Pharmacodynamics and Pharmacokinetics of Insulin Detemir and Insulin Glargine 300
U/ml in Healthy Dogs

Thesis

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By

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Abstract

Insulin glargine 300U/mL and insulin detemir are synthetic long-acting insulin analogs associated with minimal day-to-day variability or episodes of hypoglycemia in people. Here, eight healthy purpose-bred dogs each received 2.4 nmol/kg SQ injections of insulin detemir (0.1U/kg) and insulin glargine 300U/mL (0.4U/kg) on two different days, >1 week apart, in random order. Blood glucose (BG) was measured every 5 minutes and glucose was administered intravenously at a variable rate with the goal of maintaining BG within 10% of baseline BG (“isoglycemic clamp”). Endogenous and exogenous insulin were measured for up to 24 h after insulin injection. The effect of exogenous insulin was defined by glucose infusion rate or a decline in endogenous insulin. Isoglycemic clamps were generated in all eight dogs after detemir but only in four dogs after glargine. Median time to onset of action was delayed with glargine compared to detemir (4.0 h [3.3-5.8 h] versus 0.6 h [0.6-1.2 h], $P = 0.002$). There was no difference in time to peak (median [range] = 6.3 h [5.0-21.3 h] versus 4.3 h [2.9-7.4h], $P = 0.15$) or duration of action (16.3 h [6.1-20.1 h] versus 10.8 h [8.8-14.8 h], $P = 0.21$) between glargine or detemir, respectively. Glargine demonstrated a peakless time-action profile in 4/8 dogs. The total metabolic effect and peak action of detemir was significantly greater than glargine. Significant concentrations of glargine were detected in all but one dog following administration. Glargine might be better suited than detemir as a once-daily insulin formulation in some dogs based on its long duration of action and peakless time-action profile. Day-to-day variability in insulin action should be further assessed for both formulations.

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Fink H, Wennogle S, Davis WL, Von Simpson C, Lappin MR. Field comparison of a collar containing 10% imidacloprid/4.5% flumethrin (Seresto®) and a placebo collar placed on cats. J Feline Med Surg. 2016;18(12):1031-1033

Fields of Study

Major Field: Comparative and Veterinary Medicine

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Chapter 1: Diabetes Mellitus and Dogs

Diabetes mellitus is a syndrome that affects a variety of species and is characterized by hyperglycemia caused by insulin deficiency or decreased target tissue insulin sensitivity. Multiple mechanisms have been proposed that may lead to canine diabetes mellitus, including immune-mediated destruction of pancreatic β -cells, congenital abnormalities, and insulin resistance, though the latter appears to merely contribute to the severity of the disease and is not thought to be a primary pathogenic factor. The overall impact of total insulin deficiency or decreased target cell sensitivity to insulin leads to negative physiologic consequences resulting from abnormalities in metabolism of lipids, carbohydrates, and proteins. The underlying process contributing to insulin deficiency and/or resistance may fluctuate over time with comorbidities, resulting in concurrent fluctuations in degree and magnitude of hyperglycemia [1].

Diabetes mellitus classification in veterinary patients is extrapolated from definitions in human diabetic patients (American Diabetes Association). Diabetes mellitus has been traditionally classified into type I (T1DM) and type II diabetes (T2DM), with T1DM resulting from immune-mediated destruction of pancreatic β -cells, and T2DM resulting from insulin resistance in target tissues. Regardless of the primary underlying mechanism leading to development of DM, a subclinical phase occurs in all patients, in which normal blood glucose is maintained despite abnormalities in either β -cell or target cell function. This subclinical phase is referred to as “prediabetes,” in which hyperglycemia is present, but there is a lack of criteria to diagnose diabetes mellitus. Progression of the disease

results in impaired glucose tolerance, resulting in the continuation of the disease from prediabetes to the overt diabetic state that can be diagnosed based on classic clinicopathologic abnormalities. Characteristic clinicopathologic abnormalities for diagnosis of DM in dogs include persistent hyperglycemia ($BG \geq 144$ mg/dL), glucosuria, and elevated fructosamine [1].

The majority of canine diabetic patients traditionally have been classified as T1DM because they are persistently insulin deficient, do not release c-peptide in response to hormone stimulation, and require life-long insulin supplementation to prevent ketoacidosis. These criteria meet those of type 1 diabetes, but can also be seen in states of glucotoxicity in which normal β -cell mass is present but structural and functional changes have occurred in both β -cells and target tissues that cause persistent hyperglycemia. Glucotoxicity leading to a clinical diabetic state has been shown to occur in the dog [2].

There are multiple theories and possible contributing factors to the development of diabetes mellitus in dogs, including auto-immune destruction, genetic factors, pancreatitis, and hypercortisolism, among others. Conflicting evidence exists for immune-mediated β -cell destruction, as some studies have found a prevalence in up to 50% of dogs with diabetes, and others that found no evidence of autoimmune destruction of the pancreatic islets [2-11]. Serological evidence of auto-reactivity to pancreatic β -cells has been found against specific pancreatic antigens, including IA-2, GAD65, and pro-insulin [2,5,6]. Detection of auto-antibodies is dependent on relative β -cell mass, and

thus may not be detected at end-stages of disease [1]. Certain breeds of dogs are known to have either an increased (Samoyed, Cairn Terrier, Tibetan Terrier) or decreased risk (Boxer, German Shepard, Golden Retriever) of developing diabetes mellitus, indicating a likely contributing hereditary factor in the development of DM [12]. Insulin resistance occurs in obese dogs, however they can compensate appropriately, release more insulin and maintain a normal blood glucose with no evidence currently that insulin resistance is a contributing factor to development of diabetes [13,14].

Chapter 2: Treatment of Diabetes Mellitus in the Canine Patient

Diabetes mellitus occurs commonly in dogs and is controlled with daily insulin administration. Goals of insulin therapy in veterinary patients is aimed at relieving clinical signs of diabetes mellitus (weight loss, polyphagia, polyuria, and polydipsia) and preventing ketosis or hypoglycemia with therapy [15]. Unlike human patients, long-term consequences of diabetes mellitus are not routinely appreciated in canine diabetic patients, and tight control of blood glucose remains controversial. Tighter glycemic control in canine diabetic patients has a greater risk of causing iatrogenic hypoglycemia, which can be a life-threatening consequence of insulin therapy.

Limiting variability in glucose spikes during the day helps maintain control of clinical signs and reduces complications associated with persistent hyperglycemia (poorly controlled diabetes). Diabetic cataracts are reported to occur in up to 68% of dogs with diabetes, and are a result of conversion excess glucose in the eye to sorbitol, which alters lens permeability leading to formation of cataracts [16]. With current treatment strategies, cataract formation seems inevitable and there is no evidence that aiming for tight glycemic control is beneficial in reducing the risk of cataract formation. Uncommon consequences of persistent hyperglycemia due to poorly controlled diabetes include peripheral neuropathy, retinopathy, diabetic dermatopathy, gastric paresis, and diabetic diarrhea [15]. Complications such as glomerulonephropathy, atherosclerosis, and cardiovascular issues have been rarely reported, and their link with diabetes mellitus in canine patients is controversial [17,18].

Multiple insulin types have been utilized in veterinary medicine for canine diabetes with variable pharmacokinetic profiles and with each insulin type having its benefits and weaknesses. Furthermore, it has been shown that within insulin type and preparation, considerable variation exists. This results in difficulty establishing ideal doses and dosing intervals for each patient [19-27]. Specific insulin types are described in more detail in the following chapter.

Dietary therapy for diabetic canine patients should be aimed at preventing or managing obesity with limiting calories, maintaining consistency in diet, and one that is rich in soluble fiber, as it slows glucose absorption from the gut and post-prandial hyperglycemia. Though obesity has not been directly linked with development of canine diabetes mellitus, it contributes to insulin resistance in diabetic canine patients and will be a source of poor control if it is not managed effectively. Generally, dietary therapy affects glycemic control in diabetic dogs, but has a much lesser role compared to insulin therapy [15].

Chapter 3: Insulin Physiology and Pharmacology

Insulin is secreted from the β -cells of the islets of Langerhans in the pancreas. Secretion of insulin is stimulated predominately by increases in blood glucose, however it is also influenced by a variety of other factors including hormones, nutrients, and neuronal inputs. Insulin is secreted by two general phases, either by constant low-level secretion (basal phase) or large bursts (bolus phase) in response to a meal. Basal insulin secretion is necessary for limiting lipolysis and hepatic gluconeogenesis in a fasting state. Bolus insulin secretion following ingestion of carbohydrates prevents hyperglycemia by suppressing hepatic glucose output and stimulating use of glucose by muscle. In addition to insulin, postprandial glucose concentration is determined largely by the nutrient profile of a meal, effects of other hormones (glucagon, incretin hormones) and gastrointestinal transit time. Ideal exogenous insulin therapy mimics both the basal and bolus secretion of insulin [22].

Insulin, as stored in its natural state in the pancreas, tends to crystalize into hexamers in the presence of zinc. As the concentration of zinc decreases following excretion from the pancreas, insulin hexamers are broken down into monomers and dimers enabling entrance of secreted insulin into capillaries. Crystallization is enhanced in older formulations of insulin by the addition of zinc or protamine. Following subcutaneous administration, insulin crystals breakdown, allowing absorption into the bloodstream. There are multiple disadvantages of these formulations, including the likelihood of inaccurate dosing, as it is dependent on adequate re-suspension of crystals prior to

injection, and high variability of de-precipitation at injection site leading to variable pharmacokinetics that are inadequate at mimicking normal insulin physiology [22].

Types of these traditional human recombinant insulin formulations currently used in dogs for treatment of diabetes include protamine zinc insulin 40 U/ml (ProZinc) and neutral protamine hagedorn 100 U/ml (NPH) [22,26]. An additional intermediate-acting insulin used in dogs is Vetsulin®, a purified porcine insulin with an identical amino acid sequence to canine insulin. This formulation is a mixture of ultralente and semilente insulins and is precipitated with zinc to form a suspension and must be shaken vigorously before use [22,27].

Unlike traditional insulin formulations, synthetic insulin analogs are designed to mimic physiologic insulin release and to do so with minimal variability between injections. Human-recombinant insulin analogs have been genetically modified to prevent tendency of crystallization whilst maintaining its ability to activate insulin receptors. These products are available as clear solutions that do not require re-suspension prior to use. Some products, however, still form hexamers in the subcutaneous tissues following injection leading to a degree of variability in pharmacokinetics [22].

Insulin analog preparations can be divided into categories based on duration of action. Rapid-acting insulin analogs include lispro, aspart, and glulisine. These have been designed to mimic the bolus phase of insulin release in people and are used in conjunction with intermediate duration insulin in attempt to replace both phases of

normal insulin physiology [22]. Insulins lispro and aspart have both been evaluated experimentally in dogs, and were found to have similar pharmacokinetics and pharmacodynamics to regular insulin. Insulin lispro caused a nadir in blood glucose about 2 hours post-injection, and had a duration of action longer than 3 hours [28]. Lispro and aspart have both been evaluated and found to be effective as an intravenous treatment for canine diabetic ketoacidosis, however no rationale exists for its use intravenously over regular insulin [29,30]. The modifications made to insulin lispro cause greater dissociation in subcutaneous tissues, allowing for faster absorption into the bloodstream. When this insulin is injected intravenously in people, it has immediate dissociation that is identical to regular insulin [31]. There are no studies assessing the use of glulisine in dogs, or the use of any of the rapid-acting insulin analogs in conjunction with a longer acting insulin for maintenance insulin therapy in dogs with diabetes.

Long-acting insulin analogs have been designed to mimic basal insulin release. Ideal characteristics for these insulins are thus long duration of action, and minimal variability of action after a single injection (ie: peakless profile) and between injections. Currently available long-acting insulin analogs include glargine 100 U/ml, glargine 300 U/ml, detemir, and degludec. Minimal to no data exist on the use of most currently available long-acting insulin analogs in dogs.

Chapter 4: Use of Insulin Glargine and Insulin Detemir in the Canine Patient

Insulin glargine 100 U/ml (Lantus®) was the first human insulin analog, beginning the new revolution of management of diabetes mellitus in people. Following the development of glargine 100 U/ml, insulin detemir (Levemir®) was formulated, and most recently a new formulation of glargine as 300 U/ml (Toujeo®). These human insulin analogs have significantly improved glycemic control in human diabetic patients, by providing more predictable pharmacokinetic and pharmacodynamic profiles, reducing weight gain, and frequency of hypoglycemic events [32-44]. These two insulin formulations (insulin glargine 300 U/ml and insulin detemir) have similar potential in decreasing the frequency of these adverse events in diabetic canine patients. The time action profile of insulin glargine 300 U/ml has not been evaluated in dogs, and there is minimal data on the pharmacodynamics of insulin detemir in dogs [19-21].

Synthetic human insulin analogs have been modified chemically to improve their pharmacological profiles. Insulin glargine results from enzymatic removal of arginine at position A21 with replacement of glycine, and the addition of two arginine residues to the C-terminus of the B chain at position B30. This composition is completely water soluble at a pH of 4, and at physiologic pH forms microprecipitates in the subcutaneous tissues. These microprecipitates allow for slow and relatively predictable release from the tissues [22].

Glargine 100 U/ml (Lantus®) has been evaluated in a total of 22 dogs with naturally occurring diabetes mellitus and in 3 dogs with experimentally (streptozocin) induced diabetes mellitus. These studies indicate that insulin glargine 100 U/ml may be less predictable (greater variability) than NPH, but still is clinically useful as a twice daily insulin formulation in dogs [23-35]. Traditional glargine (Lantus®) is available as 100 U/ml, however new insulin glargine 300 U/ml (Toujeo®) has been evaluated in people and was found to have more predictable pharmacokinetic and pharmacodynamics profiles than glargine 100 U/ml. The metabolically active insulin is identical in both glargine 300 U/ml (Toujeo®) and glargine 100 U/ml (Lantus®), however steady state pharmacokinetic profiles are flatter and more prolonged for insulin glargine 300 U/ml compared to insulin glargine 100 U/ml. The greater concentration of glargine 300 U/ml enables a more gradual and prolonged release from the subcutaneous tissues following injection, resulting in an extended terminal half-life of 17-19 hours in people. Insulin glargine 300 U/ml has been shown to have both lower within-day and day-to-day variability in people [33]. The time action profile of insulin glargine 300 U/ml has not yet been evaluated in dogs.

Insulin detemir is also a human recombinant synthetic insulin. It differs from human insulin in that threonine at position B30 is replaced with a myristic acid residue (14-carbon fatty acid). Prolonged absorption from SQ tissues following injection results from hydrophobic interactions between fatty acids [22]. Reversible binding of albumin to the myristic acid residue allows buffering of insulin detemir in the blood and tissues, which contributes to its predictable effect. In people, insulin detemir provides more predictable

glycemic control among patients and less within-day variability in individual patients compared to insulin glargine 100 U/ml [40-44].

Insulin detemir has been evaluated in both small clinical trials and in experimental setting for assessment of insulin pharmacokinetics. The time-action profile of insulin detemir has only been evaluated in three dogs, and only at doses that are likely too high to be clinically relevant (0.5 U/kg) [20]. Clinical trials using insulin detemir in canine diabetic patients have revealed it to be efficacious as a twice daily insulin choice, but have suggested a possible increased risk of hypoglycemia due to relative high potency of the product compared to other insulin formulations [21].

Chapter 5: Isoglycemic Clamp Method

In veterinary medicine, blood glucose (BG) curves traditionally have been used to evaluate the pharmacodynamics of exogenous insulin. Glucose curves, however, reflect not only the effect of exogenous insulin but also the effect of endogenous insulin and stress hormones. The use of an isoglycemic clamp is different than using a BG curve in that it eliminates the interference of stress hormones that normally complicate the interpretation of a BG curve. Following insulin administration in a BG curve, BG decreases as insulin is absorbed into the blood. As insulin reaches its peak effect, BG should reach the nadir. However, BG reflects the sum of both insulin effect and stress hormones. Therefore, the increase in BG (following BG nadir) may be the result of insulin waning but also of the systemic counter-regulatory response while insulin concentration is high. The isoglycemic clamp eliminates the influence of endogenous hormones on insulin action by maintaining blood glucose constant. As such, this method of evaluating insulin pharmacodynamics is considered best in human medicine [45,46].

Chapter 6: Hypothesis

We hypothesized that after SQ administration of an equivalent molar dose, insulin glargine 300 U/ml (Toujeo®) would have longer duration of action and flatter time-action profile compared with insulin detemir (Levemir®), potentially making it more suitable for use as a once-daily injection in diabetic dogs.

Chapter 7: Materials/Methods

Animals

All animal use was approved by The Ohio State University Institutional Animal Care and Use Committee (Protocol number 2015A00000063). Two castrated Beagle males and 6 spayed mixed hound female dogs were used in this study. Median age was 2 years (range 1-3 years). Median body condition score (assessed by a single investigator [CG]) was 5 out of 9 (range 4 to 7 out of 9). Median body weight was 27 kg (range 10-30 kg). Dogs were housed individually in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All dogs were fed the same commercial dry dog food (Laboratory Canine Diet, CAT#5006, LabDiet®, St. Louis, MO) as recommended by the manufacturer (to maintain body weight), twice daily at 8:00 am and 4:30 pm. Dogs were deemed healthy based on the absence of clinical signs of disease, routine physical exams and a complete blood count, chemistry panel and urinalysis.

Vascular access port and peripheral catheter placement and maintenance

In each dog, a Vascular Access Port (VAPs; CompanionPort, CP 202K, Norfolk Vet Products, Skokie, IL, US) was surgically placed under general anesthesia at least 1 week before beginning the experiment. The vascular access ports were maintained by a weekly 3 ml (500 U/ml) heparin lock injection. On the morning of each experiment, a peripheral intravenous catheter (20 gauge) was placed in a cephalic vein at least 1 hour before the start of the experiment and removed at the end of it.

Pharmacodynamics study

Each dog received two SC injections of insulin: 2.4 nmol/kg of insulin detemir [a] (0.1 U/kg), and 2.4 nmol/kg of insulin glargine [b] 300 U/ml (0.4 U/kg), on two different days, at least 1 week apart (but no more than 4 weeks apart) in random order (based on a coin flip). The dose was rounded up to the nearest one unit. Insulin was injected by a single investigator (CG) on the dorsum, midway between the scapulas. Experiments always began between 8:00 and 10:00 AM on a given day. Dogs were fasted overnight for 15-17 hours before each experiment and remained fasted during the experiment.

All blood samples were collected through the vascular access ports. Cephalic catheters (Surflo, I.V. Catheter, 20 G X 1", Terumo Medical Products, Somerset, NJ) were used exclusively for glucose (dextrose) infusion. For glucose infusion, a 50% dextrose solution (50% Dextrose USP; VET ONE, MWI, Boise, ID, USA) was diluted with saline to a 20% solution. Infusion rate was set on a syringe pump (Medfusion® 3500 syringe infusion pump 4.0 software). Blood glucose concentrations (BG) were measured with a hand-held point-of-care glucometer that was previously validated for use in dogs (Alphatrak 2®, Zoetis Animal Health, range 20–750 mg/dL, intra-assay coefficient of variation [CV] 3.8%).

After insulin injection, BG was maintained (“clamped”) at a target isoglycemic concentration for the duration of the experiment by infusing glucose at a changing rate. Blood glucose concentrations were measured at -25, -15, time zero, and every 5 minutes thereafter and the glucose infusion rate (GIR) was adjusted to achieve the target BG.

Baseline BG was determined by averaging the 3 BG measurements taken before insulin injection. The target BG was defined as $0.9 \times \text{baseline BG} - 1.1 \times \text{baseline BG}$. Glucose infusions started when BG reached the low end of the target BG range. Throughout the clamp, GIR was increased when BG was lower than the lower end of the target BG range and decreased when BG was higher than the target BG range. Adjustments in GIR depended on absolute blood glucose concentration, the rate of change from previous measurement, the degree of deviation from the target range, and the estimated insulin action trajectory based on all the previous rates. The initial GIR, was based on previous experience performing isoglycemic clamps, with additional consideration regarding the time lapse from injection (longer time suggesting slower onset and therefore lower required rate). The clamp was terminated after GIR had been maintained at zero for at least 1 hour. Throughout the clamp, GIR was recorded every 5 minutes and the total amount of glucose infused was recorded at the end of each clamp. The insulin time-action curve was defined as the GIR over time. The graph from each clamp experiment was smoothed by computer software (which calculates a local polynomial regression [in this case of the 4th order] on a series of values [in this case the adjacent 9 neighbors] to determine the smoothed value for each point). Pharmacodynamic parameters were determined based on the smoothed data. The onset of action was defined as the time from insulin injection (time zero) until the low end of the target BG was first reached (i.e. 1st increase in GIR). The end of action was defined as the time from insulin injection until glucose infusion was discontinued. The duration of action was defined as (end of action – onset of action). The peak insulin action was defined as the highest GIR of the smoothed data over the duration of the clamp. Time to peak was defined as the time elapsed from

insulin injection until peak insulin action. The average time action curves for insulin detemir and insulin glargine 300 U/ml were calculated from the raw data and then smoothed. The total metabolic effect was defined as the total amount of glucose (mg/kg) infused throughout the clamp. When BG concentrations did not decrease below the low end of the BG target and glucose infusion was therefore not started, a time-action curve was not generated. In these cases, onset of action and end of action were estimated based on the suppression of endogenous insulin. A decline of endogenous insulin concentration below 10% of baseline was then considered onset of action and the last endogenous insulin concentration that was still below 10% of baseline was considered end of action.

Endogenous and exogenous insulin measurement

Blood samples (1 ml) for insulin measurement were collected in pre-chilled EDTA tubes immediately before insulin injection (time zero) and then hourly during each isoglycemic clamp study until 1 hour after clamp termination. In 4 dogs, after the insulin glargine injection, glucose did not decrease below target ($0.9 \times$ baseline BG) and therefore no glucose was infused. In these 4 instances, blood samples for insulin were collected hourly in the first 12 hours and then at 16, 20 and 24 hours post injection. Blood samples were immediately placed on ice, and remained on ice until the end of the clamp (12-24 hours). Samples were then centrifuged (4°C , 4000 rpm, 15 minutes), plasma was collected, divided into 3 aliquots and stored at -20°C until analysis. Recombinant insulin (detemir or glargine) and canine insulin were measured in all samples using an Iso-Insulin ELISA (Merckodia AB, Uppsala, Sweden) and a canine Insulin ELISA (Merckodia AB, Uppsala, Sweden) kits, respectively. The Canine Insulin ELISA was previously validated by

Mercodia with reported cross-reactivity of insulin detemir and insulin glargine of 0% and 6%, respectively. To measure the cross reactivity of recombinant insulin in the canine ELISA, an equal part mixture of plasma samples collected from all 8 healthy dogs at baseline (prior to insulin injection) with a measured concentration of canine insulin of 28 ng/L was used. Insulin glargine and, separately, insulin detemir (as supplied by the manufacturers) were spiked into the canine plasma mixture and then diluted with adding more of the canine plasma mixture to form expected total insulin concentrations of 4347 ng/L, 1304 ng/L, 434 ng/L and 130 ng/L (equivalent to the iso-insulin standards at 100, 30, 10, and 3 mU/L respectively). All samples were then measured in quadruplicates in the canine insulin ELISA. The cross-reactivity was calculated using the following formula:

$$100 * ([\text{measured insulin}] - [\text{known canine insulin}]) / [\text{expected recombinant insulin}].$$

The cross-reactivity of insulin detemir and insulin glargine were 0% and 7.9%, respectively. For canine insulin, the limit of detection (LoD) = 6 ng/L, intra-assay CV = 3.2%, inter-assay CV = 7.4%.

Iso-insulin ELISA validation

The Mercodia Iso-Insulin ELISA is a solid phase two-site ('sandwich') enzyme immunoassay that is used for measuring the concentrations of insulin analogs. The cross-reactivity of this assay with porcine insulin is 306%. To validate this assay for use in dogs, its cross reactivity with canine insulin was measured as well as the recovery upon dilution of insulin detemir and insulin glargine (separately) in canine plasma. The intra-

assay CV was 4.8% for detemir, 2.6% for glargine. The cross-reactivity of canine insulin in the Iso-Insulin ELISA was assessed by running samples with known canine insulin concentrations in the Iso-Insulin ELISA. These included standards from the canine insulin assay (as supplied for the Mercodia canine insulin assay), as well as 7 samples with known canine insulin concentrations spiked with glargine or insulin detemir as explained above (in the canine insulin ELISA section). The mean cross reactivity of canine insulin in the Iso-Insulin ELISA was 400%. The expected concentrations were compared with mathematically predicted concentrations of detemir and glargine (taking into account the background canine insulin concentration). Based on that, the recovery was 70.0% for insulin glargine and 115% for insulin detemir. Linear regression results for expected versus observed results in serial dilutions for glargine was $R^2 = 0.998$ ($P < 0.0001$), slope = 0.70 and a Y intercept = -0.004 and for detemir was $R^2 = 0.999$ ($P < 0.0001$), slope = 1.15 and a Y intercept = 0.015 (Figure 1).

Figure 1: Linear regression results for expected versus observed optical density (at 450 nm) as measured in the Iso-Insulin ELISA with serial dilutions for insulin glargine 300 U/mL (gray) and insulin detemir (black). For insulin glargine $R^2 = 0.998$ ($P < 0.0001$), slope = 0.70 and a Y intercept = -0.004 and for insulin detemir $R^2 = 0.999$ ($P < 0.0001$), slope = 1.15 and a Y intercept = 0.015.

Calculation of insulin glargine and insulin detemir concentrations

For any given sample, insulin glargine concentrations were calculated from the measured concentrations by solving the following 2 equations in which [G] = glargine concentration, [K9] = canine insulin concentration, [Mi] = measured insulin

concentration in the iso-insulin assay, [Mk9] = measured insulin in the canine assay.

Based on the information above, the cross reactivity of glargine is 70% in the iso-insulin assay and 7.9% in the canine insulin assay and the cross reactivity of canine insulin is 400% in the iso-insulin assay and 100% in the canine insulin assay:

$$\text{Equation 1: } [Mi] = 0.7[G] + 4[K9]$$

$$\text{Equation 2: } [Mk9] = 0.079[G] + 1[K9]$$

$$\text{Equation 2 rearranged: } [Mk9] - 0.079[G] = [K9]$$

Using the term for [K9] from equation 2, equation 1 is revised:

$$[Mi] = 0.7[G] + 4([Mk9] - 0.079[G])$$

$$[Mi] = 0.7[G] + 4[Mk9] - 4 \times 0.079[G]$$

$$[Mi] - 4[Mk9] = 0.384[G]$$

$$[G] = ([Mi] - 4[Mk9])/0.384 = 2.6([Mi] - 4[Mk9])$$

Similarly, for any given sample, insulin detemir concentrations [D] can be calculated given zero cross reactivity of detemir in the canine insulin assay and 115% in the iso-insulin assay:

$$\text{Equation 1: } [Mi] = 1.15[D] + 4[K9]$$

$$\text{Equation 2: } [Mk9] = 0 [D] + 1[K9]$$

Using the term for [K9] from equation 2, equation 1 is revised:

$$[\text{Mi}] = 1.15[\text{D}] + 4[\text{Mk9}]$$

$$[\text{D}] = ([\text{Mi}] - 4[\text{Mk9}]) / 1.15 = 0.87([\text{Mi}] - 4[\text{Mk9}])$$

Chapter 8: Statistical Analysis

Statistical analysis and smoothing of time-action curves were performed using commercially available computer software (GraphPad Prism; GraphPad Software Inc, La Jolla, CA, USA). All data were assessed for normal distribution using the Shapiro-Wilk test before applying parametric and nonparametric analysis where appropriate. Normally distributed data are reported as mean $\pm$ SD and non-normally distributed data are reported as median (range). To assess the quality of the isoglycemic clamps (how well BG was maintained within target range), the coefficient of variation of BG during the clamp procedure was calculated for each procedure and then compared between insulin groups. In addition, for each clamp procedure, mean absolute BG difference was calculated by averaging the absolute difference between measured BG at each 5-min time point and mean BG during the clamp. The mean absolute BG difference was compared between insulin detemir and insulin glargine clamps with a Mann Whitney test. Pharmacodynamic parameters (onset of action, peak action, time to peak action, end of action, duration of action, total metabolic effect) were compared between insulin glargine 300 U/mL and insulin detemir clamps using a Mann-Whitney U test. Data on onset of action and end of action as estimated by suppression of endogenous were not included in the comparisons between insulins glargine 300 U/mL and detemir. Mann-Whitney U test was also used to compare insulins glargine 300 U/ml and insulin detemir in terms of maximum serum concentration and total insulin concentration during the duration of each study. All statistical tests were performed as two-tailed tests and P value ≤ 0.05 was considered significant.

Chapter 9: Results

Pharmacodynamics study

Blood glucose was monitored for up to 24 hours for both insulin detemir and insulin glargine 300 U/ml in all 8 dogs, for a total of 16 isoglycemic clamp studies (Figure 2). The difference in monitoring time was due to the difference in action of the insulin preparations. Glucose infusion rate (GIR) profiles were available for 8 dogs following detemir injection and 4 dogs following glargine 300 U/ml injection. Dextrose infusion (isoglycemic clamp) was initiated in all 8 dogs following SQ administration of insulin detemir and in 4 out of 8 dogs following insulin detemir. Dextrose infusion was not initiated in the other 4 dogs following SQ injection of insulin glargine 300 U/ml because blood glucose did not decrease by 10% below baseline. These four dogs were excluded from comparison of pharmacodynamics between insulin glargine 300 U/ml and insulin detemir.

Baseline values and isoglycemic clamp quality

Comparing insulin detemir and insulin glargine 300 U/ml experiments, there was no difference in baseline BG concentrations (111.3 ± 11.3 mg/dL vs. 105.8 ± 13.0 mg/dL respectively, $P = 0.18$), baseline insulin concentrations (27.0 [0-165.0] ng/L vs 24.5 [6-109] ng/L respectively, $P = 0.7$), BG variability ($CV = 19.3\%$ [8.0-27.6] vs. 15.8% [10.2-19.7], $P = 0.2$) and mean absolute BG difference (6.4 ± 5.8 mg/dL vs. 2.8 ± 2.8 mg/dL, $P = 0.2$). In the 8 insulin glargine procedures, baseline BG was higher in dogs that did not respond appreciably to insulin (110.0 mg/dL [102.0-136.0]) and therefore did not have a

clamp procedure, compared to dogs that did have a clamp procedure (99.0 mg/dL [89.0-101.0], $P = 0.03$).

Pharmacodynamics results

Time-action profiles of each insulin in individual dogs are presented in Figure 2 with median (and interquartile range) time action profile for each insulin formulation presented in Figure 3. Box and whiskers plots of pharmacodynamic parameters for each insulin formulation are presented in Figure 4 and summarized in Table 1.

Figure 2: Exogenous and endogenous insulin concentrations, and glucose infusion rate (GIR) following subcutaneous injection of insulin detemir and insulin glargine 300 U/mL in individual dogs. Each panel of 3 graphs represents one dog. For each dog, the top (insulin detemir – black lines) and bottom (insulin glargine 300 U/mL – gray lines) graphs represent endogenous (dashed line) and exogenous (solid line) insulin measured after insulin detemir or insulin glargine injection. The middle graph represents GIRs for both insulin detemir (black line) and insulin glargine 300 U/mL (gray line). Solid lines represent smoothed GIRs and dotted lines represent raw GIR data. Black (lines, dashes and dots) represents insulin detemir (top and middle), grey (lines, dashes and dots) represents insulin glargine 300U/mL (middle and bottom). Dogs G and H are males, all others are female.

Figure 3: Median and interquartile range of exogenous and endogenous insulin concentrations, glucose infusion rates (GIR) and blood glucose concentrations following subcutaneous injection of insulin detemir and insulin glargine 300 U/mL in 8 dogs. Panels A (insulin detemir – black line) and C (insulin glargine 300 U/mL – gray lines) represent median endogenous (dashed line) and exogenous (solid line) insulin concentrations. Panel B represents median GIRs for both insulin detemir (black line) and (when available) insulin glargine 300 U/mL (gray line). Dashed lines represent the interquartile range for insulin detemir (black) and insulin glargine (gray). Panel D represents blood glucose concentrations after insulin detemir injection (black) and insulin glargine administration (gray).

Figure 4: Box and whiskers plots (median, minimum, maximum and calculated 25th and 75th percentiles) comparing medians of onset of action (OA), peak action (PA), and end of action (EA) and duration of action (DA) of insulin detemir in 8 dogs and insulin glargine 300 U/mL in 4 dogs based on isoglycemic clamps with Mann-Whitney. Insulin glargine is represented in grey, and insulin detemir is represented in black.

Table 1: Pharmacodynamic data based on isoglycemic clamps following subcutaneous injection of insulin detemir (n = 8) and insulin glargine 300 U/mL (n = 4).

Parameter	Statistic	Detemir	Glargine 300 U/ml	P value
Onset of action (min)	Median (range)	35 (35-70)	240 (195-345)	0.002
	Mean ($\pm$ SD)	39.4 (14.7)	255.0 (57.9)	
	CV%	37	23	
Time to peak action (min)	Median (range)	257.5 (175-445)	380 (299-1280)	0.15
	Mean ($\pm$ SD)	281.9 (94.8)	584.8 (402.9)	
	CV%	34	69	
Peak action (mg/kg/min)	Median (range)	17.4 (11-22.7)	7.35 (4.1-10.8)	0.004
	Mean ($\pm$ SD)	16.7 (3.8)	7.4 (2.4)	
	CV%	23	32	
Duration of action (min)	Median (range)	645 (530-890)	980 (365-1205)	0.21
	Mean ($\pm$ SD)	671.3 (129.6)	882.5 (315.9)	
	CV%	19	36	
End of action (min)	Median (range)	690 (515-930)	1260 (630-1400)	0.082
	Mean ($\pm$ SD)	710.6 (135.3)	1137.5 (298.5)	
	CV%	19	26	
Total metabolic effect (mg/kg)	Median (range)	1126.5 (484-1604)	381 (256-928)	0.016
	Mean ($\pm$ SD)	1132.9 (315.6)	485.5 (271.4)	
	CV%	28	55	

The TME (total metabolic effect = total glucose infused) was significantly greater for insulin detemir (1126.5 mg/kg, range 484-1604) compared to insulin glargine 300 U/mL (381 mg/kg, range 256-928; $P = 0.016$; Table 1, Figure 5). The time-action profile of insulin detemir was more consistent amongst dogs than insulin glargine 300 U/mL, as indicated by the lack of detectable effect in 4/8 dogs following subcutaneous administration of insulin glargine and larger coefficients of variation for all PD parameters (except time to onset of action) as compared to insulin detemir (Table 1).

Figure 5: Box and Whiskers plot (median, minimum, maximum, and calculated 25th and 75th percentiles) comparing median total metabolic effect of insulin detemir and insulin glargine 300U/mL based on isoglycemic clamps. Insulin glargine is represented in grey, and insulin detemir is represented in black.

Time to onset of action was significantly shorter for insulin detemir compared to insulin glargine 300 U/mL (Table 1). There was no significant difference in time to peak action, end of action, or duration of action between insulin detemir and insulin glargine 300 U/mL (Table 1).

Endogenous and exogenous insulin measurement

Baseline insulin concentrations (endogenous and exogenous) were calculated by averaging the results of time -15 min and 0 min and are presented in Figure 2 as time zero. Pharmacokinetic profiles of each insulin for individual dogs are presented in Figure 2. Measurable concentrations of insulin detemir and insulin glargine 300 U/mL were detected in 15/16 isoglycemic clamp studies. Dog D had minimal to no detectable insulin glargine (Figure 2). Median time to maximum serum concentration (C_{max}) of insulin detemir was 240 min (range 120-300 min) compared to 570 min (range 300-900 min) for insulin glargine ($P = <0.001$). Median total insulin concentration of detemir (24002.5 ng/L, range 20218-30804) was significantly greater than insulin glargine 300 U/mL (5676 ng/L, range 1.4-19155, $P = <0.001$).

Ten out of 16 experiments had an undetectable amount of exogenous insulin at time zero, and a concurrent rise in exogenous insulin (glargine or detemir) correlated with a decrease in endogenous insulin (Figure 5). Exogenous insulin was measured in six out of 16 experiments at time zero (range 32-697 ng/L). In dog H, a high concentration of exogenous insulin was measured prior to insulin glargine administration at time zero (697 ng/L) and remained increased above pre-injection concentrations throughout 24 h (including at 24 h post injection = 814 ng/L).

In the 4 dogs in which an BG concentrations did not decrease below the low end of the target BG and isoglycemic clamps were therefore not performed, the estimated median (range) onset and end of action of insulin glargine 300 U/mL based on endogenous

insulin suppression were 60 min (120 – 180) and 1080 min (960 – 1440) respectively (Figure 2). Combining the measured (based on GIR) and estimated (based on endogenous insulin suppression) data from all 8 dogs, the median (range) onset and end of action of insulin glargine 300 U/mL were 187.5 min (60 – 345) and 1230 min (630 – 1440) respectively.

Chapter 10: Discussion

Administered at the same molar dosage (2.4 nmol/kg), insulin detemir (Levemir®) provided a more predictable time action profile, and greater total insulin action and concentration over time, compared to insulin glargine 300 U/ml (Toujeo®). Insulin glargine appeared to have a flatter time action profile and had a longer time to onset of action than insulin detemir. Both insulin detemir and insulin glargine 300 U/ml (Toujeo®) were well-tolerated, and not associated with any adverse reactions during the study.

When assessed by the isoglycemic clamp method, insulin glargine had less predictable pharmacodynamics than insulin detemir when administered at the same dosage, given that in four dogs no clamp was generated. However, glargine was detected in the majority of these dogs during a 24-hour study period and did lead to suppression of endogenous insulin for at least 16 hours. Median total concentration of insulin detemir was significantly greater than that of glargine 300 U/mL. This suggests that the relative dose (nmol/kg and U/kg) required to achieve a similar metabolic effect is higher for insulin glargine 300 U/ml compared to insulin detemir. The reason for this is unclear, however studies in people have also consistently found a higher dosage requirement for insulin glargine 300 U/ml compared to insulin glargine 100 U/ml [33,36,37]. In clinical trials in people, the overall glucose lowering effect of insulin glargine 300 U/mL is only 12% of an equivalent U/kg dosage of insulin glargine 100 U/mL [a]. A proposed mechanism behind this difference is increased inactivation of the glargine molecule by tissue

proteases due to the prolonged amount of time insulin glargine 300 U/mL spends in the subcutaneous tissues compared to insulin glargine 100 U/mL. This greater residence time is consistent with the extended absorption and prolonged PD/PK profile of insulin glargine 300 U/mL compared to insulin glargine 100 U/mL observed in human clinical trials [32-38].

The pharmacokinetic and pharmacodynamic data of insulin glargine 300 U/mL in this study suggests its use as a 24-hour (once daily) insulin in dogs. Once daily insulin administration is a desirable feature for pet owners, as it provides a reduced time commitment from the owner and lessens owner compliance issues. The dose of glargine 300 U/ml used in this study was 0.4 U/kg, and 4 dogs did not have a significant change in their BG following subcutaneous administration. All four of the dogs that did not have a significant change in BG had a detectable amount of insulin glargine following subcutaneous injection, as well as suppression of endogenous insulin, suggesting a subclinical dosing in this subset of dogs. Based on these findings, a dose higher than 0.4 U/kg SQ once daily should be investigated for insulin glargine 300 U/mL in dogs for treatment of diabetes mellitus.

An additional contributing factor for the lack of significant change in blood glucose following SQ administration of insulin glargine is its relatively flat time action profile. It is likely that the combination of a relatively low effective dose and flat time-action profile allowed the insulin glargine concentration to remain just low enough to replace endogenous insulin while still be below glucose-lowering threshold in the subset of dogs

in which a glucose infusion was not needed. A higher baseline BG might suggest another potential contributor to perceived exogenous insulin inaction: Subclinical stress. The peakless time-action profile of insulin glargine 300 U/mL is a desirable feature of this insulin in people. This profile mimics basal insulin release, helping to reduce post-prandial hyperglycemia, in addition to limiting lipolysis and hepatic glucose production in a fasting state [32,33,44]. In people, basal insulin is supplemented with prandial insulin, either ultra-short acting insulin administered with meals in Type 1 diabetics or endogenous insulin in Type 2 diabetics. To date, there are no reports of using insulin formulations as a replacement of endogenous basal insulin in diabetic dogs. It is likely that using a basal insulin in dogs would require a paradigm shift in the overall strategy of canine diabetes treatment, including feeding strategies. Similar to people, it may be recommended to administer basal insulin along with ultra-short acting insulin, enabling a once daily injection protocol if a single large meal is fed, instead of dividing the daily caloric intake into two meals, as is currently recommended.

Our results indicate that insulin detemir (Levemir®) had minimal inter-individual variability with regards to peak action, end of action, and duration of action among clinically healthy dogs. Though detemir's time action profile was relatively consistent amongst dogs, its duration of about 11 hours action coupled with a significant peak does not allow for once daily administration without a relatively high risk of hypoglycemia. A previous clinical trial using insulin detemir in canine diabetics has highlighted similar concerns even when used every 12 hours (at the dose used in this study), with 6 episodes of clinical hypoglycemia observed in 4 dogs in a 6-month period in one study [21].

Previous studies have questioned if the higher incidence of hypoglycemic events observed with insulin detemir are secondary to its potency compared to other insulin formulations [21]. The current study eliminated this variable by administering an identical molar dose versus the more widely used, U/kg dosing.

A unit of action of insulin is an equivalency term that relates to the ability of 34.7 µg of pure crystalline insulin to reduce the concentration of blood glucose in a 2 kg fasting rabbit to 45 mg/dl. This unit of action cannot necessarily be extrapolated between species [47]. A pertinent example is insulin detemir: Because of its low potency in people, insulin detemir is formulated at a concentration that is 4 times higher (Levemir®, 2400 nmol/mL, 100U/ml) than most other insulin formulations (for example, Humulin N®, 600 nmol/ml, 100U/ml). This low potency of insulin detemir is likely related to the fact that most detemir molecules are bound in plasma to albumin, allowing only a small fraction to be biologically active [b]. However, unlike in people, the potency of detemir in dogs (and pigs) is not exceptionally lower than that of other insulins. This is thought to be a result of inter-species variation in the percentage of detemir molecules bound to albumin [48]. Thus, unlike in people, the unit action of detemir is approximately 600 nmol/ml, making the commercially available product (Levemir®) seeming 4 times more potent than other formulations. From a clinical perspective, however, it is important to emphasize that high or low potency are not determined by the relationship between concentration and dose equivalency but rather by the relationship between duration of action and peak action. A potent insulin is an insulin that peaks quickly after SQ administration and exert its total effect over a short period of time. In this context and

based on our results, insulin detemir is not a very potent insulin in dogs and should be classified as an intermediate acting formulation [47,b].

The discrepancy between concentration and unit of action also applies to insulin glargine. The concentration of insulin glargine 300 U/mL is 1800 nmol/mL, consistent with being three times more concentrated than the chemically identical insulin glargine 100 U/mL. As mentioned above, in clinical trials in people, the overall glucose lowering effect of insulin glargine 300 U/mL is only 12% of an equivalent U/kg dosage of insulin glargine 100 U/mL [b]. Based on this, insulin glargine 300 U/mL should be considered a 0.12 U/mL (U12) insulin in people despite its high molar concentration. Our results are somewhat consistent with this finding in people: In the four dogs in which a metabolic effect could be measured, the overall metabolic effect of insulin glargine 300 U/mL was about 3-fold lower than that of insulin detemir (Table 1), despite using an identical molar dose. Considering the possibility that the other 4 dogs did not have a significant change in their BG after injection of insulin glargine 300 U/mL because the insulin did not achieve high enough blood concentration, it is likely that the difference in the glucose-lowering effect between the two formulations is greater than 3-fold.

Ten out of 16 clamp experiments had no detectable exogenous insulin, as expected, prior to administration of either insulin detemir or insulin glargine 300 U/ml. Six out of the 16 clamp experiments (in four different dogs) had exogenous insulin (four in the insulin detemir clamp and two in the insulin glargine clamp) measured at time zero, and one dog had a large amount of insulin glargine detected at both time zero and at 24 hours (Dog H,

glargine injection, Figure 2). One possible explanation for this result is a high degree of matrix interference with the Mercodia Iso-Insulin ELISA assay in these four dogs. Matrix interference results from an interaction from one or more of the steps in an immunoassay procedure such that the that the measurable analyte concentration in the sample or antibody binding is altered. Interfering, endogenous substances that are natural, polyreactive antibodies or autoantibodies, together with other unsuspected binding proteins that are unique to the individual, can interfere with the reaction between the analyte and reagent antibodies in an immunoassay [49]. It is possible that plasma from Dog H contained endogenous substances that caused interference in the iso-insulin assay, making interpretation of this data difficult. In contrast, Dog D had pharmacodynamics data available (isoglycemic clamp), but did not have measurable exogenous insulin. Theoretically, that can be caused by excessive stress during the beginning of the clamp causing an elevated baseline blood glucose, leading to the false appearance that exogenous insulin is active when infact blood glucose simply normalizes as the dog calms down. This is unlikely because the baseline BG in this clamp was 99 mg/dl, which was similar to fasting BG in this dog during its other clamp and at other times. Another possible cause for this discrepancy include poor handling of the EDTA samples causing unmeasurable amounts of insulin. The latter is unlikely because the same samples were used for measuring endogenous insulin and yielded appropriate results in this dog.

Limitations of this study include a relatively small sample size and the use of healthy dogs for evaluation of the efficacy of insulin for use in dogs with diabetes mellitus.

Though healthy dogs were used to evaluate the pharmacodynamics and pharmacokinetics

of insulin detemir and insulin glargine 300 U/ml in this study, these characteristics are likely to be mostly consistent when used in dogs with diabetes mellitus. The use of an isoglycemic clamp is different than using a BG curve in that it eliminates the interference of stress hormones that normally complicate the interpretation of a BG curve. Following insulin administration in a BG curve, BG decreases as insulin is absorbed into the blood. As insulin reaches its peak effect, BG should reach the nadir. However, BG reflects the sum of both insulin effect and stress hormones. Therefore, the increase in BG (following BG nadir) may be the result of insulin waning but also of the systemic counter-regulatory response while insulin concentration is high. The isoglycemic clamp eliminates the influence of endogenous hormones on insulin action by maintaining blood glucose constant. As such, this method of evaluating insulin pharmacodynamics is considered the gold-standard in human medicine [45,46]. Ultimately, the difference in pharmacodynamics of insulin between a diabetic and a healthy dog using this method is likely negligible and has been mostly eliminated by this study by evaluating concurrent endogenous insulin. In general, diabetic dogs have a complete lack of endogenous insulin compared to a healthy dog that has a normal amount of insulin. The administration of exogenous insulin to a healthy dog, as seen in this study, results in the complete suppression of endogenous insulin release.

In conclusion, insulin detemir (Levemir®) had smaller inter-patient variability than insulin glargine 300U/ml (Toujeo®), administered at the same dosage. Insulin glargine 300U/ml might be better suited than insulin detemir as a once-daily insulin in some dogs

based on its long duration of action and pharmacokinetic profile. Day-to-day variability in insulin action should still be assessed for both formulations.

References

- 1) Gilor C, Niessen SJM, Furrow E, Dibartola SP. What's in a Name? Classification of Diabetes Mellitus in Veterinary Medicine and Why It Matters. *J Vet Int Med* 2016;30.4:927-40.
- 2) Davison LJ, Herrtage ME, Catchpole B. Autoantibodies to recombinant canine proinsulin in canine diabetic patients. *Res Vet Sci* 2011;91:58–63.
- 3) Hoenig M, Dawe DL. A qualitative assay for beta cell antibodies. Preliminary results in dogs with diabetes mellitus. *Vet Immunol Immunopathol* 1992;32:195–203.
- 4) Alejandro R, Feldman EC, Shienvold FL, Mintz DH. Advances in canine diabetes mellitus research: etiopathology and results of islet transplantation. *J Am Vet Med Assoc* 1988;193:1050–1055.
- 5) Davison LJ, Walding B, Herrtage ME, Catchpole B. Anti-insulin antibodies in diabetic dogs before and after treatment with different insulin preparations. *J Vet Intern Med* 2008;22:1317– 1325.
- 6) Davison LJ, Weenink SM, Christie MR, et al. Autoantibodies to GAD65 and IA-2 in canine diabetes mellitus. *Vet Immunol Immunopathol* 2008;126:83–90.
- 7) Gepts W, Toussaint D. Spontaneous diabetes in dogs and cats. A pathological study. *Diabetologia* 1967;3:249–265.
- 8) Haines DM. A re-examination of islet cell cytoplasmic antibodies in diabetic dogs. *Vet Immunol Immunopathol* 1986;11:225– 233.
- 9) Ling GV, Lowenstine LJ, Pulley LT, Kaneko JJ. Diabetes mellitus in dogs: a review of initial evaluation, immediate and long-term management, and outcome. *J Am Vet Med Assoc* 1977;170:521–530.
- 10) Ahlgren KM, Fall T, Landegren N, et al. Lack of evidence for a role of islet autoimmunity in the aetiology of canine diabetes mellitus. *PLoS ONE* 2014;9
- 11) Shields EJ, Lam CJ, Cox AR, et al. Extreme beta-cell deficiency in pancreata of dogs with canine diabetes. *PLoS ONE* 2015;10
- 12) Catchpole B, Kennedy LJ, Davison LJ, Ollier WE. Canine diabetes mellitus: from phenotype to genotype. *J Small Anim Pract* 2008;49:4–10.

- 13) Verkest KR, Rand JS, Fleeman LM, Morton JM. Spontaneously obese dogs exhibit greater postprandial glucose, triglyceride, and insulin concentrations than lean dogs. *Domest Anim Endocrinol* 2012; 42:103–112.
- 14) Davison, LJ. Diabetes Mellitus and Pancreatitis - Cause or Effect? *J Small Anim Pract* 2015; 56-1: 50–59.
- 15) Ettinger, SJ, et al. *Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat*. 7th ed., Elsevier, 2010:1785–1796.
- 16) Basher, Anthony W, and Steven M. Roberts. Ocular Manifestations of Diabetes Mellitus: Diabetic Cataracts in Dogs. *Vet Clin N Am: Small Animal Practice* 1995; 25.3:661–676.
- 17) Marynissen SJJ, et al. Long-Term Follow-up of Renal Function Assessing Serum Cystatin C in Dogs with Diabetes Mellitus or Hyperadrenocorticism. *Vet Clin Path* 2016; 45.2:320–329.
- 18) Verkest KR. Is the Metabolic Syndrome a Useful Clinical Concept in Dogs? A Review of the Evidence. *The Vet J* 2014; 199.1:24–30.
- 19) Mori A, Kurishima M, Oda H, Saeki K, Arai T, Sako T. Comparison of Glucose Fluctuations between Day and Night-Time Measured Using a Continuous Glucose Monitoring System in Diabetic Dogs. *J Vet Med Sci* 2013;75.1:113-17.
- 20) Sako T, Mori A, Lee P, Oda H, Saeki K, Miki Y, Kurishima M, Mimura K, Nozawa S, Mizutani H, Makino Y, Ishioka K, Arai T. Time-action Profiles of Insulin Detemir in Normal and Diabetic Dogs. *Res Vet Sci* 2011;90.3:396-403.
- 21) Fracassi F, Corradini S, Hafner M, Boretti FS, Sieber-Ruckstuhl NS, Reusch CE. Detemir Insulin for the Treatment of Diabetes Mellitus in Dogs. *J Am Vet Med Assoc* 2015;247.1:73-78.
- 22) Gilor C, Graves TK. Synthetic insulin analogs and their use in dogs and cats. *Vet Clin North Am Small Anim Pract* 2010;40.2:297-307.
- 23) Hess R, et al. Glargine Insulin for Treatment of Naturally Occurring Diabetes Mellitus in Dogs. *J Am Vet Med Assoc* 2013;243.8:1154-161.
- 24) Fracassi F, Boretti FS, Sieber-Ruckstuhl NS, Reusch CE. Use of Insulin Glargine in Dogs with Diabetes Mellitus. *Vet Rec* 2011;170.2:52.
- 25) Mori A, Sako T, Lee P, Motoike T, Iwase K, Kanaya Y, Fukuta H, Mizutani H, Arai T. Comparison of Time-action Profiles of Insulin Glargine and NPH Insulin in Normal and Diabetic Dogs. *Vet Res Commun* 2008;32.7:563-73.

- 26) Maggiore AD, et al. Efficacy of Protamine Zinc Recombinant Human Insulin for Controlling Hyperglycemia in Dogs with Diabetes Mellitus. *J Vet Int Med* 2011; 26.1:109–115.
- 27) Monroe WE, et al. Efficacy and Safety of a Purified Porcine Insulin Zinc Suspension for Managing Diabetes Mellitus in Dogs. *J Vet Int Med* 2005;19.5:675–682.
- 28) Matsuo Y, Shimoda S, Sakakida M, et al. Strict glycemic control in diabetic dogs with closed-loop intraperitoneal insulin infusion algorithm designed for an artificial endocrine pancreas. *J Artif Organs* 2003;6:55–63.
- 29) Sears KW, Drobatz KJ, Hess RS. Use of lispro insulin for treatment of dogs with diabetic ketoacidosis [abstract]. *J Vet Intern Med* 2009;23(3):696.
- 30) Walsh ES, Drobatz KJ, Hess RS. Use of intravenous insulin aspart for treatment of naturally occurring diabetic ketoacidosis in dogs. *J Vet Emergency and Critical Care* 2016;26:101-107
- 31) Horvath K, Bock G, Regittnig W, et al. Insulin glulisine, insulin lispro, and regular human insulin show comparable end-organ metabolic effects: an exploratory study. *Diabetes Obes Metab* 2008;10:484–91.
- 32) Becker RHA, Nowotny I, Teichert L, Bergmann K, Kapitza C. Low within- and between-day variability in exposure to new insulin glargine 300 U/ml. *Diabetes Obes Metab* 2015;17.3:261-267
- 33) Ritzel, R, Roussel R, Bolli GB, Vinet L, Brulle-Wohlhueter C, Glezer S, Yki-Jarvinen H. Patient-level Meta-analysis of the EDITION 1, 2 and 3 Studies: Glycaemic Control and Hypoglycaemia with New Insulin Glargine 300 U/ml versus Glargine 100 U/ml in People with Type 2 Diabetes. *Diabetes Obes Metab* 2015;17.9:859-67.
- 34) Steinstraesser A, Schmidt R, Bergmann K, Dahmen R, Becker RHA. Investigational New Insulin Glargine 300 U/ml Has the Same Metabolism as Insulin Glargine 100 U/ml. *Diabetes Obes Metab* 2014;16.9:873-76.
- 35) Yki-Järvinen H, Bergenstal RM, Bolli GB, Ziemien M, Wardecki M, Muehlen-Bartmer I, Maroccia M, Riddle MC. Glycaemic Control and Hypoglycaemia with New Insulin Glargine 300 U/ml versus Insulin Glargine 100 U/ml in People with Type 2 Diabetes Using Basal Insulin and Oral Antihyperglycaemic Drugs: The EDITION 2 Randomized 12-month Trial including 6-month Extension. *Diabetes Obes Metab* 2015.

- 36) Riddle, MC, Yki-Järvinen H, Bolli GB, Ziemien M, Muehlen-Bartmer I, Cissokho S, Home PD. One-year Sustained Glycaemic Control and Less Hypoglycaemia with New Insulin Glargine 300 U/ml Compared with 100 U/ml in People with Type 2 Diabetes Using Basal plus Meal-time Insulin: The EDITION 1 12-month Randomized Trial, including 6-month Extension. *Diabetes Obes Metab* 2015;17.9: 835-42.
- 37) Goldman J, White JR. New Insulin Glargine 300 U/mL for the Treatment of Type 1 and Type 2 Diabetes Mellitus. *Annals of Pharmacotherapy* 2015;49.10:1153-161.
- 38) Shiramoto M, et al. Single-dose New Insulin Glargine 300 U/ml Provides Prolonged, Stable Glycaemic Control in Japanese and European People with Type 1 Diabetes. *Diabetes Obes Metab* 2014;17.3:254-60.
- 39) Monami, M, Marchionni N, Mannucci E. Long-acting Insulin Analogues vs. NPH Human Insulin in Type 1 Diabetes. A Meta-analysis. *Diabetes, Obes Metab* 2009;11.4: 372-78.
- 40) Koehler G, Treiber G, Wutte A, Korsatko S, Mader JK, Semlitsch B, Pieber TR. Pharmacodynamics of the Long-acting Insulin Analogues Detemir and Glargine following Single-doses and under Steady-state Conditions in Patients with Type 1 Diabetes. *Diabetes Obes Metab* 2013;16.1:57-62.
- 41) Fakhoury W, Lockhart I, Kotchie RW, Aagren M, LeReun C. Indirect Comparison of Once Daily Insulin Detemir and Glargine in Reducing Weight Gain and Hypoglycaemic Episodes When Administered in Addition to Conventional Oral Anti-Diabetic Therapy in Patients with Type-2 Diabetes. *Pharmacology* 2008;82.2:156-63.
- 42) Danne T, Datz N, Endahl L, Haahr H, Nestoris C, Westergaard L, Fjording MS, Kordnouri O. Insulin Detemir Is Characterized by a More Reproducible Pharmacokinetic Profile than Insulin Glargine in Children and Adolescents with Type 1 Diabetes: Results from a Randomized, Double-blind, Controlled Trial. *Pediatric Diabetes* 2008;9.6:554-60.
- 43) Heise T, Losek L, Ronn BB, Endahl L, Heinemann L, Kapitza C, Draeger E. Lower Within-Subject Variability of Insulin Detemir in Comparison to NPH Insulin and Insulin Glargine in People With Type 1 Diabetes. *Diabetes. American Diabetes Association* 2004.
- 44) Moore MC, Smith MS, Turney MK, Boysen S, Williams PE. Comparison of Insulins Detemir and Glargine: Effects on Glucose Disposal, Hepatic Glucose Release and the Central Nervous System. *Diabetes Obes Metab* 2011;13.9:832-40.

- 45) Heise T, Pieber TR. Towards Peakless, Reproducible and Long-acting Insulins. An Assessment of the Basal Analogues Based on Isoglycaemic Clamp Studies. *Diabetes Obes Metab* 2007;9.5:648-59.
- 46) Heinemann L, Anderson JH. Measurement of Insulin Absorption and Insulin Action. *Diabetes Tech & Therapeutics* 2004;6.5:698-718.
- 47) Stewart GA. Historical Review of the Analytical Control of Insulin. *The Analyst* 1974;1185:913.
- 48) Havelund S, Plum A, Ribel U, Jonassen I, Volund A, Markussen J, Kurtzhals P. The Mechanism of Protraction of Insulin Detemir, a Long-Acting, Acylated Analog of Human Insulin. *Pharmaceutical Research* 2004;21.8.
- 49) Klee G. Interferences in hormone immunoassays. *Clin Lab Med* 2004;24.1:1-18.