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Comparison of Cholesterol Lipoprotein Profile and Levels of Blood Biomarker for Lipid Metabolism in Plasma of Dolphins, Horses and Cows

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Abstract: In this study, plasma glucose and lipid concentrations and cholesterol lipoprotein profile were measured and compared in 5 captive bottlenose dolphins (*Tursiops truncatus*), 6 Thoroughbred riding horses and 12 lactating Holstein cows. Plasma glucose concentrations of cows (71.36±5.05 mg dL^{-1}) were significantly lower than those of dolphins (107.00±16.20 mg dL^{-1}) and horses. Plasma TG concentrations of dolphins (22.80±8.70 mg dL^{-1}) were demonstrated significant difference compared to horses and cows (14.33±5.28 and 8.14±1.46 mg dL^{-1}), respectively. Total cholesterol concentrations of horses (73.50±2.88 mg dL^{-1}) were significantly lower than those of dolphins (195.20±61.20 mg dL^{-1}). Furthermore, NEFA values of dolphins (0.32±0.12 mEq L^{-1}) were demonstrated significant difference compared to horses and cows (0.04±0.02 and 0.09±0.03 mEq L^{-1}, respectively). All animals showed HDL-cholesterol dominant patterns in plasma and dolphins and horses showed clear LDL-cholesterol peak which was lower than HDL-cholesterol.

Key words: Dolphin, horse, cow, LDL, HDL, Japan

INTRODUCTION

Mammals have their own characteristics in glucose and lipid metabolism and they show different plasma metabolites values and energy metabolism related enzyme activities with various physical conditions (Arai *et al*., 2001, 2003; Muranaka *et al*., 2011; Li *et al*., 2012). Lipoproteins are believed to play important roles in energy and lipid metabolism of animals and their circulating levels reflect metabolic changes in animals with various metabolic disorders. It has been reported that since animal lipoprotein density profiles are divergent from humans, it is possible to characterize different species by fraction pattern (Terpstra *et al*., 1982). As such, animals are generally classified into 2 main lipoprotein groups: HDL and LDL dominant mammals. HDL dominant mammals consist of dogs, cats, horses, mice and rats; the LDL dominant group includes human, hamsters, guinea pigs, pigs and rabbits (Chapman, 1986).

The laboratory earlier reported on energy metabolism in horses and cows (Li *et al*., 2012). Practical comparison in plasma metabolite values clarifies the differences in nutrient metabolism among animal species. Horses and cows are similarly large animals as dolphins and have also been trained or domesticated by humans as well. Dolphins normally have relatively good motility and horses and cows can serve as good comparisons because horses are also quite active whereas cows exhibit little physical activity; thereby highlighting differences in energy metabolism between all 3 species. Dolphins may be closely related to even-toed ungulates such as cows, pigs and camels (Shimamura *et al*., 1997). Therefore, it is intriguing to investigate energy metabolism between dolphins and cows, in order to search for common points, between these animal species.

Aim of the present study was to examine and compare plasma lipid concentrations and cholesterol lipoprotein profile between healthy captive bottlenose dolphins, Thoroughbred riding horses and lactating Holstein cows, in order to seek any commonness or difference in energy metabolism between these animal species. Since, little is known about the health conditions of marine mammals, like dolphins, it is very difficult to discover disease development until it is in well advanced stages. Researchers expect that a better understanding of metabolism patterns can lead to more efficient management and disease prevention strategies of these animals.

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MATERIALS AND METHODS

Animals: Five captive bottlenose dolphins (*Tursiops truncatus*), 6 Thoroughbred riding horses and 12 lactating Holstein cows were used for this study. All animals were diagnosed to be healthy and exhibited no clinical signs for disease. The bottlenose dolphins were part of the population at Enoshima Aquarium in Kanagawa, Japan. The Thoroughbred riding horses were maintained and trained at Nippon Veterinary and Life Science University. The lactating Holstein cows were maintained at Koizumi Milk Farm in Tokyo, Japan. Dolphins were fed 4-8 times per day between 9:00 and 17:30 and consumed approximately 10-14 kg daily. Horses were fed a daily diet of ~5 kg in total, 3 times per day, between 6:00 and 16:00. Cows were given 29-32 kg of feed daily, administered 4 times daily between 5:30 and 10:00. Approval for this research has been given by the Nippon Veterinary and Life Science University Animal Research Committee.

Sample collection: Blood sampling from dolphins was conducted on a monthly basis as a part of their routine physical exams at Enoshima Aquarium. Briefly, blood was taken from the tail fluke of dolphins by venipuncture using a sterile 21 gauge disposable butterfly needle. Because the dolphins were trained for blood sampling and/or other routine physical exams, they can voluntarily display their tail or body when samples need to be collected. All dolphin blood samples were obtained 16-17 h after their last feeding. Blood sampling from horses and cows was obtained from the jugular or caudal vein, respectively, early in the morning before feeding (5:00-7:00 am). The animals showed no excitement or fear when blood sampling was conducted, since they were accustomed to blood sampling. Blood samples from all animals were centrifuged at 3500 rpm for 10 min at 4°C in order to obtain plasma samples which were subsequently frozen at -80°C until further use.

Plasma metabolite assays: Plasma biochemistry analysis on Glucose (Glu), Total cholesterol (T-cho), Triglycerides (TG) was performed using an AU680 auto analyzer (Beckman Coulter, CA, USA) with the manufacturer's reagents. Non-Esterified Fatty Acid (NEFA) concentration was measured using a Wako NEFA-C test commercial kit (Wako Pure Chemical Industries, Inc., Tokyo, Japan).

Lipoprotein cholesterol profiling: Plasma lipoprotein cholesterol profiles were detected by the biphasic agarose gel electrophoresis method utilizing commercial Quickgel LIPO gels (Code No. J715, Helena Laboratories, Saitama, Japan). In brief, 30 μL of plasma as sample volume were loaded into the dipping well of an Epalyzer 2 Electrophoresis Processing Analyzer (Helena Laboratories) of which 5-6 μL was loaded onto the gel and run with a 13 min set migration time at 240 V and at 15°C. After migration, the gels underwent a 14 min reaction time, followed by a 12 min and 30 sec decolorizing and fixing time, respectively. Lipoprotein cholesterol profiles were assessed and analyzed using Edbank III Analysis Software (Helena Laboratories).

Statistical analysis: Results are expressed as means±SD. Paired groups were compared using the Mann-Whitney U-test for data with non-normal distribution and statistical significance was set at $p<0.05$. All tests were performed using Sigmaplot analysis software (Sigmaplot 11.0, Build 11.0.077; Systat Software Inc., San Jose, CA).

RESULTS AND DISCUSSION

Blood biochemistry values: Plasma glucose and lipid concentrations in dolphins, horse and cows are shown in Table 1. Plasma glucose concentrations in dolphins were similar to those in horses and significantly higher than those in cows. Plasma TG and NEFA concentrations in dolphins were significantly higher than those of horses and cows. Plasma T-cho concentrations in dolphins were almost same to those in cows and significantly higher than those in horses.

Electrophoretic patterns of cholesterol lipoprotein: Cholesterol lipoprotein electrophoretic patterns in plasma of animals are shown in Fig. 1. Three animals showed HDL-cho dominant patterns and dolphin and horse showed clear fraction of LDL-cho. However, cow did not show clear fraction of LDL-cho.

The present data showed that plasma TG and NEFA values of healthy dolphins were significant higher than those of horses and cows, respectively and that dolphins, horses and cows with plasma HDL-cho dominant patterns were classified into HDL dominant mammals such as dog, cat, mouse, goat and rat (Chapman, 1986). That indicates different electrophoretic patterns differing from that of human as LDL-cho dominant animal. Dolphin and horse

Table 1: Comparisons of blod biochmistry values of dolphins, horses and cows

Parameters	Dolphins (n = 5)	Horses (n = 6)	Cows (n = 2)
Glucose (mg dL^{-1})	107.00±16.2	100.67±4.84	71.36±5.050*
TG (mg dL^{-1})	22.80±8.70	14.33±5.28*	8.14±1.460*
T-Cho (mg dL^{-1})	195.20±61.2	73.50±2.88*	217.07±40.57
NEFA (mEq L^{-1})	0.32±0.12	0.04±0.02*	0.09±0.030*

Values areshown as mean±SD; *Significant difference as compared to dolphins (Mann Whitney U-test, $p<0.05$)

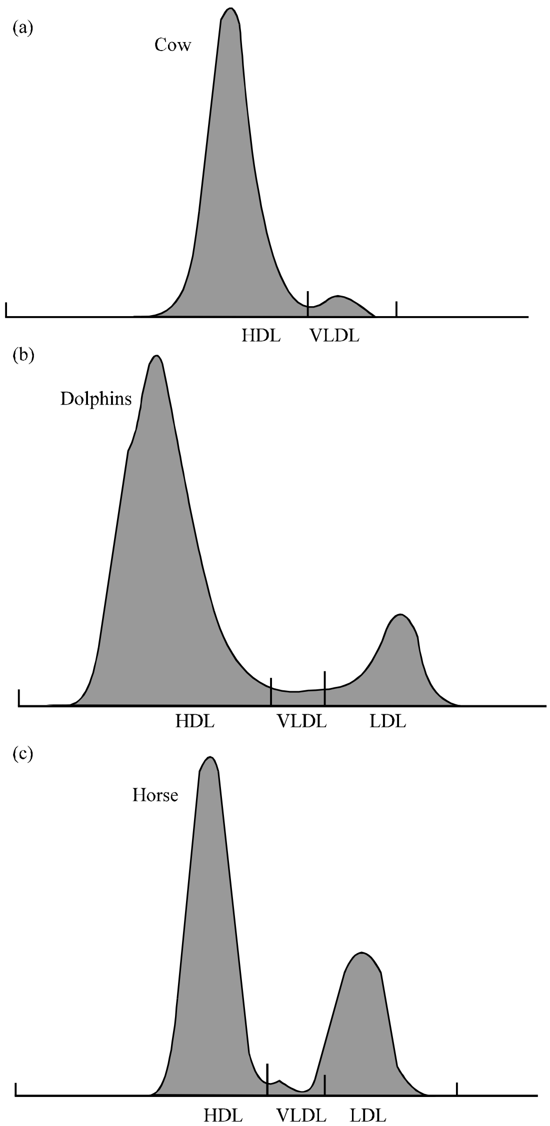

Fig. 1: Comparison of plasma cholesterol profile pattern in a) cows, b) dolphins and c) horses. Comparison of plasma cholesterol patterns between cows, horses and dolphins. Plasma cholesterol were separated by an electrophoretic technique and analyzed using Epalyzer 2 Electrophoresis Processing Analyzer (Helena Laboratories)

showed clear LDL-cho peak which was lower than HDL-cho fraction but cow did not show LDL-cho fraction in plasma. Dolphin plasma cholesterol lipoprotein profile resembles to that in horse rather than in cow. It was reported that HDL accounts for approximately 60% of horse plasma lipoprotein and LDL comprised three discrete subfractions (Watson *et al*., 1993). LDL-cho concentrations were light in the rabbit and scarce in the horse (Hollanders *et al*., 1986).

It was suggested that serum lipid value might be useful for evaluating metabolic states in Turkman horses with some kinds of disorders (Asadi *et al*., 2006). Furthermore, it was reported that the Shetland ponies had higher plasma triglyceride and VLDL concentrations than their Thoroughbred counterparts (Watson *et al*., 1991). On the other hand, hyperlipidemia of captive Bottlenose dolphins during pregnancy (Terasawa and Kitamura, 2005) and seasonal changes in T-cho value in captive Bottlenose dolphin sera (Terasawa *et al*., 2002) have been reported. Therefore, application of plasma lipid concentrations as diagnostic marker for some metabolic disorders should be considered in relation to factors to induce changes such as pregnancy, season, diseases, etc. Moreover, foods seem to mostly affect the plasma lipid concentrations and compositions. Dolphins are carnivores whereas horses and cows are herbivores. Further studies on the relationship between food compositions and lipid metabolism in dolphins are needed such as rice bran oil affects plasma lipid concentrations and lipoprotein composition in mares (Frank *et al*., 2005).

In obese animals, significant increase of plasma NEFA and LDL-cho fractions are prominent (Mori *et al*., 2011). Dolphins maintained in aquarium appear to tend to become obese with ease compared to wild dolphins. It was reported that captive dolphins indicated metabolic states that mimicked those found in human type 2 diabetes associated with some insulin resistance (Venn-Watson *et al*., 2011). Therefore, LDL-cho lipoprotein and levels of blood biomarker for lipid metabolism such as TG and NEFA are expected to be available for diagnostic marker for obesity-associated disease in dolphins.

CONCLUSION

Since, it has been regarded that high NEFA values could induce to lipotoxicity in any kinds of animals (Wu *et al*., 2008; Cheng *et al*., 2009; Jun *et al*., 2011; Yao *et al*., 2011), monitoring of plasma lipid concentrations and cholesterol profile is possibly a useful indicator for preventing metabolic disorders in dolphins.

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REFERENCES

Arai, T., A. Inoue, Y. Uematsu, T. Sako and N. Kimura, 2003. Activities of enzymes in the malate-aspartate shuttle and the isoenzyme pattern of lactate dehydrogenase in plasma and peripheral leukocytes of lactating Holstein cows and riding horses. Res. Vet. Sci., 75: 15-19.

Arai, T., M. Takahashi, K. Araki and T. Washizu, 2001. Activities of enzymes related to the malate-aspartate shuttle in the blood cells of thoroughbred horses undergoing training exercise. Vet. Res. Commun., 25: 577-583.

Asadi, F., M. Mohri, M. Adibmoradi and M. Pourkabir, 2006. Serum lipid and lipoprotein parameters of Turkman horses. Vet. Clin. Pathol., 35: 332-334.

Chapman, M.J., 1986. Comparative Analysis of Mammalian Plasma Lipoproteins. In: Methods in Enzymology, Segrest, J.P. and J.J. Alberts (Eds.). Vol. 128A, Academic Press, Boston, pp: 70-143.

Cheng, Y., J. Zhang, J. Shang and L. Zhang, 2009. Prevention of free fatty acid-induced hepatic lipotoxicity in HepG2 cells by magnesium isoglycyrrhizinate *in vitro*. Pharmacology, 84: 183-190.

Frank, N., F.M. Andrew, S.B. Elliot, J. Lew and R.C. Boston, 2005. Effects of rice bran oil on plasma lipid concentrations, lipoprotein composition and glucose dynamics in mares. J. Anim. Sci., 83: 2509-2518.

Hollanders, B., A. Mougin, F. N'Diaye, E. Hentz, X. Aude and A. Girard, 1986. Comparison of the lipoprotein profiles obtained from rat, bovine, horse, dog, rabbit and pig serum by a new two-step ultracentrifugal gradient procedure. Comp. Biochem. Physiol. Part B: Comp. Biochem., 84: 83-89.

Jun, D.W., W.K. Cho, J.H. Jun, H.J. Kwon and K.S. Jang *et al*., 2011. Prevention of free fatty acid-induced hepatic lipotoxicity by carnitine via reversal of mitochondrial dysfunction. Liver Int., 31: 1315-1324.

Li, G., P. Lee, N. Mori, I. Yamamoto and T. Arai, 2012. Long term intensive exercise training leads to a higher plasma malate/lactate dehydrogenase (M/L) ratio and increased level of lipid mobilization in horses. Vet. Res. Commun., 36: 149-155.

Mori, N., P. Lee, K. Kondo, T. Kido, T. Saito and T. Arai, 2011. Potential use of cholesterol lipoprotein profile to confirm obesity status in dogs. Vet. Res. Commun., 35: 223-235.

Muranaka, S., N. Mori, Y. Hatano, T.R. Saito and P. Lee *et al*., 2011. Obesity induced changes to plasma adiponectin concentration and cholesterol lipoprotein composition profile in cats. Res. Vet. Sci., 91: 358-361.

Shimamura, M., H. Yasue, K. Ohshima, H. Abe and H. Kato *et al*., 1997. Molecular evidence from retroposons that whales form a clade within even-toed ungulates. Nature, 388: 666-670.

Terasawa, F. and M. Kitamura, 2005. Hyperlipemia of captive bottlenose dolphins during pregnancy. J. Vet. Med. Sci., 67: 341-344.

Terasawa, F., M. Kitamura, A. Fujimoto and S. Hayama, 2002. Seasonal changes of blood composition in captive bottlenose dolphins. J. Vet. Med. Sci., 64: 1075-1078.

Terpstra, A.H., F.J. Sanchez-Muniz, C.E. West and C.J. Woodward, 1982. The density profile and cholesterol concentration of serum lipoproteins in domestic and laboratory animals. Comp. Biochem. Physiol. B, 71: 669-673.

Venn-Watson, S., K. Carlin and S. Ridgway, 2011. Dolphins as animal model for type 2 diabetes: Sustained, post-prandial hyperglycemia and hyperinsulinemia. Gen. Comp. Endocrinol., 170: 193-199.

Watson, T.D., C.J. Packard and J. Shepherd, 1993. Plasma lipid transport in the horse (*Equus caballus*). Comp. Biochem. Physiol. Part B: Comp. Biochem., 106: 27-34.

Watson, T.D.G., L. Burns, S. Love and C.J. Packard and J. Shepherd, 1991. The isolation, characterisation and quantification of the equine plasma lipoproteins. Equine Vet. J., 23: 353-359.

Wu, X., L. Zhang, E. Gurley, E. Studer and J. Shang *et al*., 2008. Prevention of free fatty acid-induced hepatic lipotoxicity by 18β-glycyrrhetinic acid through lysosomal and mitochondrial pathways. Hepatology, 47: 1905-1915.

Yao, H.R., J. Liu, D. Plumeri, Y.B. Cao and T. He *et al*., 2011. Lipotoxicity in HepG2 cells triggered by free fatty acids. Am. J. Transl. Res., 15: 284-291.