

EFFECTS OF SHORT-TERM FASTING ON LIPID AND LIPOPROTEIN CONCENTRATIONS IN HEALTHY LEAN DOGS

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Abstract

Introduction. Analysis of canine lipoprotein fractions after agarose gel electrophoresis (agEF) separation could be an important diagnostic tool in primary and secondary dyslipidemia diagnosis. The aim of this study was to measure concentrations of triglycerides and cholesterol and to analyze lipoprotein fractions in dogs after basal (12 hours) and short-term (24 and 36 hours) fasting, i.e., frequent conditions in clinical practice.

Materials and Methods. Blood samples were collected from six lean dogs of both sexes and different breeds, after 12, 24 and 36 hours of fasting. Concentrations of glucose, triglycerides and cholesterol were determined on an automated wet biochemistry analyzer, lipoprotein fractions were separated by agEF and leukocyte numbers were assessed on an automated hematology analyzer.

Results and Conclusions. Results showed there was no significant change in glucose, triglyceride and cholesterol concentrations nor in leukocyte numbers during dog fasting. Moreover, there was no change in $\alpha 1$ - and $\alpha 2$ -fractions, but there was a significant decrease in pre β - and β -fraction of lipoproteins. It is known that high density lipoproteins (HDL) have α -mobility and very low density (VLDL) and low density lipoproteins (LDL) have pre β - and β -mobility. Thus, it is possible that reverse cholesterol transport maintained by HDL is not affected during short-term fasting. On the contrary, synthesis

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of VLDL and formation of LDL are probably decreased because endogenous synthesis of triglycerides is decreased or their clearance is increased.

Key Words: agarose gel electrophoresis, lipoprotein fractions, cholesterol, triglycerides

INTRODUCTION

Lipoproteins (Lps) are a heterogeneous group of molecules involved in lipid transport between tissues. According to their hydrated density, structure, and function, Lps are divided into chylomicrons (CMs), very low density Lps (VLDL), low density Lps (LDL) and high density Lps (HDL). One of the fast and easy methods to visualize serum Lps is agarose gel electrophoresis (agEF). Fractions that can be obtained after agEF of canine serum are: α 1-, α 2-, pre β - and β -fractions, considered to be HDL-2, HDL-1, VLDL and LDL, respectively (Mahley et al., 1974). VLDL and LDL contain more triglycerides, and less cholesterol than HDL (Maldonado et al., 2001). Overnight fasted canine sera of lean, healthy dogs should not have CMs. If they are present, CMs, due to their large molecular mass, are immobile and remain at the application site in the gel. The utility of canine Lp fraction analysis in clinical and research settings is not fully investigated.

To date, investigations concerning lipid metabolism and Lps in dogs have mainly targeted primary (Xenoulis et al., 2013) or secondary lipid disorders characterized by hyperlipidemia (Behling-Kelly, 2014). Furthermore, lipids and Lps were investigated in canine obesity and different dietary strategies designed to reduce increased body weight (Mori et al., 2015; Bauer, 2004). The effect of long-term fasting has also been investigated, showing that it does not influence total plasma lipid concentration in healthy dogs (Spitzer & Miller, 1956) or in an actively wintering canid, the arctic blue fox (Mustonen et al., 2006). However, these investigations did not consider Lps. Therefore, we hypothesize that more subtle changes in lipid and Lp metabolism could be documented using agEF.

The aim of this study was to measure concentrations of triglycerides and cholesterol and to analyze agEF Lp fractions in dogs after basal (12 hours) and short-term (24 and 36 hours) fasting. To monitor the possible stress reaction during food deprivation, dogs' behavior, total numbers of leukocytes, neutrophil granulocytes, and lymphocytes were determined along with lipid and Lp concentrations.

MATERIALS AND METHODS

Animals

The study was conducted on five female (three neutered, two intact) and one intact male dog with body mass indices of 3. Body mass index was assessed by observation and palpation of the dogs and a scaling system from 1 to 5 was used. Dogs were two to six years old with a median age of 3.25 years. Two dogs were mongrel while four other dogs were of following breeds: German Shepherd, Cane Corso, German Shorthaired

Pointer and German Wirehaired Pointer. The dogs were clinically healthy, living indoors with their owners. They were habituated to have one meal of dry commercial food during the afternoon. Food was withdrawn from the dogs for 36 hours. Water was available *ad libitum*. A veterinarian familiar with the dogs enrolled them in the study and performed the blood sampling from *v. cephalica antebrachii* after 12, 24 and 36 hours of fasting. After the last blood sampling, food was introduced. Before blood sampling, the veterinarian noted the dog behavior as: normal, hyperactive – seeking food behavior or hypoactive – less agile than usual. The owners signed informed consent to participate in the study. In accordance with the Serbian Law for protection of animal welfare, permission (323-07-10264/2016-05/2 issued 21 November 2016) for the study was obtained from the Ministry of Agriculture and Forestry, Republic of Serbia.

Laboratory analysis

Blood samples were collected for hematology and biochemistry analysis in EDTA tubes and tubes with clot activator (Vacutainer BD). Complete blood count (CBC) was conducted on an impedance hematology analyzer (Abacus Junior Vet, Diatron, Austria) within two hours of blood collection. Serum was centrifuged after 30 minutes and stored at -20°C until all samples were collected. Samples were not frozen for more than two weeks. Concentrations of glucose, triglycerides and cholesterol were determined on an automated wet biochemistry analyzer (Technicon RA-XT, USA). Intrassay and interassay coefficients of variation were less than 5%.

Lps were separated by commercial agarose gels according to the manufacturer protocol (SAS lipoprotein, Helena laboratories, UK). Gels were scanned on an Epson perfection v800 scanner and evaluated using densitometry and TotalLab quant software. According to the manufacturer, the Fat red stain provided in this kit stained triglycerides and cholesterol esters (Helena Laboratories). The Lp concentration in single electrophoretic fractions (mmol/L) was obtained when the electrophoretic fraction (%) was multiplied by the sum of the cholesterol and triglyceride concentrations (mmol/L) and divided by 100. To be able to compare the change in pre β - and β -fractions, their concentrations were summed and expressed as a unique fraction named Triglyceride Rich Lipoprotein fraction (TRL).

RESULTS

Our results demonstrated that total cholesterol, triglyceride and glucose concentrations did not change significantly during fasting (Figure 1).

In four dogs, after agEF, Lps were separated into α 1-, α 2- and β -fractions (Figure 2a). Two dogs also had a pre β -fraction (Figure 2b). The concentration of lipids within the α 1- and α 2-fractions was without significant change during fasting (Figure 2a, b). Pre β -fractions disappeared after 24 and 36 hours of fasting in the two dogs that had this fraction (Figure 2b), while β -fractions decreased after 24 and 36 hours of fasting

(Figure 2ab). When pre β - and β -fractions were expressed as TRL, it was evident that fasting dogs experienced significant decreases of these Lps (Figure 3).

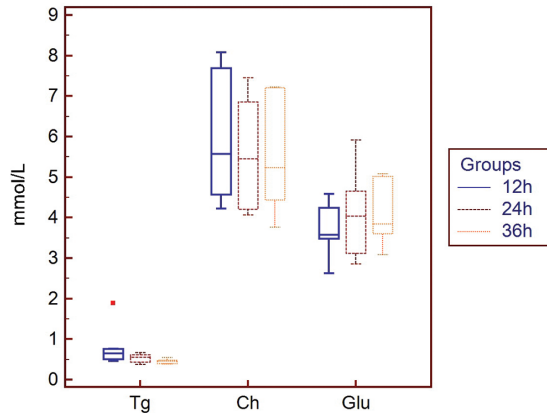


Figure 1. Total cholesterol (Ch), triglycerides (Tg) and glucose concentration in dog sera after 12 h, 24 h and 36 h of fasting.

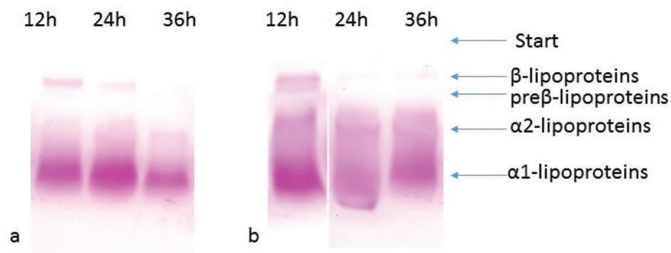


Figure 2. Representative electrophoretic pattern of canine lipoprotein fractions after 12h, 24h and 36 h of fasting. Image a) depicts a sample without pre β -lipoprotein fraction and b) depicts a sample with pre β -lipoprotein fraction that disappears after 24 h and 36 h of fasting.

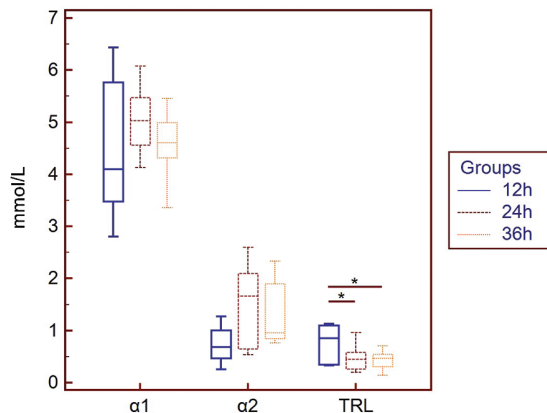


Figure 3. Concentration of $\alpha 1$ -lipoproteins, $\alpha 2$ -lipoproteins and triglyceride-rich lipoproteins (TRL) in dog sera after 12 h, 24 h and 36 h of fasting.

White blood cell counts showed there was no significant change in total leukocyte, neutrophil, granulocyte or lymphocyte numbers during fasting (Figure 4). However, a slight increase in total leukocytes and lymphocytes was observed after 24 hours of fasting. At the same time point, the veterinarian noticed slight hyperactivity in most of the dogs (Table 1).

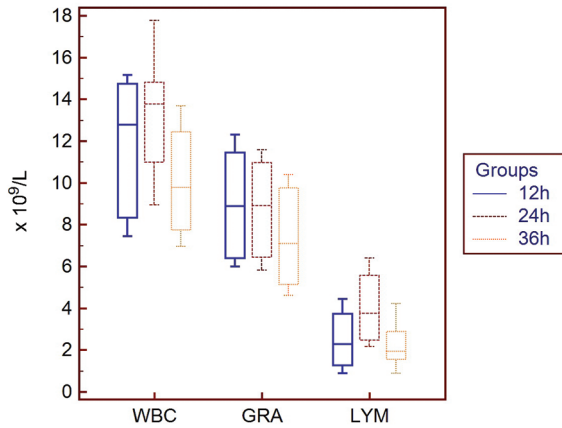


Figure 4. Number of total white blood cells (WBC), granulocytes (GRA) and lymphocytes (LYM) in dog blood after 12 h, 24 h and 36 h of fasting.

Table 1. Dog activity during fasting, as estimated by a supervising veterinarian

Fasting time (hours)	Normal activity	Hyperactivity	Hypoactivity
12	6/6	/	/
24	1/6	5/6	/
36	6/6	/	/

DISCUSSION

The main results of this study have shown that concentration of total triglycerides, cholesterol and α 1- and α 2- fractions do not change significantly during basal and short-term fasting in lean dogs. However, there is a significant decrease in pre β - and β -fractions after 24 and 36 hours of fasting. The animals were slightly hyperactive after 24 hours of fasting, searching for food at regular times during the day. The glucose concentration was within physiological limits and without change during fasting as was the number of leukocytes, indicating that animals did not experience significant stress as an effect of short-term fasting.

Our study has shown that during basal and short-term fasting, there is no substantial change in concentrations of total triglycerides and cholesterol in healthy lean dogs. This is not surprising, as a previous study had already shown that canid species can

maintain physiological levels of triglycerides after one week of fasting (de Brujine et al, 1981). For cholesterol concentration, such data from fasting canids were not found in the available literature. It is interesting to note that the most abundant agEF fraction in dogs is the α 1-fraction, which contains HDL-2 molecules involved in binding cholesterol from cells. Approximately 40% of lipids detected with Fat red stain after agEF are located at the position of HDL-2 molecules. Although not always very clearly distinct visually, the α 2-fraction contains larger HDL-1 particles, which in the body, are already loaded with cholesterol esters and are ready to deliver them to the liver for elimination or reuse (Xenoulis & Steiner, 2010). In the current study, lipid concentrations in the α 1- and α 2-fractions did not change with fasting, indicating that reverse cholesterol transport, maintained by HDL-2 and HDL-1 molecules, was not disturbed during short-term fasting.

After 24 hours of fasting, a significant decrease in lipid concentration in pre β - and β -fractions in the dogs' sera was evident. The pre β -fraction contains VLDL molecules synthesized in the liver that serve to deliver endogenous triglycerides to tissues. In that process, VLDLs are shaped to form LDLs positioned in the β -fraction. LDL molecules are rich in cholesterol, delivering it to the tissues or liver. Apparently, after 24 and 36 hours of fasting, dogs synthesize less endogenous triglyceride in the liver, less VLDL is secreted and consequently, fewer LDL molecules are formed. The other possibility is that clearance of these molecules is more efficient, decreasing their plasma concentration. In mice, after 24 hours of fasting, decreases in VLDL and LDL Lp concentrations were demonstrated, but also, decreases in total serum cholesterol and triglycerides were found (Van Ginneken et al., 2007). This fast decrease could be ascribed to higher basal metabolic rate in mice than in dogs (Singer and Morton, 2000). In feline species, Lp profiles during fasting have not been determined. In humans after 36 hours of fasting, there is a decrease in the level of serum triglycerides (Elia et al., 1999). The upper limit of the reference interval for triglycerides is the same for humans and dogs (1.5 mmol/L), while cholesterol is higher in dogs (7 mmol/L vs 5.5 mmol/L in humans). Humans have a LDL/HDL cholesterol ratio of 1.5 (Zimmer et al., 1980) while in dogs this ratio is around 0.25 (Mahley et al., 1974) due to the absence of cholesteryl ester transfer protein that is necessary for transfer of cholesteryl esters from HDLs to VLDLs and LDLs and triglycerides in the reverse direction (Ouguerram et al., 2004). The fact that during short-term fasting in humans, total triglycerides decrease, and in dogs, only triglyceride-rich Lps decrease (while total triglycerides are not decreased significantly), probably reflects specificity of metabolism in these two species.

CONCLUSION

In conclusion, these data suggest that canids have a physiologic adaptation to short-term (and probably long-term) fasting that keeps their concentration of plasma cholesterol readily available for tissues and does not provoke an important

decrease in total plasma triglycerides. We also propose that data describing changes in concentrations of triglyceride, cholesterol, VLDL, LDL and HDL, glycaemia and selected hematology parameters in healthy canines during short-term fasting are important for interpretation of changes that could be encountered during different acute diseases that are characterized by loss of appetite or to define changes in primary lipid disorders.

Authors contributions

ADD collected the samples. ADD, ZM and ABI carried out laboratory work. ZM has done statistical analysis. ADD, AR and MKF designed the study and wrote the article while all authors read and approved final version of the article.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

- Bauer, J. E. 2004. Lipoprotein-mediated transport of dietary and synthesized lipids and lipid abnormalities of dogs and cats. *Journal of the American Veterinary Medical Association*, 224: 668-675. <https://doi.org/10.2460/javma.2004.224.668>
- Behling-Kelly, E. 2014. Serum lipoprotein changes in dogs with renal disease. *Journal of veterinary internal medicine*, 28: 1692-1698. <https://doi.org/10.1111/jvim.12450>
- de Bruijne, J. J., Altszuler, N., Hampshire, J., Visser, T. J., & Hacking, W. H. 1981. Fat mobilization and plasma hormone levels in fasted dogs. *Metabolism*, 30: 190-194.
- Elia, M. R. J. S., Stubbs, R. J., & Henry, C. J. K. 1999. Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing total starvation. *Obesity*, 7: 597-604. <https://doi.org/10.1002/j.1550-8528.1999.tb00720.x>
- Mahley, R. W., Weisgraber, K. H., & Innerarity, T. 1974. Canine lipoproteins and atherosclerosis. *Circulation Research*, 35: 722-733. <https://doi.org/10.1161/01.RES.35.5.713>
- Maldonado, E. N., Romero, J. R., Ochoa, B., & Aveldaño, M. I. 2001. Lipid and fatty acid composition of canine lipoproteins. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 128: 719-729. [doi.org/10.1016/S1096-4959\(00\)00366-3](https://doi.org/10.1016/S1096-4959(00)00366-3)
- Mori, N., Yuki O., Naoto, T., Yutaka, H., Makoto, H., Shingo, I., Ichiro, Y., and Toshiro, A. 2015. Preliminary analysis of modified low-density lipoproteins in the serum of healthy and obese dogs and cats. *Frontiers in Veterinary Science*, 2: 34 <https://doi.org/10.3389/fvets.2015.00034>
- Mustonen, A. M., Pyykönen, T., Puukka, M., Asikainen, J., Hänninen, S., Mononen, J., & Nieminen, P. 2006. Physiological adaptations to fasting in an actively wintering canid, the arctic blue fox (*Alopex lagopus*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 305: 32-46. <https://doi.org/10.1002/jez.a.241>
- Ouguerram, K., Nguyen, P., Krempf, M., Pouteau, E., Briand, F., Bailhache, E., & Magot, T. 2004. Selective uptake of high density lipoproteins cholesteryl ester in the dog, a species lacking in cholesteryl ester transfer protein activity: An in vivo approach using stable

- isotopes. in Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 138: 339-345. <https://doi.org/10.1016/j.cbpc.2004.04.011>
- Singer, M. A., & Morton, A. R. 2000. Mouse to elephant: biological scaling and Kt/V. American journal of kidney diseases, 35: 306-309. [http://dx.doi.org/10.1016/S0272-6386\(00\)70341-6](http://dx.doi.org/10.1016/S0272-6386(00)70341-6)
- Spitzer, J. J., & Miller, H. I. (1956). Unesterified Fatty Acids and Lipid Transport in Dogs*. Proceedings of the Society for Experimental Biology and Medicine, 92: 124-126. <https://doi.org/10.3181/00379727-92-22406>
- Xenoulis, P. G., Cammarata, P. J., Walzem, R. L., Macfarlane, R. D., Suchodolski, J. S., & Steiner, J. M. (2013). Novel lipoprotein density profiling in healthy dogs of various breeds, healthy miniature schnauzers, and miniature schnauzers with hyperlipidemia. BMC veterinary research, 9: 47. <https://doi.org/10.1186/1746-6148-9-47>
- Xenoulis, P. G., & Steiner, J. M. (2010). Lipid metabolism and hyperlipidemia in dogs. The Veterinary Journal, 183: 12-21. <https://doi.org/10.1016/j.tvjl.2008.10.011>
- Van Ginneken, V., E. Verhey, R. Poelmann, R. Ramakers, K. Willems van Dijk, Lisanne Ham, P. Voshol, L. Havekes, M. Van Eck, and J. Van der Greef. 2007. Metabolomics (liver and blood profiling) in a mouse model in response to fasting: a study of hepatic steatosis. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1771: 1263-1270. <https://doi.org/10.1016/j.bbalip.2007.07.007>
- Zimmer F, Riebeling V, Benke B, Schuster J, Roskamm H. 1980. The LDL-HDL ratio in patients with coronary arteriosclerosis (author's transl). Z Kardiol. 69: 149-53.

EFEKAT KRATKOTRAJNOG GLADOVANJA NA KONCENTRACIJU LIPIDA I LIPOPROTEINA KOD ZDRAVIH PASA NORMALNE TELESNE KONDICIJE

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Kratak sadržaj

Uvod. Poznato je iz literature da se odnosi lipoproteinskih frakcija seruma pasa koje se dobijaju posle elektroforeze na agaroznom gelu (EFag) menjaju kod oboljenja praćenih dislipidemijama, što može biti jedan od značajnih elemenata dijagnostike pojedinih patoloških stanja. Stoga je cilj ovih ispitivanja bio da se utvrdi koncentracija triglicerida, holesterola i glukoze u serumu pasa kao i odnos lipoproteinskih frakcija dobijenih pomoću EFag, u uslovima koji se često sreću u kliničkoj praksi: posle bazalnog (12 časova) i kratkotrajnog gladovanja od 24 i 36 časova.

Materijal i metode. Uzorci krvi su sakupljeni od šest pasa različitih rasa, normalne telesne kondicije, oba pola, posle 12, 24 i 36 časova gladovanja. Koncentracija glukoze, triglicerida i holesterola je određena na automatskom biohemijskom analizatoru.

Lipoproteinske frakcije su razdvojene korišćenjem EFig. Broj leukocita je utvrđen na hematološkom analizatoru.

Rezultati i zaključak. Rezultati ovog ispitivanja su pokazali da se tokom kratkotrajnog gladovanja kod pasa koncentracija glukoze, triglicerida i holesterola kao i broj leukocita ne menjaju. Osim toga, nije bilo promena vezanih za α 1-i α 2-elektroforetske zone, ali se javio značajan pad koncentracije lipoproteina pre β -i β -zone. Poznato je da se lipoproteini velike gustine (HDL) nalaze u α -zoni, dok lipoproteini veoma male gustine (VLDL) i lipoproteini male gustine (LDL) pripadaju pre β -i β -zoni. Na osnovu naših nalaza moguće je zaključiti da reverzni transport holesterola koji se odvija posredstvom HDL-a nije poremećen tokom kratkotrajnog gladovanja. Nasuprot tome, kratkotrajno gladovanje verovatno utiče na smanjen obim sinteze triglicerida u jetri, odnosno na smanjenu sintezu VLDL-a i LDL-a ili na njihovo efikasnije uklanjanje iz plazme.

Ključne reči: elektroforeza na agaroznom gelu, lipoproteini, holesterol, trigliceridi