FULL PAPER *Biochemistry*

Enzyme-linked Immunosorbent Assays of Canine Apolipoproteins B-100 and A-I

Kenjirou MIYOSHI[1)], Eiji UCHIDA[1)]* and Masayoshi NIIYAMA[1)]

[1)]*Department of Veterinary Internal Medicine II, Rakuno Gakuen University, Ebetsu, Hokkaido 069–8501, Japan*

(Received 23 June 2000/Accepted 12 August 2000)

ABSTRACT. An enzyme-linked immunosorbent assay (ELISA) for canine blood apolipoprotein (apo) B-100 and A-I was developed. The working range for the assay was 1.8 to 28.7 *n*g/well for apoB-100 and it was 50 to 410 *n*g/well for apoA-I. The intra- and inter-assay coefficients of variation for the assay for apoB-100 were 5.4 and 6.9%, respectively, and for apoA-I they were 5.8 and 10.6%, respectively. The average concentrations of apoB-100 and A-I in 25 beagles (males, aged 3–4 years) were 0.084 +/- 0.028 (mean +/- SD) mg/m*l* and 6.29 +/- 1.55 mg/m*l*, respectively. The ratios of canine (C) apoB-100 to CapoA-I were 1.41 +/- 0.58%. The respective concentrations in one case of hyperlipidemia with systemic atherosclerosis were 0.454 mg/m*l* and 11.28 mg/m*l* (a ratio of 4.03%). These values were larger than those of the controls. These results suggest that the measurements of CapoB-100 and A-I concentrations by this newly developed ELISA are helpful for diagnosis of lipidosis.

KEY WORDS: apolipoprotein A-I, apolipoprotein B-100, arteriosclerosis, canine, enzyme-linked immunosorbent assay.

J. Vet. Med. Sci. 62(12): 1269–1274, 2000

Apolipoprotein (apo) B-100 is the major protein component of very low density lipoprotein (VLDL) and low density lipoprotein (LDL), and apoA-I is the major protein component of high density lipoprotein (HDL) in human [12, 23] and other mammals [3, 11, 17, 25, 27]. These are structural proteins that allow lipids to be transported in the aqueous plasma environment. In addition, apoB-100 and A-I bind to cell surface receptors and facilitate incorporation of lipoproteins into peripheral tissues and organs. In human, increased plasma levels of LDL and VLDL or decreased HDL are regarded as risk factors for the development of arteriosclerosis and premature coronary artery disease [22]. Therefore, the levels and dynamics of apoB-100 and A-I concentrations are routinely measured in humans.

In previous studies, we found that apoB-100 was deposited on atherosclerotic lesions in obese canines [16] and suggested that measurements of the dynamics of these proteins should be required in dogs to diagnose atherosclerosis [25]. Therefore, our purpose of this study was to develop a method for measuring serum apoB-100 and A-I concentrations in the dog. In this paper, these canine proteins are referred to as CapoB-100 and A-I.

MATERIALS AND METHODS

Samples: Twenty-five male beagles in good general health were used for this study. They were kept in our laboratory's kennel with regular normal feeding and exercise. The animals were between 3 and 4 years old and their weights, 10.3 +/- 1.2 kg (mean +/- SD), were considered to be normal for their ages and breed. Blood samples were taken from the jugular vein of dogs after fasting for at least 12 hr. Immediately after clotting, the serum was separated by centrifugation at 3,000 × g for 10 min and stored at –80°C. Serum concentrations of total cholesterol (TC) and triglycerides (TG) were measured using commercial kits (Wako Pure Chemicals, Osaka, Japan). Some sera were delipidated with diisopropylether:1-butanol (40:60 [v/v]) according to Cham and Knowles [5]. Serum from a case of hyperlipidemia with systemic atherosclerosis [16] was also used.

CapoB-100 and A-I purification and antibody production: LDL and HDL were separated from canine serum by the method of Hatch and Lees [13] as described by Uchida *et al.* [24]. CapoB-100 in LDL and A-I in HDL were purified by preparative sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), with slight modifications [17]. The obtained apoB-100 and A-I (1 mg per 200 μl of saline) were emulsified with equal volumes of Freund's complete adjuvant and each was injected intradermally into one rabbit and one mouse. After 3 weeks, the same amount of protein, emulsified with Freund's incomplete adjuvant, was injected as a booster. Blood samples were obtained from each rabbit and mouse by cardiac puncture 1 to 2 weeks after the booster injection. Serum samples were tested by immunoblot analysis (Western blot).

ELISA for CapoB-100: Polystyrene microplates with flat-bottomed wells (Nunc, Tokyo, Japan) were coated with 100 μl capturing antibody (rabbit anti-CapoB-100 serum solution) that was diluted 4,000-fold with 50 mM carbonate buffer, pH 9.6. The microplates were covered and incubated overnight at 4°C. After the incubation period, the antibody solution was aspirated and washed 3 times consecutively with phosphate-buffered saline (PBS) containing 0.5 m*l*/*l* Tween-20 (PBS-Tween20) using an ELISA plate washer (SkanWASHER400, Skatron Instruments Inc., U.S.A.). PBS (200 μl) containing 15 g/*l* gelatin and 0.2 g/*l* NaN_3 was dispensed into each well and allowed to incubate for 1 hr at 37°C to block any unbound sites. The plates were then washed 3 times and stored at 4°C. The plates were stable for up to 4 weeks.

One hundred μl of either a standard that had been diluted

* CORRESPONDENCE TO: UCHIDA, E., Department of Veterinary Internal Medicine II, Rakuno Gakuen University, Ebetsu, Hokkaido, 069–8501, Japan.

80- to 5,120-fold or a sample was added to the wells. Samples were diluted 1,000-fold in PBS containing 3 g/*l* gelatin and 0.04 g/*l* NaN_3. After incubating for 1 hr at 37°C, the solution was removed and washed with PBS-Tween20, as described above. One hundred μl of detecting antibody (mouse anti-CapoB-100 serum solution) was dispensed into each wells and the plates were incubated for 1 hr at 37°C. The wells were washed to remove any unbound antibody. One hundred μl of 3,000-fold-diluted goat anti-mouse IgG alkaline phosphatase conjugate (Bio-Rad Laboratories, Hercules, CA, U.S.A.) was added to each well and the plates were incubated for 1 hr at 37°C. After washing, 100 μl of the substrate solution (Alkaline Phosphatase Substrate Kit, Bio-Rad) was added to each well and color was allowed to develop at 37°C for 1 hr. The absorbance of each well was determined at 405 nm using a microplate reader (Bio-Rad).

ELISA for CapoA-I: Microplates wells with flat bottoms were coated with 100 μl of either a standard that had been diluted 1,280- to 40,960-fold or a sample that had been diluted 5,000-fold in 50 mM carbonate buffer, pH 9.6. The microplates were covered and incubated for 2 hr at 37°C. After the incubation period, the standard or sample solutions were aspirated and unbound proteins were removed by washing 3 times with PBS-Tween20, and the remaining binding sites on the plate were blocked as described above. After the blocking period, 100 μl of detecting antibody (rabbit anti-CapoA-I serum solution) that was diluted 4,000-fold was added to the wells. After incubating for 2 hr at 37°C, the solutions were removed and washed with PBS-Tween20, as described above. A 100 μl solution of the goat anti-rabbit IgG–alkaline phosphatase conjugate (Bio-Rad), a 3,000-fold-dilution, was added to each well and the plates were then processed, and absorbances were read as described above.

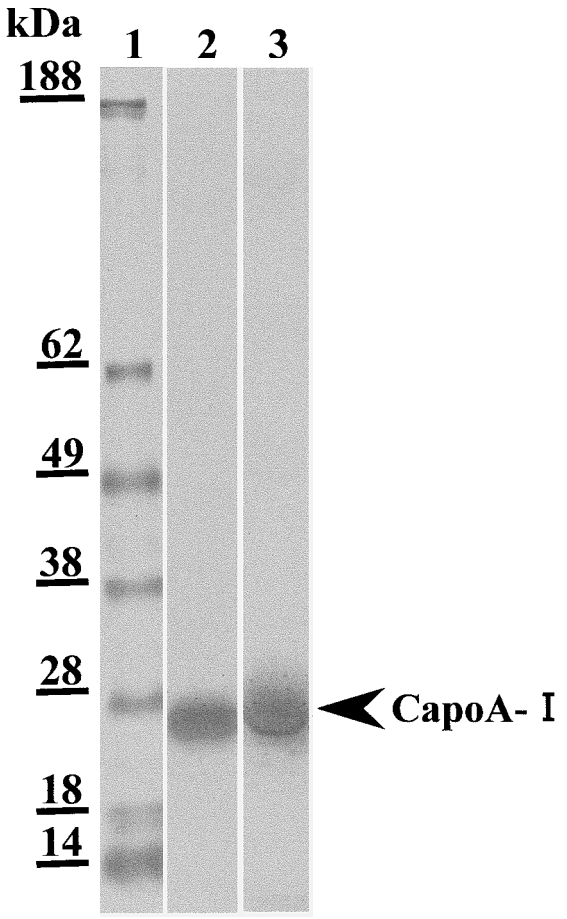

Fig. 1. Immunoblot analysis with rabbit anti-canine (C) apoA-I serum. The molecular weight markers (lane 1), purified CapoA-I (lane 2) and canine whole serum (lane 3) were electrophoresed, then blotted. An approximately 26-kDa band is labeled by rabbit anti-CapoA-I serum (arrowhead).

RESULTS

Specificity of anti-CapoA-I: Following immunopurification, rabbit polyclonal antiserum directed against CapoA-I was tested for specificity by a western blotting utilizing purified CapoA-I and whole canine serum. Immunoreactive bands were observed at a position corresponding to approximately 26 kDa (Fig. 1). We previously reported the specificity of anti-CapoB-100 serum [16].

Optimal ELISA conditions for CapoB-100: The capturing and detecting antibody concentrations for the ELISA were determined by checkerboard titration [4]. Coating of the wells with a 4,000-fold-dilution of the capturing antibody was sufficient for good adsorption of the protein (Fig. 2). The optimal concentration for the detecting antibody was evaluated by coating the wells with 4,000-fold-diluted capturing antibody, using a fixed 640-fold-diluted canine serum and different detecting antibody dilutions (2,000- to 512,000-fold). An 8,000-fold-dilution of the detecting antibody (Fig. 3) was enough for maximum color development with minimal blank value. A standard curve was obtained from several-fold-dilutions of canine serum containing apoB-100 ranging from 0.002 to 1.148 μg/m*l* at the above conditions for capturing and detecting antibody.

Analytical variables of ELISA for CapoB-100: A typical calibration curve established with canine serum is shown in Fig. 4. This ELISA has a working range of 1.8 to 28.7 *n*g CapoB-100/well. When sample sera were diluted 1,000-fold, the CapoB-100 concentrations usually ranged from 0.018 to 0.287 μg/m*l*. There was no appreciable cross-reactivity with purified canine albumin (Fig. 4). We determined the precision of the ELISA by testing the same serum sample 20 times on a day and by testing the same serum sample 12 times at different days over a period of 1 month. The intra- and inter-assay coefficients of variation (CV) were 5.4 and 6.9%, respectively. Therefore, routine assays were performed under the above conditions and a 1,000-fold-dilution of sample sera.

Optimal ELISA conditions for CapoA-I: The detecting antibody concentrations for the ELISA were determined as described above. The optimal concentration of detecting antibody was evaluated by use of a fixed 5,000-fold-diluted

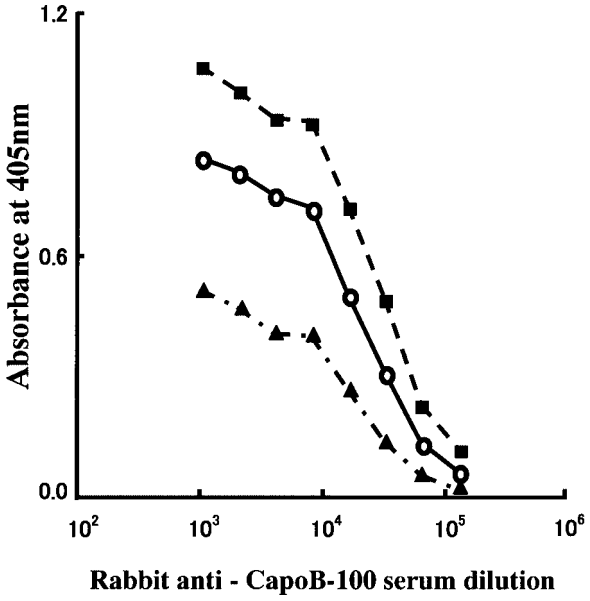

Fig. 2. Effects of the concentration of the rabbit anti-CapoB-100 serum (capturing antibody) on the assays of different concentrations of apoB-100. ▲: 1.8, ○: 7.1, ■: 28.7 *n*g CapoB-100/well.

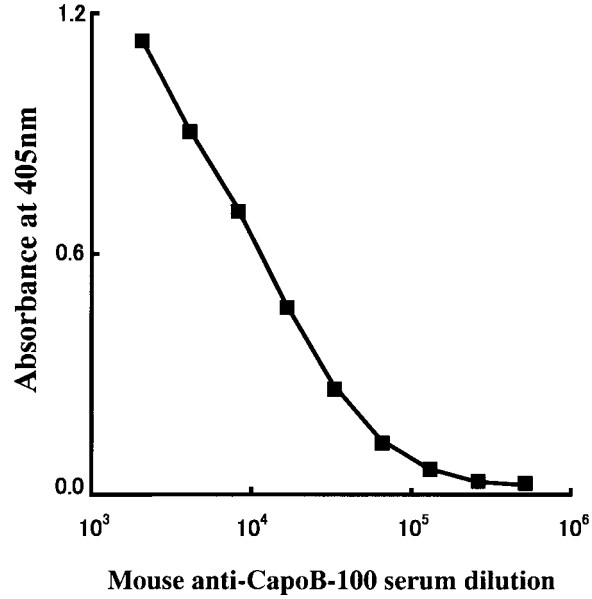

Fig. 3. Optimization of detecting antibody. CapoB-100 ELISA was performed with various dilutions of mouse anti-CapoB-100 serum to determine its appropriate concentration. An excess of rabbit anti-CapoB-100 serum (4,000-fold) and canine serum (640-fold) was used.

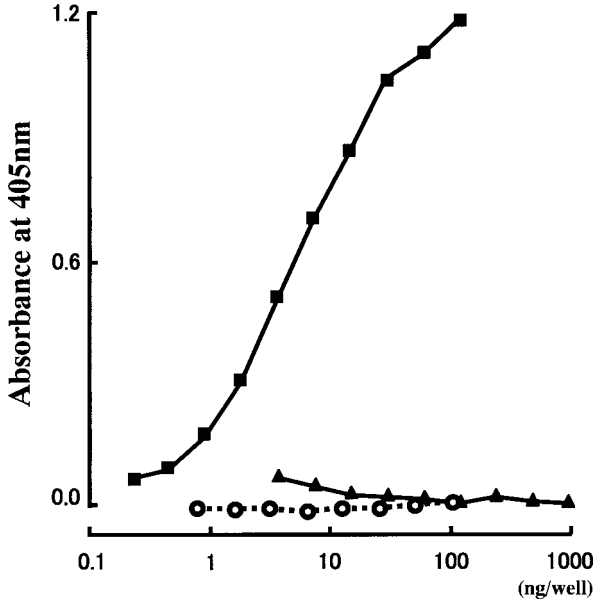

Fig. 4. Calibration curves for standard serum, delipidated serum and canine albumin. ApoB-100 concentration in standard serum was measured using single radial immunodiffusion with the purified apoB-100. The working range of the assay was from 1.8 to 28.7 *n*g CapoB-100/well. ■: standard serum, ○: delipidated serum, ▲: canine albumin.

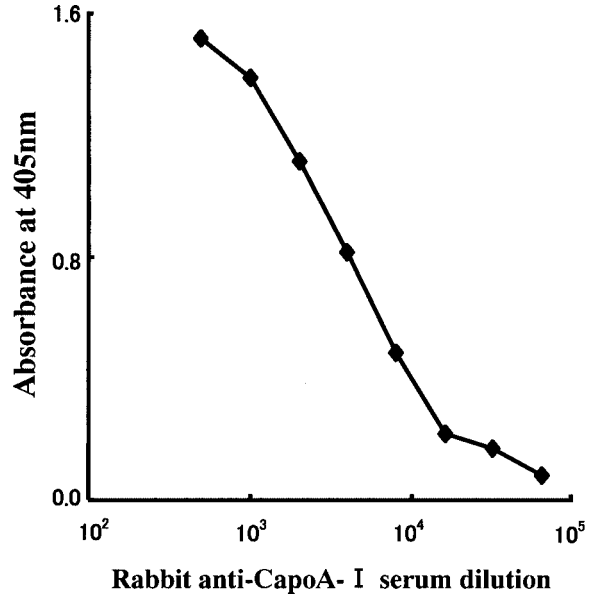

Fig. 5. Optimization of detecting antiserum. CapoA-I ELISA was performed with various dilutions of rabbit anti-CapoA-I serum to determine its appropriate concentration. An excess of canine serum (5,000-fold) was used.

canine serum and different antibody dilutions (500- to 64,000-fold). A 4,000-fold-dilution of the detecting antibody (Fig. 5) was enough for maximum color development and minimal blank value. A standard curve was obtained from several-fold-dilutions of canine serum containing apoA-I ranging from 0.13 to 16.56 μg/m*l* at the above condi-

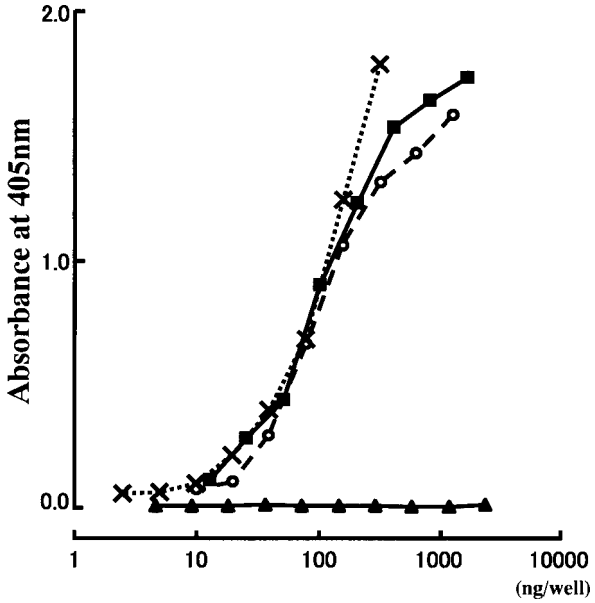

Fig. 6. Calibration curves for purified CapoA-I, standard serum, delipidated serum and canine albumin. ApoA-I concentration in standard serum was measured using the ELISA procedure described in methods section with the purified apoA-I. The working range of the assay was from 50 to 410 *n*g CapoA-I/well. ×: purified CapoA-I, ■: standard serum, ○: delipidated serum, ▲: canine albumin.

tion for detecting antibody.

Analytical variables of ELISA for CapoA-I: A typical calibration curve established with canine serum is shown in Fig. 6. This ELISA has a working range of 50 to 410 *n*g CapoA-I/well. When sample sera were diluted 5,000-fold, the CapoA-I concentrations usually ranged from 0.5 to 4.1 μg/m*l*. There was no appreciable cross-reactivity with purified canine albumin (Fig. 6). We determined the precision of the ELISA by testing the same serum sample 20 times on a day and by testing the same serum sample 10 times at different days over a period of 2 months. The intra- and inter-assay CV were 5.8 and 10.6%, respectively. Therefore, routine assays were performed under the previous conditions and a 5,000-fold-dilution of sample sera.

Sample treatment: Calibration curves were obtained from untreated serum (standard serum) and serum delipidated with diisopropylether:1-butanol (60:40 [v/v]) using the ELISA systems for CapoB-100 and A-I. The curves for CapoA-I obtained with untreated serum and delipidated serum are parallel (Fig. 6). But in the ELISA system for CapoB-100, anti-CapoB-100 did not react with the delipidated serum (Fig. 4).

Serum apoB-100 and A-I concentrations of male beagles and one case dog: Serum apoB-100 and A-I concentrations of 25 normal male beagles and one case dog were evaluated with the two developed ELISA systems. The concentrations of CapoB-100 and A-I were calculated as the ratio of CapoB-100 to CapoA-I. The results are summarized in Table 1. The average concentrations of apoB-100 and A-I in 25 beagles were 0.084 +/- 0.028 mg/m*l* and 6.29 +/- 1.55 mg/m*l* and their concentrations in the one case of hyperlipidemia with systemic atherosclerosis were 0.454 mg/m*l* and 11.28 mg/m*l*, respectively. The ratio of CapoB-100 to CapoA-I ranged from 0.60 to 2.33% (1.41 +/- 0.58%) in 25 beagles and was 4.03% in the one case dog.

DISCUSSION

We developed ELISA systems for CapoB-100 and A-I which have high sensitivity, ease of application, precision, and the potential for automation. These methods can process many samples in one assay using very little sample volume.

Studies on apolipoproteins have been reported in cow [17, 21, 28, 29], horse [26], pig [14], and dog [8, 9, 18, 19, 25] in addition to human and rat. The present study is the first to use ELISA methods for dogs, as previous measurements in dogs have been done only with an immunonephelometry assay (IPA) [8].

In this study, we developed two ELISA systems: one was a system for CapoB-100 using the sandwich method and the other was a system for CapoA-I which included a method for directly coating each well with sample sera. At first we attempted to develop a method for assaying CapoB-100 using a method similar to the one used for CapoA-I. But this method did not have sufficient sensitivity to CapoB-100 because of its low serum concentration. Therefore it was modified to the sandwich method. On the other hand, since the apoA-I concentration in dog serum is high, we could obtain a sufficiently sensitive assay for CapoA-I without using the sandwich method. In human, it was reported that undelipidated samples could not be measured due to the epitope positions when a polyclonal antibody was used for measurement of serum apoA-I [20]. But our obtained antibody against CapoA-I should provide a stable and a sufficiently sensitive ELISA system without delipidation treatment of the sample. Conversely, delipidated samples did not react with the ELISA system for CapoB-100 and their concentrations could not be measured. CapoB-100 is thought to be strongly hydrophobic, as in the human counterpart. We conclude that naked apoB-100 obtained from LDL particles by delipidation inhibited the antigen-antibody reaction due to its hydrophobic nature.

Serum apoB-100 concentrations by ELISA were reported to be 0.201 +/- 0.014 mg/m*l* in nonlactating cows [29] and 0.950 +/- 0.180 mg/m*l* in human [1]. Serum apoA-I concentrations were reported to be 0.895 +/- 0.159 mg/m*l* in cows [21], 0.551 +/- 0.043 mg/m*l* in male Wistar rats (8–12 weeks old), 0.623 +/- 0.086 mg/m*l* in male Wistar rats (36–40 weeks old) [6] and 1.18 +/- 0.16 mg/m*l* in human [2]. These serum apoB-100 and A-I concentrations are different from those in dogs. The serum apoB-100 concentration obtained from this study was approximately 1/2 that of nonlactating cows and 1/11 that of human. The serum apoA-I concentration obtained from this study was approximately 7 times that in nonlactating cows, 11 times that in male Wistar rats and 5 times that in human.

Table 1. Canine serum lipids, apo[a] B-100 and apoA-I concentrations and ratios of apoB-100 to apoA-I in male beagles (n=25) and one case of hyperlipidemia with systemic atherosclerosis

No. of canines	TC[b] mg/100 m*l*	TG[c] mg/100 m*l*	apoB-100 (B) mg/m*l*	apoA-I (A) mg/m*l*	B/A %
1	127	38	0.070	6.64	1.05
2	114	40	0.042	6.95	0.60
3	176	63	0.074	8.33	0.89
4	112	49	0.118	5.63	2.09
5	133	35	0.136	5.81	2.33
6	133	42	0.060	5.90	1.02
7	120	39	0.143	5.11	2.79
8	188	63	0.059	7.75	0.77
9	194	49	0.065	9.05	0.72
10	141	29	0.109	5.60	1.95
11	134	35	0.069	7.20	0.95
12	138	46	0.087	8.08	1.08
13	120	33	0.111	7.23	1.54
14	134	37	0.096	5.75	1.67
15	135	51	0.093	7.42	1.25
16	194	33	0.093	8.19	1.13
17	230	42	0.053	5.08	1.04
18	259	39	0.080	3.80	2.12
19	142	43	0.064	4.35	1.47
20	248	45	0.117	6.33	1.85
21	196	37	0.046	4.64	0.99
22	252	44	0.066	4.23	1.57
23	233	64	0.101	4.43	2.29
24	262	33	0.103	8.82	1.17
25	189	35	0.047	4.94	0.97
mean	172.2	42.6	0.084	6.29	1.41
SD[d]	50.7	9.6	0.028	1.55	0.58
case canine	459	173	0.454	11.28	4.03

Abbreviations: a) Apolipoprotein. b) Total cholesterol. c) Triglycerides. d) Standard deviation.

Previous measurements of apoB-100 and A-I concentrations in 12 beagles by IPA were 0.550 +/- 0.16 mg/m*l* and 3.37 +/- 0.53 mg/m*l*, respectively [8]. The difference in the apoB-100 concentrations obtained in the previous study and in our study was larger than the difference in the apoA-I concentrations. Though IPA is a rapid and inexpensive method, this method requires that the lipoproteins in the sample and the lipoprotein used for the standard are of similar sizes, and also that dyslipoproteinemic samples can seriously affect the results [10, 15]. The density range of LDL that probably contains most apoB-100 in canines is broader than the range reported for human [18]. We suggest that particles of LDL in canines have more irregular sizes than do those in human. Furthermore, in the European badger, *Meles meles*, LDL particles are present at low concentrations (<0.5 mg/m*l*) [3] and this report also suggests that serum apoB-100 concentrations are lower in the canidae than in humans.

The incidence of arteriosclerosis in human is inversely related to HDL concentrations [7]. We initially speculated that a high concentration of apoA-I and a low concentration of apoB-100 inhibit the formation of atheroma. However, our results suggest also that in dogs with lipidosis, an increase in the apoB-100 concentration to a level similar to that found in humans increases the danger of forming atheroma. In a case of hyperlipidemia with systemic atherosclerosis, the serum was turbid, and the concentrations of TC and TG were high in comparison with those in the beagles used in this study. The atherogenic index was 3.303 [25] and this hyperlipidemia type was similar to IIb type in human. In this case, the apoB-100 concentration was extremely higher, whereas the increase in apoA-I concentration is not remarkable, when compared with those in the 25 beagles. However, the ratio of apoB-100 to apoA-I in this case was higher than it was in the 25 beagles.

In conclusion, we have established ELISA systems for CapoB-100 and A-I, which can be useful for measurement of serum apoB-100 and A-I concentrations in dog and as an index of lipidosis.

REFERENCES

1. Albers, J. J., Lodge, M. S. and Curtiss, L. K. 1989. Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay as a candidate reference method for the measurement of apolipoprotein B-100. *J. Lipid Res.* 30: 1445–1458.

2. Barkia, A., Martin, C., Puchois, P., Gesquiere, J. C., Cachera, C., Tartar, A. and Fruchart, J. C. 1988. Enzyme-linked immunosorbent assay for human proapolipoprotein A-I using specific antibodies against synthetic peptide. *J. Lipid Res.* 29: 77–84.
3. Beaubatie, L., Laplaud, P. M., Rall, S. C. and Maurel, D. 1986. Isolation and characterization of the major plasma apolipoproteins, A-I and B, in the European badger, *Meles meles*. *J. Lipid Res.* 27: 140–149.
4. Bury, J. and Rosseneu, M. 1985. Quantification of human serum apolipoprotein A-I by enzyme immunoassay. *Clin. Chem.* 31: 247–251.
5. Cham, B. E. and Knowles, B. R. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. *J. Lipid Res.* 17: 176–181.
6. Chiba, H., Mitamura, T., Matsuno, K. and Kobayashi, K. 1991. A sensitive sandwich enzyme-linked immunosorbent assay of rat apolipoprotein A-I: effect of various sample treatments on apolipoprotein A-I immunoreactivity and an application to young and aged rat sera. *Biochem. Med. Metab. Biol.* 46: 380–390.
7. de Backer, G., de Bacquer, D. and Kornitzer, M. 1998. Epidemiological aspects of high density lipoprotein cholesterol. *Atherosclerosis* 137: S1–6.
8. Downs, L. G., Bolton, C. H., Crispin, S. M. and Wills, J. M. 1993. Plasma lipoprotein lipids in five different breeds of dogs. *Res. Vet. Sci.* 54: 63–67.
9. Downs, L. G., Zani, V., Wills, J. M., Crispin, S. M. and Bolton, C. H. 1994. Changes in plasma lipoprotein during the oestrous cycle of the bitch. *Res. Vet. Sci.* 56: 82–88.
10. Fievet, C., Koffigan, M., Ouvry, D., Marcovina, S., Moschetto, Y. and Fruchart, J. C. 1984. Noncompetitive enzyme-linked immunoassay for apolipoprotein B in serum. *Clin. Chem.* 30: 98–100.
11. Forgez, P., Chapman, M. J., Rall, S. C. Jr. and Camus, M. C. 1984. The lipid transport system in the mouse, *Mus musculus*: isolation and characterization of apolipoproteins B, A-I, A-II, and C-III. *J. Lipid Res.* 25: 954–966.
12. Greeve, J., Altkemper, I., Dieterich, J. H., Greten, H. and Windler, E. 1993. Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. *J. Lipid Res.* 34: 1367–1383.
13. Hatch, F. T. and Lees, R. S. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* 6: 1–68.
14. Hoff, H. F., Gerrity, R. G., Naito, H. K. and Dusek, D. M. 1983. Quantitation of apolipoprotein B in aortas of hypercholesterolemic swine. *Lab. Invest.* 48: 492–504.
15. James, R. W., Ferrer, M. and Pometta, D. 1985. A non-competitive enzyme-linked immunosorbent assay for measuring human plasma apolipoprotein B levels. *Clin. Chim. Acta* 151: 317–324.
16. Kagawa, Y., Hirayama, K., Uchida, E., Izumisawa, Y., Yamaguchi, M., Kotani, T., Niiyama, M., Yoshino, T. and Taniyama, H. 1998. Systemic atherosclerosis in dogs: histopathological and immunohistochemical studies of atherosclerotic lesions. *J. Comp. Pathol.* 118: 195–206.
17. Katoh, N., Minoura, S., Uchida, E. and Takahashi, K. 1993. Effect of estradiol administration and subsequent nonfeeding on liver estrogen receptor, serum apolipoprotein B-100, and serum triglycerides concentrations in steers. *Am. J. Vet. Res.* 54: 1476–1482.
18. Mahley, R. W. and Weisgraber, K. H. 1974. Canine lipoproteins and atherosclerosis. I. Isolation and characterization of plasma lipoproteins from control dogs. *Circ. Res.* 35: 713–721.
19. Mahley, R. W., Weisgraber, K. H. and Innerarity, T. 1974. Canine lipoproteins and atherosclerosis. II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. *Circ. Res.* 35: 722–733.
20. Marcel, Y. L., Jewer, D., Vezina, C., Milthorp, P. and Weech, P. K. 1987. Expression of human apolipoprotein A-I epitopes in high density lipoproteins and in serum. *J. Lipid Res.* 28: 768–777.
21. Oikawa, S. and Katoh, N. 1995. Enzyme-linked immunosorbent assay for apolipoprotein A-I in the serum of cattle. *Am. J. Vet. Res.* 56: 409–414.
22. Ross, R. 1986. The pathogenesis of atherosclerosis—an update. *New. Engl. J. Med.* 314: 488–500.
23. Swaney, J. B. 1980. Characterization of the high-density lipoprotein and its major apoprotein from human, canine, bovine and chicken plasma. *Biochim. Biophys. Acta* 617: 489–502.
24. Uchida, E., Katoh, N. and Takahashi, K. 1992. Induction of fatty liver in cows by ethionine administration and concomitant decreases of serum apolipoproteins B-100 and A-I concentrations. *Am. J. Vet. Res.* 53: 2035–2042.
25. Uchida, E., Kagawa, Y., Izumisawa, Y., Taniyama, H. and Niiyama, M. 1999. Plasma lipoproteins of the dog to have recognized atherosclerosis *Anim. Biochem.* 36: 29–35 (in Japanese with English Summary).
26. Watson, T. D., Burns, L., Love, S., Packard, C. J. and Shepherd, J. 1991. The isolation, characterization and quantification of the equine plasma lipoproteins. *Equine Vet. J.* 23: 353–359.
27. Watson, T. D. G. and Barrie, J. 1993. Lipoprotein metabolism and hyperlipidaemia in the dog and cat: a review. *J. Small Anim. Pract.* 34: 479-487.
28. Yamamoto, M., Katoh, N. and Oikawa, S. 1998. Evaluation of serum apolipoprotein C-III concentration by enzyme-linked immunosorbent assay and its higher concentration in cows during midlactation than during the nonlactating stage. *Am. J. Vet. Res.* 59: 1358–1363.
29. Yamamoto, O., Oikawa, S. and Katoh, N. 1995. Enzyme-linked immunosorbent assay for serum apolipoprotein B-100, a major triglyceride-transport protein in dairy cows. *Am. J. Vet. Res.* 56: 1413–1417.