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Serum Fatty Acid Contents and Their Relationships with Bone Quality Indices and Bone Metabolism Markers in Male and Female Silver Foxes (*Vulpes vulpes*)

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Fatty acids are crucial in order to obtain optimal peak bone mass. The composition of fatty acids in 8-month-old, healthy silver foxes and their interrelationships with the morphological, densitometric, and mechanical properties of the mandible and bone metabolism markers was studied. We used gas chromatography and the ELISA and EIA methods to evaluate the biochemical profile of the serum. Quantitative computed tomography, dual-energy X-ray absorptiometry, and the three-point bending test were used for mandible evaluation. In males, elaidic acid content was lower by 3.21%, while oleic acid content was higher by 4.87% when compared to females ($p<0.05$). With the exception of stearic acid found in saturated fatty acids, the concentrations of caprylic, capric, palmitic, and heptadecanoic acids and the total sum of saturated fatty acids have shown only negative correlations with bone formation markers, and the morphological and densitometric properties of the mandible. Monounsaturated fatty acids (heptadecenoic and eicosenoic acids) were both positively and negatively correlated with bone properties, while erucic, elaidic and oleic acids showed only positive correlations with bone traits and bone formation markers ($p<0.05$). Among the polyunsaturated fatty acids, linolenic and arachidonic acids were negatively correlated with densitometric parameters, while linoleic acid showed the highest number of positive correlations with densitometric and mechanical properties ($p<0.05$). Eighteen-carbon-chain fatty acids were mainly positively correlated with the morphological, densitometric and mechanical properties of the mandible. The most positive implications for bone metabolism and bone properties, in regard to fatty acids, may be ascribed to oleic and linoleic acids. The elaborated experimental model may serve for further studies on bone metabolism regulation in monogastric mammals using dietary modifications of fatty acid content.

Key words: mandible, fatty acids, gas chromatography, quantitative computed tomography, silver fox (*Vulpes vulpes*).

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Epidemiological studies in humans and animals have proven that prenatal and postnatal nutrition may have consequences for systemic growth and development and further health status (LUCAS 1991, 1998, 2005). Besides the intake of proteins, calcium and vitamins during prenatal and postnatal growth and development, fatty acids are crucial to obtaining optimal peak bone mass of the skeleton at skeletal maturity and to maintain skeletal homeostasis later in life (WATKINS *et al.* 2001; BAGGIO 2002; EASTELL & LAMBERT 2002). Reduced growth and development in early life in ani-

mals and humans are considered a potential marker of nutritional status and as a risk factor for adult diseases in mammals (GODFREY & BARKER 2000). The status of polyunsaturated fatty acids (PUFA) in humans was shown to be correlated with growth rate in early life, and their role in growth-promoting effects is ascribed to cellular membrane lipid formation, gene expression regulation, and eicosanoids synthesis (KOLETZKO & BRAUN 1991). A dietary deficiency of essential fatty acids (EFA) may cause a low birth weight and slow growth rate in early life leading to decreased bone mineral density (BMD) and bone mineral content (BMC) in the axial and peripheral skeleton in adulthood and higher risk for bone fracture in the elderly (ROBILLARD & CHRISTON 1993; COOPER *et al.* 1995, 1997, 2001). Protein deficiency during early development in rats reduces the activity of fatty acid metabolism enzymes – desaturases, impairing the biochemical conversion of EFA to PUFA. A deficiency of EFA and a reduction of their intake were observed in malnourished children and are considered to be the cause of poor fetal growth (HOLMAN *et al.* 1981; ROBILLARD & CHRISTON 1993). Dietary fatty acids (FA) may influence the bone tissue metabolism directly by eicosanoids synthesis or indirectly via the regulation of a systemic hormone release like the insulin-like growth factor 1 (IGF-1) (WATKINS *et al.* 2001; KOROTKOVA *et al.* 2005). Prostaglandin E_2 (PGE_2) is a major prostaglandin which affects the bone tissue metabolism and is synthesized from arachidonic acid. Low levels of PGE_2 stimulate bone formation processes, while higher levels accelerate bone resorption processes. Similar bone resorption effects are induced via leukotriene B_4, being another arachidonic acid metabolite (RAISZ & FALL 1990; GARCIA *et al.* 1990; TAKIGUCHI *et al.* 1999; KOROTKOVA *et al.* 2005). It was shown in rats, that a reduced dietary ratio of n-6:n-3 PUFA decreases PGE_2 synthesis and increases bone formation processes (WATKINS *et al.* 2000). A diet enriched with n-3 fatty acids was shown to prevent ovariectomy-induced osteopenia and osteoporosis development (SAKAGUCHI *et al.* 1994).

The silver fox (*Vulpes vulpes*) is a domesticated animal bred mainly for its valuable fur. The precise formulation and individual administration of caged, farm foxes' diets is relatively easy to perform during their whole breeding cycle and provides the possibility for investigations of the biological effects of experimental dietary manipulations in monogastric living animals, both prenatally and postnatally. Moreover, farm foxes are kept in the same environmental conditions for a longer period of time in a relatively large population of animals, when compared to laboratory animals such as mice, rats, or even experimental dogs. Due to the obligatory slaughter procedure at the end of the production cycle, nutritional experiments on farm foxes may be an attractive, ethical alternative to experimenting on dogs and could reduce the number of dogs that would need to undergo euthanasia for experimental purposes (ŁUSZCZEWSKA-SIERAKOWSKA *et al.* 2019).

Considering the importance of fatty acids for optimal growth and development of the skeletal system and its homeostasis maintenance, the aim of this study was to determine the serum content of the fatty acids in male and female, 8-month-old, farmed silver foxes. As diagnosed in previous investigations using quantitative computed tomography (QCT), silver foxes possess closed growth plates in their long bones at this developmental stage, indicating their recent skeletal maturity attainment. Thus, the possible existence of a relationship between serum fatty acid concentrations and the morphological, densitometric, and mechanical properties of the skeletal system, as well as biochemical bone metabolism markers were determined.

Materials and Methods

The experimental procedures used in this study were approved by The Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland (permission number 20/2015).

Experimental design and sampling procedure

The study was performed on 8-month-old male (N = 7) and female (N = 8) silver foxes (*Vulpes vulpes*) kept on a commercial farm. The animals were kept in pens under standard rearing conditions. Weaning was performed at the age of 10 weeks. The foxes were housed two to a cage (1.0 x 1.1 m). All animals were provided with drinking water *ad libitum* and were fed a standard diet twice a day. The diet was identical for males and females and was based mainly on animal-origin waste products enriched with a vitamin-mineral premix (TATARA *et al.* 2018). At the age of 8 months, the animals were slaughtered and blood samples (fasted state) were collected for serum content. After clotting and further centrifugation, the serum samples were kept frozen in 1.5 ml tubes at –25°C until biochemical analysis.

Determination of serum fatty acids composition

The serum samples were thawed immediately before analysis. 0.5 ml of serum sample was placed in amber glass tubes and 5 ml of chloroform-me-

thanol (2:1) solution was added. The tubes containing the samples were shaken for 3 h, and then centrifuged at 2000 rpm for 20 min. Afterwards an aliquot of the chloroform-methanol extract was transferred into 4 ml amber glass tubes and dried under a stream of nitrogen gas. The dried samples were next dissolved in 0.4 ml of 0.5 M potassium methoxide-methanol solution then 0.5 ml of the 14% boron trifluoride-methanol solution was added. After 35 min of incubation, the fatty acid methyl esters were determined using gas chromatography and a Clarus 600 apparatus (PerkinElmer, USA) equipped with a capillary column 60 m GC-Column Elite-5MS, 0.25 internal diameter and 0.25 μm film thickness (PerkinElmer, USA). Several fatty acids were identified by comparison of the retention times of those of a standard FAME mixture C4-C24 (SUPELCO 37 Component FAME Mix, SIGMA-ALDRICH Co.) and expressed as a percent of the total fatty acids (KUSUNOKI *et al.* 2007; POŁAWSKA *et al.* 2013; MAJEWSKA *et al.* 2016).

Determination of bone metabolism markers in serum

The serum's concentration of osteocalcin (OC) was measured in ng/ml using a N-MID® Osteocalicin ELISA kit (Immunodiagnostic Systems Ltd., Boldon, Tyne & Wear, UK), while the quantitative determination of C-terminal propeptide of type I procollagen (CICP) in ng/ml was performed using a MicroVue CICP EIA Kit Bone Health (Quidel Corp., San Diego, CA, USA). The serum's concentration of C-terminal telopeptides of type I collagen (CTX-I) was measured in ng/ml using a Serum CrossLaps® ELISA kit (Immunodiagnostic Systems Ltd., Boldon, Tyne & Wear, UK). The evaluation of biochemical bone metabolism markers in the serum was performed using a Benchmark Plus microplate spectrophotometer supplied with Microplate Manager Software Version 5.2.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Measurement of bone mineral density (BMD) and bone mineral content (BMC)

The areal bone mineral density and bone mineral content of the mandible were measured using the dual-energy X-ray absorptiometry (DEXA) method and a Norland XR-46 densitometer, equipped with Research Scan software (Norland, Fort Atkinson, WI, USA). The measurements of BMD and BMC were performed for the whole right and left parts of the mandible including teeth, and independently for the right and left mandibular body and ramus. The first region of interest (ROI) included measurements of BMD (mandibular bone mineral density – M_{BMD}) and BMC (mandibular bone mineral content – M_{BMC}) for the whole right and left halves of the mandible which were separated at the mandibular symphysis. Analogical measurements of BMD and BMC were performed in the second and the third ROIs for the mandibular ramus (MR_{BMD} and MR_{BMC}) and the mandibular body (MB_{BMD} and MB_{BMC}) (TYMCZYNA *et al.* 2012ab, TATARA *et al.* 2018). The mean values of the BMD and BMC measurements for each particular ROI from both halves (right and left) of the mandible were used for further statistical evaluation.

Densitometric and morphometric measurements using quantitative computed tomography

Using the quantitative computed tomography technique and a Somatom Emotion Siemens apparatus (Siemens, Erlangen, Germany), equipped with Somaris/5 VB10B software (version B10/2004A) the volumetric bone mineral density (vBMD) of the cortical bone (Cd), the cortical bone area (CBA), the mean volumetric bone mineral density (MvBMD) and total bone volume (Bvol) of the entire mandible including teeth were determined (Volume Evaluation application package, Siemens, Erlangen, Germany). The measurements of Cd and CBA were performed on a cross-sectional 2-mm thick scan of the mandibular body that was positioned just after the last molar tooth. Mean volumetric bone mineral density and Bvol of the mandible were determined using a volume of interest (VOI) limited by minimum and maximum density of the investigated samples at 0 and 3071 Hounsfield units (HU), respectively. The measurements of MvBMD and Bvol reflect the values obtained for all mineralized anatomical structures of whole mandible including trabecular and cortical bone compartments and teeth (SZABELSKA *et al.* 2017ab; TATARA *et al.* 2018). Except for, MvBMD and Bvol, mean values of the morphometrical and densitometric parameters from both halves of the mandible were used for further statistical evaluation.

Mechanical analysis of the mandible

The mechanical properties of the right and left mandibular halves, such as maximum elastic strength (Wy) and ultimate strength (Wf) were determined using an INSTRON 3367 apparatus supplied with Bluehill 2 software (Instron Corp., Canton, USA). The relationship between the loading force and bone displacement in the three-point bending test was determined. The right and left part of the mandible were placed separately on flat bone supports on the lateral mandibular surface and the measuring head loaded bone samples on

the medial surface at 50% of its length. The measuring head loaded bone samples at a constant speed of 50 mm/min. The distance between bone supports was set at 40% of mandibular length (TYMCZYNA *et al.* 2012ab; SZABELSKA *et al.* 2017ab). Mean values of the Wy and Wf obtained from the right and left halves of the mandible were used for further statistical evaluation.

Statistical analysis

All data are presented as means ±SEM. Statistical analysis of the results was performed using Statistica software (version 6.0) and MS Excel 2003. The comparison of the mean values of the investigated variables between males and females was performed using non-paired Student's *t*-test for non-dependent variables. P-value <0.05 was considered as statistically significant for all comparisons. Pearson's correlation coefficient (r) was determined between the serum concentration of the evaluated fatty acids and all the investigated parameters of the mandible, bone metabolism markers, and final body weight. P<0.05 was considered as statistically significant for all correlations.

Results

The mean final body weight value in 8-month-old male foxes reached 8.851 ± 0.207 kg and was significantly higher by 20.0% when compared to females (7.378 ± 0.345 kg; p = 0.003). The mean, minimum and maximal values of morphometric, densitometric and mechanical properties of the mandible, as well as serum biochemical bone metabolism markers concentration found in the 8-month-old silver foxes are presented in Table 1. The percentage content of saturated fatty acids in serum in males and females is presented in Table 2. Neither the percentage content of particular SFA in serum nor the total sum of SFA were significantly different between the males and females (p>0.05). The percentage content of monounsaturated fatty acids (MUFA) in serum of the silver foxes is presented in Table 3. The mean value of elaidic acid content in serum in males was signifi-

Table 1

Morphometric, densitometric, and mechanical properties of the mandible, and serum biochemical bone metabolism markers concentration in 8-month-old female and male silver foxes (N = 15)

Investigated parameter	Mean value	Minimum	Maximum	Confidence intervals	± SEM
Bone weight (g)	15.66	12.29	18.34	14.57-16.75	0.51
Bone length (mm)	117.06	110.00	124.00	114.39-119.74	1.25
Bone volume (cm^3)	8.94	7.45	10.63	8.37-9.52	0.27
Volumetric bone mineral density of the cortical bone (g/cm^3)	2.258	2.041	2.511	2.178-2.338	0.037
Cortical bone area (mm^2)	53.40	41.50	60.00	50.55-56.25	1.33
Mean volumetric bone mineral density (g/cm^3)	1.974	1.815	2.135	1.920-2.029	0.026
Bone mineral density of mandibular halve (g/cm^2)	0.291	0.248	0.327	0.281-0.301	0.004
Bone mineral content of mandibular halve (g)	7.95	6.55	8.85	7.57-8.32	0.17
Bone mineral density of mandibular body (g/cm^2)	0.268	0.239	0.301	0.260-0.277	0.004
Bone mineral content of mandibular body (g)	1.028	0.765	1.272	0.961-1.096	0.031
Bone mineral density of mandibular ramus (g/cm^2)	0.192	0.170	0.219	0.186-0.199	0.003
Bone mineral content of mandibular ramus (g)	1.734	1.364	2.144	1.613-1.854	0.056
Maximum elastic strength (N)	376	240	450	341-410	16
Ultimate strength (N)	657	499	811	610-704	22
Osteocalcin (ng/ml)	3.32	2.52	6.79	2.73-3.91	0.27
C-terminal propeptide of type I procollagen (ng/ml)	0.000816	0.000145	0.001783	0.000536-0.001096	0.000131
C-terminal telopeptides of type I collagen (ng/ml)	0.185	0.020	1.345	0.005-0.366	0.084

Confidence intervals values set between –95.0 and +95.0%.

Table 2

Serum saturated fatty acid content expressed as mean percent values of total fatty acid content in female and male silver foxes

Fatty acid	Females (N = 8)	Males (N = 7)	t-value	df
C8:0 (Caprylic acid methyl ester)	0.059 ±0.0011	0.060 ±0.0009	-1.100	13
C10:0 (Capric acid methyl ester)	0.247 ±0.0046	0.238 ±0.0030	1.622	13
C11:0 (Undecanoic acid methyl ester)	0.053 ±0.0004	0.053 ±0.0006	-0.520	13
C12:0 (Lauric acid methyl ester)	0.329 ±0.0041	0.333 ±0.0056	-0.632	13
C13:0 (Tridecanoic acid methyl ester)	0.037 ±0.0006	0.037 ±0.0007	0.279	13
C14:0 (Myristic acid methyl ester)	0.565 ±0.0067	0.561 ±0.0088	0.435	13
C15:0 (Pentadecanoic acid methyl ester)	0.305 ±0.0044	0.298 ±0.0038	1.311	13
C16:0 (Palmitic acid methyl ester)	16.485 ±0.172	16.350 ±0.339	0.368	13
C17:0 (Heptadecanoic acid methyl ester)	1.253 ±0.0101	1.268 ±0.0188	-0.766	13
C18:0 (Stearic acid methyl ester)	22.799 ±0.1839	22.433 ±0.3691	0.924	13
C20:0 (Arachidic acid methyl ester)	0.033 ±0.0006	0.0032 ±0.0004	1.408	13
C21:0 (Heneicosanoic acid methyl ester)	0.022 ±0.0004	0.022 ±0.0002	0.027	13
C22:0 (Behenic acid methyl ester)	0.017 ±0.0003	0.017 ±0.0002	0.690	13
C23:0 (Tricosanoic acid methyl ester)	0.012 ±0.0003	0.012 ±0.0002	0.086	13
C24:0 (Lignoceric acid methyl ester)	0.118 ±0.0018	0.119 ±0.0011	-0.592	13
Total sum of saturated fatty acids	42.333 ±0.242	41.834 ±0.178	1.626	13

Values are means ± SEM. t-value – t-value for Student's *t*-test for non-dependent variables; df – degrees of freedom.

Table 3

Serum monounsaturated fatty acid content expressed as mean percent values of total fatty acid content in female and male silver foxes

Fatty acid	Females (N = 8)	Males (N = 7)	t-value	df
C14:1 (Myristoleic acid methyl ester)	0.369 ±0.0060	0.369 ±0.0064	- 0.029	13
C15:1 (Cis-10-Pentadecenoic acid methyl ester)	0.185 ±0.0035	0.188 ±0.0032	- 0.616	13
C16:1 (Palmitoleic acid methyl ester)	2.229 ±0.0224	2.243 ±0.0259	- 0.425	13
C17:1 (Cis-10-Heptadecenoic acid methyl ester)	0.631 ±0.0108	0.608 ±0.0062	1.709	13
C20:1 (Cis-11-Eicosenoic acid methyl ester plus)	0.042 ±0.0008	0.041 ±0.0003	0.743	13
C22:1n9 (Erucic acid methyl ester)	0.003 ±0.00003	0.003 ±0.00004	- 0.215	13
C24:1 (Nervonic acid methyl ester)	0.016 ±0.0001	0.015 ±0.0002	0.707	13
C18:1n9t (Elaidic acid methyl ester)	4.576 ±0.0388	4.429* ±0.0393	2.655	13
C18:1n9c (Oleic acid methyl ester)	8.074 ±0.1534	8.467* ±0.0579	- 2.268	13
Total sum of monounsaturated fatty acids	16.124 ±0.146	16.365 ±0.076	- 1.394	13

Values are means ± SEM. t-value – t-value for Student's *t*-test for non-dependent variables; df – degrees of freedom. *$p<0.05$.

cantly lower by 3.21% when compared to females ($p = 0.019$). The mean value of oleic acid content in serum in males was significantly higher by 4.87% when compared to its content in females ($p = 0.040$). The percentage content of PUFA in serum in males and females is presented in Table 4. Neither the percentage content of particular PUFA in serum nor the total sum of PUFA were significantly different between males and females ($p>0.05$). No significant differences were found between male and female foxes when comparing serum content of n-6 and n-3 fatty acids and

Table 4

Serum polyunsaturated fatty acid content expressed as mean percent values of total fatty acid content in female and male silver foxes

Fatty acid	Females (N = 8)	Males (N = 7)	t-value	df
C18:3n3 (Linolenic acid methyl ester)	1.717 ±0.0248	1.714 ±0.0330	0.072	13
C20:4n6 (Arachidonic acid methyl ester)	19.178 ±0.2213	19.407 ±0.2116	-0.741	13
C20:5n3 (Cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester)	0.479 ±0.0047	0.481 ±0.0108	-0.241	13
C20:3n3 (Cis-11,14,17-Eicosatrienoic acid methyl ester)	0.768 ±0.0168	0.756 ±0.0085	0.602	13
C20:3n6 (Cis-8,11,14-Eicosatrienoic acid methyl ester)	0.060 ±0.0009	0.059 ±0.0009	0.766	13
C20:2 (Cis-11,14-Eicosadienoic acid methyl ester)	0.012 ±0.0002	0.012 ±0.0002	-0.692	13
C22:6n3 (Cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester)	0.142 ±0.0026	0.144 ±0.0026	-0.547	13
C22:2 (Cis-13,16-Docosadienoic acid methyl ester)	0.028 ±0.0005	0.027 ±0.0004	0.366	13
C18:2n6c (Linoleic acid methyl ester)	18.762 ±0.3476	18.801 ±0.1297	-0.098	13
C18:3n6 (Gamma-Linolenic acid methyl ester)	0.397 ±0.0067	0.401 ±0.0095	-0.347	13
n-6	38.397 ±0.225	38.667 ±0.195	-0.893	13
n-3	3.105 ±0.026	3.095 ±0.041	0.212	13
n-6:n-3	12.374 ±0.150	12.508 ±0.189	-0.560	13
Total sum of polyunsaturated fatty acids	41.541 ±0.215	41.801 ±0.191	-0.893	13

Values are means ± SEM. t-value – t-value for Student's *t*-test for non-dependent variables; df – degrees of freedom. *p<0.05.

n-6:n-3 fatty acids ratios (Table 4). The mean values of the content of SFA and PUFA did not differ in serum from male foxes (p = 0.886) and were significantly higher when compared to serum content of MUFA (p<0.001; Tables 2-4). The mean value of the content of SFA was significantly higher in serum from females than the content of PUFA (p = 0.012). Moreover, mean values of the serum content of SFA and PUFA were significantly higher when compared to serum MUFA content in females (p<0.001; Tables 2-4).

The values of Pearson's correlation coefficient between SFA content in serum and all the evaluated parameters of the mandible in the studied silver foxes are shown in Table 5. Serum content of caprylic acid was negatively correlated with MvBMD, MR_{BMD} and MR_{BMC} (p<0.05). Serum content of capric acid was negatively correlated with final body weight, bone weight and Bvol (p<0.05). A positive correlation between serum concentration of capric acid and CTX-I was found (p = 0.035). Serum content of palmitic acid was negatively correlated with CBA of the mandible. A negative correlation between the serum concentration of heptadecanoic acid and CICP was found (p = 0.009). Serum content of stearic acid in the silver foxes was positively correlated with MR_{BMC} (P = 0.047). The total sum of SFA was negatively correlated with bone weight, CBA and Bvol (p<0.05).

The values of Pearson's correlation coefficient between MUFA content in serum and all the evaluated parameters of the mandible are shown in Table 6. Serum content of cis-10-heptadecenoic acid was negatively correlated with bone weight, CBA and Bvol and positively correlated with MvMBD of the mandible (p<0.05). Serum content of cis-11-eicosenoic acid was positively correlated with MR_{BMD} and negatively correlated with Wy (p<0.05). A positive correlation between the serum content of erucic acid and Cd was stated (p = 0.046). Serum concentration of elaidic acid and CICP was positively correlated (p = 0.019). Serum content of oleic acid was positively correlated with bone weight, bone length, Bvol and MR_{BMC} (p<0.05). The total sum of MUFA in serum was positively correlated with bone weight and M_{BMC} (p<0.05). The values of Pearson's correlation coefficient between PUFA content in serum and all the evaluated parameters of the mandible in are shown in Table 7. Serum content of linolenic acid was negatively correlated with the Cd of the mandible (p = 0.049). A negative correlation between serum content of arachidonic acid and M_{BMD} and MB_{BMD} was found (p<0.05). Serum content of linoleic acid was positively correlated with M_{BMD}, M_{BMC}, MR_{BMD}, MB_{BMD} and Wy (p<0.05). Serum content of n-3 PUFA was negatively correlated with Cd and MR_{BMC} (p<0.05).

Table 5

Pearson correlation coefficient values between serum saturated fatty acid concentration and the investigated parameters of the mandible, final body weight, and serum concentration of bone metabolism markers in 8-month-old silver foxes

Investigated parameter	Body weight	Bone weight	Bone length	CBA	Bvol	Cd	MvBMD	M_{BMD}	M_{BMC}	MR_{BMD}	MR_{BMC}	MB_{BMD}	MB_{BMC}	Wy	Wf	OC	CICP	CTX-I
C8:0	-0.07	0.05	0.02	-0.10	-0.03	-0.45	-0.63*	-0.44	-0.24	-0.59*	-0.60*	-0.44	-0.18	-0.31	-0.39	0.19	-0.02	0.15
C10:0	-0.61*	-0.54*	-0.45	-0.23	-0.58*	-0.07	0.13	0.21	-0.35	0.11	-0.11	0.07	-0.40	-0.45	-0.39	0.21	-0.04	0.55*
C11:0	-0.02	0.03	0.12	-0.01	-0.09	-0.35	-0.06	0.42	0.16	0.23	-0.19	0.13	-0.04	0.02	-0.04	-0.12	0.27	0.17
C12:0	0.31	0.22	0.06	-0.04	0.13	-0.27	-0.24	0.17	0.23	-0.25	-0.40	0.01	0.01	0.33	0.34	-0.04	0.51	-0.13
C13:0	0.15	-0.07	-0.03	-0.26	-0.03	0.51	0.44	-0.09	-0.17	0.02	-0.08	0.06	0.07	-0.09	0.34	-0.26	-0.08	0.04
C14:0	-0.06	-0.13	-0.09	-0.05	-0.09	0.49	0.40	-0.21	-0.25	-0.07	-0.07	-0.05	0.03	0.01	0.45	-0.05	-0.07	-0.19
C15:0	-0.03	-0.04	-0.27	-0.12	-0.08	-0.10	0.01	0.28	0.13	0.34	-0.04	0.15	-0.02	0.22	-0.23	-0.22	0.44	-0.04
C16:0	-0.05	-0.34	-0.32	-0.55*	-0.41	-0.39	-0.29	-0.19	-0.31	-0.50	-0.47	-0.41	-0.37	-0.21	-0.49	-0.09	0.22	0.46
C17:0	-0.20	0.09	0.35	0.19	0.19	0.01	0.05	-0.16	-0.08	0.20	0.18	-0.15	-0.05	-0.06	0.01	-0.25	-0.65*	0.21
C18:0	-0.14	-0.17	0.18	-0.01	-0.06	0.38	0.34	-0.10	-0.07	0.12	0.52*	0.05	0.05	-0.05	0.14	0.01	-0.24	-0.06
C20:0	-0.09	-0.38	-0.50	-0.48	-0.43	0.09	0.04	-0.02	-0.35	-0.05	-0.34	0.02	-0.32	-0.18	-0.22	0.21	0.43	0.24
C21:0	-0.03	0.22	0.16	0.45	0.24	0.03	0.21	0.32	0.22	0.18	-0.25	0.16	0.09	0.08	0.29	-0.18	-0.10	-0.28
C22:0	-0.30	-0.23	-0.15	-0.12	-0.19	0.13	0.23	-0.09	-0.30	-0.15	-0.28	-0.17	-0.22	-0.35	-0.32	-0.12	-0.43	0.19
C23:0	-0.05	-0.03	0.28	0.06	-0.10	-0.33	-0.22	0.23	0.12	0.38	0.13	0.19	0.04	-0.45	-0.03	0.32	0.44	-0.05
C24:0	-0.06	0.02	0.07	0.19	0.04	-0.31	0.02	0.29	0.21	0.21	0.25	0.05	0.09	-0.03	0.02	-0.25	-0.33	0.36
Total sum	-0.24	-0.58*	-0.31	-0.60*	-0.53*	0.03	0.11	-0.34	-0.44	-0.38	0.11	-0.40	-0.35	-0.30	-0.35	-0.11	-0.07	0.44

*p<0.05. CBA – cortical bone area, Bvol – total bone volume, Cd – volumetric bone mineral density of the cortical bone, MvBMD – mean volumetric bone mineral density, M_{BMD} – mandibular bone mineral density, M_{BMC} – mandibular bone mineral content, MR_{BMD} – mandibular ramus BMD, MR_{BMC} – mandibular ramus BMC, MB_{BMD} – mandibular body BMD, MB_{BMC} – mandibular body BMC, Wy – maximum elastic strength, Wf – ultimate strength, OC – osteocalcin, CICP – C-terminal propeptide of type I procollagen , CTX-I – C-terminal telopeptides of type I collagen.

Table 6

Pearson correlation coefficient values between serum monounsaturated fatty acid concentration and the investigated parameters of the mandible, final body weight, and serum concentration of bone metabolism markers in 8-month-old silver foxes.

Investigated parameter	Body weight	Bone weight	Bone length	CBA	Bvol	Cd	MvBMD	M_{BMD}	M_{BMC}	MR_{BMD}	MR_{BMC}	MB_{BMD}	MB_{BMC}	Wy	Wf	OC	CICP	CTX-I
C14:1	0.11	-0.04	0.21	-0.24	-0.11	-0.26	-0.21	-0.04	-0.08	-0.05	-0.35	-0.20	-0.16	-0.19	-0.24	-0.20	0.35	0.14
C15:1	0.23	0.05	0.02	-0.18	-0.01	-0.12	-0.09	0.12	0.09	-0.34	-0.24	-0.01	-0.07	0.02	-0.11	-0.31	-0.02	0.45
C16:1	-0.04	0.06	-0.05	0.01	0.02	-0.09	-0.09	0.09	0.07	0.10	-0.02	0.12	0.16	-0.27	-0.25	-0.20	-0.18	0.38
C17:1	0.07	-0.62*	-0.36	-0.52*	-0.57*	0.43	0.53*	-0.20	-0.50	-0.19	-0.04	-0.34	-0.35	0.19	-0.06	-0.24	0.27	-0.15
C20:1	-0.37	-0.10	0.04	0.07	-0.13	-0.34	-0.16	0.31	0.12	0.53*	0.31	0.28	0.02	-0.52*	-0.31	0.42	0.10	0.11
C22:1n9	-0.06	-0.21	-0.07	-0.25	-0.20	0.52*	0.49	-0.11	-0.27	-0.18	0.03	-0.01	-0.02	-0.10	0.04	-0.18	-0.42	0.26
C24:1	-0.33	-0.29	0.05	-0.07	-0.20	0.15	0.21	-0.27	-0.45	-0.04	-0.21	-0.36	-0.29	-0.17	-0.13	-0.13	-0.33	0.02
C18:1n9t	-0.31	-0.44	-0.42	-0.01	-0.46	0.08	0.26	0.30	-0.16	0.43	0.06	0.21	-0.20	-0.06	0.07	0.45	0.60*	-0.34
C18:1n9c	0.12	0.65*	0.58*	0.49	0.58*	-0.22	-0.33	0.23	0.56*	0.17	0.14	0.35	0.45	0.01	0.16	0.19	-0.13	-0.22
Total sum	0.03	0.52*	0.46	0.48	0.43	-0.21	-0.25	0.37	0.53*	0.34	0.15	0.45	0.42	-0.07	0.13	0.31	0.08	-0.29

*p<0.05. CBA – cortical bone area, Bvol – total bone volume, Cd – volumetric bone mineral density of the cortical bone, MvBMD – mean volumetric bone mineral density, M_{BMD} – mandibular bone mineral density, M_{BMC} – mandibular bone mineral content, MR_{BMD} – mandibular ramus BMD, MR_{BMC} – mandibular ramus BMC, MB_{BMD} – mandibular body BMD, MB_{BMC} – mandibular body BMC, Wy – maximum elastic strength, Wf – ultimate strength, OC – osteocalcin, CICP – C-terminal propeptide of type I procollagen , CTX-I – C-terminal telopeptides of type I collagen.

Table 7

Pearson correlation coefficient values between serum polyunsaturated fatty acid concentration and the investigated parameters of the mandible, final body weight, and serum concentration of bone metabolism markers in 8-month-old silver foxes.

Investigated parameter	Body weight	Bone weight	Bone length	CBA	Bvol	Cd	MvBMD	M_{BMD}	M_{BMC}	MR_{BMD}	MR_{BMC}	MB_{BMD}	MB_{BMC}	Wy	Wf	OC	CICP	CTX-I
C18:3n3	-0.26	-0.28	0.02	-0.25	-0.34	-0.52*	-0.30	0.12	-0.14	0.10	0.01	-0.17	-0.41	-0.01	-0.31	0.01	0.21	0.50
C20:4n6	-0.04	-0.16	0.07	-0.20	-0.10	-0.21	-0.27	-0.61*	-0.44	-0.45	-0.30	-0.61*	-0.27	-0.28	-0.06	-0.03	-0.30	0.25
C20:5n3	-0.10	-0.12	-0.14	0.04	-0.20	-0.17	0.10	0.28	-0.01	0.02	-0.22	0.03	-0.10	0.16	0.20	0.09	0.19	-0.01
C20:3n3	-0.30	-0.24	-0.09	0.08	-0.21	-0.11	-0.02	-0.18	-0.31	-0.44	-0.47	-0.37	-0.33	-0.35	-0.22	0.05	-0.25	0.01
C20:3n6	0.19	-0.15	-0.16	-0.40	-0.18	0.21	0.05	-0.17	-0.20	-0.29	-0.31	-0.03	-0.06	-0.35	-0.11	0.04	0.30	0.06
C20:2	0.17	0.01	0.23	-0.05	0.01	-0.01	-0.25	-0.42	-0.26	-0.23	-0.31	-0.26	-0.07	-0.35	0.10	0.04	0.09	0.04
C22:6n3	-0.03	0.11	0.02	-0.04	0.02	-0.18	-0.11	0.24	0.10	0.14	-0.25	0.14	0.02	-0.09	-0.35	-0.17	0.01	0.22
C22:2	-0.14	-0.23	0.02	-0.17	-0.18	0.03	0.17	-0.08	-0.28	-0.26	-0.30	-0.31	-0.43	0.07	0.01	-0.48	-0.25	0.46
C18:2n6c	0.26	0.42	-0.01	0.48	0.38	0.30	0.27	0.60*	0.52*	0.54*	0.12	0.66*	0.39	0.53*	0.33	-0.02	0.25	-0.51
C18:3n6	0.20	0.13	0.18	-0.11	0.17	0.15	0.01	-0.29	-0.12	0.06	-0.16	-0.13	0.05	-0.03	0.20	-0.37	-0.02	0.11
n-6	0.30	0.38	0.07	0.39	0.38	0.17	0.06	0.13	0.20	0.21	0.22	0.22	-0.17	0.39	0.35	-0.07	0.01	-0.38
n-3	-0.38	-0.36	-0.05	-0.18	-0.42	-0.54*	-0.25	0.11	-0.24	-0.28	-0.52*	-0.08	-0.27	-0.13	-0.34	0.03	0.12	0.45
n-6:n-3 ratio	0.43	0.45	0.08	0.50	0.49	0.21	-0.04	-0.04	0.28	0.30	0.50	0.15	0.14	0.25	0.41	-0.06	-0.09	-0.49
Total sum	0.25	0.34	0.07	0.38	0.33	0.09	0.03	0.15	0.17	0.22	-0.21	0.17	0.14	0.38	0.31	-0.07	0.03	-0.32

*$p<0.05$. CBA – cortical bone area, Bvol – total bone volume, Cd – volumetric bone mineral density of the cortical bone, MvBMD – mean volumetric bone mineral density, M_{BMD} – mandibular bone mineral density, M_{BMC} – mandibular bone mineral content, MR_{BMD} – mandibular ramus BMD, MR_{BMC} – mandibular ramus BMC, MB_{BMD} – mandibular body BMD, MB_{BMC} – mandibular body BMC, Wy – maximum elastic strength, Wf – ultimate strength, OC – osteocalcin, CICP – C-terminal propeptide of type I procollagen , CTX-I – C-terminal telopeptides of type I collagen.

Discussion

Sex hormones may affect the enzymatic synthesis of FA that may result in sex-related differences in their content in body compartments. Experimental studies on rats indicated that estradiol and testosterone treatments change the enzymatic activity of the enzymes responsible for FA metabolism such as delta-9-desaturase, delta-5-desaturase, and delta-6-desaturase. It was shown that testosterone may decrease docosahexaenoic acid (DHA) content in plasma and liver lipids, while hormonal treatment with estrogens may decrease the availability of n-6 log-chain PUFA (GONZALEZ *et al.* 1986; MARRA *&* DE ALANIZ 1989; DECSI *&* KENNEDY 2011). It was suggested that the actions of sex hormones on a cellular level may be related to the changes of the physicochemical properties of microsomal membranes (DE ALANIZ *&* MARRA 2003). Studies on humans have shown that liver enzymatic synthesis of eicosapentaenoic acid (EPA) and DHA from alpha-linolenic acid (ALNA) in men and women may vary leading to their differentiated plasma concentrations (CHILDS *et al.* 2008).

In this study, the evaluation of FA content in serum has revealed slight differences between males and females only in the case of selected MUFA. Serum concentration of elaidic acid (C18:1n9t) was lower in males by 3.21%, while oleic acid concentration (C18:1n9c) was higher in males by 4.87%. However, the total sum of MUFA was not differentiated between males and females. Moreover, all the evaluated SFA and PUFA, as well as their total sum have shown a lack of sex-related differences. These observations were performed in relatively young animals that reached sexual and somatic maturity 2-3 months before the natural reproductive season and should be continued at the differentiated stages of the growth and sexual cycles of male and female foxes to explain whether the observed sex-related differences may appear later at the same or at a different scale (OSADCHUK 1999; OSADCHUK *et al.* 2003; SZYMECZKO *et al.* 2009). Neither the quantitative nor the qualitive changes of FA content in different body compartments in foxes may be excluded at different phases of the growth and sexual cycle, especially when one considers the significantly different hormonal milieu interior in matured male and female foxes

(OSADCHUK & TRUT 1988, 1989; OSADCHUK 1990, 1999; OSADCHUK & VOITENKO 1992; OSADCHUK *et al.* 1996). Short-term studies by LYMAN and colleagues (1967) on lipid metabolism in rats confirmed a different rate of phosphatidylcholine synthesis in males and females. Female rats had a higher proportion of stearic and arachidonic acids in phosphatidylcholine fast-moving fractions when compared to males (LYMAN *et al.* 1967). Other studies on castrated male rats have shown that estradiol administration over the course of 3 weeks increased proportions of stearic acid and polyenoic acid, increased stearic acid content, and decreased linoleic acid content, whereas testosterone had neither a stimulatory nor inhibitory effect (LYMAN *et al.* 1968). As shown by BURDGE *et al*. (2008) in their studies on rats, sex-related differences of fatty acid concentration were much more highlighted than in the current study. Fatty acid composition analysis of hepatic phosphatidylcholine in male rats have revealed a higher content of palmitic acid (by 46.7%), oleic acid (by 79.6%), linoleic acid (by 61.9%), eicosatrienoic acid (by 75.0%) and eicosapentaenoic acid (by 50.0%) when compared to females, while the content of stearic acid, gamma-linolenic acid, arachidonic acid and docosahexaenoic acid was higher in females by 34.9%, 68.4%, 13.7% and 24.2%, respectively. In case of phosphatidylethanolamine analysis, a higher content of palmitic acid (by 27.51%), linoleic acid (by 33.33%), gamma-linolenic acid (by 71.43%), eicosatrienoic acid (by 40.00%) and eicosapentaenoic acid (by 75.00%) was observed in males. In female rats, the contents of stearic acid and docosahexaenoic acid were higher by 18.00% and 23.75%, respectively than in males. However, fatty acid composition of triacyloglycerides was not differentiated between males and females (BURDGE *et al.* 2008). Studies in humans between the ages of 18 and 35 have confirmed the stimulatory effects of estrogens on lipid metabolism. It was shown that DHA concentration was significantly higher in plasma phosphatidylcholine (by 31%) and triacylglycerides (by 71%) and non-essential fatty acids (by 33%) in women when compared with men (BAKEWELL *et al.* 2006).

Regardless of the observed sex-related differences in serum FA concentrations in the current study, numerous significant correlations between the serum FA content in foxes and the morphological, densitometric and mechanical properties of the mandible, and serum bone metabolism markers were found. Capric acid was the only fatty acid that had a negative correlation with final body weight of the silver foxes. Among the assessed SFA, serum concentration of caprylic acid was negatively correlated with densitometric parameters, such as MvBMD, $MR_{BMD,}$ and MR_{BMC}. Negative correlations were also found between serum content of capric acid and morphological bone traits, such as mandibular weight and volume. The observed negative relationships of capric acid content with morphological bone properties were confirmed by their positive correlation with CTX-I in serum that is considered to be a biochemical marker of the bone resorption processes (VASIKARAN *et al.* 2011; SZULC *et al.* 2017). Negative correlations between palmitic acid and CBA, as well as between heptadecanoic acid and the serum bone formation marker concentration – CICP, indicate negative implications between the SFA, bone tissue metabolism processes, and skeletal system properties. These observations are confirmed by the evaluation of the total sum of the SFA, since this parameter was also negatively correlated with bone weight, CBA and, Bvol. Stearic acid was the only saturated fatty acid showing positive correlation with bone mineral content in the mandibular ramus. The observed negative associations of serum SFA with bone traits in the current study are in agreement with the previous studies by CORWIN *et al.* (2006) on humans with 13,572 men and women in which SFA intake was negatively associated with the BMD in the femoral neck. The negative association between dietary intake of SFA and low BMD values was more visible in men than in women, especially in the group at a mean age of 47.8 ± 19.0 years where statistically significant correlations were found for femoral neck, trochanter and total hip. In the younger group of men below the age of 50, the negative relationships between SFA consumption and femoral neck BMD were observed as a clear linear trend, while in the group over the age of 50, similar tendencies were found (CORWIN *et al.* 2006). In studies by WOHL *et al.* (1998) it was suggested that bone sites within the skeleton with more trabecular than cortical bone are more vulnerable to negative effects of dietary SFA (WOHL *et al.* 1998). Diminished intestinal calcium absorption due to calcium-fat soap formation, the atherogenic effects on blood vessels and reduced bone blood flow, and the inhibition of the osteogenic differentiation of bone marrow stromal cells with a corresponding enhancement of osteoclastic differentiation are considered as the most important mechanisms responsible for the deleterious effects of a high dietary intake of SFA on bone tissue (GACS & BARLTROP 1977; WOHL *et al.* 1998; CORWIN *et al.* 2006; PARHAMI *et al.* 2001; OH *et al.* 2010).

Our analysis of MUFA has shown both the positive and negative relationships between the evaluated skeletal properties in silver foxes. Heptadecenoic acid was negatively correlated with the morphological traits of the mandible such

as bone weight, bone volume and CBA, but positively correlated with MvBMD. Serum content of eicosenoic acid showed a positive relationship with MR_{BMD}, and a negative correlation with the maximal elastic strength of the mandible. Erucic, elaidic and oleic acids were the only MUFA that showed positive correlations with bone properties and biochemical bone formation markers. Erucic acid content was positively correlated with Cd, while elaidic acid showed a positive correlation with serum concentration of CICP. In the case of oleic acid, its concentration in serum has shown the highest number of positive correlations with bone parameters, both morphological (bone weight, bone length and Bvol) and densitometric (M_{BMC}). The total sum of MUFA in serum was also positively correlated with bone weight and M_{BMC}. Relationships between selected SFA and MUFA, and the stiffness index (SI) were investigated in Inuit women between the ages of 35 and 72 from Nunavik in Canada. It was shown that total SFA and behenic acid, as well as cis-vaccenic acid were negatively associated among MUFA with bone SI, whereas total cis-MUFA and oleic acid were positively associated with SI (PAUNESCU *et al.* 2014). In another study, this time on 22-year-old men, serum concentration of MUFA was negatively correlated with whole body BMD. Contrary to the results of the densitometric measurements obtained in current study, previous investigations on men have shown negative correlation between whole body BMD and serum content of oleic acid (HÖGSTRÖM *et al.* 2007). In experimental studies on the effects of MUFA on bone tissue metabolism, it was shown that palmitic acid enhances the receptor activator of NF-κB ligand (RANKL)-stimulated osteoclastogenesis and induces osteoclast differentiation, while oleic acid inhibits palmitic acid-induced osteoclastogenesis (DROSATOS-TAMPAKAKI *et al.* 2014).

Among PUFA, linolenic and arachidonic acids were negatively correlated with selected densitometric parameters of the mandible. Linolenic acid content was negatively correlated with Cd, while arachidonic acid had negative relationships with M_{BMD} and MB_{BMD}. Serum concentration of linoleic acid had the highest number of positive correlations with densitometric and mechanical properties of the mandible. Positive correlations of linoleic acid were found with BMD within all regions of the mandible, as well as with M_{BMC} and maximum elastic strength. It must be underlined here, that linoleic acid serves as the most common source for n-6 PUFA synthesis. Linoleic acid is present in high concentrations in vegetable oils, while EPA and DHA (n-3 PUFA) originate mainly from fish fat (VANEK & CONNOR 2007). Nutritional studies on the effects of PUFA on bone metabolism regulation and skeletal properties in humans have shown both the positive and negative associations. Higher total PUFA intake during menopausal transition has been related to a greater loss of bone mineral density (BMD) within the skeleton (MACDONALD *et al.* 2006). In a study on 167 elderly patients suffering from fragility fractures, PUFA intake was associated with an increased risk of osteoporotic fractures, whereas a high ratio of MUFA:PUFA was associated with decreased risk (MARTÍNEZ-RAMÍREZ *et al.* 2007). Conversely, a positive relationship between PUFA intake and skeletal fracture risk was shown in the study by ORCHARD and colleagues (2010) on 137,486 postmenopausal women. It was shown that SFA intake increases hip fracture risk, whereas MUFA and PUFA intakes may decrease total fracture risk. In postmenopausal women with a low intake of n-3 PUFA, a higher intake of n-6 PUFA was shown to modestly decrease total fracture risk in the skeleton. A higher intake of EPA and DHA (long-chain n-3 PUFA) was also associated with increased skeletal fracture risk (ORCHARD *et al.* 2010). Similarly to the results obtained in the current study on foxes, the clinical studies on humans have found no association with fracture risk for either total n-3 intake or EPA+DHA intake (MARTÍNEZ-RAMÍREZ *et al.* 2007; VIRTANEN *et al.* 2010; FARINA *et al.* 2011). Positive correlations of serum linoleic acid with densitometric and mechanical properties of the mandible in silver foxes correspond with the results obtained in cohort studies on 46,476 men and 75,878 women, where low linoleic acid intake was implicated with increased hip fracture risk in women. Similar outcomes were found analyzing total PUFA and n-6 PUFA in the previous study (VIRTANEN *et al.* 2012). In an experimental study on postmenopausal women at a mean age of 67.9 ± 1.9, dietary intakes of total PUFA, linoleic and linolenic acids, and total n-3 and n-6 fatty acids were positively associated with BMD of the lumbar spine (JÄRVINEN *et al.* 2012).

Regardless of the chemical form of FA (SFA, MUFA or PUFA) it must be underlined that, in this study, fatty acids possessing 18-carbon chains showed mainly positive correlations with the morphological, densitometric and mechanical properties of the skeletal system in foxes. This observation results from the analysis of stearic acid, elaidic acid, oleic acid and linoleic acid; however, neither positive nor negative relationships between serum content of gamma-linolenic acid and bone tissue properties were found. Linolenic acid (C18:3n3) was the only one among the 18-carbon chain fatty acids that had a negative relationship with the vBMD of the cortical bone compartment. Moreover, n-3 fatty acids were

negatively correlated with MR_{BMC} and the Cd of the mandible. Similarly to most of the 18-carbon-chain FA, erucic acid possessing a 22-carbon chain (C22:1n9), also showed a positive correlation with the Cd of the mandible. All other analyzed fatty acids within each chemical group showed no significant correlations or showed a negative relationship with the evaluated bone properties and bone tissue metabolism indices.

Conclusions

This study presents the sex-related differences of serum SFA, MUFA and PUFA in 8-month-old, farmed silver foxes. In males, elaidic acid concentration was lower by 3.21%, while oleic acid concentration was higher by 4.87%, when compared to females. The total sum of SFA, MUFA and PUFA showed a lack of sex-related differences at this growth stage of silver foxes. Except for stearic acid within the group of SFA, serum concentrations of caprylic acid, capric acid, palmitic acid, heptadecanoic acid and the total sum of SFA showed only negative relationships with the biochemical markers of bone formation processes and the morphological and densitometric properties of the mandible. Monounsaturated fatty acids (heptadecenoic acid and eicosenoic acid) were both positively and negatively correlated with the evaluated skeletal system properties. Serum concentrations of erucic, elaidic and oleic acids showed only positive associations with bone properties and bone formation markers. Among PUFA, linolenic and arachidonic acids were negatively correlated with densitometric parameters, while linoleic acid showed the highest number of positive correlations with the densitometric and mechanical properties of the mandible. Except for gamma-linolenic acid and linolenic acid, fatty acids with 18-carbon chains have shown mainly positive correlations with the morphological, densitometric and mechanical properties of the skeletal system of foxes. Moreover, n-3 fatty acids were negatively correlated with MR_{BMC} and the Cd of the mandible.

As shown in this study, the most positive implications for bone metabolism regulation and skeletal system properties in silver foxes may be ascribed to oleic and linoleic acids. The elaborated experimental model may serve for further studies on bone metabolism regulation in monogastric mammals with the use dietary modifications of fatty acids content.

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Author Contributions

Research concept and design: M.R.T., I.Ł.-S.; Collection and/or assembly of data: M.R.T., I.Ł.-S.; Data analysis and interpretation: M.R.T., I.Ł.-S., R.P.-Ł.; Writing the article: M.R.T., I.Ł.-S.; Critical revision of the article: M.R.T., I.Ł.-S., R.P.-Ł.; Final approval of article: M.R.T., I.Ł.-S., R.P.-Ł.

Conflict of Interest

The authors declare no conflict of interest.

References

BAGGIO B. 2002. Fatty acids, calcium and bone metabolism. J. Nephrol. **15**: 601-604.

BAKEWELL L., BURDGE G.C., CALDER P.C. 2006. Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. Br. J. Nutr. **96**: 93-99. https://doi.org/10.1079/BJN20061801

BURDGE G.C., SLATER-JEFFERIES J.L., GRANT R.A., CHUNG W.S., WEST A.L., LILLYCROP K.A., HANSON M.A., CALDER P.C. 2008. Sex, but not maternal protein or folic acid intake, determines the fatty acid composition of hepatic phospholipids, but not of triacylglycerol, in adult rats. Prostaglandins Leukot. Essent. Fatty Acids **78**: 73-79. https://doi.org/10.1016/j.plefa.2007.10.028

CHILDS C.E., ROMEU-NADAL M., BURDGE G.C., CALDER P.C. 2008. Gender differences in the n-3 fatty acid content of tissues. Proc. Nutr. Soc. **67**: 19-27. https://doi.org/10.1017/S0029665108005983

COOPER C., CAWLEY M., BHALLA A., EGGER P., RING F., MORTON L., BARKER D. 1995. Childhood growth, physical activity, and peak bone mass in women. J. Bone Miner. Res. **10**: 940-947. https://doi.org/10.1002/jbmr.5650100615

COOPER C., ERIKSSON J.G., FORSÉN T., OSMOND C., TUOMILEHTO J., BARKER D.J. 2001. Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporos. Int. **12**: 623-629. https://doi.org/10.1007/s001980170061

COOPER C., FALL C., EGGER P., HOBBS R., EASTELL R., BARKER D. 1997. Growth in infancy and bone mass in later life. Ann. Rheum. Dis. **56**: 17-21. http://dx.doi.org/10.1136/ard.56.1.17

CORWIN R.L., HARTMAN T.J., MACZUGA S.A., GRAUBARD B.I. 2006. Dietary saturated fat intake is inversely associated with bone density in humans: analysis of NHANES III. J. Nutr. **136**: 159-165. https://doi.org/10.1093/jn/136.1.159

DE ALANIZ M.J., MARRA C.A. 2003. Steroid hormones and fatty acid desaturases. Prostaglandins Leukot. Essent. Fatty Acids **68**: 163-170. https://doi.org/10.1016/S0952-3278(02)00266-1

DECSI T., KENNEDY K. 2011. Sex-specific differences in essential fatty acid metabolism. Am. J. Clin. Nutr. **94**(6 Suppl): 1914S-1919S. https://doi.org/10.3945/ajcn.110.000893

DROSATOS-TAMPAKAKI Z., DROSATOS K., SIEGELIN Y., GONG S., KHAN S., VAN DYKE T., GOLDBERG I.J., SCHULZE P.C., SCHULZE-SPÄTE U. 2014. Palmitic acid and DGAT1 deficiency enhance osteoclastogenesis, while oleic acid-induced triglyceride formation prevents it. J. Bone Miner. Res. **29**: 1183-1195. https://doi.org/10.1002/jbmr.2150

EASTELL R., LAMBERT H. 2002. Diet and healthy bones. Calcif. Tissue Int. **70**: 400-404. https://doi.org/10.1007/s00223-001-0047-9

FARINA E.K., KIEL D.P., ROUBENOFF R., SCHAEFER E.J., CUPPLES L.A., TUCKER K.L. 2011. Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults. J. Nutr. **141**: 1146-1153.

GACS G., BARLTROP D. 1977. Significance of Ca-soap formation for calcium absorption in the rat. Gut **18**: 64-68. https://doi.org/10.3945/jn.110.133728

GARCIA C., BOYCE B.F., GILLES J., DALLAS M., QIAO M., MUNDY G.R., BONEWALD L.F. 1996. Leukotriene B_4 stimulates osteoclastic bone resorption both *in vitro* and *in vivo*. J. Bone Miner. Res. **11**: 1619-1627. https://doi.org/10.1002/jbmr.5650111105

GODFREY K.M., BARKER D.J. 2000. Fetal nutrition and adult disease. Am. J. Clin. Nutr. **71**(Suppl 5): 1344S-1352S. https://doi.org/10.1093/ajcn/71.5.1344s

GONZALEZ S., NERVI A.M., PELUFFO R.O. 1986. Effects of estradiol and environmental temperature changes on rat liver delta 6 microsomal desaturase activity. Lipids **21**: 440-443. https://doi.org/10.1007/BF02536400

HÖGSTRÖM M., NORDSTRÖM P., NORDSTRÖM A. 2007. n-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO_2 Study. Am. J. Clin. Nutr. **85**: 803-807. https://doi.org/10.1093/ajcn/85.3.803

HOLMAN R.T., JOHNSON S.B., MERCURI O., ITARTE H.J., RODRIGO M.A., DE TOMAS M.E. 1981. Essential fatty acid deficiency in malnourished children. Am. J. Clin. Nutr. **34**: 1534-1539. https://doi.org/10.1093/ajcn/34.8.1534

JÄRVINEN R., TUPPURAINEN M., ERKKILÄ A.T., PENTTINEN P., KÄRKKÄINEN M., SALOVAARA K., JURVELIN J.S., KRÖGER H. 2012. Associations of dietary polyunsaturated fatty acids with bone mineral density in elderly women. Eur. J. Clin. Nutr. **66**: 496-503. https://doi.org/10.1038/ejcn.2011.188

KOLETZKO B., BRAUN M. 1991. Arachidonic acid and early human growth: is there a relation? Ann. Nutr. Metab. **35**: 128-131. https://doi.org/10.1159/000177636

KOROTKOVA M., OHLSSON C., GABRIELSSON B., HANSON L.A., STRANDVIK B. 2005. Perinatal essential fatty acid deficiency influences body weight and bone parameters in adult male rats. Biochim. Biophys. Acta **1686**: 248-254. https://doi.org/10.1016/j.bbalip.2004.10.006

KUSUNOKI M., TSUTSUMI K., NAKAYAMA M., KUROKAWA T., NAKAMURA T., OGAWA H., FUKUZAWA Y., MORISHITA M., KOIDE T., MIYATA T. 2007. Relationship between serum concentrations of saturated fatty acids and unsaturated fatty acids and the homeostasis model insulin resistance index in Japanese patients with type 2 diabetes mellitus. J. Med. Incest. **54**: 243-247. https://doi.org/10.2152/jmi.54.243

LUCAS A. 1991. Programming by early nutrition in man. Ciba Found. Symp. **156**: 38-50.

LUCAS A. 1998. Programming by early nutrition: an experimental approach. J. Nutr. **128**(Suppl 2): 401S-406S. https://doi.org/10.1093/jn/128.2.401S

LUCAS A. 2005. Long-term programming effects of early nutrition – implications for the preterm infant. J. Perinatol. **25**(Suppl 2): S2-S6. https://doi.org/10.1038/sj.jp.7211308

LYMAN R.L., HOPKINS S.M., SHEEHAN G., TINOCO J. 1968. Effects of estradiol and testosterone on the incorporation and distribution of [Me-14C]methionine methyl in rat liver lecithins. Biochim. Biophys. Acta **152**: 197-207.

LYMAN R.L., TINOCO J., BOUCHARD P., SHEEHAN G., OSTWALD R., MILJANICH P. 1967. Sex differences in the metabolism of phosphatidyl cholines in rat liver. Biochim. Biophys. Acta **137**: 107-114. https://doi.org/10.1016/0005-2760(67)90013-6

ŁUSZCZEWSKA-SIERAKOWSKA I., TATARA M.R., SZPETNAR M., KURZEPA J. 2019. Free amino acid concentration in serum and trapezius muscle from male and female silver foxes (*Vulpes vulpes*). Czech J. Anim. Sci. **64**: 130-140. https://doi.org/10.17221/42/2018-CJAS

MACDONALD H.M., NEW S.A., GOLDEN M.H., CAMPBELL M.K., REID D.M. 2004. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am. J. Clin. Nutr. **79**: 155-165. https://doi.org/10.1093/ajcn/79.1.155

MAJEWSKA D., SZCZERBIŃSKA D., LIGOCKI M., BUCŁAW M., SAMMEL A., TARASEWICZ Z., ROMANISZYN K., MAJEWSKI J. 2016. Comparison of the mineral and fatty acid profiles of ostrich, turkey and broiler chicken livers. Br. Poult. Sci. **57**: 193-200. https://doi.org/10.1080/00071668.2016.1154136

MARRA C.A., DE ALANIZ M.J. 1989. Influence of testosterone administration on the biosynthesis of unsaturated fatty acids in male and female rats. Lipids **24**: 1014-1019. https://doi.org/10.1007/BF02544071

MARTÍNEZ-RAMÍREZ M.J., PALMA S., MARTÍNEZ-GONZÁLEZ M.A., DELGADO-MARTÍNEZ A.D., DE LA FUENTE C., DELGADO-RODRÍGUEZ M. 2007. Dietary fat intake and the risk of osteoporotic fractures in the elderly. Eur. J. Clin. Nutr. **61**: 1114-1120. https://doi.org/10.1038/sj.ejcn.1602624

OH S.R., SUL O.J., KIM Y.Y., KIM H.J., YU R., SUH J.H., CHOI H.S. 2010. Saturated fatty acids enhance osteoclast survival. J. Lipid Res. **51**: 892-899. https://doi.org/10.1194/jlr.M800626

ORCHARD T.S., CAULEY J.A., FRANK G.C., NEUHOUSER M.L., ROBINSON J.G., SNETSELAAR L., TYLAVSKY F., WACTAWSKI-WENDE J., YOUNG A.M., LU B., JACKSON R.D. 2010. Fatty acid consumption and risk of fracture in the Women's Health Initiative. Am. J. Clin. Nutr. **92**: 1452-1460. https://doi.org/10.3945/ajcn.2010.29955

OSADCHUK L.V., BRAASTAD B.O., HOVLAND A.L., BAKKEN M. 2003. Reproductive and pituitary-adrenal axis parameters in normal and prenatally stressed prepubertal blue foxes (*Alopex lagopus*). Anim. Sci. **76**: 413:420. https://doi.org/10.1017/S135772980005863X

OSADCHUK L.V., GUL'TIAEVA V.V., FILIMONENKO A.A., JALKANEN L. 1996. Spermatogenesis and the testosterone level in young male silver foxes in their reproductive period. Fiziol. Zh. S. S. S. R. Im. I. M. Sechenova **82**: 102-108.

OSADCHUK L.V., TRUT L.N. 1989. Gonadal endocrine function in female silver foxes selected for domesticated behavior. Zh. Obshch. Biol. **50**: 189-198.

OSADCHUK L.V., TRUT L.N. 1988. Photoperiodic control of gonadal endocrine function in silver-gray foxes and its al-

teration during domestication. Fiziol. Zh. S. S. S. R. Im. I. M. Sechenova **24**: 176-183.

OSADCHUK L.V., VOITENKO N.N. 1992. Sex steroid hormones and brain serotonin in the estrous cycle of silver foxes. Fiziol. Zh. S. S. S. R. Im. I. M. Sechenova **78**: 118-123.

OSADCHUK L.V. 1990. Hormones, reproductive behavior and fertility in male silver foxes. Fiziol. Zh. S. S. S. R. Im. I. M. Sechenova **76**: 446-452.

OSADCHUK L.V. 1999. The effect of the photoperiod on steroid hormone secretion, on seasonal reproductive rhythms and on fertility in female silver foxes. Izv. Akad. Nauk Ser. Biol. 191-200.

PARHAMI F., TINTUT Y., BEAMER W.G., GHARAVI N., GOODMAN W., DEMER L.L. 2001. Atherogenic high-fat diet reduces bone mineralization in mice. J. Bone Miner. Res. **16**: 182-188. https://doi.org/10.1359/jbmr.2001.16.1.182

PAUNESCU A.C., AYOTTE P., DEWAILLY E., DODIN S. 2014. Saturated and monounsaturated fatty acid status is associated with bone strength estimated by calcaneal ultrasonography in Inuit women from Nunavik (Canada): a cross-sectional study. J. Nutr. Health Aging **18**: 663-671. https://doi.org/10.1007/s12603-014-0461-0

POŁAWSKA E., HORBAŃCZUK J.O., PIERZCHAŁA M., STRZAŁKOWSKA N., JÓŹWIK A., WÓJCIK A., POMIANOWSKI J., GUTKOWSKA K., WIERZBICKA A., HOFFMAN L.C. 2013. Effect of dietary linseed and rapeseed supplementation on fatty acid profiles in the ostrich. Part 1. Muscles. Anim. Sci. Pap. Rep. **31**: 239-248.

RAISZ L.G., FALL P.M. 1990. Biphasic effects of prostaglandin E2 on bone formation in cultured fetal rat calvariae: interaction with cortisol. Endocrinology **126**: 1654-1659. https://doi.org/10.1210/endo-126-3-1654

ROBILLARD P.Y., CHRISTON R. 1993. Lipid intake during pregnancy in developing countries: possible effect of essential fatty acid deficiency on fetal growth. Prostaglandins Leukot. Essent. Fatty Acids **48**: 139-142. https://doi.org/10.1016/0952-3278(93)90101-2

SAKAGUCHI K., MORITA I., MUROTA S. 1994. Eicosapentaenoic acid inhibits bone loss due to ovariectomy in rats. Prostaglandins Leukot. Essent. Fatty Acids **50**: 81-84. https://doi.org/10.1016/0952-3278(94)90151-1

SZABELSKA A., TATARA M.R., KRUPSKI W. 2017a. Morphological, densitometric and mechanical properties of mandible in 5-month-old Polish Merino sheep. BMC Vet. Res. **13**: 12. https://doi.org/10.1186/s12917-016-0921-3

SZABELSKA A., TATARA M.R., KRUPSKI W. 2017b. Interrelationships between bone metabolism markers and deciduous teeth and mandible properties in Polish Merino sheep. J. Hard Tissue Biol. **26**: 249-256. https://doi.org/10.2485/jhtb.26.249

SZULC P., NAYLOR K., HOYLE N.R., EASTELL R., LEARY E.T. 2017. Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. Osteoporos. Int. **28**: 2541-2556. https://doi.org/10.1007/s00198-017-4082-4

SZYMECZKO R., MAĆKOWIAK P., PIOTROWSKA A., BOGUSŁAWSKA-TRYK M., BURLIKOWSKA K., PRUSZYŃSKA-OSZMAŁEK E., SASSEK M., SZCZEPANKIEWICZ D. 2009. The level of selected hormones in peripheral blood in female polar foxes (*Alopex lagopus L.*) in relation to age. Folia Biol. (Kraków) **57**: 213-218.

TAKIGUCHI T., KOBAYASHI M., NAGASHIMA C., YAMAGUCHI A., NISHIHARA T., HASEGAWA K. 1999. Effect of prostaglandin E_2 on recombinant human bone morphogenetic protein-2-stimulated osteoblastic differentiation in human periodontal ligament cells. J. Periodontal Res. **34**: 431-436. https://doi.org/10.1111/j.1600-0765.1999.tb02278.x

TATARA M.R., ŁUSZCZEWSKA-SIERAKOWSKA I., KRUPSKI W. 2018. Serum concentration of macro-, micro-, and trace elements in Silver fox (*Vulpes vulpes*) and their interrelationships with morphometric, densitometric, and mechanical properties of the mandible. Biol. Trace Elem. Res. **185**: 98-105. https://doi.org/10.1007/s12011-017-1221-x

TYMCZYNA B., BACHANEK T., TATARA M.R., KRUPSKI W., TYMCZYNA-SOBOTKA M., ŁUSZCZEWSKA-SIERAKOWSKA I. 2012a. Interrelationships between morphometric, densitometric, and mechanical properties of mandible in 6-month-old male pigs. Bull. Vet. Inst. Pulawy **56**: 177-181.

TYMCZYNA B., TATARA M.R., KRUPSKI W., TYMCZYNA-SOBOTKA M., ŁUSZCZEWSKA-SIERAKOWSKA I., BACHANEK T. 2012b. Relationships between biochemical bone metabolism indices and morphometric, densitometric and mechanical properties of mandible in 6-month-old pigs. Ann. Agric. Environ. Med. **19**: 535-539.

VANEK C., CONNOR W.E. 2007. Do n-3 fatty acids prevent osteoporosis? Am. J. Clin. Nutr. **85**: 647-648. https://doi.org/10.1093/ajcn/85.3.647

VASIKARAN S., EASTELL R., BRUYÈRE O., FOLDES A.J., GARNERO P., GRIESMACHER A., MCCLUNG M., MORRIS H.A., SILVERMAN S., TRENTI T., WAHL D.A., COOPER C., KANIS J.A. 2011. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos. Int. **22**: 391-420. https://doi.org/10.1007/s00198-010-1501-1

VIRTANEN J.K., MOZAFFARIAN D., CAULEY J.A., MUKAMAL K.J., ROBBINS J., SISCOVICK D.S. 2010. Fish consumption, bone mineral density, and risk of hip fracture among older adults: the cardiovascular health study. J. Bone Miner. Res. **25**: 1972-1979. https://doi.org/10.1002/jbmr.87

VIRTANEN J.K., MOZAFFARIAN D., WILLETT W.C., FESKANICH D. 2012. Dietary intake of polyunsaturated fatty acids and risk of hip fracture in men and women. Osteoporos. Int. **23**: 2615-2624. https://doi.org/10.1007/s00198-012-1903-3

WATKINS B.A., LI Y., ALLEN K.G., HOFFMANN W.E., SEIFERT M.F. 2000. Dietary ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats. J. Nutr. **130**: 2274-2284. https://doi.org/10.1093/jn/130.9.2274

WATKINS B.A., LI Y., SEIFERT M.F. 2001. Nutraceutical fatty acids as biochemical and molecular modulators of skeletal biology. J. Am. Coll. Nutr. **20**(Suppl 5): 410S-416S.

WOHL G.R., LOEHRKE L., WATKINS B.A., ZERNICKE R.F. 1998. Effects of high-fat diet on mature bone mineral content, structure, and mechanical properties. Calcif. Tissue Int. **63**: 74-79.