Effect of age on reference intervals of serum biochemical values in kittens

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Objective—To determine the effect of age on reference intervals of serum biochemical values in kittens.

Design—Prospective clinical trial.

Animals—55 kittens from 12 specific-pathogen–free queens.

Procedure—Kittens were allocated at birth into colostrum-fed (n = 27) and colostrum-deprived (28) groups. Blood was collected at birth and on days 1, 2, 4, 7, 14, 28, and 56. Serum samples were analyzed for activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, amylase, and lipase and for concentrations of albumin, total protein, bilirubin, urea nitrogen, creatinine, cholesterol, glucose, calcium, phosphorus, and triglycerides by use of an automated analyzer. Total serum solids concentrations were determined by use of refractometry. Serum IgG concentrations were quantified by use of radial immunodiffusion.

Results—For several analytes, reference intervals changed rapidly, most notably during the first few days of life. Reference intervals for alkaline phosphatase, creatine kinase, lactate dehydrogenase, and triglycerides were higher from birth to 8 weeks than adult reference intervals. Aspartate aminotransferase, bilirubin, urea nitrogen, and creatinine were higher than in adults at birth but were similar to or lower than adult reference intervals by 8 weeks. Compared with adult reference intervals, reference intervals for calcium and phosphorus concentrations were higher and for albumin and total protein concentrations were lower throughout the study period.

Conclusions and Clinical Relevance—Important differences exist between reference intervals for serum biochemical values of neonatal and adult cats. Age-appropriate reference intervals should be used for accurate assessment of serum biochemical test results in cats. (J Am Vet Med Assoc 2006;228:1033–1037)

It is well established that neonates of many species have serum biochemical values and reference intervals that are different from those of adults. This is likely attributable to the transition from fetal to neonatal life, ingestion of colostrum, maturation of metabolic processes, growth, and changes in volume of distribution and body composition.

In addition to developmental effects on serum biochemical values, nutrition also plays a role. The neonatal intestine has high absorptive capacity for ingested macromolecules during the first day of life. The composition of feline colostrum is different from that of feline milk or artificial milk replacer. Kittens may be intentionally deprived of colostrum for therapeutic reasons, such as prevention of neonatal isoerythrolysis, or because of unintentional events, such as poor maternal care or orphaning. Thus, the biochemical analytes measured in kitten sera early in life are likely to be affected by the diet consumed immediately after birth.

Age-appropriate reference intervals are essential for accurate assessment of serum biochemical test results in cats. Reference intervals have been reported for kittens for only a few analytes at selected ages. Notably absent is a detailed evaluation of the rapid changes that occur shortly after birth. The purpose of the study reported here was to establish a comprehensive set of reference intervals for commonly used serum biochemical analytes in neonatal cats from birth through 8 weeks of age, including colostrum-fed and colostrum-deprived kittens.

Materials and Methods

Cats—Twelve blood type A, specific-pathogen–free queens and their 55 kittens were enrolled in the study. Queens were under constant observation during the last days of pregnancy, and all deliveries were attended. At the conclusion of the study, all kittens were neutered and adopted by private individuals. The research protocol was approved by the University of Florida Institutional Animal Care and Use Committee and was conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Treatment groups—Kittens were removed from the queens immediately after birth to prevent ingestion of colostrum. After collection of blood samples, kittens were assigned to a colostrum-deprived group or a colostrum-fed group. Group assignments were made such that littermates were allocated into both treatment groups, and both groups had equivalent sex and birth weight distributions. Colostrum-deprived kittens (n = 28) were fed a kitten milk replacer by bottle every 4 hours for the first 48 hours of life, then returned to the queens and allowed to nurse normally. Colostrum-fed kittens (n = 27) were returned to the queens.

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Sample collection—Blood (0.5 mL) was collected from kittens on the day of parturition via jugular venipuncture for determination of baseline serum IgG and biochemical values prior to feeding and again on days 1, 2, 4, 7, 14, 28, and 56. Food was not withheld from any of the kittens prior to sample collection. Serum was harvested by centrifugation in serum separator tubes within 1 hour of collection. None of the samples were hemolyzed. Serum samples were stored in cryovials at –80°C pending analysis. All samples were analyzed within 28 days of collection.

Sample analysis—Serum IgG concentrations were determined via radial immunodiffusion with a commercial kit according to the manufacturer’s instructions. The analytical interval was 32 to 2,000 mg of IgG/dL; results < 32 mg/dL were recorded as 0 for purposes of statistical analysis, whereas results > 2,000 were diluted with phosphate-buffered saline solution and analyzed again. Total serum solids concentration was estimated by use of a refractometer. Analyses of ALP, ALT, AST, CK, LDH, GGT, amylase, lipase, albumin, total protein, bilirubin, urea nitrogen, creatinine, cholesterol, glucose, calcium, phosphorus, and triglycerides were performed in a commercial reference laboratory by use of an automated analyzer. Lipemic samples (n = 6) were cleared by ultracentrifugation prior to analysis. Serum IgG concentrations were significantly (P < 0.001) lower in kittens in the colostrum-deprived group than in the colostrum-fed group from day 1 through day 28, similar at day 42, and higher in the colostrum-deprived group at day 56 (P = 0.007). In colostrum-fed kittens, serum IgG concentration peaked 1 day after parturition, decreased to a nadir at 4 to 6 weeks, and increased toward the adult reference interval at 8 weeks. These data indicated that the colostrum-deprived group was effectively deprived of colostrum, whereas the colostrum-fed group received

Results

Treatment groups—The colostrum-deprived treatment group was composed of 28 kittens (68% males) with mean ± SD birth weight of 106 ± 10 g. The colostrum-fed group was composed of 27 kittens (59% males) with mean ± SD birth weight of 103 ± 13 g. The sex and birth weight composition of the groups was not significantly (P = 0.3) different.

Serum IgG concentration—None of the kittens had detectable serum IgG concentrations prior to nursing. Serum IgG concentrations were significantly (P < 0.001) lower in kittens in the colostrum-deprived group than in the colostrum-fed group from day 1 through day 28, similar at day 42, and higher in the colostrum-deprived group at day 56 (P = 0.007). In colostrum-fed kittens, serum IgG concentration peaked 1 day after parturition, decreased to a nadir at 4 to 6 weeks, and increased toward the adult reference
adequate transfer of immunoglobulins, which would be expected to be accompanied by other uncharacterized macromolecules contained in colostrum.

Serum biochemical analyses—Reference intervals for kittens from 0 to 56 days of age were determined (Table 1). For most analytes, the significant differences observed between the groups were minor and of little clinical importance. Notable exceptions were detected at 1 day of age, and to a lesser degree at 2 days of age, when reference intervals for certain enzyme activities (ALP, CK, GGT, LDH, and lipase) were substantially higher for colostrum-fed kittens, compared with...
colostrum-deprived kittens. These differences resolved by day 4; thereafter, the use of a single reference interval for all kittens, regardless of their initial diets, would provide accurate clinical assessment of serum biochemical results for neonatal cats. In addition, marked differences were identified for several serum biochemical analytes measured in kittens, compared with reference intervals reported for adult cats.

Discussion

Several variables associated with plasma volume, including albumin, creatinine, urea nitrogen, and total protein, were high in the immediate postpartum period and then rapidly decreased over several days. This has also been reported in human newborns and suggests that plasma volume expansion occurs shortly after birth.

The most striking changes associated with age were in enzyme activities. Similar to human newborns, reference intervals for kittens at birth for ALP, AST, CK, and LDH were higher than in adults and often increased greatly during the following 24 hours. The ALP, CK, and LDH reference intervals remained higher than those of adults at 8 weeks of age, whereas AST reference interval was similar to those of adults by 8 weeks of age. In contrast, serum activities of ALT, amylase, and lipase were consistently lower than in adults. Serum activities of AST, CK, and LDH are increased by muscle injury, and it is possible that early increases in these enzyme activities were associated with the trauma of birth. The osseous isoenzyme of ALP is increased in serum during bone growth in young animals. Serum activity of ALP is further increased after ingestion of colostrum, a rich source of ALP.

Several analytes affected by liver function had variable changes during the first 8 weeks of life. Albumin, total protein, and urea nitrogen concentrations were lower than in adults during most of the study period. Glucose, which had a wide reference interval on the day of birth, was similar to adults thereafter. Similar to human newborns, the upper limit of the reference interval for bilirubin was higher than that of adults during the first week; in 3 kittens, the serum was grossly icteric on the day of birth but this resolved thereafter. After the first week, the bilirubin reference interval was similar to that of adults. The mechanism for neonatal physiologic hyperbilirubinemia is not completely understood but may involve increased bilirubin load because of relative polycythemia, decreased life span of fetal RBCs, increased enterohepatic circulation, and the sudden transition from maternal bilirubin metabolism to dependence on immature neonatal hepatic uptake and conjugation.

Reference intervals for analytes associated with renal function also varied from those of adults. Urea nitrogen and creatinine were higher at birth than in adults, then decreased to equivalent or less than adult reference intervals through 8 weeks of age. These changes are explained by an increase in plasma volume after birth, accompanied by immature hepatic production (urea nitrogen) and reduced relative muscle mass (creatinine).

Calcium and phosphorus, analytes associated with growth and bone development, were higher in kittens than in adults throughout the study period, as is true for virtually all species studied. Parathyroid-hormone-related peptide is present in high amounts in milk throughout lactation and may affect transport of calcium into milk as well as calcium metabolism in nursing neonates. Growth hormone, which is high in juveniles, enhances renal phosphate reabsorption.

Cholesterol (after day 0) and triglyceride (at all time points) concentrations were also consistently higher in kittens of all ages, compared with adults. Kittens were not removed from their queens to withhold food prior to blood collection, so it is likely that postprandial lipidemia increased these 2 reference intervals in affected kittens.

The effect of ingestion of colostrum versus kitten milk replacer was clinically important only during the first days of life. Feline colostrum is a rich source of the enzymes ALP, ALT, AST, LDH, GGT, amylase, and lipase, which was reflected in higher activities for those enzymes in colostrum-fed kittens at 1 and 2 days of age, compared with kittens fed milk replacer.

Reference intervals are generally calculated to include 95% of healthy individuals. By definition, 5% of healthy individuals have values outside of the reference interval, and the clinician must use other information when deciding how much weight to assign to abnormal laboratory values for clinical decision making. The IFCC recommends that a minimum of 120 observations be used for calculation of human reference intervals. Although larger sample sizes are preferred, it is often impractical or too costly to perform such large sampling. The ASVCP recommends a minimum of 40 observations for estimated 95% reference intervals in animals. The present study included 55 kittens, but differences between colostrum-deprived and colostrum-fed kittens reduced some samples to 27 or 28 observations. In most instances, the statistical differences between the 2 groups were small and clinically unimportant.

Because of lack of standardization among reference laboratories, it is recommended that each laboratory develop its own reference intervals with samples from clinically normal individuals. Ideally, separate reference intervals should be established for each life stage and for each population (breed, sex, age, and lifestyle). Although reference intervals are expected to vary somewhat among laboratories, the values of and relationships among analytes should follow a similar pattern. For this reason, it would be inappropriate to adopt the reference intervals reported here as reference intervals for other facilities. However, practitioners who care for neonatal cats may use these reference intervals as a guideline for accurate interpretation of serum biochemical test results.

c. Iams Kitten, The Iams Co, Dayton, Ohio.
d. Feline IgG RID assay, VMRD Inc, Pullman, Wash.
e. Clinical refractometer 3711-2020, Schulke, Toledo, Ohio.
f. IDEXX Reference Laboratories, Sacramento, Calif.
g. Hitachi 747, Roche Diagnostics Corp, Indianapolis, Ind.
Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Hemodynamic effects of methylprednisolone acetate administration in cats
Trasida Ployngam et al

Objective—To investigate the mechanisms by which corticosteroid administration may predispose cats to congestive heart failure (CHF).

Animals—12 cats receiving methylprednisolone acetate (MPA) for the treatment of dermatologic disorders.

Procedure—The study was conducted as a repeated-measures design. Various baseline variables were measured, after which MPA (5 mg/kg, IM) was administered. The same variables were then measured at 3 to 6 days and at 16 to 24 days after MPA administration. Evaluations included physical examination, systolic blood pressure measurement, hematologic analysis, serum biochemical analysis, thoracic radiography, echocardiography, and total body water and plasma volume determination.

Results—MPA resulted in a substantial increase in serum glucose concentration at 3 to 6 days after administration. Concurrently, RBC count, Hct, and hemoglobin concentration as well as serum concentrations of the major extracellular electrolytes, sodium and chloride, decreased. Plasma volume increased by 13.4% (> 40% in 3 cats), whereas total body water and body weight slightly decreased. All variables returned to baseline by 16 to 24 days after MPA administration.

Conclusions and Clinical Relevance—These data suggest that MPA administration in cats causes plasma volume expansion as a result of an intra- to extracellular fluid shift secondary to glucocorticoid-mediated extracellular hyperglycemia. This mechanism is analogous to the plasma volume expansion that accompanies uncontrolled diabetes mellitus in humans. Any cardiovascular disorders that impair the normal compensatory mechanisms for increased plasma volume may predispose cats to CHF following MPA administration. (Am J Vet Res 2006;67:583–587)