Measurement of glycated hemoglobin percentages for use in the diagnosis and monitoring of diabetes mellitus in nonhuman primates

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Objective—To identify a technique for measurement of glycated hemoglobin percentage in blood samples obtained from various species of nonhuman primates (NHPs), to determine whether these percentages varied with respect to glycemic control, and to assess whether this physiologic variable provided a suitable test for diagnosing diabetes mellitus in NHPs.

Sample Population—166 blood samples collected from 121 NHPs comprising 22 species from the Haplorhine and Strepsirhine suborders and including nondiabetic, treated-diabetic, and diabetic animals in 23 zoologic institutions throughout the United States.

Procedure—Hemoglobin A_{1c} percentage was measured in 154 samples by use of high-performance liquid chromatography. Total glycated hemoglobin percentage was measured in 159 samples by use of a boronate-affinity chromatographic assay. Glucose concentration was measured in 157 samples with an autochemical analyzer by use of a hexose kinase method.

Results—The boronate-affinity chromatographic technique for measurement of total glycated hemoglobin percentage was the most suitable method. Nondiabetic Haplorhines had percentages higher than those in nondiabetic Strepsirhines. In Haplorhines, diabetic animals had percentages higher than those in treated-diabetic animals, which had percentages higher than those in nondiabetic animals. In Strepsirhines, this pattern was less pronounced.

Conclusions and Clinical Relevance—Measurement of total glycated hemoglobin percentage provides useful information for diagnosing diabetes mellitus in Haplorhines and, possibly, in Strepsirhines. Until reference ranges are established for each species, it is recommended that results for samples from NHPs without clinical signs of diabetes mellitus be compared with results of samples collected concomitantly from NHPs with clinical signs of this condition. (*Am J Vet Res* 2003;64:562–568)

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Diabetes mellitus is a disease that affects numerous **nonhuman primates (NHPs)**.[1] It can develop naturally or be induced experimentally by the destruction of pancreatic β cells. Type-2 diabetes mellitus is most common and reflects the problem of obesity in captive populations. Clinical signs vary, and there is often a gradual progression of the disease.

Traditional tests for the detection of diabetes mellitus include measurement of fasted plasma glucose concentrations, measurement of urine glucose concentrations, oral and IV glucose tolerance tests, measurement of urine ketone concentrations, assessment of serum fructosamine concentrations, and measurement of fasted plasma insulin concentrations.[1-5] Diagnostic criteria are ideally based on the risk of developing long-term microvascular complications.[6-9] However, such tests present challenges in NHPs. These include difficulty in sample collection, necessity for anesthesia with the potential for drug interactions, multiple confounding factors (eg, activity, duration of food withholding, or diet), stress hyperglycemia attributable to restraint (ie, catecholamine release suppressing insulin secretion), and lack of established reference ranges.[10-12]

Glycated **hemoglobin (Hb)** is produced when a carbohydrate, such as glucose, attaches to an Hb molecule, such as HbA_o, and undergoes intermolecular transformation to form a stable glycated ketoamine product.[13] This process is dependent on the current glucose concentration and occurs continuously throughout the lifespan of RBCs. Consequently, in humans, whose RBCs have a lifespan of 120 days, glycated Hb percentage represents the integrated value for glucose of the preceding 6 to 8 weeks.[4] A number of forms of glycated Hb have been identified and vary with respect to the attached sugar residue. They include HbA_{1a} (fructose-1,6-diphosphate or glucose-6-phosphate), HbA_{1b} (ketamine-linked pyruvic acid), and HbA_{1c} (glucose).[14] This last form is the most common, constituting approximately 80% of HbA_1.

Methods routinely used for measurement of glycated Hb percentage separate the molecule on the basis of its charge, structure, or antigenic properties.[4] The most popular methods rely on the increased negative charge found in the glycated Hb molecule to distinguish it from its nonglycated form. These assays include electrophoresis and ion-exchange chromatography. The latter is commonly performed on **high-performance liquid chromatography (HPLC)** instruments that will measure HbA_1, HbA_{1c}, or both. Alternatively, boronate-affinity chromatography separates glycated Hb on the basis of its structure rather than its charge.

In this assay, separation is the result of carbohydrate moieties on the glycated Hb molecule binding by condensation to the affinity reagent, di-hydroxyboronate. This method is specific for all glycated Hbs, irrespective of molecular charge or the site of glycation on the Hb molecule. In addition, it is also able to detect the glycated portion of Hb in patients with Hb variants such as Hb S, C, or F. Thus, the term total glycated Hb has been used to describe this type of assay. Finally, immunoassays have been developed for the measurement of HbA_{1c}. Monoclonal or polyclonal antibodies have been raised against the 6- to 8-amino acid peptide of the glycated amino terminus of the β-globin chain.

Advantages for the measurement of glycated Hb percentage include the simplicity of the technique; need for only a single blood sample; the fact that it is relatively unaffected by recent food intake, activity, illness, and stress; and the fact that it reflects glycemic control during the preceding few weeks. Unfortunately, there are also a number of disadvantages associated with measurement of glycated Hb percentage and use of this assessment.[4] Fundamentally, there is lack of standardization among the various methods or instruments that are used to measure the various forms of glycated Hb (ie, HbA_1, HbA_{1c}, or total glycated Hb), and even methods that purport to measure the same analyte can have widely differing reference ranges and yield varying results for patient samples. Other confounding factors include hemoglobinopathies, Hb variants (eg, fetal Hb), Hb derivatives (eg, acetylated Hb), age-related changes, labile Hb (eg, Schiff-base), and altered lifespan of RBCs. For example, processes that decrease the mean lifespan of RBCs will reduce the availability of Hb for glycation and, therefore, the percentage of glycated Hb, irrespective of glucose concentrations. Similarly, conditions that increase the lifespan of RBCs (eg, iron deficiency) will increase the amount of glycated Hb.

Measurement of glycated Hb percentage is currently not recommended for the diagnosis of diabetes in humans, although the frequency distributions for HbA_{1c} have characteristics similar to those of the fasted plasma glucose concentration and the plasma glucose concentration in samples obtained at 2 hours during the oral glucose tolerance test.[7] Moreover, these results have defined a HbA_{1c} percentage above which the likelihood of having or developing macro- or microvascular disease increases sharply. This equates to a value ≥ 1% above the upper limit of the reference range for clinically normal people.[15] Furthermore, measurement of HbA_{1c} percentages and fasted plasma glucose concentrations (in type-2 diabetes) has become the choice for use in monitoring treatment of diabetic humans, and decisions on when and how to implement treatment are often made on the basis of HbA_{1c} percentages. These observations have led some to recommend measurement of HbA_{1c} percentage as a diagnostic test.[7,16,17] Nevertheless, the lack of standardization remains a problem.

Glycated Hb percentages have also been measured, to a limited degree, in nondiabetic, borderline-diabetic, and confirmed-diabetic NHPs.[12,18-20] This has involved a relatively low number of species and various methods. Consequently, it is difficult to make firm conclusions that will reflect all species; however, a few points can be made. Quantities of HbA_1 and HbA_{1c} in nondiabetic baboons (*Papio anubis*), measured by use of cation-exchange chromatography, are approximately half of those found in humans.[18] It has been suggested that this may be explained on the basis of differences in the lifespan of RBCs in humans (100 to 120 days) and baboons (30 to 60 days). Variations may also be related to differences in permeability of RBCs to glucose.[12] At least 2 studies[19-20] have documented an increase in HbA_{1c} percentages in borderline-diabetic or confirmed-diabetic NHPs. In 1 group of Celebes crested macaques (*Macaca nigra*), the HbA_{1c} value increased from 2.6% in nondiabetic macaques to 7.9% in diabetic macaques, as measured by use of electrophoresis.[19] In addition in that study, macaques without overt hyperglycemia but with impaired glucose clearance, impaired insulin secretion, and increased postprandial glucose concentrations had a significant increase in HbA_{1c} content to 3.5%. Reference ranges for glycated Hb percentages have not been determined in many NHPs, but values that increase by ≥ 10% are generally considered to be abnormal.[1] Measurement of glycated Hb percentages remains useful in determining the efficacy of treatment.

Comparison of the various methods for measuring glycated Hb percentages in NHPs is difficult because of the lack of published results. It is worth mentioning, however, that some tests are more likely to be accurate in several species of NHPs, compared with other tests. For example, the boronate-affinity chromatographic technique is probably, through its attraction to a broad range of carbohydrate moieties, less species-specific than an immunoassay, which relies on attraction to a specific amino acid sequence.

Objectives of the study reported here were to identify a technique for measurement of glycated Hb percentage in blood samples obtained from various species of NHPs, to determine whether these percentages varied with respect to glycemic control (ie, changed in animals with diabetes mellitus), and to assess whether this physiologic variable provided a suitable test for use in diagnosing diabetes mellitus in NHPs. This would allow changes in management and treatment of diabetic animals before onset of clinical signs.

Materials and Methods

Blood samples were collected opportunistically from NHPs in zoologic institutions throughout the United States. They encompassed various species from the Haplorhine and Strepsirhine suborders and included nondiabetic, treated-diabetic, and diabetic animals. For each sample, biological data, restraint method, health status, duration of food withholding, activity before collection of the sample, and blood vessel from which the sample was collected, as well as the person who collected the sample, were recorded. A single blood sample was obtained from most animals, but some animals contributed more than 1 sample, which were obtained on different days.

Immediately after collection of a sample, at least 1 mL of blood was transferred into a tube containing EDTA, which was used for measurement of total glycated Hb and HbA_{1c} percentages, and 1 mL was transferred into a tube containing a glycolytic inhibitor and anticoagulant (sodium fluoride and potassium oxalate), which was used for determination of glu-

cose concentration. The EDTA-containing tube was maintained at 4°C and submitted for analysis, which was performed within 7 days after collection. The fluoride-oxalate tube was centrifuged within 4 hours after collection, and plasma was decanted and tested immediately or frozen (–20°C) for subsequent analysis, which was performed within 7 days. Samples were transported in insulated containers on ice packs.

Hemoglobin A_{1c} percentage was initially measured by use of an immunoassay[a] and an HPLC system. The immunoassay was quickly discontinued, because the HbA_{1c} percentage was consistently less than the lower limit of the test (< 2.5%). The HPLC technique was performed on an automated analyzer[b] and used an assay certified by the National Glycohemoglobin Standardization Program traceable to the Diabetes Control and Complications Trial Reference Method.[c] Total glycated Hb percentage was measured by use of an affinity-based chromatography assay,[d] which is reported[21,22] in humans to correlate well with results of other methods. Total interassay precision was determined by including quality-control samples each time the assay was performed.[e] Glucose concentration was measured in an autochemical analyzer[f] by use of a hexose kinase method.

A commercially available technical computing program[g] was used for graphic analyses. Dot plots of glucose concentration versus diabetic classification, subdivided on the basis of suborder or duration of withholding of food, were prepared to evaluate the diabetic classification (nondiabetic, treated-diabetic, or diabetic) used by the submitting institutions. Suggested criteria for diabetic classification of subjects were derived from other studies[1,10] that involved the use of NHPs (**Appendix**).

A commercially available statistical program[h] was used for statistical analyses. The primary factor of interest was the relationship of total glycated Hb percentage and diabetic classification. However, because of the diversity of animal characteristics in our sample, we included a number of additional variables in our model to protect against inaccurately attributing a significant correlation between total glycated Hb content and diabetic classification that was actually attributable to an imbalance of another important covariate among the diabetic groups. Therefore, total glycated Hb percentage was modeled as a function of suborder (Haplorhine or Strepsirhine), superfamily or family within suborder (great apes, lesser apes, New World monkeys, Old World monkeys, or lemurs), species within superfamily or family within suborder, age, body weight, sex (male or female), contraception within sex (sexually intact, castrated, ovariohysterectomized, administered estrogen, or administered progestin), method of contraception within contraception within sex (eg, medroxyprogesterone acetate[i] within progestin within females), restraint method (manual or chemical), diabetic classification, duration of food withholding (< 8 hours or ≥ 8 hours), activity before collection of sample (calm, active, or excited), and animal identity. Multiple samples from the same animal were modeled as random repeated measures, and all other variables were treated as fixed effects in a mixed-model ANOVA.[23] Values of $P < 0.05$ were considered significant.

Results

One hundred sixty-six blood samples were collected from 121 NHPs in 23 zoologic institutions. The NHPs represented 18 species of Haplorhines and 4 species of Strepsirhines. There were 107 nondiabetic

Table 1—Diabetic classification of nonhuman primates from which samples were obtained

Taxon	Diabetic classification	Number of animals
Haplorhines	52 ND, 5 D, 1 D/TD, 3 TD	61
Great apes	10 ND, 1D	11
Chimpanzee (*Pan troglodytes*)	2 ND, 1 D	3
Sumatran orangutan (*Pongo pygmaeus abelii*)	6 ND	6
Western lowland gorilla (*Gorilla gorilla gorilla*)	2 ND	2
Lesser apes	7 ND, 1D	8
Agile gibbon (*Hylobates agilis*)	3 ND	3
Siamang (*Hylobates syndactylus*)	4 ND, 1 D	5
New World monkeys	7 ND	7
Black-handed spider monkey (*Ateles geoffroyi*)	1 ND	1
Black howler monkey (*Alouatta caraya*)	1 ND	1
Cotton- top tamarin (*Saguinus oedipus*)	3 ND	3
Pale-headed saki monkey (*Pithecia pithecia*)	2 ND	2
Old World monkeys	28 ND, 3D, 1D/TD, 3 TD	35
Black and white colobus monkey (*Colobus guereza*)	4 ND	4
DeBrazza's monkey (*Cercopithecus neglectus*)	3 ND, 1 D/TD	4
L'hoest's monkey (*Cercopithecus lhoesti*)	2 ND, 1 TD	3
Lion-tailed macaque (*Macaca silenus*)	12 ND, 1 D	13
Mandrill baboon (*Mandrillus sphinx*)	1 TD	1
Schmidt's spot-nosed guenon (*Cercopithecus ascanius schmidti*)	1 ND	1
Spectacled langur (*Presbytis obscurus*)	2 ND	2
White-collared/red-capped mangabey (*Cercocebus torquatus*)	4 ND, 2 D, 1 TD	7
Strepsirhines	55 ND, 1 D/TD, 4 TD	60
Lemurs	55 ND, 1 D/TD, 4 TD	60
Black and white ruffed lemur (*Varecia variegata variegata*)	8 ND, 1 TD	9
Black lemur (*Eulemur macaco macaco*)	7 ND, 1 TD	8
Red ruffed lemur (*Varecia variegata rubra*)	1 ND	1
Ring-tailed lemur (*Lemur catta*)	39 ND, 1 D/TD, 2 TD	42
Total	**107 ND, 5 D, 2 D/TD, 7 TD**	**121**

D = Diabetic. D/TD = Sample obtained from diabetic animal before treatment, then samples obtained from same animal after treatment was initiated. TD = Treated-diabetic. ND = Nondiabetic.

animals, and 14 diabetic or treated-diabetic animals. Eighty-eight nondiabetic animals contributed a single sample, 14 contributed 2 samples, and 5 contributed between 3 and 6 samples. Eight diabetic or treated-diabetic animals contributed a single sample, and 6 contributed between 2 and 5 samples. The number of nondiabetic, diabetic, and treated-diabetic animals represented within each species and taxonomic group wascalculated (**Table 1**). Two of the diabetic animals contributed blood samples before and after initiation of treatment for diabetes.

Data were not available for all variables for all samples. Glucose concentration was measured in 157 samples from 116 animals, HbA_{1c} percentage was measured in 154 samples from 119 animals, and total glycated Hb percentage was measured in 159 samples from 118 animals. Total interassay precision for the total glycated Hb assay was 11% (mean, 5.47%; n = 67 assays).

Dot plots of glucose concentration versus diabetic classification, subdivided on the basis of suborder or duration of food withholding, were created (**Fig 1 and**

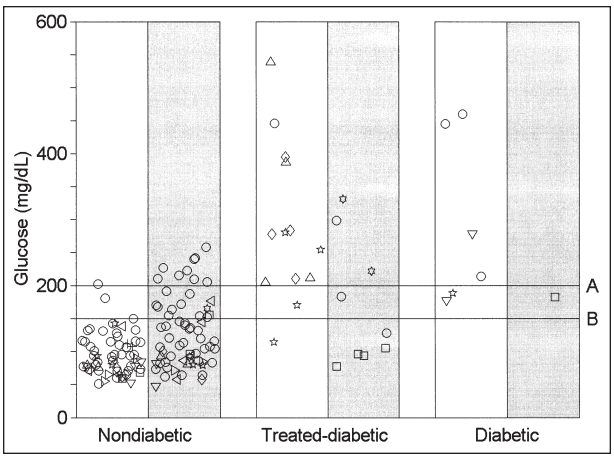

Figure 1—Dot plot of glucose concentration versus diabetic classification assigned by the submitting institution for samples obtained from nonhuman primates, subdivided on the basis of suborder (Haplorhine [white area] or Strepsirhine [gray-shaded area]). Results are reported for 157 samples obtained from 116 animals. Animals from which a single sample was obtained are denoted by open circles, whereas each animal from which ≥ 2 samples were obtained is denoted by another unique symbol. Cutoff values for glucose concentration are indicated by horizontal lines (A, 200 mg/dL; B, 150 mg/dL).

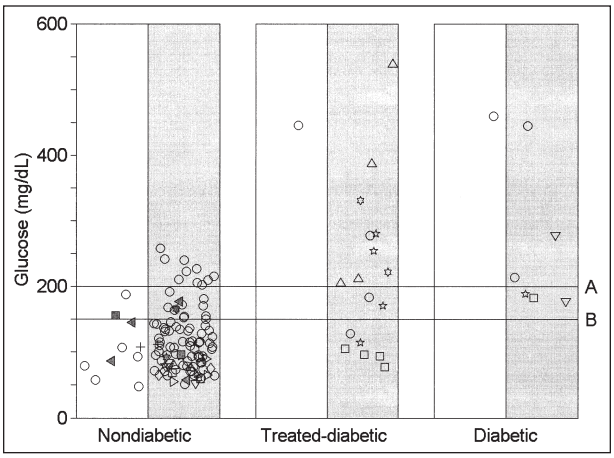

Figure 2—Dot plot of glucose concentration versus diabetic classification assigned by the submitting institution for samples obtained from nonhuman primates, subdivided on the basis of duration of withholding of food (< 8 hours [white area] or ≥ 8 hours [gray shaded area]) prior to collection of the sample. Results are reported for 138 samples obtained from 108 animals. Animals from which a single sample was obtained are denoted by open circles, whereas each animal from which ≥ 2 samples were obtained is denoted by another unique symbol. Cutoff values for glucose concentration are indicated by horizontal lines (A, 200 mg/dL; B, 150 mg/dL).

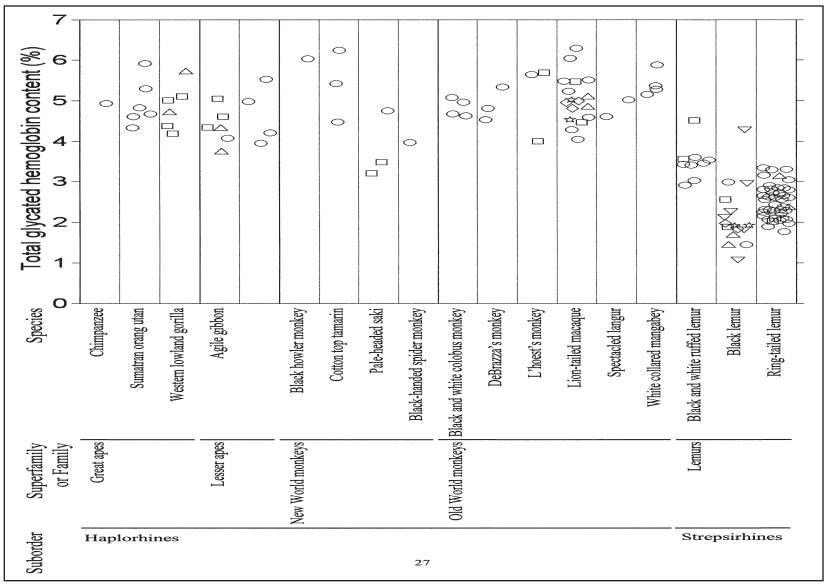

Figure 3—Dot plot of total glycated hemoglobin percentage in samples obtained from nondiabetic, nonhuman primates, subdivided on the basis of suborder, superfamily or family, and species. Results are reported for 130 samples obtained from 104 animals. Animals from which a single sample was obtained are denoted by open circles, whereas each animal from which ≥ 2 samples were obtained is denoted by another unique symbol.

2). All samples (7/7; 100%) from diabetic animals, 16 of 22 (73%) samples from treated-diabetic, and 104 of 128 (81%) samples from nondiabetic animals met the suggested criteria. Those that did not were predominantly from Strepsirhines (ie, 5 of 6 [83%] of the nonconforming samples from treated-diabetic animals and 21 of 24 [88%] of the nonconforming samples from nondiabetic animals). Only 18 of 22 samples from treated-diabetic animals were used, because 4 samples did not have information on the duration of food withholding. However, each of those 4 samples had glucose values > 200 mg/dL and, therefore, met the suggested criteria irrespective of duration of food withholding.

Tests of fixed effects from the mixed-model ANOVA relating total glycated Hb percentage to diabetic classification and other possible covariates were performed. One hundred twenty-three samples obtained from 98 animals had complete data for all variables included in the model. Results of tests revealed significant effects for suborder ($P < 0.001$), superfamily or family within suborder ($P = 0.025$), species within superfamily or family within suborder ($P < 0.001$), and diabetic classification ($P < 0.001$).

A dot plot of total glycated Hb percentages in nondiabetic animals, subdivided on the basis of suborder, superfamily or family, and species, was created (**Fig 3**).

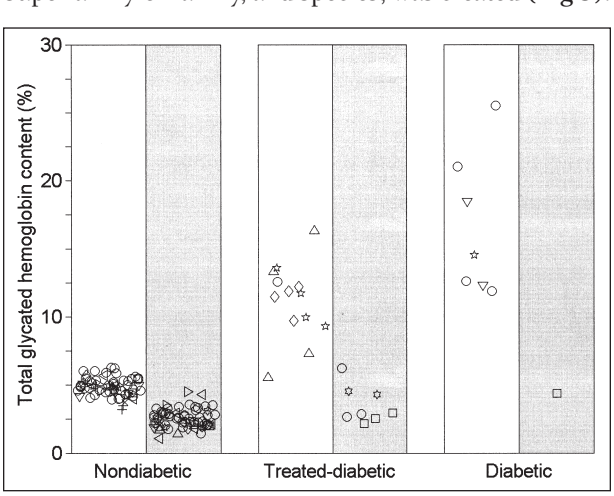

Figure 4—Dot plot of total glycated hemoglobin percentage versus diabetic classification assigned by the submitting institution for samples obtained from nonhuman primates, subdivided on the basis of suborder (Haplorhine [white area] or Strepsirhine [gray-shaded area]). Results are reported for 159 samples obtained from 118 animals. Animals from which a single sample was obtained are denoted by open circles, whereas each animal from which ≥ 2 samples were obtained is denoted by another unique symbol.

Table 2—Total glycated hemoglobin percentage in nondiabetic, nonhuman primates, stratified on the basis of suborder

Suborder*	Mean	SD	Minimum	Maximum	95th percentile
Haplorhines (n = 50)	4.94	0.65	3.20	6.29	6.04
Strepsirhines (n = 54)	2.59	0.54	1.45	3.60	3.53

*Samples were collected from 107 nondiabetic, nonhuman primates, but measurements of total glycated hemoglobin percentage were not obtained for 3 animals.

There were clear differences in the distribution of total glycated Hb percentages between Haplorhines and Strepsirhines, with less pronounced variation between superfamily or family and species.

A dot plot of total glycated Hb percentage versus diabetic classification, subdivided on the basis of suborder, was created (**Fig 4**). In Haplorhines, diabetic animals had percentages higher than those in treated-diabetic animals, which had percentages higher than those in nondiabetic animals. In Strepsirhines, differences in total glycated Hb percentages between diabetic animals, treated-diabetic animals, and nondiabetic animals were much less pronounced.

Values characterizing the distribution of total glycated Hb percentages in nondiabetic animals, stratified on the basis of suborder, were calculated (**Table 2**). In the 19 NPHs from which we collected > 1 sample, only the first sample obtained from each animal was included in the calculations.

Discussion

Samples obtained from diabetic NHPs, as classified by each of the submitting institutions, had glucose concentrations that corresponded extremely well (7/7 [100%]) with the suggested criteria derived from other studies with NHPs.[1,10] The same was true for samples obtained from nondiabetic Haplorhines (60/63 [95%]) and treated-diabetic Haplorhines (12/13 [92%]). In contrast, 21 of 65 (32%) samples obtained from nondiabetic Strepsirhines had glucose concentrations that were higher than expected, and 5 of 9 (56%) samples obtained from treated-diabetic Strepsirhines had glucose concentrations that were lower than expected. This may reflect the relatively small size and, therefore, higher basal metabolic rate of Strepsirhines; the quality and quantity of carbohydrates in the diet of Strepsirhines (with the potential for wide but normal fluctuations in blood glucose concentrations); the fact that a higher proportion of samples obtained from Strepsirhines were collected by use of manual restraint (18/74 [24%] in Strepsirhines and 1/78 [1%] in Haplorhines) or when animals were anesthetized with isoflurane following manual restraint (39/74 [53%] in Strepsirhines and 4/78 [5%] in Haplorhines), which resulted in associated stress; or the fact that a higher proportion of samples obtained from Strepsirhines were collected from animals from which food was withheld for < 8 hours (9/63 [14%] in Strepsirhines and 3/75 [4%] in Haplorhines). Indeed, in another unpublished study conducted by our laboratory group in which blood glucose concentrations were assessed in 6 healthy golden-headed lion tamarins (*Leontopithecus chrysomelas*), mean ± SEM blood glucose concentration decreased significantly ($P < 0.001$) from a postprandial value of 381 ± 76 mg/dL to a value of 77 ± 9.9 mg/dL after food was withheld for 8 hours. This raised questions about the appropriate duration of food withholding in NHPs to obtain a fasted blood glucose concentration without causing overt hypoglycemia and about its relationship to species or body weight. It exemplifies the difficulties of the use of blood glucose measurement as a diagnostic test for diabetes mellitus in NHPs.

For the measurement of glycated Hb percentages, it

quickly became apparent that the immunoassay did not work for samples obtained from NHPs, because the HbA_{1c} percentage was consistently less than the lower limit of the test (< 2.5%). This was not surprising, because the assay recognizes the first 6 to 8 amino acid residues of the glycated amino-terminus of the βchain of human HbA, which often differs from that of NHPs. Consequently, we stopped conducting this test. Similarly, the HPLC system yielded results that were often impossible to interpret (ie, the chromatogram did not resemble that of a blood chromatogram in humans, and its shape sometimes varied substantially between repeat samples obtained from the same NHP). This may have been attributable to the fact that HPLC separation has been optimized for human Hb, and NHPs have differing Hb amino acid structures. In contrast, measurement of total glycated Hb percentage by use of the affinity-based assay was simple to perform, and results were easy to interpret and apparently consistent when repeated for the same NHP or with the same sample.

Nondiabetic Haplorhines had total glycated Hb percentages higher than those in nondiabetic Strepsirhines. This difference between suborders was possibly related to RBC permeability to glucose and the lifespan of RBCs. Little is known about permeability to glucose; however, the lifespan of RBCs reportedly[12] is proportional to body weight. Consequently, it may be hypothesized that because the mean ± SEM body weight of 53 of the Strepsirhines used in the study reported here (2.54 ± 0.06 kg) was significantly lower than that of 59 of the Haplorhines (21.2 ± 3.55 kg; body weights were not recorded for 7 Strepsirhines and 2 Haplorhines), the lifespan of their RBCs would be shorter, resulting in lower total glycated Hb contents. Unfortunately, this did not appear to be the case, because there was not a significant difference in total glycated Hb percentage on the basis of body weight.

Overall, diabetic animals had total glycated Hb percentages higher than those in treated-diabetic animals, which had percentages higher than those in nondiabetic animals. The magnitude of the differences between diabetic classes was substantially greater in the Haplorhines than in the Strepsirhines. These differences in total glycated Hb content with respect to diabetic classification confirmed that percentages vary with glycemic control in Haplorhines and possibly in Strepsirhines, although additional samples would be needed to support this contention.

In the study reported here, the boronate-affinity chromatographic technique was the most suitable method for measurement of total glycated Hb percentages in NHPs. It is simple, inexpensive, and can be rapidly performed, and it requires only a single blood sample, which may be stored at 4°C for up to 7 days. Total glycated Hb percentage varies on the basis of glycemic control, and its measurement provides a useful test for diagnosing diabetes mellitus in Haplorhines and possibly Strepsirhines. This will allow caretakers to make changes in management and treatment before the onset of clinical signs and will allow monitoring of the subsequent treatment. However, until reference ranges are established for each species, it is recommended that samples from NHPs without clinical signs of diabetes mellitus be collected and tested concomitantly with samples obtained from NHPs with clinical signs of this condition.

[a]DCA 2000 hemoglobin A_{1c} reagent kit, Bayer Corp, Elkhart, Ind.
[b]Variant hemoglobin testing system, Bio-Rad Laboratories, Hercules, Calif.
[c]Variant hemoglobin A_{1c} program, Bio-Rad Laboratories, Hercules, Calif.
[d]Glyc-Affin GHb, Perkin-Elmer Wallace Inc, Norton, Ohio.
[e]Lyphochek, Bio-Rad Laboratories, Hercules, Calif.
[f]Roche/Hitachi 747, Hitachi Instruments Inc, San Jose, Calif.
[g]MATLAB, The MathWorks Inc, Natick, Mass.
[h]SAS, release 8.2, SAS Institute Inc, Cary, NC.
[i]Depo-Provera contraceptive injection, Pharmacia & Upjohn Co, Kalamazoo, Mich.

Appendix

Suggested criteria for classification of diabetes mellitus that were derived from studies[1,10] involving nonhuman primates

	Plasma glucose (mg/dL)			
Classification	Food withheld < 8 hours	Food withheld > 8 hours	Treated	Clinical signs*
Nondiabetic	< 150	< 150	No	No
Treated-diabetic	≥ 200	≥ 150	Yes	Yes
Diabetic	≥ 200	≥ 150	No	Yes

*Clinical signs include hyperglycemia, glucosuria, hyperlipidemia, polyuria, dysuria, polydipsia, polyphagia, weight loss, inappetence, and lethargy.

References

1. Walzer C. Diabetes in primates. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine. Current therapy 4*. Philadelphia: WB Saunders Co, 1998;397–400.

2. Burtis CA, Ashwood ER. Carbohydrates. In: Burtis CA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*. 3rd ed. Philadelphia: WB Saunders Co, 1999;750–808.

3. Cefalu WT, Wagner JD, Bell-Farrow AD. Role of glycated proteins in detecting and monitoring diabetes in cynomolgus monkeys. *Lab Anim Sci* 1993;43:73–77.

4. Kilpatrick ES. Problems in the assessment of glycaemic control in diabetes mellitus. *Diabet Med* 1997;14:819–831.

5. Wagner JD, Bagdade JD, Litwak KN, et al. Increased glycation of plasma lipoproteins in diabetic cynomolgus monkeys. *Lab Anim Sci* 1996;46:31–35.

6. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979;28:1039–1057.

7. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997;20:1183–1197.

8. World Health Organization. WHO Expert Committee on Diabetes Mellitus. Second Report. *Tech Rep Ser* 1980;646:8–12.

9. World Health Organization. Diabetes mellitus: report of a WHO Study Group. *Tech Rep Ser* 1985;727.

10. Howard CF, Yasuda M. Diabetes mellitus in non-human primates: recent research advances and current husbandry practices. *J Med Primatol* 1990;19:609–625.

11. Kemnitz JK, Baker A, Shellabarger W. Glucose tolerance and insulin levels of captive orangutans. In: Ogden JJ, Perkins LA, Sheeran L, eds. *Proceedings of the international conference on orangutans: the neglected ape*. San Diego: Zoological Society of San Diego, 1994;250–256.

12. Richter NA. Percentage of glycosylated hemoglobin and serum concentration of glucose in the blood of Japanese macaques and in three exotic ruminant species. *Am J Vet Res* 1986;47:1783–1784.

13. Huisman W, Kuijken JP, Tan-Tjiong HL, et al. Unstable gly-

cosylated hemoglobin in patients with diabetes mellitus. *Clin Chim Acta* 1982;118:303–309.

14. John WG. Glycated hemoglobin analysis. *Ann Clin Biochem* 1997;34:17–31.

15. Davidson MB, Schriger DL, Peters AL. An alternative approach to the diagnosis of diabetes with a review of the literature. *Diabetes Care* 1995;18:1065–1071.

16. Knowler WC. Screening for NIDDM: opportunities for detection, treatment and prevention. *Diabetes Care* 1994;17:445–450.

17. Peters AL, Davidson MB, Schriger DL, et al. A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycated hemoglobin levels. *JAMA* 1996;276:1246–1252.

18. Alperin JB, Dow PA, Stout LC. A comparison of hemoglobin A_{1c} in human and baboon blood. *Acta Haematol* 1979;61:334–338.

19. Howard CF. Correlations of hemoglobin A_{1c} and metabolic status in non-diabetic, borderline diabetic, and diabetic *Macaca nigra*. *Diabetes* 1982;31:1105–1108.

20. Widness JA, Schwartz R, Thompson D, et al. Hemoglobin A_{1c} in the glucose-intolerant, streptozotocin-treated or pancreatectomized macaque monkey. *Diabetes* 1978;27:1182–1188.

21. Abraham EC, Perry RE, Stallings M. Application of affinity chromatography for separation and quantitation of glycosylated hemoglobins. *J Lab Clin Med* 1983;102:187–197.

22. Willey DG, Rosenthal MA, Caldwell S. Glycosylated hemoglobin and plasma glycoprotein assays by affinity chromatography. *Diabetologia* 1984;27:56–58.

23. Littell RC, Milliken GA, Stroup WW, et al. A setting for mixed models applications: randomized blocks designs. In: Littell RC, Milliken GA, Stroup WW, et al, eds. *SAS system for mixed models*. Cary, NC: SAS Institute Inc, 1996;1–29.

Correction: Quantification, risk factors, and health impact of natural congenital infection with bovine viral diarrhea virus in dairy calves.

In "Quantification, risk factors, and health impact of natural congenital infection with bovine viral diarrhea virus in dairy calves," published in the March 2003 (2003;64:358–365) issue of the *American Journal of Veterinary Research*, the authors regret that there were several errors. On page 359, column 1, line 16, the sentence should read, "Vaccine programs for cows in herd A consisted of a BVDV modified-live virus (MLV) vaccine[a] (containing BVDV type 1) administered 1 to 2 months prior to the first breeding. Cows in herd B were administered a BVDV MLV vaccine[b] (BVDV type 1) 1 to 2 months prior to the first breeding and 20 to 30 days postpartum." In the same column, line 27, the sentence should read, "For calves in herd A, a killed BVDV vaccine[c] was given at 16 and 47 days of age, a BVDV MLV vaccine[d] was given at 60 days, and a different BVDV MLV vaccine[a] was given at 240 days. For calves in herd B, a killed BVDV vaccine[c] was given at 15 days of age and a BVDV MLV vaccine[b] was given at 45 and 180 days."

In addition, the product indicated by superscript e in the footnotes was not used in these cattle.