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Fructosamine Reference Ranges in Rhesus Macaques (*Macaca mulatta*)

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Naturally occurring diabetes mellitus (DM) is common in several species of Old and New World nonhuman primates. Fructosamine values provide important information about recent glycemic control and can be useful in the diagnosis and management of DM. However, despite an abundance of reports in the literature describing spontaneous and induced DM in monkeys, few reference ranges are available for fructosamine. Reference ranges have been published for woolly monkeys (*Lagothrix lagotricha*), cynomolgus macaques (*Macaca fascicularis*), and stumptail macaques (*Macaca arctoides*) but currently are not available for rhesus macaques. At our institution, DM is a common diagnosis in aging rhesus macaques. Here we report a reference range for fructosamine in rhesus macaques. The overall range was 157 to 230 µmol/L, with male rhesus and macaques 10 y or older having significantly higher values than do female rhesus and macaques younger than 10 y, respectively. This range provides clinical veterinarians with an additional tool for evaluating glycemic control in rhesus macaques.

Abbreviation: DM, diabetes mellitus.

Diabetes mellitus (DM) occurs spontaneously and can be induced experimentally in many species, including nonhuman primates.[6] Diabetes has been reported to occur in cynomolgus, rhesus, bonnet, Formosan rock, pig-tailed, and Celebes macaques, as well as African green monkeys, greater white-nosed guenons, woolly monkeys, and baboons.[1,4,7,8,10,12] In nonhuman primates, spontaneous type II diabetes is more common than type I disease and, as in humans, is associated with increased age and body weight.[6,8,11,12] Clinically, monkeys with DM exhibit polydipsia, polyuria, weight loss, polyphagia, and lethargy. Fasting serum hyperglycemia, transient hyperinsulinemia followed by insulin deficiency, hypertriglyceridemia, decreased glucose clearance response after an intravenous glucose tolerance test, hypercholesterolemia, glucosuria, and ketonuria are indicative of the disease. Islet amyloidosis and loss of pancreatic beta cells may be evident on histopathology in nonhuman primates with overt diabetes.[1,8,11,12]

Despite characteristic alterations in clinical chemistry values, diagnosis of DM in nonhuman primates is not always straightforward. Early diagnosis and clinical management of diabetes in nonhuman primates presents several challenges: (1) onset of type 2 DM is often gradual and clinical signs such as weight loss may not be obvious, (2) polyphagia, polydipsia, and polyuria may go unnoticed depending on husbandry and housing practices, (3) hyperglycemia is an inconsistent finding and is not specific to DM, because food consumption and stress associated with handling and restraint for sample collection may result in transient hyperglycemia, (4) serum insulin levels are not monitored routinely, (5) triglycerides may be elevated in overweight, nondiabetic animals, (6) the intravenous glucose tolerance test requires prolonged physical or chemical restraint; therefore, this test may not be completely accurate or feasible, (7) obtaining representative urine samples to test for glucosuria can be technically difficult or dependent on caging type and husbandry practices, and (8) fructosamine reference standards for comparison are available for only a few nonhuman primates species.[4,6,8]

Measurement of serum glycated proteins, such as glycated hemoglobin (HbA1c) and fructosamine, provides consistent information regarding long-term glycemic control and is used routinely to detect and monitor DM in humans and veterinary species.[3,4,6] HbA1c and fructosamine have both been measured in various nonhuman primate species and are elevated consistently in diabetic animals. HbA1c can provide information regarding average glycemic control for the preceding 1.5 to 3 mo, and fructosamine allows assessment of the average blood glucose concentration for the preceding 2 to 3 wk.[4,6,12] The relative cost of these assays makes fructosamine analysis a more desirable option.

Values for serum fructosamine have been established in woolly monkeys, cynomolgus macaques, stump-tailed macaques, and rhesus macaques.[1,4] In a previous study,[4] rhesus macaques were fed a high-cholesterol diet, which may have resulted in elevations in fructosamine values. In addition, the reference values for stump-tailed and rhesus macaques were calculated by using a historical technique (first-generation fructosamine assay), thus obviating their comparison with fructosamine values obtained by the currently used second-generation assay.[4] The objective of the current study was to determine normal fructosamine levels in healthy rhesus macaques to aid in the diagnosis and medical management of DM.

Materials and Methods

Animals. Sixty rhesus macaques of both sexes ranging from 2 to 28 y old were included in this study. Few animals (approximately 5 to 7) were related. Macaques were maintained in an AAALAC-accredited facility and enrolled in neurobiology or breeding protocols approved by the Yale University Institutional Animal Care and Use Committee. Physical examinations were performed semiannually, and *Mycobacterium tuberculosis* and *M. bovis* screening was performed by using the intradermal tuberculin test, in conjunction with PrimaGam (Prionics AG,

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Table 1. Summary of fructosamine (µmol/L) ranges by sex and age

	Observed range	5th to 95th percentile	Mean ± 1.96 SD	Mean ± SEM
Female (n = 38)	159–243	160–218	157–221	188.7 ± 2.6[a]
Male (n = 22)	153–232	165–231	156–243	199.7 ± 4.7
<10 y old (n = 22)	153–205	160–204	155–214	184.5 ± 3.2[b]
≥10 y old (n = 38)	159–243	166–232	159–236	197.5 ± 3.2

[a]P = 0.03 compared with mean ± SEM value for male rhesus
[b]P = 0.01 compared with mean ± SEM value for rhesus older than 10 y

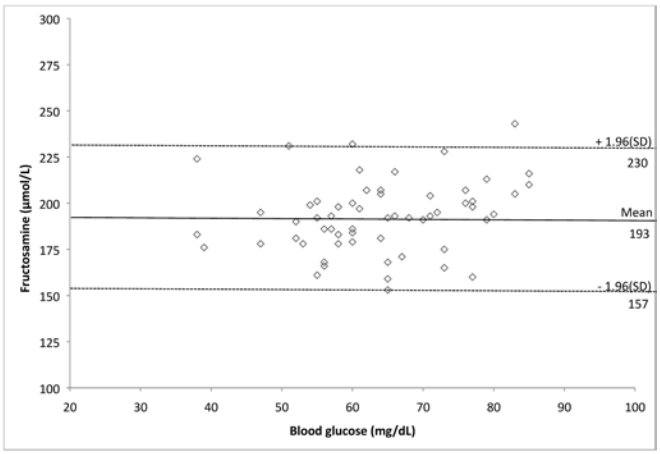

Figure 1. Individual blood glucose (mg/dL) and corresponding fructosamine (µmol/L) values. The solid line represents fructosamine population mean, dotted lines represent fructosamine population mean ± 1.96 SD, numeric values represent overall fructosamine range.

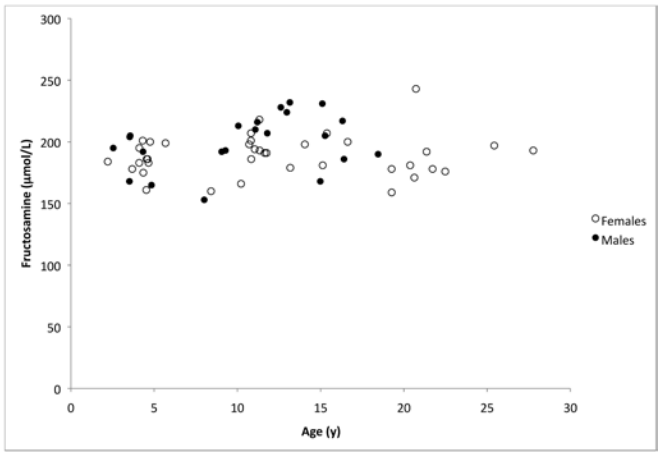

Figure 2. Individual fructosamine values (µmol/L) by age (y) and sex.

Schlieren-Zurich, Switzerland) or PrimaTB Stat Pak (Chembio Diagnostic Systems, Medford, NY). Serology was performed annually by BioReliance Corporation (Rockville, MD) for simian retrovirus type D (1 macaque was positive), simian T-cell leukemia virus (3 macaques were positive), and *Macacine herpesvirus 1* (8 macaques were positive); 4 animals were seropositive for at least 2 viruses. None showed clinical signs of disease. Facility containment was at Biosafety Level 2. Macaques were fed a commercial standard primate diet (Teklad 8714, Harlan Laboratories, Indianapolis, IN) supplemented daily with fresh fruits and vegetables. In addition, animals were offered food enrichment for enhancement of psychologic wellbeing or behavioral testing (for example, dried fruit, seed and nut mix, cereals, peanuts, granola bars, peanut butter, Prima-Treats [BioServ, Frenchtown, NJ], honey, chocolate chips). Supplemental food enrichment did not make up a substantial portion of the daily diet. Drinking water was provided through an automatic watering system (hyperchlorinated) or water bottle (tap water) according to the research protocol in which the macaque was enrolled. All animals were housed in stainless steel caging and pair-housed whenever possible to allow for social enrichment. A variety of toys, such as rubber kongs, flexi-keys, and Hercules Dental Devices (BioServ), were provided at all times on a rotating basis for enhancement of psychologic wellbeing. Room temperature and humidity were maintained at 72 ± 2 °F (22.2 ± 1.1 °C) and 50% ± 10%, respectively.

Samples. After an overnight fast, macaques were sedated, usually in the morning, by using 7 to 10 mg/kg ketamine intramuscularly. Blood samples were collected from the saphenous vein into serum separator blood tubes, centrifuged, separated immediately into a conical tube without additive, and stored at 4 °C until submission to the diagnostic laboratory. Serum samples were analyzed within 24 h by Antech Diagnostics (Lake Success, NY) by using an Olympus AU5400 Chemistry Analyzer (Beckman Coulter, Brea, CA) for determination of blood glucose and fructosamine levels. Healthy macaques with blood glucose values ranging from 30 to 91 mg/dL were included in the analyses. Hyperglycemic and known diabetic monkeys were excluded from the reference range calculations. Macaques were assigned to 1 of 5 groups: female macaques younger than 10 y; female macaques 10 to 20 y old; female macaques older than 20 y; male macaques younger than 10 y old; and male macaques 10 to 20 y old.

Statistical analyses. Data were analyzed by using SAS software (version 9.2, SAS Institute, Cary, NC). Reference range values for fructosamine were obtained by calculating the fifth and 95th percentiles, observed range, and sample mean ±1.96 SD. Age and sex were compared by ANOVA, linear regression, and t tests. Histograms did not reveal outliers. The data appeared normally distributed, but this assumption was not subjected to a formal statistical test.

Results

Table 1 summarizes the observed range, fifth through 95th percentile range, mean ± 1.96 SD, and mean ± SEM of fructosamine values by sex and age. Male rhesus macaques had higher (P = 0.03) fructosamine values than did female macaques, and animals 10 y or older had higher fructosamine values than did those younger than 10 y (P = 0.01). Individual blood glucose and corresponding fructosamine values are shown in Figure 1. Figure 2 shows fructosamine values of individual macaques by age. The calculated range of fructosamine values for the entire sampled population (n = 60) was 157 to 230 µmol/L. Table 2 shows how this range compares with those of other primate species.

Table 2. Fructosamine reference range values (µmol/L) by species

	From reference no.	Sample size	Reference range	Mean
Homo sapiens	3	230	157–297	227
Macaca mulatta	current study	60	157–230	193
Macaca fascicularis	12	24	168–202	185
Lagothrix lagotricha	1	6	133–273	203

Discussion

Diabetes mellitus is a common metabolic disease in both humans and animals, including nonhuman primates. Monitoring serum glycated proteins, such as fructosamine, allows more consistent assessment of glycemic control than does blood glucose alone.[4,12] Measuring serum fructosamine has numerous other advantages. Whereas blood glucose measurement represents a snapshot in time and may transiently be elevated or decreased with stress, activity, or illness, fructosamine is unaffected by these factors, and the concentration represents an average blood glucose level for the 2 to 3 wk preceding blood sample collection.[4,6] In addition, HbA1c and fructosamine concentrations are unaffected by recent food intake, because surfactants have been added to the second-generation assay to overcome lipemia, thus eliminating the need for presampling fasting.[4,6] Furthermore, measurement of both fructosamine and glucose may allow diagnosis of DM earlier in the course of the disease, thereby allowing earlier therapeutic intervention. Concentrations of fructosamine and HbA1c depend on the half-life of the bound protein in the tissue and the glucose concentration. In the glycosylation reaction, glucose is bound nonenzymatically to hemoglobin. Because the half-life of a human erythrocyte is approximately 60 to 90 d, HbA1c reflects glycemic control over the previous 1.5 to 3 mo. Fructosamine is formed by the attachment of glucose to serum albumin, which has a half life of 14 to 20 d. Therefore fructosamine levels reflect glycemic control over the antecedent 2 to 3 wk.[1,3,6] Because fructosamine concentrations do not depend on hemoglobin or RBC, this test is useful in patients with blood loss, hemolytic anemia, or hemoglobinopathies.[4] Finally, fructosamine is a more cost-effective test for confirming DM than is HbA1c.

In the current study, rhesus macaques 10 y or older had higher fructosamine values than did younger animals. This finding is consistent with recent human data indicating the prevalence of type II DM in humans older than 60 y is 23.1%, compared with 10.7% in people older than 20 y.[5] In humans, glucose intolerance increases with age, a result that may be related to obesity or inactivity.[11] The same phenomena may have contributed to the elevated fructosamine ranges in rhesus macaques in the current study, although body weight, musculoskeletal condition, and activity levels were not evaluated. In addition, we learned that male macaques had higher fructosamine values than did female macaques, another finding that is consistent with human data.[5] Although significant age- and sex-associated differences in the fructosamine levels of rhesus macaques were detected in the current study, ranges overlapped greatly. Therefore the differences in reference values probably are not clinically significant.

When measuring serum fructosamine levels, appropriate sample storage is important. Serum samples kept in standard self-defrosting freezers at 4 °C are subject to freeze–thaw cycles, which may affect fructosamine values, presumably due to breakdown of the protein. Indeed, analysis of a large group of samples stored for as long as 5 y in a self-defrosting freezer revealed a fructosamine range higher than the range established in the current study (data not shown). We therefore recommend evaluating fresh refrigerated samples within 24 h of collection or storing samples at −80 °C until fructosamine analysis can be performed.

Lack of chemistry profile data for the macaques evaluated is a limitation of this study. Fructosamine concentrations may be decreased falsely in animals with hypoproteinemia, hypoalbuminemia, hypervitaminosis C, or hyperthyroidism and in samples that are hemolyzed.[2,9] However, a review of routine health screening results, including periodic serum chemistry analysis, revealed no evidence of these serum abnormalities.

Here we report a serum fructosamine range of 157 to 230 µmol/L for a population of healthy rhesus macaques of various ages and both sexes. The values for *M. mulatta* reported here are similar to published values for cynomolgus macaques and humans and are slightly higher than published values for *L. lagotricha*.[3,4] Our findings provide clinical veterinarians with species-specific values with which to monitor glycemic control in rhesus macaques.

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