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Involvement of glucagon-like peptide 1 in the glucose homeostasis regulation in obese and pituitary-dependent hyperadrenocorticism affected dogs

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ABSTRACT

The incretin glucagon-like peptide 1 (GLP-1) enhances insulin secretion. The aim of this study was to assess GLP-1, glucose and insulin concentrations, Homeostatic Model Assessment ($HOMA_{insulin\ sensitivity}$ and $HOMA_{\beta\text{-}cell\ function}$) in dogs with pituitary-dependent hyperadrenocorticism (PDH), and compare these values with those in normal and obese dogs. The Oral Glucose Tolerance Test was performed and the glucose, GLP-1 and insulin concentrations were evaluated at baseline, and after 15, 30, 60 and 120 minutes. Both basal concentration and those corresponding to the subsequent times, for glucose, GLP-1 and insulin, were statistically elevated in PDH dogs compared to the other groups. Insulin followed a similar behaviour together with variations of GLP-1. $HOMA_{insulin\ sensitivity}$ was statistically decreased and $HOMA_{\beta\text{-}cell\ function}$ increased in dogs with PDH. The higher concentrations of GLP-1 in PDH could play an important role in the impairment of pancreatic β-cells thus predisposing to diabetes mellitus.

1. Introduction

The incretin glucagon-like peptide 1 (GLP-1) is a potent insulinotropic peptide secreted by intestinal L cells in response to food ingestion. Its effects on the endocrine pancreas are to stimulate the synthesis and secretion of insulin, inhibit the secretion of glucagon and somatostatin, and maintain the functional mass of pancreatic β-cells (Kieffer and Habener, 1999; Meier and Nauck, 2005). In turn, in muscle and adipose tissue, it promotes glucose uptake and metabolism (Baggio and Drucker, 2007; Sandhu et al., 1999). The half-life of GLP-1 is very short, as dipeptidyl peptidase enzyme 4 (DPP-4) rapidly inactivates it (Deacon, 2005).

In the last few years, studies of GLP-1 have increased significantly due to its relationship with the development of type II diabetes mellitus and the implementation of new therapies for this disease (Tahrani et al., 2011). It is known that in obese human patients and those with impaired glucose, GLP-1 levels are decreased, postulating that this reduction may reduce insulin secretion and predispose to the development of type II diabetes mellitus (Madsbad, 2013; Ranganath et al., 1996). In veterinary medicine, it has been shown that obese dogs present basal concentrations of GLP-1 that are similar to those of normal-weight dogs (Verkest et al., 2011). In contrast, it has been reported that obese cats show lower concentrations of GLP-1 compared to those of control cats, similar to what is seen in humans (Hoenig et al., 2010). Recently, it has been reported that administration of dexamethasone increases GLP-1 concentrations (Jensen et al., 2012). It is feasible that cortisol in PDH affected dogs could play the same role in GLP-1 metabolism. However, to the best of our knowledge, there are no publications on GLP-1 levels in dogs with PDH.

Hypercortisolism, either due to chronic stress, the administration of exogenous glucocorticoid, or pituitary-dependent hyperadrenocorticism (PDH), induces a state of insulin resistance because it stimulates hepatic glycogenolysis, interferes in the recruitment of GLUT-4 (glucose transporter type 4), inhibits insulin secretion, and can even induce apoptosis of pancreatic β-cells (Ranta et al., 2006; Vegiopoulos and Herzig, 2007). These events promote the development of glucose intolerance and subsequently the establishment of diabetes mellitus (Di Dalmazi et al., 2012; Pivonello et al., 2010). In a previous study, we observed that both basal glucose and insulin were high in dogs with PDH (Miceli et al., 2012). It is estimated that between 8% and 10.5% of dogs with PDH suffer from secondary diabetes mellitus (Eigenmann and Peterson, 1984; Miceli et al., 2012) which represents a severe complication to the specific treatment of PDH.

Homeostatic Model Assessment indices (HOMA) are used to assess both peripheral insulin sensitivity ($HOMA_{insulin\ sensitivity}$) and the

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function of pancreatic β-cells to produce insulin ($HOMA_{\beta\text{-cell function}}$) (Matthews et al., 1985; Verkest et al., 2010). In turn, insulin sensitivity can be calculated by the HOMA-IR index, which records insulin resistance (Levy et al., 1998). HOMA-IR was found to be high in dogs with PDH (Miceli et al., 2012).

Alterations in the levels of GLP-1 and HOMA indices might be involved in the development of metabolic disorders present in PDH, thus favouring the establishment of diabetes mellitus. Therefore, the objective of this study was to evaluate the concentrations of GLP-1 (basal and post-glucose load), glucose, insulin, $HOMA_{insulin\ sensitivity}$ and $HOMA_{\beta\text{-cell function}}$ in dogs with PDH compared with those found in obese and healthy normal-weight dogs.

2. Materials and methods

2.1. Animals

Eighteen dogs, divided into three groups (n = 6 each group), were studied:

Group A: healthy normal-weight dogs (mean age: 7.3 ± 2.4 years; 4 spayed females and 2 males (1 neutered), mean weight: 15.6 ± 4.5 kg, all mongrel) from the kennel of the Faculty of Veterinary Sciences, University of Buenos Aires (FVSc-UBA).

Group B: obese dogs (mean age: 10.3 ± 3.2 years; 5 females (3 spayed and 2 intact in anoestrus) and 1 neutered male, mean weight: 25.7 ± 7.5 kg, 3 mongrels and 3 of different breeds) taken to the Endocrinology Unit at School Hospital of Veterinary Medicine, FVSc-UBA. The possibility of these dogs having any concurrent endocrine disease (hypothyroidism, PDH, polycystic ovary) or other systemic diseases (heart disease, liver disease, malignant or infectious disease), or had received any medication (including corticosteroids and anticonvulsants) in the 60 days prior to the incorporation of the dogs in the study had been previously ruled out. The diagnosis of obesity was performed considering the body condition score (BCS) on a 9-point scale (Laflamme, 1997). Only dogs with a BCS of 8 or 9 were included.

Group C: dogs with PDH (mean age: 9.3 ± 4.1 years; 4 females (1 spayed and 3 intact in anoestrus since 2 years ago) and 2 males (1 neutered), mean weight: 10.7 ± 5.5 kg, 3 mongrels and 3 of different breeds) who were referred to the aforementioned institution. The diagnosis was made taking into account: clinical symptoms of PDH, cortisol/creatinine ratio in urine before and after oral dexamethasone (Galac et al., 1997), stimulation with ACTH (basal cortisol measurement and 1 hour post intravenous 0.25 mg ACTH), measurement of plasma ACTH, ultrasound of the adrenal glands, and magnetic resonance imaging of sellar region (Gallelli et al., 2010; Kooistra and Galac, 2012). Dogs with PDH were exclusively included (Kooistra and Galac, 2012). Dogs with diabetes mellitus, with an infectious disease (systemic or local), the presence of tumours (except pituitary adenoma) or any other situation capable of generating insulin resistance or an atypical hypercortisolism situation, were excluded. None of these dogs were undergoing any treatment prior to conducting the studies.

2.2. Oral glucose tolerance test (OGTT)

Dogs maintained a solid 12-hour fast. After the first blood sample (basal time), glucose was administered orally (4 g/kg body weight, 50% w/v solution) (Church, 1980; Irvine et al., 2002). The extraction protocol after glucose ingestion was as follows: 15, 30, 60 and 120 minutes (min) to assess glucose, insulin and GLP-1 levels. All extractions were performed in the saphenous vein. No dogs presented diarrhoea or vomiting after the ingestion of glucose.

Blood glucose was assessed using an automated laboratory method (Metrolab Autoanalizer Merck) according to the manufacturer's instructions. Blood for glucose analysis was collected in glass tubes with sodium fluoride and EDTA as the anticoagulant (Anticoagulant G; Wiener Laboratory, Argentina).

2.3. Determining GLP-1 and insulin

Samples for insulin and GLP-1 were taken concurrently with the glucose but in different tubes with anticoagulant (EDTA), analysed in duplicate. In the case of GLP-1, the tubes were chilled and appropriate amounts of DPP-4 inhibitor (also known as adenosine deaminase complexing protein-2 or T-cell activation antigen CD26) were added immediately after the blood taking. Within 5 minutes of completing the extraction, the sample was centrifuged for three minutes. The plasma obtained was immediately frozen at –80 °C until processing.

Insulin concentrations were measured by means of specific enzyme immunoassay (EIA) for dogs and pigs (ALPCO Insulin Porcine/Canine EIA Alpco Diagnostic Immunoassays, USA), with intra- and inter-assay variation coefficients (canine performance) of 4.2% and 4.3%, respectively, and sensitivity of 0.05 pmol/L.

Concentrations of GLP-1 (7-36 and 7-37 active forms amide) were tested by immunofluorescent ELISA (Glucagon-like peptide-1 [active] ELISA Kit, Linco PLGA-35K, Linco Research, St. Charles, MO, USA). The reading of the plate was performed in a fluorescence plate reader at excitation/emission wavelengths of 355/460 nm. The sensitivity of the kit was 1.5 pM; intra- and inter-assay variation coefficients were 7.4 ± 1.1 and 8 ± 4.8, respectively.

2.4. $HOMA_{\text{insulin sensitivity}}$ and $HOMA_{\beta\text{-cell function}}$

Both insulin sensitivity fasting and the role of pancreatic β-cells were assessed by HOMA indices. $HOMA_{insulin\ sensitivity}$ and $HOMA_{\beta\text{-cell function}}$ were calculated with nonlinear formulas (Levy et al., 1998) in HOMA Calculator Version 2.2.2; Diabetes Trial Unit, University of Oxford, UK (http://www.dtu.ox.ac.uk/index.php?maindoc_/homa/), and validated in dogs (Verkest et al., 2010). In those cases in which insulin concentrations exceeded the maximum value allowed by the HOMA calculator, the mathematical formula (Matthews et al., 1985) was used to calculate the aforementioned indices:

$$HOMA_{insulin\ sensitivity}: ([Glucose] \times [Insulin])/22.5$$

$$HOMA_{\beta\text{-cell function}}: (20.[Insulin]/[Glucose] - 3.5)$$

2.5. Statistical analyses

The intra- and inter-group comparisons were performed using the nonparametric ANOVA test followed by the median multiple comparison test (Dunn's test). In addition, whether there was any correlation between the studied variables was also evaluated (Spearman test). Data are expressed as median and range or median and interquartile ranges as indicated. The level of significance was $P < 0.05$ and a tendency (T) is indicated when $P \geq 0.05$ and ≤ 0.07.

By means of the mixed-model (two-way ANOVA) the effects of sex and body/health status (control, obese/PDH) on glucose, insulin and GLP-1 were analysed. Prior to performing this study, all variables were normalised into the logarithm form.

The statistical analyses were performed by means of GraphPad Prism 5.0 software.

2.6. Ethical approval

The Ethics Committee of the School of Veterinary Science and the Office of Science at the University of Buenos Aires approved the present study, according to the laws on experimentation in animals