Praca oryginalna *Original paper*

Lipid metabolism indices in the blood serum of reproductive female polar foxes in relation to age of animals

KATARZYNA BURLIKOWSKA, ROMAN SZYMECZKO, CHRISTINE IBEN*, ANNA PIOTROWSKA, MONIKA BOGUSŁAWSKA-TRYK, STANISŁAW KUBACKI**

Department of Animal Physiology, Faculty of Animal Breeding and Biology, University of Technology and Life Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland
*Institute of Nutrition, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria
**Division of Horse and Fur Animal Breeding, Faculty of Animal Breeding and Biology, University of Technology and Life Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland

Burlikowska K., Szymeczko R., Iben C., Piotrowska A., Bogusławska-Tryk M., Kubacki S.

Lipid metabolism indices in the blood serum of reproductive female polar foxes in relation to age of animals

Summary

The study was carried out on 50 female polar foxes, which were divided into 5 experimental groups with respect to age (n – 10): from 1 to 5-years-of-age. The content of triglycerides, total cholesterol, HDL, LDL, total, free and estrified L-carnitine and the composition of fatty acids was determined in blood serum samples. The lowest content of triglycerides, total cholesterol and HDL fraction was found in one-year-old females ($p < 0.05$). It was observed that there was a tendency towards an increase in the content of triglycerides and LDL until 3-years-of-age and in total cholesterol and HDL until 4-years-of age. No significant, age-dependant changes in the content of individual fatty acids and L-carnitine were observed. Among fatty acids, the dominant ones were C16:0; C18:0; C16:1; C18:1 and n-6 PUFA, where C16:2 n-6 prevailed.

Keywords: polar fox, age, lipid metabolism indices

The study of biochemical blood parameters, including those related to lipid metabolism, allows for the drawing of conclusions about the state of health of animals and it shows any intensification of the metabolic processes in their organism. Basic lipid metabolic indices in the blood include the content of triglycerides (TG), total cholesterol, high-density-lipoprotein (HDL) and low-density-lipoprotein (LDL), as well as the fatty acid profile (3, 4, 15, 16, 18, 24, 27). Another important parameter is the level of L-carnitine (total, free and estrified) in the blood, indicating proper processes of β-oxidation of fatty acids in cells (22). Factors that influence the level of the above mentioned indices in the blood include species, breed, sex, age, physiological state of animals and also the diet composition and type of fat used in it (7, 11, 15, 18, 24-26).

The polar fox (*Alopex lagopus L.*) is a typical representative of carnivorous animals. Its life span is about 8-10 years, but for reproduction it is used mainly until 6-years-of-age. It reaches sexual maturity after completed growth and development at the age of 9-11 months (2). Available literature does not contain any data concerning lipid metabolism indices in the blood of mature foxes in relation to their age. It would seem essential to know these values to distinguish age-related changes from those indicating pathological processes in the animal's organism (4, 12).

The aim of the study was to determine the values of selected lipid metabolism indices of female polar foxes in relation to their age, three months after a lactation period.

Material and methods

The experiment was conducted on 50 polar fox females coming from a reproductive farm in the non-mating period, three months after a lactation period. The females were divided into 5 experimental groups (n = 10): one-year-old females (group I), two-year-old females (group II), three-year-old females (group III), four-year-old females (group IV) and five-year-old females (group V). After the veterinary examination the foxes were considered clinically healthy. The number of reared kits in the last reproductive period was from 7 to 10 per female. The diet used on the

farm in this period included poultry and fish offal, meat-and-bone meal, extruded cereals, rapeseed oil and vitamin-mineral mixture. The concentration of metabolizable energy in 1 kg of the diet was 1700 kcal, and its distribution of protein, fat and carbohydrates was in agreement with recommended feeding standards (1).

Blood samples for analysis were taken from a cephalic vein in the morning (8:00-10:00), before feeding the animals. In the blood serum samples stored until the time of analysis at the temperature of –20°C, the content of TG, total cholesterol and LDL fraction was determined by an enzymatic method, and the content of HDL fraction by a spectrophotometric method. All the above mentioned determinations were made with an Advia 1650 analyzer using original Bayer reagents and application notes. The content of fatty acids in the blood serum was determined by liquid chromatography (HPLC) according to the method described by Osterroht (20) with the use of an ÄKTA Purifier instrument (Pharmacia LKB). The content of L-carnitine (total, free and short-chain carnitine esters) was determined with the radiochemical method by Cederblad &Lindstedt, modified by Bøhmer et al., & Iben (10) using the Tri-Carb 2100 TR Liquid Scintillation Analyzer Packard Instrument at the Institute of Physiology of Veterinary Medicine in Vienna. The obtained results were analysed statistically with analysis of variance using the Statistica 5.5 PL program. The level of significance was set at $p < 0.05$.

Results and discussion

The content of the total cholesterol in the blood serum of experimental female polar foxes was around 4.55-5.55 mmol/l (tab. 1) which is similar to the mean cholesterol content in adult polar foxes (5.17 mmol/l) presented by Brandt (4). The lowest content of TG, total cholesterol, HDL and LDL fraction was found in one-year-old females (0.49; 4.55; 3.56; 0.78 mmol/l respectively) (tab. 1). Only in the case of LDL fraction the difference was not statistically significant. There was a tendency for the described parameters to increase, which in the case of TG and LDL fraction was until 3-years-of-age (0.70 and 0.96 mmol/l), and in the case of total cholesterol and HDL fraction until 4-years-of-age (5.55 and 4.31 mmol/l). Studies on dogs and cats showed age-dependant changes in the cholesterol content in the blood serum. The changes were most distinct before the animals reached sexual maturity, reflecting their intensive growth and development, and increased steroidogenesis at the time of maturation (7, 9, 18). After reaching sexual maturity and ending growth and development, most of the biochemical blood parameters concerning lipid metabolism reach values close to standards typical of a given animal species and stay at a relatively stable level until old age (4, 15, 25).

The HDL fraction in the blood of the experimental female foxes amounted to about 80% of the total cholesterol (tab. 1), which agrees with results obtained in investigations on foxes, mink (23) and dogs (3, 15, 16). The HDL/LDL ratio in the examined female foxes (4.38-4.90) was similar to the value obtained by Maldonado et al. (15) in mice (4.3), remarkably lower than in dogs (8.3) and considerably higher compared to cats (1.8). It is generally known that various lipoprotein fractions have different functions in the transport of lipids. A higher content of high-density lipoproteins (HDL) is beneficial for the organism, for it enables a reverse transport of cholesterol (from peripheral cells to the liver), protecting the organism against cholesterol deposition in the blood vessels. Moreover, the HDL fraction takes away lipid peroxides that are cytotoxic to endothelial cells. These peroxides are released during decomposition of lipoproteins rich in TG (15-17, 23).

The level of TG in serum of experimental female foxes showed a tendency to increase until 3-years-of-age and then there was a decrease in their content to a significantly lower level (0.57 mmol/l) in 5-year-old females (tab. 1). An increase in the TG content in the blood until a certain age and their subsequent decrease was also observed in rats and dogs (12, 27). In other experiments on dogs, no direct influence of age on the level of TG in the blood was observed (18, 21).

Carnitine (trimethyl-γ-amino-β-hydroxybutyrate) plays an important role in the production of metaboli-

Tab. 1. Lipid metabolism indices in serum of polar fox females ($\bar{x}$ ± SD)

Index	Group					p <
	1	2	3	4	5	
Triglyceride (mmol/l)	0.49^{a} ± 0.10	0.61^{bc} ± 0.10	0.70^{b} ± 0.16	0.64^{bd} ± 0.06	0.57^{acd} ± 0.13	0.05
Total cholesterol (mmol/l)	4.55^{a} ± 0.66	5.21^{b} ± 0.60	5.46^{b} ± 0.58	5.55^{b} ± 0.86	5.37^{b} ± 0.33	0.05
HDL cholesterol (mmol/l)	3.56^{a} ± 0.53	4.12^{b} ± 0.50	4.21^{b} ± 0.44	4.31^{b} ± 0.69	4.21^{b} ± 0.26	0.05
LDL cholesterol (mmol/l)	0.78 ± 0.10	0.84 ± 0.11	0.96 ± 0.20	0.90 ± 0.21	0.91 ± 0.10	NS*
Total L-carnitine (µmol/l)	136.06 ± 25.66	126.42 ± 18.82	111.51 ± 22.19	138.39 ± 28.27	136.84 ± 28.01	NS
Free L-carnitine (µmol/l)	94.77 ± 20.30	88.97 ± 19.18	88.91 ± 17.54	96.39 ± 13.86	95.84 ± 14.07	NS
Estrified L-carnitine (µmol/l)	38.04 ± 18.53	35.85 ± 18.47	29.34 ± 5.28	43.34 ± 22.09	40.62 ± 22.14	NS
Estrified L-carnitine/Free L-carnitine	0.42 ± 0.23	0.44 ± 0.33	0.35 ± 0.12	0.45 ± 0.21	0.42 ± 0.21	NS

Explanations: a, b, c, d – mean values in the same row with different postscripts are significantly different ($p < 0.05$); * – not significant

zable energy enabling transport of long-chain fatty acids through the inner mitochondrial membrane. Moreover, it facilitates removal of short- and medium--chain fatty acids which are accumulated in mitochondria as a result of metabolic processes (10, 22). In these studies, no influence of age on the level of L-carnitine in serum of experimental female foxes was observed. The lowest, but statistically not significant, content of total, free and estrified carnitine was observed in 3-year-old female foxes (111.51; 88.91; 29.34 µmol/l respectively) and the highest in 4-year--old females (138.39; 96.39; 43.34 µmol/l respectively) (tab. 1). It should be noted that the level of L-carnitine in the serum of female polar foxes determined in this experiment was remarkably higher in comparison with values obtained in humans (8), dogs (19) and rats (26). The estrified/free carnitine ratio was inversely related to carnitine availability and, being an early marker for impending carnitine deficiency in tissue, ranged from 0.35 (3-year-old females) to 0.45 (4-year--old females). Studies on humans and animals have given quite diversified results as far as the influence of age on the level of L-carnitine in the blood is concerned (8, 11, 13, 14, 26). The lack of unequivocal data on this subject is a problem, because plasma carnitine levels are often used as an index of body carnitine status (26).

The content of fatty acids in serum is shown in tab. 2. The present experiment shows a relatively constant, age-independent composition of fatty acids in the blood of adult female foxes, which agrees with results in dogs (15).

Saturated fatty acids (SFA) constituted from 26% (5-year-old females) to 35% (2-year-old females) of the total fatty acids (TFA) in the blood. There was a tendency for this group of fatty acids to decrease with age. Among SFA, palmitic acid (C16:0) and stearic acid (C18:0) prevailed and together they constituted over 78% of SFA. Similar results were obtained for adult dogs, in which about 30% of TFA were SFA with the majority of C18:0 and C16:0 (15, 24).

Monounsaturated fatty acids (MUFA) were accounted for 40% of all determined fatty acids with the highest content of palmitoleic acid (C16:1) and oleic acid (C18:1), which agrees with results in dogs (15, 24). A large amount of these fatty acids in the serum of the examined animals results most probably from their large content in the foxes' diet and their high ileal digestibility and absorption shown in our previous experiments (5, 6).

In the present study polyunsaturated fatty acids (PUFA) ranged from 25% (2-year-old females) to 34% (5-year-old females) of TFA. There was an insignificant tendency towards an increase in the PUFA content until 4th year of age (1-year-old females: 419.03 µmol/l; 4-year-old females 552.53 µmol/l). More than 80% of PUFA in serum was constituted by linoleic acid (C18:2 n-6), which is an essential unsaturated fatty acid for foxes (1). Our previous study also showed that in standard diets for polar foxes the C18:2 n-6 acid definitely predominates in PUFA (5, 6). The content of arachidonic acid (C20:4 n-6), was 8-11% of PUFA. A characteristic feature of dogs' plasma is a relatively large content of long-chain n-6 PUFA, with the majority of C18:2 n-6 and C20:4 n-6 (15, 16). A considerably lower amount of n-3 PUFA was observed in the blood of experimental foxes, which also agrees with the results in dogs (15, 16, 24). Linoleic acid (C18:3 n-3) was dominant among n-3 PUFA. Long-chain PUFA are formed by desaturation and elongation of dietary C18:2 n-6 and C18:3 n-3 acids and they have many biologically important functions in the organism

Tab. 2. Fatty acids in serum of polar fox females (µmol/l) ($\bar{x}$ ± SD)

Fatty acid	Group				
	1	2	3	4	5
14:0	117.52 ± 32.67	106.90 ± 34.75	109.42 ± 53.19	112.72 ± 35.47	85.02 ± 45.83
16:0	226.02 ± 79.98	196.72 ± 44.43	217.86 ± 75.23	216.20 ± 45.69	168.02 ± 59.98
18:0	211.78 ± 52.77	205.94 ± 71.82	195.26 ± 125.18	170.36 ± 89.01	102.82 ± 40.19
20:0	0.41 ± 0.41	0.36 ± 0.16	0.40 ± 0.44	0.10 ± 0.17	0.13 ± 0.16
22:0	0.45 ± 0.37	0.13 ± 0.13	0.21 ± 0.29	0.23 ± 0.28	0.20 ± 0.23
14:1	105.58 ± 32.67	118.40 ± 33.88	143.26 ± 32.08	141.32 ± 40.87	130.46 ± 87.91
16:1	303.46 ± 63.32	286.12 ± 81.91	324.64 ± 151.36	298.52 ± 55.60	211.58 ± 102.12
18:1	246.20 ± 113.88	185.64 ± 30.95	212.32 ± 43.49	225.68 ± 66.94	188.10 ± 66.08
22:1	3.93 ± 4.41	2.73 ± 2.19	5.66 ± 4.06	6.05 ± 3.49	7.09 ± 6.67
18:2 n-6	338.80 ± 157.77	299.28 ± 50.67	401.32 ± 103.88	452.92 ± 190.28	397.26 ± 196.50
γ-18:3 n-6	2.13 ± 2.37	1.95 ± 1.43	1.47 ± 1.19	3.66 ± 3.89	2.21 ± 3.32
20:2 n-6	7.63 ± 3.81	7.86 ± 3.14	7.81 ± 4.28	8.74 ± 4.18	4.96 ± 3.59
20:3 n-6	13.06 ± 6.81	8.51 ± 6.01	12.13 ± 11.80	13.54 ± 6.20	5.39 ± 6.54
20:4 n-6	48.58 ± 25.25	31.94 ± 5.79	39.92 ± 9.65	55.52 ± 18.95	38.52 ± 16.36
22:2 n-6	0.33 ± 0.67	0.06 ± 0.04	0.24 ±0 .43	0.05 ± 0.02	0.04 ± 0.04
α-18:3 n-3	8.05 ± 7.57	9.09 ± 2.62	9.66 ± 3.02	14.15 ± 10.90	10.79 ± 8.87
22:5 n-3	0.89 ± 1.04	1.63 ± 2.04	1.09 ± 0.73	4.94 ± 2.01	5.81 ± 1.45
SFA	555.73 ± 143.81	509.92 ± 147.09	522.94 ± 250.28	499.38 ± 141.81	355.97 ± 132.65
MUFA	659.17 ± 192.92	592.89 ± 143.21	685.88 ± 213.73	671.57 ± 136.49	537.23 ± 243.86
PUFA	419.03 ± 187.70	359.99 ± 62.31	473.34 ± 110.33	552.53 ± 212.76	461.06 ± 232.31

(17). The results of the present investigations show that foxes, like dogs, have a relatively large amount of polyunsaturated fatty acids in the blood, related to the high level of HDL fraction which is composed mainly of phosphatidylcholine and cholesterol esters containing large amount of long-chain PUFA (15, 16).

The present study showed a higher level of TG, total cholesterol, HDL and LDL fraction in the blood serum of older polar fox females compared to one-year-old females. No age-dependant changes in the content of L-carnitine and fatty acids were observed.

References

1. *Anon*.: NRC. Nutrient Requirement of Mink and Foxes. National Academy Press, Washington D.C. 1982.
2. *Berestov V. A., Kozhevnikova L*.: Biology of farmed fur bearing animals, [in:] Brandt A. (Eds.): Haematology and Clinical Chemistry of Fur Animals. Scientifur Finland 1989, 10-18.
3. *Beynen A. C., Kappert H. J., Lemmens A. G., van Dongen A. M*.: Plasma lipid concentrations, macronutrient digestibility and mineral absorption in dogs fed dry food containing medium-chain triglycerides. J. Anim. Physiol. Anim. Nutr. 2002, 86, 306-312.
4. *Brandt A*.: Lipids, [in:] Brandt A. (Eds.): Haematology and clinical chemistry of fur animals. Scientifur Finland 1989, 57-63.
5. *Burlikowska K*.: Apparent ileal digestibility of fat and fatty acids in polar foxes fed diets used over the non-mating period. Pol. J. Natur. Sc. 2006, 21, 637-645.
6. *Burlikowska K., Szymeczko R., Błaszyk J*.: Apparent ileal digestibility of fat and fatty acids in polar foxes. Scientifur 2003, 27, 71-77.
7. *Butterwick R. F., McConnel M., Markwell P. J., Watson T. D. G*.: Influence of age and sex on plasma lipid and lipoprotein concentrations and associated enzyme activities in cats. Am. J. Vet. Res. 2001, 62, 331-336.
8. *Chiu K. M., Schmidt M. J., Havighurst T. C., Shug A. L., Daynes R. A., Keller E. T., Gravenstein S*.: Correlation of serum L-carnitine and dehydroepiandrosterone sulphate levels with age and sex in healthy adults. Age Ageing 1999, 28, 211-216.
9. *Harper E. J., Hackett R. M., Wilkinson J., Heaton P. R*.: Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers. J. Am. Vet. Med. Assoc. 2003, 223, 1436-1442.
10. *Iben Ch*.: Effects of L-carnitine administration on treadmill test performance of untrained dogs. J. Anim. Physiol. Anim. Nutr. 1999, 82, 66-79.
11. *Jacobs G., Keene B., Cornelius L. M., Rakich P., Shug A*.: Plasma, tissue, and urine carnitine concentrations in healthy adults cats and kittens. Am. J. Vet. Res. 1990, 51, 1345-1348.
12. *Lowseth L. A., Gillett N. A., Gerlach R. F., Muggenburg B. A*.: The effects of aging on hematology and serum chemistry values in the beagle dog. Vet. Clin. Pathol. 1990, 19, 13-19.
13. *Maccari F., Arseni A., Chiodi P., Ramacci M. T., Angelucci L*.: Levels of carnitines in brain and other tissues of rats of different ages: effect of acety--L-carnitine administration. Exp. Gerontol. 1990, 25, 127-134.
14. *Maebashi M., Imamura A., Yoshinaga K*.: Effect of aging on lipid and carnitine metabolism. Tohoku J. Exp. Med. 1982, 138, 231-236.
15. *Maldonado E. N., Casanave E. B., Aveldaño M. I*.: Major plasma lipids and fatty acids in four HDL mammals. Comp. Biochem. Physiol. 2002, 132A, 297-303.
16. *Maldonado E. N., Romero J. R., Ochoa B., Aveldaño M. I*.: Lipid and fatty acid composition of carnitine lipoproteins. Comp. Biochem. Physiol. 2001, 128B, 719-729.
17. *Michajlik A., Bartnikowska E*.: Serum lipids and lipoproteins. PZWL, Warszawa 1999 (in Polish).
18. *Mundim A. V., Coelho A. O., Hortêncio S. M., Guimarães E. C., Espindola F. S*.: Influence of age and sex on the serum biochemical profile of Doberman dogs in the growth phase. Comp. Clin. Pathol. 2007, 16, 41-46.
19. *Neumann S., Welling H., Thuere S., Kaup F. J*.: Plasma L-carnitine concentration in healthy dogs and dogs with hepatopathy. Vet. Clin. Pathol. 2007, 36, 137-140.
20. *Osterroht C*.: Determination of particulate fatty acids as p-bromophenacyl- or phenylphenacylesters using HPLC. Chromatographia 1987, 23, 419-422.
21. *Piccione G., Fazio F., Giudice E., Grasso F., Caola G*.: Blood lipids, fecal fat and chymotrypsin excretion in the dog: influence of age, body weight and sex. J. Vet. Med. Sci. 2004, 66, 59-62.
22. *Rebouche Ch. J*.: Carnitine function and requirements during the life cycle. Faseb J. 1992, 6, 3379-3386.
23. *Regerand T. L., Lizenko E. I., Sidorov V. S*.: Lipoproteins in the blood serum in mink and polar fox, the main representatives of farmed fur-bearing animals. Scientifur 1999, 23, 142 (abstract).
24. *Sævik B. K., Thoresen S. I., Taugbøl O*.: Fatty acid composition of serum lipids in atopic and healthy dogs. Res. Vet. Sci. 2002, 73, 153-158.
25. *Swanson K. S., Kuzmuk K. N., Schook L. B., Fahey G. C.* Jr.: Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. J. Anim. Sci. 2004, 82, 1713-1724.
26. *Tanaka Y., Sasaki R., Fukui F., Waki H., Kawabata T., Okazaki M., Hasegawa K., Ando S*.: Acetyl-L-carnitine supplementation restores decreased tissue carnitine levels and impaired lipid metabolism in aged rats. J. Lipid Res. 2004, 45, 729-735.
27. *Uchida K., Nomura Y., Kadowaki M., Takase H., Takano K., Takeuchi N*.: Age-related changes in cholesterol and bile acid metabolism in rats. J. Lipid Res. 1978, 19, 544-552.

Author's address: Dr Katarzyna Burlikowska, University of Technology and Life Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland; e-mail: kasiab@utp.edu.pl