Cushing's Syndrome and Other Causes of Insulin Resistance in Dogs

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KEYWORDS

• Dog • Canine • Diabetes mellitus • Insulin resistance • Cushing's syndrome
• Hypercortisolism • Basal-bolus insulin • Diestrus

KEY POINTS

- Cushing syndrome is the most common cause of insulin resistance in diabetic dogs in populations where neutering of young dogs is routine.
- The most pronounced effects of hypercortisolism on glucose metabolism are insulin resistance, perceived short duration of insulin action, excessive postprandial hyperglycemia, and substantial within-day and day-to-day glycemic variability.
- Successful strategies to manage excessive glycemic variability include basal insulin monotherapy and combined basal-bolus insulin treatment.
- Diestrus is the most common cause of insulin resistance in diabetic dogs in populations where neutering of young dogs is not routinely recommended.
- Obesity and other causes of insulin resistance have an additive effect on insulin requirements and the risk of progression to clinical diabetes in dogs.

INSULIN RESISTANCE

Definition of Insulin Resistance

Project ALIVE (Agreeing Language In Veterinary Endocrinology)[1] recommends using the term "insulin resistance" "to describe the presence of varying degrees of interference of insulin action on target cells. The term is not defined by the exogenous insulin dose required or by the change of blood glucose following insulin injection. However, when there is concern over the need for a high insulin dose, the presence of insulin resistance should be considered among other potential causes."[1]

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[1] Project ALIVE (Agreeing Language In Veterinary Endocrinology) was founded by the European Society of Veterinary Endocrinology in 2016 and endorsed by the Society for Comparative Endocrinology in 2017 and focuses on creating agreement over the definition of common terminology in veterinary endocrinology.

Vet Clin Small Anim 53 (2023) 711–730
https://doi.org/10.1016/j.cvsm.2023.01.009 **vetsmall.theclinics.com**

Causes of Insulin Resistance in Dogs

Causes of insulin resistance in dogs are listed in **Table 1**. The 3 most common causes of insulin resistance in dogs are discussed in the following section.

CUSHING SYNDROME

Definition of Cushing Syndrome

Project ALIVE defines Cushing syndrome as "the umbrella term for a range of *clinical syndromes* that are caused by a chronic excess of glucocorticoid activity, which can be due to a range of endogenous or exogenous steroid hormones."[1]

How Glucocorticoids Cause Impairment of Glucose Metabolism

- Impairment in glucose metabolism caused by glucocorticoids is multifactorial and includes increased glucogenesis, decreased glycogenosis, decreased glucose uptake and oxidation, impaired insulin secretion, and insulin resistance (reduced sensitivity of tissues to insulin). Insulin resistance may be manifested by direct changes in insulin receptor signaling or via indirect mechanisms through changes in lipid, carbohydrate, and protein metabolism.[2] The main organs involved are the liver, skeletal muscle, endocrine pancreas, and adipose tissue. Glucocorticoids cause increased glucogenesis in the liver. Glucocorticoid response elements within the promoter region of genes are activated, and this leads to increased expression of enzymes involved in the glucogenesis pathway. In addition, increased lipolysis and proteolysis augment this effect with increased substrates available for glucogenesis. Glucocorticoids also inhibit the suppressive effects of insulin on this pathway.
- There is interindividual variability in the susceptibility of people to the adverse effects of glucocorticoids and to the degree of insulin resistance they induce. The situation is likely similar in dogs. Dogs that develop unexpectedly pronounced side effects to treatment with systemic or topical glucocorticoids might have concurrent spontaneous hypercortisolism and experience additive effects of exogenous and endogenous glucocorticoids.
- Basal and postprandial insulin resistance occur but in nondiabetic human patients the diabetogenic effects of prednisolone were most prominent in the postprandial, hyperinsulinemic state.[3,4]

Table 1
Causes of insulin resistance in dogs

Cause	Mechanism
Cushing syndrome (hypercortisolism/glucocorticoid treatment)	Excess cortisol or cortisone
Diestrus/pregnancy/progestogen treatment	Predominantly due to progestogens and growth hormone
Obesity	Via hormonal signals from adipose tissue (eg, leptin)
Hypothyroidism	Excess growth hormone induced by thyrotropin-releasing hormone
Stress hyperglycemia	Increased counterregulatory hormones (cortisol, catecholamines)
Inflammatory, traumatic, and other health conditions	Via increased stress hormones (cortisol, catecholamines)
Pheochromocytoma/paraganglioma	Increased catecholamines

- Within nonhepatic tissues, insulin resistance is associated with impaired glucose metabolism and decreased glucose uptake; this may involve a direct effect on receptor signaling pathways and reduced glucose transporters, but not all of the pathways and molecular mechanisms of insulin resistance are understood.[5]
- Within the acute phase of corticosteroid use, insulin-stimulated glucose uptake is inhibited in skeletal muscle. More chronically, decreased muscle mass and inhibition of capillary recruitment leads to a decreased surface area for exchange and reduced glucose uptake.[6,7]
- Hormones released from adipose tissue are termed adipokines. Glucocorticoids influence the synthesis and release of adipokines, which leads to a more diabetogenic profile (increased leptin and resistin, which then decrease insulin sensitivity). Increased lipolysis also increases the substrates for glucogenesis, although this glucocorticoid-associated dyslipidemia has not been proved to cause insulin resistance on its own.[8]
- In the endocrine pancreas, insulin synthesis and secretion from beta cells is impaired, and this especially affects glucose-stimulated insulin secretion.[9]
- Glucocorticoids potentiate the action of glucagon and cause incomplete suppression of glucagon after meals. High-dose (30 mg) prednisolone treatment in healthy humans leads to an increase in fasting and postprandial glucagon concentrations.[10]

Canine Cushing Syndrome and Insulin Resistance

- Insulin resistance associated with Cushing syndrome in dogs has been recognized for more than 40 years.[11]
- The gold standard for assessment of insulin sensitivity is the euglycemic-hyperinsulinemic glucose clamp. Using this gold standard, dogs with spontaneous Cushing syndrome have severe insulin resistance, even more severe than bitches in diestrus.[12]
- Nondiabetic dogs with Cushing syndrome secrete more insulin from beta cells in response to the insulin resistance.[13] If this compensation fails, diabetes ensues.
- Most dogs with Cushing syndrome remain euglycemic (146/235 [62%] in a prospective study); however, those that developed mild hyperglycemia (100–180 mg/dL; 5.6–10.0 mmol/L) had increased risk for becoming more severely hyperglycemic within a year.[14]

Association of Hypercortisolism and Diabetes Mellitus in Dogs

- For dogs that have both Cushing syndrome and diabetes, it is logical that Cushing usually precedes the onset of diabetes. Clinical signs consistent with Cushing are often reported to have been present for several years before diagnosis of diabetes. However, as the diagnosis of diabetes is much more straightforward than the diagnosis of hypercortisolism in dogs, diabetes will often be diagnosed before Cushing syndrome.
- Clinical experience suggests that in populations where neutering of young dogs is routine, Cushing syndrome is the most common cause of insulin resistance in diabetic dogs.
- A major limitation for reporting data on the prevalence of Cushing syndrome in diabetic dogs is the unreliability of diagnostic tests for hypercortisolism in this population with both false-positive and false-negative results occurring commonly. Nevertheless, a positive association between Cushing syndrome and diabetes is well recognized. Diabetic dogs were 6 times more likely than controls to be diagnosed with hypercortisolism in first-opinion practices[15]; this aligns

with a reported 23% prevalence of hypercortisolism in diabetic dogs[16] and 13.6% prevalence of diabetes in dogs with hypercortisolism.[14]

- Insulin resistance typically causes compensatory increase of beta cell function. Therefore, progression to diabetes mellitus in insulin-resistant individuals always requires concurrent loss of beta cell function. In diabetic dogs with all forms of insulin resistance including Cushing syndrome, the mechanisms of beta cell loss are likely the same as for diabetic dogs without insulin resistance.
- Risk factors associated with the diagnosis of diabetes in dogs with Cushing syndrome included hypercholesterolemia, hypertriglyceridemia, pituitary-dependent hypercortisolism, and nonspayed females.[14] However, significantly increased plasma cholesterol and triglycerides occurred after the onset of diabetes, and not in the euglycemic or mildly hyperglycemic dogs with hypercortisolism, indicating that these factors were likely a consequence rather than a cause of the diabetic state.[14]

Iatrogenic Cushing Syndrome in Diabetic Dogs

- The effect of systemic glucocorticoid treatments on insulin requirement in diabetic dogs is predictable. Such treatments will often increase insulin requirement and protect against insulin-induced hypoglycemia. Conversely, insulin requirement can markedly decrease when glucocorticoid treatment is withdrawn.
- The effect of topical glucocorticoid treatments on insulin requirement in diabetic dogs is less predictable. Twice daily application of prednisolone acetate eye drops to healthy dogs for 2 weeks suppressed the hypothalamic-pituitary-adrenal axis,[17] yet application 4 times daily for 4 weeks to diabetic dogs was associated with no detectable differences in diabetic control compared with topical diclofenac.[18] However, dogs with hypercortisolism and poorly controlled diabetes were excluded from the latter study, and our experience is that patients from those specific populations often seem to have increased insulin requirement when treated with prednisolone acetate eye drops and decreased insulin requirement when the treatment is discontinued. Therefore, although there is no contraindication for treatment of diabetic dogs with prednisolone acetate eye drops, this might affect diabetic control in dogs with poorly controlled diabetes and/or hypercortisolism.

Clinical Presentation of Cushing Syndrome in Diabetic Dogs

- Underlying Cushing syndrome can be difficult to recognize in diabetic dogs because there is overlap of clinical signs, laboratory abnormalities, age of diagnosis, and breed predispositions. An important difference is that diabetes typically causes weight loss whereas Cushing does not. Therefore, once glycemic control is sufficient to arrest weight loss, persistent polyphagia, polyuria, polydipsia, and lethargy might be indicators of Cushing syndrome.
- The Project ALIVE Cushing's Clinical Score[1] will often be difficult to apply in diabetic dogs because of the overlap of clinical signs. It is recommended to instead focus on signs specific to Cushing syndrome.
- As it is presumed that in dogs affected concurrently by Cushing syndrome and diabetes, Cushing typically precedes the onset of diabetes, owners may have become accustomed to the Cushing signs and describe their dog as "normal" once the diabetes is controlled.
- There are marked interindividual and breed differences in the clinical presentation of Cushing syndrome. For example, endocrine alopecia presents differently in short- versus long-haired breeds and in those with curly versus straight hair

coats. Similarly, deep-chested breeds are less likely to present with a pot-bellied appearance than those breeds with relatively broad chests. As awareness of canine hypercortisolism has increased over time, more subtle case presentations have been recognized.[19] Specific clinical signs of Cushing syndrome include endocrine alopecia, a pot-bellied appearance and muscle wasting that persist after diabetes is controlled, heat and exercise intolerance, increased anxiety or behavioral changes, and signs reflecting premature aging. Hepatomegaly, hypertriglyceridemia, and an alkaline phosphatase that is much more elevated than alanine transaminase can also be indicators of underlying Cushing syndrome, especially if these persist or increase after diabetes is controlled. Dogs with Cushing syndrome are also at increased risk for cruciate ligament rupture.

- The more clinical abnormalities identified that are specific for Cushing syndrome, the stronger the suspicion that hypercortisolism might be present.[19] However, there is no requirement to identify multiple indicators, and suspicion of underlying Cushing syndrome may be based on the presence of only one specific clinical sign (eg, endocrine alopecia) in addition to diabetes. Caution is advisable considering the overlap of clinical signs of the 2 conditions.
- Suspicion of underlying Cushing syndrome may also be based solely on persistently poor response to high doses of insulin not attributable to another cause.[19]

THE EFFECT OF CUSHING-ASSOCIATED DYSGLYCEMIA IN DIABETIC DOGS

- The ALIVE project states that insulin resistance is not defined by the exogenous insulin dose. Therefore, insulin resistance is not necessarily associated with requirement for a higher exogenous insulin dose than is typical for the general diabetic dog population. It follows that there is no insulin dose "threshold" that defines a state of insulin resistance.
- Diabetes in dogs with hypercortisolism is not necessarily more difficult to manage than in dogs with uncomplicated diabetes; this is because glycemic control depends on the interaction in the individual between residual beta cell function and the degree of insulin resistance. These factors may change over time because of ongoing beta cell loss and/or increased or decreased influence of endogenous glucocorticoids. Therefore, there may be good diabetic control for a variable period with subsequent deterioration.
- Diabetes can be very difficult to manage when substantial Cushing-associated dysglycemia is present. The most pronounced effects are typically
  - Excessive postprandial hyperglycemia,
  - Perceived short duration of insulin action,
  - Substantial within-day and/or day-to-day glycemic variability.
- Glycemic variability is best appreciated using continuous glucose monitoring systems (**Figs. 1–5**).
- Glucocorticoids confer protection against insulin-induced hypoglycemia. However, unexpected neuroglycopenia can occur when there is substantial day-to-day glycemic variability necessitating a more cautious approach to insulin treatment (see **Fig. 3**A).

Insulin Treatment to Manage Cushing-Associated Dysglycemia

- Strategies to manage the excessive glycemic variability can provide immediate clinical benefit for diabetic dogs; this can also minimize the confounding effect of poor diabetic control on the diagnostic investigation for the underlying cause of the insulin resistance.

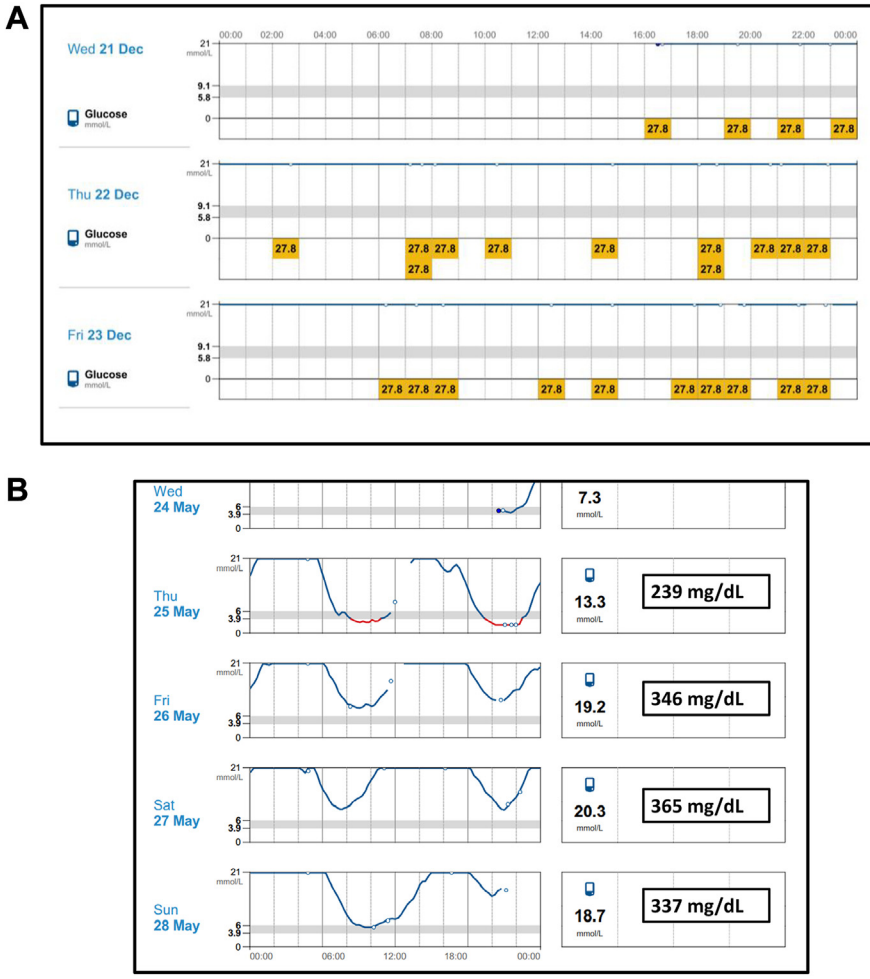


Fig. 1. Example of the effect of Cushing-associated insulin resistance on glycemic control in diabetic dogs: within-day glycemic variability. (*A*) Continuous glucose graph from a diabetic dog soon after commencing treatment with an intermediate-acting insulin formulation at 0.5 U/kg q12 h. All interstitial glucose results were greater than 500 mg/dL (>27.8 mmol/L). (*B*) Continuous glucose graph from the same dog in Fig. 1A 6 months later when treated with the same intermediate-acting insulin formulation at 2.0 U/kg q12 h. Average daily interstitial glucose results are provided in the right panel. Note that there is large within-day glycemic variability and apparent short duration of insulin action.

- The most successful approaches mimic the pattern of endogenous insulin secretion of nondiabetic, insulin-resistant individuals:
  - Basal insulin requirements, and hence basal insulin secretion, typically increase in response to insulin resistance (although this might be either a constant or an intermittent effect).
  - Glucocorticoids additionally increase postprandial insulin requirement by inhibiting the suppressive effects of insulin on glucogenesis, along with incomplete suppression of glucagon after meals.

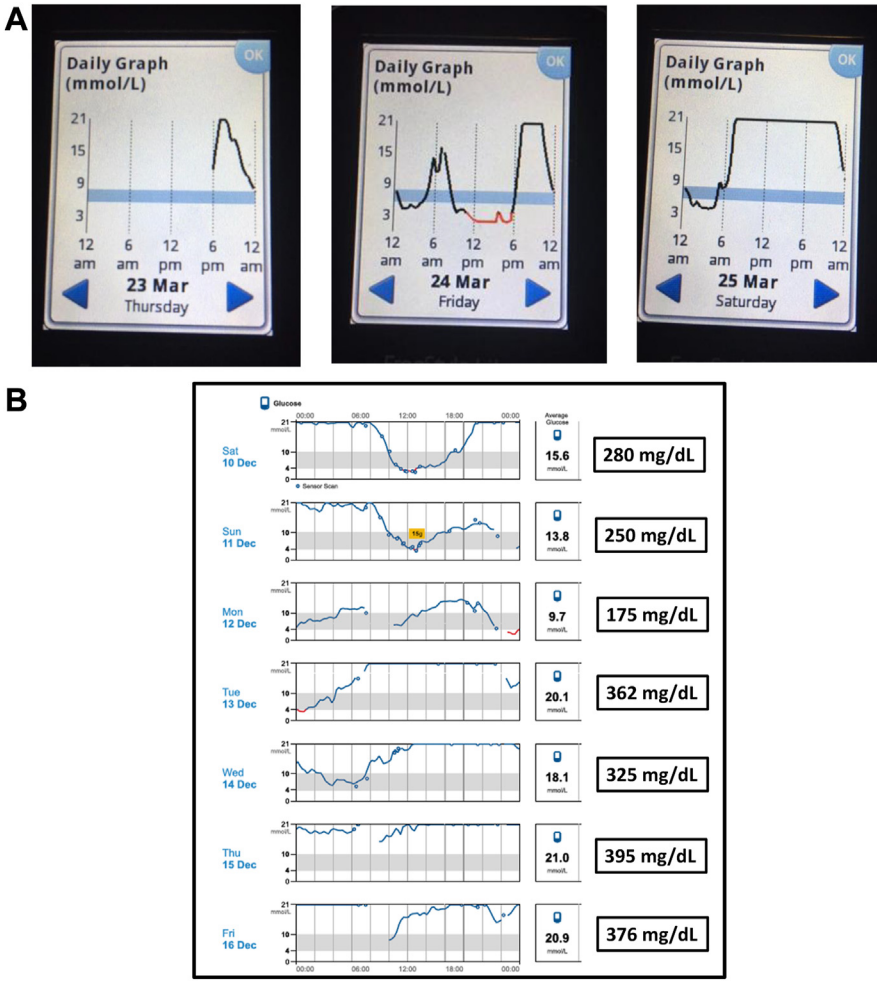
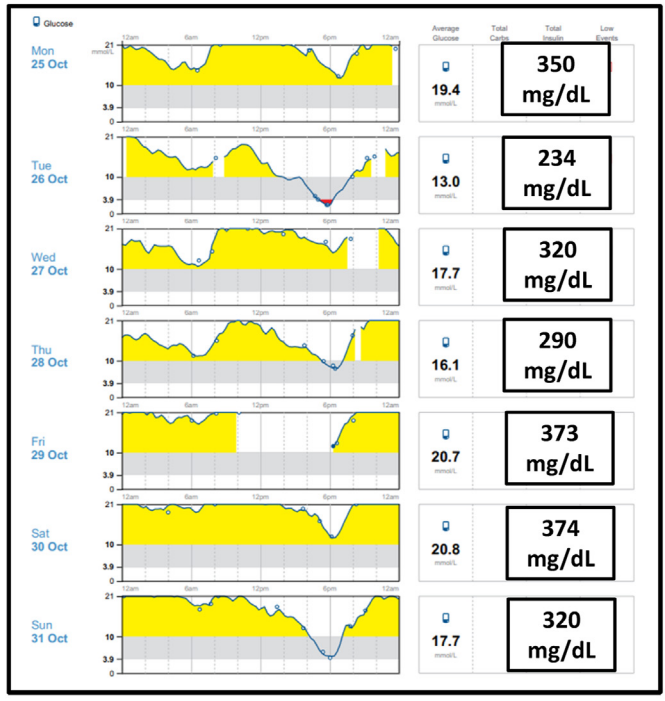


Fig. 2. Examples of the effect of Cushing-associated insulin resistance on glycemic control in diabetic dogs: day-to-day glycemic variability. (*A*) Daily graphs from consecutive days showing marked day-to-day glycemic variability despite a consistent insulin dosing and feeding routine. (*B*) Continuous glucose graph from a different dog showing marked day-to-day glycemic variability despite a consistent insulin dosing and feeding routine. Average daily interstitial glucose results are provided in the right panel.

- *Basal insulin monotherapy* aims to address background insulin resistance without specifically addressing postprandial hyperglycemia. This simple approach provides good-to-excellent control in most of the dogs with Cushing-associated diabetes (Fig. 6). Ideally, a basal insulin formulation should have a similar action at every hour of the day and minimal day-to-day variability. There are only 2 options that fulfill these criteria in dogs: insulin glargine U300 and insulin degludec insulin (see Chapter 9, Insulin therapy in dogs).[20,21]
- As there is large variability in the severity of insulin resistance in dogs with Cushing-associated diabetes, there is corresponding large variability of the required dose of basal insulin. Therefore, dose titration is only feasible if response to treatment is monitored using a continuous glucose monitor.[22]

A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Total Carbs
Total Insulin
Low Events
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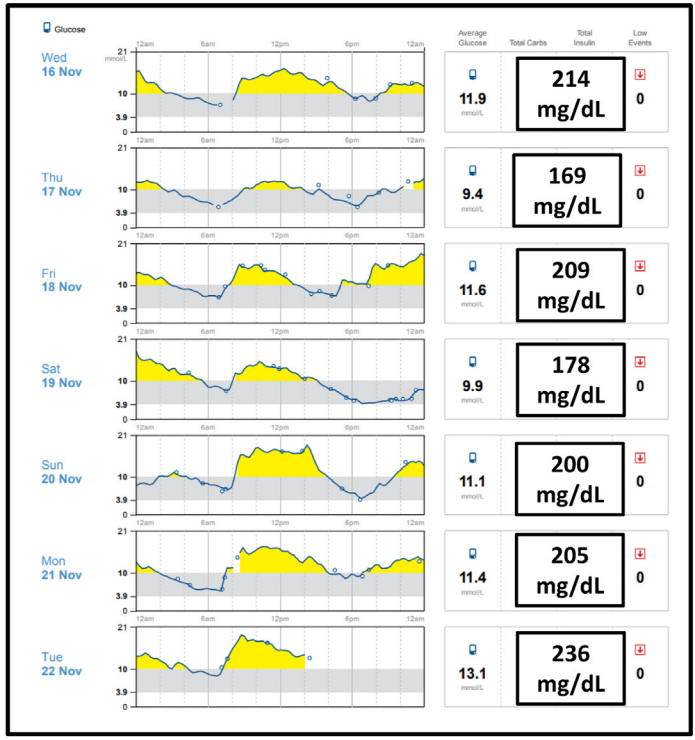
350 mg/dL
Tue 26 Oct
13.0 mmol/L
234 mg/dL
Wed 27 Oct
17.7 mmol/L
320 mg/dL
Thu 28 Oct
16.1 mmol/L
290 mg/dL
Fri 29 Oct
20.7 mmol/L
373 mg/dL
Sat 30 Oct
20.8 mmol/L
374 mg/dL
Sun 31 Oct
17.7 mmol/L
320 mg/dL

B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Total Carbs
Total Insulin
Low Events
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0
Thu 17 Nov
9.4 mmol/L
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0
Fri 18 Nov
11.6 mmol/L
209 mg/dL
0
Sat 19 Nov
9.9 mmol/L
178 mg/dL
0
Sun 20 Nov
11.1 mmol/L
200 mg/dL
0
Mon 21 Nov
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205 mg/dL
0
Tue 22 Nov
13.1 mmol/L
236 mg/dL
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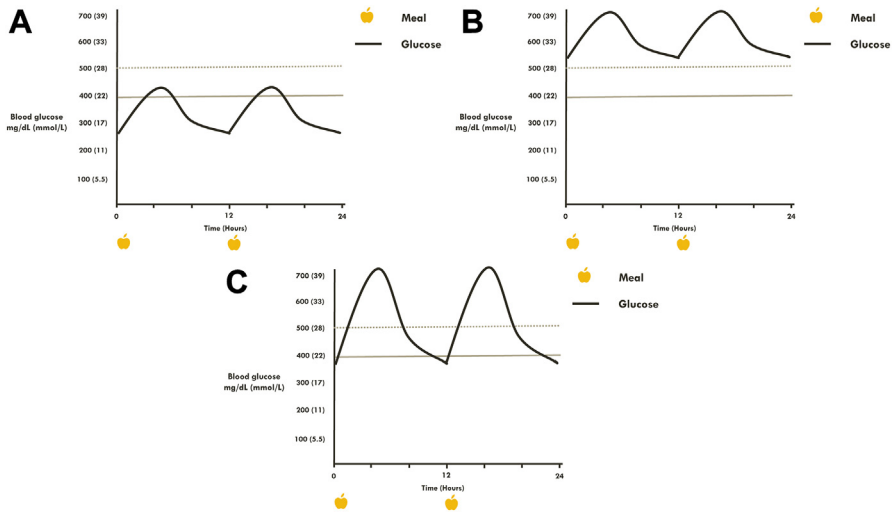

Fig. 4. Graphical illustration of the influence of insulin resistance on glucose control in diabetic dogs. Graphs show examples of 24-hour glucose data. The x-axis shows time, and the y-axis shows blood/interstitial glucose concentrations. The solid gray line denotes the upper limit of the Freestyle Libre graph (400 mg/dL; 22 mmol/L) and the broken gray line the highest glucose concentration measured by that device (500 mg/dL; 27.8 mmol/L). (*A*) Example of a 24-hour glucose graph in a diabetic dog with no underlying insulin resistance. The same meal is consumed every 12 hours, and no insulin treatment is administered. Note that the typical postprandial hyperglycemic period is 6 to 9 hours. (*B*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance that is relatively constant over the day. The same meal is consumed every 12 hours, and no insulin treatment is administered. (*C*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance associated with excessive postprandial hyperglycemia, for example, due to Cushing syndrome. The same meal is consumed every 12 hours, and no insulin treatment is administered.

- *Combined basal-bolus insulin treatment* aims to address both background insulin resistance and postprandial hyperglycemia. This approach typically requires that 2 different insulin formulations are administered with a meal twice daily (**Fig. 7**). Intermediate-acting insulin formulations are the most appropriate for bolus insulin treatment of dogs, rather than the short-acting formulations used for this purpose in diabetic people (see Chapter 9, Insulin therapy in dogs).

Fig. 3. Example of the effect of Cushing-associated insulin resistance on glycemic control in diabetic dogs: response to trilostane treatment. (*A*) Continuous glucose graph from a diabetic dog with hypercortisolism 4 months after starting trilostane treatment. There was still marked postprandial hyperglycemia and day-to-day glycemic variability despite good control of hypercortisolism. The low glucose event on Tuesday October 26 was associated with mild signs of neuroglycopenia. Average daily interstitial glucose results are provided in the right panel. (Treatment: insulin glargine U300 at 1 U/kg BID; trilostane at 3 mg/kg BID). (*B*) Continuous glucose graph from the same dog in Fig. 3A 15 months after starting trilostane treatment. Glycemic variability has substantially decreased, so the insulin dose could be safely increased to improve overall glycemic control without associated increased risk of neuroglycopenia. Average daily interstitial glucose results are provided in the right panel. (Treatment: insulin glargine U300 at 2 U/kg BID; trilostane at 3 mg/kg BID).

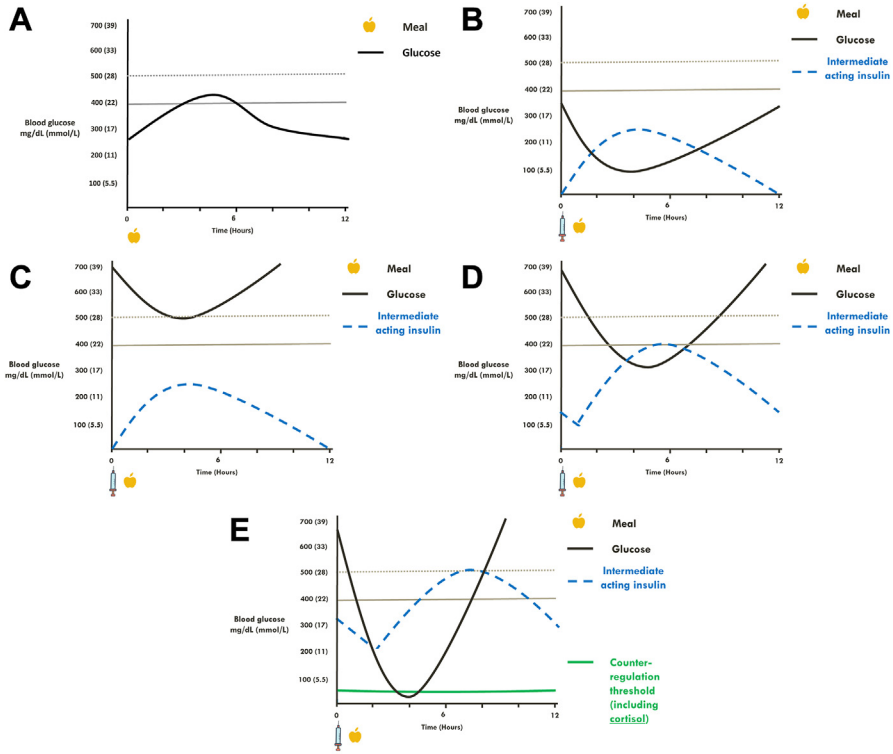

Fig. 5. Graphical illustration of the effect of treatment of a diabetic dog with insulin resistance using an intermediate-acting insulin. Graphs show examples of 12-hour glucose data. The x-axis shows time, and the y-axis shows blood/interstitial glucose concentrations. The solid gray line denotes the upper limit of the Freestyle Libre graph (400 mg/dL; 22 mmol/L) and the broken gray line the highest glucose concentration measured by that device (500 mg/dL; 27.8 mmol/L). The same meal is consumed every 12 hours. (*A*) Example of a 12-hour glucose graph in a diabetic dog with no underlying insulin resistance. No insulin treatment is administered. (*B*) Example of a 12-hour glucose graph in a diabetic dog with no insulin resistance treated with a standard dose of intermediate-acting insulin. Note that the expected (yet not always achieved) graph is U-shaped. (*C*) Example of a 12-hour glucose graph in a diabetic dog with insulin resistance treated with a standard dose of intermediate-acting insulin q12 h. (*D*) Example of a 12-hour glucose graph in a diabetic dog with insulin resistance treated with 1.5 times a standard dose of intermediate-acting insulin q12 h. Note that the duration of action of the insulin increases as the dose increases. (*E*) Example of a 12-hour glucose graph in a diabetic dog with insulin resistance treated with 2 times a standard dose of intermediate-acting insulin q12 h. The insulin decreases the blood glucose to the hypoglycemic range, which triggers a counterregulatory response. Dogs with Cushing syndrome might have excessive cortisol counterregulation.

- When switching to a basal-bolus protocol, it is recommended that the same total insulin dose (per unit) is used every 12 hours by reducing the dose of the intermediate-acting insulin by 50%, and the remainder of the total insulin dose is divided and given by basal insulin twice daily.[23] The doses can then be adjusted based on the dog's response. In this situation, it is crucial that there is very clear communication with owners regarding the dose of each of the insulin preparations. The response to treatment is much more predictable with this

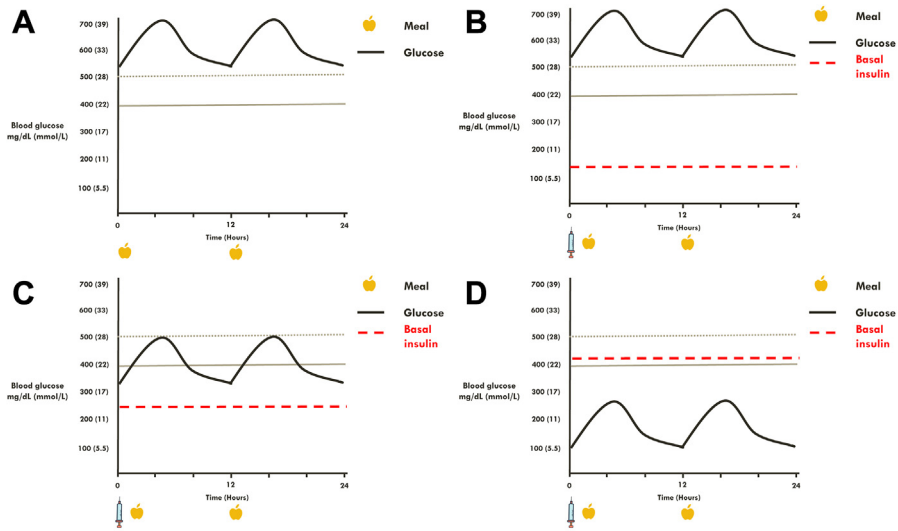

Fig. 6. Graphical illustration of the effect of treatment of a diabetic dog with insulin resistance using a basal insulin. Graphs show examples of 24-hour glucose data. The x-axis shows time, and the y-axis shows blood/interstitial glucose concentrations. The solid gray line denotes the upper limit of the Freestyle Libre graph (400 mg/dL; 22 mmol/L) and the broken gray line the highest glucose concentration measured by that device (500 mg/dL; 27.8 mmol/L). The same meal is consumed every 12 hours. (*A*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance. No insulin treatment is administered. (*B*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance treated with a standard dose of basal insulin q24 h. Note the postprandial increase of glucose that causes the graph to be an inverse of the U-shape expected following intermediate-acting insulin. (*C*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance treated with 1.5 times a standard dose of basal insulin q24 h. (*D*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance treated with 3 times a standard dose of basal insulin q24 h.

approach compared with basal insulin monotherapy and so it is not necessary to use a continuous glucose monitor during the changeover.

DIAGNOSIS OF CANINE HYPERCORTISOLISM

- Details regarding the diagnosis of hypercortisolism in dogs are available in the Project ALIVE resources.[1] The "General definitions for diagnostic tests for Cushing's and Hypoadrenocorticism" section provides information on the various tests.
- The ALIVE criteria for the diagnosis of naturally occurring Cushing syndrome are as follows:
  - "Identification of a set of clinical features attributable to Cushing's Syndrome including supportive history, physical examination findings, and clinicopathologic test results;
  - AND demonstration of an excess of cortisol through dynamic testing of pituitary-adrenal function; dynamic testing of pituitary-adrenal function can include a dexamethasone suppression test based on blood or urine or an adrenocorticotropic hormone (ACTH) stimulation test."[1]
- Importantly, Project ALIVE provides a definition for "subdiagnostic" Cushing syndrome:

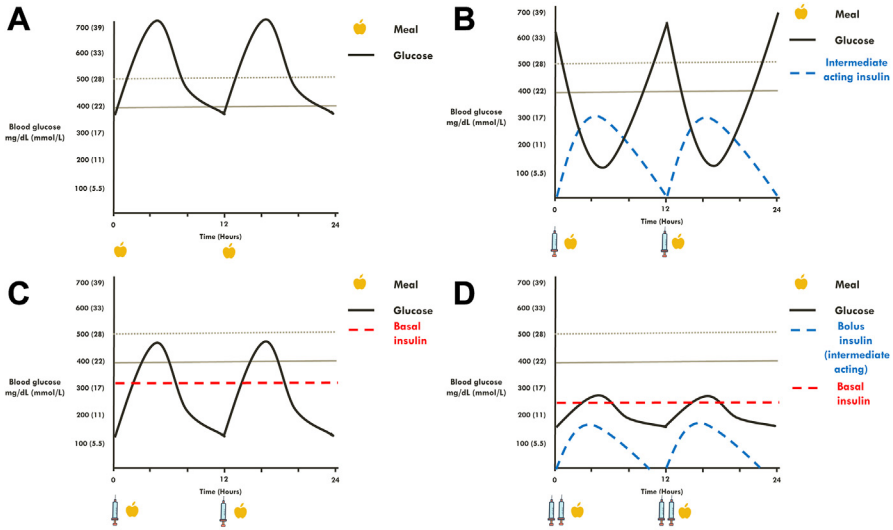

Fig. 7. Graphical illustration of the effect of treatment of a diabetic dog with insulin resistance using basal-bolus insulin. Graphs show examples of 24-hour glucose data. The x-axis shows time, and the y-axis shows blood/interstitial glucose concentrations. The solid gray line denotes the upper limit of the Freestyle Libre graph (400 mg/dL; 22 mmol/L) and the broken gray line the highest glucose concentration measured by that device (500 mg/dL; 27.8 mmol/L). The same meal is consumed every 12 hours. (*A*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance associated with excessive postprandial hyperglycemia, for example, due to Cushing syndrome. No insulin treatment is administered. (*B*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance associated with excessive postprandial hyperglycemia treated with an intermediate-acting insulin q12 h. (*C*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance associated with excessive postprandial hyperglycemia treated with a basal insulin q12 h. The same pattern can be achieved in many dogs with q24 h dosing. (*D*) Example of a 24-hour glucose graph in a diabetic dog with insulin resistance associated with excessive postprandial hyperglycemia treated with basal-bolus insulin treatment q12 h. The same pattern can be achieved in many dogs with q24 h dosing of the basal insulin.

  - "A clinical syndrome in which a dog or cat appears to have Cushing's syndrome, yet the results of dynamic testing of pituitary-adrenal function fall into appropriate (normal) reference intervals.
  - Testing requires a normal dexamethasone suppression test (based on blood or urine estimates of corticoid activity) and a normal ACTH stimulation test.
  - "Subdiagnostic Cushing's syndrome" has previously been referred to as Atypical or Occult Cushing's/Hyperadrenocorticism."[1]
- "Subdiagnostic Cushing syndrome" therefore defines the relatively common situation when there are false-negative results for both a dexamethasone suppression test and an ACTH stimulation test for an individual case.

Diagnosis of Hypercortisolism in Diabetic Dogs: Specificity, Sensitivity, and the Relationship Between Pretest Probability and the Positive and Negative Predictive Values of Diagnostic Tests

- Reported sensitivities and specificities for both the low-dose dexamethasone suppression test and the ACTH stimulation test for diagnosis of canine

hypercortisolism were mostly determined decades ago with cortisol assays that are no longer used. Nevertheless, although updated and current sensitivity and specificity data are scarce, it is recognized that false-positive and false-negative results still commonly occur with both tests.[24]

- It is recommended that individual features of each case are carefully considered to assist with assessment of the likelihood of false-positive and false-negative results because this will vary from case to case and over time for the one case:
  - False-positive results are typically associated with stress and/or nonadrenal illness, including diabetes; this means that it is necessary to achieve control of the diabetes before testing for hypercortisolism to improve the specificity of the tests and reduce the likelihood of a false-positive result. It is the authors' experience that a practical guideline is to first achieve sufficient control of the diabetes to arrest weight loss before performing diagnostic tests for hypercortisolism. It is also advisable to ensure that there are no signs of malaise or inappetence on the day the test is performed and that the dog is housed in a low-stress environment during the test.
  - Pretest probability is influenced by disease prevalence. There is a positive association between hypercortisolism and diabetes in dogs, which means that a diabetic dog is more likely to have hypercortisolism than a nondiabetic dog. If care is taken to minimize the likelihood of false-positive results as outlined earlier, then this increased prevalence affects the pretest probability in diabetic dogs as follows:
    - The negative predictive value of a diagnostic test for hypercortisolism is decreased compared with nondiabetic dogs.
    - The positive predictive value of a diagnostic test for hypercortisolism is increased compared with nondiabetic dogs.
- Negative (normal) test results cannot exclude a diagnosis of hypercortisolism.
- If it is suspected that the first test returned a false-negative result, then it is recommended that the other alternative test is performed. For example, if the first test was an ACTH stimulation test, then a low-dose dexamethasone suppression test is recommended (and vice versa). If it is suspected that both tests returned false-negative results, then subdiagnostic Cushing syndrome may be present.
- The situation where there is no perfectly reliable test for hypercortisolism and there is a possibility of subdiagnostic Cushing syndrome can result in a frustrating situation where hypercortisolism is strongly suspected but diagnosis cannot be confirmed. An appropriate approach in many cases will be to retest after 3 to 6 months if clinical signs persist. However, if clinical signs are severe and negatively affecting the dog's and/or the owner's quality of life, and there is no other likely reason for the dog's clinical signs, then a carefully monitored trilostane treatment trial should be considered. Improvement in response to treatment can thus be used as a diagnostic test. Dogs with normal adrenal function seem to be more resistant to adverse effects associated with trilostane treatment than dogs with hypercortisolism[25,26]; this means that a carefully monitored trilostane treatment trial might be unlikely to cause problems in dogs that do not have hypercortisolism.

Treatment of Cushing Syndrome in Diabetic Dogs

- Glucocorticoid treatment should ideally be withdrawn if iatrogenic Cushing syndrome is present. However, it is acknowledged that this might not always be possible.

- If the cause of ACTH-dependent or ACTH-independent hypercortisolism cannot be removed, then medical treatment to control excess adrenal hormone secretion is indicated.
- If trilostane treatment is used, a twice-daily administration protocol is typically recommended for diabetic dogs.
- Use of clinical signs to monitor the response to treatment of Cushing syndrome is likely less reliable in diabetic dogs than in nondiabetic dogs. Therefore, direct measurement of cortisol response is recommended, for example, with an ACTH stimulation test.
- Short-acting glucocorticoid tablets should be dispensed when Cushing treatment commences. Administration of these tablets is recommended whenever there is potential for absolute or relative hypocortisolism, for example, when there is inappetence, malaise, a stressful event, or surgery. Administration will also protect diabetic dogs against the potential for hypoglycemia. It is additionally noted that one of the differential diagnoses for owners reporting inappetence in trilostane-treated dogs is resolution of polyphagia.

What Happens to the Insulin Resistance When Cushing Syndrome Is Treated?

- Insulin resistance and associated within-day and/or day-to-day glycemic variability seems to persist for many months after very good control of hypercortisolism is achieved with trilostane treatment (see **Fig. 3**). Resolution of insulin resistance and glycemic variability seems to correlate with resolution of hepatomegaly.
- Therefore, there is no requirement to decrease the insulin dose at the same time as commencing trilostane treatment.
- This is in contrast to treatment methods that eliminate the cause of hypercortisolism, such as surgical removal of a functional adrenal tumor. In those cases, insulin resistance and dysglycemia seem to resolve rapidly after treatment.

DIESTRUS

Why Diestrus Is Associated with Insulin Resistance

- Entire female dogs undergo cyclical changes in insulin sensitivity associated with their estrous cycle. Insulin resistance occurs during diestrus,[12] which, in dogs, is a prolonged, progesterone dominant luteal stage that lasts for 2 to 3 months. Many of the hormonal changes during diestrus mimic those that occur during pregnancy.
- In women, no single hormone or metabolic mechanism has been found to explain insulin resistance during pregnancy.[27] Instead insulin resistance results from the summation of multiple effects and increases as pregnancy progresses.[28] However, unlike people, progesterone-induced growth hormone secretion from the mammary glands is an important hormonal change in dogs.
- Progesterone and growth hormone are 2 diabetogenic hormones that are associated with insulin resistance. Growth hormone and progesterone concentrations are comparable in diestrus and pregnancy in dogs, with reduced insulin sensitivity during both stages. However, pregnant dogs are more insulin resistant and have a 43% reduction in insulin sensitivity in late pregnancy.[12,29]
- Progesterone can stimulate growth hormone production and release from the mammary gland of entire bitches. In some individuals, hypersecretion of growth hormone is induced, causing hypersomatotropism, an acromegalic phenotype, and greater insulin resistance compared with normal diestrus.[30]

- In susceptible dogs, diabetes mellitus develops in association with insulin resistance during diestrus.[31] This subtype of canine diabetes is likely comparable to gestational diabetes in women in that progression from insulin resistance to diabetes requires concurrent loss of beta cell function (because insulin resistance alone does not directly cause beta cell loss). The mechanisms of beta cell loss are likely the same as for diabetic dogs without insulin resistance.
- There is interindividual variability of the diabetogenic effects of diestrus with recognized breed predispositions, including Border Collies and Swedish Elkhounds.[31] The increased risk of Swedish Elkhounds does not seem to be associated with beta cell autoimmunity because all affected dogs were negative for GAD-65-autoantibodies.[32]

The Effect of Routine Neutering of Young Female Dogs on Risk Factors for Diabetes in Dog Populations

- In contrast to the female predisposition for diabetes in populations where neutering of young female dogs is not routinely recommended (eg, 72% female predisposition for diabetes in Swedish dogs),[31] there tends to be a marginal male predisposition for diabetes where neutering is routine.[15,33]
- There is an interaction of neutering status with common causes of insulin resistance in dogs. Compared with intact dogs, neutered dogs have an increased risk of obesity and for developing Cushing syndrome.[34] However, entire female dogs with hypercortisolism have increased risk for diabetes compared with males and neutered females.[14]

Treatment and Remission of Diestrus Diabetes

- Ovariohysterectomy can promptly resolve progesterone-associated insulin resistance. The chance of diabetic remission is likely much higher if neutering is performed as soon as practical after the diagnosis of diabetes. In addition, prompt insulin treatment is important to attempt to preserve pancreatic beta cell function. This approach has been reported to achieve diabetic remission in about 10% of cases.[35] Remission usually occurs within 4 to 39 days after ovariohysterectomy, but the time to resolution of insulin resistance and decreased exogenous insulin requirements is unpredictable.[30]
- Remission may spontaneously occur at the end of diestrus.[31] Ovariohysterectomy should be performed before subsequent estrus cycles to prevent relapse of diabetes and risk of progression to permanent diabetes.

OBESITY

Obesity Results in Compensatory Increase of Insulin Secretion in Dogs

- Obesity causes insulin resistance in dogs as it does in all species, with the degree of insulin resistance positively correlated with the severity of adiposity.[36]
- Spontaneously obese dogs compensate for reduced insulin sensitivity with hyperinsulinemia, with the result that glucose tolerance is maintained. In one study, obese dogs had approximately half the insulin sensitivity of lean dogs, with a 4-fold increase in insulin concentrations, while maintaining euglycemia.[37]
- Adipose tissue is an active endocrine organ that secretes adipokines that modify insulin sensitivity. In spontaneously obese dogs, leptin seems to be the main adipokine associated with obesity-associated changes in insulin sensitivity and compensatory hyperinsulinemia. Glucagon-like peptide 1 also likely has a role in compensatory hyperinsulinemia.[38]

- Similar to other species, obesity is associated with hypertriglyceridemia in dogs, but, unlike other species, obesity-associated hypertriglyceridemia might not contribute to insulin resistance in dogs.[39]

Obesity and Canine Diabetes Mellitus

- Type 2 diabetes mellitus, a condition strongly associated with obesity in people, is not recognized in dogs.
- Disease processes that cause progressive loss of beta cells, such as immune-mediated destruction or chronic pancreatitis, will limit the capacity of obese dogs to compensate for obesity-associated insulin resistance; this might result in earlier presentation with diabetes than if the dog was lean.
- Insulin is an anabolic hormone and so treatment with exogenous insulin promotes weight gain. If an insulin-treated diabetic dog becomes obese, the resulting insulin resistance will increase basal insulin requirement; this can lead to a cycle of increasing insulin doses and increasing adiposity. Conversely, if an obese diabetic dog loses weight, insulin sensitivity will improve and exogenous insulin requirement will decrease.
- Obesity and other causes of insulin resistance have an additive effect on insulin requirements and the risk of progression to clinical diabetes in dogs. Dogs with Cushing syndrome had greater risk for developing diabetes if they were entire females and/or were overweight or obese on initial presentation.[14] The same cumulative insulin resistance occurs in obese female dogs in diestrus.[40]

SUMMARY

- CUSHING SYNDROME is the most common cause of insulin resistance in diabetic dogs in populations where neutering of young dogs is routine.
- Suspicion of underlying Cushing syndrome may be based solely on persistently poor response to high insulin doses not attributable to another cause. There is no requirement to identify multiple indicators; although, the more abnormalities identified, the stronger the suspicion for hypercortisolism.
- The most pronounced effects of Cushing syndrome on glucose metabolism are insulin resistance, excessive postprandial hyperglycemia, perceived short duration of insulin action, and/or substantial within-day and/or day-to-day glycemic variability.
- Strategies to manage excessive glycemic variability can provide immediate clinical benefit for diabetic dogs and minimize the confounding effect of poor diabetic control on the diagnostic investigation for the underlying cause of the insulin resistance.
- Successful strategies include basal insulin monotherapy and combined basal-bolus insulin treatment.
- False-positive and false-negative results commonly occur with diagnostic tests for hypercortisolism in dogs.
- False-positive results are typically associated with stress and/or nonadrenal illness, including diabetes. A practical guideline before testing for hypercortisolism to reduce the likelihood of a false-positive result is to first achieve sufficient control of the diabetes to arrest weight loss.
- Diabetic dogs are more likely to have hypercortisolism than nondiabetic dogs. Therefore, if care is taken to minimize the likelihood of false-positive results as outlined above:
  - The negative predictive value of a diagnostic test for hypercortisolism is decreased compared with nondiabetic dogs.

  - The positive predictive value of a diagnostic test for hypercortisolism is increased compared with nondiabetic dogs.
- The situation where there is no perfectly reliable test for hypercortisolism and there is a possibility of subdiagnostic Cushing syndrome can result in a frustrating situation where hypercortisolism is strongly suspected but diagnosis cannot be confirmed. If clinical signs are severe and negatively affecting the dog's and/or the owner's quality of life, and there is no other likely reason for the dog's clinical signs, then a carefully monitored trilostane treatment trial may be considered.
- DIESTRUS is the most common cause of insulin resistance in diabetic dogs in populations where neutering of young dogs is not routinely recommended.
- Ovariohysterectomy and insulin treatment can achieve diabetic remission in about 10% of cases of diestrus diabetes.
- OBESITY: spontaneously obese dogs compensate for reduced insulin sensitivity with hyperinsulinemia, with the result that glucose tolerance is maintained. There is no evidence that obesity is associated with type 2 diabetes mellitus in dogs.
- Obesity and other causes of insulin resistance have an additive effect on insulin requirements and the risk of progression to clinical diabetes in dogs.

CLINICS CARE POINTS

- Suspicion of underlying Cushing syndrome may be based solely on persistently poor response to high insulin doses not attributable to another cause. There is no requirement to identify multiple indicators, although, the more abnormalities identified, the stronger the suspicion for hypercortisolism.
- Strategies to manage excessive glycemic variability can provide immediate clinical benefit for diabetic dogs and minimize the confounding effect of poor diabetic control on the diagnostic investigation for the underlying cause of the insulin resistance.
- Successful strategies include basal insulin monotherapy and combined basal-bolus insulin treatment.
- False positive and false negative results commonly occur with diagnostic tests for hypercortisolism in dogs.
- The situation where there is no perfectly reliable test for hypercortisolism and there is a possibility of subdiagnostic Cushing syndrome can result in a frustrating situation where hypercortisolism is strongly suspected but diagnosis cannot be confirmed. If clinical signs are severe and negatively affecting the dog's and/or the owner's quality of life, and there is no other likely reason for the dog's clinical signs, then a carefully monitored trilostane treatment trial may be considered.
- Ovariohysterectomy and insulin treatment can achieve diabetic remission in about 10% of cases of diestrus diabetes.

DISCLOSURE

L. Fleeman has received honoraria for educational seminars for MSD Animal Health, Zoetis, Royal Canin, Nestle Purina, and consulting fees from Dechra.

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