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Time-action profiles of insulin detemir in normal and diabetic dogs

T. Sako^a, A. Mori^{a,*}, P. Lee^b, H. Oda^a, K. Saeki^a, Y. Miki^a, M. Kurishima^a, K. Mimura^a, S. Nozawa^a, H. Mizutani^b, Y. Makino^a, K. Ishioka^a, T. Arai^b^a Department of Veterinary Nursing and Technology, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino, Tokyo 180-8602, Japan^b Department of Veterinary Science, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino, Tokyo 180-8602, Japan

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ABSTRACT

Insulin detemir is the first member of a new class of long-acting soluble insulin analogues capable of maintaining the basal level of insulin in humans. In this preliminary study, we investigated the time-action profiles of insulin detemir in normal and diabetic dogs since the use of insulin detemir in canines has yet to be determined. Eight animals were used in our study (three normal and five insulin dependent diabetic dogs). Time-action profiles of insulin detemir were monitored in normal dogs using an artificial pancreas apparatus under euglycemic condition. Blood sampling was performed at 2 h intervals post feeding, with insulin administration, in insulin dependent diabetic dogs. Time-action profiles of insulin detemir, in normal dogs, demonstrated that insulin detemir is a long-lasting preparation similar to what has been observed in humans. A pronounced peak was detected at 8–10 h while the glucose-lowering effect lasted for over 24 h after insulin injection, thus illustrating its longer prolonged peak activity time. Furthermore, intensive glycemic control was achieved with insulin detemir in insulin dependent diabetic dogs, using a lower dosage than NPH insulin and insulin glargine therapeutic doses. Our results indicate that insulin detemir has a greater effect than either NPH insulin or insulin glargine in canines, requiring a lower dose than either insulin preparation. However, using insulin detemir also carries a higher risk of inducing hypoglycemia as compared to either NPH insulin or insulin glargine.

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1. Introduction

Diabetes mellitus (DM) is a commonly encountered endocrine disease with increasing prevalence affecting dogs (Feldman and Nelson, 2004). Insulin dependent type of diabetes mainly affects dogs and insulin injections are commonly used as an effective treatment for long-term glycemic control (Feldman and Nelson, 2004). Thus far, Neutral Protamine Hagedorn (NPH) insulin and insulin glargine are two injectable insulin preparations commonly used with diabetic dogs (Behrend, 2006; Feldman and Nelson, 2004; Mori et al., 2008). Alternatively, insulin detemir [Lys^{B29}(N^c-tetradecanoyl) des(B30) human insulin] is the first of a new class of long-acting soluble insulin analogues. Its prolonged duration of action is attributable to a combination of increased self-association (hexamer stabilization and hexamer-hexamer interaction) and albumin binding due to acylation of the amino acid lysine in position B29 with a ¹⁴C fatty acid (myristic acid). Insulin detemir is highly albumin bound in the interstitial fluid and in plasma, and has been shown to elicit a protracted metabolic action, with a slow onset of action and a less pronounced peak of action compared with that observed

for NPH insulin in humans (Heinemann et al., 1999; Kurtzhals et al., 1997). Absorption of insulin detemir, which is presented as a clear neutral solution (Kurtzhals et al., 1997), is dependent on neither appropriate resuspension before injection and dissolution of crystals in the subcutaneous tissue, as is the case for NPH insulin, nor on formation and dissolution of microprecipitates, as is the case for insulin glargine, making it easier to handle and administer.

Previously, our laboratory demonstrated a clear difference between NPH insulin and insulin glargine by examining time-action profiles for both insulin preparations in normal dogs. Our results confirmed that NPH insulin is an intermediate-acting preparation whereas insulin glargine is a long-lasting preparation in canines which was similar to what has been observed previously in humans (Mori et al., 2008). Moreover, NPH insulin has an approximate time of onset of 1 h and a 12 h duration of action period with a peak of 4–6 h in normal dogs. Alternatively, insulin glargine is a developed, long-acting, human synthetic basal insulin analogue. It has an over 24 h duration of action period with a peak of 6–10 h in normal dogs, thus it mimics the basal insulin secretion levels of normal pancreatic β -cells, as is the case with humans. In addition, we determined that co-administration of NPH insulin and insulin glargine results in intensive glycemic control in diabetic dogs (Mori et al., 2008). Insulin detemir was designed to

* Corresponding author. Tel.: +81 422314151; fax: +81 422317841.

E-mail addresses: amori@nvl.u.ac.jp, amori1981@gmail.com (A. Mori).

provide a basal or background level insulin concentration, with the intention that a short-duration acting insulin be administered at meal times to achieve optimal glycemic control than either insulin preparation alone (Heise et al., 2004; Pieber et al., 2007). Time-action profiles for insulin detemir have been previously documented for humans (Heise and Pieber, 2007), however these time-action profiles are unclear in dogs. While NPH insulin and insulin glargine have been used in dogs, insulin detemir has not. Therefore, the aim of this study was twofold. First, we wanted to determine the time-action profile of subcutaneously injected insulin detemir in normal dogs, since it has never been previously done. Second, we sought to investigate the glucose-lowering effect of insulin detemir by comparing it against the two most commonly used insulin preparations, NPH insulin and insulin glargine, in insulin dependent diabetic dogs.

2. Materials and methods

2.1. Animals

In the present study, eight dogs maintained in our laboratory, were split into two groups and used. Three spayed female beagles (6–8 years old) were grouped into the normal control group, while three streptozotocin experimentally induced (Mori et al., 2008) diabetic dogs (male beagles, 6–8 years old) and two juvenile onset diabetic dogs (4 year old castrated male miniature dachshund and 3 year old female miniature schnauzer) represented the diabetic group (Table 1). All dogs were fed on a commercial diet (Select Protein, Royal canine Japon, Tokyo, Japan) twice a day (8 am and 8 pm), and caloric intake was set at $1/2 \times 2.0 \times \text{RER} (\text{BW}^{0.75} \times 70)$ for each feeding period, whereby RER means a resting energy requirement and BW means body weight, for the normal control and diabetic dog groups.

All diabetic dogs had confirmed diabetes mellitus for over 3 years and were defined as DM positive with clinical signs (polyuria and polydipsia) and documentation of persistent fasting hyperglycemia (over 250 mg/dL) and glucosuria. Before use in our study, diabetic dogs were treated with insulin detemir injections twice daily (8:10 am and pm), to maintain serum GA percentages at 13–17% (Sako et al., 2008, 2009) while being maintained in our laboratory. Approval for this work has been given by the Nippon Veterinary and Life Science University Animal Research Committee.

2.2. Time-action profiles of insulin detemir in normal control dogs

For this portion of the study, three normal control dogs were utilized. All animals underwent overnight fasting prior to use. On the subsequent day, a catheter (20GX1" BD Insyte Autoguard Shielded IV Catheter, Becton Dickinson, Tokyo, Japan) was inserted

into the cephalic vein for blood sampling, and connected to an artificial pancreas apparatus (Nikkisio STG-22 artificial pancreas apparatus, Nikkiso, Tokyo, Japan) by coupling the other end to a tube (Nikkisio FS-D2G blood sampling, Tokyo, Japan). Blood glucose concentration was monitored by glucose-oxidase methods using the Nikkisio artificial pancreas apparatus. Subsequently, catheter (22GX1" BD Insyte Autoguard Shielded IV Catheter, Becton Dickinson, Tokyo, Japan) was inserted into another cephalic vein for injection of glucose with physiological saline. The catheter was connected to the Nikkiso artificial pancreas apparatus by coupling to an injecting tube (Nikkisio FS-F3 injecting tube, Nikkiso, Tokyo, Japan). The artificial pancreas apparatus was set to maintain blood glucose concentration at a normal range of 68–90 mg/dL after insulin injections. In this study, intravenous insulin injection using the artificial pancreas apparatus was not required, since insulin detemir was subcutaneously administered. After connecting the artificial pancreas apparatus, each dog was given 30 min to settle down while blood glucose concentrations were stabilized between 68 and 90 mg/dL. Then each dog received a single subcutaneous injection (0.5 IU/kg) of insulin detemir (Levemir, Novo Nordisk, Tokyo, Japan) under euglycemic glucose clamp conditions. Glucose infusion rates (GIR) were recorded for up to 24 h, GIR (mg/kg/min) means the rate of administered glucose (mg) per body weight (kg) per minute.

2.3. NPH insulin, insulin glargine and insulin detemir comparison in diabetic dogs

Before proceeding with this portion of this study, we needed to determine the optimal insulin doses for the various insulin preparations for each diabetic dog. We therefore implemented a pre-clinical study before beginning this portion of our study. The pre-clinical study lasted for 15 days with 5 days being given for each insulin preparation. Two hour interval blood sampling was performed to determine the appropriate dose of each insulin preparation with each diabetic dog. The dose of insulin preparation was determined to be ideal if it could meet the following conditions: (1) does not induce clinical hypoglycemia, and (2) maintains blood glucose concentration to <300 mg/dL. As such, optimal dose of each insulin preparation is in accordance with dose required for optimal glycemic control on an individual dog basis.

This comparative study portion of the our study employed five insulin dependent diabetic dogs, and was carried out in three phases with each phase lasting for up to 7 days continuously. In the first phase (Days 1–7), diabetic dogs were tested with varying amounts of NPH insulin (0.41–0.63 IU/kg) provided as a single injection twice daily at a 12 h interval (8:10 am and pm). It has been reported that the normal recommended dose of NPH for diabetic doses ranges between 0.4 and 0.7 IU/kg twice a day (Mori et al., 2008). In the second phase (Days 8–14), varying amounts of insulin glargine (0.34–0.54 IU/kg) were administered as a single

Table 1
Profiles of the insulin dependent diabetic dogs used in this study.

	Breeds	Type of diabetes	Sex	Years	Body weight (kg)	NPH insulin (IU/kg)	Insulin glargine (IU/kg)	Insulin detemir (IU/kg)
Dog 1	Beagle	Experimentally induced	Male	6	13.1	0.61	0.46	0.23
Dog 2	Beagle	Experimentally induced	Male	6	13.5	0.59	0.48	0.19
Dog 3	Beagle	Experimentally induced	Male	8	11.3	0.49	0.40	0.09
Dog 4	Miniature dachshund	Juvenile onset	Castrated male	4	5.6	0.63	0.54	0.18
Dog 5	Miniature schnauzer	Juvenile onset	Female	3	7.3	0.41	0.34	0.07

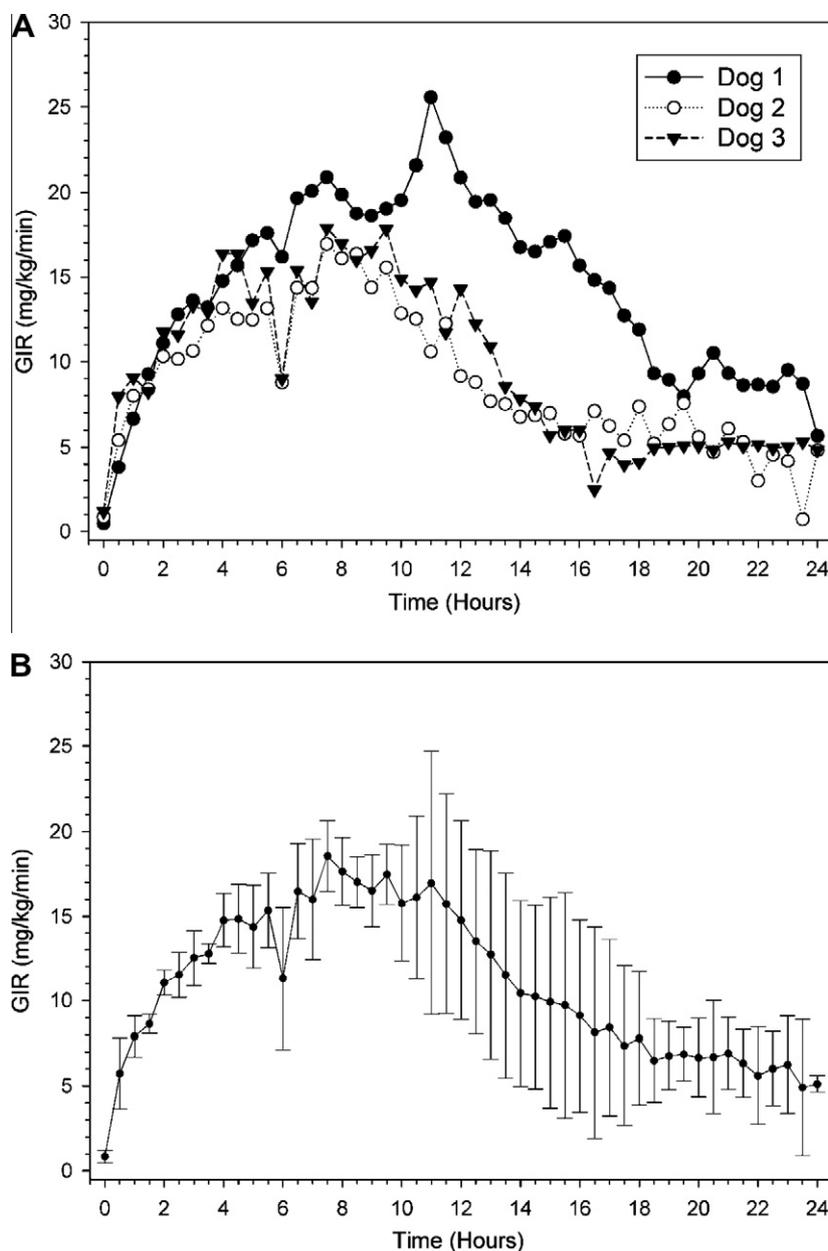


Fig. 1. Time-action profile of insulin detemir over a 24 h period in three individual (A) and mean \pm SD ($n = 3$) (B) results in normal control dogs. Results are presented as GIR (Glucose Infusion Rate) over time being treated with either 0.5 IU/kg insulin detemir. Higher values of GIR indicate stronger insulin action.

injection twice daily at a 12 h interval (8:10 am and pm). The glargine IU was approximately 20% lower than standalone NPH IU administered in the first phase of the study. In the third and final phase (Days 15–21), varying insulin detemir amounts (0.09–0.23 IU/kg) were injected twice daily at a 12 h interval (8:10 am and pm). Insulin detemir IU was approximately 73% lower as compared to standalone NPH IU administered in the first phase of the study. Throughout the three phases, all dogs were fed on a commercial diet (Select Protein, Royal canine Japon, Tokyo, Japan) twice daily (8 am 8 pm), and caloric intake was set at $1/2 \times 2.0 \times \text{RER} (\text{BW}^{0.75} \times 70)$ for each feeding period.

2.4. Assaying serum glucose concentration

Blood (1 ml) was obtained on day 7, 14 and 21 from the jugular vein prior to and 2, 4, 8, 10, 12 h after feeding, with insulin injections. Blood samples were transferred to 5 ml polypropylene tubes

and allowed to clot for up to 30 min. at room temperature. Subsequently, the blood samples were centrifuged (1700g) for 10 min at 4 °C to obtain serum which was immediately stored at –80 °C until further use. Serum glucose concentrations were measured by an enzymatic method using a commercially available kit (Glucose C2 test-WAKO, Wako Pure Chemical industries, Tokyo, Japan).

2.5. Statistics

For statistical analysis, values were expressed as mean \pm standard deviation (SD). Statistical significance was determined by Kruskal–Wallis one-way analysis of variance by ranks (Sigmapast analysis software Version 3.5, Build, 3.5.0.54, Systat Software Inc., San Jose, CA. USA). The significance level was set at $p < 0.05$. Total area under the curve (AUC) during 0–24 h represents Glucose Infusion Values (mg/kg) and was calculated by the trapezoidal rule.

Table 2

Pharmacodynamics of GIR (Glucose Infusion Rate) AUC (Area Under Curve) in 2 h intervals and total (0–24 h) time for normal dogs after insulin injection.

	Insulin detemir	
AUC _{0–2h}	846	96
AUC _{2–4h}	1490	131
AUC _{4–6h}	1728	252
AUC _{6–8h}	1964	343
AUC _{8–10h}	2031	234
AUC _{10–12h}	1921	702
AUC _{12–14h}	1510	694
AUC _{14–16h}	1190	714
AUC _{16–18h}	971	620
AUC _{18–20h}	821	261
AUC _{20–22h}	782	296
AUC _{22–24h}	676	314
AUC _{0–24h}	15,930	4340

Values represent mean \pm standard deviation of glucose infusion amount (mg/kg) to maintain targeting blood glucose concentrations post insulin injections, and are representative of three dogs ($n = 3$). Higher values indicate stronger insulin action.

3. Results

3.1. Time-action profiles of insulin detemir in normal dogs

Since insulin detemir has never been utilized in canines, there is a requirement to determine whether its action profile is similar to that in humans. Insulin detemir preparation was utilized at 0.5 IU/kg administered subcutaneously as a preliminary assessment in normal control dogs. Time-action profiles for individual animals are presented in Fig. 1A to demonstrate the variability of the insulin detemir preparations, and as a group in Fig. 1B. Half hourly interval GIR means \pm SD were plotted whereas Table 2 shows the GIR AUC results in 2 h intervals and 24 h basis after insulin injection. After subcutaneous injection of insulin detemir, GIR demonstrated significant changes ($p < 0.05$, Kruskal–Wallis test). A pronounced peak was detected at 8–10 h post injection of insulin detemir illustrating its prolonged and extended peak activity time. Furthermore, the insulin action effect was persistent for over 24 (Table 2). As such, our results clearly demonstrate that insulin detemir is a long-acting insulin preparation, and exhibits a similar action profile in canines as it does in humans.

3.2. Efficacy comparison of NPH insulin, insulin glargine and insulin detemir on post-prandial serum glucose concentration in diabetic dogs

Since the insulin detemir time-action profiles exhibited a positive effect in normal control dogs, we were interested in evaluating insulin detemir efficacy in treating insulin dependent diabetic dogs as compared to NPH insulin and insulin glargine, two treatments commonly used with diabetic dogs. Since insulin preparation amounts varied in accordance with glycemic control for each respective animal, a comparison between insulin treatments overall cannot be done since no standardized amount was used between all three treatments. However, for each respective animal, it is possible to compare the efficacy between treatments using ideal dosages on an individual basis. As such, post-prandial serum glucose concentration results of the five individual dogs undergoing all three different insulin preparations at optimal doses for each individual animal are presented in Fig. 2. An insulin preparation summary for NPH insulin, insulin glargine, and insulin detemir is presented in Fig. 3 to demonstrate the mode of action trends of each preparation.

Looking at the first dog (Fig. 2), when NPH insulin (0.61 IU/kg) was used, glucose concentration increased from 106 to 301 mg/dl after 4 h and decreased back down to 178 mg/dl by 12 h post injection.

In comparison, insulin glargine (0.46 IU/kg) resulted in a starting glucose concentration of 99 mg/dl, increasing up to 445 mg/dl by 4 h, and subsequently decreasing back down to 93 mg/dl by 12 h post injection. Meanwhile, with insulin detemir (0.23 IU/kg), glucose concentration decreased from 54 to 27 mg/dl as quick as 2 h, before peaking up to 191 mg/dl by 8 h post injection, and gradually decreasing to 98 mg/dl after 12 h post injection. When average glucose concentrations were determined and compared within the 12 h period for all three different insulin treatments, insulin detemir induced the lowest glucose concentration, at 108.92 mg/dl. NPH was in between at 218.42 mg/dl, and insulin glargine produced the highest value at 279.17 mg/dl. Therefore, insulin detemir was the best treatment for glycemic control with the first dog.

Looking at the second dog, NPH insulin (0.59 IU/kg) controlled glucose level maintaining it <200 mg/dl (55–155 mg/dL) throughout the whole 12 h monitoring period. Alternatively, insulin glargine's (0.48 IU/kg) effect on glucose level started at 44 mg/dl and was stabilized at 38–59 mg/dl for up to 10 h before rising up to 124 mg/dL at 12 h post injection. Insulin detemir's (0.19 IU/kg) glucose level started off at 57 mg/dL and increased to 112 mg/dL by 2 h subsequently decreasing to 24 mg/dl at 6 h post injection. Thereafter, glucose concentration gradually increased up to 63 mg/dl by 12 h post injection. When average glucose concentrations resulting from all three different insulin treatments were compared, insulin detemir was lowest at 54.87 mg/dl, with insulin glargine being very similar at 57.50 mg/dl and NPH being highest at 94.50 mg/dl. So, NPH appeared to be the best treatment for glycemic control with dog 2 since insulin detemir and insulin glargine brought about hypoglycemic conditions.

With the third dog, all three insulin preparations resulted in similar glucose concentration curves from 2 h and on post injection. NPH insulin (0.49 IU/kg), insulin detemir (0.09 IU/kg), and insulin glargine (0.40 IU/kg) consistently maintained glucose level at 67–186, 59–149 mg/dl, and to 55–176 mg/dl throughout the whole 12 h monitoring period. Since similar glucose curves were observed between the three treatments, average glucose levels were also similar with insulin glargine being lowest at 108.83 mg/dl, and NPH and insulin detemir being very similar at 114.0 and 114.25 mg/dl, respectively. Hence, all three insulin treatments were equally as effective in dog 3.

The fourth and fifth dogs responded in a similar manner to the three different insulin preparations. NPH insulin (0.63 IU/kg-dog 4, 0.41 IU/kg-dog 5) maintained consistent glucose levels at 54–160 and 72–175 mg/dL in dog 4 and 5, respectively throughout the whole 12 h monitoring period. Insulin detemir (0.18 IU/kg-dog 4, 0.07 IU/kg-dog 5) also stabilized glucose levels to 50–200 and 77–182 mg/dl in dog 4 and 5, respectively throughout the whole 12 h monitoring period. In contrast to NPH and insulin detemir, insulin glargine's glucose curve was different. With dog 4, insulin glargine's (0.54 IU/kg) glucose level started at 45 mg/dl, increasing up to 251 mg/dl within 2 h, remaining there for up to 4 h post injection, thereafter gradually decreasing to 45 mg/dl at 8 h and maintained there up to 12 h post injection. With dog 5, insulin glargine's (0.41 IU/kg) glucose level started off at 52 mg/dl, increasing up to 291 mg/dl by 4 h post injection, then subsequently decreasing to 35 mg/dl by 10 h, before rising back up 66 mg/dl at 12 h post injection. Average glucose concentrations were similar between NPH and insulin detemir, with insulin glargine being greatest in both animals. In dog 4, NPH was 101 mg/dl, insulin detemir was 94.58 mg/dl and insulin glargine was 141.5 mg/dl. In dog 5, NPH was 109.58 mg/dl, insulin detemir was 104.75 mg/dl, and insulin glargine was 170.83 mg/dl.

Overall, using optimal doses for each insulin treatment, insulin detemir maintained a glucose concentration of 95.47 ± 23.82 mg/dl, whereas average glucose level under NPH was 127.50 ± 51.38

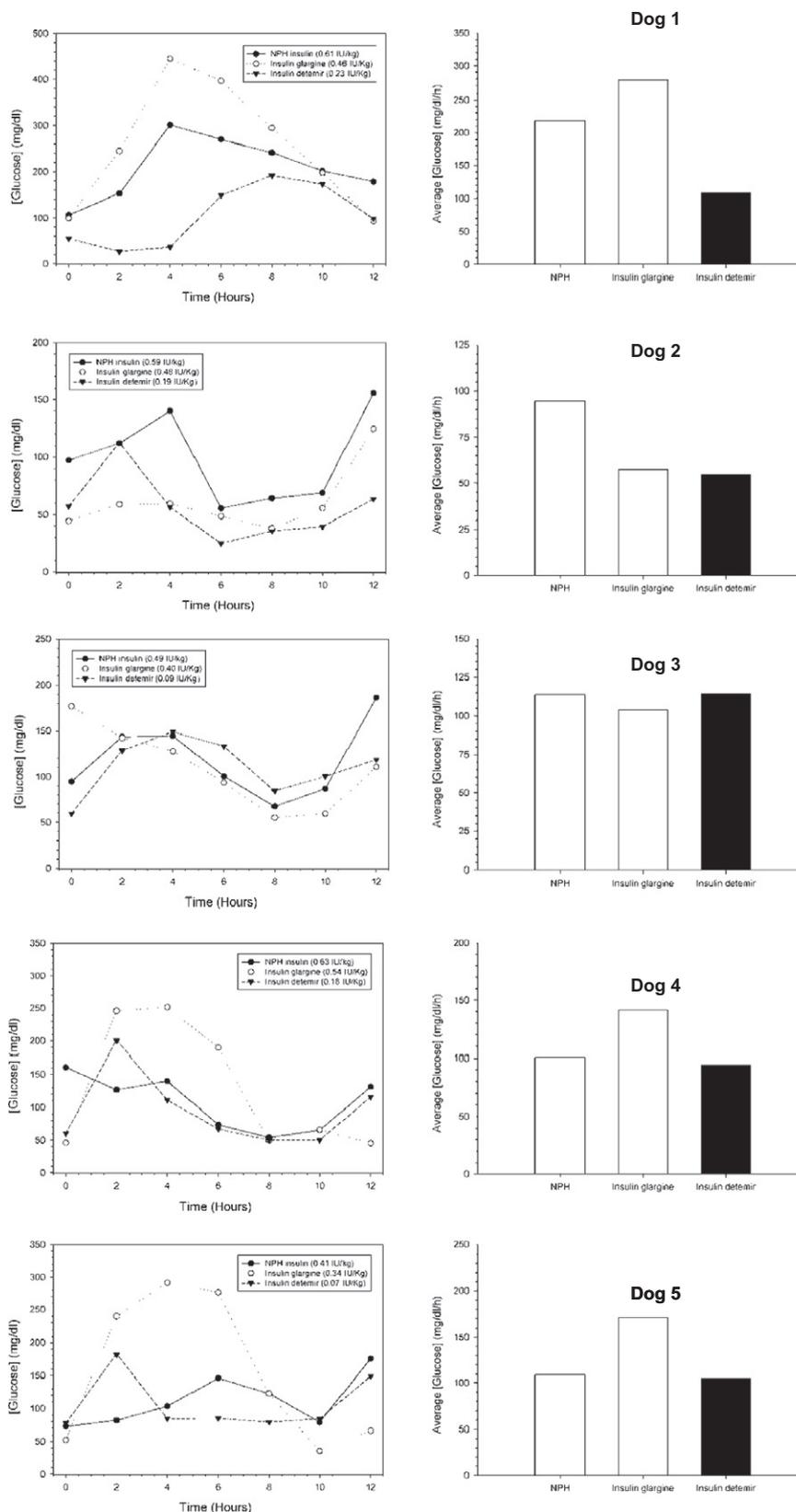


Fig. 2. Individual diabetic dog 12 h serum glucose curves after insulin injection of NPH insulin, insulin glargine, or insulin detemir. Following insulin injection, blood samples were collected at 2 h intervals for up to 12 h post feeding. Dog 1, dose of insulin IU (NPH Insulin, insulin glargine and insulin detemir) was as follows: 0.61, 0.46 and 0.23 IU/kg, respectively. Dog 2, dose of insulin IU (NPH Insulin, insulin glargine and insulin detemir) was as follows: 0.59, 0.48 and 0.19 IU/kg, respectively. Dog 3, dose of insulin IU (NPH Insulin, insulin glargine and insulin detemir) was as follows: 0.49, 0.40 and 0.09 IU/kg, respectively. Dog 4, dose of insulin IU (NPH Insulin, insulin glargine and insulin detemir) was as follows: 0.63, 0.54 and 0.18 IU/kg, respectively. Dog 5, dose of insulin IU (NPH insulin, insulin glargine and insulin detemir) was as follows: 0.41, 0.34 and 0.07 IU/kg, respectively.

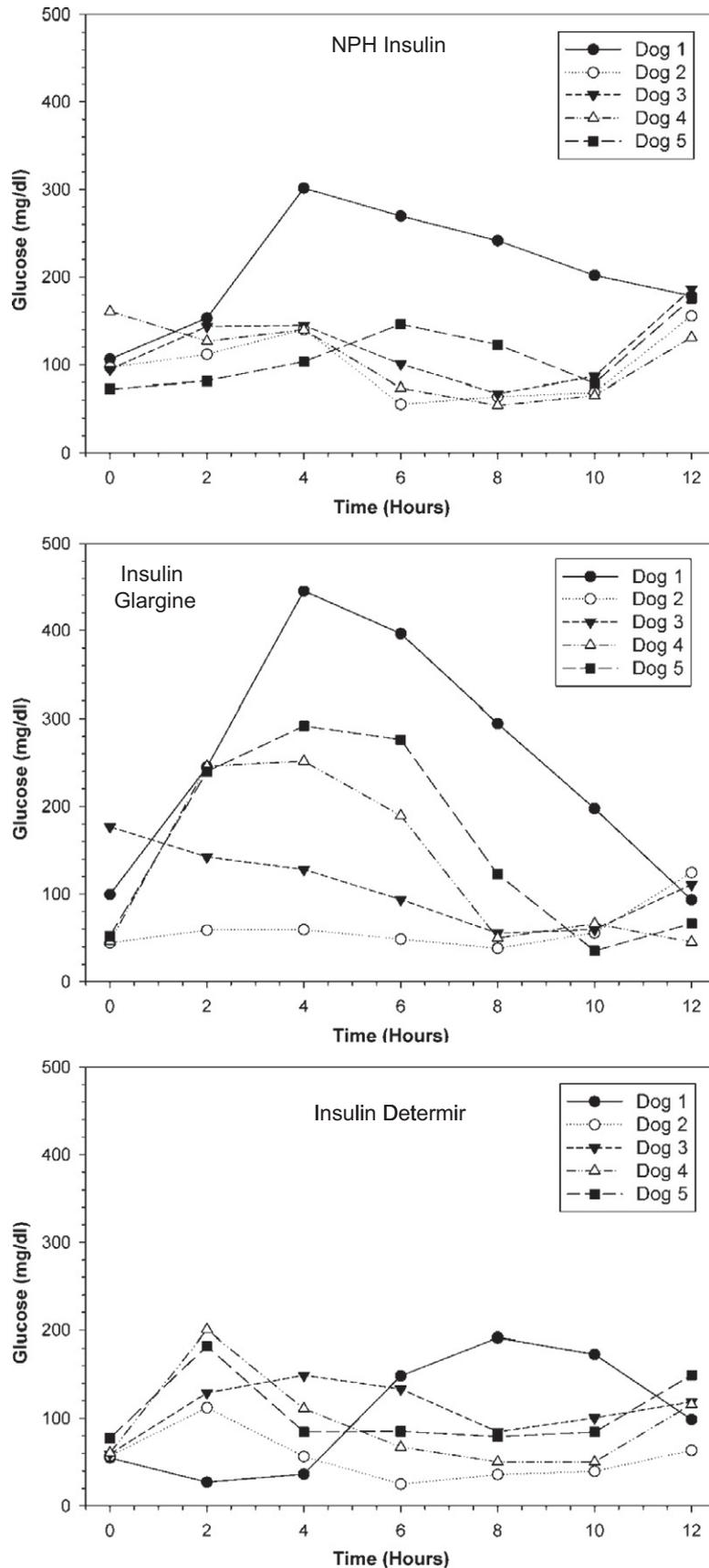


Fig. 3. Comparison of 12 h serum glucose curves after insulin injection of insulin preparation for NPH insulin, insulin glargine, and insulin determir in five insulin dependent diabetic dogs.

and 150.57 ± 83.48 mg/dl under insulin glargine, respectively for all five animals.

4. Discussion

When testing the effect of any type of administered insulin, it is recommended that serial glucose curves, established at 2 h intervals, be carried out to establish time to peak effect and duration of effect. We carried out our time-action profiles for insulin detemir in normal control dogs using a Nikkiso STG-22 artificial pancreas apparatus, which has been considered a gold standard to measure insulin time-action profiles in humans and dogs (Heinemann et al., 2000; Heise et al., 2004; Mori et al., 2008). As previously described, this device has two lines: one for blood sampling in order to measure blood glucose concentration, and one used for injecting glucose solution. Thus, this device can automatically maintain blood glucose concentration within a target range by making adjustments, as necessary, to the amount of glucose solution being injected by monitoring real-time changes in blood glucose concentration. We used the apparatus to maintain blood glucose concentration at a normal range between 68 and 90 mg/dL after insulin administration in normal control dogs. By doing so, we were able to prevent possible side effects such as hypoglycemia while simultaneously measuring the direct action of insulin injection in dogs.

Our time-action profiles in normal dogs affirmed the pharmacodynamic role of insulin detemir is long-lasting. Its time to peak effect was 8–10 h and a pronounced peak was detected at 2–11 h for insulin detemir after injection. It has been reported that insulin detemir has no time to peak effect in human studies. However, insulin action for humans was approximately equivalent between insulin glargine and insulin detemir (Heise et al., 2004). Also, it appears that insulin detemir has a different glucose-lowering effect in dogs as compared to humans. In our study, using optimal different doses for each insulin treatment with five diabetic dogs (average \pm SD; NPH insulin, 0.55 ± 0.09 IU/kg; insulin glargine, 0.44 ± 0.07 IU/kg, and insulin detemir, 0.15 ± 0.07 IU/kg), insulin detemir maintained a glucose level of 95.47 ± 23.82 mg/dl, whereas average glucose level under NPH was 127.50 ± 51.38 and 150.57 ± 83.48 mg/dl with insulin glargine, respectively. However, one reason why insulin detemir may have had a greater insulin effect in dogs could have been due to the fact that insulin detemir was produced at a high concentrate (24 mmol/L), which was 4 \times greater than other insulin preparations (6 mmol/L for NPH insulin and insulin glargine), to adjust the insulin action for human usage. Actually, there is no significant difference in glucose-lowering effect between equimolar insulin detemir and human insulin in normal control dogs (Dea et al., 2002). As such, taking this into consideration, when we administered the different insulin preparations into diabetic animals, we adjusted IU dosage for the different insulin preparations and on an individual animal basis.

Since the time-action profiles for insulin detemir were determined, we evaluated the efficacy of insulin detemir for treating insulin dependent diabetic dogs as compared to NPH insulin and insulin glargine. When NPH insulin was evaluated, the 4 out of 5 animals (Dogs 2–5) maintained glycemic control under 200 mg/dL, which is considered to be excellent glycemic control. In fact, their glucose concentration curves were flatlined. However, glucose concentrations decreased under 60 mg/dL for the 2 out of 4 animals under glycemic control (Dog 2 and 4) which was detrimental since hypoglycemia occurred. Furthermore, one out of the five dogs (Dog 1) demonstrated no glycemic control with the NPH insulin amount tested, having a higher glucose concentration (over 200 mg/dL). Overall, NPH insulin was able to consistently sta-

bilize glucose levels with minor variation resulting in good glycemic control. When insulin glargine was evaluated (Fig. 2, middle panel), the 3 out of 5 animals (Dog 1, 4 and 5) had a higher glucose concentration (over 200 mg/dL) at 2–6 h after insulin administration showing no glycemic control. Therefore, insulin glargine was unable to prevent post-prandial hyperglycemia at the optimal dosages for each respective animal tested. Meanwhile, the 4 out of 5 dogs (Dog 2–5) experienced hypoglycemia (under 60 mg/dL) just after initial injection and 8–10 h after. Overall, insulin glargine appears to have a high variability and individual specificity on glycemic control. Lastly, when insulin detemir was evaluated (Fig. 3, bottom panel), all five diabetic dogs maintained excellent glycemic control (under 200 mg/dL) with glucose concentration levels being flatlined. However, the 4 out of 5 (Dog 1–4) animals experienced hypoglycemia (under 60 mg/dL) at various time points, concurrently. This is in spite of the fact that we used a lower amount of insulin IU than either of the other two insulin preparations which highlights the strength and potency of insulin detemir. Overall, insulin detemir displays a similar mode of action to NPH insulin, but appears to be more effective using lower amounts and carries an increased risk of inducing hypoglycemia.

Hypoglycemia is the most serious condition seen with insulin-treated diabetic humans and dogs and should always be avoided (DCCT Research Group). Since insulin detemir had never been used in dogs, we decided to use small doses (0.09–0.23 IU/kg) to avoid inducing textbook hypoglycemia (<60 mg/dl). Fortunately, we did not observe any clinical hypoglycemia in diabetic dogs used in this study. Clinical hypoglycemia can be defined when blood glucose concentration is <35 mg/dl. However, lower blood glucose in the diabetic animals tested was consistently observed with insulin glargine and insulin detemir as compared to NPH insulin. This point was one limitation of our study; if blood glucose drops to less than 60 mg/dl, the Somogyi phenomenon can occur. In this scenario, the body tries to correct the hypoglycemia through release of counter-regulatory hormones, such as epinephrine or glucagon, and the blood glucose concentration quickly rises to high levels, falsely decreasing the apparent insulin duration effect (Behrend, 2006). Another limitation of our study is the low statistical power attributed to the small number of diabetic animals used in this preliminary study. As such, due to the large biological variability amongst animals and the small sample number used in our study, it is not possible to accurately assess true efficiency of insulin detemir. Lastly, it is important and critical that glycemia and insulin concentration are both measured to accurately determine the mode of action of any insulin treatment in a clinically diabetic dog. Unfortunately, our past experience with commercial canine insulin ELISA kits available and used in Japan falsely react for insulin glargine thereby preventing us to accurately measure insulin levels for all three of our insulin treatments. Therefore, although we did measure insulin concentration in this study, we chose not to present the data in this study because we cannot authenticate nor determine what % is *in vivo* produced insulin versus synthetic insulin.

In conclusion, our study demonstrates that the time-action profile of insulin detemir in normal control dogs is long-acting effects. This would be the first report purporting the use of insulin detemir in dogs. In addition, insulin detemir exerted a greater effect with a smaller dose, due to a higher concentration, than either NPH insulin or insulin glargine. An overall lower and stabilized blood glucose concentration resulted from insulin detemir administration, thus illustrating its potential as a possible alternative to using NPH since they both have a similar mode of action and duration of effect in diabetic dogs. However, a side effect of possible combination insulin therapy with insulin detemir and a faster onset insulin preparation, as shown by our preliminary results, is the increased risk of hypoglycemia. Veterinary clinicians would need

to thoroughly explain the risk of hypoglycemia to diabetic dog owners, before ever prescribing the aforementioned insulin preparations, especially insulin detemir in combination insulin therapy. Furthermore, since insulin action is different between diabetic and nondiabetic dogs, additional experiments are required for evaluating time-action profiles of insulin detemir of clinically encountered diabetic dogs in the future to better gauge insulin detemir efficiency. Especially, it would be of great interest to determine the time-course of insulin detemir action in naturally diabetic dogs with and without variation in body condition score, as diabetic dogs are often overweight or obese and insulin-resistant.

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