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Low dose of insulin detemir controls glycaemia, insulinemia and prevents diabetes mellitus progression in the dog with pituitary-dependent hyperadrenocorticism

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ABSTRACT

Diabetes is often associated with pituitary-dependent hyperadrenocorticism (PDH). Hypercortisolism causes insulin resistance and affects β-cell function. The purpose of this study was to test if daily administration of a long-acting insulin analogue during the first month of anti-PDH treatment can prevent progress to diabetes in these animals. Twenty-six PDH dogs were divided into three groups: one group with glycaemia <5.83 mmol/L and two groups with glycaemia >5.83 mmol/L and <9.35 mmol/L, one of which received insulin detemir during 4 months. Dogs with glycaemia <5.83 mmol/L and those with glycaemia >5.83 mmol/L which received insulin did not develop diabetes. In the non-insulin group, 6/7 dogs developed diabetes after the third month. There is a 13-fold higher risk of diabetes in dogs with glycaemia >5.83 mmol/L and no insulin treatment. Administering insulin detemir to dogs with PDH and glycaemia >5.83 mmol/L could prevent progression to diabetes.

1. Introduction

It is widely reported that glucocorticoids affect carbohydrate and lipid metabolism (Vegiopoulos and Herzig, 2007). Both in humans and dogs with PDH, diabetes mellitus is one of the associated endocrine diseases (Eigenmann and Peterson, 1984; Hess et al., 2000; Resmini et al., 2009).

Chronic increase of cortisol triggers insulin resistance in muscle and adipose tissue and in the hepatocyte (Andrews and Walker, 1999; Ruzzin et al., 2005), with high insulin concentrations. In muscle tissue, cortisol interferes both with the insulin signalling cascade (Giorgino et al., 1993; Saad et al., 1993; Ruzzin et al., 2005) and with glucose uptake (Weinstein et al., 1998; Vestergaard et al., 2001; Glass, 2005). In the visceral adipose tissue, it promotes preadipocyte differentiation (Gregoire et al., 1998; Drapeau et al., 2003; Walker, 2006) and stimulates glyceroneogenesis (Beale et al., 2003; Forest et al., 2003), favouring the accumulation of abdominal fat. On the other hand, in the peripheral fat, it reduces lipoprotein lipase activity and stimulates hormone-sensitive lipase, increasing triglyceride hydrolysis (Ottosson et al., 1994; Slavin et al., 1994).

Cortisol activates glyconeogenesis, lipogenesis and glyceroneogenesis in the hepatocyte by diverse mechanisms (Kalhan et al., 2001; Beale et al., 2004; Vegiopoulos and Herzig, 2007). One of these is to stimulate phosphoenol pyruvate carboxykinase activity, a limiting step of glyconeogenesis and glyceroneogenesis (Reshef et al., 2003; Beale et al., 2004), resulting in greater glycaemia and triglyceride synthesis. The consequences are glucose intolerance, both when fasting (fast hyperglycaemia) and postprandial, and hypertriglyceridemia, typical of insulin resistance (Lebovitz, 2001; Catchpole et al., 2005; Landsberg, 2005).

In the endocrine pancreas, cortisol affects both insulin synthesis and secretion in the pancreatic β-cell, and induces its apoptosis (Delaunay et al., 1997; Lambillotte et al., 1997; Schacke et al., 2002; Davani et al., 2004; Ranta et al., 2006). This reduces circulating insulin, gradually increases glycaemia and alters lipid fractions even more. Thus, individuals who suffer from PDH have a greater predisposition to develop diabetes as a concurrent disease (Hess et al., 2000; Peterson et al., 1984).

During a posthypophysectomy follow up in PDH patients, both human and canine, it has been proved that the adverse effects of cortisol persist for a long time (Colao et al., 1999; Meij et al., 2002; Pivonello et al., 2007; Barahona et al., 2009, 2010; Webb et al., 2010). Drug therapies for PDH reduce cortisol levels (Kintzer and Peterson, 1991; Sieber-Ruckstuhl et al., 2006; Castillo et al., 2006, 2008a); therefore, its adverse effects would persist during a lapse of time. In a retrospective study carried out on 152 PDH cases treated at the Endocrinology Unit of the School Hospital, of the

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Faculty of Veterinary Sciences, Buenos Aires University, between 2004 and 2006, 10.5% (16/152) progressed to diabetes within 3–4 months (mo) after diagnosis and initiation of the corresponding therapy (personal communication), in agreement and similar with were previously reported (Hess et al., 2000; Katherman et al., 1980; Peterson et al., 1981).

The aim of this study was to test if daily administration of a long-acting insulin analogue during the first months of PDH treatment can control the above mentioned metabolic imbalances and prevent progress to diabetes in these animals.

2. Materials and methods

2.1. Study population

During a period of 2 years (2008–2010), were attended in the institution mentioned a total of 148 dogs with PDH. From this group, 26 dogs were included in the present study. Twelve male and 14 female (6 of the latter were spayed and the other with anoestrus since 2 years ago), average age 9.2 ± 2.6 years (yr), 7 crossbreeds and 18 of different breeds. All dogs were feeding with a recognised commercial diet. Diagnosis was based on the typical clinical signs for the disease, and according to the above mentioned institution's protocol (Gallelli et al., 2010), consisting of a urinary cortisol/creatinine ratio (C/CR) test performed before and after inhibition by dexamethasone (Galac et al., 1997), adenocorticotropin (ACTH) measurement and confirmation of the adenoma was carried out by magnetic resonance imaging.

As routinely administered in Argentina, all dogs received retinoic acid as treatment in the form of 2 mg/kg/day of 9-cis isotretinoin (9-cis-RA) (Castillo et al., 2006).

Exclusion criteria for the study were: dogs with clinical diabetes (glycaemia $\geqslant$9.35 mmol/L and glycosuria at the time of PDH diagnosis); dogs weighing less than 7 kg (due to hypoglycaemia risk as they were close to the minimum insulin dose to be used); dogs with infectious diseases (either systemic or local), presence of neoplasia (except for a pituitary adenoma), or any other situation capable of producing insulin resistance other than hypercortisolism.

Dogs were grouped based on their blood glucose concentrations after 12 h fasting, taking glycaemia <5.83 mmol/L or glycaemia $\geqslant$5.83 mmol/L and <9.35 mmol/L (hyperglycaemic state). According to our previous observations, the cut-off value was set at 5.83 mmol/L (dogs which developed diabetes had a fasting glycaemia $\geqslant$5.83 mmol/L), as it was an abnormality high fasting glycaemia for the period of time since the last ingestion. Moreover, the ADA (American Diabetes Association) recommendations for humans were also considered (Shaw et al., 2000; Borthery et al. 2002), as the PDH dog has the risk factors mentioned by the ADA and by Peterson et al. (1984). Dogs with glycaemia $\geqslant$5.83 mmol/L were randomly distributed in two groups (alternately 1 to 1 to each group in order of arrival during the period mentioned and simple blind) according to whether or not they received the long-acting insulin analogue Detemir (Levemir®, Novo Nordisk). This analogue was chosen because of its pharmacokinetics (Chapman and Perry, 2004) and our and other authors experience with this kind of insulin in diabetic dogs (Sako et al., 2010). Hyperglycaemia between 5.83–9.35 mmol/L was used as the interval for selection of the dogs to be given Detemir and to act as their paired non Detemir controls.

Thus three groups were formed: Group 1 (G1, n = 10; mean age:8.6 ± 1.7 yr; 5 male and 5 female being 2 castrated, 3 crossbreed and 7 different breeds) with glycaemia <5.83 mmol/L (as control group, formed with the first 10 dogs diagnosed with PDH at the beginning of the study and glycaemia less than the values mentioned); Group 2 (G2, n = 9; mean age: 10.33 ± 3 yr; 4 male and 5 female being 2 castrated, 3 crossbreed and 6 different breeds) with glycaemia $\geqslant$5.83 mmol/L and <9.35 mmol/L, which received insulin detemir once a day for the first 4 mo of treatment. The insulin dose used was 0.1 IU/kg/day (the insulin detemir dose for diabetic dogs in our institution is 0.3 to 0.5 IU/kg/day), administered between 08.00 and 09.00 am. Thus the following total dose/day was established based on weight range: 7 to14 kg, 1 IU/day; 15 to 24 kg, 2 IU/day, and 25 to 35 kg, 3 IU/day. Finally, Group 3 (G3, n = 7; mean age: 8.5 ± 3.1 yr; 3 male and 4 female being 1 castrated, 2 crossbreed and 5 of different breeds) was formed by dogs with glycaemia $\geqslant$5.83 mmol/L and <9.35 mmol/L but without insulin detemir.

Dogs belonged to the groups 2 and 3 were recruited during the first 2 years. The three groups were followed for a total of 12 mo (4 mo under study and 8 mo of follow up) to identify new cases of diabetes during that period, taking into account clinical signs and glycaemia values (evaluated monthly).

2.2. Measurement of ACTH, C/CR, insulin, glycaemia, triglyceride and total cholesterol

Blood samples to measure the different values were taken at the same time and with a 12 h fast of solid food, both at the time of diagnosis (baseline time) and at 4 mo after starting treatment, except for glycaemia values, which were tested monthly.

Plasma ACTH concentration was measured by means of an enzyme immunoassay (EIA) using an available commercial kit (ACTH Alpco immunoassays, Alpco Diagnostics). In order to obtain our reference range of ACTH (5–60 pg/mL) and determine the precision of the test, we previously took samples from 30 healthy dogs and ran them by duplicate. The inter- and intra-assay coefficients of variation were 3.1 and 5.8%, respectively, and sensitivity was 0.1 pmol/L. ACTH values were considered high when >15.4 pmol/L or close to the maximum cut-off value (>Percentile 75 [P75] = 11 pmol/L) in relation to increased values of C/CR.

Urinary cortisol was measured by means of radioimmunoassay (RIA), using a commercial kit (DPC Corporation, San Diego, California, USA). The inter- and intra-assay coefficients of variation for cortisol were 8% and 5%, respectively. Creatinine was measured by an automated method (Metrolab Autoanalizer Merck, Germany) according to the manufacturer's indications. A C/CR <70 (reference values for our laboratory) was considered normal.

Insulin was measured by means of a canine and porcine-specific EIA (ALPCO Insulin Porcine/Canine EIA, Alpco Immunoassays Diagnostic, USA), where the inter- and intra-assay coefficients of variation (canine performance) were 4.2% and 4.3%, respectively, and sensitivity was 0.05 pmol/L. The blood sample used to test insulin was the same as that used to test glycaemia. To measure insulinemia at 4 months in G2, dog owners were told to stop administration of insulin detemir 1 day before the sample was taken.

The insulin resistance was inferred by Homeostatic Model Assessment (HOMA-A) using the following formula: Insulin (μUI/mL) × Glycaemia (mmol/L)/22.5 (Matthews et al., 1985; Verkest et al., 2010), where the reference value for our laboratory was HOMA-A <2.5 (calculated in 100 normal weight dogs, 50 male and 50 female, r = 0.96; P < 0.0001 with serum insulin concentration, unpublished data). The Insulin/glucose ratio (In/G) was also estimated (reference values for our lab: 6–18).

Glycaemia, triglyceride and total cholesterol were measured by a laboratory automated method (Metrolab Autoanalizer Merck) according to the manufacturer's instructions.

2.3. Statistical analysis

Groups were compared using a one-way non-parametric ANOVA test (Kruskal–Wallis) followed by Dunn's test. The intra-group

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evaluation was performed using Wilcoxon *U* test or Mann–Whitney's test, as appropriate. Values are expressed as median and range. Variables were correlated by means of Spearman's correlation test. A contingency table and Fisher's exact test was used to analyse if there were differences amongst groups as regards progression to diabetes depending on glycaemia values and Insulin detemir administration (between G2 and G3), and the relative risk (RR) was calculated. A P value of <0.05 was considered significant.

2.4. Ethical approval

The Ethics Committee of the Faculty of Veterinary Science (CICUAL) and the Secretary of Science of the University of Buenos Aires (UBACYT-V006) approved the present study, according to the laws on experimentation in animals in Argentina and World Health Organization recommendations. The signed consent of the dog owners was obtained for participation in the present project.

3. Results

3.1. ACTH and C/CR

ACTH concentration (Fig. 1) did not show significant differences between groups, both at baseline concentrations and at 4 mo. When analysing intra-group variations, 4 mo ACTH decreased significantly when compared to the time of diagnosis in all three groups (G1, $P = 0.004$; G2, $P < 0.01$; G3, $P = 0.01$).

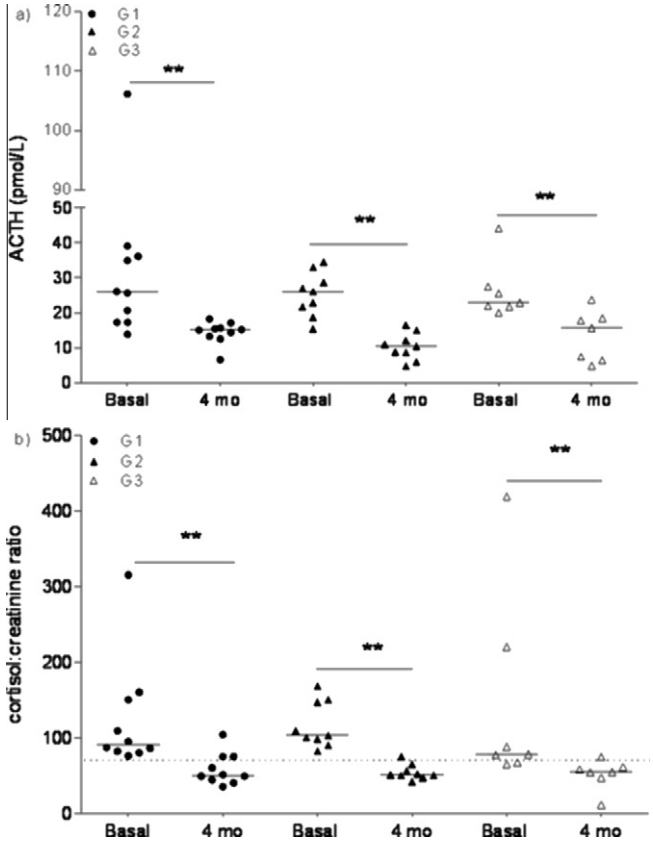

Fig. 1. ACTH (a) and C/CR (b) of the dogs undergoing treatment with 9-cis RA and G <5.83 mmol/L (G1) and >5.83 mmol/L with insulin detemir (G2) and without insulin detemir (G3). In the three groups, the ACTH and C/CR decrease at the 4 mo, indicating that at that time the 9-cis RA has been effective in controlling the PDH. (a) G1: $^{**}P = 0.04$, G2: $^{**}P < 0.01$; G3: $^{**}P = 0.01$, 4 mo vs Basal time. (b) G1: $^{**}P = 0.002$; G2: $^{**}P = 0.004$; G3: $^{**}P = 0.01$, 4 mo vs Basal time. Values are expressed as median and ranges.

C/CR (Fig. 1) did not show significant differences between groups either, both at baseline time and at 4 mo. Intra-group C/CR decreased significantly in the three groups (G1, $P = 0.002$; G2, $P = 0.004$; G3, $P = 0.01$) compared to baseline values. C/CR after dexamethasone test (only performed at the time of diagnosis) was inhibited in all three groups with respect to the basal values (data not shown).

Decreases in ACTH and C/CR resulted in improved clinical signs, except for 6/7 in G3 were increased fluid intake and urination was registered after the third month. For the rest of the study, both variables did not show significant changes compared to the values at 4 mo (data not shown).

3.2. Glycaemia, Insulinemia, HOMA-A and lipids

When comparing glycaemia values (Fig. 2) amongst the groups, no significant differences were observed between baseline G2 and G3, but, as expected, there were significant differences with G1 (G2, $P < 0.001$ and G3, $P < 0.01$). At 4 mo, intra-group glycaemia values did not differ significantly compared to their baseline concentrations in G1, decreased significantly in G2 ($P = 0.003$) and

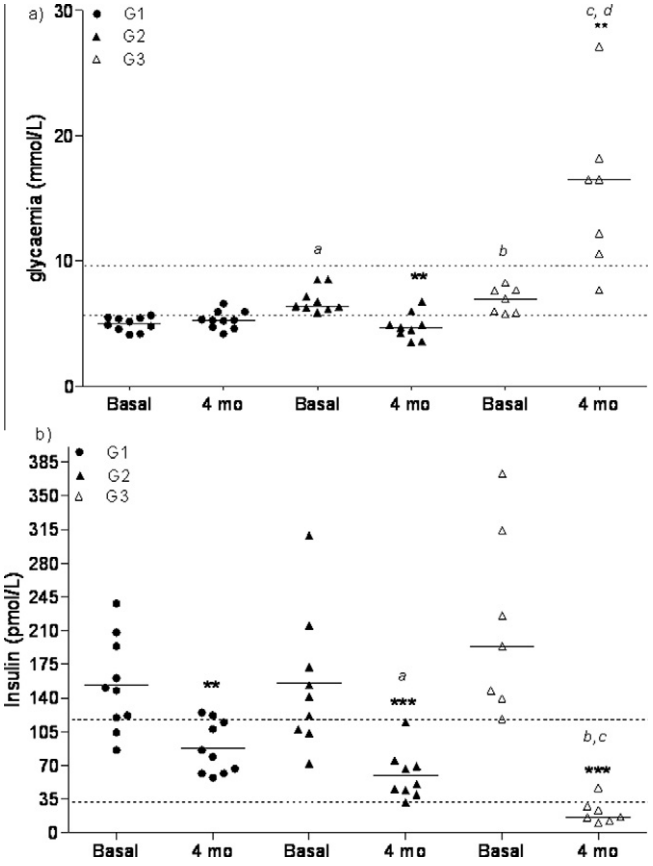

Fig. 2. Glucose (a) and insulin concentration (b) in dogs treated with 9-cis RA and (G2) or without insulin detemir (G1 and G3). Both G and insulin decrease to their reference values in the majority of the dogs of G1 and G2 (G <5.83 mmol/L, In: 34.8–120 pmol/L). On the contrary, G in G3 increases its values over 9.44 mmol/L in 6/7 dogs and insulin decreases below the minimum value, indicating inhibition of β cell. (a) ^{a}G1 vs G2 $P < 0.01$ and ^{b}G3 vs G1 $P < 0.001$ between baseline values; ^{c}G1 vs G3 $P < 0.01$ and ^{d}G3 vs G2 $P < 0.001$ amongst the 4 mo of each group. G2: $^{**}P = 0.003$; G3: $^{**}P = 0.004$, 4 mo vs Baseline. There are not differences in G1 between the compared times. (b) ^{a}G1 vs G2 $P = 0.03$ and ^{b}G3 vs G1 $P = 0.0008$, ^{c}G2 vs G3 $P = 0.002$, between the 4 mo of each group. G1: $^{**}P = 0.004$; G2: $^{***}P = 0.0005$; G3: $^{***}P = 0.0006$, 4 mo vs basal time. The dotted lines indicate the cut-off value of G (a) from which insulin detemir is supplied (lower line) and from which the dogs are considered diabetic (top line). For insulin (b) it indicates the reference values for the method. Values are expressed as median and ranges.

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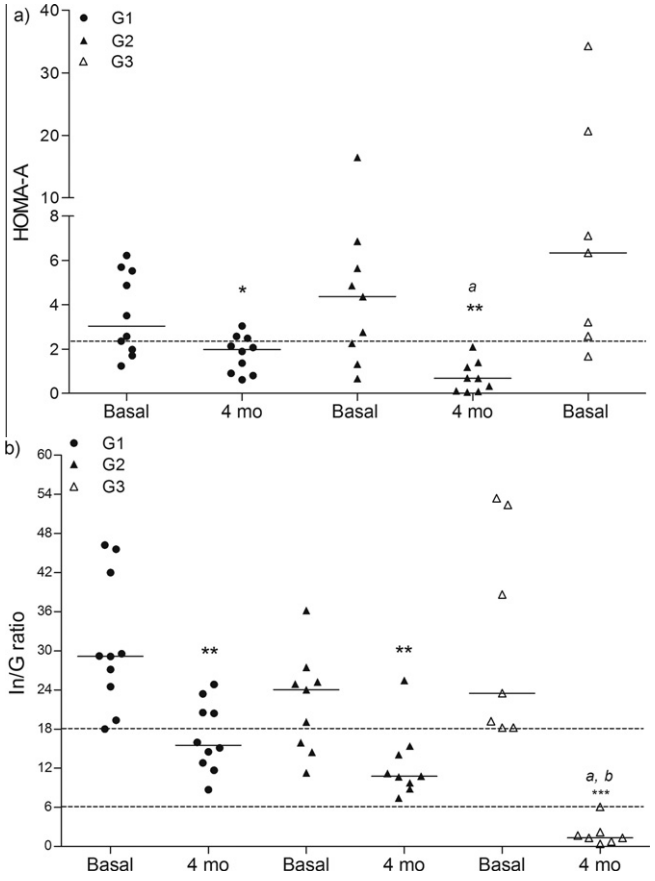

Fig. 3. HOMA-A (a) and In/G ratio (b) in dogs with PDH and treated with 9-cis RA and (G2) or without insulin detemir (G1 and G3). The HOMA-A index (not assessed in G3) decreases at 4 mo in both G1 and G2. This decrease is higher in G1 vs G2, indicating lower insulin resistance in G2. The In/G ratio also decreases in the three groups, but the biggest decrease is in G3, associated to the greater decrease of insulin. In the other two groups the relationship is within the reference values. (a) G1: $^{*}P = 0.02$, G2: $^{**}P = 0.004$, 4 m vs Basal. G2 vs G1: $^{a}P = 0.04$, between the 4 mo (b) ^{a}G3 vs G1 $P < 0.001$ and ^{b}G2 vs G3 $P = 0.0002$, between the 4 mo of each group. G1: $^{**}P = 0.001$; G2: $^{**}P = 0.005$; G3: $^{***}P < 0.0001$, 4 mo vs Basal time. Dotted lines indicate cut-off values of reference (minimum and maximum). Values are expressed as median and ranges.

increased significantly in G3 ($P = 0.01$), with 6/7 cases of clinical diabetes in this group, whereas glycaemia was 7.77 mmol/L in the other dog. These dogs developed diabetes between the evaluation of the 3 mo and 4 mo (glycaemia before and in the 3 mo were within the values previously mentioned, data not shown), being under PDH's established therapy.

When comparing glycaemia values at 4 mo amongst groups, there were no differences between G1 and G2, but in G3 were significantly higher when compared to G2 ($P < 0.001$) and G1 ($P < 0.01$). The 6 diabetic dogs of G3 started with insulin detemir treatment (diabetes doses: 0.3–0.5 IU/kg once a day).

In the following 8 mo, and as the dogs continued with 9-cis-RA as the only treatment, no dog in G1 developed clinical diabetes. Only 1/9 dogs in G2 developed clinical diabetes 8 mo after suspending insulin detemir and in coincidence with the recovery of oestrus. The dog of the G3 with hyperglycaemia state maintained permanently elevated glycaemia values (ranged between 6.9 and 8.49 mmol/L) for the following 8 mo at the end of the study (data not shown). Taken into account the total dogs that were attended during 2 years (148), only 7/148 dogs (1/9 from the G2 at the end of the study and 6/7 from the G3) developed diabetes (4.7%).

Insulin assessed at the time of PDH diagnosis (Fig. 2) did not differ significantly between groups, although its concentrations were above reference ranges determined in dogs with normoglycaemia. At 4 mo of treatment, intra-group values decreased significantly in G1 ($P = 0.004$), G2 ($P = 0.0005$) and G3 ($P = 0.0006$); and in this last group they were below cut-off value. When comparing values at 4 mo in the three groups, the reduction was significant in G2 vs G1 ($P = 0.03$), G3 vs G1 ($P = 0.0008$) and G3 vs G2 ($P = 0.002$).

Baseline HOMA-A values (Fig. 3) did not differ significantly amongst groups when compared, but values were high. At 4 mo of treatment, intra-group values decreased significantly in G1 ($P = 0.02$) and G2 ($P = 0.004$) compared to their baseline values. When comparing G1 and G2 values at 4 mo, HOMA-A was significantly lower in the latter ($P = 0.04$). HOMA-A was not calculated for G3 at 4 mo because it is not representative in diabetes.

Insulin/Glucose ratio (Fig. 3) was elevated in the three groups at the time of PDH diagnosis. At 4 mo, a significant reduction was observed in all groups: G1 ($P = 0.001$), G2 ($P = 0.005$) and G3 ($P < 0.0001$), compared to their baseline values. In/G ratio did not differ significantly when comparing G2 with G1 at 4 mo. On the contrary, this relationship is significantly reduced in G3 compared with G2 ($P = 0.0002$) and G1 ($P < 0.001$).

There were no differences in triglyceride baseline concentrations (Table 1) between the three groups. As regards intra-group performance, there was a significant reduction in G2 ($P = 0.04$) compared to its baseline value, but there were no differences in G1. At 4 mo, triglyceride showed a significant increase in G3 ($P = 0.002$) in relation to its baseline values and compared with G2 ($P < 0.01$) and G1 ($P < 0.05$).

Baseline total cholesterol (Table 1) was significantly higher in G2 ($P < 0.05$) compared with the other two groups. At 4 mo, total cholesterol in G3 was significantly higher than in G1 and G2 ($P < 0.05$). In the intra-group analysis, at 4 mo a significant decrease in total cholesterol was observed only in G2 ($P = 0.004$) compared to its baseline values, and there were no significant changes in the other two groups.

3.3. Correlation and relative risk

In our study, insulin ($r = 0.68$; $P < 0.0001$), insulin/glucose ratio ($r = 0.57$; $P = 0.0002$) and HOMA-A ($r = 0.60$; $P < 0.001$) correlated with ACTH (Fig. 4). Glycaemia ($r = 0.35$; $P = 0.03$) and triglyceride ($r = 0.37$; $P = 0.02$) correlated weak but significant with ACTH.

Table 1
Triglyceride and total cholesterol in dogs with PDH and treated with 9-cis RA and with (Group 2) or without insulin detemir (Group 1 and Group 3).

	Tryglyceride (mmol/L)		Total cholesterol (mmol/L)	
	Basal time	4 month	Basal time	4 month
Group 1	1.2 (0.5–9.7)	1.1 (0.4–2.8)	7.02 (4.8–13.08)	5.5 (4.1–11.2)
Group 2	1.3 (0.6–6.6)	0.8* (0.4–1.7)	11.5[c] (6.2–16.3)	6.4** (4.2–11)
Group 3	1.7 (1.2–3.2)	2.2**;[a,b] (1.1–12.7)	8.05 (6.2–12.2)	8.2[d] (6–14.1)

Tryglyceride: Intra-group: G2:$^{*}P = 0.04$, G3: $^{**}P = 0.002$; 4 mo vs basal time. Inter-group ^{a}G3 vs G1 $P < 0.05$; ^{b}G3 vs G2 $P < 0.01$, between the 4 mo of each group.
Total Cholesterol: Intra-group: G2: $^{**}P = 0.004$, 4 mo vs basal time. Inter-group: ^{c}G2 vs G1 and G3, $P < 0.05$ amongst baseline values and ^{d}G3 vs G2 and G1, $P < 0.05$ amongst the 4 mo of each group. Values are expressed as median and ranges.

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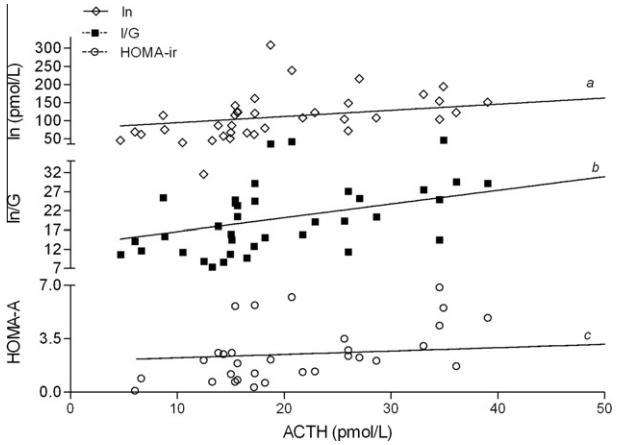

Fig. 4. Correlation between ACTH vs Insulin, In/G ratio and HOMA-A. The analysed variables are positively correlated with the ACTH concentration. [a]$r = 0.68$, $P < 0.0001$; [b]$r = 0.57$, $P = 0.0002$, [c]$r = 0.6$, $P < 0.001$. In: Insulin; In/G: Insulin/glucose ratio.

When analysing glycaemia, triglyceride and total cholesterol in relation to insulin only glycaemia ($r = 0.37$; $P = 0.04$) and triglyceride ($r = 0.37$; $P = 0.02$) were weakly correlated.

Contingency table analysis (Chi–square test for trend) showed significant differences between the three groups ($P = 0.0002$) regarding progression to clinical diabetes according to their glycaemia. When comparing G2 to G3, the RR of progressing to a clinical diabetes when insulin detemir was not administered was 0.13 ($P = 0.008$; 95%, CI = 0.02–0.84).

4. Discussion and conclusions

The reduction in ACTH and C/CR is an indication of the efficacy of the implemented therapy and of a trend towards normal cortisol secretion (Castillo et al., 2008b). According to our previous experience with 9-cis-RA, normal concentrations of ACTH and C/CR are achieved after 3 mo, and are maintained thereafter (Castillo et al., 2006). During that lapse, however, the adverse effects of cortisol will be present and may persist even longer, as reported in humans after hypophisectomy (Pivonello et al., 2007; Barahona et al., 2009; Webb et al., 2010).

Glycaemia values at the time of diagnosis are important and should be considered. Behaviour will be different depending on whether glycaemia is >or <5.83 mmol/L. We have seen that dogs with untreated PDH and long-standing disease at the time of diagnosis and treatment did not develop diabetes as long as their fasting glycaemia values were lower than 5.83 mmol/L (personal communication). Lower glycaemia values suggest that β-cells would not be affected in this group of animals, at least during that time. Possibly, there are differences both in intracellular β-cell mechanisms and in PDH molecular aetiology and this determines the different behaviours, as shown by the fact that glycaemia values in G1 dogs were not altered throughout the time of evaluation. Bone morphogenetic protein 4 (BMP-4) has been seen related with the corticotroph development (Giacomini et al., 2006; Nudi et al., 2005) and with the β-cell function and insulin synthesis (Goulley et al., 2007). Taking this data all together, it is feasible that PDH dogs which develop diabetes would have a lower BMP-4 both in the corticotroph cell and in the β-cell, remained this hypothesis to be explored in a future.

Considering that insulin concentration, HOMA-A and insulin/glucose ratio do not differ between the three groups at the time of diagnosis, it becomes clear that 9-cis-RA as the only treatment improves these variables in individuals with glycaemia <5.83 mmol/L. On the contrary, in G3 dogs it fails to prevent progress to diabetes in spite of PDH control. Noticeably, when adding insulin detemir to PDH therapy, insulin concentration is normalised and its peripheral sensitivity (HOMA-A) is improved, as is reflected in the decrease of glycaemia and in insulin/glucose ratio, this normalisation being much better than in G1. Administering insulin detemir would protect β-cells from exhaustion until 9-cis-RA exercises its described effects on the corticotroph cell (Kang et al., 2000; Paez-Pereda et al., 2001; Giacomini et al., 2006).

The beneficial effect of incorporating insulin detemir can be seen in the behaviour of triglyceride and total cholesterol, which decreased more in G2. Hepatic steatosis caused by hypercortisolism persists in spite of PDH early control, and it is one of the residual effects of cortisol observed post-therapy in humans (Colao et al., 1999; Barahona et al., 2009, 2010). Both hepatic steatosis and abdominal or visceral fat (present in PDH) cause insulin resistance (McGarry, 2002; Landsberg, 2005; Vegiopoulos and Herzig, 2007). This would account for the different behaviours between G2 dogs that received insulin detemir and G1 dogs in which triglyceride and total cholesterol do not vary significantly compared to their baseline values. Administering insulin manages to regulate lipid metabolism in G2 dogs. The presence of associated diabetes in G3 explains the increase in lipids.

It is important to consider the existing correlation between variables. In principle, HOMA-A would be useful to infer insulin resistance in dogs with PDH. The correlation observed between ACTH and insulin is interesting. Extra-adrenal ACTH actions have been described, including its effect on β-cell inducing insulin synthesis (Ohsawa et al., 1967; Gagliardino et al., 1995; Al-Majed et al., 2004) and on the adipocyte causing lipolytic effects (Boston and Cone, 1996). These actions would explain ACTH correlation with insulin, and therefore with HOMA-A, In/G ratio, glycaemia and triglyceride (although was weak on these later variables). To our knowledge, this would be the first time that ACTH correlation with the above mentioned variables is reported in PDH dogs, as the study by Ohsawa et al. (1967) was performed in healthy animals with exogenous ACTH. The consequences of installed insulin resistance, together with β-cell stimulation by ACTH, end in hyperinsulinism. This sustains the normoglycemic individual at the expense of future β-cell exhaustion. On the other hand, chronically elevated cortisol causes an effect opposite to that of ACTH on β-cells, inhibiting insulin synthesis (Delaunay et al., 1997; Lambillotte et al., 1997; Van Raalte et al., 2009). We do not know whether there is a moment in which ACTH action predominates over β-cell and with time cortisol inhibiting action prevails or if it is a consequence of an aggregation and cascade of events. These would start with a corticotrophinoma and PDH development, followed by hyperinsulinism (due to insulin resistance and ACTH action), and ending with the inhibition of insulin synthesis and progressive exhaustion of β-cells. At this point, dogs would have a glycaemia >5.83 mmol/L and would be at risk of developing diabetes. As 9-cis-RA decreases ACTH, not only does plasma cortisol decrease, but also circulating insulin concentration, as was observed in G1. The administration of insulin detemir would help in these cases to protect the β-cell (Chapman and Perry, 2004) by preventing its exhaustion and allowing for a greater improvement of the studied parameters, as proved in G2. On the other hand, the lack of this protection in G3 would be one of the causes leading to β-cell exhaustion and consequent diabetes. All this determines a higher risk of diabetes developing in dogs with PDH and glycaemia $\geqslant$5.83 mmol/L, a risk which is 13-fold higher if insulin detemir is not administered as associated treatment, at least during the first 4 mo. This higher risk would be a consequence of β-cell exhaustion and impairment.

Although the number of dogs studied was low and the follow-up period consisted in only one year, it was evident in this study that Insulin detemir may be effective in preventing progression to diabe-

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tes in dogs with PDH and glycaemia ⩾5.83 mmol/L; and it would be important to administer it together with 9-cis-RA or other prescribed therapies. The rate of diabetic dogs associated with PDH decreased to 4.7% from our previous rates (10.5%). Further experiences with a bigger population under study and including molecular techniques, would be useful to improve the obtained results.

5. Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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