Protein-Deficient Diet Alters Serum Alkaline Phosphatase, Bile Acids, Proteins and Urea Nitrogen in Dogs[1]

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EXPANDED ABSTRACT

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• dog • protein • liver • bile acids • albumin

Inadequate dietary protein intake in dogs can occur in a number of clinical situations, including prolonged voluntary anorexia, feeding of improperly formulated homemade diets (i.e., vegetarian) and overzealous protein restriction for the management of hepatic disease, renal insufficiency or struvite urolithiasis. Preliminary data in our laboratory and others (Osborne et al. 1985) suggested that restricted protein diets (8% dry matter basis (DMB)) can result in mild abnormalities in a number of common serum biochemical assays including serum albumin, urea nitrogen and alkaline phosphatase. For that reason, a study was undertaken to assess the effect of an overtly protein-deficient diet on serum biochemical assays and hepatic function tests.

Materials and methods. A prospective randomized study was designed to evaluate the effects of a protein-deficient diet on serum proteins, urea nitrogen, hepatic enzymes and bile acids. This experiment was one component of a larger study evaluating the effects of protein deficiency on bone healing in dogs. The experimental protocol was reviewed and approved by the institutional Animal Care and Use Committee of the Akron City Hospital.

Sixteen healthy adult female beagles were utilized in the study. Dogs were determined to be healthy before the study on the basis of a physical examination, complete blood count, serum biochemistry panel and urinalysis. Eight of the dogs consumed a control diet for the entire 6-mo course of the study. The remaining eight dogs were fed a protein-deficient diet. Dogs were randomly assigned to each feeding group. Dogs were housed individually and were meal fed an amount calculated to maintain their body weight. To detect external evidence of protein malnutrition, physical exams were performed on each dog weekly. Body weights were also recorded weekly.

Table 1 outlines the nutrient profile and metabolizable energy content of the two experimental diets used in the study. **Table 2** provides the ingredient composition of the two diets. Both diets were formulated and manufactured by the Experimental Food Laboratory at Mark Morris Associates (Topeka, KS) using a twin screw extruder (APV Baker, Grand Rapids, MI) for the cooking and forming process.

The amino acid sources in both the control and protein-deficient diets were egg and food grade cornstarch. Egg was an important source of protein in both the protein-deficient (80%) and control (95%) diets. Amino acids contained in the cornstarch supplied ~20% and 5% of the protein in the protein-deficient and control diets, respectively. The amino acid profile of the control diet exceeded the canine amino acid requirements established by Association of American Feed Control Officials (1994) and the National Research Council (1985).

Jugular venipuncture was performed after withholding food for 12 h at 0 (baseline), 4, 8, 12, 16 and 24 wk of the study. Venous blood was also sampled pre- and postprandially (2 h) during weeks 12 and 24 for serum bile acid analysis. Serum was harvested immediately and frozen at −70°C pending analysis.

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TABLE 1

Nutrient profile and metabolizable energy content of experimental diets (expressed as percent dry matter)

Nutrient	Protein-deficient diet	Control diet
Protein, %	4.07	15.20
Fat, %	19.42	19.58
NFE[1], %	69.73	58.61
Crude fiber, %	2.07	2.07
Metabolizable energy as fed, *kJ/g*	17.64	17.64

[1] NFE = Nitrogen-free extract.

Serum urea nitrogen (SUN),[3] serum alanine aminotransferase (SALT), serum aspartate aminotransferase (SAST), serum alkaline phosphatase (SAP), serum albumin (ALB), serum globulin and total protein (TP) analyses were performed by standard methods (Kaneko 1989). Serum bile acids (SBA) were assayed using an enzymatic method validated in dogs and cats (Center 1984).

Data were analyzed by repeated measures analysis of variance. A priori contrasts were used. Planned orthogonal contrasts were used to address specific variable effects. Statistical significance was defined as $P \leq 0.05$.

[3] Abbreviations: ALB, albumin; SALT, serum alanine aminotransferase; SAP, serum alkaline phosphatase; SAST, serum aspartate aminotransferase; SBA, serum bile acids; SUN, serum urea nitrogen; TP, total protein.

TABLE 2

Composition of experimental diets (expressed as g/kg diet as fed)

Ingredient	Protein-deficient diet	Control diet
Cornstarch	671.9	555.7
Animal fat (pork)	167.9	275.1
Egg, whole dried	61.2	86.1
Cellulose	26.9	26.9
Dicalcium phosphate	25.9	16.2
Sugar	21.6	21.6
Potassium chloride	10.8	8.1
Sodium chloride, iodized	6.5	3.0
Mineral supplement[1]	4.3	4.3
Choline chloride	2.2	2.2
Vitamin supplement[2]	0.5	0.5
Ethoxyquin	0.2	0.2

[1] The mineral supplement included zinc oxide, ferrous sulfate, manganese oxide, copper oxide, ethylenediamine dihydroiodide, cobalt carbonate and sodium selenite. Mineral levels met or exceeded established standards (National Research Council 1985).

[2] The vitamin supplement included vitamin A, vitamin D-3, vitamin E, niacin, calcium pantothenate, thiamin mononitrate, riboflavin, pyridoxine HCl, folic acid, biotin and vitamin B-12. Vitamin levels met or exceeded established standards (National Research Council 1985).

Results. Throughout the 6-mo study, all dogs were judged healthy on the basis of weekly physical examinations. Clinical signs of protein deficiency were minimal and were manifested in the protein deficient group by delayed hair regrowth and hair loss on distal extremities such as ear tips and tail. Body weights remained unchanged throughout the study (10.2 ± 1.3 kg; means ± SD).

Marked changes in multiple biochemical parameters were noted in dogs fed the protein-deficient diet and confirmed that these dogs were in a state of protein malnutrition. SUN values (0.98 ± 0.65 mmol/l) fell below the reference range (2.14–17.14 mmol/l) and were significantly different from baseline (2.81 ± 0.84 mmol/l) by the first sampling interval (4 wk) in all dogs in the protein-deficient group. Serum ALB (24.6 ± 2.5 g/l, normal range 25–40 g/l) and TP (47.1 ± 2.6 g/l, normal range 55–75 g/l) fell from baseline (ALB 35.0 ± 1.7 g/l, TP 56.6 ± 3.6 g/l) by 4 wk and remained below normal in all dogs in the protein-deficient group. Dogs fed the control diet developed no significant changes in serum TP or ALB over the course of the study. Globulin levels remained within normal reference ranges in both groups.

Two serum enzymes reflective of hepatocellular injury, SALT and SAST, were unchanged in all dogs throughout the course of the study. In contrast, as demonstrated in **Figure 1,** SAP values rose above baseline in dogs consuming the protein-deficient diet by the 8 wk of the feeding trial and mean SAP values exceeded the reference range by week 12. SAP levels remained abnormal through week 24 of the trial. Dogs consuming the control diet developed no significant changes in SAP at any sampling interval.

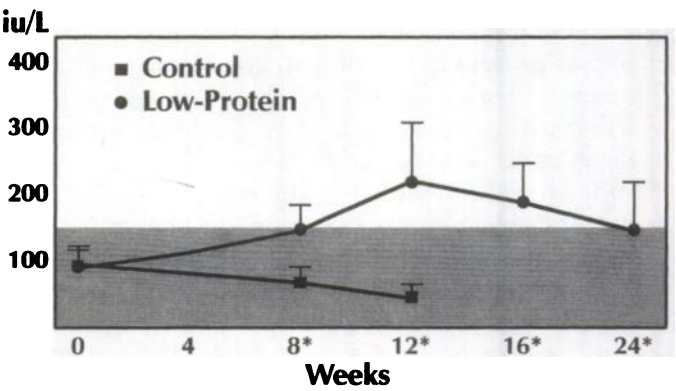

FIGURE 1 Serum alkaline phosphatase values (IU/l) in dogs fed a control (■) or a protein-deficient diet (●). All values are expressed as means ± SD for n = 8 dogs. * Sampling intervals where values differed from baselines in dogs consuming the protein-deficient diet. The reference range for SAP is shaded.

Figure 2 represents the effect of the protein-deficient diet on SBA concentrations. Postprandial SBA determinations were abnormal in the protein-deficient group, whereas control dogs were normal pre- and postprandially. Postprandial values in excess of 30 μmol/l are indicative of significant hepatic disease in dogs (Center 1985).

Discussion. The feeding of a protein-deficient diet results in rapid and biologically significant clinicopathologic abnormalities in dogs. Alterations in measurements such as SUN, serum ALB and TP are not unexpected. SUN levels are dependent on dietary protein intake; thus, protein-deficient diets result in reduced SUN concentrations. In protein-deficient states, amino acid precursors of albumin synthesis are depleted, resulting in reduced albumin production. In this study, TP values paralleled ALB values in the protein-deficient group, whereas globulins remained normal. Thus, it appears that the decrease in TP was due to reduced albumin in these experimental subjects.

SAP elevations are very common abnormalities in canine biochemical screens. Many hepatic and extrahepatic conditions can result in increased production of SAP iosenzymes from bone and hepatobiliary sources. Isoenzyme analysis was not performed in this study. However, the concommitant SBA abnormalities strongly suggest the liver as the source of SAP. Osborne et al. (1985) implicated protein-restricted diets as a cause of increased SAP. However, this finding is not discussed in commonly used veterinary reference texts on hepatobiliary disease (Center 1989, Strombeck & Guilford 1990). Based on these data, protein deficiency should be considered as a cause of mild to moderate SAP elevations.

SBA measurements are a sensitive and specific test of hepatic function (Center 1989). The finding that four of eight dogs consuming a protein-deficient diet had postprandial SBA > 30 μmol/l is strongly suggestive of hepatocellular dysfunction. However, alterations of bile flow and secretion secondary to hypoalbuminemia cannot be ruled out.

Based on these findings, protein-deficient diets can result in rapid and biologically significant alterations in SAP and SBA, parameters commonly used to evaluate hepatocellular function, as well as SUN, ALB and TP. Clinicians should consider dietary protein intake when assessing these assays.

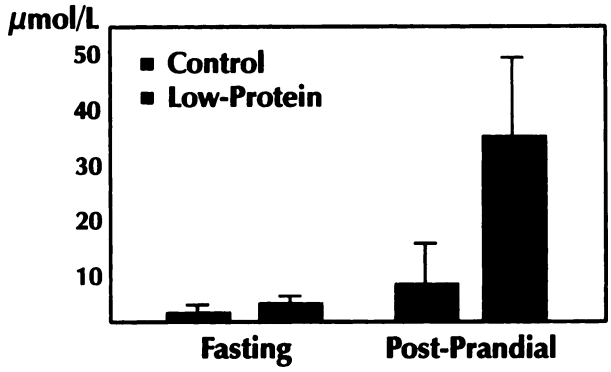

FIGURE 2 Serum bile acid concentrations in dogs fed a control (■) or a protein-deficient (□) diet. Values are expressed as means ± SD for n = 8 dogs.

LITERATURE CITED

Association of American Feed Control Officials (1994) Official Publication. AAFCO, Atlanta, GA.

Center, S. A. (1984) Direct spectrometric determinations of serum bile acids in the dog and cat. Am. J. Vet. Res. 45: 2043–2047.

Center, S. A. (1985) Bile acid concentrations in the diagnosis of hepatobiliary disease in the dog. J. Am. Vet. Med. Assoc. 187: 935–940.

Center, S. A. (1989) Pathophysiology and laboratory diagnosis of liver disease. In: Textbook of Veterinary Internal Medicine. Vol. 2 (Ettinger, S. J., ed.), pp. 1421–1479. W. B. Saunders, Philadelphia, PA.

Kaneko, J. J. (1989) Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA.

National Research Council (1985) Nutrient Requirements of Dogs. National Academy Press, Washington, D.C.

Osborne, C. A., Polzin, D. J., Abdullahi, S. U., Leininger, J. R., Clinton, C. W., & Griffith, D. P. (1985) Struvite urolithiasis in animals and man: Formation, detection, and dissolution. Adv. Vet. Sci. 29: 1–101.

Strombeck, D. R. & Guilford, W. G. (1990) Laboratory evaluation for hepatic disease. In: Small Animal Gastroenterology (Strombeck, D. R. & Guilford, W. G., eds.), pp. 529–551. Stonegate Publishing, Davis, CA.