Exploring fructosamine beyond diabetes mellitus

Kimberly M. Pattullo, DVM, MVetSc, and Beverly A. Kidney, DVM, PhD

Fructosamine is the common name of 1-amino-1-deoxyfructose, a ketoamine that results from posttranslational nonenzymatic linking of glucose or mannose to proteins.1 Formation of fructosamine is proportional to the degree and duration of hyperglycemia within the patient.2 Determination of and monitoring of serum fructosamine concentrations has become an important part of the diagnosis and management of diabetes mellitus in dogs and cats,3,4 and its use has also been explored in numerous other species, both as a marker of diabetes mellitus as well as other diseases and conditions resulting in or from derangements in carbohydrate intake and metabolism.5–22 It has also been shown to be a useful marker for differentiating nondiabetic causes of glucosuria.23 Although the use of fructosamine concentration as a marker of chronic hyperglycemia is now considered commonplace within veterinary medicine, much remains to be elucidated about the formation, lifespan, and role of fructosamine in diabetic and nondiabetic disease.

Formation and Degradation of Fructosamine

Fructosamine formation begins with the condensation of the free aldehyde group of a glucose or mannose molecule with the amino group of a protein, with lysine being the preferred site of glycation.5 This results in the formation of aldimine, an unstable Schiff base compound. Schiff bases may undergo spontaneous disassociation back to their original sugar and protein structures but more frequently undergo slow isomerization (ie, Amadori rearrangement), resulting in the formation of a stable ketoamine, in this case, fructosamine24 (Figure 1). The structure of the resulting carbon backbone is identical to that of fructose, hence the name. In humans, approximately 80% of ketoamines in serum are composed of glycated albumin24; reports of the percentage of albumin in serum that is glycated in healthy adult humans have been variable, ranging from 1.4% to 16%.25,26 Which proteins are favored for glycation in animals has not been established; numerous proteins in the body, intra- and extracellular as well as intra- and extravascular, are susceptible to glycation.24 Individual variation as well as the health status of the patient may also affect which proteins are preferentially glycated, with various shifts found in healthy and diabetic cats.27 It has not been determined what percentage of proteins are normally glycated in serum samples from healthy patients.

The lifespan of fructosamine typically reflects the lifespan of its parent protein and is therefore affected by the rate of protein turnover.28 Human and canine fructosamine is thought to last 2 to 3 weeks in the body,24,29 but this has yet to be confirmed in dogs. Given that canine albumin has a lifespan of only 8.2 days,30 compared with 19 days in humans,31 this figure may be an overestimation, although other proteins in canine serum have been reported to last up to 23 days.32 The lifespan of feline proteins has yet to be determined, but fructosamine concentrations are estimated to represent blood glucose concentrations from the previous 7 to 10 days.33 Equine and bovine albumin last 19.4 days and 16.5 days, respectively, in circulation34,35; the lifespans of other plasma proteins are unknown. Aside from the individual lifespan of each protein, the rate of protein turnover in the body affects fructosamine concentrations.

Glycation of proteins was originally thought to be an irreversible process, resulting in a structural change of the protein.24 However, findings from a study36 in humans revealed the presence of an enzyme, fructosamine 3-kinase. This enzyme has the ability to destabilize fructosamine through phosphorylation of its third carbon, resulting in the spontaneous detachment of the sugar from its protein37 (Figure 2). However, the tertiary structure of the protein component of fructosamine limits the access of fructosamine 3-kinase to glycation sites, resulting only in partial repair of glycated proteins.38 Fructosamine 3-kinase requires ATP as a cofactor and is therefore only found intracellularly.39 To date, an extracellular enzyme has not been discovered. Fructosamine 3-kinase has been found in numerous tissues, but the brain, heart, kidneys, and skeletal muscles have been discovered to contain the highest concentrations.40

Role of Fructosamine in Disease

The structure that fructosamine attains following Amadori rearrangement may not be its final form. Fructosamine as well as proteins glycated by various other sugars are also known as Amadori products, which can

ABBREVIATIONS

<table>
<thead>
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<tr>
<td>AGE</td>
<td>Advanced glycation end product</td>
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<tr>
<td>dK</td>
<td>Critical difference</td>
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<td>NBT</td>
<td>Nitroblue tetrazolium</td>
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No funding was received for this publication.

The authors have no conflict of interest.

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gradually undergo progressive oxidation, dehydration, cyclization, and isomerization to form AGE (Figure 1); Amadori products can therefore also be referred to by the contradictory term of early AGEs. Advanced glycation end-products are thought to be truly irreversible, lasting the entire life of the protein. The kinetics of their formation with respect to blood glucose concentration has yet to be characterized, although typically the blood glucose concentration is important in the initiation of a nonoxidative reaction, followed by an oxidative pathway that is influenced by the degree of lipid peroxidation and glucose autoxidation in the body, resulting in AGE creation.

Increased concentrations of AGEs have been observed in dogs with diabetes mellitus, with no substantial differences in concentrations between well-controlled and poorly controlled diabetes. Both Amadori products, including fructosamine, and AGEs have been implicated as a cause of many diabetes mellitus–associated diseases in people, including diabetic retinopathy, nephropathy, neuropathy, coronary heart disease, and inhibition of certain coagulation proteins, and have been suspected as a cause of diabetic neuropathy in cats.

In humans, fructosamine, specifically glycated albumin, has been shown to upregulate proinflammatory TGF-β and stimulate the expression of extracellular matrix proteins in glomerular cells, damaging glomerular filtration and thereby increasing urinary excretion of albumin and type IV collagen. These molecules are also the focus of intensive research as an inciting or contributing cause of numerous other chronic conditions in nondiabetic patients, regardless of blood glucose concentrations. Fructosamine and other Amadori products induce cross-linking of proteins in arterial walls as well as reduce the rate of protein turnover, resulting in increased matrix stiffness, a major factor in the development of atherosclerosis. Both Amadori products, including fructosamine, and AGEs are also implicated in the pathogenesis of myocardial infarctions, Alzheimer's disease, and various neoplasms, including colorectal, prostate, and pancreatic cancers. They have also been found to be important in proinflammatory processes and reactive oxygen species production.

Production of reactive oxygen species results in further oxidation of albumin; oxidated bovine albumin has shown to be more readily glycated than native albumin at all blood glucose concentrations, resulting in a never-ending cycle of reactive oxygen species and Amadori product and AGE production, further exacerbating the disease state within the body.

Analysis of Fructosamine

The NBT assay is the most commonly used detection method in human and veterinary medicine, with numerous commercial kits available for use in an automated analyzer. Fructosamine acts as a reducing agent for NBT under alkaline conditions, resulting in the generation of formazane at a rate directly proportional to fructosamine concentration. Formazane production is measured spectrophotometrically, typically between 530 and 550 nm. A pH between 10.5 and 11 is often used to limit interference by free glucose, which also reduces NBT at alkalinity outside of this range. The assay appears to have high accuracy and precision, with low within-run and between-run coefficients of variation. The original NBT assay was found to be affected...
by hyperlipidemia as well as the presence of other reducing agents normally found in serum; a modified NBT assay was developed and standardized, which uses surfactants, enzymes, and various stabilizers to limit these interferences. Measurements do not begin until 10 minutes after assay initiation, to allow faster-acting reducing agents to react, thereby limiting their influence on the results. To date, the modified NBT assay has been validated for use in numerous species, including dogs, cats, ponies, horses, and cattle. Hyperbilirubinemia was reported to have no effect on serum fructosamine measurements in dogs and cats, although false low measurements have been observed in humans with a total bilirubin concentration > 68 µmol/L. No studies into the effects of hemolysis on the modified NBT assay have been reported in veterinary medicine; in humans, hemoglobin concentrations > 62 µmol/L cause false findings of hypofructosaminemia. An ELISA version of the modified NBT assay has been verified for use in plasma samples of cattle, dogs, and chickens, with good correlation to the automated NBT assay (r = 0.94 to 0.98), although based on examination of the literature, its use appears to be limited.

Several studies in veterinary medicine have evaluated the use of various enzymatic assays specific for detecting fructosyl-lysine bonds. These assays seem to limit the interference by bilirubin and other reducing agents, with high specificity for glycated proteins. Each commercial enzymatic method uses slightly different methodologies and reagents, but briefly, the test involves the use of various proteases and other enzymes that digest fructosamine into low-molecular weight fragments. Another enzyme, such as amadoriase, catalyzes these fragments, resulting in the production of hydrogen peroxide. Hydrogen peroxide is measured colorimetrically, with the production of hydrogen peroxide proportional to the concentration of fructosamine. A study of its use in Beagles resulted in fructosamine concentrations that were one-quarter to one-third less than those obtained by the modified NBT assay. Although correlation was not determined in that study, another study in humans found excellent agreement between the 2 methods (r² = 0.98). Its use appears limited in both human and veterinary clinical medicine, with most fructosamine studies making use of the modified NBT assay.

Other tests, such as high-performance liquid chromatography and affinity chromatography, are used primarily for research purposes. Older assays, which have generally fallen out of favor because of time- and labor-intensive procedures, include 2-thiobarbituric acid colorimetric procedure, the furosine procedure, and the phenylhydrazine procedure.

Reports of fructosamine stability in animal and human samples have been inconsistent; fructosamine has been reported to be stable for 3 to 7 days at 25°C, and 5 to 14 days at 4°C. The most variability has been identified at –20°C, with the largest disputes involving storage > 30 days. Both serum and heparinized plasma have been found to be reliable samples in dogs, whereas results were found to be more variable in ponies by use of serum versus heparinized plasma.

Recently, expired heparinized plasma and serum separator tubes, up to 11 months after the expiration date, were found to cause mild false elevations in fructosamine concentrations. Most variations were observed with fructosamine concentrations that were within reference limits, although some of these differences may have led to misclassification of the results.

Critical difference—The dk of fructosamine, or the minimal change an analyte concentration must change in a certain patient to represent a true fluctuation versus normal individual and analytic variation, has been evaluated in several species (Table 1). This con-

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Table 1—Critical differences reported for serum fructosamine concentrations in domestic veterinary species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Critical difference (µmol/L)</th>
<th>Reference No.</th>
<th>Hyperfructosaminemia</th>
<th>Hypofructosaminemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>32.4–33.5</td>
<td>38,70,71</td>
<td>Diabetes mellitus</td>
<td>Hypoalbuminemia</td>
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<td></td>
<td></td>
<td></td>
<td>Obesity</td>
<td>Insulinomas</td>
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<td></td>
<td></td>
<td></td>
<td>Hypothyroidism</td>
<td>Azotemia</td>
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<td></td>
<td></td>
<td></td>
<td>Monoclonal IgA gammopathy</td>
<td>Hyperlipidemia</td>
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<td></td>
<td></td>
<td></td>
<td>Diestrus or pregnancy</td>
<td>(hypertriglyceridemia and hypercholesterolemia)</td>
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<td></td>
<td></td>
<td></td>
<td>Drugs (cyclosporine and prednisolone)</td>
<td>Metabolic epidermal</td>
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<td></td>
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<td></td>
<td>necrosis secondary to α-cell pancreatic tumors</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Infections (Angiostrongylus vasorum and leishmaniasis)</td>
</tr>
<tr>
<td>Feline</td>
<td>33</td>
<td>40</td>
<td>Diabetes mellitus</td>
<td>Hyperproteinemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Preeclampsia diabetes mellitus</td>
<td>Hyperthyroidism</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Obesity</td>
<td>Hyperthyroidism</td>
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<td></td>
<td></td>
<td></td>
<td>Male cats</td>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Drugs (dexamethasone, methylprednisolone, and triamcinolone)</td>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td>Equine</td>
<td>Unknown</td>
<td>None</td>
<td>Laminitis</td>
<td>Cyathostomias</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pituitary pars intermedia dysfunction</td>
<td>Subclinical rumen acidosis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Late gestation</td>
<td>Infection (Teladorsagia circumcincta)</td>
</tr>
<tr>
<td>Bovine</td>
<td>20.8</td>
<td>72</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ovine</td>
<td>Unknown</td>
<td>None</td>
<td>Not reported</td>
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Conditions for which hyperfructosaminemia or hypofructosaminemia have been found are listed.
cept is based on the idea that 2 independent variables with the same expected result and same SD should not vary more than $2\sqrt{2}\sigma$, where $\sigma$ represents the SD. If the difference between the 2 results is $> 2\sqrt{2}\sigma$, a 95% probability exists that the parameter has changed as a result of treatment or disease progression and resolution. The formula has since been expanded to include the SD of the analytic method: $dk = 2\sqrt{2}(\sigma_i + \sigma_f)$, where $\sigma_i$ represents the SD of the results within dogs and $\sigma_f$ represents the SD of the test method. Therefore, $dk$ is helpful in situations where a difference exists between 2 consecutive results, yet both concentrations remain outside of the reference interval. If $dk$ is greater than the difference between these results, the change in concentration merely reflects normal individual and analytic variation of the analyte versus a true alteration in the disease status of the patient. The $dk$ of fructosamine has only been determined in healthy dogs, cats, and cattle; the effect of diabetes mellitus and non–diabetes mellitus conditions and the effect of species variation on $dk$, if any, has not been determined.

**Hyperfructosaminemia**

Alterations in fructosamine, determined by the modified NBT assay, have been associated with various conditions, both related and not related to diabetes mellitus (Table 1). Hyperfructosaminemia is frequently the result of derangements in carbohydrate metabolism, particularly increased peripheral insulin resistance or insulin deficiency, often resulting in hyperglycemia as well as increased time for interaction between glucose and proteins.41

**Diabetes mellitus**—Not surprisingly, hyperfructosaminemia is commonly found in diabetic patients. As a diagnostic test, determination of fructosamine concentration has been found to have a sensitivity in cats and dogs of 89% to 93% and 88%, respectively, and a specificity of 55% to 86% and 99%, respectively.68,69 It is an important aid in the differentiation of well-controlled diabetes in dogs and cats from those with moderately to poorly controlled diabetes. The test is considered important for longer-term monitoring of diabetic patients; it is not affected by short-term cortisol-induced hyperglycemia in cats and dogs or the transitory epinephrine-induced hyperglycemia in cats that is commonly found in a veterinary hospital setting.35,70

Decreased protein turnover and hyperproteinemia—Hypothyroidism is a well-known cause of hyperfructosaminemia in people. Thyroxine is an important stimulus for metabolism; decreased production of thyroxine results in a decreased metabolic rate, with a subsequent decrease in the rate of albumin turnover. Glycated proteins therefore have a longer half-life, resulting in an increase in fructosamine concentration. Although the precise mechanism has not been determined, similar affects have been observed in dogs, with 43% to 82% of untreated normoglycemic, normoalbuminemic hypothyroid dogs reported to have mild to moderate hyperfructosaminemia; however, all fructosamine concentrations were $< 500 \mu$mol/L.71,72 Following initiation of appropriate treatment, hyperfructosaminemia took 8 to 24 weeks to return to within reference intervals despite rapid normalization of thyroxine concentrations.72

Hyperfructosaminemia secondary to monoclonal IgA gammopathy associated with multiple myeloma is recognized in people, despite the fact that a compensatory hypoalbuminemia is often present in these patients.73 It has also been described in a case report74 involving 2 dogs with monoclonal IgA gammopathy, with fructosamine concentrations of 654 and 506 mmol/L (normal, 200–370 mmol/L). Interestingly, polyclonal IgA gammopathy as well as monoclonal IgG or IgM gammopathies have not been associated with hyperfructosaminemia.73 The mechanism for the preferential glycation of IgA versus IgG or IgM is unknown.

Given its association with a protein parent molecule, it is logical that hyperproteinemia or hyperalbuminemia would result in hyperfructosaminemia; however, studies33,41,57,76,77 in humans and animals have demonstrated inconsistent results, possibly because of variable durations of hyperproteinemia as well as variations in albumin-to-globulin ratios. The rate of protein turnover also has a strong influence on fructosamine, which can vary greatly with various physiologic and pathological changes. Therefore, most authors have found that correcting fructosamine in hyperproteinemic and hyperalbuminemic patients failed to improve the diagnostic value of fructosamine concentrations, with 1 study75 demonstrating even wider confidence limits and greater SD with corrected fructosamine concentrations versus the noncorrected values.

**Cortisol-induced hyperglycemia**—Although transitory cortisol-induced hyperglycemia has been shown to have no effect on fructosamine concentrations in both dogs and cats,33,69,78–81 preclinical or reversible diabetes mellitus secondary to an underlying disease process has been found to cause substantial increases in fructosamine concentration, compared with that of healthy cats, with 2 of 14 cats having concentrations above the reference limit. Blood glucose concentrations were as high as 28 mmol/L (505 mg/dL), yet the correlation between blood glucose and fructosamine measurements was poor ($r = 0.14$). Following resolution of their clinical disease, all of these cats had at least 2 subsequent blood glucose measurements within reference range, confirming reversible diabetes mellitus.80

A study33 investigating the effect of duration and magnitude of experimentally induced hyperglycemia on fructosamine concentrations in healthy cats demonstrated significant differences in fructosamine dynamics between moderate and marked hyperglycemia. Fructosamine concentrations in cats with marked hyperglycemia (blood glucose concentration, approx 29 mmol/L; 523 mg/dL) took 3 to 5 days to exceed the upper reference limit, 20 days to plateau, and 5 days to return to baseline following cessation of the glucose infusion. Although cats with moderate hyperglycemia (blood glucose concentration, approx 17 mmol/L; 306 mg/dL) took 7 days to exceed the upper reference limit, fructosamine concentrations only took 8 days to plateau and 1 to 2 days to return to baseline. One cat in the marked hyperglycemia group became ketoacidotic, which had been preceded by a sudden spike in fruc-
tosamine concentration of 50 µmol/L. 24 hours before developing ketonuria. Other cats that developed keto- nuria had a mean decrease in fructosamine concentration of 120 µmol/L 24 hours after institution of low-dose insulin treatment, despite a lack of change in blood glucose concentration; these cats had not been assessed for presence of ketoacidosis prior to initiation of treatment. Although the half-life of proteins in feline serum has yet to be determined, it is unlikely that all major proteins turn over this rapidly, suggesting that there may be temporary bonds between glucose and the amino groups or extracellular enzymes that cause their dissociation, although this has yet to be investigated.55

Drugs—Not unexpectedly, given their known role in peripheral insulin resistance, several corticosteroids as well as other immunosuppressive agents have been associated with hyperfructosaminemia in dogs and cats. Administration of cyclosporine following label protocol for 6 weeks caused a significant increase in fructosamine concentration with no change in albumin or blood glucose concentrations in 14 of 16 healthy dogs.56 A study57 performed by the same authors failed to find significant differences using anti-inflammatory dosages of prednisolone in healthy dogs, despite 11 of 16 dogs having posttreatment hyperfructosaminemia. Long-term therapeutic dosages of methylprednisolone and triamcinolone in atopic cats resulted in a total mean increase in fructosamine concentration of 49.1 µmol/L, although albumin concentrations also significantly increased in both treatment groups.84 Immunosuppressive doses of dexamethasone and prednisolone for 56 days in healthy cats resulted in hyperfructosaminemia; although the differences were not significant, a median increase of 40 and 82 µmol/L was observed with prednisolone and dexamethasone, respectively. Although fructosamine concentration increased significantly over time in both treatment groups, the magnitude of this increase was greater in the dexamethasone-treated cats, compared with those treated with prednisolone. Albumin and blood glucose concentrations were not measured.85

Parenteral administration of glucosamine has been shown to cause peripheral insulin resistance in skele- tal muscles of normoglycemic rats86; therefore, its use has been questioned in both diabetic patients as well as those considered high-risk for the development of diabetes mellitus. Insulin resistance is thought to develop through drug-induced activation of the hexokinase pathway, which typically is only activated when cells are receiving adequate glucose, thereby limiting further influence by insulin.86 However, these effects have not been observed in dogs or cats, or in normoglycemic or diabetic people.87-92

Obesity—As obesity rates in humans and companion animals continue to increase, investigations into the effects of obesity on the body have multiplied in number. In both humans and rodents, the amount of body fat has been positively correlated with the concentrations of cortisol, insulin, and insulin growth fac- tor-1, with concurrent negative correlation with growth hormone secretion, consistent with peripheral insulin resistance.50 These effects have also been similarly ob- served in dogs,91 with normoglycemic obese dogs having significantly higher fructosamine concentrations, compared with that of normal weight dogs (254 ± 18.43 µmol/L vs 198 ± 11.9 µmol/L, respectively)94; similar findings have been observed in obese cats.93 Another study found almost two-thirds of obese dogs had fruc- tosamine concentrations above the reference limit with no corresponding alterations in blood glucose concentra- tion.93 Body weight loss resulted in significant reductions in fructosamine (mean initial fructosamine concentra- tion, 726.3 µmol/L; following a 3-month weight loss program, mean fructosamine concentration, 422 µmol/L), despite the lack of significant changes in blood glucose concentration.86

Laminitis—Although the exact mechanism of laminitis in horses has yet to be determined, one of the current theories involves increased peripheral insulin resistance, leading to vascular disease and decreased blood flow to the hoof.97 Laminitic horses had significantly higher fructosamine concentrations versus that of clinically normal unaffected horses (median of 288 vs 253 µmol/L, respectively), with no significant differences in total protein or albumin concentrations between laminitic horses and unaffected horses; 23% of laminitic horses had fructosamine concentrations above the upper reference limit. Fructosamine concentra- tion failed, however, to be correlated with outcome, history of previous episodes of laminitis, or grade and duration of laminitis prior to testing.22

Pituitary pars intermedia dysfunction—Hyper- fructosaminemia has been observed in horses with hirsutism and in horses with positive dexamethasone sup- pression tests (median fructosamine concentrations, 2.2 and 2.3 mmol/L, respectively); however, abundant overlap was found between these 2 groups and the age-matched control horses. Pars pituitary intermedia dysfunction was not confirmed, and the study failed to rule out other diseases that can also result in a posi- tive dexamethasone suppression test result. Although not specifically stated, given the results were reported in mmol/L, it is likely that the original NBT assay was used, therefore the effect of interfering substances on test results must be questioned.15

Sex—When matched on the basis of weight, body condition score, and age, male cats were shown to have significantly higher fructosamine concentrations versus females (279.9 ± 42.6 µmol/L and 249.0 ± 36.0 µmol/L, respectively), with no significant differences in total protein concentration between male and female cats.95 Significantly higher fructosamine concentrations were also found in male Rhesus macaques (Macaca mulatta)98 and in male silver foxes (Vulpes spp),99 compared with females. However, serum protein concentrations were not evaluated in the macaques, and male silver foxes also had higher total protein, albumin, and β-globulin concentrations. No sex-related differences in fructosamine were found in Gottingen minipigs, despite evi- dence of obesity and peripheral insulin resistance in young female pigs.100

Pregnancy and diestrus—It has been well estab- lished that Swedish and Norwegian Elkhounds are
predisposed to the development of gestational and diestrus-induced diabetes mellitus.\textsuperscript{104} However, it has recently been demonstrated that healthy nondiabetic Norwegian Elkhounds had significantly higher concentrations of fructosamine and insulin and decreased insulin sensitivity during diestrus, compared with that during anestrous, consistent with increased peripheral insulin resistance, whereas non-Elkhound breeds did not, and no significant difference was found in progesterone concentration between Elkhounds and non-Elkhounds.\textsuperscript{105} The mechanism for this is unknown. Hyperfructosaminemia has also been observed in late-gestation mares and is thought to reflect adaptation to the higher metabolic demands of late-stage pregnancy.\textsuperscript{103}

### Hypofructosaminemia

Most clinicians have experience interpreting hyperfructosaminemia, yet many fail to recognize factors that may artifactually decrease both normal and abnormal fructosamine concentrations, or understand the causes of true hypofructosaminemia. Again, both pathological and physiologic factors have been recognized to decrease fructosamine concentrations.

**Hypoproteinemia and hypoalbuminemia**—Although hyperproteinemia and hyperalbuminemia do not appear to have substantial effects on fructosamine concentration, pathological hypoproteinemia and hypoproteinemia have been directly correlated with hypofructosaminemia in dogs and cats, respectively.\textsuperscript{57,76–78,104,105} Given the half-life of albumin, the presence of hypofructosaminemia in a hypoalbuminemic dog suggests persistent hypoalbuminemia of > 7 days' duration.\textsuperscript{105} Authors have debated the use of a correction formula to correct fructosamine concentrations (ie, in dogs with dysproteinemias\textsuperscript{57}):

\[
\text{Corrected [FRA]} = \frac{\text{observed [FRA]} \times \text{normal median [albumin]}}{\text{observed [albumin]}}
\]

where [FRA] represents fructosamine concentration.

*For cats, total protein concentrations are used in the place of albumin.\textsuperscript{57}

However, the formula implies a completely linear relationship between albumin (or total protein) and fructosamine concentrations without regard for any alterations in protein half-life that have taken place as a result of the associated disease. Any condition that results in decreased serum albumin concentrations, either through decreased production or increased losses, results in a compensatory reduction of albumin turnover, therefore, increasing its half-life.\textsuperscript{28} Some authors determined that only 10% to 20% of the changes in fructosamine concentration were the result of hypoproteinemias in dogs and cats.\textsuperscript{33,57} Therefore, there appears to be no consensus or guidelines as to when the use of this formula is appropriate, with 1 author only recommending fructosamine correction when the total protein concentration is markedly outside of the reference interval in cats.\textsuperscript{33} Generally, assessing fructosamine concentration is not recommended when albumin concentration is < 30 g/L in humans.\textsuperscript{33}

Hyperthyroidism in untreated cats has been associated with a significantly lower fructosamine concentration, compared with that of clinically normal age-matched control cats (254.0 ± 27.6 µmol/L vs 295.0 ± 18.5 µmol/L, respectively), likely secondary to increased protein turnover.\textsuperscript{106} Interestingly, following radioactive iodine treatment, only cats that became hypothyroid had a significant increase in fructosamine concentration by 30 days after treatment. Unfortunately, patients were not followed past 30 days, so it is conceivable that fructosamine concentration may require a longer time period to normalize, as found in hypothyroid dogs.

**Insulinoma and α-cell pancreatic tumors**—Not surprisingly, hypofructosaminemia has been associated with insulinomas in both humans and dogs, but this finding has also been observed even in a persistently normoglycemic dog with an insulinoma.\textsuperscript{107} This may be due to intermittent cortisol-induced peripheral insulin resistance while at the veterinary clinic; blood glucose concentration is in the normoglycemic range during these visits, yet it is usually in the hypoglycemic range otherwise, resulting in decreased formation of fructosamine.\textsuperscript{108,109}

Metabolic epidermal necrosis, also known as hepato- cutaneous syndrome, is primarily associated with severe hepatopathies in dogs but is also rarely associated with productive α-cell pancreatic adenocarcinomas. Productive glucagonomas have been known to result in secondary metabolic epidermal necrosis and have been linked to hypofructosaminemia, likely secondary to the hypoaminoacidemia which results from the action of glucagon. Blood glucose concentration is typically within reference limits, owing to a compensatory increase in insulin secretion; this may complicate differentiation from an insulinoma, although hyperinsulinemia tends to be mild to moderate in cases of α-cell pancreatic tumors, compared with the marked increase observed with insulinomas. The relationship between α-cell pancreatic tumors, metabolic epidermal necrosis, and hypofructosaminemia is unknown.\textsuperscript{110}

**Azotemia**—In 1 study,\textsuperscript{37} 47% of dogs with azotemia alone (with urea or creatinine or both above the upper reference limit and all other results of biochemical analysis within normal reference limits) had significantly decreased fructosamine concentration, which the authors suggested may be secondary to denaturation of albumin. However, the type of azotemia, such as renal or prerenal, was not stated, so the importance of this finding is unknown. In contrast, humans with end-stage renal failure secondary to either diabetic or nondiabetic nephropathies often have mild to moderate hyperfructosaminemia.\textsuperscript{25} A multifactorial etiology is presumed, with decreased glomerular filtration rate, increased reactive oxygen species, and increased excretion of nonglycated albumin resulting in a relative increase in the glycated protein fraction versus nonglycated proteins.\textsuperscript{23,47,104,112} Azotemia had no effect on fructosamine concentration in cats.\textsuperscript{37}

**Hyperlipidemia**—Although the original NBT assay displayed interference secondary to hyperlipidemia, 38% of dogs with hyperlipidemia, characterized by hypertriglyceridemia (triglycerides concentration > 1.7 mmol/L) or hypercholesterolemia (cholesterol concentration > 9.9 mmol/L), were also found to have significantly decreased fructos-
ame based on modified NBT analysis. All other biochemical results were within normal reference intervals. Whether this was due to interference with the assay or a true pathological effect was not discussed. Cats with hypertriglyceridemia (triglyceride concentration > 1.2 mmol/L) or hypercholesterolemia (cholesterol concentration > 6.5 mmol/L) had no significant differences in fructosamine concentration, compared with that in healthy control cats.

**Pregnancy toxemia**—Fructosamine has been suggested as a prognostic indicator in ovine pregnancy toxemia, given that hypoglycemia has been directly correlated with a poorer prognosis. However, subsequent studies have failed to demonstrate alterations in fructosamine in cases of pregnancy toxemia; all affected ewes had either normo- or hyperfructosaminemia, despite normal total protein and albumin concentrations, regardless of the response to treatment and clinical outcome of the patient.

**Subclinical rumen acidosis**—High-producing dairy cattle require a readily available and high-quality carbohydrate supply to prevent a negative energy balance. Fructosamine has been proposed as a marker that may be used on a herd basis to assess energy balance, given that hypofructosaminemia has been correlated with subclinical rumen acidosis in dairy herds; although hypofructosaminemia was related to decreased rumen pH, no direct correlation was found between the degree of hypofructosaminemia and the severity of acidosis.

**Infectious agents**—Various parasitic and protozoal infections have been associated with hypofructosaminemia, typically due to their effect on serum protein concentrations, as the result of direct losses (eg, protein-losing enteropathy associated with *Teladorsagia circumcincta* infection in lambs) or *Polymorphus minutus* infection in common eider ducklings) or decreased albumin production (eg, leishmaniasis in dogs). However, other mechanisms are not as clear; only 5 of 9 of hypofructosaminemic ponies with experimentally induced cyathostomiasis were hypoaalbuminemic, suggesting an increase in protein turnover (and therefore a decrease in protein half-life) or alterations in the proteins, which prevented glycation. Studies have demonstrated that 74.5% to 100% of dogs with naturally induced *Angiostrongylus vasorum* infections were found to have hypofructosaminemia, despite concurrent normo- or hyperproteinaemia; fructosamine concentrations significantly increased following treatment. No mechanism for this finding was proposed.

**Diet**—To date, there have been no reports of diet-induced hypofructosaminemia in healthy animals; however, high-protein diets have been found to increase protein turnover in dogs, cats, and humans and therefore may cause decreased fructosamine concentrations. In cattle, hypofructosaminemia has been found with starvation. As normofructosaminemia was observed in cattle with marked loss of body condition secondary to chronic abomasal displacement and ketosis, hypofructosaminemia in the former scenario is most likely reflective of starvation-induced hypoproteinaemia and hypoalbuminemia. However, blood glucose concentrations were not reported.

In healthy human adults, fructosamine is negatively correlated with monounsaturated fatty acid intake, suggesting a role of monounsaturated fatty acids in improving insulin sensitivity.

**Summary**

Fructosamine is not merely a marker of chronic hyperglycemia. Concentrations of glycated serum proteins can be both increased and decreased in various disease states, and, along with AGEs, appear to have an important role in disease development and progression, in both diabetic and nondiabetic patients.

Still much needs to be learned about fructosamine. From an analytic perspective, inquiry into the effect of potential interfering substances in various species is still required, and further evaluation of assays other than the modified NBT test may be considered. Structurally and physiologically, investigation into fructosamine and protein half-lives in serum, factors that lead to protein glycation in normoglycemic animals, which proteins are preferentially glycated, and whether these preferences shift with various disease states is required. Another potential area of research involves determination of the presence of temporary bonds between glucose and proteins and, if present, how factors lead to stable, more permanent bonds. Examination of the role of fructosamine 3-kinase and identification of other deglycating enzymes would allow for development of assays that can aid in diagnosis and determination of prognosis of diseases, as scientists are beginning to uncover in human medicine.

As researchers discover more details about fructosamine and its role in the body, differentiating permanent and irreversible diabetes mellitus from a preclinical or reversible diabetic state may become more complex. However, this information is likely to aid in the development of potential intervening treatments that may slow disease progression and improve quality of life of our patients.

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