters was as described above except that maintenance of anesthesia was not necessary.

*Catheter maintenance*

At 8-h intervals, patency of catheter lumens was checked. Only after patency had been verified by drawing some blood, catheter lumens were flushed with 1.5 mL 0.9% saline solution with added heparin (Leo Pharma BV, Breda, the Netherlands) in a final concentration of 50 $IU \cdot mL^{-1}$. Arterial blood drawn to check patency of the arterial catheter was returned through the venous catheter.

*Arterial blood pressure measurement*

The distal lumen of the catheter was connected to a disposable pressure transducer (Gabarith™, catalogue number 682001, Becton Dickinson BV, Alphen aan den Rijn, The Netherlands). The pressure transducer was connected to a patient monitor (AS/3, Datex Ohmeda BV, Hoevelaken, The Netherlands) and a pressurised (300 mmHg) bag of 0.9% saline solution. The pressure transducer was taped to the inside of the cat's cage with the transducer at the height of the heart when the cat was in lateral recumbency. The system was zeroed electronically at atmospheric pressure. Cats could move freely throughout their cage, while ABP was recorded. Whenever a cat was standing or sitting, the measured ABP was corrected for the difference in height between the cat's heart (length measured between the tip of the olecranon and the floor) and the height of the pressure transducer.

Video camera images of the cat and of the screen of the patient monitor, respectively, were mixed into one image and recorded using a video recorder. Thus, the cat's ABP and behaviour were recorded simultaneously on a single videotape. Recordings took place while no individual was present in the same room as the cats. Video recordings were reviewed after collection of all data. Videotapes were assessed by the same person. Systolic, diastolic and

mean blood pressure (SBP, DBP and MBP, respectively) were determined every minute and collected in a spreadsheet. During petting, ABP was recorded every 30 seconds. The behaviour and the position of the cats were continuously recorded. The monitoring results of both cats were displayed on the same monitor. During darkness, a small red light allowed for the recording of the behaviour of the cats.

Depending on the time of day, the cat's behaviour and other circumstances (Table 1, categories 1 - 21), 21 categories were defined and all measurements of SBP, DBP, and MBP were ordered per cat into these categories. After preliminary assessment some categories were combined into new categories (Table 1, categories C1 - C3). A cat at rest was lying quietly with its eyes closed. To ensure that measurements were performed with the cat at complete rest, measurements were only used for analyses after the cat had been resting for at least two minutes (category 1, 4 or 9). Attentive, but inactive behaviour was defined as a cat sitting or lying down while looking around, aware of its surroundings. Active behaviour included walking, eliminating, eating, drinking, grooming and smelling at surroundings. When cats demonstrated different expressions of active behaviour during the same minute, the measurement was not used for analyses.

*Statistics*

Statistical analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, USA), and R [16] using NLME library version 3.1-78.[17] Using SPSS, correlations between MBP and SBP and DBP were investigated by partial correlation controlling for cat and category. To assess the influence of the order of petting, differences in MBP within category 7 and 12 were analysed using full factorial linear mixed-effects modelling (LMM) with random cat effect, and petting order and category as fixed effect, allowing a different variance for each petting order and category. Then, all data for MBP were analysed together using full factorial LMM with random cat effect and fixed category effect, allowing a different variance for each category. After preliminary assessment and redefining some categories into new categories (Table 1), last mentioned LMM was repeated with the redefined categories. For LMM, R was used and MBP data were transformed using the natural logarithm. A transformation of the data was necessary judged by the quantile-quantile plot of the residuals. Post hoc Bonferroni correction was applied. Values of $P<0.05$ (two tailed) were considered statistically significant.

## Results

Arterial catheterisations were uneventful in 16 out of 21 cats. In 5 cats, the bevel of the introductory needle hit the arterial wall without entering the arterial lumen. The resulting hemorrhage was controlled by application of pressure to the insertion site and catheterisation was discontinued. The cats were allowed to recover from anesthesia and were monitored for 24 hours in individual cages before returning to their group housing. Catheterisation was re-attempted after at least two weeks. In one cat, in which the initial catheterisation was successful, loss of blood occurred at the insertion site when blood pressure rose substantially

because of being petted by the familiar person. In this cat, the catheters were removed and the cat was re-catheterised several weeks later without reoccurrence of bleeding. There were no other complications of arterial catheterisations and venous catheterisations were uneventful in all cats. Cats tolerated the collar bandage well.

Signal loss (n=1), a leaking pressure bag (n=1), loss of a videotape (n=1) and video-operator error (n=1) resulted in 17 usable recordings for the analyses of ABP while being petted by an unfamiliar person and 18 usable recordings of ABP while being petted by the familiar person.

A total of 18 recordings at night time (24.00 – 3.00) were used for analyses. Of the 21 recordings made, one was excluded because the ABP signal was lost during the period of measurement. Another videotape, with the recording of two cats, was lost by accident.

As SBP and DBP correlated strongly to MBP (both: r=0.98, $P<0.001$), differences between categories were calculated using MBP as dependent variable.

In Table 1 the ABPs for each category are listed. The first LMM analysis demonstrated that within both category 7 and category 12 there were no significant differences with respect to the order in which the cats were petted ($P=0.64$ and $P=0.68$, respectively). Therefore, the order in which the cats were petted was not included as a factor in further analyses. In addition, the LMM analysis demonstrated that the MBP during petting by a familiar person (144.7 mmHg) tended to be higher ($P=0.065$) than that during petting by an unfamiliar person (139.4 mmHg).

There were no significant differences between the MBPs in categories 1, 4 and 9, categories 2, 5 and 10, and categories 3, 6 and 11, respectively (all combinations $P>0.15$). Therefore, these groups of similar categories were combined into three new categories (C1, C2, C3; Table 1) which were used for further analyses. The MBP in resting cats (114.6 mmHg) was lower ($P<0.001$) than in alert cats (122.7 mmHg), which was lower ($P<0.001$) than that of active cats (136.8 mmHg).

The MBP during petting by a familiar person (144.7 mmHg) was significantly higher ($P=0.003$) than the MBP of active cats (136.8 mmHg). This difference did not exist ($P=1.00$) when comparing cats petted by an unfamiliar person and active cats. After having been petted by a familiar person, MBP returned to resting values (i.e. was not statistically different from resting values anymore) between 16 and 20 minutes after the person had left. For the unfamiliar person this was true between 11 and 15 minutes after the unfamiliar person had left (Figure 1).

Subjectively, cats actively approached the familiar person, whereas the unfamiliar person was less actively approached. Judged by their behaviour, all cats seemed to enjoy company from both the familiar and the unfamiliar person.

| Category | Time | Behaviour | MBP | | | | SBP | | | | DBP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean | median | SD | N | mean | median | SD | N | mean | median | SD | N |
| **1** | 24.00 - 3.00 | Resting | 113.2 | 115.0 | 7.2 | 17 | 130.9 | 132.1 | 8.2 | 17 | 94.4 | 95.4 | 6.9 | 17 |
| **2** | 24.00 - 3.00 | Attentive, but inactive | 122.7 | 121.8 | 10.2 | 18 | 140.8 | 140.0 | 12.2 | 18 | 103.4 | 102.9 | 9.6 | 18 |
| **3** | 24.00 - 3.00 | Active | 136.3 | 131.0 | 16.5 | 15 | 157.2 | 151.3 | 19.1 | 15 | 114.6 | 114.1 | 15.5 | 15 |
| **4** | 17.00 - 17.30 | Resting | 114.4 | 114.0 | 11.3 | 15 | 129.9 | 127.3 | 12.8 | 15 | 98.2 | 97.2 | 11.0 | 15 |
| **5** | 17.00 - 17.30 | Attentive, but inactive | 122.9 | 123.2 | 10.0 | 12 | 139.4 | 140.5 | 10.8 | 12 | 105.1 | 103.5 | 9.7 | 12 |
| **6** | 17.00 - 17.30 | Active | 143.0 | 142.7 | 12.0 | 6 | 161.6 | 161.1 | 14.7 | 6 | 121.9 | 120.3 | 9.4 | 6 |
| **7** | 17.30 | Being petted by unfamiliar person | 139.4 | 141.2 | 21.5 | 17 | 159.0 | 161.8 | 23.5 | 17 | 119.1 | 119.3 | 21.1 | 17 |
| **8** | 17.30 | Unfamiliar person present | 134.8 | 135.5 | 20.7 | 17 | 153.9 | 156.4 | 24.4 | 17 | 114.9 | 112.7 | 18.9 | 17 |
| **9** | 23.00 - 23.30 | Resting | 114.0 | 113.9 | 12.1 | 10 | 132.6 | 133.9 | 14.5 | 10 | 94.8 | 92.9 | 11.7 | 10 |
| **10** | 23.00 - 23.30 | Attentive, but inactive | 119.5 | 120.1 | 15.2 | 13 | 137.6 | 136.8 | 15.5 | 13 | 100.4 | 100.9 | 14.9 | 13 |
| **11** | 23.00 - 23.30 | Active | 133.6 | 140.3 | 16.3 | 5 | 152.0 | 161.7 | 18.7 | 5 | 114.0 | 119.3 | 14.5 | 5 |
| **12** | 23.30 | Being petted by familiar person | 144.7 | 142.7 | 19.4 | 18 | 167.3 | 165.5 | 21.0 | 18 | 121.2 | 120.1 | 18.8 | 18 |
| **13** | 23.30 | Familiar person present | 144.6 | 145.7 | 19.1 | 18 | 166.5 | 169.5 | 22.4 | 18 | 122.6 | 121.4 | 18.5 | 18 |
| **14** | 23.30 - 24.00 | 0' - 5' after familiar person | 130.4 | 127.8 | 11.4 | 18 | 149.1 | 147.2 | 11.9 | 18 | 109.5 | 107.5 | 11.1 | 18 |
| **15** | 23.30 - 24.00 | 6' - 10' after familiar person | 123.7 | 121.5 | 13.5 | 18 | 141.5 | 140.6 | 14.3 | 18 | 103.8 | 100.9 | 13.3 | 18 |
| **16** | 23.30 - 24.00 | 11' - 15' after familiar person | 117.9 | 114.6 | 12.2 | 18 | 135.3 | 132.2 | 13.0 | 18 | 98.4 | 95.5 | 11.8 | 18 |
| **17** | 23.30 - 24.00 | 16' - 20' after familiar person | 113.5 | 112.9 | 9.6 | 18 | 130.7 | 129.2 | 10.4 | 18 | 94.5 | 94.1 | 9.5 | 18 |
| **18** | 17.30 - 18.00 | 0' - 5' after unfamiliar person | 127.2 | 130.1 | 18.0 | 17 | 143.7 | 150.1 | 19.7 | 17 | 109.6 | 108.5 | 18.0 | 17 |
| **19** | 17.30 - 18.00 | 6' - 10' after unfamiliar person | 120.2 | 122.3 | 14.3 | 17 | 136.0 | 139.6 | 16.2 | 17 | 103.7 | 104.4 | 15.0 | 17 |
| **20** | 17.30 - 18.00 | 11' - 15' after unfamiliar person | 116.5 | 115.9 | 14.6 | 17 | 131.6 | 133.3 | 16.2 | 17 | 99.8 | 95.4 | 14.5 | 17 |
| **21** | 17.30 - 18.00 | 16' - 20' after unfamiliar person | 115.2 | 115.3 | 16.6 | 17 | 130.2 | 131.8 | 18.4 | 17 | 98.1 | 93.6 | 15.8 | 17 |
| **C1** | | Combination of categories 1, 4 & 9 | 114.6 | 116.1 | 7.6 | 20 | 132.2 | 132.5 | 8.9 | 20 | 96.0 | 95.7 | 7.6 | 20 |
| **C2** | | Combination of categories 2, 5 & 10 | 122.7 | 123.1 | 10.6 | 18 | 140.8 | 140.8 | 12.6 | 18 | 103.6 | 104.4 | 10.0 | 18 |
| **C3** | | Combination of categories 3, 6 & 11 | 136.8 | 134.3 | 16.2 | 16 | 157.4 | 151.8 | 18.9 | 16 | 115.5 | 115.4 | 15.1 | 16 |

**Table 1** Definition of categories and measured ABP values in different categories. Mean, median, standard deviation (SD) and number of cats contributing to these values (N) of mean blood pressure (MBP), systolic blood pressure (SBP), and diastolic blood pressure (DBP) for all categories. The presented values of each category were calculated as follows: for each cat the average ABP results per category were calculated; these averages were used to calculate the mean, median and standard deviation for each category.

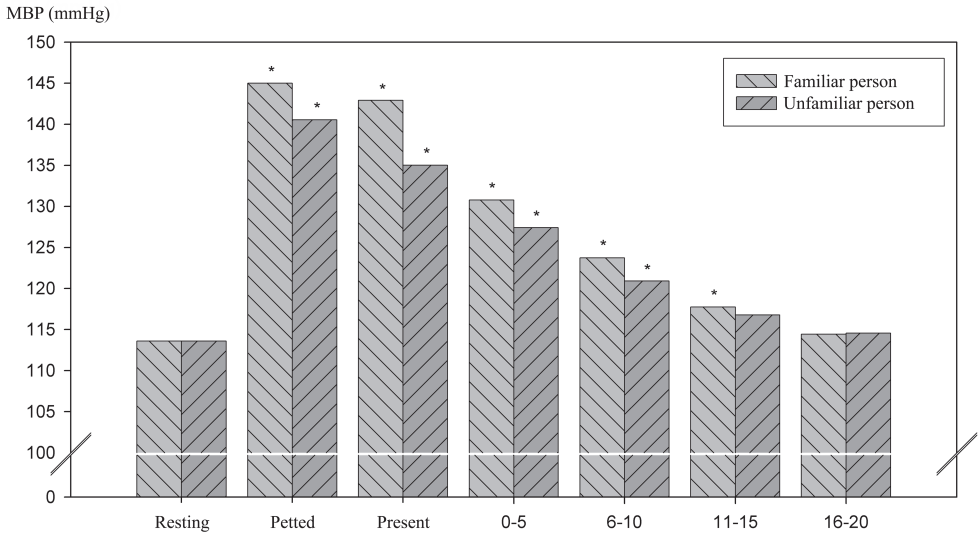


**Figure 1** Mean blood pressure (MBP) of cats, when resting, when being petted by a familiar or unfamiliar person, and when a (familiar or unfamiliar) person was present in the same room. Also, the recovery phase (minutes after the person had left) is depicted. The presented values of each category are the predicted means provided by linear mixed-effects modelling. * Different from resting MBP ($P$<0.001)

## Discussion

Catheterisation of the aorta through a common carotid artery is advocated as the preferred technique for long-term (>48 h) arterial access in dogs and cats.[18] However, arterial catheterisation, maintenance of patency, and catheter removal require great care.[18,19] Complications that can be expected are hemorrhage, thrombosis or (thrombo-)arteritis of the catheterised vessel, thrombo-embolism, infection, and loss of patency. In this study, most complications were probably prevented by pre-set guidelines. In agreement with earlier reports, placement of the catheter under aseptic conditions helped preventing infections,[18] whereas frequent flushing of catheters and adequate fixation of catheters helped preventing loss of patency.[14,19,20] Flushing a non-patent arterial catheter can lead to thrombo-embolism.[21] Therefore, patency was always checked before flushing an arterial catheter by drawing some blood. When blood is returned arterially, there is the risk of introducing (micro-) thrombi into the arterial vasculature. Therefore, in our cats blood was returned intravenously. Digital pressure to the puncture site prevented serious hemorrhage after catheter removal.[19]

In addition to the pre-set guidelines, we were also guided by experiences from pilot arterial catheterisations. If during catheterisation the needle hit the arterial wall without entering the arterial lumen, the needle was removed, digital pressure was applied for at least 10 minutes and no further attempts were made to enter the carotid artery to prevent excessive hemorrhage and associated complications. As reported earlier,[14,20] advancing the catheter tip

into the brachiocephalic trunk or the descending aorta (instead of leaving it in the common carotid artery) strongly reduced catheter patency problems.

For long-term continuous monitoring of ABP in cats 2 methods have been described.[13,14] Both techniques use a traumatic surgical approach and involve ligation of the catheterised vessel. Therefore these techniques are not suited for repeated catheterisations, which may be required in a follow-up study. Using ultrasonography to approach an artery reduces the invasiveness of the catheterisation, when compared to a cut-down technique or when compared to the implantation of radiotelemetry devices. This has the advantage of less trauma to the tissues surrounding the common carotid artery. Less trauma further reduces the risk of complications resulting from insertion (e.g. local infections and (thrombo-)arteritis). Furthermore, it enables repeated performance of the procedure in the same cat over time as the catheterised artery is not severely damaged or permanently ligated. This allows for research designs in which ABP is measured repeatedly in the same individual, with long intervals between measurements. Ultrasound-guided puncturing of the common carotid artery can be considered a technical challenge as demonstrated by the 5 out of 21 cats in which catheterisation was not successful the first time. However, in these cats excessive hemorrhage could easily be prevented by not reattempting to enter the carotid artery at that time and by applying digital pressure for at least 10 minutes.

In one cat, the study had to be repeated because of bleeding at the catheter insertion site. When this cat was re-catheterised later, loss of signal occurred whenever the cat laid down, probably due to kinking of the catheter or obstruction of the tip of the catheter. The catheterisation technique described here can be considered useful for the measurement of feline ABP as it only posed measurement problems related to the catheterisation technique in one cat. However, continuous, direct arterial blood pressure measurement requires clear protocols with pre-set guidelines and well-trained, experienced personnel to prevent the potentially severe complications of arterial catheter introduction, maintenance, and removal.

The resting MBP values (category C1) in this study compare well with those of other studies. In one study in which the MBP of five conscious cats was measured using radiotelemetry catheters implanted in the femoral artery, the mean baseline MBP was 103 mmHg with a standard error of 5 mmHg.[22] In another study, the same technique was used in 12 conscious cats to measure the mean 24-hour MBP, resulting in a mean MBP of 107.1 mmHg with a standard error of 3.3 mmHg.[13] In a third study, in which the aorta was catheterised through the common carotid artery, resting MBP ranged from 100 to 110 mmHg in 1 conscious cat.[14]

ABPs of cats vary less than 3 mmHg between night and day.[23] The absence of differences between afternoon and night MBPs in our cats, support this finding. However, environmental synchronisers such as the presence of laboratory personnel and (the anticipation of) feeding have been hypothesised to induce fluctuations, irrespective of the time of day these environmental synchronisers occur.[23,24] Our data confirm that certain activities (eating, grooming, eliminating etc.) and the presence of personnel result in MBP elevations. This supports the notion that feeding at set times acts as an environmental synchroniser and induces the patterns in the ABP of cats reported by others.[23,24]

Surprisingly, while it is generally assumed that familiarity with people reduces stress in cats during studies, being familiar to a cat tended to result in a higher MBP during the short petting sessions reported here. The difference between a familiar and an unfamiliar person is supported by the finding that MBP while being petted by a familiar person was significantly higher than that while being active, whereas this difference was not found for the unfamiliar person. Furthermore, the return of the MBP values to resting values took longer after being petted by a familiar person than after being petted by an unfamiliar person. All the abovementioned differences demonstrate that the technique described in this study allows detection of relatively small differences in ABP, making it suited for application in studies where the researched variable is expected to induce only slight ABP changes.

Others have described similar effects of familiarity on cardiovascular variables, although the exact causes remain speculative. In humans, entry of a dog with which a companion bond had been established resulted in significantly higher blood pressures than entry of an unknown dog.[25] People to which a dog is attached have the greatest effect on the dog's heart rate.[26] In cats, human presence has been shown to increase heart rate. Petting results in a further increase of the cat's heart rate.[27] We can also only speculate why there was a different response to familiar and unfamiliar persons in our cats. In the present study the familiar person probably caused more excitement, due to the expectation of for instance being fed or petted, whereas not knowing a person may have resulted in a different, more reserved emotional and motor response.

Differences in MBP observed between resting, inactive but attentive, and active cats together with differences between petting by familiar or unfamiliar persons illustrate the potential problems associated with ABP measurement in conscious cats, as the outcome of a measurement may depend on interactions between the cat and the researcher. A so called white-coat effect has been described in cats and transportation and physical examination have been identified as possible contributors to this effect.[11] Therefore, measurement of ABP in conscious cats is ideally performed without handling the cat and requires the absence of any individual at the time of measurement. Non-invasive ABP measurements obviously cannot fulfil these requirements, although being the most practical solution for routine clinical ABP measurements at this time. For research purposes, however, measurement techniques are required that have a minimal influence on ABP.[12] The method described here meets abovementioned requirements and also allows for comparison of ABP with the animal's behaviour as well as sampling of arterial blood. Using this technique, relatively small differences in ABP can be detected. Additionally, because of the reduced invasiveness during catheter insertion, this technique is suited for research designs requiring repeated catheterisation. Therefore, the technique described here is a valuable tool for the study of ABP in unrestrained, conscious cats.

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# Calcium stimulation test for the assessment of β-cell function in cats

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## Abstract

To assess the potential of an intravenous calcium stimulation test (CST) as an indicator of insulin secretion in cats, indices calculated from CST results were compared with indices of insulin secretion derived from an intravenous glucose tolerance test (ivGTT) and hyperglycemic glucose clamp (HGC) in 11 healthy, normal glucose tolerant, conscious cats.

Intravenous administration of 2.5 mg • $kg^{-1}$ $Ca^{2+}$ resulted in a significant increase in plasma free $Ca^{2+}$ ($P$<0.001) and plasma insulin ($P$=0.047) concentrations but did not affect the plasma glucose concentration. The indices of insulin secretion based on the CST did not correlate significantly with corresponding indices based on the ivGTT and HGC.

In conclusion, the CST is not a useful test for assessing insulin secretion in cats. Other indices of insulin secretion, such as fasting insulin concentrations and the homeostasis model assessment of β-cell function (HOMA-B), are easier to obtain and correlate better with indices of insulin secretion derived from the HGC, the gold standard technique for assessing insulin secretion.

# Introduction

In-vitro studies have demonstrated that an increase in the extracellular $Ca^{2+}$ concentration causes a transient increase in insulin secretion by rat and human pancreatic β-cells, followed by an inhibition of insulin secretion, which is $Ca^{2+}$ concentration dependent, prolonged and reversible.[1,2] We investigated the potential of an intravenous calcium stimulation test (CST) as a relatively simple test for evaluating insulin secretion in cats. We compared indices of insulin secretion immediately after intravenous administration of calcium with those of insulin secretion derived from an intravenous glucose tolerance test (ivGTT) and hyperglycemic glucose clamp (HGC) in 11 healthy, conscious, normal glucose tolerant cats.

# Materials and methods

The cats (5 castrated males, 6 spayed females) were healthy according to physical examination and routine biochemical and hematological variables. Housing, feeding, care for the cats, (anesthesia for) catheter placement and management, performance of the ivGTT and HGC, sample handling and analysis (glucose, insulin) and calculations of variables from the ivGTT and HGC were as described previously.[3] The research protocols and care for the cats were approved by the Committee for the Ethical Treatment of Animals of Utrecht University (the Netherlands) according to Dutch and European law.

The HGC, the gold standard technique for assessing insulin secretion, was performed on average 3 months after the CST. The CST was performed 1 day before (6 cats) or 1 day after the ivGTT (5 cats), using the same jugular catheter. After collection of 2 fasting blood samples at –15 and –2 minutes, 2.5 mg • $kg^{-1}$ body weight of $Ca^{2+}$ (Calcii Gluconas, Pharmachemie BV, Haarlem, the Netherlands) was administered intravenously in 30 seconds through a catheter lumen that had not been used for blood sampling or administration of glucose for the ivGTT; two other samples were collected 1 and 2 minutes after $Ca^{2+}$ administration. Blood samples (0.2 mL) collected with a dry, heparin-containing syringe (PICO 30, Radiometer Nederland BV, Zoetermeer, the Netherlands) were analysed immediately after collection by an automated analyser (EML 105, Radiometer Nederland BV, Zoetermeer, the Netherlands) to measure free $Ca^{2+}$.

The measure of insulin secretion derived from the CST, $INS_{CST}$, was calculated as the area under the curve (AUC) for insulin from 0 to 2 minutes, using the trapezoidal rule. The insulin concentration at 0 minutes was determined as the mean of the two fasting insulin samples collected at –15 and –2 minutes. First-phase insulin secretion and total insulin secretion during the ivGTT (Phi-1 and Phi-1+2, respectively) were calculated, as were first-phase insulin secretion (AUC-Ins1) and the β-cell response to glucose during steady state hyperglycemia (I) during HGC. In addition to these values calculated above the zero-level, values above the baseline were calculated (incremental values). The baseline was determined as the average fasting plasma insulin concentration measured directly before each separate test.

Statistical analyses were performed with Microsoft Excel 2000 (Microsoft NL BV, Amsterdam, the Netherlands) and SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, USA). Some data from the HGC and the ivGTT have been published earlier and were tested for outliers and distribution type as described.[3] All other data (including incremental ivGTT and incremental HGC variables) were tested for outliers and distribution in a similar fashion, using Grubb's T-statistic and Kolmogorov-Smirnov tests with Lilliefors significance correction. Phi-1, Phi-1+2, incremental Phi-1 and incremental I were log transformed. Data for I (but not incremental I) were not parametric after log transformation, and therefore the non-transformed data for I were used and the correlation between $INS_{CST}$ and I was calculated using Spearman's rank correlation test. In all other cases, Pearson's correlation coefficient was calculated (Table 1). To assess whether the intravenous $Ca^{2+}$ administration led to an increase in plasma-free $Ca^{2+}$ concentrations and plasma insulin concentrations, a repeated measures ANOVA with post hoc Bonferroni correction was used.

## Results and discussion

The fasting glucose concentrations and the glucose half-life during ivGTT indicated that the cats were normal glucose tolerant.[4] The mean plasma glucose concentration (± SD) during the CST (baseline and 1 and 2 minutes after $Ca^{2+}$ administration) was 4.7 (0.3), 4.7 (0.3), and 4.8 (0.4) mmol • $L^{-1}$, respectively. The intravenous administration of 2.5 mg • $kg^{-1}$ $Ca^{2+}$ led to a significant increase in the mean plasma concentrations of free $Ca^{2+}$ ($P$<0.001) and insulin ($P$=0.047) (Figure 1). The $Ca^{2+}$-induced increase in plasma insulin concentration was probably caused by an increase in insulin secretion, as demonstrated by in-vitro tests with β-cells from humans and rats.[1,2]

| | $INS_{CST}$ | | Incremental $INS_{CST}$ |
|---|---|---|---|
| Phi-1 | 0.52 ($P$=0.10) | Incremental Phi-1 | 0.48 ($P$=0.13) |
| Phi-1+2 | 0.47 ($P$=0.14) | Incremental Phi-1+2 | 0.29 ($P$=0.39) |
| AUC-Ins1 | 0.45 ($P$=0.16) | Incremental AUC-Ins1 | 0.28 ($P$=0.40) |
| I | 0.45 ($P$=0.17)$^{\Theta}$ | Incremental I | 0.34 ($P$=0.30) |

**Table 1** Pearson's correlation coefficients between the indices of insulin secretion from the calcium stimulation test and those of the intravenous glucose tolerance test and hyperglycemic glucose clamp in 11 healthy cats. Θ: Spearman's rank correlation coefficient.

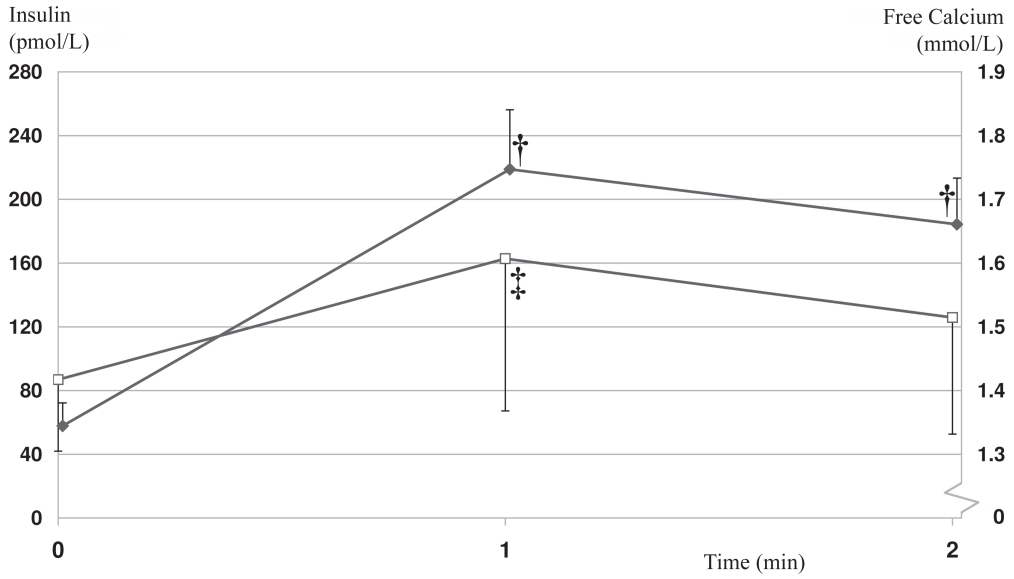


**Figure 1** Mean (± SD) plasma insulin concentrations (□) and plasma free $Ca^{2+}$ concentrations (◆) in 11 healthy cats during the calcium stimulation test. The average fasting concentrations of $Ca^{2+}$ and insulin are depicted as the concentration at 0 minutes. †: Significantly different from fasting plasma free $Ca^{2+}$ concentrations ($P < 0.001$). ‡: Significantly different from fasting plasma insulin values ($P = 0.047$).

The mean (± SD; range) $INS_{CST}$ was 269 (± 141; 102 to 469) pmol • $L^{-1}$ • min, but the mean incremental $INS_{CST}$ was 95 (± 119; -48 to 295) pmol • $L^{-1}$ • min. The plasma insulin concentration did not increase but instead decreased in 3 of 11 cats. Of these 3 cats, 2 underwent the CST before the ivGTT and 1 underwent the CST after the ivGTT, which makes it unlikely that the test order was a causative factor. These cats may not have had a 'readily releasable pool' of insulin granules in their β-cells [5] and therefore were unable to respond to the increase in $Ca^{2+}$ with an increase in insulin secretion. This readily releasable pool of insulin may not be present if fasting insulin secretion is increased due to an increased fasting glucose concentration. However, in our cats incremental $INS_{CST}$ was not correlated with fasting glucose concentrations or fasting insulin concentrations measured immediately before administration of $Ca^{2+}$. Moreover, our 3 cats responded with a decrease in insulin concentrations and not with an attenuated or absent increase. Therefore, the reason for this lack of an insulin response to increased free $Ca^{2+}$ levels in these 3 cats remains unclear. This inter-individual variation in the response to an intravenous bolus of $Ca^{2+}$ limits the usefulness of the CST to population studies only.

The CST variables did not correlate significantly with the corresponding ivGTT or HGC variables (Table 1). In contrast, the mean fasting insulin concentration immediately before the HGC correlated significantly with AUC-Ins1 and I.[3] Whereas the incremental

$INS_{CST}$ did not correlate with the incremental ivGTT and HGC indices either (Table 1), the incremental measures of first-phase insulin secretion during the ivGTT (incremental Phi-1) and the HGC (incremental AUC-Ins1) were correlated (Pearson's r=0.79; *P*=0.004), as were the incremental measures of overall β-cell response to glucose during the ivGTT (incremental Phi-1+2) and HGC (incremental I, Pearson's r=0.76; *P*=0.006).

The homeostasis model assessment of β-cell function (HOMA-B), another measure of insulin secretion, is derived from fasting plasma glucose and fasting plasma insulin concentrations.[6] This index correlated significantly with AUC-Ins1 and I (Pearson's r=0.94; *P*<0.001 and Pearson's r=0.81; *P*<0.001, respectively) after HGC in healthy glucose tolerant cats.[3]

In addition to the HOMA-B, indices of insulin sensitivity can be calculated from fasting plasma glucose and fasting plasma insulin levels. Of these, the homeostasis model assessment of insulin resistance (HOMA-R)[6] and the quantitative insulin sensitivity check index (QUICKI)[7] are used most often in humans and have also been used in cats.[3,8] The calculation of the HOMA-B, HOMA-R and QUICKI requires only the measurement of the plasma glucose and insulin concentrations and does not require intravenous administration of stimulating substances. Thus, HOMA-B, HOMA-R and QUICKI are easier to obtain than the CST index of insulin secretion. Additionally, the indices based on fasting samples allow the assessment of β-cell function in relation to insulin sensitivity, which is necessary for accurately assessing β-cell function.[9]

In conclusion, our results indicate that the CST is not a valuable test for assessing insulin secretion in cats. Indices derived from fasting samples are easier to obtain and correlate better with results from the gold standard technique for assessing insulin secretion, the HGC.

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# II

# Risk factors

# Growth hormone excess and the effect of octreotide in cats with diabetes mellitus

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Submitted

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## Abstract

In this prospective study 16 cats with diabetes mellitus were examined for concurrent acromegaly by measuring plasma growth hormone (GH) and insulin-like growth factor-I concentrations, and magnetic resonance imaging (MRI) of the pituitary fossa. Additionally, the effects of octreotide administration on the plasma concentrations of glucose, GH, α-melanocyte-stimulating hormone (α-MSH), adrenocorticotrophic hormone (ACTH), and cortisol were measured.

Five cats were diagnosed with hypersomatotropism. The pituitary was enlarged in these 5 cats and in 2 other cats. Six cats that required a maximum lente insulin dosage >1.5 IU/kg body weight per injection had pituitary enlargement and 5 of these cats had acromegaly. Plasma concentrations of GH, ACTH, and cortisol decreased significantly after octreotide administration in the acromegalic cats. The effect on GH concentrations was more pronounced in some of the acromegalic cats than in others. In the non-acromegalic cats only ACTH concentrations decreased significantly. In both groups plasma glucose concentrations increased slightly but significantly, whereas α-MSH concentrations were not significantly affected.

In conclusion, the incidence of hypersomatotropism with concomitant pituitary enlargement appears to be high among diabetic cats with severe insulin resistance. Some of these cats responded to octreotide administration with a pronounced decrease in the plasma GH concentration, which suggests that octreotide administration could be used as a pre-entry test for treatment with somatostatin analogues.

# Introduction

Feline diabetes mellitus and human type 2 diabetes mellitus share several clinical and pathological characteristics, such as age of onset in midlife or later, variable but at least residual insulin secretion at the time of diagnosis, relative resistance to ketoacidosis, significant but incomplete loss of ß cells, and deposition of amyloid in the pancreatic islets.[1] The main pathogenetic mechanisms of type-2 diabetes mellitus in humans are impaired insulin secretion and insulin resistance, which both may have a genetic background.[2,3] Genetic predisposition also seems to play a role in feline diabetes.[4] In analogy to the situation in humans, it is generally accepted that in (genetically predisposed) cats, acquired factors such as obesity and physical inactivity may precipitate the disease by inducing insulin resistance.[5-7]

Certain diseases, such as acromegaly and hypercortisolism, also induce insulin resistance and consequently may lead to "other specific types" of diabetes mellitus. Affected cats are often first presented with manifestations of diabetes mellitus.[8,9] There is less clarity on the occurrence of these diseases among diabetic cats. In one review these diseases are not mentioned as potential factors involved in the development of feline diabetes mellitus.[10] In another review these conditions were considered to be rare, albeit with the remark that the prevalence of these cases tends to be higher in referral institutions than in general practice.[11] From one referral institution there is a report on a study in 16 diabetic cats with insulin resistance. Computed tomography revealed that all these cats had an enlarged pituitary gland.[12]

In case of acromegaly or pituitary-dependent hypercortisolism, treatment directed at the pituitary lesion may alleviate the diabetes mellitus considerably or even lead to complete cure. The three treatment modalities are radiation therapy, hypophysectomy, and medical treatment. Radiation therapy shrinks the pituitary tumour [13,14] and improves diabetic control but this may take weeks to months and relapse may occur 6-18 months after treatment.[15] Disadvantages of radiation therapy include limited availability, extended hospitalisation, frequent anesthesia, high expense, and unpredictable outcome.[16] Microsurgical transsphenoidal hypophysectomy is an effective treatment for pituitary-dependent hypercortisolism, with some cats no longer requiring insulin treatment.[9] Although, like in human medicine, it is probably the method of choice, experience is limited so far.

Against this background it is worthwhile considering medical treatment options, which play an important role in the management of pituitary disease in humans. One such option is the use of somatostatin receptor ligands such as octreotide and lanreotide. These drugs improve symptoms of acromegaly in most human patients, with normalisation of circulating insulin-like growth factor-I (IGF-I) concentration and tumour shrinkage occurring in approximately 50% of cases.[17] Somatostatin analogues have also been used in the treatment of Cushing's disease.[18] There is little experience with these drugs in cats. One cat with acromegaly responded to octreotide administration with normalisation of plasma growth hormone (GH) concentration,[19] but in 4 acromegalic cats treatment with octreotide had little or no effect on serum GH concentrations.[8] Pre-entry tests to select cases for effectiveness of octreotide have not been reported in cats.

Here we report on a series of 16 diabetic cats that were examined for the presence of GH excess at the Utrecht University Clinic for Companion Animals. We also investigated the effects of a single intravenous injection of octreotide on plasma concentrations of glucose, GH, α-melanocyte-stimulating hormone (α-MSH), adrenocorticotrophic hormone (ACTH), and cortisol.

## Materials and methods

*Protocol*

In this prospective study, the only inclusion criteria were that the cats were known to have diabetes mellitus and that the owners were willing to participate in the study. The owners were asked to bring a urine sample collected on the morning of the day the cats were to be examined for routine urinalysis and not to give food or insulin. If the owners did not succeed in collecting urine, the cats underwent cystocentesis. Following informed client consent and recording of the cat's disease history, blood was collected for the assessment of routine biochemical and hematological variables. An octreotide suppression test was performed in the morning, followed by MRI of the pituitary fossa and abdominal ultrasonography in the afternoon. After completion of the octreotide test, cats received one third of their usual dose of insulin. At least 1 week after the visit to the university clinic, the owners collected morning urine samples on two consecutive days for the measurement of the basal urinary corticoid/creatinine ratio (UCCR). Then the cats were referred back to their primary-care veterinarians, who could contact one of the authors (LIS) for additional advice.

*Cats*

The ages of the 11 castrated males and 5 spayed females ranged from 6 to 15 years and their body weights ranged from 4.1 kg to 7.1 kg. There were 13 domestic shorthairs, 1 Maine Coon, 1 Burmese, and 1 Abyssinian cat. At the time of examination the cats were in good bodily condition and there were no physical signs of hypercortisolism or acromegaly. In all cats there was glucosuria and hyperglycemia and the plasma fructosamine concentrations ranged from 425 to 661 μmol/L (reference range 156-240 μmol/L). None of the cats were treated with glucocorticoids or progestagens at the time of admission. Two cats had a plantigrade posture when standing or walking. There were no other nervous system signs or locomotor disturbances. None of the cats were deemed to be hyperthyroid on the basis of the plasma total thyroxine concentration. Other routine hematological and biochemical variables were unremarkable for diabetic cats. A complete history was taken, with special attention being paid to the duration of insulin treatment, hypoglycemic events, and previous use of glucocorticoids and progestagens. The list of used insulin dosages and resulting blood glucose concentrations was checked against information provided by the veterinarian, and the maximum dose of insulin that did not lead to symptoms of hypoglycemia and blood glucose concentrations <4 mM (as demonstrated by blood glucose day curves) was established.

*Octreotide suppression test*

Before intravenous administration of octreotide (5 µg/kg body weight, Sandostatine®, Novartis Pharma BV, Arnhem, NL), two basal blood samples (at –15 and –2 minutes) were collected from the jugular vein for the determination of the plasma concentrations of glucose, GH, IGF-I, α-MSH, ACTH, and cortisol. Additional blood samples were collected 15, 30, 60, 90 and 120 minutes after administration of octreotide for the determination of glucose, GH, α-MSH, ACTH, and cortisol.

*Sample handling and hormone measurements*

Blood for the measurement of glucose was transferred immediately to fluoride-coated tubes, and blood for the measurement of hormone concentrations was immediately transferred into pre-chilled EDTA-coated tubes, kept on ice until the end of the octreotide test, and then centrifuged for 12 minutes at 4°C at 5500 g. Plasma was stored at -20°C until assayed. The assays for the determination of α-MSH, ACTH, cortisol, and GH have been described elsewhere.[20,21] Total plasma IGF-I was measured after acid-ethanol extraction, using a mixture of 87.5% (v/v) ethanol and 12.5% 2 M formic acid. Tubes containing 100 µL of plasma and 400 µL of the acid-ethanol mixture were incubated at room temperature for 30 minutes. After centrifugation at 5500 g and 4°C for 30 minutes, 50 µL of the supernatant was diluted 1:50 with assay buffer containing 63 mM $Na_2HPO_4$ (pH 7.4), 13 mM $Na_2EDTA$, and 0.25% (w/v) bovine serum albumin. The mean extraction efficiency was 75% with a standard deviation of 7%. After extraction, plasma IGF-I concentrations were measured in a heterologous radioimmunoassay. IGF-I antiserum AFP4892898 was obtained from the National Hormone and Peptide Program (Harbor-UCLA Medical Center, Torrance CA) and human IGF-I for iodination was obtained from Novozymes GroPep (Adelaide, Australia). Dilutions of a feline sample were parallel to the standard curve; the sensitivity of the assay was 10 µg/L. The intra-assay and inter-assay coefficients of variation were 4.7% and 12.4% respectively, at a plasma concentration of 700 µg/L. The urine samples for the determination of the UCCR were sent to the laboratory by regular mail and were analysed upon arrival, as reported earlier.[22,23]

*Diagnostic imaging*

MRI was performed in anesthetised cats in sternal recumbence using a 0.2-Tesla open magnet (Magnetom Open Viva, Siemens AG, Erlangen, Germany) with a small multipurpose coil, using a T1-weighted gradient echo (flash 3D) sequence with a slice thickness of 1 mm and a flip-angle of 30º, before and after the intravenous injection of 0.2 mL of contrast medium (Dotarem, Guerbet Nederland BV, Gorinchem, The Netherlands, containing 377 mg megluminegadoterate per mL) per kg of body weight, a dose which was earlier found to be optimal for examination of the pituitary gland in dogs.[24] In each cat the pituitary gland was judged to be 'enlarged' or 'not enlarged' on the basis of whether or not the dorsal contour of the gland protruded above the suprasellar extensions of the intercrural cistern. The height and width of the pituitary gland were measured on the images of the original transverse series before injection of contrast medium. Length was measured on the sagittal multiplanar reconstruction series from the original transverse series. On the image of the transverse series that contained the largest cross-section of the pituitary gland, the edges of the brain were traced and the enclosed area was calculated. The pituitary

height/brain area (P/B) value was calculated by dividing the height of the pituitary (mm x $10^2$) and the area of the brain ($mm^2$), as described previously for CT measurements of the pituitary gland in dogs.[25] Ultrasonography was done in anesthetised cats in dorsal recumbence through a ventral abdominal approach using a high-definition ultrasound system (HDI 3000, Philips, Eindhoven, NL) equipped with an 8-5 MHz broadband curved array transducer.

*Data analysis*

Statistical analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, USA), and R [26] using NLME library version 3.1-80.[27] A Mann-Whitney U test was used to compare the P/B values for two categories of maximum insulin dosage (<1.5 IU / kg body weight per injection (category 1) and >1.5 IU / kg body weight per injection (category 2)). The (natural logarithm transformed) results of the hormone measurements during the octreotide test were analysed using linear mixed-effect modelling, with random cat effect. The random effects were modelled based on the Akaike Information Criterion (AIC). A group variable was created that differentiated between acromegalic and non-acromegalic cats. Depending on the AIC, models could include a factor time, a factor group, and interactions between these two factors. If beneficial to the model, variances were allowed to differ between different strata and an autoregressive correlation structure (AR1) was used to model the dependence in time. Bonferroni correction was applied and $P<0.05$ was considered significant.

# Results

Acromegaly was diagnosed in 5 cats (4 males, 1 female) with elevated plasma GH concentrations and an enlarged pituitary gland on MRI (Table 1; cats 2, 8, 11, 15, and 16). Plasma IGF-I concentrations were also elevated in these cats (Table 1). Cats 8, 15, and 16 died or were euthanised because of diabetic complications (hypoglycemia, ongoing problems with regulation). On post-mortem examination, a pituitary adenoma that was immunohisto-chemically positive for GH was found in cats 8 and 16. In cat 15 two pituitary adenomas, one staining for GH and the other for ACTH, were found. In the latter cat, which died before collection of urine for UCCR measurements was completed, post-mortem examination also revealed that the zona fasciculata of both adrenal glands was hyperplastic.

The UCCR was elevated in 3 acromegalic cats (cats 2, 11, and 16) and 2 non-acromegalic cats (cats 7 and 14). Abdominal ultrasonography was unremarkable in 15 cats, but multiple hypoechoic foci in the pancreas were detected in cat 11. Microscopic examination of fine-needle aspirates of these foci led us to diagnose (focal) purulent necrotising pancreatitis. Cat 16 was accustomed to urinating outdoors, but for collection of urine samples for UCCR determination it was locked up in the owner's bathroom.

| | | Cat 1 | Cat 2 | Cat 8 | Cat 9 | Cat 11 | Cat 15 | Cat 16 | Mean (SD; range) of the other 9 cats | Reference ranges |
|---|---|---|---|---|---|---|---|---|---|---|
| **GH (µg/L)** | **t=-15** | 1.5 | 35 | 415 | 3.8 | 18 | 16 | 20 | 3.2 (1.9; 0.6-7.2) | 1.5 - 7.9 |
| | **t=-2** | 9.4 | 25 | 230 | 2.9 | 16 | 16 | 22 | 3.5 (2.7; 1.1-9.5) | |
| **IGF-I (µg/L)** | **t=-15** | 496 | 2420 | 1908 | 229 | 3378 | 1648 | 956 | 476 (158; 270-770) | 196 -791 |
| | **t=-2** | 412 | 2662 | 1942 | 252 | 2960 | 1648 | 1203 | 478 (159; 274-784) | |
| **α-MSH (ng/L)** | **t=-15** | ND | 58 | 235 | 31 | 293 | 391 | 283 | 208 (146; 80-497) | 29 - 503 |
| | **t=-2** | ND | 32 | 87 | 36 | 267 | 131 | 526 | 180 (138; 74-486) | |
| **ACTH (ng/L)** | **t=-15** | 109 | 68 | 55 | 145 | 108 | 206 | 37 | 67 (55; 21-156) | 15 - 358 |
| | **t=-2** | 100 | 68 | 43 | 114 | 88 | 126 | 41 | 67 (53; 16-158) | |
| **Cortisol (nmol/L)** | **t=-15** | 93 | 177 | 379 | 146 | 255 | 298 | 132 | 131 (83; 35-303) | 12 - 263 |
| | **t=-2** | 85 | 250 | 440 | 97 | 238 | 331 | 90 | 165 (79; 55-306) | |
| **UCCRs (x $10^{-6}$)** | **day 1** | 20 | 66 | 3.4 | 25 | 77 | ND | 57 | 26 (15; 10-51) | 8 - 42 |
| | **day 2** | 22 | 92 | 6.5 | 25 | 61 | ND | 33 | 33 (24; 15-85) | |
| **Pituitary Height (mm)** | | 5.2 | 8.8 | 9.6 | 4.7 | 6.2 | 4.8 | 8.2 | 3.8 (0.4; 3.2-4.4) | |
| **Pituitary Width (mm)** | | 6.2 | 8.7 | 8.9 | 6.2 | 6.4 | 5.3 | 6.9 | 4.9 (0.8; 3.3-5.8) | |
| **Pituitary Length (mm)** | | 6.5 | 10.2 | 9.4 | 6.8 | 8.3 | 7.7 | 11.4 | 5.6 (0.5; 4.6-6.3) | |
| **P/B value** | | 0.53 | 0.94 | 0.91 | 0.48 | 0.58 | 0.44 | 0.73 | 0.38 (0.03; 0.32-0.42) | |

**Table 1** Basal endocrine variables and measures of pituitary size in 16 cats with diabetes mellitus. The 5 cats in which growth hormone excess was demonstrated are presented with individual data, together with two other cats in which there was pituitary enlargement. The data of the cats without indications of growth hormone excess and/or pituitary enlargement are presented as mean (± SD; range). Reference ranges for α-MSH, ACTH, and cortisol are derived from Javadi et al.[20] The reference ranges for GH and IGF-I are derived from Reusch et al.[21] The reference range for the UCCR is derived from de Lange et al.[23] ND: not determined.

In cats 7 (Abyssinian, UCCRs: 42 and 85 x $10^{-6}$) and 14 (Burmese, UCCRs: 51 and 34 x $10^{-6}$) MRI of the pituitary fossa did not reveal an enlarged pituitary gland. The insulin requirements of cat 7 steadily decreased from 0.8 to 0.3 IU insulin/kg body weight twice daily. In cat 14 diabetes mellitus had been diagnosed one month after treatment with glucocorticoids and it went into remission 9 months after diagnosis. The (moderately) elevated UCCR suggested the presence of (mild?) hypercortisolism in the two cats, but the decreasing insulin requirements and a non-demonstrable pituitary lesion did not seem to justify a definite diagnosis of hypercortisolism.

Cats 1 and 9 had pituitary enlargement, but no evidence of either GH excess or hypercortisolism was found (Table 1). Cat 1 had a history of treatment with glucocorticoids and progestagens. When diabetes mellitus was diagnosed, these treatments were discontinued and the diabetes went into remission. When the disease recurred the cat was entered into this study. Remission and recurrence alternated several times at intervals of a few months. With recurrence the maximum insulin requirement was >1.5 IU/kg, twice daily. Cat 9 did not have a history of treatment with glucocorticoids or progestagens. The maximum recorded insulin dosage was 0.8 IU/kg body weight, twice daily. From the plasma glucose day curves made by the owner, it was clear that the lowest plasma glucose levels frequently were in the desired range (5-9 mmol/L).

All cats had been treated with insulin for periods ranging between 2 weeks and 27 months (median: 4 months and 3 weeks). Fifteen cats were treated with a 30/70 lente porcine insulin preparation (Caninsulin®, Intervet Nederland BV, Boxmeer, the Netherlands) and 1 cat was treated with human regular insulin (Actrapid®, Novo Nordisk Farma BV, Alphen aan den Rijn, the Netherlands). At the time of investigation, cats on lente insulin received 0.3–2.4 (median 1.0) IU insulin/kg body weight twice daily, whereas the cat on regular insulin received 0.4 IU/kg body weight in the morning and 0.2 IU/kg body weight 12 hours later. The maximum insulin dosages that did not lead to signs of hypoglycemia and plasma glucose concentrations <4 mmol/L ranged from 0.3 to 2.5 (median 0.8) IU insulin/kg body weight twice daily for the cats on lente insulin, and was 4.1 IU insulin/kg body weight, twice daily, for the cat on regular insulin.

Nine cats received a maximum insulin dosage <1.5 IU lente insulin/kg body weight (category 1) and 6 cats received >1.5 IU lente insulin/kg body weight (category 2). Acromegaly was diagnosed in none of the cats in the first category. Only 1 cat in category 1 had pituitary enlargement, albeit without demonstrable hormonal abnormalities (cat 9). In contrast, acromegaly was diagnosed in 5 of the 6 cats in category 2. The sixth cat in this group had an enlarged pituitary but no hormonal abnormalities were found (cat 1). A Mann-Whitney U test revealed a significant difference ($P$=0.002) in the P/B values of the 15 cats in category 1 and category 2 (Figure 1). Neither pituitary enlargement nor pituitary hormone abnormalities were detected in the one cat treated with regular insulin (cat 12).

Administration of octreotide caused a statistically significant decrease in plasma GH concentrations in the acromegalic cats ($P$<0.001 at time points 15, 30, 60; $P$<0.01 at time point 90; $P$<0.05 at time point 120) but only a slight, not significant decrease in the non-acromegalic cats. Individually, the decrease in plasma GH concentrations was clear-cut in 2 of 5 acromegalic cats but less distinct in the other acromegalic cats (Figure 2).

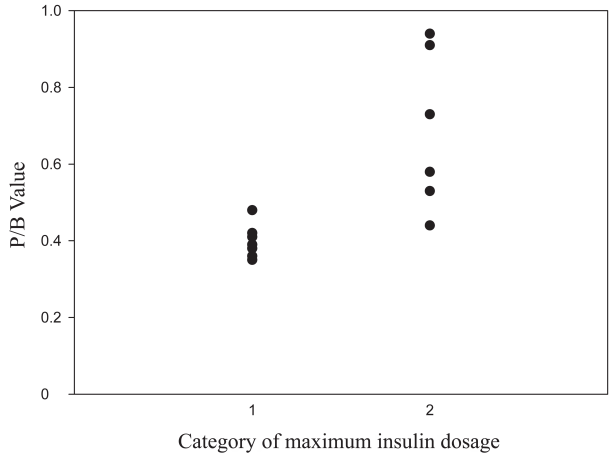


**Figure 1**

The pituitary height / brain area (P/B) values of diabetic cats, depicted per category of maximum insulin dosage. Cats in category 1 all received < 1.5 IU lente insulin/kg body weight per injection. All other cats treated with lente insulin are in category 2.

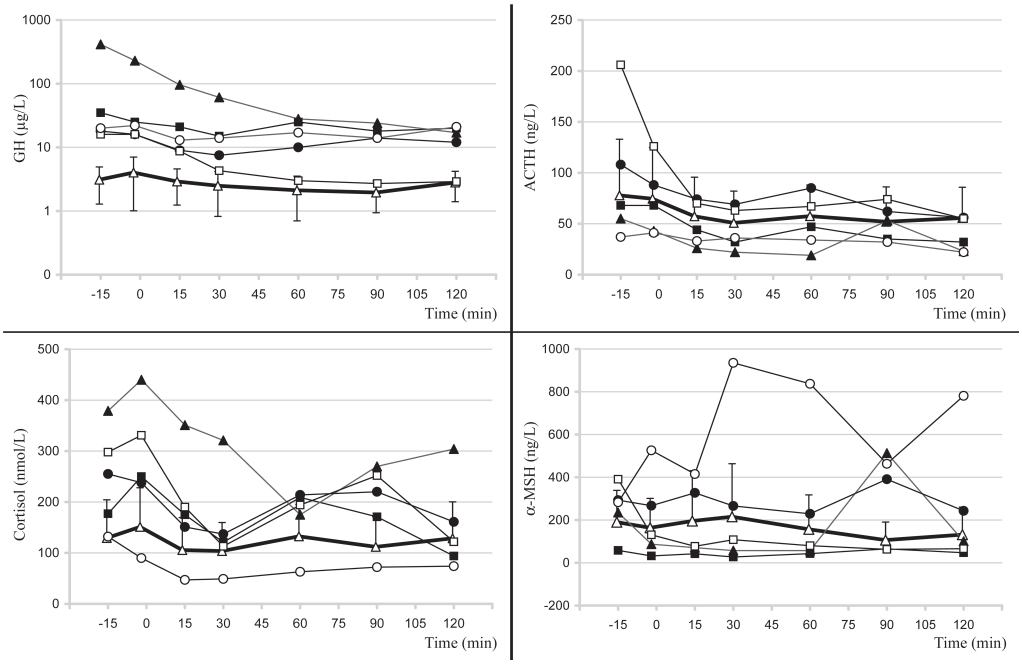


**Figure 2** Plasma concentrations of GH, ACTH, cortisol and α-MSH in 16 diabetic cats, as influenced by the administration of the somatostatin analogue octreotide (time zero). For reasons of readability the results of the acromegalic cats are presented individually (cat 2 ■; cat 8 ▲; cat 11 ●; cat 15 □; cat 16 ○; all cats with thin lines) and the data of the non-acromegalic cats are presented as mean (▵, thick lines) with the SD depicted on error bars. Note the logarithmic scale for GH.

Octreotide also significantly decreased plasma ACTH concentrations in both the acromegalic and non-acromegalic cats (Figure 2), and in the acromegalic cats this decrease was associated with a statistically significant decrease in plasma cortisol concentrations. This decrease was most pronounced in the two acromegalic cats that also had a pronounced decrease in plasma GH concentrations. Plasma cortisol concentrations in the non-acromegalic cats were not significantly affected by octreotide administration. In neither group was there a statistically significant effect of octreotide administration on plasma α-MSH concentrations.

There were no statistically significant differences between the plasma glucose concentrations of acromegalic and non-acromegalic cats at any time point. When all cats were analysed together in one group, a slight but statistically significant increase in the plasma glucose concentrations (from 25.3 to 26.7 mmol/L) occurred after octreotide administration.

## Discussion

Five of the 16 diabetic cats in this study were diagnosed with concurrent acromegaly. Owners were probably more willing to participate in the study if they experienced the regulation of their cat's diabetes mellitus as troublesome. This may explain the high prevalence of acromegaly among diabetic cats in this study. Four of the 5 acromegalic cats were male, consistent with an earlier report in 14 acromegalic cats of which 13 cats were male.[8] Interestingly, there is no sex predilection for acromegaly in humans.[28]

The cases highlight the difficulty of establishing a definite diagnosis, especially when the features of an underlying or associated disease are dominated by another disease, such as diabetes mellitus. In general, disease usually starts as a slight deviation from health and the diagnostic characteristics may not have surpassed the sensitivity threshold of the available detection methods. Apart from this common problem, the presenting disease -in this case diabetes mellitus- may affect variables used to diagnose the underlying/associated disease.

The latter has been suggested to be the case for the diagnosis of hypersomatotropism in cats with diabetes mellitus when plasma IGF-I concentrations are used as a diagnostic criterion. One report of 8 diabetic cats concluded that diabetes mellitus *per se* may increase total plasma IGF-I concentrations;[29] however, no information was provided on whether the diabetic cats had been treated with insulin or had been left untreated before measurement of IGF-I and plasma GH concentrations were not measured, so that the co-existence of hypersomatotropism cannot be excluded. Studies of humans and cats indicate that concentrations of IGF-I are low in untreated and short-term treated diabetics and increase to (near-)normal during treatment with insulin.[21,30,31] Insulin deficiency is known to lower IGF-I concentrations in other species as well.[32,33] This phenomenon may lead to non-elevated IGF-I concentrations in untreated diabetic cats with hypersomatotropism. When such cats are treated with insulin, plasma IGF-I concentrations may rise considerably, as demonstrated in 11 previously untreated diabetic cats in which the plasma IGF-I concentration was monitored for 16 weeks after initiation of insulin therapy.[21] In the present cats, the diagnosis of hypersomatotropism was primarily based on the elevated plasma GH concentrations. In the five cats diagnosed with acromegaly in this way, the pituitary was enlarged and the plasma

IGF-I concentrations exceeded the reference range. All cats were treated with insulin and therefore an effect of insulin deficiency on plasma IGF-I concentrations will not have played a role.

Two cats had a relatively large pituitary gland but no evidence of either hypersomatotropism or hypercortisolism. In these cats there may have been a functional abnormality that was not searched for or that was undetectable at the time of investigation. The combination of normosomatotropism, normocortisolism, and a melanotroph pituitary adenoma has been reported in a diabetic cat.[34] Unfortunately information about basal plasma α-MSH concentrations in one cat (cat 1) was not available and the basal plasma α-MSH concentrations in the other cat (cat 9) were well below the upper limit of the reference range.

The enlarged pituitaries in cat 1 and cat 9 could be "pituitary incidentalomas". In humans, pituitary incidentalomas are pituitary abnormalities that are discovered incidentally, and with increasing frequency, due to the increasing use of modern imaging. Some human pituitary incidentalomas are found to be pituitary adenomas causing hormonal abnormalities that had gone unnoticed.[35,36] However, some human pituitary incidentalomas remain unchanged in size, never lead to tumoral or hormonal symptoms,[37,38] and may be caused by pituitary hypertrophy.[38] It is not clear whether pituitary incidentalomas occur in cats, and if so, whether they remain asymptomatic.

Hypercortisolism is rare in cats, and thus there is limited experience with diagnostic testing. For the biochemical diagnosis of hypercortisolism there are two approaches: (1) demonstration of increased production of cortisol, and (2) demonstration of decreased sensitivity to glucocorticoid feedback.[39] The latter poses problems in cats because 15–20% of healthy cats fail to demonstrate suppression after intravenous administration of 0.01 mg dexamethasone per kg body weight.[40] Consequently such cats could be falsely diagnosed as having hypercortisolism. Probably the stress caused by test procedures in cats [41] overrides the suppressive effect of dexamethasone in some cats. For this reason, the test dose of dexamethasone has been increased to 0.1 mg/kg.[40] However, even with this dose cats with hypercortisolism may go undetected because some animals are relatively sensitive to the suppressive effect of dexamethasone. In some cats with hypercortisolism, plasma cortisol concentrations at 8 h after dexamethasone administration (0.01 mg/kg) are lower than the commonly held criterion of 40 nmol/l.[9] As stated earlier, the low-dose dexamethasone suppression test cannot be considered a definitive test for the diagnosis of hypercortisolism in cats.[42]

In the present study, cortisol production was assessed by measuring the UCCR. This test is regarded as a highly sensitive diagnostic aid for distinguishing between cats with and without hypercortisolism.[40] The high sensitivity of the test means that it can detect cortisol responses to other diseases and to stress.[23,43,44] This should be taken into consideration when interpreting the results. In two of our cats, necrotizing pancreatitis and the stress of confinement (an "outdoor" cat was kept indoors for urine collection) were probably responsible for the elevated UCCR. Growth hormone plays an important role in the peripheral interconversion of cortisol and cortisone through its IGF-I-mediated inhibitory effect on 11ß-hydroxysteroid dehydrogenase I, which leads to partial inhibition of the conversion of cortisone to cortisol. In humans this does not seem to significantly influence urinary cortisol

excretion,[45,46] and if this also holds for cats, the GH excess would not have influenced the UCCR. In two other cats persistent hypercortisolism was somewhat unlikely because of the course of the diabetes mellitus. Repeated measurements of UCCRs would have been indicated. Thus the elevated UCCR of only one diabetic cat (cat 2) could be explained by hypercortisolism. This acromegalic cat may have had two pituitary adenomas, that is a somatotroph adenoma and a corticotroph adenoma, as described earlier.[47] In fact, the only cat in which the UCCR was not measured (cat 15) was found to have a double adenoma at necropsy. Thus we diagnosed hypersomatotropism in five cats, two of which had concurrent hypercortisolism. In some of the other diabetic cats there remained too many diagnostic uncertainties to allow a definite diagnosis of either hypercortisolism or hypersomatotropism.

Somatostatin analogues are highly effective in 60–70 % of humans with acromegaly, dependent upon the receptor profile of the individual tumours.[48] Drug resistance is related to an overall reduction in somatostatin receptor (SSTR) density or to a differentiated expression of SSTR subtypes.[49] In a recent prospective multicentre study there was some increase of the number of responders after 24–38 weeks of treatment. It was concluded that long-acting octreotide analogues lead to progressive regression of tumour volume, sustained control of biochemical abnormalities, and adequate relief of disease symptoms.[50] Surgery is still regarded as the first choice for microadenomas. For large and/or invasive tumours, first-line therapy is somatostatin analogue treatment with debulking surgery as adjunct.[51] In the two cats with acromegaly and hypercortisolism, octreotide administration caused some lowering of the plasma ACTH concentrations, but without a concomitant decrease in plasma cortisol concentration. Octreotide analogues also have little effect on these hormones in humans with pituitary-dependent hypercortisolism.[51] It appears that hypercortisolism causes decreased expression of SST-2 receptors on ACTH-producing pituitary adenoma cells.[52] Corticotroph adenomas predominantly express SSTR-5. The newly developed multiligand somatostatin analogue SOM230 may be more effective than currently available analogues that preferentially target SSTR-2.[18,53] We found octreotide administration not to signficiantly affect plasma α-MSH concentrations, comparable to findings in rats. *In situ* hybridisation demonstrated only low levels of mRNA for SSTR 1, 2, and 4 in melanotrophs in the intermediate lobe.[54]

Although a statistically significant increase in plasma glucose concentrations occurred during the 2-hour octreotide suppression test, the clinical relevance of this rise in hyperglycemic cats is questionable. Incubation of rat pancreatic islets with octreotide suppresses insulin secretion dose dependently, without consistently affecting glucagon secretion.[55] If these results can be extrapolated to cats, an octreotide-induced decrease in the insulin to glucagon ratio may indeed lead to an increase in the plasma glucose concentration. However, it is doubtful whether octreotide can significantly influence the insulin to glucagon ratio in diabetic cats, because these animals already have impaired insulin secretion. More likely, withholding the regular morning administration of insulin before the octreotide suppression test may have led to the slight increase in plasma glucose concentrations.

Most cats diagnosed with diabetes mellitus are assumed to have type 2 diabetes mellitus. However, some cats may suffer from another specific type of diabetes mellitus, e.g. caused by acromegaly or hypercortisolism. Clinical experience has demonstrated that severely

insulin-resistant (insulin requirement >1.5 IU/kg body weight per injection) diabetic cats should be screened for concurrent acromegaly or hypercortisolism.[56] Our results support these recommendations because all cats diagnosed with concurrent acromegaly and/or hypercortisolism required >1.5 IU lente insulin/kg body weight per injection. Measurements of feline pituitary hormones are not done routinely in most laboratories, and therefore it may take some time before results of these measurements become available. In the meantime, diagnostic imaging of the pituitary fossa may be indicated, because the results of the present study showed that all cats requiring >1.5 IU lente insulin/kg body weight had pituitary enlargement. Cat 12 was not treated with Caninsulin®, but was treated with Actrapid®. Because the cut-off points for severe insulin resistance may differ for different insulin preparations as a result of different pharmacokinetics, cat 12 was not classified (with regard to its maximum insulin dosage) together with the other 15 cats in this study. However, a cat requiring a dosage of 4.1 IU regular insulin/kg body weight may still be considered severely insulin resistant. This cat did not have acromegaly or pituitary enlargement, and at the time it was examined, its insulin requirements had decreased to <0.5 IU regular insulin/kg body weight, twice daily. It is unclear what caused the varying insulin demand in this cat.

In conclusion, the results of this study indicate that the incidence of hypersomatotropism (with or without co-existence of hypercortisolism) with concomitant pituitary enlargement is high among diabetic cats with severe insulin resistance. In some acromegalic cats octreotide administration lowered plasma GH concentrations considerably, which suggests that a single injection of octreotide could be used as a pre-entry test for treatment with somatostatin analogues.

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# Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors in the development of feline type 2 diabetes mellitus

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## Abstract

With domestication and urbanisation, cats have transformed from hunting animals eating protein-rich prey to often sedentary animals eating a carbohydrate-rich diet. It was hypothesised that a high intake of dry cat food and a lack of physical activity play a role in the development of feline type 2 diabetes mellitus.

Information on dietary history and physical activity of 96 cats with diabetes mellitus and 192 matched controls was collected retrospectively, using a telephone questionnaire. Conditional logistic regression analysis was used to investigate the association between questionnaire-derived variables and the development of diabetes mellitus. The energy percentage of dry food in the diet was not significantly correlated with the development of diabetes mellitus ($P$=0.29), whereas both indoor confinement ($P$=0.002) and low physical activity ($P$=0.004) were.

In conclusion, the results of the present study indicate that the proportion of dry food in a cat's diet may not be an independent risk factor for the development of type 2 diabetes mellitus, whereas physical inactivity and indoor confinement are.

# Introduction

Diabetes mellitus is a common disorder among domestic cats. Its estimated incidence is 2.45 cases /1000 cat-years-of-risk.[1] In most cases, the condition closely resembles human type 2 diabetes mellitus (type 2 DM),[2] which is characterised by both decreased pancreatic beta cell function and insulin resistance.[3] The similarities between feline and human type 2 DM are also reflected by changes in the pancreatic islets and in some risk factors. In both species, pancreatic islet amyloidosis is an almost invariant feature of the disease.[2,4] Commonly recognised risk factors for the development of type 2 DM in humans and cats include old age and obesity.[1,5]

Physical activity greatly influences the development of type 2 DM in humans. Cross-sectional studies have demonstrated that the prevalence of type 2 DM among physically inactive people is two to three times higher than that among physically active people.[6] With respect to diet as risk factor for the development of human type 2 DM, it may not just be the total caloric content that is important, but also the composition of the diet and the individual's energy expenditure. In some reports on type 2 DM in humans diets with a high glycemic load and a high glycemic index are regarded as a risk factor, whereas in other publications conflicting results have been reported. These controversies were reviewed recently, together with possible mechanisms by which glycemic load and the glycemic index might influence the development of DM, e.g. by influencing insulin secretion and (indirectly) insulin sensitivity.[7] In cats, different glycemic responses were demonstrated when diets with different carbohydrate sources were fed.[8,9]

Cats are obligate carnivores and their natural diet mainly consists of protein-rich prey animals, with a low intake of dietary carbohydrate.[10,11] With domestication and urbanisation, the cat has transformed from an active hunting animal to an often sedentary animal fed on an industrially produced diet. Industrially produced dry diets usually have a much higher carbohydrate content than the cat's natural diet, with carbohydrates accounting for up to 50% of metabolisable energy (ME), which makes them a high carbohydrate diet. Canned cat food usually contains less than 10% carbohydrate on ME basis and can be regarded as a low carbohydrate diet.[12,13]

We hypothesised that dry cat food may be a risk factor for the development of feline DM because of its high carbohydrate content. So far, the possible roles of the proportion of dry food in the cat's diet and physical activity in the development of feline DM have not been fully investigated. Here we report on a questionnaire-based retrospective case-control study of 288 cats. Each cat diagnosed with diabetes was matched with two healthy controls for age, gender, body weight, and body condition. The proportion of dry food (on ME basis) in the cat's diet and information regarding physical activity were derived from data collected with the questionnaire.

## Materials and methods

*Subject selection*

Of the 76 animal clinics that were asked to participate in this project, 28 practices provided information about cats with DM. Twenty-three of these practices also participated in the selection of healthy control cats, with additional control animals being recruited through 17 other practices. Practices were spread all over the Netherlands. During the process of recruiting cases and controls for this study, we requested participating veterinarians to provide us with cats without concurrent disease and with no history of treatment with glucocorticoids and/or progestagens.

The owners of 109 cats diagnosed with DM were interviewed using a telephone questionnaire. Six cases were excluded due to lack of eligible matches of the same age and body weight, one case was excluded because the cat was the only sexually intact cat in the study, two cases were excluded because of concurrent hyperthyroidism, and four cases were excluded because of uncertainty about the type of DM (one cat was diagnosed at five months of age, and the three others were being treated with > 1.5 IU lente insulin per kg body weight twice daily).[14]

Each DM cat included in the study was matched to two healthy controls. Thus, 96 diabetic cats and 192 controls were included in the study. The controls were matched to the cases based on age, gender (neutered male or spayed female), body weight, and body condition (underweight, correct weight, or overweight). Through matching, known risk factors (age and obesity) were controlled and could not confound results regarding physical activity and dietary factors. The body weight and body condition prior to the diagnosis of DM were used for matching, with a maximum of 10% difference in body weight and 20% difference in age being permitted.

*Questionnaire*

The questionnaire included questions about signalment, diet, general information, medical history, and physical activity of the cat. Signalment included sex, neutered or intact status, age, age at gonadectomy, and breed. Dietary history included questions about the type of food (dry, canned, or home-made), brand names, amount fed per day, feeding pattern (restricted or ad libitum), duration of the time period in which the stated dietary combinations were fed, and use of treats or snacks, all during the current ownership of the cat. General questions comprised body weight and body condition, duration of ownership, origin of the cat, and presence of other cats in the household. Medical questions concentrated on the existence of concurrent hyperthyroidism and the administration of drugs, specifically glucocorticoids or progestagens. Questions on the cat's physical activity included whether the cat had outdoor access (outdoor/indoor status), owner perceived activity level (low, medium, or high), playing time, sleeping time, and time spent outdoors. For diabetic cats, these questions pertained to the period before DM was diagnosed.

The owners of diabetic cats were asked additional questions on the DM history, such as time of diagnosis and use of insulin or other diabetic treatment. The veterinary practitioners

were asked to verify the body weight and body condition, medical history, and DM history of the eligible cases and the controls.

*Data analysis*

The amount of dry and canned food in the cats' diet was calculated in terms of the percentage of total daily energy intake. Homemade diets, such as fish and meat, were considered as canned food as they are rich in protein and poor in carbohydrates. Treats and snacks were defined as food given by the owner in small amounts and irregularly and were not added to the total amount of daily food in the dietary analysis. Instead they were used as a categorical variable (treats fed or no treats fed) in the analysis.

The amount of canned food consumed was known in all cases. However, the exact amount of dry food remained unclear in some interviews, as a result of ad libitum feeding, frequent disposal of leftovers, and/or multiple cat households. Two methods of calculation were applied depending on whether the amount of dry food was known or not. First, for an unknown amount of dry food, the quantity was calculated from the daily ME requirement of 300 kJ ME/kg body weight.[15] The energy content of dry food was assumed to be 1500 kJ ME/100 g, and that of canned food 330 kJ ME/100 g.[16] The daily energy derived from dry food was estimated by subtracting the energy supplied by the canned food from the total ME requirement. The energy from the dry food was then expressed as a percentage of the daily energy intake ($ME_{dry}$). Second, when the amount of dry food was mentioned by the owner, the declared quantity was checked against the daily ME requirement of the cat with margins of ± 25%. If the quantity stated by the owner did not match these margins, the amount was recalculated using the previously established daily energy requirement of the cat. If the amount of dry food mentioned by the owner was plausible, this figure was used in the final analysis. Whenever an owner mentioned different feeding regimens during a cat's life, the duration of the periods in which these regimens were given was expressed as a percentage of the total time covered by the diet history and was used as a weighting factor to calculate the 'average exposure' to dry and canned foods, expressed as a percentage of daily metabolisable energy intake.

Summary statistics were performed for all the variables including general information, diet history, medical history, and physical activity of the cats. The Kolmogorov-Smirnov test with Lilliefors significance correction was used to assess the distribution of continuous data. A binomial test was used to test whether or not males and females develop DM with equal frequency. Differences in playing time, sleeping time, and time spent outdoors between cats with different levels of physical activity were assessed with Kruskal-Wallis tests. Cats with complete indoor confinement were excluded from analyses of the time spent outdoors. Spearman's rank correlation tests were used to identify correlations between variables. Univariate conditional logistic regression was used to identify which variables should be included in multivariate regression analysis, with all variables with $P<0.15$ being included. Multivariate backward stepwise (based on the likelihood ratio) conditional logistic regression was used to assess the relationship between the development of DM and several variables derived from the questionnaire. All statistical analyses were performed with SPSS 12.0.1 for Windows (SPSS Inc.). $P<0.05$ (two-tailed) was considered statistically significant.

## Results

The owners' information on dry food intake could be used for 27 of the 96 diabetic cats and 32 of the 192 control cats. The exact amount of dry food given in the past could not be recalled by 38% of the owners of the diabetic cats and by 52% of the owners of the control cats. Additionally, approximately one third of the owners (34% of the cases and 32% of the controls) provided us with data that deviated strongly from the energy requirements of their cats. Although veterinarians had been asked not to include cats with a history of treatment with glucocorticoids and/or progestagens, some cats did have such a history, as indicated by the owners and subsequently confirmed by the veterinarians (Table 1).

| | Cases (n=96) | Controls (n=192) |
|---|---|---|
| **Gender (n (%))** [a] | | |
| male (castrated) | 61 (64%) | 122 (64%) |
| female (spayed) | 35 (36%) | 70 (36%) |
| **Age (years)** | | |
| median | 12 | 11 |
| range | 6 - 18 | 6 - 21 |
| **Body weight (kg)** | | |
| median | 6.0 | 5.9 |
| range | 3.5 - 10.0 | 3.2 - 10.0 |
| **Body condition (n (%))** | | |
| underweight | 0 | 0 |
| correct weight | 22 (23%) | 44 (23%) |
| overweight | 74 (77%) | 148 (77%) |
| **Number of other cats in the household (n (%))** | | |
| 0 | 23 (24%) | 37 (19%) |
| 1 | 39 (41%) | 73 (38%) |
| 2 - 3 | 23 (24%) | 51 (27%) |
| > 3 | 11 (11%) | 31 (16%) |
| **Corticosteroid and/or progestagen use (n (%))** [b] | | |
| no history of use | 72 (75%) | 165 (86%) |
| history of use | 24 (25%) | 27 (14%) |

**Table 1** General information of diabetic cats (cases) and healthy controls. [a]: Significant difference between male and female diabetic animals ($P$=0.010). [b]: Conditional logistic regression analysis indicates that a history of corticosteroid and/or progestagen use is a risk factor for the development of DM ($P$=0.040).

Assuming an equal distribution of males and females in the entire cat population, males developed DM more often than females ($P$=0.010) (Table 1). Kruskal-Wallis tests revealed that playing time ($P$<0.001), sleeping time ($P$<0.001), and time spent outdoors ($P$=0.015) differed significantly in cats with low, moderate, and high activity levels. Physical activity level was positively correlated with playing time and time spent outdoors, and was negatively correlated with sleeping time (Spearman's rho of 0.33 ($P$<0.001), 0.28 ($P$=0.004), and -0.48 ($P$<0.001) respectively) (Table 2).

| | Cases (n=96) | Controls (n=192) |
|---|---|---|
| **Outdoor / Indoor status (n (%))** [a] | | |
| only inside | 38 (40%) | 42 (22%) |
| only outside | 0 | 0 |
| both | 58 (60%) | 150 (78%) |
| **Physical activity level (n (%))** [bc] | | |
| low | 53 (55%) | 65 (34%) |
| moderate | 37 (39%) | 97 (51%) |
| high | 6 (6%) | 30 (16%) |
| **Playing time** [c] | | |
| median (hours / day) | 0.15 | 0.17 |
| range (hours / day) | 0 - 1 | 0 - 4 |
| unknown (n (%)) | 18 (19%) | 59 (31%) |
| **Sleeping time** [c] | | |
| median (hours / day) | 18 | 18 |
| range (hours / day) | 3 - 24 | 7.5 - 22 |
| unknown (n (%)) | 43 (45%) | 83 (43%) |
| **Time spent outside** | | |
| cats confined indoors | | |
| n (%) | 38 (40%) | 42 (22%) |
| cats not confined indoors [c] | | |
| median (hours / day) | 1.5 | 2.0 |
| range (hours / day) | 0.03 - 14 | 0.08 - 14 |
| unknown (n (%)) | 28 (29%) [d] | 78 (41%) [d] |

**Table 2** Physical activity of diabetic cats (cases) and healthy controls. [a]: Indoor confinement is a risk factor for the development of DM ($P$=0.002). [b]: The physical activity level is an explanatory variable for the development of DM ($P$=0.004). [c]: The physical activity level is correlated with playing time ($P$<0.001), sleeping time ($P$<0.001), and time spent outdoors ($P$=0.004). [d]: The percentages presented here are calculated as percentages of the total number of cases and the total number of controls, respectively.

On the basis of the results of the univariate analyses, the following variables were included in the multivariate conditional logistic regression analysis: $ME_{dry}$, activity level, outdoor / indoor status, use of glucocorticoids or progestagens, and diet category. Correlations between these variables did not exceed 0.20 (Spearman's rho). Multivariate conditional logistic regression analysis of the collected data indicated that $ME_{dry}$ was not significantly correlated with the development of DM (*P*=0.29) (Figure 1), whereas both indoor confinement (odds ratio 2.56; *P*=0.002, 95% confidence interval 1.41-4.65) and the cat's activity level (*P*=0.004) were highly significant risk factors for the development of DM (Table 2). Compared with cats with a low activity level, moderately active cats had an odds ratio of 0.46 (*P*=0.006, 95% confidence interval 0.26-0.80) for the development of DM and highly active cats an odds ratio of 0.26 (*P*=0.007, 95% confidence interval 0.10-0.69). A history of treatment with glucocorticoids or progestagens increased the likelihood of the development of DM (odds ratio of 2.02; *P*=0.040, 95% confidence interval 1.03-3.93). Whether cats were fed on industrially produced food only or a combination of industrially produced food and homemade food (diet category) was not significantly correlated with the development of DM (*P*=0.17) (Table 3). Q-Q plots of the residuals of the multivariate conditional logistic regression revealed no abnormalities.

| | **Cases (n=96)** | **Controls (n=192)** |
|---|---|---|
| **Diet category (n (%))** | | |
| only industrially produced food | 80 (83%) | 174 (91%) |
| only homemade food | 0 | 0 |
| both | 16 (17%) | 18 (9%) |
| **Type of industrially produced food (n (%))** | | |
| dry | 19 (20%) | 41 (21%) |
| canned | 1 (1%) | 2 (1%) |
| both | 76 (79%) | 149 (78%) |
| **Ad libitum feeding (n (%))** | | |
| no | 34 (35%) | 81 (42%) |
| yes | 62 (65%) | 111 (58%) |
| **Treats fed (n (%))** | | |
| no | 35 (37%) | 81 (42%) |
| yes | 61 (64%) | 111 (58%) |

**Table 3** Dietary variables of diabetic cats (cases) and healthy controls. None of these variables were risk factors for the development of DM.

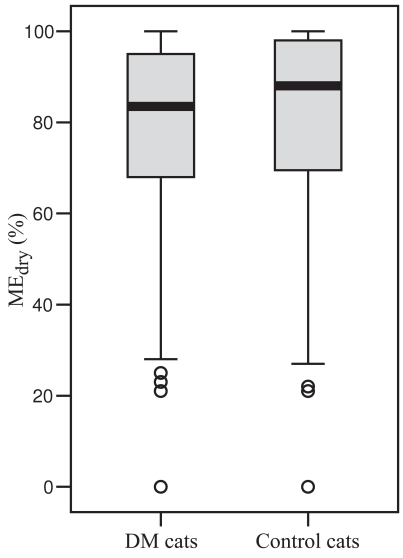


**Figure 1**

Box plot of the estimated metabolisable energy% of dry food in the diet ($ME_{dry}$) of 96 diabetic cats and 192 control cats.

## Discussion

The results of this study do not support the hypothesis that the proportion of dry food in the cat's diet is a risk factor for the development of DM. When calculated as a percentage of ME, regular dry cat food has a four to five times higher carbohydrate content compared to canned cat food. If the energy provided by dry food versus that provided by canned food is used as an index of a high versus a low carbohydrate intake, our results do also not support the hypothesis that high carbohydrate intake is a risk factor for developing feline DM. However, dry and canned foods do not only differ in their carbohydrate content but also in other aspects including their fibre content and how they are produced. Most likely the glycemic index of dry cat food is different from that in canned cat food. Nonetheless, the difference in carbohydrate content between dry and canned cat food is of such magnitude that it is unlikely that the glycemic index could fully compensate for its effects on postprandial blood glucose and insulin concentrations. Therefore, our results at least suggest that a high carbohydrate intake should not be considered a risk factor for the development of feline DM.

Canned foods may contain up to 10% of their metabolisable energy in the form of carbohydrates, which is the lower limit of carbohydrate intake of the cats included in this study. It could be suggested that in this study the range of carbohydrate intakes would not discriminate as to DM development. However, the range of carbohydrate intakes reflects that of the practice of feeding domestic cats.

Our observations are in agreement with the results of a recent study involving lean and obese cats fed on a high carbohydrate/low protein and a high protein/low carbohydrate diet. Glucose and fat metabolism were studied during euglycemic hyperinsulinemic clamps.[17] The authors found that obesity, but not the dietary protein or carbohydrate content, led to insulin resistance and decreased glucose effectiveness. The results of Hoenig et al. do not

directly support the concept that high carbohydrate diets promote the induction of insulin resistance and thereby the development of feline DM.

Cats seem to be able to adapt their metabolism to their diet. Studies using a glucose tracer, indirect calorimetry, and euglycemic hyperinsulinemic clamping have demonstrated that cats fed on a high protein diet increase heat production by increasing net protein oxidation, and that a high-carbohydrate diet leads to greater fatty acid disappearance, probably due to increased lipogenesis.[17-19] These data indicate that the sensitivity to insulin may be substrate-specific, and have led to the suggestion that cats on a high carbohydrate diet may be more prone to weight gain and fat accumulation over time.[19] Thus a high-carbohydrate diet may be an indirect risk factor for the development of DM by promoting the development of obesity. In the present study, diabetic cats were matched to control cats for body weight and body condition, thereby negating possible effects of diet composition on the development of DM through the development of obesity.

Several clinical reports indicate that low carbohydrate / high protein diets may be beneficial to the management of feline DM.[20-22] While this may seem to be contradictory to our results, it should be borne in mind that managing feline DM and preventing feline DM do not necessarily require the same dietary approach. Although the exact pathogenesis is incompletely understood, insulin secretion is impaired in diabetic cats. Whereas reducing the amount of dietary carbohydrate does not influence insulin sensitivity,[17] in a study by Bennett et al,[21] the number of diabetic cats that went into remission was higher in cats on a low carbohydrate diet. Reducing the amount of carbohydrate in diets tended to reduce the amount of insulin required for 'disposal' of nutrients in normal cats.[23] Thus, the beneficial effects of low carbohydrate / high protein diets in the management of feline DM may be caused by decreasing the demand for insulin. In normal cats, however, insulin secretion is not impaired, and thus an increased demand for insulin caused by dietary carbohydrate can be met. Whether or not this increased demand for insulin is a risk factor for the development of diabetes mellitus remains to be determined. However, the results of this study do not support this particular hypothesis.

Because a greater proportion of the control cats were able to spend time outdoors, control cats may have had higher energy requirements than the DM cats. However, because we calculated energy intake using a standard formula for energy requirement in relation to body weight, the present data are not suited to assess a difference in energy intake between cases and controls. Most likely, the energy intake of the controls versus the cases was underestimated, thus underestimating the dry food intake of the control cats. This would only strengthen our conclusion that the proportion of dry food in a cat's diet is not a risk factor in the development of feline DM.

Our results seem to confirm that treatment with glucocorticoids or progestagens (G&P) is a risk factor for the development of DM. While it is recognised that these drugs induce glucose intolerance,[24,25] only one research abstract has reported on glucocorticoids as a risk factor for the development of DM in cats; in a case-control study of 33 Burmese cats glucocorticoids were found to increase the risk of developing DM.[26] We specifically tried to recruit cats not treated with G&P, which may have introduced bias as to the effects of treatment with G&P on the development of DM. Thus, caution is needed when interpreting the

results of this study with respect to the possible function of G&P treatment as risk factor for the development of DM. We used a history of treatment with G&P as a variable in our analyses, thus correcting for any influence the treatment may have had on the assessment of the role of other factors in the development of DM.

Caution is also needed regarding the results of ad libitum feeding as a risk factor. If it has a role, ad libitum feeding will likely exert its effects on the development of DM through the development of obesity. We matched our cases and controls for body weight and body condition, which may have introduced bias in the assessment of ad libitum feeding as a risk factor for the development of DM.

We quantified the physical activity of the cats using several variables. A lower physical activity level was correlated with less playing time, more sleeping time, and less time spent outside. The abovementioned correlations indicate that the owners were able to correctly assess their cat's activity level. We chose to enter the physical activity level into the regression analysis rather than the time spent playing, time spent sleeping, and time spent outside, because of the number of "unknowns" in these variables. Regression analysis revealed that physical inactivity is a risk factor for the development of feline DM. This is consistent with results from human studies, in which physical activity is negatively correlated with the risk of developing type 2 DM.[27,28] Also, the outdoor / indoor status of the cat appeared to have a significant influence on the risk of developing DM, with the risk being significantly lower in cats that spent time roaming outdoors. Our findings are in agreement with those of a case-control study of Burmese cats, in which both indoor confinement and low physical activity were found to be risk factors for the development of DM.[26]

Insulin resistance is a major contributor to the development of DM, and in humans it has been demonstrated that the turnover rate of the intramuscular triacylglycerol pool is an important determinant of insulin sensitivity.[29] The turnover rate is the consequence of the oxidative capacity of skeletal muscle and therefore directly dependent on physical activity.[30,31] Regular physical activity also changes muscle composition in favor of type-1 fibres, which are more sensitive to insulin than type-2 fibres.[32] In addition, exercise increases muscle mass, improves muscle vascularisation, and promotes the expression of the gene encoding glucose-transporter protein 4.[33] Lastly, exercise induces glucose-transporter protein 4 translocation to the plasma membrane in skeletal muscle.[34] There is little reason to assume that these mechanisms are not operational in cats, especially because it has been demonstrated in cats that an ad libitum food intake results in an increase in both intra- and extramyocellular lipid. Once obese, these cats have a decreased insulin sensitivity, for which the increased intramuscular triacylglycerol pool can be held responsible.[35]

In conclusion, the results of the present study indicate that the proportion of dry food intake may not be an independent risk factor for the development of type 2 DM in cats, whereas physical inactivity and indoor confinement are.

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# Effects of dietary macronutrients on glucose disposal, β-cell function, insulin sensitivity, arterial blood pressure, and body composition in healthy cats fed for weight stasis

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## Abstract

The aim of this study was to determine whether glucose homeostasis, blood pressure, and body composition in cats are influenced by dietary macronutrients. Over a 9-month period, the effects of a high-fat, a high-carbohydrate, and a high-protein diet were determined in a longitudinal, parallel group approach in 3 groups of 7 healthy cats fed for weight stasis. β-Cell function, glucose disposal, and insulin sensitivity were measured using hyperglycemic glucose clamps (HGC), arterial blood pressure by direct pressure measurements, and body composition by dual-energy x-ray absorptiometry. In addition, homeostasis model assessment (HOMA-R and HOMA-B)) was performed.

The cats' weights were stable (coefficient of variation <5%) and body composition (fat percentage and lean body mass) did not change. Exchanging dietary protein for fat or carbohydrate increased glucose-induced second phase insulin secretion during HGCs ($P$=0.003 and $P$<0.001, respectively). In the high-fat group this was associated with increased glucose disposal ($P$=0.051). Insulin sensitivity decreased in all groups ($P$=0.005). HOMA-B and HOMA-R correlated with their counterparts derived from the HGC: HOMA-B correlated with second phase insulin secretion (r=0.74; $P$<0.001) and HOMA-R correlated with insulin sensitivity index (r=-0.79; $P$<0.001). Decreasing dietary fat led to lower arterial blood pressure. The decrease in blood pressure was not correlated with any measure of insulin sensitivity or glucose-induced insulin secretion.

The results of this study indicate that a high-fat or high-carbohydrate diet improves β-cell function (insulin secretion) in cats. They do not support the hypothesis that high carbohydrate consumption leads to β-cell exhaustion and loss of β-cells in cats.

# Introduction

With an estimated incidence of 2.45 cases per 1,000 cat-years-of-risk, diabetes mellitus (DM) is a common disease in cats. Risk factors include obesity, age, physical inactivity, and indoor confinement.[1-3] The majority of diabetic cats has a type of DM that closely resembles human type 2 DM. Central hallmarks of human and feline type 2 DM are insulin secretory dysfunction and insulin resistance.[4,5]

Insulin resistance, prediabetic states [impaired glucose tolerance (IGT), impaired fasting glucose (IFG)] and type 2 DM are criteria used in humans for the diagnosis of the metabolic syndrome, also referred to as "Syndrome X" or the "Insulin Resistance Syndrome". This metabolic syndrome has been proposed to be a clustering of risk factors for atherosclerotic macrovascular disease. There are several definitions of the metabolic syndrome with different cut-off points, but the definitions always include ❶ a measure of body composition (elevated waist circumference, waist:hip ratio, and/or body mass index), ❷ a measure of insulin resistance and/or disturbed glucose homeostasis (IGT, IFG, type 2 DM), ❸ arterial hypertension, and ❹ a measure indicating dyslipidemia (elevated triglycerides, low HDL-cholesterol).[6]

It has been proposed that the risk factors for atherosclerotic macrovascular disease in humans share a "common soil",[7] as they tend to coexist more than they occur in isolated form and because the clustering of the risk factors exceeds coincidental clustering.[8,9] Presumably obesity and the accompanying insulin resistance are at the root of these disturbances. A lack of physical exercise and improper nutrition increase the risk of developing obesity. As a result, recommendations with regard to physical exercise and nutrition have been made for the prevention and treatment of the metabolic syndrome in humans.[10,11]

Based on the similarities between human and feline DM, we hypothesised that certain risk factors of the human metabolic syndrome can be influenced in cats by nutritional interventions. We investigated the effects of three diets with different macronutrient compositions (high fat, high carbohydrate, and high protein) on glucose disposal, β-cell function, insulin sensitivity, arterial blood pressure, and body composition in 21 healthy, middle-aged cats fed for weight stasis. By comparing the three diets, it was possible to investigate the effects of exchanging dietary fat with carbohydrate or protein and carbohydrate with protein.

# Materials and methods

*Protocol*

In a prospective, longitudinal, parallel group approach the effects of three different test diets (high fat, high carbohydrate, and high protein) on β-cell function, glucose disposal, insulin sensitivity, arterial blood pressure, and body composition were assessed in 3 groups of 7 healthy, middle-aged cats fed for weight stasis. The cats' body weights were measured every week and food intake was adjusted to maintain stable body weight.

After a flush-out period of at least 10 weeks on a constant mixture of commercially manufactured dry and canned cat food (described below), baseline values for the above-mentioned variables were collected (time point 1). After completion of baseline measurements, each group of cats was assigned to 1 of the 3 test diets for 9 months, after which all parameters were measured again (time point 2).

At both time points, the measurements of variables were collected over a 5-week period for each cat. Typically, on day 1 (9.00–12.00 h) arterial and venous catheters were inserted. During the night (24.00–3.00 h) between day 1 and day 2, arterial blood pressure was measured. On day 3 or 4 a hyperglycemic glucose clamp (HGC) was performed (13.00–16.30 h). The catheters were removed under anesthesia on day 5. Body composition was determined 4 weeks after removal of the catheters.

The cats were housed in individual cages after catheters were inserted and were returned to their groups 3 days after catheters were removed.

*Cats*

Ten castrated male and 11 spayed female cats were studied. At the beginning of the study their ages were estimated to range from 5 to 9 years. Findings by physical examination and routine biochemical and hematological examination were unremarkable and all cats were accustomed to daily handling. The cats were housed in three groups of equal size in enclosures with natural daylight. The groups had been formed before the measurements at time point 1, in such a way that they comprised similar numbers of males and females (Table 1). The enclosures were enriched with shelves, (rotation of) toys and other structures. Human-cat socialisation was provided by dedicated caretakers and each group of cats had free access to a playground for at least two hours per day.

*Diets*

During the flush-out period, all cats were fed a constant mixture of commercially manufactured dry and canned cat food, resulting in a macronutrient composition of 38% of metabolisable energy from protein, 29% from fat, and 33% from carbohydrate (start diet). The cats were fed for weight stasis and had been fed this diet for at least 10 weeks prior to testing.

| | High fat group | High CHO group | High protein group |
|---|---|---|---|
| Estimated age[1] | 7 (5 - 9) | 7 (6 - 9) | 8 (6 - 9) |
| Sex | 3 M, 4 F | 3 M, 4 F | 4 M, 3 F |
| Breed | all DS | 1 MB, rest DS | all DS |
| Body weight[1] | 4.3 (2.9 - 5.2) | 4.3 (3.4 - 5.1) | 4.0 (2.3 - 4.7) |
| Percentage fat[1] | 24.8 (18.0 - 34.7) | 27.7 (19.8 - 31.6) | 20.8 (18.3 - 29.2) |

**Table 1** Description of the three groups of cats (median and (range)). CHO = carbohydrate; M = male; F= female; DS = domestic shorthair; MB = mixed breed (persian x DS). [1]: at time point 1

After completion of the data collection at time point 1, the food was changed to the test diets. The analysis of these (dry, extruded) diets is listed in Table 2. The metabolisable energy of the diets was calculated using modified Atwater factors [12] and results from proximate analysis (Weende analysis). Test diets were formulated to contain different amounts of macronutrients but to have similar carbohydrate, amino acid, and fatty acid profiles (i.e., the subtypes of a macronutrient expressed as percentages of the total amount of that macronutrient). In addition, the diets were formulated to contain equal amounts of vitamins, minerals and trace elements per unit of metabolisable energy.

*Anesthesia and catheter insertion*

The insertion of catheters has been described elsewhere in detail.[13] Briefly, a double lumen catheter was inserted into a common carotid artery under general anesthesia, sterile conditions, and guided by ultrasonography. Then, guided by fluoroscopy, the tip of the catheter was manoeuvred into the descending aorta or the brachiocephalic trunk. An identical catheter was inserted 8 cm into the contralateral external jugular vein.

For insertion of the catheters, the cats were premedicated with medetomidine (80 µg/kg body weight, IM) and anesthesia was induced with propofol (2-4 mg/kg body weight, IV) and maintained with a mixture of oxygen, air, and isoflurane via an endotracheal tube. Upon completion of the procedure, medetomidine was antagonised with atipamezole (200 µg/kg body weight, IM). For removal of the catheters, anesthesia was induced with propofol (3-5 mg/kg body weight, IV) and maintained by inhalation anesthesia as described above.

*β-Cell function, glucose disposal, and insulin sensitivity*

Hyperglycemic glucose clamps were performed in 18-h-fasted, conscious cats with the arterial plasma glucose concentration clamped at 9 mmol/L. First phase insulin secretion (AUC-Ins1), second phase insulin secretion (AUC-Ins2), the glucose disposal rate (M), and the insulin sensitivity index ($ISI_{HGC}$) were calculated from the HGC data as described previously.[14]

Fasting plasma glucose concentration and fasting plasma insulin concentration were measured in 4 arterial blood samples collected immediately before the HGC. Measures of β-cell function and insulin sensitivity (homeostasis model assessment (HOMA-B and HOMA-R) were calculated from these fasting plasma glucose and insulin concentrations.[14,15]

*Arterial blood pressure and body composition*

Assessment of arterial blood pressure and body composition have been described elsewhere in detail.[13,14] Briefly, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured in resting, conscious cats through the arterial catheter (see above). The percentage of total body weight from fat (fat percentage) and the lean body mass were measured using dual energy x-ray absorptiometry (DXA), using a pencil beam DXA machine (Hologic QDR-1000/W, Hologic Europe, Zaventem, Belgium) previously validated for use in cats.[16,17]

*Statistics*

Statistical analyses were performed with R [18] using NLME library version 3.1-80,[19] and SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, USA). Data were analysed using linear

| | High fat | High CHO | High protein |
|---|---|---|---|
| **Nutrient composition, g/100 g** | | | |
| Protein | 31.64 | 25.08 | 49.13 |
| Fat | 21.88 | 10.24 | 11.72 |
| Carbohydrate | 33.40 | 52.09 | 26.28 |
| Fiber | 1.64 | 1.89 | 1.62 |
| Ash | 4.41 | 3.73 | 4.83 |
| **Nutrient composition, % of calculated ME** | | | |
| Protein | 26.8 | 24.6 | 47.3 |
| Fat | 45.0 | 24.4 | 27.4 |
| Carbohydrate | 28.3 | 51.0 | 25.3 |
| **Components, %** | | | |
| Chicken meal | 22.85 | 15.63 | 39.88 |
| Yellow corn | 19.39 | 32.63 | 16.21 |
| Sorghum | 19.39 | 32.63 | 16.21 |
| Fat, chicken | 18.83 | 4.26 | 0.82 |
| Casein, dried | 11.42 | 7.81 | 19.94 |
| Beet pulp | 4.20 | 4.08 | 4.16 |
| Cat digest | 1.17 | 0.96 | 0.98 |
| Dicalcium phosphate | 0.22 | - | - |
| Potassium chloride | 0.21 | - | - |
| Vitamin mix | 0.28 | 0.23 | 0.24 |
| Mineral mix | 0.28 | 0.17 | 0.17 |
| Calcium carbonate | 0.29 | 0.60 | - |
| Choline chloride | 0.40 | 0.27 | 0.31 |
| DL-Methionine | 0.27 | 0.23 | 0.47 |
| Salt | 0.26 | 0.20 | - |
| Magnesium sulphate | 0.18 | - | - |
| Taurine | 0.36 | 0.30 | 0.61 |

**Table 2** Specifics of the three test diets. CHO = carbohydrate; ME = metabolisable energy

mixed-effect modelling, with random cat effect. Modeling of random effects was based on the Akaike Information Criterion (AIC).[20] For modeling of fixed effects a group variable was created that differentiated among the three diet groups. Depending on the AIC, models could include a factor time, a factor group, and interactions between these two factors. Differences between the AICs of models were assessed by ANOVA. If the ANOVA indicated that the AICs of two models were not significantly different, the most extended model providing significant results was used. If beneficial to the model, variances were allowed to differ between different strata and an autoregressive correlation structure (AR1) was used to model the dependence in time. Whenever Q-Q-plots indicated that transformation of data was necessary, natural logarithmic transformation was used. Bonferroni correction was applied and $P<0.05$ was considered significant. The values presented in the figures are unadjusted outcomes.

Partial correlations, controlling per cat and diet group, were calculated between arterial blood pressure and HGC variables and also between HOMA and HGC variables. For calculation of correlations, data were tested for normality and outliers using the Kolmogorov-Smirnov test with Lilliefors significance correction and Grubb's T-statistic, respectively.[21,22] If necessary, data were transformed using the natural logarithm.

*Ethics*

The research protocols and care for the cats were reviewed and approved by the Utrecht University Committee for the Ethical Treatment of Animals, according to Dutch and European law.

## Results

*Procedures*

At the first time point all procedures were completed without complications. However, at the end of the study 2 cats in the high-protein (HP) diet group had to be euthanised within three days after removal of the arterial catheter because of posterior paralysis due to a saddle thrombus in the abdominal aorta and external iliac arteries. No indications of macrovascular atherosclerotic disease were found during necropsy. Consequently, the body composition of these cats was not measured. In addition, the arterial catheterisation (and thus the HGC and the measurement of blood pressure) was cancelled for another cat (the last cat to be tested, also in the HP group).

*Body weight and diet consumption*

On average, the consumption of metabolisable energy per kg body weight per day was 156 kJ in the high-fat (HF) group, 172 kJ in high-carbohydrate (HC) group, and 163 kJ in the HP group The body weights of the cats remained stable, with a coefficient of variation <5% for each individual cat. Analysis of the fat percentage and lean body mass revealed no significant time*group interactions, nor were there any main time or group effects (all $P>0.20$) (Figure 1).

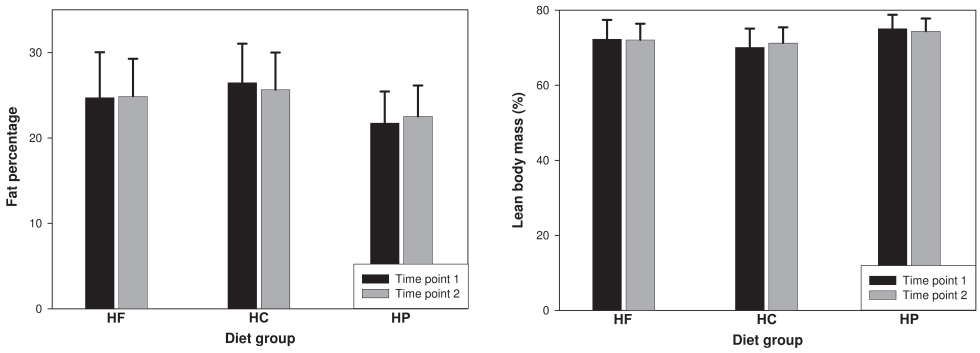


**Figure 1** Mean values (unadjusted outcomes) of the fat percentage and lean body mass in the three groups at time points 1 and 2. Error bars represent the standard deviations. HF = high-fat diet, HC = high-carbohydrate diet, HP = high-protein diet. There were 7 cats per group at all points except for the HP group at time point 2, in which there were 5.

*Glucose-induced insulin secretion*

Analysis of the first phase insulin secretion (AUC-Ins1; Figure 2) results revealed no significant time*group interactions or a main group effect (all $P>0.20$), but analysis with all cats grouped together revealed an increase with time ($P<0.001$).

Second phase insulin secretion (AUC-Ins2; Figure 2) increased with time in the HF group ($P=0.003$) and the HC group ($P<0.001$), but not in the HP group ($P>0.20$). There were no significant differences between the groups at either time point ($P>0.20$).

*Glucose disposal*

Differences in the glucose disposal rate (M; Figure 2) between dietary groups at the beginning of the study were not significant (all combinations $P>0.20$). At the end of the study, M was higher in the HF group than in the HP group ($P=0.017$), but the difference between the HF and HC groups and between the HC and HP groups were not significant ($P>0.20$). There was a significant increase in M between time points 1 and 2 in the HF group ($P=0.051$), but the differences in M between the two time points in the HC and HP groups were not significant ($P>0.20$).

*Insulin sensitivity index*

Analysis of the insulin sensitivity indices ($ISI_{HGC}$; Figure 2) revealed no significant time*group interactions or main group effect ($P>0.20$). However, analysis of the grouped results in all cats revealed a significant decrease in $ISI_{HGC}$ with time ($P=0.005$).

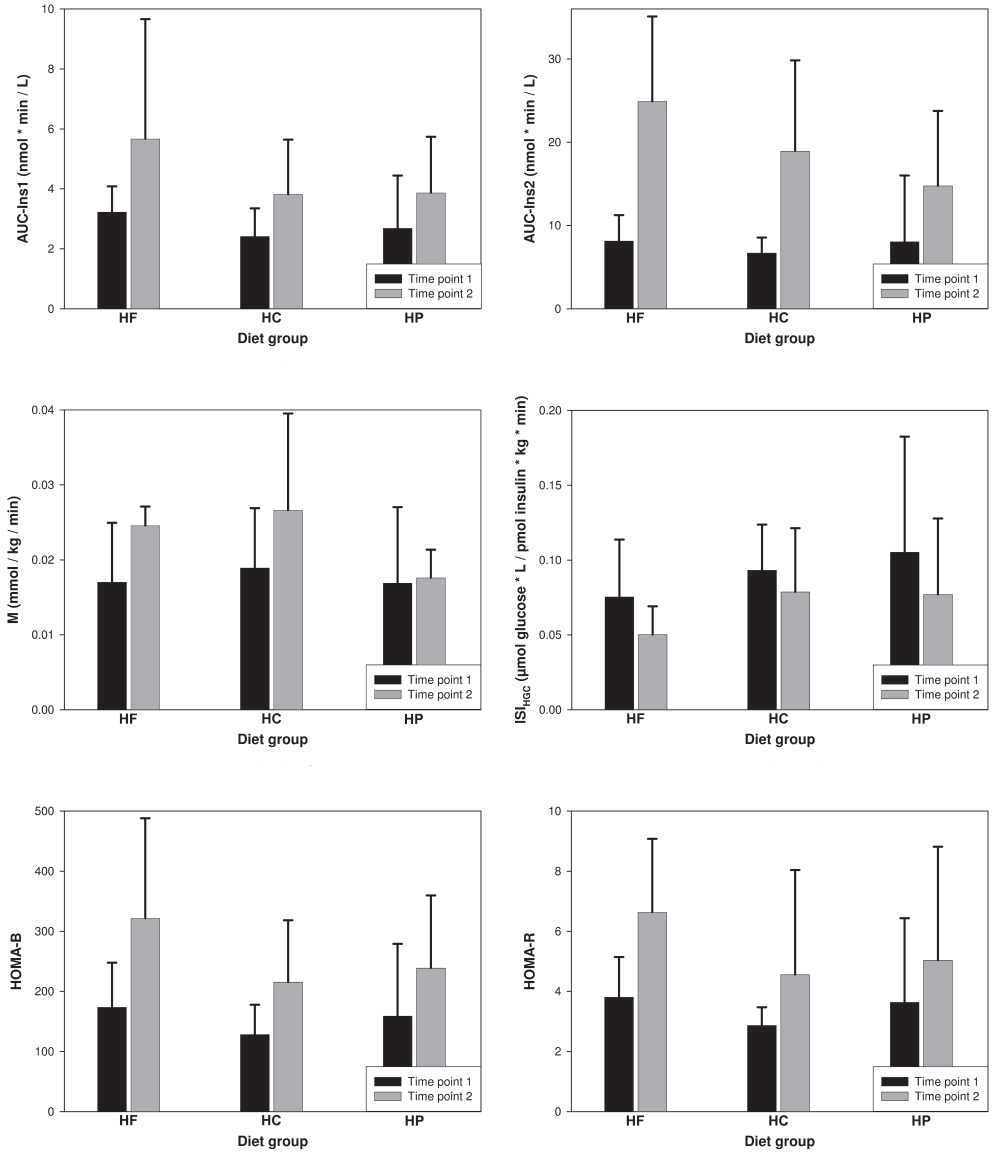


**Figure 2** Mean values (unadjusted outcomes) of AUC-Ins1, AUC-Ins2, M, $ISI_{HGC}$, HOMA-B, and HOMA-R in the three dietary groups at time points 1 and 2. Error bars represent standard deviations. HF = high-fat diet, HC = high-carbohydrate diet, HP = high-protein diet. There were 7 cats per group at all points except for the HP group at time point 2, in which there were 6.

*HOMA*

Differences in the measure of β-cell function provided by homeostasis model assessment (HOMA-B; Figure 2) among the three groups were not significant at either time point ($P$>0.20 for all combinations). There was a significant increase with time in the HF group ($P$=0.040), but the increases in the HC and HP groups were not significant ($P$>0.20).

Analysis of the measure of insulin sensitivity provided by homeostasis model assessment (HOMA-R; Figure 2) revealed no significant time*group interactions or main group effect ($P$>0.20). Analysis of the grouped results in all cats revealed an increase in HOMA-R with time ($P$=0.007).

HOMA-B and HOMA-R were correlated with their counterparts derived from the HGC: HOMA-B was correlated with AUC-Ins2 (r=0.74; $P$<0.001) and HOMA-R was correlated with $ISI_{HGC}$ (r=-0.79; $P$<0.001).

*Arterial blood pressure*

Diastolic blood pressure (DBP; Figure 3) decreased significantly with time in the HC group ($P$=0.035), but not in the HF and HP groups ($P$>0.20). Differences between the groups at either time point were not significant (all combinations $P$>0.20).

Systolic blood pressure (SBP; Figure 3) tended to decrease in the HC group ($P$=0.053), but differences in the HF and HP groups were not significant ($P$>0.20). None of the differences between groups at either time point was significant (all combinations $P$>0.20).

When the HC and HP groups were combined (HCHP group) for comparison with the HF group, the differences in DBP and SBP were not significant at time point 1 ($P$>0.20) or at time point 2 ($P$=0.133 and $P$>0.20, respectively). The decrease in DBP with time in the HCHP group was significant ($P$=0.023) but the decrease in SBP was not ($P$=0.127). Neither DBP nor SBP decreased significantly in the HP group ($P$>0.20).

There was no significant correlation between arterial blood pressure and $ISI_{HGC}$, AUC-Ins1, AUC-Ins2, fasting plasma insulin concentration, fasting plasma glucose concentration, HOMA-R, or HOMA-B.

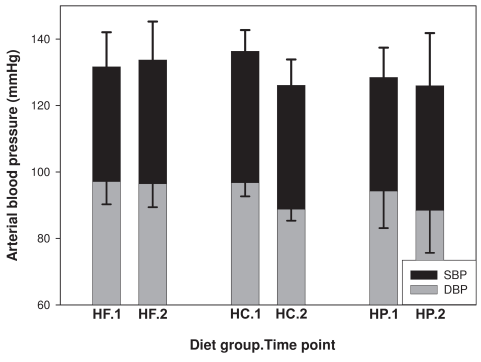


**Figure 3**

Mean values (unadjusted outcomes) of the arterial blood pressure in the three groups at time points 1 and 2. Error bars represent standard deviations. HF = high-fat diet, HC = high-carbohydrate diet, HP = high-protein diet, SBP = systolic blood pressure, DBP = diastolic blood pressure. There were 7 cats per group at all points except for the HP group at time point 2, in which there were 6.

# Discussion

In this study we investigated in detail the effects of a 9-month dietary intervention on measures of insulin secretion, glucose disposal (as measured by HGCs), the insulin sensitivity index, ABP, and body composition in 3 groups of 7 cats. Insulin secretion was assessed with the HGC, the use of which we introduced in cats.[14] In humans, the HGC has become the gold standard for evaluation of parameters of β-cell function.[23-27] Insulin secretion normally consists of a rapid so-called first phase (in this study: AUC-Ins1), lasting approximately 10-15 minutes, followed by a slowly evolving second phase (in this study: AUC-Ins2), which lasts as long as plasma glucose concentration is elevated. The results of this study demonstrate that second phase insulin secretion was clearly augmented in the HF and HC groups.

We can only speculate about the mechanisms of augmentation of glucose-induced insulin secretion. An explanation may be found in the action of the incretin hormones Glucagon-Like Peptide 1 (GLP-1) and/or Glucose-dependent Insulinotrophic Polypeptide (GIP). Ingestion of fat and carbohydrate is the primary physiologic stimulus for incretin secretion in the intestine [28,29] and greater incretin secretion may have been stimulated by the HF and HC diets than by the HP diet. Incretins have both acute and chronic biological effects. The acute effects include glucose-dependent insulin secretion and inhibition of glucagon secretion, while the chronic effects include increasing glucose-induced insulin biosynthesis, improved capacity of glucose-resistant ß-cells to sense and respond to glucose, stimulation of ß-cell proliferation and neogenesis, and inhibition of ß-cell apoptosis.[30] While the acute effects cannot have played a role in the present study, using intravenous glucose stimulation, the chronic effects may well have come into play. Our results suggest that, within the parameters of this study, the exchange of protein for either fat or carbohydrate leads to increased glucose-induced insulin secretion during a HGC.

The glucose disposal rate (M) increased significantly in the HF group but not in the HC and HP groups. In addition, the increase in the HF group led to a significant difference in M between the HF and HP groups at time point 2. Although M increased in the HC group over time, the increase was not significant, possibly because of the larger variation in M. The glucose disposal rate is a result of insulin secretion (AUC-Ins2 in this study) and the prevailing insulin action (insulin sensitivity index or $ISI_{HGC}$ in this study).[31] The increase in M in the HF group (and less clearly in the HC group) was small in relation to the increase of AUC-Ins2. Hence in addition to the improvement in glucose-induced insulin secretion, there appears to have been diminished insulin sensitivity.

The macronutrient differences among the three diets had no demonstrable influence on $ISI_{HGC}$. Others have studied the effects of high-carbohydrate and high-protein diets on whole body insulin sensitivity in cats using a crossover design with 12 lean and 16 obese cats and the euglycemic hyperinsulinemic glucose clamp (EGC) to measure insulin sensitivity.[32] They concluded that exchanging carbohydrates for protein (or vice-versa) in cats fed for weight stasis did not influence whole body insulin sensitivity after 4 months of feeding each diet.[32] Our results appear to support these conclusions and may add the observation that exchanging fat for either protein or carbohydrate also does not seem to influence whole body insulin sensitivity in cats. However, the relatively low number of cats in each group separately

limits our conclusions. Exchanging dietary fat for dietary carbohydrate isoenergetically also did not influence whole body insulin sensitivity assessed by EGC in 6 healthy men, fed three different diets for 6 days each.[33]

The overall decrease in $ISI_{HGC}$ may have had several causes, including prolonged physical inactivity. Skeletal muscle accounts for approximately 75% of whole body insulin-stimulated glucose uptake in humans.[34,35] Physical inactivity decreases the lipid turnover in muscle tissue, which is related to decreases in muscle tissue insulin sensitivity.[36] Also, it is possible that nonmacronutrient differences between the initial diet and the experimental diets (e.g., different fatty-acid profiles) influenced $ISI_{HGC}$. Increased consumption of saturated fatty acids and trans (unsaturated) fatty acids may decrease insulin sensitivity.[36]

The metabolic syndrome has been proposed as a clustering of risk factors that increase the risk of atherosclerotic macrovascular disease in humans, but the syndrome does not occur in cats [37] and atherosclerosis per se only occurs under specific conditions in this species.[38-40] In contrast, the saddle thrombi causing posterior paralysis in two of the cats in this study were most probably complications of the catheter inserted into the descending aorta. It is possible that a thrombus had formed around the catheter and was dislodged during removal of the catheter.

In a previous study, we demonstrated that HOMA-R correlates well with the hyperglycemic glucose clamp derived $ISI_{HGC}$ and that HOMA-B correlates well with its hyperglycemic glucose clamp derived counterpart, AUC-Ins2.[14] In the present study, repeated measurements of these variables also demonstrated that changes in AUC-Ins2 and $ISI_{HGC}$ within cats were correlated with changes in HOMA-B and HOMA-R.

Cats are true carnivores and their original diet of prey animals contained very little carbohydrate.[41,42] Their glucose disposal rate is also lower than that of man, due to lower insulin sensitivity and lower glucose-induced insulin secretion.[14] Against this background, it was hypothesised that consumption of HC diets may lead to DM through exhaustion and loss of insulin-producing β-cells.[43] However, this hypothesis was not supported by the results of a recent study of the role of the proportion of dry food in the diet as a risk factor for the development of feline DM. Dry cat food contains a larger proportion of carbohydrate than does canned cat food.[44,45] The proportion of dry food in a cat's diet was not found to be an independent risk factor for the development of type 2 DM, whereas physical inactivity and indoor confinement were.[1]

The duration of this study was too short to determine whether any of the three test diets promotes the development of DM in cats but the findings do not support the hypothesis that consumption of HC diets could lead to DM through via exhaustion and loss of β-cells. On the contrary, glucose-induced insulin secretion during a HGC increased with the consumption of a HF or a HC diet. The question arises whether this is a primary dietary effect (possibly through induction of incretins) or whether it pertains to a "secondary" compensatory effect related to the decrease in whole body insulin sensitivity. Although it is well known that in various species β-cells rapidly adapt their function to the prevailing insulin sensitivity, to our knowledge "overcompensation" of insulin secretion has not been described. This would suggest that the improvement in insulin secretion is a primary dietary effect. It would thus seem that the HF and possibly also the HC diet (both with only ~25% of metabolisable energy

from protein) increase a healthy cat's ability to maintain normal blood glucose levels in comparison with the HP diet. However, given the design of this study, this may only be true when body weight is kept constant, as obesity is a well-known risk factor for the development of diabetes mellitus.[2] It was recently reported that HF diets are more likely than HC diets to induce obesity when fed ad libitum.[46] The duration of this study was too short for any prediction of long-term consequences of the diets, but it does not support of the hypothesis that consumption of HC diets leads to DM through exhaustion and loss of β-cells.

Decreasing total dietary fat leads to a decrease of arterial blood pressure in humans.[47] In these cats, DBP decreased and SBP tended to decrease in the HC diet group. Comparison of results in the HCHP group (essentially a low-fat group) with those in the HF group indicated that, as in humans, lower dietary fat leads to lower diastolic blood pressure.

Reducing dietary sodium reduces arterial blood pressure in humans,[48] but this relation has not been substantiated in cats.[49-51] In the cats described here body weight and body composition remained constant and SBP and DBP were not found to be correlated with any of the measures of carbohydrate metabolism provided by fasting or HGC data (AUC-Ins1, AUC-Ins2, M, $ISI_{HGC}$, fasting plasma glucose concentration, fasting plasma insulin concentration, HOMA-B, and HOMA-R). It thus remains unclear how differences in macronutrient composition influenced arterial blood pressure in this study. Changes in body weight or body composition and changes in insulin sensitivity or insulin secretion do not seem to be likely mechanisms.

In another study in cats, dietary macronutrient composition influenced the amount of fat lost during weight loss.[32] There was a smaller decrease of fat mass during weight loss on a HC diet than on a HP diet, even though total weight loss was the same. In our study body composition was not influenced by dietary macronutrient composition, probably because the cats were fed for weight stasis.

In conclusion, in cats fed for weight stasis, exchanging dietary protein for either fat or carbohydrate led to increased glucose-induced insulin secretion and an increased glucose disposal rate in cats on the high-fat diet. It is hypothesised that the improvement in glucose-induced insulin secretion was due to increased diet-induced incretin secretion. Body condition was not affected. Reducing the total fat content of the diet led to a decrease of the diastolic arterial blood pressure.

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# 9

# Summarising discussion and conclusions

Type 2 DM is the most common form of DM in cats, being responsible for an estimated 80-95% of cases.[1] Obesity and old age have been shown to be risk factors for the development of feline DM.[2] In humans, more risk factors for the development of type 2 DM are known, such as a family history of DM or low birth weight.[3] The aim of this thesis was to increase our knowledge of the risk factors for development of DM in cats. The studies presented in **Part I** of this thesis describe the development of techniques that can be used in the assessment of risk factors.

**Chapter 3** describes the use of the hyperglycemic glucose clamp (HGC) in conscious cats, using arterial blood for plasma glucose measurements. The HGC is the gold standard for the assessment of β-cell function in humans,[4-8] but had not previously been used in cats. The HGC technique breaks the feedback loop between plasma glucose concentration and insulin secretion, allowing measurement of β-cell function in a steady state. In the HGCs described in **Chapter 3**, a steady state glucose infusion was achieved in cats during the third hour of hyperglycemia. The glucose disposal rate was lower in normal glucose-tolerant cats than in normal glucose-tolerant humans, apparently related to the cat's lower insulin secretion and lower insulin sensitivity. This is thought to be compatible with the carnivorous nature of cats, for which insulin resistance would be advantageous during periods of restricted food availability.[9,10]

The measurements of β-cell function and insulin sensitivity derived from HGCs was found to be correlated with their counterparts derived from intravenous glucose tolerance tests (ivGTT) and homeostasis model assessment of fasting plasma glucose and insulin concentrations (HOMA). It is concluded that the HGC allows for measurement of β-cell function and insulin sensitivity in cats and for the following reasons it is to be preferred over the ivGTT whenever possible: ❶ Cats tolerate the HGC procedure well. ❷ The magnitude of the glucose stimulus during an ivGTT varies greatly between cats (**Chapter 3**). Variation in glucose exposure influences the results of the ivGTT in cats,[11] whereas the stimulus is fixed during the HGC. The use of the HGC in preference to the ivGTT will reduce the variation in results introduced by the measuring technique. ❸ In contrast to the ivGTT, the HGC breaks the feedback loop between plasma glucose concentration and insulin secretion. The HGC thereby allows multiple measurements of insulin secretion and glucose disposal in a steady state. The measurements can be averaged to provide reproducible determinations of these variables.

To stimulate the β-cells with a fixed glucose stimulus during the HGC, it is necessary to frequently measure the plasma glucose concentration in systemic arterial blood without causing stress. Unwanted variation is introduced when venous blood is used.[12,13] In addition, it is necessary to perform the HGC in conscious cats, because glucose homeostasis in cats is influenced by several anesthetics.[14] For these reasons it was necessary to catheterise an artery in cats. Repeated arterial catheterisation is also required in a longitudinal study. In **Chapter 4** a new technique for arterial catheterisation is described that does not sacrifice the catheterised vessel. Instead of surgically exposing and ligating the vessel, a common carotid artery is catheterised under ultrasonographic guidance. The tip of the catheter is then advanced into the brachiocephalic trunk or the descending aorta under fluoroscopic guidance. In general,

catheters were removed 5 days after insertion and until that time it was possible to collect arterial blood samples quite easily.

These catheters also allowed continuous measurement of systemic arterial blood pressure (ABP), even without the physical presence of a person. In **Chapter 4**, differences in ABP in resting cats, inactive but attentive cats, and active cats are described. Resting cats had lower ABP than inactive but attentive cats, and active cats had higher ABP than resting or inactive but attentive cats. The effect on ABP of short-term interaction with a familiar and an unfamiliar person was also studied. Interaction with a familiar person led to a higher ABP than interaction with an unfamiliar person. The time required for the ABP to return to resting values was shorter after interaction with the unfamiliar person than after interaction with the familiar person. This method of measuring ABP enabled measurements in undisturbed cats as well as the detection of relatively small differences in ABP.

Although the HGC may well be the gold standard test to assess β-cell function in cats, the technique is time-consuming and costly and is therefore not suited for routine clinical use. For the latter an easy to perform, simple, and inexpensive method is required that also minimises patient discomfort. The universal secretogogue $Ca^{2+}$ has been shown to cause a transient increase in β-cell insulin secretion *in vitro* and *in vivo*. An attempt was thus made to develop a calcium stimulation test (CST) (**Chapter 5**). The results were compared with those of the HGC and the ivGTT. The index of first phase insulin secretion in the CST was not well correlated with results of the HGC or ivGTT. In contrast, measures of β-cell function provided by HOMA were well correlated with HGC results (**Chapter 3**). In addition, HOMA is easier to perform than the CST, as it does not require the intravenous administration of $Ca^{2+}$. Therefore, it is concluded that the CST is not a valuable tool for the assessment of the β-cell function.

In **Part II** of the thesis, several risk factors for the development of DM were investigated. As mentioned above, most diabetic cats have type 2 DM. However, a significant minority of feline DM patients are diagnosed as having an "other specific type" of DM. A well-known cause of feline DM in this category is a functional pituitary tumour. In the study described in **Chapter 6**, 16 diabetic cats were examined for the presence of concurrent pituitary disease (acromegaly and pituitary-dependent hypercortisolism). Acromegaly was diagnosed in 5 of the 16 diabetic cats and pituitary-dependent hypercortisolism was diagnosed in 2 of the 5 acromegalic cats. In all of the acromegalic cats, the maximum recorded insulin requirement exceeded 1.5 IU lente insulin/kg body weight per injection. In addition, in all of the acromegalic cats an enlarged pituitary was found. In 2 of the remaining cats, pituitary enlargement was observed without conclusive evidence for the presence of hypersomatotropism or hypercortisolism. In 1 of these 2 cats, the maximum recorded insulin requirement also exceeded 1.5 IU lente insulin/kg body weight per injection. In all other cats being treated with lente insulin, the dose requirement remained below 1.5 IU/kg body weight per injection. These findings support the recommendation to carry out examinations for concurrent disease in all diabetic cats requiring >1.5 IU lente insulin/kg body weight per injection.[15]

**Chapter 6** also describes the effects of a single intravenous injection of octreotide—an analogue of the hypophysiotrophic hormone somatostatin—on the secretion of several pituitary hormones, insulin-like growth factor-1, and cortisol (octreotide suppression test). In the acromegalic cats the plasma concentrations of growth hormone, adrenocorticotrophic hormone, and cortisol decreased significantly during the octreotide suppression test. Examined individually, the effect on plasma growth hormone concentration was found to be more pronounced in some acromegalic cats than in others. In the non-acromegalic cats only plasma adrenocorticotrophic hormone concentrations decreased significantly.

There has been little expereience with pharmacological treatment of acromegaly in cats. Treatment with the dopamine agonist L-deprenyl was reported to have unsatisfactory results in one cat.[16] In another cat with acromegaly, octreotide administration resulted in normalization of plasma growth hormone concentration.[17] However, treatment with octreotide in 4 other acromegalic cats was reported to have little or no effect on serum growth hormone concentration.[18] Based on the results in **Chapter 6**, the use of the octreotide suppression test is suggested as a pre-entry test to select acromegalic cats as candidates for treatment with somatostatin analogues. Finally, the results demonstrate that pituitary enlargement is not always accompanied by demonstrable pituitary hormone abnormalities. As in humans, it is possible that pituitary hormone abnormalities may eventually be detected in these cats [19,20] or that the pituitary will remain unchanged in size and never lead to symptoms of hormone excess or symptoms of mass effects.[21,22]

Cats are true carnivores and their original diet of prey animals contained only small amounts of carbohydrate. High carbohydrate intake has been proposed to be a risk factor for the development of feline DM.[1] The increased demand for insulin secretion associated with a high carbohydrate intake is thought by some to lead to exhaustion and loss of β-cells. Industrially manufactured dry cat food may be a risk factor for the development of feline DM, as it contains 4-5 times more carbohydrate than canned cat food (when expressed as percent of metabolisable energy).[23,24] In addition, domestication and urbanisation have reduced physical exercise considerably, which is known to be a risk factor for DM in humans. These two risk factors were studied in a questionnaire-based retrospective case-control study (**Chapter 7**). Results from this study reveal that physical inactivity (as assessed by cat owners) and indoor confinement are risk factors for the development of DM in cats, independent of age, body weight or body condition. The percentage of metabolisable energy provided by dry food had no demonstrable effect.

To further investigate the role of dietary macronutrients in the development of feline DM, the effects of feeding 3 different diets (high-fat, high-carbohydrate, and high-protein) for 9 months on glucose-induced insulin secretion, glucose disposal, insulin sensitivity, ABP, and body composition were studied in a prospective, longitudinal study in healthy cats fed for weight stasis (**Chapter 8**), using the techniques described in **Chapters 3** and **4**. The results of this study indicate that diets with a low fat content lower the ABP. Body composition was not affected. Glucose homeostasis, however, was influenced by the macronutrient composition of the diets. The exchange of protein for fat or carbohydrate led to an increase in glucose-induced insulin secretion during the HGC. Glucose disposal increased when protein was exchanged for fat. The macronutrient differences did not influence insulin sensitivity. It is proposed that the

increase in glucose-induced insulin secretion is a primary dietary effect mediated by the chronic effects of the incretins Glucagon-like peptide-1 and Glucose-dependent Insulinotrophic Polypeptide.[25]

Obesity is a risk factor for the development of feline DM.[2] It should therefore be emphasised that the results in **Chapters 7** and **8** were obtained in studies that excluded the influence of the studied parameters on the development of obesity. Recently, it has been demonstrated that high-fat diets increase the risk of developing obesity.[26] Additionally, the effects of different types of dietary carbohydrate, fat, or protein—for example, differences in glycemic index—on the studied variables need to be elucidated in cats. Nonetheless, the results from **Chapters 7** and **8** do not support the concept that high-carbohydrate diets lead to exhaustion and loss of β-cells.

From these studies is can be concluded that:

- During a hyperglycemic glucose clamp, a steady state glucose infusion can be achieved in conscious cats.
- A hyperglycemic glucose clamp allows for measurement of β-cell function and insulin sensitivity in cats.
- Glucose disposal measured by a hyperglycemic glucose clamp is lower in normal glucose-tolerant cats than in normal glucose-tolerant humans.
- The relatively low glucose disposal rate in cats appears to be related to low insulin secretion and low insulin sensitivity, which may be compatible with the carnivorous nature of cats.
- The technique for arterial catheterisation used in these studies allows for repeated catheterisations of the common carotid artery, easy collection of arterial blood samples, and direct measurement of arterial blood pressure in undisturbed, conscious cats.
- Relatively small changes in arterial blood pressure can be detected with this technique in cats.
- Short-term interaction with a familiar person leads to a higher arterial blood pressure in cats than does interaction with an unfamiliar person.
- The calcium stimulation test introduced in chapter 5 is not a valuable tool for the assessment of β-cell function in cats.
- Making a definite diagnosis of pituitary-dependent hypercortisolism in cats with diabetes mellitus may be difficult.
- Diabetic cats requiring >1.5 IU lente insulin/kg body weight per injection should be screened for concurrent pituitary disease.
- Intravenous administration of octreotide (a somatostatin analogue) results in a decrease in plasma growth hormone concentration in acromegalic cats, although this effect may be more pronounced in some acromegalic cats than in others.
- An octreotide suppression test may be useful in acromegalic cats as a pre-entry test for the pharmacological treatment with (long-acting) octreotide.

- In cats, pituitary enlargement can occur without demonstrable pituitary hormone abnormalities.
- Indoor confinement and low physical activity, as assessed by cat owners, are independent risk factors for the development of feline diabetes mellitus.
- The percent metabolisable energy provided by dry cat food in a cat's diet is not an independent risk factor for the development of feline diabetes mellitus.
- The exchange of dietary protein for dietary fat or carbohydrate during 9 months increases glucose-induced insulin secretion during hyperglycemic glucose clamps in cats fed for weight stasis.
- Changes in the macronutrient composition of diets do not seem to influence whole-body insulin sensitivity as measured by hyperglycemic glucose clamps.
- Low-fat diets lower arterial blood pressure in cats.
- The results of these studies do not support the hypothesis that feeding high-carbohydrate diets to cats leads to exhaustion and loss of β-cells.

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# 10

# Samenvattende discussie en conclusies

Type 2 diabetes mellitus (DM) is de meest voorkomende vorm van DM bij katten; naar schatting heeft 80-95% van de diabetische katten type 2 DM.[1] Overgewicht en toenemende leeftijd zijn bekende risicofactoren voor het ontstaan van DM bij katten.[2] In de humane geneeskunde zijn er meer risicofactoren voor het ontstaan van DM bekend.[3] Zo verhogen zowel het voorkomen van DM in de familie als een laag geboortegewicht de kans op het ontstaan van DM. Het doel van het onderzoek dat is beschreven in dit proefschrift was om de kennis omtrent de risicofactoren voor DM bij katten te vergroten. De hoofdstukken in **Deel I** van dit proefschrift beschrijven de ontwikkeling van methoden die kunnen worden gebruikt bij het beoordelen van deze risicofactoren.

In **Hoofdstuk 3** wordt de toepassing van de hyperglycemische glucose clamp (HGC) bij katten beschreven. Tijdens deze test waren de katten bij bewustzijn en werd arterieel bloed gebruikt voor het meten van de plasmaglucoseconcentratie. De HGC is de gouden standaard voor de beoordeling van β-celfunctie in mensen,[4-8] maar was niet eerder toegepast bij katten. De HGC-techniek doorbreekt het terugkoppelingsmechanisme tussen de plasmaglucose-concentratie en de insulinesecretie, waardoor de β-celfunctie gemeten kan worden in een stabiele situatie. Tijdens de in **Hoofdstuk 3** beschreven HGCs was de glucose-infusie bij katten constant gedurende het derde uur van de hyperglycemische fase. In vergelijking tot mensen met een normale glucosetolerantie was de snelheid waarmee glucose werd opgenomen in katten met een normale glucosetolerantie laag. De gegevens wijzen op een combinatie van relatief lage insulinesecretie en lage insulinegevoeligheid. Dit lijkt te passen bij de strikt carnivore kat, voor wie wordt verondersteld dat insulineresistentie voordelig is tijdens periodes van voedselschaarste.[9,10]

De van de HGC afgeleide variabelen van β-celfunctie en insulinegevoeligheid correleerden met overeenkomstige variabelen afgeleid van zowel intraveneuze glucosetolerantietesten (ivGTT) als van "homeostasis model assessment" van de plasmaglucose- en insulineconcentraties na vasten (HOMA). Er wordt geconcludeerd dat het mogelijk is met HGCs de β-celfunctie en insulinegevoeligheid bij katten te meten. Indien mogelijk verdient HGC de voorkeur boven de ivGTT, omdat: ❶ Katten de HGC goed doorstaan. ❷ Bij de ivGTT de grootte van de glucosestimulus sterk tussen katten varieert **(Hoofdstuk 3)**, hetgeen de resultaten van ivGTTs in katten beïnvloedt.[11] Daarentegen is de stimulus constant tijdens HGCs. Om deze reden wordt de variatie in de resultaten als gevolg van de meetmethode verminderd door de HGC te verkiezen boven de ivGTT. ❸ In tegenstelling tot de ivGTT doorbreekt de HGC het terugkoppelingsmechanisme tussen de plasma glucoseconcentratie en de insulinesecretie. Hierdoor is het mogelijk om in een stabiele situatie meerdere metingen van de insulinesecretie en de opnamesnelheid van glucose te verrichten. Met berekeningen uit herhaalde metingen wordt een reproduceerbare maat voor beide variabelen verkregen.

Om de β-cel tijdens een HGC te stimuleren met een constante glucosestimulus is het noodzakelijk om zonder stress bij de kat te veroorzaken de plasmaglucoseconcentratie regelmatig te meten in arterieel bloed uit de systemische circulatie. Het gebruik van veneus bloed introduceert ongewenste variatie.[12,13] Tevens is het noodzakelijk om de HGC bij ongeanestheseerde katten uit te voeren, omdat anesthetica het glucosemetabolisme van katten beïnvloeden.[14] Om voornoemde redenen was het noodzakelijk om bij katten arteriën te kunnen

katheteriseren. Een longitudinale studie vereist bovendien dat deze katheterisaties herhaalbaar zijn. In **Hoofdstuk 4** wordt een vernieuwde techniek geïntroduceerd om arteriën te katheteriseren, waarbij de arterie niet verloren gaat. In plaats van het bloedvat chirurgisch te benaderen en te ligeren wordt een arteria carotis communis gekatheteriseerd onder begeleiding van echografie. Het distale puntje van de katheter wordt vervolgens onder begeleiding van fluoroscopie opgeschoven tot in de truncus brachiocephalicus of de aorta descendens. In vrijwel alle gevallen werden deze katheters 5 dagen na plaatsing weer verwijderd en in die periode was het mogelijk om arterieel bloed via deze katheters te verzamelen.

Via deze katheters was het ook mogelijk om de systemische arteriële bloeddruk (AB) te meten, zelfs zonder de fysieke aanwezigheid van een persoon. In **Hoofdstuk 4** worden de verschillen in de AB van rustende katten, inactieve maar attente katten, en actieve katten beschreven. De AB van rustende katten was lager dan die van inactieve maar attente katten terwijl de AB van actieve katten hoger was dan die van inactieve maar attente katten. Ook werd de verandering van de AB bij een kortdurende interactie met een bekend en een onbekend persoon bestudeerd. In vergelijking met de onbekende persoon was de stijging van de AB groter bij interactie met de bekende persoon. Ook duurde het bij de onbekende persoon minder lang voordat de AB weer tot rustwaarden was teruggekeerd dan bij de bekende persoon. Deze manier van meten van de AB laat metingen toe zonder de kat te storen en maakt tevens het aantonen van relatief kleine verschillen in AB mogelijk.

Ook al is de HGC waarschijnlijk de gouden standaard voor de beoordeling van β-celfunctie bij katten, het is een tijdrovende en kostbare methode die daarom minder geschikt is voor routinematige klinische toepassing. Voor laatstgenoemd doel is een methode nodig die gemakkelijk uitvoerbaar, simpel en goedkoop is en die daarnaast de patiënt zo min mogelijk belast. Het is bekend dat het universele secretagoog $Ca^{2+}$ *in vitro* en *in vivo* een voorbijgaande toename van insulinesecretie door de β-cel veroorzaakt. Om die reden werd een calciumstimulatietest (CST) ontwikkeld en werden de resultaten hiervan vergeleken met resultaten van de HGC en de ivGTT (**Hoofdstuk 5**). De maat voor de eerste fase insulinesecretie die werd berekend uit de CST correleerde niet met de bijbehorende maten verkregen uit de HGC of de ivGTT. Daarentegen correleerde de maat voor β-celfunctie verkregen uit HOMA wel met HGC resultaten (**Hoofdstuk 3**). Daarbij is de HOMA makkelijker uit te voeren en vereist deze benadering geen intraveneuze toediening van $Ca^{2+}$. De conclusie van dit onderzoek is dat de CST geen geschikte methode is voor de beoordeling van de β-celfunctie.

In de onderzoeken beschreven in **Deel II** van dit proefschrift werden enkele risicofactoren voor het ontstaan van DM onderzocht. De meeste katten met DM hebben type 2 DM, maar een belangrijke minderheid valt onder de “andere specifieke types” DM. In deze categorie is een functionele hypofysetumor een bekende oorzaak van DM bij katten. In het onderzoek dat in **Hoofdstuk 6** wordt beschreven, werden 16 katten met spontane DM onderzocht op de aanwezigheid van hypofysaire aandoeningen (acromegalie en/of hypofyse-afhankelijk hypercortisolisme). Acromegalie werd bij 5 van de 16 katten met DM gediagnosticeerd en bij 2 van deze 5 katten met acromegalie werd tevens hypofyse-afhankelijk hypercortisolisme vastgesteld. Bij alle acromegale katten overschreed de maximaal waargenomen insuline-

behoefte 1,5 IE lente insuline / kg lichaamsgewicht / injectie en werd met diagnostische beeldvorming een vergrote hypofyse gevonden. Twee van de 11 overige katten hadden ook een vergrote hypofyse, maar zonder definitieve bewijzen voor het bestaan van acromegalie of hypercortisolisme. Bij 1 van deze 2 katten overschreed de maximaal waargenomen insulinebehoefte ook 1,5 IE lente insuline / kg lichaamsgewicht / injectie. De insulinebehoefte van alle overige katten die werden behandeld met lente insuline was minder dan 1,5 IE lente insuline / kg lichaamsgewicht / injectie. De resultaten van dit onderzoek ondersteunen de aanbeveling om diabetische katten met een insulinebehoefte van meer dan 1,5 IE lente insuline / kg lichaamsgewicht / injectie te onderzoeken op de aanwezigheid van acromegalie en hypercortisolisme.[15]

In **Hoofdstuk 6** worden ook de effecten van een intraveneuze injectie van een analogon van het hypofysiotrope hormoon somatostatine (octreotide) op de secretie van diverse hypofysehormonen, insulin-like growth factor-1 en cortisol (octreotide-suppressietest) beschreven. Bij de acromegale katten daalden de plasmaconcentraties van groeihormoon, adrenocorticotroop hormoon en cortisol significant tijdens de octreotide-suppressietest. Individueel beoordeeld was de daling van groeihormoonconcentraties bij sommige acromegale katten duidelijker dan bij anderen. Alleen de plasmaconcentraties van adrenocorticotroop hormoon daalden significant bij de niet-acromegale katten.

Er is niet veel bekend over de farmacologische behandeling van acromegalie bij katten. Behandeling van een acromegale kat met de dopamine-agonist L-deprenyl leidde niet tot bevredigende resultaten.[16] Een andere acromegale kat reageerde op toediening van octreotide met een normalisering van de plasmaconcentratie van groeihormoon.[17] Daarentegen wordt in een derde publicatie gerapporteerd dat behandeling van 4 acromegale katten met octreotide weinig tot geen effect had op de plasmaconcentraties van groeihormoon.[18] Op grond van de in **Hoofdstuk 6** beschreven resultaten wordt het gebruik van de octreotide-suppressietest geopperd als test om te selecteren welke acromegale katten kandidaat zouden kunnen zijn voor behandeling met een somatostatine-analogon. Dit onderzoek laat voorts zien dat vergroting van de hypofyse niet altijd samengaat met aantoonbare afwijkingen van de plasmaconcentratie van hypofysehormonen. Net als bij mensen zouden laatstgenoemde afwijkingen in deze katten met het verloop van tijd mogelijk alsnog kunnen worden aangetoond.[19,20] Ook is het theoretisch mogelijk dat de grootte van de hypofyse onveranderd blijft en de vergrote hypofyse nooit zal leiden tot symptomen van hormoonovermaat of symptomen van massa-effecten.[21,22]

Katten zijn obligate carnivoren wier originele dieet van prooidieren maar weinig koolhydraten bevat. Een koolhydraatrijke voeding is daarom genoemd als risicofactor voor het ontstaan van DM bij katten.[1] De toename van de insulinebehoefte door de opname van veel koolhydraten wordt verondersteld te leiden tot uitputting en verlies van β-cellen. Industrieel geproduceerd droog kattenvoer zou een risicofactor kunnen zijn voor het ontstaan van DM bij katten, aangezien droog kattenvoer 4 tot 5 keer meer koolhydraten bevat dan kattenvoer in blik (wanneer het koolhydraatgehalte wordt uitgedrukt als percentage van de metaboliseerbare energie).[23,24] Ook heeft domesticatie en urbanisatie ertoe geleid dat katten minder bewegen. Geringe lichaamsbeweging is bij de mens een bekende risicofactor voor het ontstaan van DM. In een retrospectief patiënt-controle-onderzoek werden deze twee mogelijke risicofactoren met

telefonische enquêtes bestudeerd (**Hoofdstuk 7**). Hierbij bleek dat geringe lichamelijke activiteit (beoordeeld door de katteneigenaar) en uitsluitend binnenshuis leven risicofactoren zijn voor het ontstaan van DM bij katten, onafhankelijk van leeftijd, lichaamsgewicht en lichaamsconditie. Er konden geen effecten van het percentage droog kattenvoer in de voeding (op basis van metaboliseerbare energie) worden aangetoond.

Om de rol van macronutriënten in de voeding bij het ontstaan van DM bij katten verder te onderzoeken, kregen 3 groepen van 7 katten 9 maanden lang verschillende voeders: vetrijk, koolhydraatrijk en eiwitrijk. Met de methoden beschreven in de **Hoofdstukken 3 en 4** werden de effecten op glucose-gestimuleerde insulinesecretie, glucoseverwerkingssnelheid, insulinegevoeligheid, AB en lichaamssamenstelling bestudeerd (**Hoofdstuk 8**). In dit prospectieve, longitudinaal opgezette onderzoek werden gezonde katten zodanig gevoerd dat ze hetzelfde lichaamsgewicht hielden. Bij de katten met een voeding met een laag vetgehalte daalde de AB. De lichaamssamenstelling werd niet beïnvloed door de macronutriëntensamenstelling van de voeders, terwijl de glucosehomeostase wel werd beïnvloed. De vervanging van eiwit door vet of koolhydraten leidde tot een toename van de door glucose gestimuleerde insulinesecretie tijdens een HGC. De glucoseverwerkingssnelheid nam toe door de vervanging van eiwit door vet, maar de macronutriëntverschillen tussen de voeders hadden geen invloed op de insulinegevoeligheid. Er wordt geopperd dat de toename van de door glucose gestimuleerde insulinesecretie een direct gevolg is van de voeders via de chronische effecten van de incretines "Glucagon-like peptide-1" en "Glucose-dependent Insulinotrophic Polypeptide".[25]

Overgewicht is een risicofactor voor het ontstaan van DM bij katten.[2] Daarom moet worden benadrukt dat de in de **Hoofdstukken 7 en 8** vermelde resultaten werden verkregen met onderzoeken die de invloed van de bestudeerde parameters op de ontwikkeling van overgewicht uitsloten. Recent is aangetoond dat vetrijke voeding bij katten het risico op de ontwikkeling van overgewicht vergroot.[26] Daarnaast is het noodzakelijk de invloeden van verschillende typen koolhydraten (of vetten of eiwitten), bijvoorbeeld verschillen in glycemische index, op de onderzochte variabelen op te helderen. Niettemin is in de onderzoeken beschreven in de **Hoofdstukken 7 en 8** geen steun gevonden voor het idee dat koolhydraatrijke diëten leiden tot uitputting en verlies van β-cellen.

Uit de in dit proefschrift beschreven onderzoeken kan worden geconcludeerd dat:

- Er tijdens een hyperglycemische glucose clamp (HGC) een constante glucose infusie kan worden bereikt in ongeanestheseerde katten.
- Het mogelijk is om met een HGC de β-celfunctie en insulinegevoeligheid van katten te meten.
- De glucoseverwerkingssnelheid gemeten tijdens een HGC lager is bij katten met een normale glucosetolerantie dan bij mensen met een normale glucosetolerantie.
- De relatief lage glucoseverwerkingssnelheid bij katten lijkt te berusten op een lage insulinesecretie gecombineerd met een lage insulinegevoeligheid, hetgeen past bij de carnivore aard van katten.

- De techniek voor arteriële katheterisatie die tijdens deze onderzoeken is gebruikt het mogelijk maakt de arteria carotis communis herhaald te katheteriseren, gemakkelijk arteriële bloedmonsters af te nemen en de systemische arteriële bloeddruk op directe manier te meten bij ongeanestheseerde katten zonder de katten te hoeven benaderen.
- Relatief kleine veranderingen van de arteriële bloeddruk bij katten kunnen worden vastgesteld met deze techniek.
- Kortdurende interactie met een bekend persoon bij katten leidt tot een hogere arteriële bloeddruk dan interactie met een onbekend persoon.
- De in dit proefschrift geïntroduceerde calcium-stimulatietest geen geschikte methode is voor de beoordeling van de β-celfunctie bij katten.
- Het met zekerheid diagnosticeren van hypofyse-afhankelijk hypercortisolisme bij katten met DM moeilijk kan zijn.
- Katten met DM, die een insulinebehoefte > 1,5 IE lente insuline / kg lichaamsgewicht / injectie hebben, onderzocht dienen te worden op mogelijk tevens aanwezige (hypofyse) aandoeningen.
- Intraveneuze toediening van octreotide (een somatostatine analogon) leidt tot afname van de plasmaconcentratie van groeihormoon bij acromegale katten, hoewel dit effect bij sommige acromegale katten duidelijker is dan bij andere.
- Een octreotide-suppressietest nuttig kan zijn om te beoordelen of acromegale katten in aanmerking komen voor de behandeling met (langwerkend) octreotide.
- Er bij katten vergroting van de hypofyse kan voorkomen zonder aantoonbare afwijkingen in (hypofysaire) hormoonconcentraties.
- Uitsluitend binnenshuis leven en een lage lichamelijke activiteit (beoordeeld door de katteneigenaar) onafhankelijke risicofactoren zijn voor het ontstaan van diabetes mellitus bij katten.
- Het percentage droog kattenvoer (op basis van metaboliseerbare energie) in de voeding van een kat geen onafhankelijke risicofactor voor het ontstaan van diabetes mellitus is.
- Bij katten met een stabiel lichaamsgewicht de door glucose gestimuleerde insulinesecretie tijdens HGCs toeneemt wanneer gedurende 9 maanden eiwit in de voeding door vet of koolhydraat wordt vervangen.
- Veranderingen in de macronutriëntensamenstelling van voeders bij katten de algehele insulinegevoeligheid zoals die door de HGC wordt gemeten niet beïnvloeden.
- Vetarme diëten bij katten tot een daling van de arteriële bloeddruk leiden.
- De bevindingen van deze onderzoeken de hypothese niet ondersteunen dat koolhydraatrijke voeding bij katten leidt tot uitputting en verlies van β-cellen.

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# Ganzenbord, een fabelachtig dankwoord

# Curriculum vitae

# Publications

# List of abbreviations

# Ganzenbord, een fabelachtig dankwoord

Je opent de doos, haalt het speelbord eruit en zet de ganzen neer. Je wilt graag zelf je zetten bepalen, maar helaas…. dobbelstenen! Gelukkig brengt de eerste worp meteen veel goeds: er ontstaat een samenwerkingsverband tussen een aantal ganzen die aan een Grote Reis zullen beginnen. De leider van de groep, een echte Anser canagicus, kent de risico's van het spel. Een gansje dat net heeft leren vliegen heeft veel geluk als het in zijn groep mee mag. Al meteen krijgt het jonge gansje verantwoordelijkheden: tijdens deze reis zal een groep krullenkrabbers veilig over het Diëtica-gebergte moeten worden getransporteerd. Vol goede moed begint de groep aan deze Grote Reis. De leider van de groep neemt zijn rechtergans mee, die geregeld de koppositie in de V-formatie overneemt. Voor extra informatie over de reisbestemming Mellona zorgen deze twee leiders ervoor dat regelmatig het advies wordt ingewonnen van een derde gans. Deze gans is bekend met de bestemming en vliegt met regelmaat een stuk mee om te helpen de meest efficiënte route te kiezen.

Tijdens de volgende beurten worden hoge ogen gegooid en slaagt de ganzengroep erin om de lading krullenkrabbers op te halen, samen met enkele ganzen die het op zich nemen om voor de krullenkrabbers te zorgen. Dit dreamteam maakt dat de krullenkrabbers zich goed voelen en meewerken tijdens het vervoer. Ook wordt een gans aangetrokken die in het oversteken van het Diëtica-gebergte gespecialiseerd is. Samen met weer een andere, intensieve gans wordt een nieuw systeem ontwikkeld, dat moet bijdragen aan een succesvolle afloop van de reis. De slaapganzen staan hun dons af; dit is zo zacht dat het gebruikt kan worden om krullenkrabbers slaperig te maken. Tevens werkt een groep zichtganzen mee, die met hun speciale kijkers meer kunnen zien. Maar ook de ganzen die werken met de koe die geen melk geeft, de ganzengroep "Ria & Irma", hun benedenburen, een bloedzuigend gansje en de ganzen die roeren in het vet, zorgen er allemaal voor dat de V-formatie op koers blijft.

Maar zoals is te verwachten bij een spelletje ganzenbord, zijn er tegenslagen. Na een fatale misworp steekt vlak voor het Diëtica-gebergte een zware storm de kop op die de ganzengroep weer helemaal terug naar af blaast. Terwijl de ganzen weer op krachten komen, wordt druk gewerkt aan een snelle herstart van de reis. Samen wordt een nieuw reisschema opgezet, met nieuwe pleisterplaatsen verzorgd door dezelfde cateraar. Al snel wordt de Grote Reis hervat. Deze keer lukt het wel om het gebergte te passeren. Lawines, luchtzakken en bergvossen worden ontweken en de eerste reisverslagen worden verstuurd.

Eenmaal in Mellona aangekomen wachten nieuwe uitdagingen. De laatste reisverslagen moeten worden geschreven en de krullenkrabbers aan hun nieuwe gezinnen afgestaan. Maar als dat eenmaal is gebeurd, heeft het jonge gansje even de tijd om rustig rond te dobberen in een Melloons meertje. Hij realiseert zich dat de Grote Reis zonder alle hulp anders had kunnen aflopen en is dankbaar voor de samenwerking.

De basis voor deze bundel van reisverslagen werd al gelegd in het nest waar het jonge gansje uit zijn ei kroop. Ook sommige gansjes waarmee hij leerde vliegen, de ganzen die hem leerden vliegen en weer andere gansjes die tegelijk met hem aan hun eerste Grote Reis begonnen, hebben het jonge gansje gesteund. De twee ganzen die het jonge gansje in zijn kraampje op de komende vliegreizenbeurs bijstaan, zijn hun gewicht in goud waard. En lest best de zwaan, waar het jonge gansje niet meer zonder wil.

# Curriculum Vitae

The author of the thesis was born in Willemstad (Curaçao, Netherlands Antilles) on the 30th of May 1974. After a primary school education in Cobham (United Kingdom), Abu Dhabi (United Arab Emirates) and Woerden (the Netherlands), he finished his secondary school education at the Minkema College (Woerden) in 1992. He started to study veterinary medicine at the Faculty of Veterinary Medicine at Ghent University (Belgium) and after 1 year he continued his veterinary education at the Faculty of Veterinary Medicine at Utrecht University. He graduated in 2000 and started to work as a PhD-student at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University.

De schrijver van dit proefschrift werd geboren in Willemstad (Curaçao, Nederlandse Antillen) op 30 mei 1974. Hij volgde in Cobham (Verenigd Koninkrijk), Abu Dhabi (Verenigde Arabische Emiraten) en Woerden (Nederland) de basisschool en behaalde zijn VWO-diploma aan het Minkema College (Woerden) in 1992. Daarna begon hij aan de studie diergeneeskunde aan de Faculteit Diergeneeskunde van de Universiteit Gent (België). Na 1 jaar zette hij deze studie voort aan de Faculteit Diergeneeskunde van de Universiteit Utrecht, waar hij in 2000 afstudeerde. Vanaf 2000 was hij als assistent-in-opleiding verbonden aan het Departement Geneeskunde van Gezelschapsdieren van de Faculteit Diergeneeskunde van de Universiteit Utrecht.

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# List of abbreviations

| | |
|---|---|
| α-MSH | α-melanocyte stimulating hormone |
| AB | arteriële bloeddruk |
| ABP | arterial blood pressure |
| ACTH | adrenocorticotrophic hormone |
| ADP | adenosine diphosphate |
| ANOVA | analysis of variance |
| AIC | Akaike Information Criterion |
| ATP | adenosine triphosphate |
| AUC | area under the curve |
| $AUC_{glucose}$ | area under the glucose curve |
| AUC-Ins1 | measure of the first phase of insulin secretion during a HGC |
| AUC-Ins2 | measure of the second phase of insulin secretion during a HGC |
| cAMP | cyclic adenosine monophosphate |
| CHO | carbohydrate |
| CST | calcium stimulation test |
| DBP | diastolic arterial blood pressure |
| DM | diabetes mellitus |
| DXA | dual-energy X-ray absorptiometry |
| EDTA | ethylene diamine tetraacetic acid |
| EGC | euglycemic, hyperinsulinemic glucose clamp |
| GH | growth hormone |
| GIP | glucose-dependent insulinotrophic peptide |
| GLP-1 | glucagon-like peptide-1 |
| GLUT2 | glucose transporter 2 |
| GLUT4 | glucose transporter 4 |
| GTT | glucose tolerance test |
| HC | high-carbohydrate |
| HDL | high density lipoprotein |
| HF | high-fat |
| HFi | high-fibre |
| HGC | hyperglycemic glucose clamp |
| HOMA-B | homeostasis model assessment of β-cell function |
| HOMA-R | homeostasis model assessment of insulin resistance |
| HP | high-protein |
| I | β-cell response during a HGC |
| IAPP | islet amyloid polypeptide |
| IE | internationale eenheid |
| IFG | impaired fasting glucose |
| IGF-I | insulin-like growth factor-I |
| IGT | impaired glucose tolerance –or– impaired glucose tolerant |
| IM | intramuscular |

| | |
|---|---|
| $INS_{CST}$ | measure of insulin secretion during a CST |
| $ISI_{HGC}$ | measure of whole body insulin sensitivity (insulin action) from the HGC |
| IU | international units |
| IV | intravenous |
| ivGTT | intravenous glucose tolerance test |
| K value | % glucose disappearance per minute during an ivGTT |
| LCLFi | low carbohydrate, low fibre |
| LFi | low fibre |
| LMM | linear mixed-effects modelling |
| LPL | lipoprotein lipase |
| M | glucose disposal rate during a HGC |
| MBP | mean arterial blood pressure |
| MCHFi | medium carbohydrate, high fibre |
| ME | metabolisable energy |
| $ME_{dry}$ | metabolisable energy from dry food expressed as a percentage of the total daily ME-intake |
| $M_{EGC}$ | glucose disposal rate during an EGC (measure of insulin sensitivity) |
| $M/I_{EGC}$ | measure of insulin sensitivity during an EGC |
| MRI | magnetic resonance imaging |
| mRNA | messenger ribose nucleic acid |
| MUFA | cis-monounsaturated fatty acid |
| NGT | normal glucose tolerance –or– normal glucose tolerant |
| oGTT | oral glucose tolerance test |
| P/B value | pituitary height/brain area value |
| Phi-1 | measure of the first phase insulin secretion during an ivGTT |
| Phi-2 | measure of the second phase insulin secretion during an ivGTT |
| Phi-1+2 | measure of the total insulin secretion during an ivGTT |
| PUFA | cis-polyunsaturated fatty acid |
| QUICKI | quantitative insulin sensitivity check index |
| Q-Q-plot | quantile-quantile plot |
| SBP | systolic arterial blood pressure |
| SC | subcutaneous |
| SD | standard deviation |
| $SI_{ivGTT}$ | sensitivity index for insulin during an ivGTT |
| SSTR | somatostatin receptor |
| $T_{1/2}$ | glucose half life during an ivGTT |
| UCCR | urinary corticoid/creatinine ratio |
| VC | variation coefficient |