BRIEF COMMUNICATION

The Lipid Composition of Subcutaneous Adipose Tissue of Brown Bears (*Ursus arctos*) in Croatia

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ABSTRACT

The composition of adipose tissue in brown bears (*Ursus arctos*) is highly variable and depends on an individual's feeding habits. Fatty acid composition of subcutaneous adipose tissue (SAT) may provide insight into brown bear feeding habits, for which data are scarce. The aim of this study was to determine the lipid composition of SAT and variations in the composition of fatty substances with regard to gender and to assess SAT relative to season and body mass (BM) of brown bears in Croatia. Seventy-six tissue samples of brown bear SAT were analyzed in this study. We found that gender, season, and BM significantly affected the lipid composition of SAT. Both females and males had higher percentages of saturated fatty acids and monounsaturated fatty acids (MUFAs) in SAT in spring than in autumn, while the percentage of polyunsaturated fatty acids (PUFAs) was higher in autumn. The prevalence of MUFAs in SAT and the greater presence of PUFAs in autumn, together with the presence of odd-chain saturated fatty acids, indicate the importance of these fatty acids in brown bear physiology. We suggest that the lipid content of adipose tissue may provide valuable information on changes in brown bear condition in response to feeding habits and the effects of supplemental feeding.

Keywords: fatty acid composition, season, supplemental feeding, phospholipids, total cholesterol, triacylglycerols.

Introduction

It is essential to have accurate information about wildlife foraging behavior so that populations can be well managed and conserved. The study of wild-animal foraging patterns can be performed by direct observation of feeding, identification of prey remains, fecal analysis, and determining the fatty acid composition of adipose tissue (Thiemann et al. 2008). In Croatia, research on brown bear nutritional ecology has been based mainly on analysis of feces collected from unknown free-ranging individuals (Cicnjak et al. 1987). Estimates of foraging patterns based on fecal analysis are limited to the population or subpopulation level (Iverson et al. 2001), and feces composition shows only the composition of a relatively recent meal eaten by an individual animal. Conversely, analysis of fatty acids can elucidate feeding habits of individuals over an extended period of time and provide more reliable information about how individuals meet their energy needs (Iverson et al. 2001). However, fatty acid tissue composition is not entirely determined by food; there is also endogenous modification (Tosi et al. 2014) by selective retention and mobilization of certain fatty acids (Florant et al. 1990).

Fatty acids are the primary components of most lipids. Fatty acids without double bonds are considered to be saturated (SFAs) because they contain the maximum number of hydrogen atoms. Fatty acids with one or more double bonds are referred to as monounsaturated (MUFAs) or polyunsaturated (PUFAs) fatty acids, respectively. Fatty acid composition of adipose tissue is based on the physiological incorporation of food ingredients into adipose tissue. In monogastric predators, fatty acids longer than 14 carbon atoms are absorbed from the gastrointestinal tract into the circulation in the form of triacylglycerols in chylomicrons, as fatty acids arrive at the tissues unmetabolized (Galli and Risé 2006). Thus, the fatty acid composition of the diet is

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reflected in the fatty acid composition stored in the adipose tissue, for instance, in canids (Pond et al. 1995) and ursids (Thiemann et al. 2007). In some ecosystems, fatty acids are available in only a few types of food, can be used as a nutritional marker, and can thus provide detailed information on animal's feeding habits (Thiemann et al. 2007).

Fatty acids in an organism are rarely found in free form because of their detergency and cytotoxic effects (Gibbons et al. 2000). Nonetheless, fatty acids are esterified to glycerol in triacylglycerols and phospholipids. Triacylglycerols are the main form of storage fat. The amount of storage fat is dynamic. Storage fat is quite responsive to diet fatty acid composition, while the fatty acid composition of membrane fats is relatively unresponsive (Hulbert and Abbott 2011). Phospholipids spontaneously form lipid bilayers, consisting of a multitude of intracellular membrane elements, and represent the dominant source of fatty acids for the cell signaling reactions. Cholesterol is a molecule found in the cell membranes of all tissues (representing about 50% of the lipid membrane mass fraction) and is required for the normal function of an organism (Alberts et al. 2002). The proportions of phospholipids and cholesterol in the membranes are particularly important because they determine overall cell membrane function (Arnold et al. 2015).

Changes in fatty acid composition of subcutaneous adipose tissue (SAT) associated with feeding habits have been documented in polar bears (*Ursus maritimus*; Thiemann et al. 2006). The nutritional ecology of American black bears (*Ursus americanus*; Iverson et al. 2001) has been studied by examining changes in fatty acid composition of milk, while for brown bears (*Ursus arctos*), data are scarce. It is well known that PUFAs are necessary in the diet before wintering (Florant et al. 1993), although the effects on hibernation are due not to PUFAs in general but to shifts in the ratio of n-6 PUFAs to n-3 PUFAs in membrane phospholipids (Ruf and Arnold 2008). Furthermore, SFAs are released and PUFAs are selectively retained in adipose tissue (Florant et al. 1990). In Croatia, some brown bears are active throughout the winter (Huber et al. 2008), and the influence such behavior has on adipose tissue metabolism remains unknown.

The purpose of this study was to determine lipid profile of SAT in female and male brown bears, variations in the composition of fatty acids, total cholesterol, tryacylglycerols, and phospholipids with regard to the season of sampling and body mass (BM). In addition, this study improves our knowledge about the nutritional and physiological status of brown bears in Croatia.

Methods

Animals

The study was conducted on 76 brown bears (19 females, 57 males), of which 71 were killed during the hunting season in the periods from March 1 to May 15 and from September 16 to December 15, 2013. Of the remainder, one was killed by a train, two were shot outside of a desirable bear zone, one was killed in an intervention shooting, and one was euthanized. The average BM of females and males was 109 ± 35 and 155 ± 62 kg, respectively (mean ± standard deviation). The average age of females was 7.07 ± 1.08 yr, while in males it was 6.94 ± 2.34 yr. Age was determined by counting the cementum annuli on the teeth radix (Matson et al. 1993), which was done by Matson's Laboratory (Manhattan, MT).

Croatian brown bear habitat is located in the Central European climate zone, which is strongly influenced by the Mediterranean climate. This climatic regime is characterized by long, snowy winters, abrupt weather changes, a short vegetation period, and low average annual temperatures. High humidity, early and late frosts and fog, abundant rainfall and snowfall, and strong winds from the northeast and the southeast are additional characteristics of the climate in this area. There are more than 120 frost days, and temperatures reach below −5°C on more than 40 days, with more than 20 days below −10°C. Average days of snow cover exceed 85 (Huber et al. 2008). The bear habitat in Croatia is largely located in the high karst area and extends over altitudes of 0–1,700 m. Bears in Croatia may be found in forest communities typical of the mountainous-hilly area of the Dinaric mountain range. The most important forest communities overlapping bear home ranges in Croatia are mountain spruce forest, prealpine and Dinaric beech forest, fir forest with ribbed fern and feather reed grass, Illyrian mountain beech forest with dead nettle, and forests of downy oak and hop hornbeam (Huber et al. 2008).

Although brown bears are classified as carnivores, about 95% of the nutritional needs of Croatian brown bears are met from plant foods (Huber et al. 2008). The animal protein used by brown bears consists mainly of invertebrates and carrion. The plant foods used by bears in the spring and summer months are mainly green plants and grasses. During summer months, bears find and consume a variety of soft fruits, while in the autumn, acorns and beechnuts are the two main food sources for building adipose tissue reserves for wintering (Huber et al. 2008).

Brown bears are considered a game species and are hunted in Croatia. Consequently, management includes controlled supplemental feeding in designated hunting grounds from January 1 to April 30 and from September 1 to December 15, which includes spring and fall hunting seasons (Bišćan et al. 2014). Food uneaten at the end of the autumn season is not removed. Supplemental foods are derived from plant and animal origins. Grainy, fodder, and meat products and special annual and perennial crops are used for supplemental feeding. Grains fed to bears are primarily corn, oats, and barley. Sugar or fodder beets and various fruits are also used (Bišćan et al. 2014). Since Croatian inclusion to the European Union in 2013, feeding of brown bears with food of animal origin has been restricted, and it is forbidden to exhibit by-products of domestic ruminants (such as cattle, sheep, and goats; category 1). Supplemental feeding is allowed with meat in categories 2 and 3, including by-products of monogastric animals (e.g., domestic pigs), poultry, fish, and parts of wild game species (Bišćan et al. 2014).

Sampling and Preparation of Samples for Lipid Extraction

Samples of approximately 50 g of brown bear SAT were taken in the lumbar-sacral area and stored at −20°C until analyzed. Sampling was done shortly (within hours) after the time of death of each bear, and this protocol was consistent across all

animals in the study. The layer of SAT in bears consists of several anatomically distinct depots; however, it appears to be uniform in its fatty acid composition (Thiemann et al. 2006). After thawing of samples on ice, 1 g of tissue, sampled from depth, was weighed and homogenized for 60 s (3 × 20 s, with 10-s cooling intervals) with an Ultra-Turrax T25 Basic homogenizer (IKA, Staufen, Germany) at 9,500 rpm.

Extraction of Total Lipids

Extraction of total lipids was conducted with a modified method developed by Folch et al. (1957). Extraction of total lipids was performed with a solvent mixture of chloroform that is methanol of different polarities. The ratio of extraction solvent was 15 cm^3/g of tissue, divided into a three-part composition: chloroform : methanol at 2 : 1, chloroform : methanol at 1 : 1, and chloroform : methanol at 1 : 2. Total lipid homogenates in each solvent were extracted for 30 min with stirring (700 rpm) and then centrifuged for 10 min at 3,000 rpm at 20°C. Total lipid extracts were combined and concentrated in a UNIVAPO 100H rotary evaporator, equipped with a UNICRYO MC 2L cooling unit (Uniequip, Planegg, Germany). Total lipid extracts were subdivided into two aliquots, one for methyl esters and a second for phospholipids, cholesterol, and triacylglycerols, and were stored at −20°C until analyzed.

Preparation of Fatty Acid Methyl Esters

Fatty acids from the total lipid extract were converted to methyl esters via transesterification with methanolic HCl according to international standard procedure ISO 5509 (2000). The resulting methyl esters of fatty acids were prepared for analysis with gas chromatography. In the sample of the total lipid, methyl nonadecanoic acid (C19:0) was used as an internal standard.

Gas Chromatography

Analysis of fatty acid methyl esters was performed with an SRI 8610 C gas chromatograph (SRI, Torrance, CA) equipped with a flame ionization detector. Temperatures of the injector and detector were 150° and 240°C, respectively. Chromatography was performed on an 007 Carbowax 20 M capillary column (007-CW-60–0.25 F Quadrex fused, Woodbridge, CT; length 60 m, with internal column diameter 0.25 mm, active-layer thickness of 0.25 μm, serial no. 140122 D). Initial column temperature was 150°C for 3 min, and it was then increased to 230°C by heating for 8°C/min and held at this temperature for 5 min. The carrier gas, hydrogen, was used at a flow rate of 60 mL/min in the split mode. Collection and processing of results were conducted with the computer program PeakSimple3D, version 2.97.

Total Cholesterol, Triacylglycerol, and Phospholipid Determination

From the total lipid extract, the concentration of phospholipids was determined with the original reagent of bioMérieux (Marcy-l'Étoile, France), while concentration of total cholesterol was determined with the original reagents of Herbos (Sisak, Croatia), and concentrations of triacylglycerols were determined with the original reagents of Human (Wiesbaden, Germany) on a Helios Delta spectrophotometer (Thermo Spectronic, Cambridge, UK).

Statistical Data Analysis

Bears were grouped according to gender and season killed (autumn from September to December and spring from March to May). Collected data are presented as mean and standard error of mean. Statistical comparison was performed by computing general linear models (GLMs) for ANOVA with Type III sums of squares. The effects of gender, season, and the interaction of gender and season were tested. BM was added to the model as a covariate. Therefore, significance in model terms refers to the changes in fatty acid composition or distribution relative to BM. A visual inspection of quantile-quantile plots was used to assess approximate normal distribution of the model's residuals. Post hoc Tukey's tests were performed to test which groups differed from each other. Differences were considered statistically significant if $P \leq 0.05$. The software STATISTICA (data analysis software system), version 12.0 (StatSoft, Tulsa, OK), was used for data analyses.

Results

Gender and season affect BM in Croatian brown bears (fig. 1). Females have significantly lower BM in comparison to males in both seasons ($F_{1,72} = 7.745$, $P < 0.007$).

Table 1 shows the percentages of fatty acids in females and males with respect to the presence and number of unsaturated bonds, together with the most prevalent fatty acid. The SAT of brown bears was dominated by MUFAs, of which the most common was oleic acid (C18:1n-9; table 3). SFAs were the second most prevalent, with palmitic acid (C16:0; table 3) having the highest percentage. The least represented were PUFAs, of which linoleic acid (C18:2n-6; table 3) was the most common. Table 2 shows the effect of gender and season on the distribution of fatty

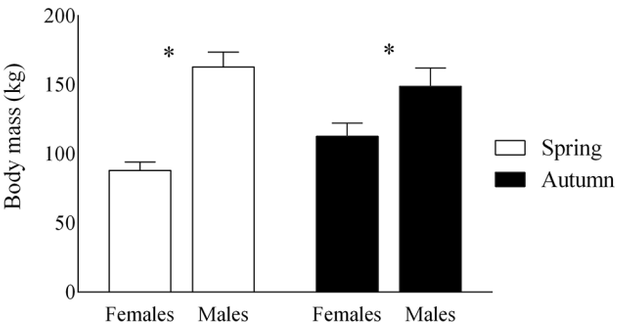

Figure 1. Average body mass with regard to gender and season of brown bears (kg). Results are presented as mean ± SEM; an asterisk indicates a significant difference between genders and seasons ($P < 0.007$).

Table 1: Distribution of fatty acids in subcutaneous adipose tissue of brown bears (%)

	Females		Males	
	Spring	Autumn	Spring	Autumn
SFA	30.10 ± 3.85	28.13 ± 2.59	39.40 ± 3.63	36.08 ± 3.98
MUFA	54.42 ± 4.80	47.11 ± 2.05	42.31 ± 2.79	39.77 ± 3.24
PUFA	15.48 ± 2.69	24.77 ± 3.09	18.30 ± 2.46	24.15 ± 3.77

Note. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

acids in the SAT of brown bears. Season had a significant effect on PUFAs.

In our GLMs, the effects of gender, season, and gender × season were the fixed factors tested. BM was always included as a covariate. Tables 2, 4, and 6 show that BM has a statistically significant effect on the following traits: concentrations of triacylglycerols, phospholipids, and total cholesterol and percentages of SFAs, MUFAs, PUFAs, C8:0, C14:0, C16:0, C16:1, C17:0, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:0, C20:1, and C20:4n-6. In females in autumn, the concentration of total cholesterol and the percentages of PUFAs, C8:0, C16:0, C16:1, C17:0, C18:0, C18:2n-6, and C18:3n-3 increased with BM (tables 1, 3, 5; fig. 1). Moreover, in males in spring, the concentration of phospholipids and the percentages of SFAs, MUFAs, C14:0, C16:0, C16:1, C17:0, C18:0, C18:1n-9, and C20:4n-6 increased with BM (tables 1, 3, 5; fig. 1).

Table 3 presents the data concerning the fatty acid composition of SAT of female and male brown bears. Table 4 shows the significant effect of season on C8:0, C18:3n-3, and C20:4n-6. Table 4 also shows the significant effect of season and the mixed effect of gender and season on C15:0 and C18:2n-6. The percentage of C20:0 was affected by all the effects tested.

Table 5 shows concentrations of triacylglycerols, phospholipids, and total cholesterol in the SAT of brown bears. Table 6 shows the significant effect of season on triacylglycerol concentration.

Discussion

In this study, we determined differences in variations in the composition of fatty acids and triacylglycerols with regard to BM, season of sampling, and interactions between gender and season. We determined that bears, regardless of gender, had higher percentages of SFAs and MUFAs in SAT in spring than in autumn, possibly because brown bears in Croatia do not winter solely in the den and because supplemental feeding is performed.

The SAT of brown bears in Croatia was dominated by MUFAs (oleic acid, C18:1n-9; tables 1, 3), which reflects the results of another study of brown bears (Käkelä and Hyvärinen 1996) and a study of badgers (*Meles meles*; Zalewski et al. 2007). The SAT of beavers (*Castor fiber*) contains PUFAs in the highest percentage (Zalewski et al. 2009). There is selective release of fatty acids from fat storage; for example, the arterial composition has been found to reflect the release of fatty acids from the abdominal SAT within subjects (Halliwell et al. 1996). Jacobsen et al. (1983) reported significant correlations between adipose tissue and fasting plasma nonesterified fatty acids for PUFAs and MUFAs. We propose that there could be a potential relationship between the abundance of MUFAs in the SAT of brown bears and the lack of incidence of atherosclerosis in bears, despite the increased concentration of cholesterol in plasma during hibernation (Arinell et al. 2012). In humans, MUFAs and PUFAs have been associated with a reduced risk in the incidence of atherosclerosis (Simopoulos 1991). This phenomenon requires further research.

Odd-chain saturated (OCS) fatty acids (of which C15:0 and C17:0 are of the most research interest) are thought to originate mainly from dairy fat (Brevik et al. 2005). In this study, males had higher prevalence of OCS fatty acids than females (table 3). Fatty acids like C15:0 and C17:0 have a positive association with an individual's health, and OCS fatty acids may increase the fluidity of membranes (Holman et al. 1989) to a similar degree as PUFAs. One hypothesis on the mechanisms by which unsaturated fatty acids affect membrane fluidity suggests that double bonds in a *cis* configuration create kinks in the fatty acyl chain, which, as fatty acids move and rotate, decrease order and increase fluidity of the bilayer (Arnold et al. 2015). The increase in OCS fatty acids may increase fluidity of acyl chains, because of alternation that occurs in melting points of

Table 2: General linear model testing for effects on gender and season on distribution of fatty acid of subcutaneous adipose tissue of brown bears

		Gender		Season		Gender × season		Covariate BM	
Trait (response variable)	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
SFA	1, 72	1.796	NS	1.419	NS	2.983	NS	127.948	<.0001
MUFA	1, 72	.869	NS	.092	NS	.814	NS	79.222	<.0001
PUFA	1, 72	.149	NS	14.353	<.001	1.579	NS	63.560	<.0001

Note. BM = body mass; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; NS = not significant.

Table 3: Fatty acid composition of subcutaneous adipose tissue of brown bears (%)

	Females		Males	
Fatty acid	Spring	Autumn	Spring	Autumn
C8:0	.10 ± .48	.65 ± .35	.63 ± .22	.84 ± .29
C14:0	1.45 ± .33	1.32 ± .63	1.97 ± .24	1.33 ± .21
C15:0	2.08 ± 1.68	.37 ± .16	1.93 ± 1.04	2.73 ± 1.15
C16:0	13.05 ± 6.62	14.75 ± 2.28	20.55 ± 1.81	11.54 ± 8.53
C16:1	2.01 ± .13	4.40 ± 1.22	4.10 ± .51	1.99 ± .55
C17:0	.90 ± .34	1.60 ± .69	4.19 ± 2.36	2.10 ± .86
C18:0	5.87 ± 2.71	6.35 ± 1.11	13.03 ± 3.50	13.01 ± 3.44
C18:1n-9	42.18 ± 15.40	41.72 ± 2.48	38.77 ± 2.86	34.88 ± 2.88
C18:2n-6	7.77 ± 3.20	25.99 ± 3.11	12.06 ± 1.52	25.06 ± 3.61
C18:3n-3	.75 ± .68	2.47 ± .91	1.22 ± .48	3.14 ± .75
C20:0	6.85 ± 6.85	.16 ± .07	.30 ± .22	.99 ± .65
C20:1	9.95 ± 9.93	.15 ± .07	.53 ± .21	1.69 ± .71
C20:4n-6	6.14 ± 6.13	.09 ± .04	.73 ± .64	.72 ± .64

SFA homologs. The presence of OCS fatty acids in some phospholipid molecules may thus be expected to increase the fluidity of membrane phospholipids (Holman et al. 1989).

In this study, the higher percentages of SFAs and MUFAs in the SAT of bears in spring (table 1) could be because brown bears in Croatia do not spend winter solely in the den (Huber et al. 2014) and because of supplemental feeding (Huber et al. 2008). Supplemental feeding in Croatia is conducted from January 1 to April 30 and from September 1 to December 15 (Bišćan et al. 2014). Almost throughout the bear range, bears are additionally fed as a game species, and most of grain used for bear supplemental fodder is corn (Huber et al. 2008). Corn contains around 20% SFAs, with more than 50% palmitic acid (C16:0), and approximately 26% MUFAs, with oleic acid (C18:1n-9, around 25%) being the most common (Kostik et al. 2013). The higher percentage of PUFAs found in this study in autumn (table 1) could be explained by a greater need for linoleic and α-linolenic fatty acid during the autumn period for fattening and preparing for hibernation (Florant et al. 1990). In addition, increased PUFAs could also be due to a greater use of, and need for, PUFAs for the preparation of cell membrane integrity that follows seasonal body temperature fluctuations, even with a less dramatic drop, as is the case with brown bears during winter. Dietary intake of PUFAs influences the phospholipid fatty acid composition of membranes in mammals and birds (Maillet and Weber 2006; Valencak and Ruf 2011), which in turn influences transmembrane protein activity and thus can compensate for temperature effects (Arnold et al. 2015). In this study, females had a higher percentage of MUFAs than males did (table 1). The percentage of MUFAs may boost female's reproductive success, with its influence on membrane properties, fluidity, and cell proliferation of oocytes and granulosa

Table 4: General linear model testing for effects on gender and season of fatty acid composition of subcutaneous adipose tissue of brown bears

		Gender		Season		Gender × season		Covariate BM	
Trait (response variable)	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
C8:0	1, 72	3.686	NS	4.082	<.045	3.750	NS	9.597	<.003
C14:0	1, 72	.006	NS	1.523	NS	.090	NS	4.866	<.03
C15:0	1, 72	.232	NS	5.337	<.024	7.662	<.01	3.109	NS
C16:0	1, 72	.223	NS	.002	NS	3.951	NS	66.124	<.0001
C16:1	1, 72	1.004	NS	.199	NS	3.601	NS	13.259	<.001
C17:0	1, 72	.017	NS	2.111	NS	.0137	NS	5.144	<.03
C18:0	1, 72	1.650	NS	5.927	<.02	4.516	<.04	128.490	<.0001
C18:1n-9	1, 72	.144	NS	.991	NS	1.748	NS	72.147	<.0001
C18:2n-6	1, 72	.838	NS	19.706	<.0001	4.345	<.04	65.014	<.0001
C18:3n-3	1, 72	1.262	NS	8.047	<.006	1.660	NS	21.450	<.0001
C20:0	1, 72	6.635	<.012	6.159	<.015	10.652	<.002	12.826	<.001
C20:1	1, 72	.367	NS	1.784	NS	1.319	NS	36.205	<.0001
C20:4n-6	1, 72	1.289	NS	4.980	<.03	3.558	NS	38.135	<.0001

Note. BM = body mass; NS = not significant.

Table 5: Concentrations of tryacylglycerols, phospholipids, and total cholesterol in subcutaneous adipose tissue of brown bears (μmol/g tissue)

	Females		Males	
	Spring	Autumn	Spring	Autumn
Tryacylglycerols	964.71 ± 73.04	733.05 ± 39.24	662.84 ± 34.21	754.76 ± 60.53
Phospholipids	38.18 ± 16.44	38.01 ± 7.41	31.73 ± 2.77	25.93 ± 3.08
Total cholesterol	4.36 ± 2.51	7.17 ± 2.23	4.57 ± .60	4.89 ± .80

cells, which in turn are important for blastocyst development (Zeron et al. 2001).

The vegetative foods that bears find at lower elevations during spring and early summer are wild garlic (*Allium ursinum*), lords and ladies (*Arum maculatum*), grasses (*Gramineae* spp.), clovers (*Trifolium* spp.), and sorrels (*Rumex* spp.), with additional animal by-products and corn where feeding is supplemented (Kusak and Huber 1998). *Allium* species contain 20%–23% C16:0 and 4%–13% C18:1n-9 (Tsiaganis et al. 2006). Fatty acid composition of lords and ladies are dominated by C18:1n-9, C18:2n-6, and C16:0 (Christie 2003). Bears feeding on green vegetation in this study could be identified by having a higher percentage of C18:1n-9 in spring than in the autumn (table 3). In late summer, bears feed on fruits and various kinds of berries: raspberry (*Rubus idaeus*), bramble (*Rubus fruticosus*), common buckthorn (*Rhamnus cathartica*), and blueberry (*Vaccinium myrtillus*; Kusak and Huber 1998). Berries supply most of the essential fatty acids for bears (Hissa et al. 1998). During the autumn period the most common foods are nuts, predominantly beech nuts (Kusak and Huber 1998), and the higher percentage of C18:2n-6 in the autumn than in the spring (table 3) in bears in this study reflects this. Beech nuts contain high percentages of C18:1n-9 and C18:2n-6 (Munro et al. 2005). The processes by which dietary fatty acids are stored in adipose tissue are complex, as is shown in a study where a radiolabeled fatty acid was given orally. The radiolabeling continued to appear in adipose tissue over several weeks, reaching a maximum at 1 mo (Mårin et al. 1990). Such results imply that dietary fatty acids may enter other body pools and perhaps recycle through very low-density lipoproteins many times before eventually being stored in the adipose tissue (Summers et al. 2000).

In spring in this study, higher concentrations of triacylglycerols were found in females than in males (table 5). While Schroeder (1987) found no seasonal difference in triacylglycerols in black bears, Hellgren et al. (1993) found the highest concentration of triacylglycerols in the autumn and during bear hibernation. Furthermore, Lohuis et al. (2005) found a significantly higher concentration of triacylglycerols during the late denning season than in summer.

Unlike brown bears in Scandinavia, who overwinter in dens for some months (Swenson et al. 2007), wintering of brown bears in Croatia is not spent exclusively in the den (Huber et al. 2014). Hence, further research should focus on changes in the metabolism of fat tissue during the denning of brown bears. Also, determination of trans-fatty acids could be an indication of a bear using anthropogenic food sources (Thiemann et al. 2008). The study of the fatty acid composition of SAT of brown bears in Croatia, combined with fatty acid composition of their diet, could prove useful in assessing their habitat quality.

Conclusions

1. Gender, season, and BM significantly affect lipid composition of subcutaneous adipose tissue (SAT).
2. In the SAT of brown bears in Croatia, the dominant fatty acids are oleic (C18:1n-9), palmitic (C16:0), and linoleic (C18:2n-6) acids.
3. Individuals of both genders have higher percentages of SFAs and MUFAs in SAT in spring than in autumn, possibly because wintering of brown bears in Croatia is not spent solely in the den and because of supplemental feeding.
4. Higher percentages of C18:2n-6 and PUFAs in the SAT in the autumn indicate the importance of these fatty acids for brown bear wintering in Croatia.

Acknowledgments

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Table 6: General linear model testing for effects on gender and season on concentrations of triacylglycerols, phospholipids, and total cholesterol in subcutaneous adipose tissue of brown bears

		Gender		Season		Gender × season		Covariate BM	
Trait (response variable)	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Triacylglycerols	1, 72	.465	NS	6.094	<.02	3.200	NS	167.236	<.0001
Phospholipids	1, 72	.006	NS	2.905	NS	3.905	NS	110.529	<.0001
Total cholesterol	1, 72	.012	NS	3.586	NS	1.704	NS	48.543	<.0001

Note. BM = body mass; NS = not significant.

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