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Source / Izvornik: **Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, 2016, 11, 128 - 137**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:473625

IZVORNI ZNANSTVENI RAD / ORIGINAL SCIENTIFIC PAPER

Effect of dietary fatty acid variation on mice adipose tissue lipid content and phospholipid composition

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Abstract

The objective of our research was to determine the effects of five different diets as a source of various groups of fatty acids on mice adipose tissue lipid content, phospholipid composition and fatty acid composition of adipose tissue total lipids and phospholipids. Beside the standard diet, diets enriched either with pumpkin-seed, olive or fish oil or lard were used for feeding wild type C57Bl/6 male mice aged 8-10 weeks for 3 weeks. In mice fed with diets enriched with oils/lard, relative adipose tissue mass decreased, comparing to standard diet fed mice. Adipose tissue total lipid content decreased significantly in olive and fish oil diet comparing to pumpkin-seed oil and standard diet. Analysis of the phospholipid classes showed that phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin were common components in all diet types. The dominant storage form of fatty acids in adipose tissue total lipids were unsaturated fatty acids (oleic and linoleic acid prevailed), while in phospholipids, C18 acids prevailed (stearic acid was the main fatty acid) in all diets. Enrichment of the diet with specific oil or lard significantly changed the n-6/n-3 ratio. Fish oil diet provoked the highest reduction of that ratio, both in adipose tissue total lipids and in phospholipids. Obtained results imply that diet modification has an influence on adipose tissue lipid content and composition as well as remodelling process occurring in the adipose tissue phospholipids.

Key words*: adipose tissue, diet, lipids, fatty acids*

Sažetak

U ovom se istraživanju pratio utjecaj pet tipova prehrana obogaćenih različitim masnim kiselinama na sadržaj lipida i sastav fosfolipida masnog tkiva te masnokiselinski sastav ukupnih lipida i fosfolipida masnog tkiva miševa. Uz standardnu prehranu korištena je prehrana obogaćena bučinim, maslinovim ili ribljim uljem, ili svinjskom mašću za prehranu divljeg tipa mužjaka miševa C57Bl/6, starosti 8-10 tjedana u periodu od tri tjedna. U svim obogaćenim prehranama, smanjila se masa masnog tkiva. U odnosu na standardnu prehranu i prehranu obogaćenu bučinim uljem, udjel ukupnih lipida masnog tkiva značajno se smanjio kod prehrana obogaćenih maslinovim ili ribljim uljem. U svim analiziranim prehranama identificirani su fosfatidilkolin, fosfatidiletanolamin, fosfatidilserin i sfingomijelin. U ukupnim lipidima masnog tkiva prevladavaju nezasićene masne kiseline (oleinska i linoleinska), dok u fosfolipidima prevladavaju C18 (prvenstveno stearinska) masne kiseline. U svim obogaćenim prehranama značajno je promjenjen omjer n-6/n-3 masnih kiselina. Kod prehrane obogaćene ribljim uljem taj je omjer značajno smanjen i u ukupnim lipidima i u fosfolipidima masnog tkiva.

Dobivenim se rezultatima potvrđuje da se prehranom može utjecai na sadržaj i sastav lipida masnog tkiva kao i procese remodeliranja u fosfolipidima masnog tkiva.

Ključne riječi: *masno tkivo, prehrana, lipidi, masne kiseline*

Introduction

Adipose tissue (AT) is a specialized connective tissue that functions as the major storage site for lipids. 60-85% of the mass of white AT are lipids, with 90-99% being triacylglycerols (TAG), with small amounts of free fatty acids, diglycerides, cholesterol, phospholipids (PL) and cholesterol-esters (Albright, 1998). Each adipocyte is filled with a single large droplet of TAG that occupies most od the cell volume. As every other cell, adipocyte is enclosed by the plasma membrane (PM). The major structural lipids in eukaryotic PM are glycerophospholipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylserine (PtdSer), phosphatidylinositol (PtdIns) and phosphatidic acid (PtdOH). All of them have a common structure including a polar phosphate head and two hydrophobic hydrocarbon chains derived from fatty acids (FA) and numbered sn-1 and sn-2. The FA are usually between 14 and 24 carbon atoms in length, whilst the

chain at the sn-2 position is usually unsaturated. PtdCho and PtdEtn are two major phospholipids that are asymmetrically distributed in PM: the majority of PtdCho is localized in the outer leaflet, whereas PtdEtn can mostly be found in the inner leaflet. Membrane is spontaneously self-organized as planar bilayers in which each PtdCho has a nearly cylindrical molecular geometry, with the polar head groups interfacing with the aqueous phase and the lipid tails facing each other. PtdEtn assumes a conical molecular geometry due to small size of its polar head group. The sphingolipids constitute another class of membrane structural lipids, with sphingomyelin (CerPCho) as the major one. Because of the saturated tails, they are able to form taller and narrower cylinders than PtdCho lipids of the same chain length and pack more tightly, adopting solid-like phases. Sphingolipids are fluidized by sterols, the major non-polar lipids of the cell membrane (van Meer et al., 2008).

FA composition of membrane PL is determined by both the *de novo* pathway and the remodelling pathway. In the *de novo* pathway, originally described by Kenedy and Weiss, glycerol-3-phosphate is sequentially acylated to form PtdOH, which serves as a general precursor for all PL. The newly synthesized PL possess saturated and monounsaturated FA (MUFA) at sn-1 position and mono- or di-unsaturated FA at sn-2 position. These PL receive polyunsaturated FA (PUFA) to form mature membrane PL by the remodelling pathway (Lands cycle) in which the sn-2 acyl chain is replaced with PUFA by deacylation (phospholipases) and reacylation (lysophospholipid acyltransferases) reactions. Mammals possess a number of lysophospholipid acyltransferases that exhibit distinct acyl-CoA donor and lyso-PL acceptor specificities. In the last few years, enzymes involved in Lands' remodelling cycle have been identified, contributing to the knowledge of membrane asymmetry and diversity (reviewed in Shindou & Shimizu, 2009). According to Hulbert *et al*. (2005), some classes of PL are more responsive than the others to alterations in the FA profile and the response varied between different tissues (liver PL are the most, while AT PL is the least diet-responsive tissue), suggesting the existence of sophisticated regulation of FA composition mechanism in membrane bilayers.

Six FA, myristic, palmitic, palmitoleic, stearic, oleic and linoleic acid (LNA), make up app. 90% of total adipocyte lipids. AT FA have been known to reflect, to a certain degree, the type of dietary fed FA. Varying the composition of FA in diet can vary the AT FA' and PL' profile (Albright, 1998). Multiple factors can influence the incorporation of dietary FA into the PL of the cell membrane. Growth, the type of tissue and differing energy levels in the diet are some of these factors (Soriguer et al., 2000). Besides the composition, the quantity of different FA in AT is also subject to change.

According to Hulbert et al. (2005; 2014), much attention has to be given to both, n-3 and n-6 PUFA, because mammals cannot synthesise them *de novo*. The content of these PUFA in the diet has a greater influence on membrane composition and, consequently, on membrane function and metabolism than do either saturated or monounsaturated dietary fatty acids.

Proposed model of the "membrane pacemaker theory" of the metabolism considers PM as the peacemaker site and the membrane associated processes as a determinant of overall metabolic rate. Both the amount of membranes and their acyl composition, especially the relative balance between monounsaturated and long-chain polyunsaturated acyl-chains in membrane bilayers, are important for metabolic homeostasis. Special emphasis is done on docosahexaenoic acid (DHA) – its high content is associated with increased activity of the membrane-associated processes (Hulbert, 2003; Hulbert et al., 2014). The key is in the DHA molecular structure and its ability to alter bilayer elastic properties (Hulbert, 2003; Bruno et al., 2007)

The aim of this study was to determine the effects of five different diets, as a source of different groups of FA, on mice AT PL content and composition and investigate their possible role in membrane PL remodeling. For that purpose, beside the standard diet, the pumpkin-seed oil (as the source of n-6 and n-9 FA), olive oil (n-9 FA), lard (n-9 and saturated FA) and fish oil (MUFA and n-3 FA) enriched diets were used. The content of total AT lipids, composition of total AT PL, different classes of PL and the composition of the FA chains of total AT lipids and PL were analysed in mice groups fed with different types of diets.

Material and methods

Chemicals and reagents

The used chemicals and reference compounds for gas chromatography were purchased from Sigma (St. Louis, MO, USA) and J.T.Baker (Phillipsburg, NJ, USA) and were of the highest reagent grade available.

The compound used for the formulation of diets, pumpkin-seed oil, lard and extra-virgin olive oil were obtained from individual producers while fish oil was Cod Liver Oil (Child-Life Essentials, CA, USA).

Animals

Wild type C57Bl/6 male mice from our breeding colony (Laboratory Mouse Breeding and Engineering Centre, University of Rijeka, School of Medicine), aged 8-10 weeks, weighing between 21-25 g were used in the experiment. All experiments were approved by the Ethical Committee of the School of Medicine and conducted in accordance with the international guidelines for animal care and experimental use. The animals were housed in five groups of 4-6 animals, in plastic cages and fed experimental diets in a period of three weeks. The control group named standard diet (SD) was fed a standard mice diet obtained from Mucedola (Italy), containing 3 % of lipids (3270 Kcal/kg). All experimental diets were designed in a way that 5 % (w/w) of pumpkin-seed, olive and fish oil or lard were added to the standard chow to obtain four different enriched diets: pumpkin-seed oil enriched diet (PSOD), olive oil enriched diet (OOD), fish oil enriched diet (FOD) and lard enriched diet (LD). The diets enriched with specific oils/lard contained 8 % of lipids (approximately. 3720 Kcal/kg). Table 2 shows the FA analyses for all of the experimental diets. All diets were prepared weekly and stored under nitrogen atmosphere at 4 °C. The animals were maintained under 12 hours light/dark cycle, in a constant temperature (20 ± 1 °C) and humidity (50 ± 5 %) in a controlled facility. After three weeks of feeding, all five groups of animals were sacrificed under anesthesia by cervical dislocation. Epididymal AT (abdominal fat in the lower part

of abdomen, connected to the epididymis) were removed and washed with saline solution, weighted and stored at -75 °C until analysis.

Lipid analysis

Total lipids extracted from experimental feed and AT total lipids were prepared according to Folch *et al*. (1957) with chloroform-methanol (2:1 v/v) containing 0,01 % butylated hydroxytoluene as antioxidant. AT PL were separated and purified by solid phase extraction using an aminopropylsilica column, according to the method of Giacometti (2002).

Phospholipid analysis

PL classes were separated by two-dimensional thin-layer chromatography on silica gel 60 plates, 20 x 20 cm, 0.2 mm. Chloroform:methanol:ammonia (13:7:1, v/v/v) was used as the first solvent system and chloroform:acetone:methanol: acetic acid:water (10:4:2:2:1, v/v/v/v/v) as the second one. PL were visualised by iodine staining, scraped off the plate and quantified spectrophotometrically (Cary 100 Bio, UV-Vis Spectrophotometer, Varian Medical systems, Inc., CA, USA) as inorganic phosphorous, according to the method of Broekhuyse (1968).

Fatty acid analysis

FA composition of AT lipid fractions, as well as of lipids extracted from diets used in the study, was determined by gas chromatography (GC) analysis of the corresponding methyl esters. FA of total lipids were transmethylated with methanol:n-hexane:sulphuric acid (75:25:1, v/v/v) at 90 ºC for 90 minutes, extracted in petrolether and analysed by GC. GC analyses of fatty acid methyl esters (FAMEs) were carried out using an Auto System XL from Perkin-Elmer with flame-ionization detection (FID). Chromatography Software from Perkin-Elmer Nelson (Turbochrom 4) was used for data acquisition. An SP-2330 capillary column (Supelco, USA), 30 m x 0.32 mm x 0.2 μm was used. Hydrogen was obtained with a hydrogen gas generator (Parker domnick hunter, Parker Hannifin LTD, England). Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in the programmed temperature mode from 140 ºC to 220 ºC at 5 ºC/min and then isothermal for 25 min. The temperature of the injector and detector were 300 ºC and 350 ºC, respectively. FAMEs were identified by comparison with the standard mixture obtained by adding individual FAME-standards to the commercial standard mixture (Lipid standard FAMES mixture, cat. no. 189-18, Sigma-Aldrich Chemie Gmbh, Germany). The results were expressed as percentage of particular FA in total identified FA in each lipid fraction.

The degree of unsaturation was expressed as unsaturation index (UI) and represents the sum of the percentages of each FA *x* number of double bonds. The UI was calculated according to following expression: UI = [%monoenes + 2 (%dienes) + 3 (%trienes) + 4 (%tetraenes) +5 (%pentaenes) + 6 (%hexaenes)] / 100

Fatty acid analysis of oils/Fats

Pumpkin-seed oil, was characterized with high proportion of linoleic acid (LNA) (54% app.), oleic acid (32%, app.) and palmitic acid (10%). Analysis of lard showed that it contained 45% of oleic acid and 38% of total SFA (mainly palmitic acid, comprising 21% of total FA). Dietary olive oil, used in the study mainly contained oleic acid (76% of total FA), and equal amounts of LNA and palmitic acid (10% app.). Analysis of fish oil showed that it contained more than 50% (of total FA) of MUFA, comprised of oleic, 20:1 n-9 and palmitoleic acid and that the content of Σn-3 PUFA was 24% app., comprised mainly of DHA and eicosapentaenoic acid (EPA).

Statistical Methods

The collected data were statistically evaluated using the data analysis software system Statistica, version 8.0. (Woiss, 2007). The results are shown as mean ± standard deviation. Differences among groups were tested by Fisher's Least Significant Difference (Fisher LSD) test, and the level of significance was set at $p < 0.05$.

Results and discussion

Diets enriched with the specific oils or lard were well tolerated and the animals gained mass at a steady and comparable rate during the experimental course. Comparing with SD, relative AT mass decreased in diets enriched with oil/lard, with the lowest value in FOD (Table 1). In Table 1, the values of mass percent of total AT lipids for all experimental diets are also presented. OOD and FOD had the lowest and significantly altered values, compared to SD and PSOD. In AT total PL content, there were significant changes in all diets when compared to each other, with the lowest value in PSOD, and the highest in OOD.

Consumption of PUFA of marine sources rich in n-3 long-chain FA reduces mice AT growth and adipocyte lipid content, which can explain the lowest value of relative AT mass in FOD (Raclot, 1997; Fickova et al., 1998; Hynes et al., 2003). Dietary fish oil reduction in rat AT growth resulted in part from the inhibition of fat cell proliferation through the reduction in hyperplasia of AT cells and hypertrophy of adipocytes (Parrish et al., 1990; 1991). Additionally, n-3 PUFA, particularly from marine sources, provokes preferential partitioning of ingested energy towards oxidation at the expense of storage (Flachs et al., 2005).

PL composition of AT for all experimental diets (expressed in percents of total PL) is presented in Figure 1. Analysis of the PL classes showed that PtdCho, PtdEtn, PtdSer and CerPCho were common components in all diet types. In the SD group as in FOD group, PtdOH was also determined. PtdIns was found in all tested diets, except in the FOD. The main constituents of all analyzed diet types were PtdCho and PtdEtn. Clandinin et al. (1991) discussed that membrane polar lipid composition, as well as membrane cholesterol content, is carefully regulated by the cell and that the content of these constituents varies in the response to a variety of stimuli or changes in environment and physiological state, as it is the diet. Regarding individual PL classes, presented in this work, they respond to dietary FA manipulation to different degrees and some classes of PL are more responsive than others to diet variations. Both, PtdCho

and CerPCho (choline-containing, predominant constituents of the outer leaflet of the bilayer) were more responsive to variation in dietary SFA+MUFA content than to other dietary administered FA. LD is characterized with the highest level of CerPCho and the lowest level of PtdCho, comparing to other diets. According to Barenholz and Thompson (1980), although the total amount of those two lipids is constant, the membrane content of each of them may vary greatly and they are in certain measure interchangeable as membrane lipid components. As LD is characterized with the highest level of SFA, one of the cellular answers could be the synthesis of higher amounts of CerPCho, due to the fact that the CerPChos in biological membranes are mainly more saturated than PtdChos (Brown, 1998). Moreover, in LD the content of PtdEtn significantly increased when compared to other diets, resulting with the lowest ratio of PtdCho/PtdEtn in that diet (Figure 2). That ratio is crucial for membrane integrity maintenance. Recalculating the results as the ratio of two choline-containing PL (PtdCho and CerPCho) to PtdEtn, the decreasing of the ratio in LD is not so fatal. The highest PtdCho/PtdEtn, (PtdCho+CerPCho)/PtdEtn and significantly altered ratio were noticed in FOD, mainly as a consequence of the lowest content of PtdEtn, comparing to other diets (Figure 2). According to Li et al. (2006) and his research on rat liver PtdCho/PtdEtn ratio, changes in membrane structure induced by increased level of PtdEtn and consequently lowered ratio, caused a loss of membrane integrity. An increased content of cone-shaped lipid, PtdEtn, in the outer leaflet of the PM may result in membranes that are not as closely packed as those formed by cylindrically shaped PtdCho. As a consequence of altered liver PM PtdCho/PtdEtn ratio, the progression of steatosis to steatohepatitis and liver failure occurred (Li et al., 2006). Regarding AT PM, where insulin receptor was embedded, the changes in PtdCho/PtdEtn ratio may also result with some severe sickness or metabolic disorder. Field et al. (1989a) reported that the relationship between insulin receptor binding and insulin action with membrane alterations exist. Increasing PUFA content of the diet enhances adipocyte insulin binding by changing the surrounding lipid environment and improving insulin-stimulated action in AT.

Dietary fat composition influences the fatty acyl composition of PM through several mechanisms: 1) changing the rate of *de novo* PL synthesis, 2) influencing the redistribution of FA chains via phospholipases or acyltransferases and methyltransferases, (Field et al., 1989a; Hulbert et al., 2014) and 3) by direct desaturation of membrane PL-linked FA (Field et al., 1989a).

AT total lipids, represented mainly by TAG, contain a complex mixture of FA which largely reflects the dietary intake of FA that are not synthesised *de novo*. Thus, dietary FA are an important source of AT FA and have a significant effect upon both the composition and the quantity of different FA in AT (Fernandez-Quintela et al., 2007; Hulbert et al., 2014). This is consistent with the results obtained in our work because each diet mainly reflects the FA composition of administered food (Table 3). Comparing the SD, total AT lipids in PSOD had lower value of palmitic acid and total SFA and higher value of oleic acid, LNA and total n-6 FA. LD, OOD and FOD showed similar pattern with lower value of SFA, LNA and total n-6 FA and higher value of oleic and total MUFA content, compared to the standard diet. AT total lipid fraction in FOD is characterised with the highest value of EPA and DHA, comparing all diets, which is consistent with the highest values of these two FA in fish oil added to the feed. Opposite to that, a discrepancy exists in LD, regarding the proportion of palmitic and stearic acid and total SFA, because their values are much lower in total lipids extracted from the AT than are in feed enriched with lard. One of the explanations could be the existence of AT TAG in a dynamic state: they are continuously formed by an influx of FA from the TAG-rich plasma lipoproteins and are also continuously hydrolysed to efflux FA into the plasma bound to albumin. Under conditions of stimulated lipolysis, FA stored as TAG in AT are selectively mobilised. The mobilisation of individual FA is specific for each FA, influenced by their molecular structure, more than their content in AT: it depends on chain length, unsaturation and *cis*-positional isomerism (Raclot and Groscolas, 1993). The mobilisation generally correlated positively with unsaturation and negatively with the chain length of the FA. According to that, SFA are mobilised the least and PUFA the most, MUFA remaining intermediate. Highly mobilised FA include those with 16-20 carbon atom chains and 4-5 double bonds, whereas weakly mobilised FA include those with 20-24 carbon atom chains and 0-1 double bond (Raclot, 1997; Fernandez-Quintela et al., 2007; Hulbert et al., 2014). Taking that into consideration, each diet administration in this research possesses different sets of FA for incorporation in cellular lipids, as well as for mobilization (which depend on current metabolic state), so the incorporation of dietary specific FA in cell lipids is expected. Moreover, incorporation of healthier n-3 FA in cell lipids by diets can be fulfilled. Another possible reason for lower content of SFA than is expected in LD AT total lipids and generally the highest values of MUFA in AT total lipids in all types of diets used in the study, could be the conversion of SFA to MUFA by the action of stearoyl-CoA desaturase. Stearoyl-CoA desaturase introduces a single double bond into its substrates, palmitic and stearic acid and generate palmitoleic and oleic acid, the major MUFA in AT (Miyazaki and Ntambi, 2003). According to Tallman and Taylor (2003), AT total lipids also work as a reservoar of essential FA, LNA and alpha-linolenic acid. High proportion of these two FA in AT total lipids in all tested diets is expectable and consistent with the literature. LNA and alpha-linolenic acid are also precursors for the synthesis of other highly unsaturated n-3 and n-6 FA. In contrast to MUFA and precursor PUFA, all highly unsaturated FA are poor substrates for TAG synthesis and are mainly incorporated into PL, contributing to maintenance of membrane fluidity. However, maintaining membrane fluidity is not the main function of these FA in mammals. They are required for many other functions, such as eicosanoid signaling, pinocytosis and ion channel modulation (Nakamura and Nara, 2004). n-3 and n-6 PUFA, play a key role in the progression or prevention of human diseases such as obesity, diabetes, neurological and heart disease and cancer, mainly by affecting cellular membrane lipid composition, signal-transduction pathways and by direct control of gene expression. Gene expression is regulated by PUFA in several tissues, including AT (Ntambi and Bene, 2001).

Regarding FA profile in AT phospholipids (Table 4), presented in our study, it does not correlate with administered diet in so far as is noticed in AT total lipids. Relative MUFA and

PUFA proportion varied more within diets than did SFA proportion. In the fraction of SFA (comprising more than 41% in all diets), stearic and palmitic FA were the main acids and their proportion slightly varied with the administered diet. Regarding UFA, n-3 FA varied the most, particularly in FOD. The results are consistent with results of Soriguer et al. (2000). They found that AT PL was not very sensitive to dietary changes, except for n-3 FA, and correlate with the higher affinity of the n-3 for AT with higher oxidative and lipolytic capacity and lower fat mass in diets enriched with long chain PUFA. As is referred in Catta-Preta et al. (2012) PL FA composition of membranes seems to be a direct determinant of the metabolic rate in rodents. Increasing levels of PUFA/SFA ratio in experimental food resulted with increased level of PUFA in AT total lipids as well as PL. According to Field et al. (1989b) increasing the PUFA content of the diet enhances adipocyte insulin binding by changing the surrounding lipid environment, thereby demonstrating a clear physiological mechanism by which a high PUFA/SFA ratio may improve insulin-stimulated action in AT. It is known that the amount of insulin binding increases with the content of PUFA in membrane PL, while insulin binding decreases with increasing content of SFA and MUFA (Fernandez-Quintela et al., 2007).

Conclusions

Obtained results imply that diet modification has an influence on AT lipid content and composition as well as remodelling process occurring in the AT PL. With healthier diet assortment, highly unsaturated PUFA from marine origin, mediate their beneficial effects by reducing AT lipid content, increasing PtdCho/PtdEtn and (PtdCho+CerPCho)/PtdEtn ratio and decreasing n-6/n-3 ratio in both, AT lipids and phospholipids.

Acknowledgment

The investigation was supported by the Ministry of Science, Education and Sports, grant no. 062-0621341-006. Authors wish to thank M. Hrvoje Križan, mag.med.lab.diag. for his excellent technical assistance.

Abbreviations

AT – adipose tissue; CerPCho – sphingomyelin; DHA - docosahexaenoic acid; EPA – eicosapentaenoic acid; FA – fatty acids; FOD – fish oil enriched diet; LD – lard enriched diet; LNA – linoleic acid; MUFA – monounsaturated fatty acids; OOD – olive oil enriched diet; PL - phospholipids; PM - plasma membrane; PSOD – pumpkin-seed oil enriched diet; PtdCho – phosphatidylcholine; PtdEtn – phosphatidylethanolamine; PtdIns – phosphatidylinositol; PtdOH – phosphatidic acid; PtdSer – phosphatidylserine; PUFA – polyunsaturated fatty acids; SD – standard diet; SFA – saturated fatty acids; TAG – triacylglycerols; UFA – unsaturated fatty acids;

Table 1. *Effects of diets on food intake, mice body weight, relative adipose tissue weight, the content of adipose tissue total lipids and phospholipids of mice fed with standard diet (SD), pumpkinseed oil enriched diet (SPOD), lard enriched diet (LD), olive oil enriched diet (OOD) and fish oil enriched diet (FOD).*

	SD	PSOD	LD	OOD	FOD
Relative AT weight (g/100g)	1.68 ± 0.12 c,d,e,	1.44 ± 0.12	1.33 ± 0.06 a	1.35 ± 0.03 a	1.17 ± 0,15 a
AT total lipids wlipids (mg/100 mg tissue)	77.71 ± 1.57 d,e	77.10 ± 0.85 d,e	74.87 ± 1.77	72.94 ± 3.05 a,b	72.07 ± 1.36 a,b
AT phospholipids Wphospholipids (µmol/ g tissue)	31.69 ± 2.69 d,e	30.15 ± 1.31 c,d,e	35.41 ± 1.20 b,d,e	39.81 ± 3.23 a,b,c	37.67 ± 2.83 a,b,c

Data are shown as means ± SD. The level of significance was set to $p<0.05$. ap<0.05 versus SD (standard diet); bp<0.05 versus PSOD (pumpkin-seed oil enriched diet; cp<0.05 versus LD (lard enriched diet); dp<0.05 versus OOD (olive oil enriched diet); ep<0.05 versus FOD (fish oil enriched diet)

Table 2. *Fatty acid composition of total lipids extracted from standard diet (SD) feed and feed enriched with pumpkin-seed oil (PSOD), lard (LD), olive oil (OOD) and fish oil (FOD).*

FAME	TOTAL FATTY ACIDS (%)				
	SD	PSOD	LD	OOD	FOD
12:0	0.01	0.23	0.26	0.37	0.05
14:0	0.93	0.39	1.54	0.51	2.96
15:0	0.08	0.08	0.05	0.05	0.30
16:0	16.21	11.12	25.07	14.35	12.65
17:0	0.31	0.26	1.03	0.19	0.28
18:0	2.20	2.13	8.61	1.25	1.56
20:0	0.93	0.91	1.13	0.08	0
22:0	0.33	0.12	0.04	0.23	1.62
24:0	0.07	0.06	0.03	0.02	0.09
Σ SFA	**21.07**	**15.25**	**37.77**	**17.04**	**19.51**
14:1	0	0	0	0	0.39
16:1 n-7	1.51	1.06	2.82	1.17	8.41
17:1 n-7	0.02	0.04	0.33	0.05	0.11
18:1 n-9	21.37	28.93	43.05	52.27	23.67
20:1 n-9	1.00	1.09	0	2.22	10.02
24:1 n-5	0	0	0.02	0.03	0.08
ΣMUFA	**23.90**	**31.12**	**46.22**	**55.74**	**42.69**
18:2 n-6	46.72	48.02	14.89	23.82	20.16
20:2 n-6	0.87	2.25	0.29	0.99	0.16
20:3 n-6	0.11	0	0.03	0	0
20:4 n-6	0.06	0.04	0	0	0.18
Σ n-6	**47.76**	**50.31**	**15.21**	**24.81**	**20.50**
18:3 n-3	5.27	2.44	0.66	1.50	1.50
20:5 n-3	1.23	0.50	0.12	0.53	8.63
22:5 n-3	0	0	0	0	0.65
22:6 n-3	0.77	0.38	0.03	0.38	6.51
Σ n-3	**7.27**	**3.32**	**0.81**	**2.41**	**17.29**
Σ PUFA	**55.03**	**53.63**	**16.01**	**27.22**	**37.79**
Σ PUFA/ Σ SFA	**2.61**	**3.52**	**0.42**	**1.60**	**1.94**
n-6/n-3	**6.57**	**15.16**	**18.88**	**10.31**	**1.18**

FAME – fatty acid methyl esters; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; IU – index of unsaturation

Table 3. *Fatty acid composition of adipose tissue total lipids in mice fed with standard diet and diets enriched with specific oil or lard. Values are area per cent. Data are shown as means ± SD. The level of significance was set to p<0.05. ap<0.05 versus SD (standard diet); bp<0.05 versus PSOD (pumpkin-seed oil enriched diet; cp<0.05 versus LD (lard enriched diet); dp<0.05 versus OOD (olive oil enriched diet); ep<0.05 versus FOD (fish oil enriched diet)*

FAME	TOTAL FATTY ACIDS (%)				
	SD	PSOD	LD	OOD	FOD
12:0	0.06 ± 0.01 *b,c,d*	0.12 ± 0.03 *a,d,e*	0.11 ± 0.04 *a*	0.09 ± 0.01 *a,b*	0.09 ± 0.02 *b*
14:0	1.13 ± 0.12 *b,c,d,e*	0.92 ± 0.07 *a,c,e*	1.11 ± 0.09 *b,d,e*	0.97 ± 0.11 *a,c,e*	1.30 ± 0.22 *a,b,c,d*
16:0	20.68 ± 0.71 *b,c,d,e*	17.52 ± 0.95 *a,c*	18.85 ± 1.42 *a,b,d*	17.72 ± 0.54 *a,c*	18.09 ± 0.69 *a*
18:0	1.68 ± 0.22 *d*	1.62 ± 0.14 *d*	1.79 ± 0.34 *d,e*	0.76 ± 0.78 *a,b,c,e*	1.36 ± 0.45 *c,d*
22:0	0.04 ± 0.06 *b,c,e*	0.01 ± 0.01 *a*	0.00 *a*	0.01 ± 0.01	0.00 *a*
Σ SFA	**23.59 ± 0.92** *b,c,d,e*	**20.19 ± 1.05** *a,c*	**21.85 ± 1.81** *a,b,d*	**19.54 ± 1.09** *a,c,e*	**20.84 ± 0.97** *a,d*
14:1 n-5	0.08 ± 0.05 *e*	0.07 ± 0.02 *e*	0.09 ± 0.01 *e*	0.07 ± 0.02 *e*	0.13 ± 0.05 *a,b,c,d*
16:1 n-7	8.90 ± 0.59 *b,c,d*	6.42 ± 0.93 *a,c,e*	8.17 ± 0.90 *a,b,d*	6.79 ± 0.84 *a,c,e*	8.68 ± 0.35 *b,d*
18:1 n-9	34.28 ± 0.73 *b,c,d,e*	35.88 ± 0.99 *a,c,d*	39.67 ± 0.78 *a,b,d,e*	43.71 ± 1.73 *a,b,c,e*	35.79 ± 1.37 *a,c,d*
20:1 n-9	0.50 ± 0.24 *e*	0.59 ± 0.33 *e*	0.49 ± 0.29 *e*	0.69 ± 0.42 *e*	2.03 ± 0.61 *a,b,c,d*
22:1 n-9	0.01 ± 0.03 *e*	0.01 ± 0.02 *e*	0.00 *e*	0.00 *e*	0.21 ± 0.08 *a,b,c,d*
24:1 n-9	0.01 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.00	0.00 ± 0.00
Σ MUFA	**43.78 ± 0.79** *b,c,d,e*	**42.98 ± 0.62** *a,c,d,e*	**48.43 ± 0.49** *a,b,d,e*	**51.24 ± 0.95** *a,b,c,e*	**46.84 ± 0.94** *a,b,c,d*
18:2 n-6	29.33 ± 1.23 *b,c,d,e*	34.12 ± 1.31 *a,c,d,e*	26.85 ± 1.51 *a,b*	25.58 ± 2.00 *a,b,e*	27.77 ± 1.28 *a,b,d*
20:2 n-6	0.52 ± 0.19 *d*	0.40 ± 0.21 *d*	0.79 ± 0.43 *d*	1.48 ± 1.05 *a,b,c,e*	0.66 ± 0.67 *d*
20:4 n-6	0.14 ± 0.08	0.19 ± 0.04	0.19 ± 0.12	0.21 ± 0.12	0.18 ± 0.08
22:3 n-6	0.04 ± 0.04 *d,e*	0.03 ± 0.02 *d,e*	0.06 ± 0.02 *d*	0.10 ± 0.05 *a,b,c*	0.09 ± 0.05 *a,b*
Σ n-6	**30.01 ± 1.16** *b,c,d,e*	**34.75 ± 1.20** *a,c,d,e*	**27.89 ± 1.33** *a,b*	**27.38 ± 1.25** *a,b,e*	**28.69 ± 0.58** *a,b,d*
18:3 n-3	2.03 ± 0.18 *b,c,d*	1.76 ± 0.30 *a,c*	1.50 ± 0.33 *a,b,e*	1.56 ± 0.31 *a,e*	1.85 ± 0.36 *c,d*
20:5 n-3	0.10 ± 0.04 *e*	0.04 ± 0.01 *e*	0.04 ± 0.02 *e*	0.03 ± 0.02 *e*	0.50 ± 0.13 *a,b,c,d*
22:5 n-3	0.08 ± 0.02 *b,c,d,e*	0.05 ± 0.01 *a,e*	0.05 ± 0.01 *a,e*	0.04 ± 0.01 *a,e*	0.15 ± 0.02 *a,b,c,d*
22:6 n-3	0.37 ± 0.11 *b,c,d,e*	0.23 ± 0.05 *a,e*	0.25 ± 0.04 *a,e*	0.20 ± 0.04 *a,e*	1.12 ± 0.21 *a,b,c,d*
Σ n-3	**2.57 ± 0.23** *b,c,d,e*	**2.08 ± 0.28** *a,e*	**1.83 ± 0.36** *a,e*	**1.83 ± 0.31** *a,e*	**3.62 ± 0.56** *a,b,c,d*
Σ PUFA	**32.62 ± 1.27** *b,c,d*	**36.84 ± 1.22** *a,c,d,e*	**29.72 ± 1.61** *a,b,e*	**29.21 ± 1.43** *a,b,e*	**32.32 ± 0.95** *b,c,d*
ΣUFA/ ΣSFA	**3.24 ± 0.17** *b,c,d,e*	**3.97 ± 0.26** *a,c*	**3.61 ± 0.40** *a,b,d*	**4.13 ± 0.30** *a,c,e*	**3.81 ± 0.22** *a,d*
ΣPUFA/ ΣMUFA	**0.75 ± 0.03** *b,c,d,e*	**0.86 ± 0.04** *a,c,d,e*	**0.61 ± 0.03** *a,b,d,e*	**0.57 ± 0.04** *a,b,c,e*	**0.69 ± 0.03** *a,b,c,d*
Σ n-6/ Σ n-3	**11.74 ± 0.86** *b,c,d,e*	**16.92 ± 2.10** *a,e*	**15.65 ± 2.55** *a,e*	**15.26 ± 2.26** *a,e*	**8.08 ±1.19** *a,b,c,d*
IU	**1.13 ± 0.02** *b,e*	**1.20 ± 0.02** *a,c,d*	**1.11 ± 0.04** *b,e*	**1.13 ± 0.03** *b,e*	**1.20 ± 0.02** *a,c,d*

Standard diet (SD), pumpkinseed oil enriched diet (PSOD), lard enriched diet (LD), olive oil enriched diet (OOD) and fish oil enriched diet (FOD)

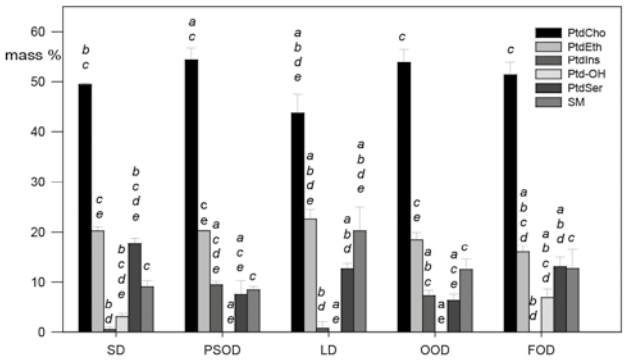
FAME – fatty acid methyl esters; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; UFA – unsaturated fatty acids, IU – index of unsaturation

Table 4. *Fatty acid composition of adipose tissue phospholipids in mice fed with standard diet and diets enriched with specific oil or lard. Values are area per cent.*
Data are shown as means ± SD. The level of significance was set to p<0.05. ap<0.05 versus SD (standard diet); bp<0.05 versus PSOD (pumpkin-seed oil enriched diet; cp<0.05 versus LD (lard enriched diet); dp<0.05 versus OOD (olive oil enriched diet); ep<0.05 versus FOD (fish oil enriched diet)

FAME	TOTAL FATTY ACIDS (%)				
	SD	PSOD	LD	OOD	FOD
14:0	1.02 ± 0.34 *d*	0.97 ± 0.17	0.79 ± 0.22	0.65 ± 0.13 *a*	0.82 ± 0.07
16:0	18.50 ± 0.86	19.93 ± 0.73 *c,d,e*	17.92 ± 1.71 *b*	18.17 ± 0.60 *b*	17.66 ± 1.05 *b*
18:0	20.12 ± 0.90 *d*	18.68 ± 3.26 *d*	20.86 ± 2.05	24.44 ± 3.51 *a,b*	22.13 ± 2.15
20:0	0.29 ± 0.08	0.29 ± 0.14	0.17 ± 0.09	0.26 ± 0.01	0.25 ± 0.14
24:0	2.20 ± 0.83 *d*	1.89 ± 0.46	1.68 ± 0.60	1.21 ± 0.66 *a*	1.73 ± 0.50
Σ SFA	**42.14 ± 1.40**	**41.77 ± 2.23** *d*	**41.41 ± 0.70** *d*	**44.72 ± 2.13** *b,c*	**42.59 ± 1.81**
14:1 n-5	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.04	0.02 ± 0.00 *e*	0.06 ± 0.02 *d*
16:1 n-7	0.94 ± 0.11	0.79 ± 0.41 *c*	1.42 ± 0.76 *b*	0.97 ± 0.03	0.95 ± 0.23
18:1 n-9	15.58 ± 2.58	15.71 ± 2.65	16.67 ± 1.19	17.07 ± 1.53	14.50 ± 0.99
20:1 n-9	0.32 ± 0.09 *e*	0.33 ± 0.21 *e*	0.20 ± 0.04 *e*	0.42 ± 0.17 *e*	0.76 ± 0.39 *a,b,c,d*
22:1 n-9	11.34 ± 1.36 *c*	7.94 ± 2.56 *c,d*	15.05 ± 4.41 *a,b,e*	13.81 ± 0.41 *b,e*	8.71 ± 1.87 *c,d*
24:1 n-9	4.91 ± 0.33 *c*	4.07 ± 0.88 *c,e*	6.40 ± 0.52 *a,b,d*	4.67 ± 0.50 *c*	5.45 ± 1.13 *b*
Σ MUFA	**33.13 ± 2.43** *c*	**28.87 ± 4.17** *c,d*	**39.79 ± 3.77** *a,b,e*	**36.95 ± 1.24** *b,e*	**30.44 ± 3.18** *c,d*
18:2 n-6	10.76 ± 0.74	12.55 ± 3.27 *c,d,e*	8.76 ± 1.73 *b*	7.91 ± 0.33 *b*	8.26 ± 1.56 *b*
20:2 n-6	1.07 ± 0.64	1.93 ± 1.03 *c*	0.55 ± 0.28 *b,d,e*	1.78 ± 1.08 *c*	1.72 ± 0.27 *c*
20:3 n-6	0.60 ± 0.47	0.25 ± 0.08	0.49 ± 0.20	0.40 ± 0.15	0.36 ± 0.23
20:4 n-6	11.08 ± 1.99	13.35 ± 1.26 *c,d*	8.14 ± 2.52 *b,e*	7.28 ± 1.46 *b,e*	13.90 ± 4.79 *c,d*
Σ n-6	**23.51 ± 1.09** *c,d*	**28.07 ± 4.91** *c,d*	**17.95 ± 3.66** *a,b,e*	**17.36 ± 0.84** *a,b,e*	**24.24 ± 5.07** *c,d*
18:3 n-3	0.65 ± 0.14	0.92 ± 0.53 *c*	0.43 ± 0.13 *b,e*	0.67 ± 0.24	0.91 ± 0.21 *c*
22:6 n-3	0.59 ± 0.06 *e*	0.37 ± 0.09 *e*	0.42 ± 0.12 *e*	0.29 ± 0.21 *e*	1.82 ± 0.42 *a,b,c,d*
Σ n-3	**1.24 ± 0.12** *e*	**1.29 ± 0.47** *c,e*	**0.85 ± 0.15** *b,e*	**0.96 ± 0.22** *e*	**2.73 ± 0.39** *a,b,c,d*
Σ PUFA	**24.74 ± 1.05** *c,d*	**29.36 ± 5.12** *c,d*	**18.80 ± 3.55** *a,b,e*	**18.32 ± 0.77** *a,b,e*	**26.97 ± 4.95** *c,d*
ΣUFA/ ΣSFA	**1.38 ± 0.08**	**1.40 ± 0.13** *d*	**1.42 ± 0.04** *d*	**1.24 ± 0.11** *b,c*	**1.35 ± 0.10**
ΣPUFA/ΣSFA	**0.59 ± 0.01** *d*	**0.71 ± 0.14** *c,d*	**0.45 ± 0.08** *b,e*	**0.41 ± 0.04** *a,b,e*	**0.64 ± 0.14** *c,d*
Σ n-6/Σ n-3	**19.15 ± 2.50** *e*	**23.42 ± 4.47** *e*	**22.23 ± 5.94** *e*	**18.70 ± 4.25** *e*	**9.09 ± 2.37** *a,b,c,d*
ΣPUFA/ΣMUFA	**0.75 ± 0.08** *b*	**1.05 ± 0.31** *a,c,d*	**0.48 ± 0.14** *b,e*	**0.50 ± 0.01** *b,e*	**0.91 ± 0.25** *c,d*
IU	**1.08 ± 0.04** *c,d*	**1.17 ± 0.09** *c,d*	**0.96 ± 0.07** ***a,****b,e*	**0.90 ± 0.06** *a,b,e*	**1.21 ± 0.14** *c,d*

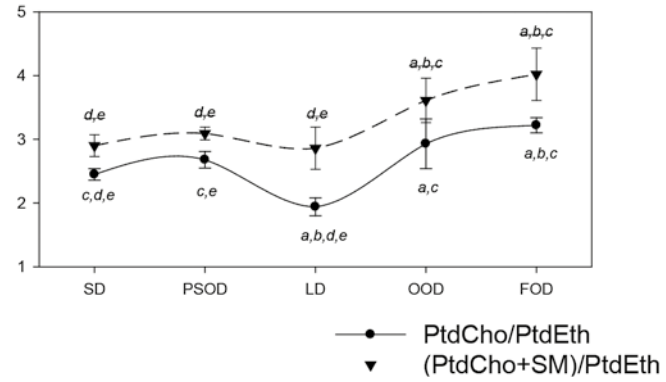
Standard diet (SD), pumpkinseed oil enriched diet (PSOD), lard enriched diet (LD), olive oil enriched diet (OOD) and fish oil enriched diet (FOD)
FAME – fatty acid methyl esters; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids SFA – saturated fatty acids; UFA – unsaturated fatty acids, IU – index of unsaturation

Figure 1. *Phospholipid composition of adipose tissue expressed as mass percent of main phospholipids in mice fed with standard diet and diets enriched with specific oil or lard*



Data are shown as means ± SD. The level of significance was set to $p<0.05$. [a]$p<0.05$ versus SD (standard diet); [b]$p<0.05$ versus PSOD (pumpkin-seed oil enriched diet; [c]$p<0.05$ versus LD (lard enriched diet); [d]$p<0.05$ versus OOD (olive oil enriched diet); [e]$p<0.05$ versus FOD (fish oil enriched diet)

Figure 2. *Mass ratio of PtdCho/PtdEtn and (PtdCho+SM)/PtdEth in adipose tissue phospholipids in mice fed with standard diet and diets enriched with specific oil or lard*



Data are shown as means ± SD. The level of significance was set to $p<0.05$. [a]$p<0.05$ versus SD (standard diet); [b]$p<0.05$ versus PSOD (pumpkin-seed oil enriched diet; [c]$p<0.05$ versus LD (lard enriched diet); [d]$p<0.05$ versus OOD (olive oil enriched diet); [e]$p<0.05$ versus FOD (fish oil enriched diet)

References

1. Albright AL, Stern JS. (1998) Adipose Tissue**.** in: Encyclopedia of Sports Medicine and Science (F.TD ed). Internet Society for Sport Science.
2. Barenholz Y., Thompson T.E. (1980) Sphingomyelins in bilayers and biological membranes. *Biochimica et Biophysica Acta,* 604**,** 129-158
3. Broekhuyse R.M. (1968) Phospholipids in tissues of the eye. I. Isolation, characterization and quantitative analysis by two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. *Biochimica et Biophysica Acta,* 152, 307-315
4. Brown R.E. (1998) Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *Journal of Cell Science* 111, 1-9
5. Bruno M.J., Koeppe R.E., Andersen O.S. (2007) Docosahexaenoic acid alters bilayer elastic properties. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 9638-9643
6. Catta-Preta M., Martins M.A., Brunini T.M.C., Mendes-Ribeiro A.C., Mandarim-de-Lacerda C.A., Aguila M.B. (2012) Modulation of cytokines, resistin, and distribution of adipose tissue in C57BL/6 mice by different high-fat diets. *Nutrition,* 28, 212-219
7. Clandinin M.T., Cheema S., Field C.J., Garg M.L., Venkatraman J., Clandinin T.R. (1991) Dietary-Fat - Exogenous Determination of Membrane-Structure and Cell-Function. *FASEB Journal,* 5**,** 2761-2769
8. Fernandez-Quintela A., Churruca I., Portillo M.P. (2007) The role of dietary fat in adipose tissue metabolism. *Public Health Nutrition,* 10**,** 1126-1131
9. Fickova M., Hubert P., Cremel G., Leray C. (1998) Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. *Journal of Nutrition,* 128**,** 512-519
10. Field C.J., Goruk S.D., Wierzbicki A.A., Clandinin M.T. (1989a) The Effect of Dietary-Fat Content and Composition on Adipocyte Lipids in Normal and Diabetic States. *International Journal of Obesity,* 13, 747-756
11. Field C.J., Toyomizu M., Clandinin M.T. (1989b) Relationship between Dietary-Fat, Adiopcyte Membrane-Composition and Insulin Binding in the Rat. *Journal of Nutrition,* 119, 1483-1489
12. Flachs P., Horakova O., Brauner P., Rossmeisl M., Pecina P., Franssen-van Hal N., Ruzickova J., Sponarova J., Drahota Z., Vlcek C., et al. (2005) Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia,* 48, 2365-2375
13. Folch J., Lees M., Stanley G.H.S. (1957) A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *Journal of Biological Chemistry,* 226**,** 497-509
14. Giacometti J., Milosevic A., Milin C. (2002) Gas chromatographic determination of fatty acids contained in different lipid classes after their separation by solid-phase extraction. *Journal of Chromatography A,* 976, 47-54

15. Hulbert A.J. (2003) Life, death and membrane bilayers. *Journal of Experimental Biology,* 206, 2303-2311
16. Hulbert A.J., Kelly M.A., Abbott S.K. (2014) Polyunsaturated fats, membrane lipids and animal longevity. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology,* 184**,** 149-166
17. Hulbert A.J., Turner N., Storlien L.H., Else P.L. (2005) Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews,* 80, 155-169
18. Hynes G.R., Heshka J., Chadee K., Jones P.J. (2003) Effects of dietary fat type and energy restriction on adipose tissue fatty acid composition and leptin production in rats. *Journal of Lipid Research,* 44**,** 893-901
19. Li Z.Y., Agellon L.B., Allen T.M., Umeda M., Jewel L., Mason A., Vance D.E. (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metabolism,* 3, 321-331
20. Miyazaki M., Ntambi J.M. (2003) Role of stearoyl-coenzyme A desaturase in lipid metabolism. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 68**,** 113-121
21. Nakamura M.T., Nara T.Y. (2004) Structure, function, and dietary regulation of Delta 6, Delta 5, and Delta 9 desaturases. *Annual Review of Nutrition,* 24**,** 345-376
22. Ntambi J.M., Bene H. (2001) Polyunsaturated fatty acid regulation of gene expression. *Journal of Molecular Neuroscience,* 16**,** 273-278
23. Parrish C.C., Pathy D.A., Angel A. (1990) Dietary Fish Oils Limit Adipose-Tissue Hypertrophy in Rats. *Metabolism-Clinical and Experimental,* 39**,** 217-219
24. Parrish C.C., Pathy D.A., Parkes J.G., Angel A. (1991) Dietary Fish Oils Modify Adipocyte Structure and Function. *Journal of Cellular Physiology*, 148**,** 493-502
25. Raclot T. (1997) Selective mobilization of fatty acids from white fat cells: Evidence for a relationship to the polarity of triacylglycerols. *Biochemical Journal,* 322**,** 483-489
26. Raclot T., Groscolas R. (1993) Differential Mobilization of White Adipose-Tissue Fatty-Acids According to Chain-Length, Unsaturation, and Positional Isomerism. *Journal of Lipid Research*, 34**,** 1515-1526
27. Shindou H. and Shimizu T. (2009) Acyl-CoA:lysophospholipid acyltransferases. *Journal of Biological Chemistry,* 284**,** 1-5
28. Soriguer F.J., Tinahones F.J., Monzon A., Pareja A., Rojo-Martinez G., Moreno F., Esteva I., Gomez-Zumaquero J.M. (2000) Varying incorporation of fatty acids into phospholipids from muscle, adipose and pancreatic exocrine tissues and thymocytes in adult rats fed with diets rich in different fatty acids. *European Journal of Epidemiology,* 16**,** 585-594
29. Tallman D.L., Taylor C.G. (2003) Effects of dietary fat and zinc on adiposity, serum leptin and adipose fatty acid composition in C57BL/6J mice. *Journal of Nutritional Biochemistry,* 14**,** 17-23
30. van Meer G., Voelker D.R., Feigenson G.W. (2008) Membrane lipids: where they are and how they behave. *Nature Reviews: Molecular Cell Biology,* 9**,** 112-124
31. Woiss C.H. (2007) StatSoft, Inc., Tulsa, OK.: STATISTICA, version 8. *Asta-Advances in Statistical Analysis,* 91**,** 339-341