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Glycosylated Hemoglobin Concentrations in Dogs with Hyperadrenocorticism and/or Diabetes Mellitus Compared to Clinically Healthy Dogs

Yu-Hsin Lien, DVM, MVM; Hui-Pi Huang, DVM, PhD

High-fasting blood glucose concentrations are commonly associated with insulin antagonism in hyperadrenocorticism. The aim of this study was to investigate glycosylated hemoglobin concentrations in clinically healthy dogs and in dogs with pituitary-dependent hyperadrenocorticism and/or diabetes mellitus. The effect of trilostane treatment on glucose and glycosylated hemoglobin concentration in dogs with pituitary-dependent hyperadrenocorticism was also evaluated. A total of 162 client-owned dogs were investigated. Subjects were 65 clinically healthy dogs, 72 non-diabetic hyperadrenocorticism dogs, and 25 pre-treated diabetic dogs. Hemoglobin A1c concentration in blood was determined by immunoturbidimetrical assay. Hemoglobin A1c concentrations in the clinically healthy group, dogs with non-diabetic hyperadrenocorticism, and diabetic dogs were 4.5 ± 0.4%, 4.7 ± 0.5%, and 7.7 ± 2.1%, respectively. Hemoglobin A1c concentrations were significantly different among the three groups (P < 0.001). Trilostane treatment decreased hemoglobin A1c concentrations of 20 dogs with non-diabetic hyperadrenocorticism (before treatment: 4.8 ± 0.5%, after treatment: 4.6 ± 0.5%; P < 0.001), but not glucose concentrations (before treatment: 103.2 ± 17.3 mg/dL, after treatment: 103.0 ± 15.3 mg/dL; P = 0.16). Glycosylated hemoglobin A1c concentrations correlated strongly with glucose levels (P < 0.001, r = 0.85) and moderately with pack cell volume (P = 0.004, r = 0.63) and hemoglobin concentrations (P = 0.009, r = 0.62). The applicability of glycosylated hemoglobin A1c concentrations may be of clinical value in assessment of PDH management. In addition to blood glucose, interpretation of glycosylated hemoglobin concentration should take into consideration pack cell volume and hemoglobin concentration.

Key Words: glycosylated hemoglobin, hyperadrenocorticism, dog

Introduction

High-fasting blood glucose concentrations are commonly associated with insulin antagonism in hyperadrenocorticism. Depending on the severity of insulin resistance, diabetes mellitus (DM) may eventually develop. Th reported incidence of DM in canine hyperadrenocorticism has been reported to range from 0–10%. From the Graduate Institute of Veterinary Medicine and Department of Veterinary Medicine, National Taiwan University, Taipei 106, Taiwan. Correspondence: Dr. Hui-Pi Huang E-mail: hphuang@ntu.edu.tw Presented in part as a poster at the 2008 Congress of the European College of Veterinary Internal Medicine-Companion Animal, Ghent, Belgian.

trations and erythrocyte lifespan, hemoglobin reacts with glucose to become glycosylated after it is synthesized. Among hemoglobin variants, levels of the hemoglobin A1c (HbA1c) correlate positively with plasma glucose concentrations. Hemoglobin A1c concentrations are not significantly altered by acute or transient hyperglycemia, however they are changed by chronic hyper- or hypo-glycemia. Levels of HbA1c are retrospective indices of the average glucose concentration over a period of 2 to 3 months, and have been used extensively in humans as a long-term monitor of diabetic control.

The aims of this study were to investigate HbA1c concentrations in clinically
healthy dogs and in dogs with pituitary-dependent hyperadrenocorticism (PDH) and/or DM. The effect of treatment on HbA1c concentration in dogs with PDH was also evaluated.

Materials and Methods

Criteria for selection of cases
A total of 162 dogs from the Section of Small Animal Internal Medicine, National Taiwan University Veterinary Hospital were investigated with informed consent obtained from the dogs’ owners. Study subjects included 65 clinically healthy dogs (16 intact females, 16 spayed females, 24 intact males, 9 neutered males; mean age: 6.3 ± 4.4 years), 72 non-diabetic dogs with PDH (15 intact females, 27 spayed females, 19 intact males, 11 neutered males; mean age 11.4 ± 2.4 years), and 25 pre-treated diabetic dogs (3 intact females, 11 spayed females, 8 intact males, 3 neutered males; mean age 9.7 ± 3.4 years). Among the 25 diabetic dogs, 13 had DM only and 12 had DM with concurrent PDH. Dogs with PDH and/or DM were identified prospectively.

Pituitary-dependent hyperadrenocorticism diagnostic criteria and prerequisites for study inclusion were: (1) clinical signs consistent with canine hyperadrenocorticism, such as polydipsia, polyuria, polyphagia, decreased activity, panting, potbellied appearance, dermatologic problems; (2) routine biochemical test results consistent with canine hyperadrenocorticism, such as elevated hepatic enzyme levels; (3) conclusive adrenocorticotropic hormone (ACTH) stimulation test results (0.25 mg, intramuscular injection); and (4) abdominal ultrasonographic findings.

Only dogs with PDH were included in this study. Dogs with inconclusive ACTH stimulation test results were excluded from the study. Likewise, dogs in which ultrasonography revealed adrenal glands with a nodular (or mass) appearance and ultrasonography revealed hyperrechoic foci were excluded from the study because such foci may be indicative of adrenal tumors.

Diagnosis criteria of DM and the prerequisites for study inclusion were: (1) clinical signs consistent with canine DM, such as polydipsia, polyuria, polyphagia, and weight loss; (2) persistent and 24-hour fasting hyperglycemia (> 200 mg/dL) for more than 2 days; (3) persistent glycosuria for more than 2 days; and (4) insulin supplementation required to maintain normal glucose levels.

Sample collection
Twelve hours fasting blood samples were collected by jugular or cephalic venipuncture and placed in upright EDTA and polypropylene tubes. Total hemoglobin, pack cell volume and HbA1c concentrations were measured from the samples. Serum was then harvested by centrifugation and aspirated for glucose concentration measurement.

Analytical procedures
Concentrations of HbA1c were determined using a commercial in vitro immunoturbidimetric assay carried out in an automatic analyzer. The intra-assay coefficients of variation during the study ranged from 1.5–4.8%, and inter-assay coefficients of variation ranged from 2.1–5.3%. Total hemoglobin concentration and pack cell volume were calculated automatically using an autoanalyzer. Serum glucose concentrations were determined using a commercial procedure on an autoanalyzer. Cortisol concentrations were measured using a validated radioimmunoassay.

Effects of Trilostane on glucose and HbA1c concentrations in dogs with pituitary-dependent hyperadrenocorticism
Twenty dogs affected with pituitary-dependent hyperadrenocorticism were administered trilostane orally for 90 days. Concentrations of HbA1c were measured prior to and 90 days after treatment.

Statistical analysis
Comparison of pack cell volume, hemoglobin, glucose, and HbA1c concentrations between clinically healthy, PDH, and DM dogs were analyzed using an analysis of variance (ANOVA) for repeat measures. Regression analysis between HbA1c concentrations and pack cell volume, as well as hemoglobin and glucose concentrations were evaluated using general linear model procedures. Effects of hyperadrenocorticism treatment on HbA1c were determined using paired t-tests. All statistical analyses were performed using SPSS software. Data are presented as mean ± standard deviation and statistical significance was set at \( P \leq 0.05 \).

**Results**

Mild hyperglycemia (120-140 mg/dL) was found in 14 of 65 clinically healthy dogs and 13 of 72 dogs with non-diabetic PDH. Glucose concentrations were significantly different among the three groups \( (P < 0.001) \). Table 1 shows the results for pack cell volume, and hemoglobin, serum glucose, and HbA1c concentrations in dogs with PDH and DM compared to clinically healthy dogs.

Hemoglobin A1c concentrations were significantly different among the three groups \( (P < 0.001) \). Glycosylated hemoglobin concentrations correlated strongly with glucose concentration \( (P < 0.001, r = 0.85; \text{Figure } 1) \), moderately with both pack cell volume \( (P = 0.004, r = 0.63; \text{Figure } 2) \), and hemoglobin levels \( (P = 0.009, r = 0.62; \text{Figure } 3) \).

**Effects of Trilostane on Glucose and HbA1c concentrations** — The pre-ACTH cortisol concentrations of 20 dogs with PDH were 3.7 ± 2.1 \( \mu g/dL \) (range: 1.0–7.9 \( \mu g/dL \); reference range: 0.5–3 \( \mu g/dL \)); post-ACTH concentrations were 27.4 ± 8.7 \( \mu g/dL \) (range: 17.6–53.1 \( \mu g/dL \); reference range: < 16 \( \mu g/dL \); > 16 \( \mu g/dL \) for diagnosing hyperadrenocorticism). Suppressed adrenal function was

![Figure 1 — Relationship between concentrations of blood glucose and glycosylated hemoglobin HbA1c \( (P < 0.001, r = 0.85) \)](image1)

![Figure 2 — Relationship between pack cell volume and concentrations of glycosylated hemoglobin HbA1c \( (P = 0.004, r = 0.63) \)](image2)

![Figure 3 — Relationship between concentrations of hemoglobin and glycosylated hemoglobin HbA1c \( (P = 0.009, r = 0.62) \)](image3)
Table 1. Mean pack cell volume, and hemoglobin, glucose, and glycosylated hemoglobin (HbA1c) concentrations in dogs with pituitary-dependent hyperadrenocorticism (PDH) and diabetes mellitus (DM) compared to clinically healthy dogs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Healthy (n = 65)</th>
<th>PDH (n = 72)</th>
<th>DM (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pack cell volume (%)</td>
<td>44.3 ± 6.2</td>
<td>43.3 ± 6.0</td>
<td>40.1 ± 9.5</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.3 ± 2.2</td>
<td>15.1 ± 2.2</td>
<td>13.9 ± 3.3</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>107.1 ± 15.6</td>
<td>103.2 ± 24.8</td>
<td>298.1 ± 63.6*+</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.5 ± 0.4</td>
<td>4.7 ± 0.5*</td>
<td>7.7 ± 2.2*+</td>
</tr>
</tbody>
</table>

* P < 0.001 compared to healthy dogs; + P < 0.001 compared to PDH dogs.

Table 2. Effects of trilostane treatment for pituitary-dependent hyperadrenocorticism on pack cell volume, and hemoglobin, glucose, and glycosylated hemoglobin (HbA1c) levels (n = 20).

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pack cell volume (%)</td>
<td>46.0 ± 4.7</td>
<td>44.9 ± 4.8</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>16.1 ± 2.0</td>
<td>15.6 ± 1.8</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>103.2 ± 17.3</td>
<td>103.0 ± 15.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8 ± 0.5</td>
<td>4.6 ± 0.5</td>
</tr>
</tbody>
</table>

found in all 20 cases after 90 days of trilostane treatment (1.8 ± 0.4 mg/kg [0.8 ± 0.2 mg/lb], q 24 h, ranged 0.8–3.4 mg/kg [0.4–1.5 mg/lb]); the mean cortisol concentration before ACTH stimulation was 2.2 ± 1.0 μg/dL (range: 1.1–4.9 μg/dL), and the mean cortisol concentration after ACTH stimulation was 7.8 ± 4.0 μg/dL (range: 2.6–15.4 μg/dL). Trilostane treatment significantly decreased cortisol concentrations both before and after ACTH stimulation (P < 0.001, P < 0.001, respectively).

Concentrations of HbA1c in the 20 dogs with non-diabetic PDH were assessed both before and after treatment for hyperadrenocorticism with oral trilostane for 90 days. Trilostane treatment did not affect glucose concentrations (P = 0.16, Table 2), but decrease HbA1c concentrations (P < 0.001, Table 2).

Discussion

In this study, mean serum glucose concentrations of dogs with non-diabetic PDH did not differ from clinically healthy dogs, although the incidence of mild hyperglycemia was slightly higher in the latter (21.5%) than the former (18.1%). Nevertheless, concentrations of HbA1c were significantly increased in PDH dogs compared to healthy dogs, a finding that contradicted a previous study.3

A single blood glucose measurement may not be sufficient to identify mild but prolonged hyperglycemia caused by insulin antagonism in dogs with PDH. On the other hand, HbA1c is an index of mean blood glucose concentrations across weeks and glycosylated levels would not be affected by acute or transient hyperglycemia15,17 due to sudden physiological stress, such as that induced by visiting a veterinary hospital. Therefore elevated HbA1c concentrations reflect prolonged hyperglycemia. Hence, this measure might provide an objective marker of propensity to develop hyperglycemia and DM in dogs with PDH.

In this study, a significant trend of decreased HbA1c concentrations was detected after 3 months of trilostane treatment for PDH. However, significant changes of glucose concentrations were not detected. Dogs with non-diabetic PDH in this study did not receive treatment for hyperglycemia, and none of the non-
diabetic PDH dogs developed hypoglycemia during the study period. Glycosylated hemoglobin A1c reflects mean blood glucose concentrations across weeks and glycosylated levels would not be affected by acute hyperglycemia or hypoglycemia.\textsuperscript{15,17,18} This finding suggests that the applicability of HbA1c concentrations may be of clinical value in assessment of PDH management.

Our results showed that HbA1c levels correlated with blood glucose concentration, and significantly increased in diabetic dogs. These findings corroborate the findings of previous studies.\textsuperscript{10,12,13} HbA1c concentrations were also correlated to pack cell volume and hemoglobin levels. Because the relationship between HbA1c and plasma glucose concentration is multi-faceted, interpretation of HbA1c levels should consider pack cell volume and hemoglobin concentration data in addition to blood glucose data.

\section*{Footnotes}
\begin{itemize}
  \item[a.] Cortrosyn; Organon, Oss, The Netherlands.
  \item[b.] Cobas Integra System, F. Hoffmann-La Roche Ltd, Basel, Switzerland.
  \item[c.] VetAutoread analyzer, IDEXX Laboratories, Inc, Westbrook, MA, USA.
  \item[d.] VetTest Chemistry Analyzer, IDEXX Laboratories, Inc, Westbrook, MA, USA.
  \item[e.] Coat-A Count Cortisol; Diagnostic Products Corporation, Los Angeles, CA, USA.
  \item[f.] Vetoryl, Dechra Ltd, Shrewsbury, UK, USA.
  \item[g.] Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL, USA.
\end{itemize}

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\end{enumerate}