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Evaluation of preprandial and postprandial serum bile acids and plasma ammonia concentrations in healthy domestic cats and the effects of frozen storage on plasma ammonia concentrations*

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Avaliações dos níveis pré e pós-prandiais de ácidos biliares séricos e amônia plasmática em felinos sadios e o efeito de tempo de congelamento do plasma nas concentrações de amônia

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SUMMARY

The evaluation of hepatic diseases in cats requires many laboratorial tests, and those routinely used are less conclusive. Considering the little availability of data and controversies related to the subject, we decided to study the preprandial and postprandial serum bile acids and plasma ammonia levels, and the effects of storage on these values. Preprandial (fasting) and postprandial serum bile acid (SBA) levels of 20 adult, healthy cats were determined using the enzymatic method. The measured values were 0.5 ± 0.1 and 3.6 ± 1.0 µmol/L, respectively. The preprandial and postprandial plasma ammonia (PA) levels, obtained 30 minutes after blood collection, 157.0 ± 18.7 µg/dL or 89.5 ± 10.7 µmol/L and 295.3 ± 28.2 µg/dL or 165.2 ± 17.3 µmol/L, were determined using the ion-specific electrode method, showing significant difference. However, this difference was not observed when the ammonia levels were measured in plasma samples kept under frozen storage (-20°C) during 24 and 48 hours. These results suggest that the plasma samples should not be stored for further ammonia level determination. The SBA and PA levels measured for healthy cats in this study can be compared to those obtained from cats under suspicion of liver disease, helping to establish the diagnosis and prognosis.

UNITERMS: Bile acids; Ammonia; Cats.

INTRODUCTION

Domestic cats are extremely susceptible to toxic or infectious liver diseases. This predisposition seems to be related to a lower ability of the liver in metabolizing drugs and chemicals, as well as to a higher susceptibility to various bacterial and viral infections[7,12,20].

Biochemical tests used for the evaluation of functional and morphological changes in Veterinary Medicine include several enzyme determinations, as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transferase (GGT) and bilirrubin and serum protein determinations[6,10,17].

In cats, evaluation of the activity of AP and GGT is not recommended as a diagnostic tool for hepatobiliary diseases, since the half-life of these enzymes is very short[20]. As to serum bile acid levels, however, there may be detectable alteration in cases of cholestasis, while alkaline phosphatase levels are usually maintained unchanged. Thus, bile acid levels may be a useful and sensitive tool for the early diagnosis of feline hepatobiliary disease[1,8].

Bile acids are produced in liver cells from conjugation of cholesterol with glycine and taurine, excreted into the bile and from then on into the small intestine. Most of the produced

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bile acids are actively absorbed in the ileum, and a small fraction reaches the colon. Liver cells remove, through the sinusoidal membrane, bile acids present in entero-hepatic circulation. Changes in the membrane, hepatic fibrosis and portal hypertension may result in increased systemic circulation of bile acids[1].

Center *et al.*[5] report that serum bile acid levels greater than 20 µmol/L may be highly suggestive of hepatobiliary disease in cats. However, fasting values ranging from 8 to 20 µmol/L do not exclude existing hepatic disease, due to physiological variations. According to these authors, the comparison fasting and postprandial values would allow a more accurate interpretation of the results.

Measuring ammonia plasma levels may also be helpful in diagnosing hepatobiliary disease. In severe hepatic disorders of acute or chronic progression plasma ammonia levels may be affected, while other laboratory values may be inconclusive. Ammonia is one of the compounds involved in clinical signs of hepatic encephalopathy (HE). This condition is a clinical syndrome leading to consciousness disorders and neurological disturbances nor only in animals with advanced hepatic disease (chronic or acute) as well as in animals with portosystemic shunts[6,14,18].

Different factors are involved in HE, as toxin build-up in the brain (ammonia, mercaptanes, short chain fatty acids, gamma-amino-butyric acid - GABA), changes in plasma aminoacids, and increased brain sensitivity. Among the above mentioned factors, the most feasible parameter to be measured is ammonia level determination, considering the need for more complex laboratory procedures for other toxin measurements. Thus, plasma ammonia level determination is advisable in veterinary practice settings[6,18].

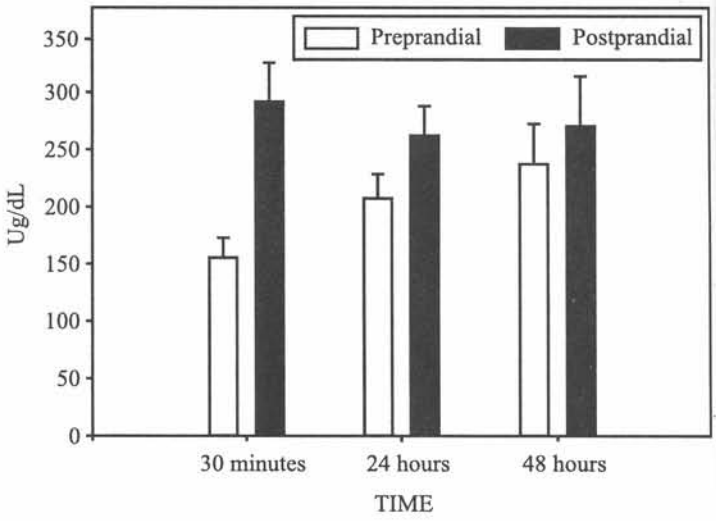

Figure 1

Preprandial and postprandial mean ± standard error of mean of plasma ammonia concentrations (µg/dL) determined up to 30 minutes after blood collection and in samples stored for 24 and 48 hours (at -20°C), obtained from 20 healthy domestic cats, using the ion specific electrode method.

The increased numbers of urease-producing bacteria in the colon is the major source of ammonia production from protein[19]. Once absorbed, the ammonia is transported to the liver through the portal circulation, and converted into urea through the Krebs-Hensleit system[6].

The ammonia tolerance test is usually recommended in dogs[6,10]. However, the use of this procedure in animals with hepatic disorders may trigger or worsen clinical signs of HE.

Table 1

Mean, standard error of the mean (SEM), maximum and minimum values of preprandial and postprandial (2 hours) plasma ammonia concentrations (µg/dL) determined up to 30 minutes after blood collection and in samples stored for 24 and 48 hours at - 20°C, obtained from healthy domestic cats. São Paulo, 1997.

Plasma ammonia (µg/dL)						
Time of measurement	30 minutes		24 hours		48 hours	
Collection time	Preprandial	Postprandial	Preprandial	Postprandial	Preprandial	Postprandial
Mean	157[a]	295.3[b]	208.4	264.6	238.7	285.9
SEM	18.7	28.2	25.0	30.2	48.4	28.4
Maximun	420.0	590.0	400.0	580.0	825.0	485.0
Minimun	58.0	123.0	62.0	72.0	66.0	82.0

a≠b Mean preprandial and postprandial values obtained from samples assayed up to 30 minutes after collection were statistically different ($p<0.05$). To convert µg/dL to mmol/L, multiply by 0.57 (Bush[4]).

[a]Whiskas - meat and vegetables flavor (Waltham Feeds) - 20 grams/kg body weight (supplied by Éffem Produtos Alimentícios)

In an attempt to prevent such risks, postprandial plasma ammonia determination is recommended[11].

In cats, as in dogs, fasting and postprandial plasma ammonia determinations in healthy animals may become an effective tool for the evaluation of liver function in suspected animals of having hepatic disorders.

As ammonia levels in blood plasma are maintained stable during a very short period, it is recommended for dogs to perform the assay within 30 minutes after blood collection[6,13]. However, Ogilvie *et al.*[15], Bush[4], and Bunch[3] report the possibility of determining ammonia levels in cats up to 48 hours after blood sampling, without any changes in plasma levels.

In this study, fasting and postprandial of serum bile acids and plasma ammonia were determined in healthy domestic cats, as well as the effect of frozen storage on the plasma ammonia levels.

MATERIAL AND METHOD

Animals - Twenty (20) healthy domestic short-hair-cats were included in this study. The selected animals, being 6 males and 14 females ranging from 1 to 3 years of age, were obtained from Experimental Animal Holding Facility of *Departamento de Clínica Médica / Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo* or from the cattery of the *Universidade de São Paulo* and also from domiciled cats. Health was assessed by history, clinical examination and confirmed by routine biochemical liver functions tests (alanine transaminase, alkaline phosphatase, total protein and albumin).

Plasma ammonia determination - Plasma ammonia concentration was determined in fresh plasma sample (kept in ice bath) obtained within 30 minutes after blood collection, and in samples stored at -20°C for 24 and 48 hours, fasting (12-hour) and postprandial (2 hours) plasma samples. The blood was centrifuged for 10 minutes at 2,000 *g* in heparinized Vacutainer® tubes. All cats received a standardized meal based on moist commercial feed[a].

Ammonia plasma levels were determined using the method proposed by Attili *et al.*[2], using Orion® electrode - model 95-12. Plasma sample was diluted with pH 12 buffer solution (Titrisol n. 9892 Merck), homogenized with magnetic stirrer, and the mV readings were measured with SA 720 Meter (Procyon). Standard solutions (0.1 to 1,000 ppm of ammonia) were diluted with pH 10.5 buffer solution (8.1 parts of pH 10 buffer Titrisol n. 9890 + 1.9 parts of pH 12 buffer Titrisol n. 9892), processed likewise and the results in mV were plotted in monolog paper. Ammonia concentration was calculated according to standard logarithmic curve referred above.

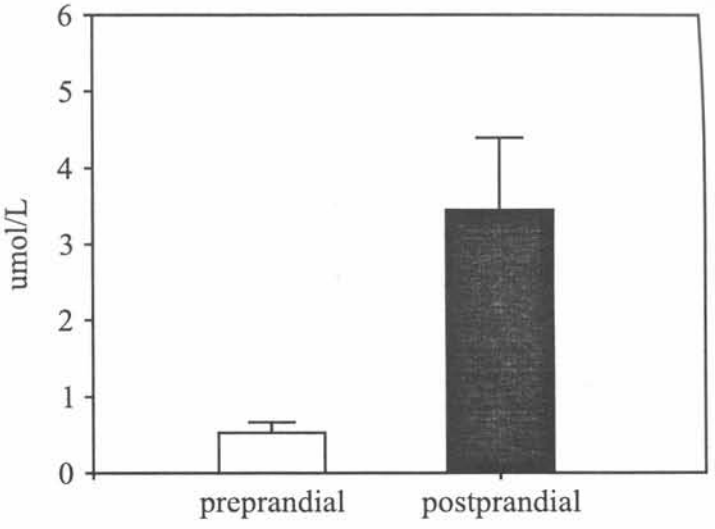

Figure 2
Mean ± standard error of mean of preprandial and postprandial bile acids values (µmol/L) obtained from 20 healthy cats, using the enzymatic method.

Serum bile acids determination - Fasting and postprandial (2 hours) serum bile acids levels were determined according to the enzymatic procedure, using commercial reagents (Enzibile® Prod. n. 1002682 / Standards Prod. n. 1002657 - NYCOMED PHARMA AS).

Statistical analysis - Means and standard error of mean were calculated considering individual values of preprandial and postprandial plasma ammonia levels and preprandial and postprandial serum bile acids levels. Variance Analysis (ANOVA) was used to evaluate the effect of frozen storage time of the samples on plasma ammonia values, followed by Dunnet's Multiple Comparison Analysis ($p<0.05$). The non-paired Student's *t* test was used at a significance level of $\alpha = 5\%$[b] for the evaluation of the difference between preprandial and postprandial plasma ammonia levels measured at 30 minutes, 24 and 48 hours, and between serum bile acids values.

RESULTS

Plasma ammonia - Fig. 1 shows mean values and standard errors of the mean for preprandial and postprandial ammonia levels, obtained 30 minutes after blood collection and in samples stored for 24 and 48 hours at -20°C.

Mean values and standard errors of the mean were 157 ± 18.7 and 295.3 ± 28.2 µg/dL (or 89.5 ± 10.7 and 165.2 ± 17.3 µmol/L), pre and postprandial respectively, for measurements

performed up to 30 minutes after blood collection; fasting and postprandial values were statistically different. However, the difference between preprandial and postprandial mean values was not observed when ammonia levels were measured in samples stored at -20°C for 24 and 48 hours (Tab. 1).

Serum bile acids - Mean values and standard error of the mean for preprandial and postprandial serum bile acids levels were 0.5 ± 0.1 and 3.6 ± 1.0 µmol/L, respectively. Fig. 2 shows mean values ± standard error of the mean for fasting and postprandial serum bile acids levels.

DISCUSSION

Serum bile acids levels observed in this study were similar, although slightly lower to those observed by Center *et al.*[5] that described 1.6 ± 0.3 and 8.2 ± 2.2 µmol/L for preprandial and postprandial, respectively. However, the increase in postprandial values compared to preprandial time was the same as that observed by the above mentioned authors, proving the efficiency and integrity of the entero-hepatic circulation as well as the liver functional capacity related to synthesis and excretion of bile acids in response to the digestive stimulus[9,16]. Lower levels observed after both time periods, when compared to those observed by Center *et al.*[5] may be related to differences in the used measuring procedure, as it may be the case with the measured ammonia levels, higher than those observed by Olgivie *et al.*[15], Bush[4], and Bunch[3].

The significant difference between fasting and postprandial plasma ammonia levels observed when the measurement was performed up to 30 minutes after blood collection has not been maintained when the samples were stored at -20°C during 24 or 48 hours. The values obtained for preprandial and postprandial stored frozen samples were very close, and the previously observed difference disappeared. This changes in the results were possibly caused by the instability of ammonia, as it was reported by Hitt; Jones[13] and Center[6], not recommending the storage of samples for 24 or 48 hours, even at very low temperatures (-20°C), opposed to the results reported by Olgivie *et al.*[15], Bush[4], and Bunch[3], where no differences were found in the plasma ammonia levels between fresh and stored samples.

The ion specific electrode method is fast and easy to perform. Thus, determination of pre and postprandial plasma ammonia concentration using this method can be considered a very safe laboratory test when compared to the risks to the patient involved in the ammonia tolerance test.

Preprandial and postprandial plasma ammonia and serum bile acids values, obtained in the present study, can be used for comparison purposes with those obtained from cats with liver disease or HE. These tests constitute extremely useful tools for the establishment of diagnosis and prognosis of hepatobiliary diseases in cats.

CONCLUSIONS

· Plasma ammonia measurement should be performed up to 30 minutes after blood collection;

· Preprandial (fasting) and postprandial plasma ammonia and serum bile acids levels, using the methods described in the present study, may allow a more accurate evaluation of liver function.

RESUMO

A avaliação das doenças hepáticas nos felinos requer uma série de provas laboratoriais e as rotineiramente empregadas ainda são pouco elucidativas. Em face da escassez de dados e das controvérsias, foi realizada a determinação dos valores pré e pós-prandiais de ácidos biliares séricos (ABS) e de amônia plasmática (AP) e a avaliação da influência do período de estocagem. Os valores de ABS pré e pós-prandiais, por método enzimático-colorimétrico, de vinte gatos sadios, adultos, foram de 0,5 ± 0,1 e 3,6 ± 1,0 µmol/l, respectivamente. No que tange aos valores pré e pós-prandiais de AP obtidos 30 minutos após a colheita de sangue (157,0 ± 18,7 µg/dl ou 89,5 ± 10,7 µmol/l e 295,3 ± 28,2 µg/dl ou 165,2 ± 17,3 µmol/l), determinados pelo método eletrodo íon-específico, observou-se diferença estatisticamente significante, mas esta diferença deixou de existir ao se determinarem os níveis de amônia nas mesmas amostras estocadas (a -20°C) por 24 e 48 horas. Esses resultados sugerem que a amostra de plasma não deve ser armazenada para posterior mensuração de amônia. Os valores observados para os ABS e AP poderão ser cotejados com aqueles obtidos em felinos com suspeita de afecção hepática, e assim auxiliar no diagnóstico e prognóstico.

UNITERMOS: Ácidos biliares; Amônia; Gatos.

REFERENCES

1- ANWER, M.S.; HABIL, V.M.; MEYER, D.J. Bile acids in the diagnosis, pathology, and therapy of hepatobiliary diseases. **The Veterinary Clinics of North America: Small Animal Practice**, v.25, n.2, p.503-17, 1995.

2- ATTILI, A.F.; AUTIZI, D.; CAPACACCIA, L. Rapid determination of plasma ammonia using an ion specific electrode. **Biochemical Medicine**, v.14, n.1, p.109-16, 1975.

3- BUNCH, S.E. Distúrbios hepatobiliares e pancreáticos exócrinos - Manifestações clínicas da doença hepatobiliar. *In*: NELSON, R.W.; COUTO, C.G. **Fundamentos de medicina interna de pequenos animais**. Rio de Janeiro : Guanabara Koogan, 1994. p.273-303.

4- BUSH, B.M. **Interpretation of laboratory results for small animal clinicians**. Oxford, Blackwell Scientific, 1991. p.290-3.

5- CENTER, S.; BALDWIN, B.H.; ERB, H.; TENNANAT, B.C. Bile acid concentration in the diagnosis of hepatobiliary disease in the cat. **Journal of American Veterinary Medical Association**, v.189, n.8, p.891-6, 1986.

6- CENTER, S.A. Pathophysiology and laboratory diagnosis of liver disease. *In*: ETTINGER, S.J. **Textbook of veterinary internal medicine**. Philadelphia : W.B. Saunders, 1989. p.1421-527.

7- CENTER, S.A.; CRAWFORD, M.A.; GUIDA, L.; ERB, H.N.; KING, J. A Retrospective study of 77 cats with severe hepatic lipidosis: 1975-1990. **Journal of Veterinary Internal Medicine**, v.7, n.6, p.349-59, 1993.

8- CENTER, S.A.; ERB, H.N.; JOSEPH, S.A. Measurement of serum bile acids concentrations for diagnosis of hepatobiliary disease in cats. **Scientific Reports**, v.207, n.8, p.1048-53, 1995.

9- CENTER, S.A.; MAN WARREN, T.; SLATER, M.R.; WILENTZ, E. Evaluation of twelve-hour preprandial and two-hour postprandial bile acid concentration for the diagnosis of hepatobiliary disease in dogs. **Journal of American Veterinary Medical Association**, v.199, n.2, p.217-26, 1991.

10- CORNELIUS, C.E. Liver function. *In*: KANEKO, J.J. **Clinical biochemistry of domestic animals**. 4.ed. San Diego : Academic Press, 1989. p.365-91.

11- DIAL, S.M. Clinicopathologic evaluation of the liver. **The Veterinary Clinics of North America: Small Animal Practice**, v.25, n.2, p.257-74, 1995.

12- HARDY, W.; ROJKO, J.L. Feline leukemia virus and other retroviruses. *In*: SHERDING, R.G. **The cat**. Diseases and clinical management. New York : Churchill Livingstone, 1989. p.229-32.

13- HITT, M.E.; JONES, B.D. Effects of storage temperature and time on canine plasma ammonia concentrations. **American Journal of Veterinary Research**, v.47, n.2, p.363-4, 1986.

14- MEYER, D.J. Liver Tests: Reflections of Hepatic Pathophysiology. *In*: WALTHAM/OSU SYMPOSIUM / GASTROENTEROLOGY, 17., Columbus, Ohio, 1993. **Proceedings**. p.43-50.

15- OGILVIE, G.K; ENGELKING, L.R.; ANWER, M.S. Effects of plasma sample storage on blood ammonia, bilirrubin, and urea nitrogen concentrations: cats and horses. **American Journal of Veterinary Research**, v.46, n.12, p.2619-22, 1985.

16- ROYER, D.; DURVILLE, A. Les acides biliaires du chien et du chat. Établissement de valeurs usuelles. **Pratique Medicale et Chirurgicale de l'Animal de Compagnie**, v.27, n.6, p.805-10, 1992.

17- SUTHERLAND, R.J. Biochemical evaluation of the hepatobiliary system. **The Veterinary Clinics of North America: Small Animal Practice**, v.19, n.5, p.899-927, 1989.

18- TABOADA, J.; DIMSKI, D.S. Hepatic encephalopathy: clinical signs, pathogenesis, and treatment. **The Veterinary Clinics of North America: Small Animal Practice**, v.25, n.2, p.337-55, 1995.

19- TAMS, T.R. Hepatic encephalopathy. **The Veterinary Clinics of North America: Small Animal Practice**, v.15, n.1, p.177-95, 1985.

20- ZAWIE, D.A.; SHAKER, E. Diseases of the liver. *In*: SHERDING, R.G. **The cat**. Diseases and clinical management. New York : Churchill Livingstone, 1989. p.1015-36.

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