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Partial Hepatectomy and Diets Enriched with Olive and Corn Oil Altered the Phospholipid Fatty Acid Profile in the Spleen

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Abstract

The purpose of this study was to examine changes in the polar fatty acid (PL FAs) profile in mice spleen after a one-third partial hepatectomy (PHx) and a diet enriched with olive and corn oil.

Fatty acids (FAs) were determined by gas chromatography (GC) after previous fractionation of polar fatty acids by solid-phase extraction using an aminopropyl (NH_2) column. The data were analysed using the nonparametric Kruskal-Wallis test and linear regression analysis.

A diet supplemented with corn oil (FCO) increased palmitic acid, while an olive oil-enriched diet (FOO) increased arachidonic and docosahexaenoic acid in the spleen PL FAs during PHx.

Based on the FAs profile of PL FAs in the spleen during PHx, in the FCO diet group stearoyl CoA desaturase (SCD1) activity showed a positive correlation (R=0.58) with 18:2n-6 as the major FAs in corn oil, while in the FOO group, SCD1 and elongase-6 (Elovl6) activities positively correlated (R=0.84, R=0.55, respectively) with 18:1n-9 as the major FAs in olive oil.

To conclude, despite the beneficial effect of diet, lipid homeostasis in the spleen was regulated more by PHx than the n-6 and n-9 diet.

Keywords: Fatty acids; Phospholipids; Corn oil diet; Olive oil diet; Partial hepatectomy

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Abbreviations

ACL: Acyl Chain Length Index; ALA: Alpha-Linolenic Acid, 18:3n-3; AA: Arachidonic Acid, 20:4n-6; D5D (FADS1): Desaturase-5 or Fatty Acid Desaturase 1; D6D (FADS2): Desaturase-6 or Fatty Acid Desaturase 2; D9C16: Delta-9-desaturase of the C16; D9C18: Delta-9-desaturase of the C18; DBI: Double Bond Index; DGLA: Dihomo-Gamma-Linolenic Acid, 20:3n-6; DHA: Docosahexaenoic Acid, 22:6n-3; EGF: Epidermal Growth Factor; Elovl5: Elongase-5; Elovl6: Elongase-6; EPA: Eicosapentaenoic Acid, 20:5n-3; EVOO: Extra Virgin Olive Oil; FAMEs: Fatty Acid Methyl Esters; FAs: Fatty Acids; FASN: Fatty Acid Synthase; FCO: Standard Diet Enriched with Corn Oil; FOO: Standard Diet Enriched with Olive Oil; GLA: Gamma-linolenicacid, 18:3n-6; HGF: Hepatocyte Growth Factor; IL-6: Interleukin-6 Cytokine; LN: Linoleic Acid, 18:2n-6; MUFA: Monounsaturated Fatty Acids; n-3 FAs: Fatty Acids n-3 Series; n-3 PL FAs: Fatty Acids n-3 Series in Polar Lipids; n-3 PUFAs: Polyunsaturated Fatty Acids n-3 Series; n-6 FAs: Fatty Acids n-6 Series; n-6 PL FAs: Fatty Acids n-6 Series in Polar Lipids; n-6 PUFAs: Polyunsaturated Fatty Acids n-6 Series; n-9 FAs: Fatty Acids n-9 Series; n-9 PL FAs: Fatty Acids n-9 Seriesin Polar Lipids; NK: Natural Killer; OA: Oleic Acid, 18:1n-9; PHx: Partial Hepatectomy; PI: Peroxidizability Index; PL FAs: Polar Lipid Fatty Acids; PL: Polar Lipid Or Phospholipids; PUFA: Polyunsaturated Fatty Acids; SCD1: Stearoyl-CoA Desaturase; SFA: Saturated Fatty Acids; TGF-α: Transforming Growth Factor-α; TGF-β1: Transforming Growth Factor-β1; TNF-α: Tumour Necrosis Factor-α.

Introduction

The liver and spleen are closely linked *via* the portal vein system. The spleen probably influences the hepatic immune microenvironment by cell migration or the secretion of splenic soluble factors [1]. Select studies reported that the spleen plays an inhibitory role in liver regeneration through spleen-derived transforming growth factor-β1 (TGF-β1) that inhibited the growth of hepatocytes

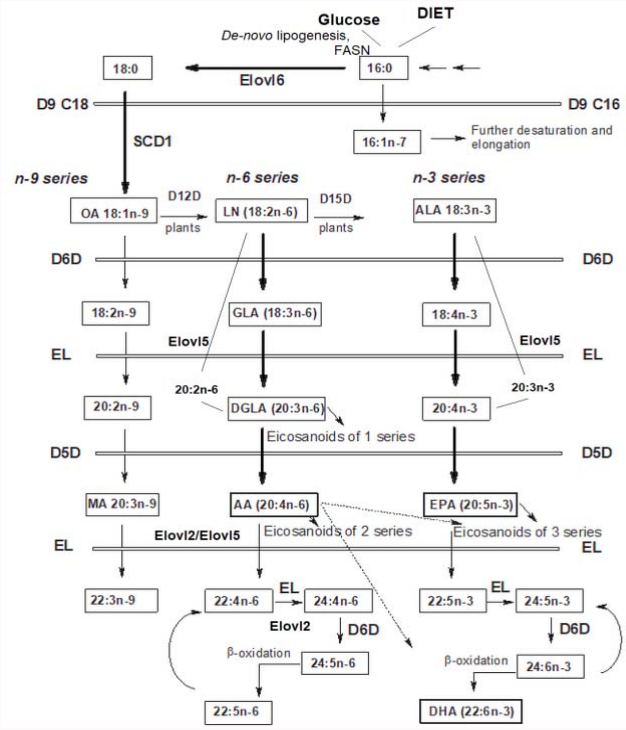

Figure 1: The biosynthesis of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).

Table 1: The fatty acid composition of the used diets and dietary oils (g/100g).

Fatty acid	Control diet	Olive oil	FOO diet	Corn oil	FCO diet
14:0	n.d.	n.d.	n.d.	0.01	n.d.
16:0	18.42	11.36	14.01	13.63	15.43
18:0	2.83	2.66	2.72	1.44	1.96
20:0	n.d.	0.43	0.27	0.14	0.09
22:0	n.d.	n.d.	n.d.	0.05	0.03
24:0	n.d.	0.09	0.06	0.07	0.04
16:1n-7	0.85	0.93	0.9	0.06	0.36
18:1n-9	21.19	74.48	54.5	25.37	23.8
20:1n-9	n.d.	0.38	0.24	0.97	0.61
22:1n-9	n.d.	n.d.	n.d.	0.76	n.d.
18:2n-6	51.8	9.00	25.05	57.06	55.09
18:3n-3	4.9	0.67	2.26	0.3	2.03
Σ n-3	*4.9*	*0.67*	*2.26*	*0.3*	*2.03*
Σ n-6	***51.8***	***9***	***25.05***	***57.06***	***55.09***
Σ n-9	***21.19***	***74.86***	***54.74***	***27.1***	***24.41***
n-9/n-6	***0.41***	***8.32***	***2.18***	***0.48***	***0.44***
Σ SFA	***21.25***	***14.54***	***17.06***	***15.49***	***17.55***
Σ MUFA	***22.04***	***75.79***	***55.63***	***27.16***	***24.77***
Σ PUFA	***56.7***	***9.67***	***27.31***	***57.36***	***57.11***

in animals [2,3] and in this way, promoted tissue fibrosis in the liver [4,5]. Although the liver has a great regenerative capacity, it is reduced during liver damage or resection and causes changes in tissue architecture [6]. Liver regeneration induces the proliferation of the main non-parenchymal cells of the liver [7]. In this process are involved many growth factors and cytokines such as the hepatocyte growth factor (HGF), the tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), the transforming growth factor-α (TGF-α), and the epidermal growth factor (EGF) [8]. During liver injury, several other organs, such as the kidney, the lung and the spleen produce HGF in response to HGF-inducers [5]. These changes alter the cell membrane and its lipid composition, and also modify the fatty acid metabolism.

The membrane proliferation implies major changes in the metabolism, traffic and re-modelling of intracellular membranes and their lipid constituents such as phospholipids (PLs). The PL fatty acid composition contributes significantly to the physical and biochemical properties of membranes. Their changes have a wide range of effects regarding the integrity of cellular membranes, fluidity, the activity of membrane enzymes and the affinity of growth factor receptors. Membrane PLs are in a constant process of degradation/resynthesis, and the amount of each PLs depends on the cell type and has characteristic fatty acids (FAs) [9,10]. The biosynthesis of unsaturated fatty acids is presented in Figure 1.

Dietary fats affect several roles in the body; as a source of energy and structural components of cells, for cell growth and differentiation and in the regulation of gene expression of lipid, and for carbohydrate and protein metabolism [11,12]. These roles attributed to FAs and their metabolites, which act directly on specific transcription factors and regulate gene transcription, and indirectly on gene expression through their effects on specific enzyme-mediated pathways or pathways that involve changes in membrane lipid/lipid raft composition [13,14]. Several studies reported their effect on immuneresponse and immunomodulation [15-17] and changes in gut microbiota control the occurrence of metabolic diseases [18-20]. PUFAs and MUFAs act on immuno-modulation in lymphocyte proliferation [21], production of cytokines [22], modification of cell surface molecules [23], change phagocytic activity [24,25], and alter natural killer (NK) cell activity [26]. Fatty acids of n-6 series are immunosuppressors, while n-9 FAs and n-3 FAs are immunomodulators [25-28].

As reported previously, a partial hepatectomy (PHx) induced the changes in liver PLs FAs, while olive and cornoil supplemented diet saffected PL Fas alteration during PHx [29].

This study explores the effect of one-third PHx on changes in splenic PL FAs and the effect of corn and olive oil supplemented diets on splenic PL FAs during PHx. Our hypothesis is based on the physiological and pathophysiological interconnection of the liver and spleen due to an increase of portal hypertension after liver surgery, increased HGF, the increased pooling of platelets in the spleen as well as a response to oxidative stress due to PHx in the spleen.

Materials and Methods

Chemicals and reagents

The used chemicals and reference compounds for gas chromatography (GC) were purchased from Sigma (St. Louis, MO, USA). Solvents were of the highest reagent grade available. The extra-virgin olive oil (EVOO) used for the preparation of the diet was obtained from Agroprodukt, d.o.o. (Vodnjan, Croatia) and the refined corn oil was purchased from Oleifico Zucchi S.p.A, Cremona, Italy. Total phenols in EVOO were 279 GAE mg/kg.

The solid-phase extraction cartridges, NH_2 (Bond Elut, 3 ml volume, 500 mg sorbents) used for the lipid classes separation were

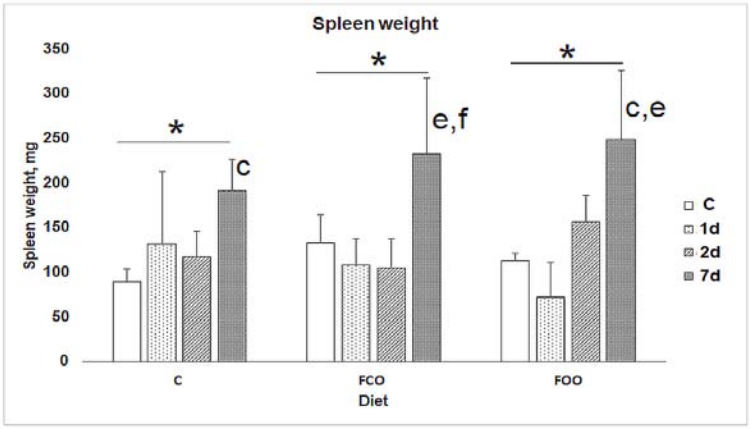

Figure 2: The effect of a standard, FCO and FOO diets on the spleen weight (SW) on the 1st, 2nd and 7th day after partial hepatectomy (PHx).
Values are area per cent (mean ± SD of 6-8 mice/group); *significant difference during PHx among same diet using *Kruskal-Wallis* Anova by Ranks test; [c]significant difference between the control and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups (P<0.05); [e]significant difference between the 1st day and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups (P<0.05); [f]significant difference between the 2nd and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups (P<0.05).

purchased from Varian (Harbor City, CA, USA).

Diets and animals

A stock standard diet (pellet, type 4RF21 GLP, Mucedola, Settimo Milanese, Italy) was used as the standard diet in all experiments. Olive and corn oil were added to the stock standard diet to a 5% w/w, (5 g of oil to 100 g of standard diet pellets) for preparation of the olive oil (FOO) and corn oil (FCO) enriched diets. Diets were freshly prepared once a week by the addition of the appropriate amounts of oils, gasses with N_2 and were stored at 0-4 °C to minimize fatty acid degradation. The FAs composition of dietary oils and diets is shown in Table 1.

Male Balb/c mice (Medical Faculty, Rijeka, Croatia), at the age of 2-3 months and weight of at least 25 g to 30 g, were acclimated for 1 week at a temperature of (21-23 °C) and in a humidity controlled facility on a 12h light/dark cycle. After the acclimatisation period, animals were divided into three groups as follows: Group 1 (Control) standard diet fed mice; Group 2 (FOO) olive oil fed mice; Group 3 (FCO) corn oil fed mice. Body weight and food intake were monitored during the study. Control group animals (standard diet, FCO, FOO) without surgical hepatectomy were sacrificed after three weeks. The remaining animals of each group were subjected to 1/3 PHx under anaesthesia and were then sacrificed on days 1, 2 and 7 after PHx. The spleen and a portion of the same region of regenerated liver in all cases were removed by plastic instruments, washed several times with a saline solution (0.9% w/w, NaCl) to remove blood, and immediately weighed and stored at -80°C until the analysis. To avoid possible diurnal variability, all operations were performed between 8:00–9:00 a.m.

All experimental procedures were performed in compliance with the Declaration of Helsinki and were approved by the Ethical Committee of the Medical faculty, University of Rijeka.

Lipid analyses

The FAs composition of the dietary oil was determined according to the modified EC Regulation 2568/91 (EEC) [30] by gas chromatographic analysis. In brief, fatty acid methyl esters (FAMEs) were prepared at 100°C by acid-catalyzed transesterification of oils under reflux over a period of four hours with 2M methanol-hydrochloric acid containing 0.01% (w/v) butylated hydroxyl toluene (BHT). The obtained mixture was extracted with petroleum ether and FAMEs were evaporated in a rotating evaporator to dryness. The same procedure was used for the preparation of FAMEs extracted from pellets and the pellet enriched FCO and FOO diets. Test portions, in the form of the FAMEs, were performed in duplicate and 1 µl of each sample solute in hexane was injected.

Total lipids from tissues were extracted from tissues with chloroform/methanol (CM, 2:1, v/v) according to Folch et al. [31] containing 0.01% BHT as an antioxidant. The PL FA composition in the spleen tissue was determined after lipid class fractionation according to Giacometti et al. [32]. Briefly, the total lipid extracts were fractionated and purified by solid-phase extraction (SPE), and the polar lipids were separated on the NH_2 column. Next, FAs of the polar lipids were transmethylated with methanol/*n*-hexane/sulphuric acid (75:25:1, v/v) at 90°C for 90 min, extracted using petroleum ether and analysed using GC.

GC analyses were carried out using an Auto system XL (Perkin-Elmer, Norwalk, CT, USA) with a flame-ionization detector (FID). Chromatography software from Perkin-Elmer Nelson (Turbochrom 4, rev. 4.1.) was used for data acquisition from the FID. A capillary SP-2330 column (Supelco, Bellefonte, PA, USA), 30m x 0.32mm I.D. of 0.2mm film thickness was used. Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in programmed temperature mode from 140 to 220°C, at 5°C/min and then isothermal for 25 min. The detector temperature was 350°C and the injector temperature was 300°C. The results of individual FAs were expressed as a percentage of the polar lipid fraction.

Calculations and statistics

SFAs were calculated as SFAs = Σ% (14:0+16:0+18:0+20:0+22:0 +24:0), MUFAs as MUFAs = Σ% (14:1+16:1+18:1+20:1) and PUFAs as PUFAs = Σ% (PUFA*n*-3 + PUFA*n*-6). PUFAs *n*–3 were calculated as PUFAs, *n*–3 = % (20:5*n*-3+22:5*n*-3+22:6*n*-3) and PUFAs *n*–6 as PUFAs, *n*–6 = Σ% (18:2*n*-6+18:3*n*-6+20:2*n*-6+20:3*n*-6+20:4*n*-6+22:4*n*-6). The delta-9-desaturation ratio of the C16 was calculated as D9C16 = 16:1*n*-7/16:0 and the delta-9-desaturation ratio of the C18 as D9C18=18:1*n*-9/18:0. The delta-6 desaturation ratio was calculated as D6D=[(18:3*n*-6+20:3*n*-6)/18:2*n*-6] and the delta-5 desaturation ratio was calculated as D5D=[(20:4*n*-6/(20:3*n*-6)]

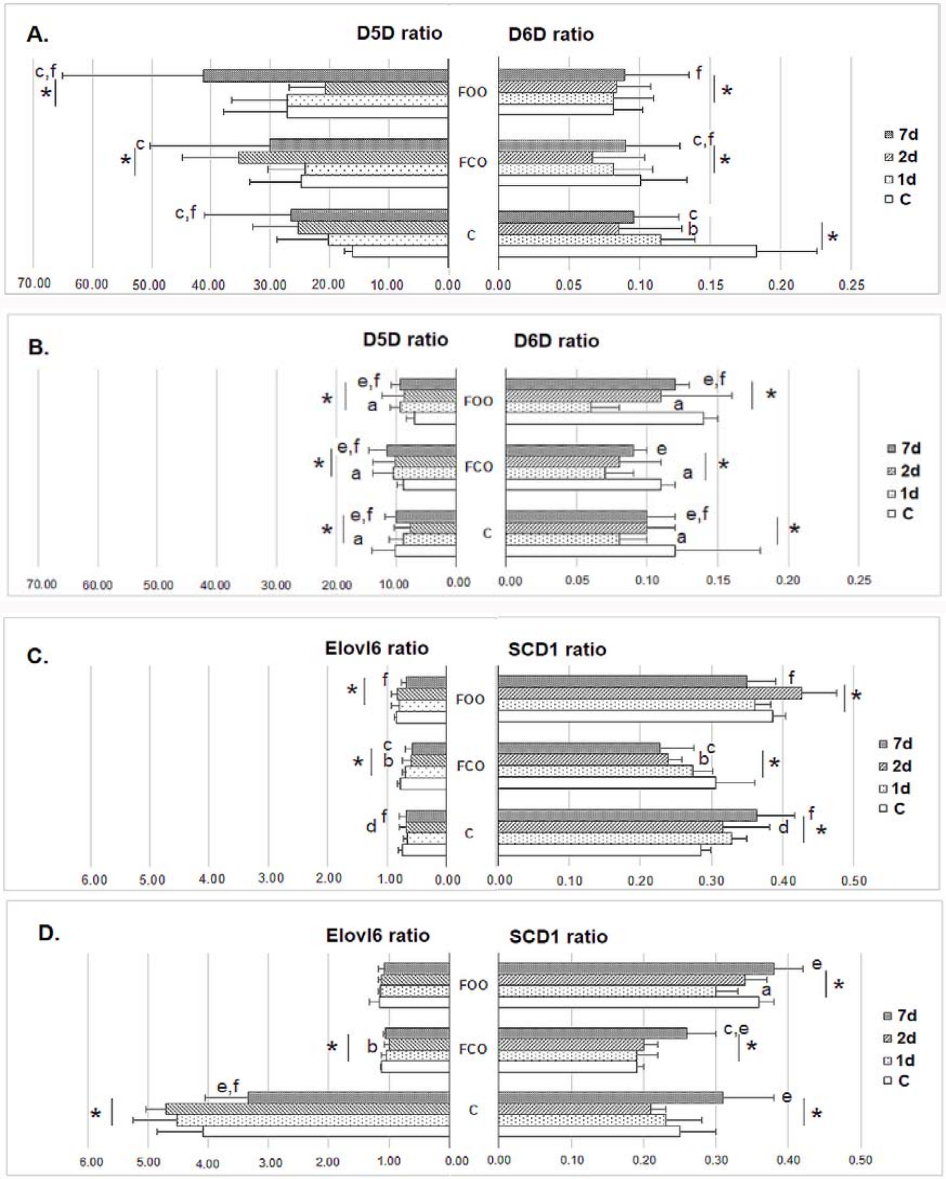

Figure 3: The desaturase-5 (D5D), desaturase-6 (D6D), stearoyl-CoAdesaturase (SCD1) and elongase-6 (Elovl6) ratiosinthe spleen (A and C), and in the liver (B and D) on the 1st, 2ndand 7th day after partial hepatectomy (PHx).
Values are area per cent (mean ± SD of 6-8 mice/group); *significant difference during PHx among same diet using *Kruskal-Wallis* Anova by Ranks test; [a]significant difference between the control and 1st day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [b]significant difference between the control and 2nd day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [c]significant difference between the control and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [d]significant difference between the 1st and 2nd day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [e]significant difference between the 1st day and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [f]significant difference between the 2nd and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$).

[33]. Stearoyl-CoA desaturase 1 (SCD1) activity has been estimated using precursor fatty acid ratios 16:1*n*7/16:0 or 18:1*n*9/18:0 and was calculated as (18:1*n*9+16:1*n*7)/(18:0+16:0), and the elongase-6 ratio (Elovl6) was calculated as (18:1*n*9+18:0)/(16:1*n*7+16:0). The ratio of 18:2*n*-6/20:4*n*-6 represents the activity of enzymes in the biosynthetic pathway of 20:4*n*-6 from 18:2*n*-6, and the 22:6*n*-3/20:4*n*-6 ratio represents the activity of enzymes in the biosynthetic pathway of 22:6*n*-3 from 20:4*n*-6.

The average chain length (ACL) was calculated as ACL = [Σ% $Total_{14}$ x 14) + …+ (Σ% $Total_n$ x n)]/100 (n=carbon atom number). The double bond (DBI) index was calculated as DBI=Σmol% of unsaturated fatty acids of an x number of double bonds of each unsaturated fatty acids. The peroxidizability index (PI) was calculated as PI = [(%Monoenoic x 0.025) + (%Dienoic x 1) + (%Trienoic x 2) + (%Tetraenoic x 4) + (%Pentaenoic x 6) + (%Hexaenoic x 8)] [34].

GC data were evaluated with Statistica (data analysis software system), version 13 (TIBCO Software Inc., 2017). The statistical analysis was performed using the nonparametric Kruskal-Wallis Anova by Ranks and Kruskal-Wallis Multiple Comparisons p values (2-tailed) among PHx in each diet group, and between time points of PHx in each diet group, respectively. Statistical significance was assumed, given $P < 0.05$, and the data are reported based on the mean (SD).

A linear regression analysis used the least square method used in significant correlation determination of individual parameters in the liver and spleen tissues.

Results and Discussion

The liver has the unique capability to restore lost tissue mass after resection by compensatory liver growth. That process is recognized as a liver regeneration. The liver grows rapidly, immediately after resection of more than 50% of its mass. The potential for liver regeneration is different and depends on the degree of liver damage; the response to one-third PHx is moderate and maximal for two-third PHx [35,36]. Because of the close connection between the liver and the spleen, *via* the portal vein system, after liver injury, the destroyed blood cells accumulate in the spleen and thus result in an enlarged spleen [5]. However, the volume of liver resection does not affect the spleen hypertrophy, and then it depends on common regulatory factors [37]. Moreover, it is reported that a hepatectomy stimulates DNA synthetic activity in the spleen [38] by confirming the hypothesis on stimulating growth factors [39]. It is implied that liver regeneration affects signalling pathways, thus ensuring the synchronized proliferation of liver cells and modulation of their cell membranes and the membranes of other associated tissues as the spleen.

As shown in Figure 2, the spleen weight was significantly altered in all used diets during PHx. In the group fed a standard diet it was increased, while it was reduced in the groups FCO and FOO. Although in 1/3 pH a minor degree of liver damage was observed, which can induce moderate stimulus for liver regeneration, we found that the spleen significantly enlarged on day 7 after PHx in all examined groups. A diet supplemented with olive oil has the most effect on spleen enlargement (Figure 1).

It is well established that FAs and their metabolites are implicated in essential aspects of cellular signalling including controlling cell growth, the inducing and regulating of apoptosis as well as having a protective effect on immunological response.

The dynamics of 1/3 PHx affected the PL FAs profile in the spleen tissue as shown in Table 2. In the group fed a standard diet, the n-3 and MUFAs class changed statistically during PHx. Although SFAs and PUFAs did not change significantly, their fatty acids as 18:0, 20:0, 24:0, 20:5n-3 and 22:6n-3 were altered. However, when the splenic PL FAs profile was compared, marked changes were found in the liver previously as reported [29]. The main n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the spleen changed markedly during PHx and decreased on day 7 (Table 2). Furthermore, the PHx changed significantly: 18:0, 20:0, 24:0, as well as 16:1n7, 18:1n9 and 20:1n-9.

The n-3 PL FAs in the spleen negatively correlated with MUFAs in the group fed a standard diet and the FOO group (R=-0.63, R=-0.79, respectively), identically as with the liver [29].

Diets enriched with olive and corn oil changed significantly PL PUFAs in the spleen, as well as n-6 PUFAs during PHx. As shown in Table 1, a diet enriched with 5% FCO contains 55.09% of n-6 FAs, while a 5%-enriched FOO diet contains 55.63% MUFAs. The major FA in an FCO diet is linoleic acid (18:2n-6) at 55.09%, and oleic acid at 54.50% was the largest FAs in the 5%-enriched FOO diet. The application of the FOO diet increased 5.32-fold the n-9 FAs as compared to the standard diet, and 4.95-fold compared to the FCO diet.

Arachidonic acid (AA, 20:4n-6) was the largest in PUFA type in the spleen, ranging from 13.34 to 19.91% in the group FCO diet, and from 9.50 to 18.11% in the group FOO diet. In addition, it was observed that 20:4n-6 decreased in the FCO group during PHx, while it increased in the FOO group on day 7. The FCO diet is rich in 18:2n-6 FA, however, its intake increased 18:2n-6 PL FAs in spleen in the group without PHx. In addition, 18:2n-6 PL FA decreased significantly during PHx in the group of the FCO diet as well as in other groups with and without supplementation. However, although 18:1n-9 is the highest in the FOO diet, their increase in the splenic PL FAs was observed on the 2nd days and decreased on day 7 following initiation of PHx (Table 2). Palmitic acid (16:0) was practically unchanged during PHx in the group fed a standard diet, while the group FCO and the group FOO diet showed a decrease and then an increase, respectively. Stearic acid (18:0) showed a similar trend (see Table 2). More changes in PL FAs was found in the liver after consumption of all studied diets during PHx [29] (data in additional Table S2).

A hyperlipidic diet that contained saturated FAs induces more death in splenic lymphocytes *via* apoptosis than the diet that contained unsaturated FAs [40]. Oleic acid is recognized as a trigger for cell death in mammary epithelial cells [41], while palmitate is a trigger for cell death in macrophages [42-44]. Oleic acid also prevented apoptosis by induction with *t*10, *c*12-linoleic acid [45], reduced cell growth *via* stearic acid-induced inhibition and suppressed the pro-inflammatory responses [46] as well as decreasing lipid accumulation and apoptosis in cultured hepatocytes compared to palmitic acid [47]. However, recent studies indicate the important roles of lipids and lipid metabolism in both triggering and executing non-apoptotic regulated cell death [48].

The repair of the lipid membrane is essential for liver regeneration and is associated with apoptosis. Apoptotic cells and cellular components are not removed because they are probably used in the synthesis of lipids, the formation of double-membrane intracellular vesicles and in helping in stabilization or repair of damaged membranes through autophagy [48]. One relevant aspect of membrane proliferation during cell death is that phospholipids specific to different organelles may become intermixed within newly formed membrane structures such as vacuoles [48,49].

Dietary 18:2n-6 promotes cell apoptosis in the hepatocytes in rats fed palm oil compared to rats receiving palm oil alone [50], however, apoptosis was not observed if hepatoma cells were co-treated with palmitic acid [51]. Oleic acid promoted the formation of triglyceride-enriched lipid droplets that induced autophagy and had a minimal effect on apoptosis. Palmitic acid suppressed autophagy due to poor conversion into triglyceride-enriched lipid droplets and thus, significantly induced apoptosis [52]. That implies that diets supplemented with corn and olive oil have perhaps a different effect on autophagy, and thus on apoptosis. Given the different effect on the PL FAs in the spleen, and especially in the liver, these diets can differently affect liver regeneration.

For the betterment of knowledge of the potential benefits of dietary fatty acid composition, Svahn et al. [53] investigated the dietary effect on the transcriptome profile of some tissues including the spleen. They found that the greatest effect of a high-fat diet rich in PUFAs on tissue transcriptomics was observed in the spleen, while by

Table 2: Fatty acid composition (%) of the total phospholipids (PL) in the mice spleen in the group fed a standard diet, FCO and FOO diets on the 1st, 2nd and 7th day after partial hepatectomy (PHx).

Fatty acids	Control diet				FCO diet				FOO diet			
	Control	pHx			Control-FCO	pHx			Control-FOO	pHx		
		1d	2d	7d		1d	2d	7d		1d	2d	7d
18:2*n*-6	8.90±1.38	9.09±0.66	9.54±1.16	8.53±0.88	12.10±2.40*	10.55±1.23	10.56±0.55	8.63±1.50[c]	7.78±0.50	8.08±0.50	7.41±0.92	6.54±1.37
18:3*n*-6	0.73±0.10*	0.34±0.06	0.25±0.11	0.15±0.03[c,e]	0.61±0.12*	0.14±0.11	0.22±0.20	0.06±0.08[c]	1.00±0.25*	0.14±0.15	0.12±0.14	0.03±0.05[c]
20:2*n*-6	0.81±0.09	0.96±0.14	0.72±0.13	0.95±0.35	1.25±0.17	1.09±0.15	1.07±0.17	1.00±0.29	1.02±0.23	0.87±0.32	0.66±0.25	0.77±0.26
20:3*n*-6	0.86±0.09	0.73±0.36	0.55±0.20	0.65±0.21	0.56±0.04	0.70±0.15	0.48±0.19	0.72±0.45	0.73±0.03	0.53±0.20	0.50±0.27	0.54±0.24
20:4*n*-6	13.74±1.35	12.20±1.14	12.53±2.13	14.90±2.77	19.91±1.08*	15.96±1.01	13.34±3.57	14.90±0.93	11.91±1.52*	13.01±1.39	9.50±3.59	18.11±6.47[f]
Σ*n*-6	**25.03±2.21**	**23.32±1.63**	**23.60±3.17**	**25.19±2.69**	**28.42±2.73***	**28.43±0.72**	**25.68±2.82**	**25.32±1.99**	**22.44±1.29***	**22.62±0.92**	**18.19±4.34**	**25.99±5.61[f]**
20:5*n*-3	0.83±0.13*	1.41±0.64	0.99±0.28	0.40±0.11[e,f]	0.68±0.14*	0.68±0.10	0.45±0.10	0.71±0.51	0.84±0.19*	1.33±0.90	0.51±0.28	0.40±0.13[c,e]
22:6*n*-3	10.46±1.43*	10.14±0.77	10.08±3.19[b]	6.63±2.32[c,e]	9.89±1.50	10.24±0.49	9.34±3.54	7.53±2.87	8.81±1.20	10.16±1.44	6.14±3.38[d]	10.71±5.77
Σ*n*-3	**11.29±1.41***	**11.55±1.07**	**11.07±3.30**	**7.03±2.42[c,e]**	**10.57±1.59**	**10.92±0.50**	**9.79±3.58**	**8.23±2.56**	**9.66±1.33**	**11.49±1.74**	**6.65±3.63[d]**	**11.11±5.79**
Σ PUFA	**36.33±2.84**	**34.87±2.09**	**34.68±4.86**	**32.21±4.89**	**39.00±4.23***	**39.35±0.63**	**35.47±6.35**	**33.55±2.93[e]**	**32.10±1.30***	**34.11±1.95**	**24.84±7.94[d]**	**37.10±11.22**
14:0	0.49±0.08	0.65±0.17	0.56±0.16	0.71±0.12	0.47±0.08	0.61±0.16	0.58±0.22	0.74±0.05	0.51±0.14	0.74±0.11	0.75±0.33	0.60±0.20
16:0	33.86±2.26	35.65±0.60[a]	35.68±2.75[b]	37.75±4.11	32.39±3.46*	33.62±1.52	38.43±6.79	40.86±4.23[e]	34.42±3.95	34.09±2.61	37.96±4.69	31.53±12.52
18:0	14.03±1.70*	12.34±1.73	12.76±1.29	10.82±0.89[c]	13.35±0.81	12.71±0.55	12.22±1.12	11.75±1.76	13.34±2.55	13.00±1.26	13.12±1.45	13.62±4.21
20:0	0.54±0.26*	0.10±0.14[a]	0.14±0.11	0.06±0.03[c]	0.30±0.08*	0.11±0.07	0.10±0.05[b]	0.14±0.15	0.50±0.11*	0.10±0.10[a]	0.09±0.06[b]	0.12±0.06[c]
24:0	1.12±0.47*	0.42±0.07	0.35±0.10[b]	0.34±0.11[c]	0.47±0.18	0.41±0.18	0.50±0.19	0.55±0.23	0.78±0.66	0.46±0.16	0.91±1.04	0.75±1.65
Σ SFA	**50.04±2.58**	**49.17±1.98**	**49.57±4.12**	**49.68±3.60**	**46.98±3.86***	**47.47±1.47**	**52.06±6.08**	**54.04±4.36**	**49.55±2.76**	**48.38±1.93**	**52.83±6.40**	**46.61±9.23**
16:1*n*-7	1.63±0.46*	2.86±0.48	2.38±0.21	2.06±0.35	1.20±0.30*	1.25±0.25	1.43±0.39	0.19±0.37[e,f]	1.66±0.59	1.91±1.07	1.98±0.54	1.40±0.96
18:1*n*-9	12.00±0.49*	12.86±1.74	12.88±0.93	15.53±2.57[c]	12.73±0.97*	11.37±0.76	10.59±0.53[b]	11.60±1.33	16.68±2.17*	15.02±1.43	19.70±2.22[d]	14.22±2.04[f]
20:1*n*-9	0.00*	0.24±0.20	0.47±0.14	0.50±0.15[c]	0.09±0.19*	0.54±0.07[a]	0.42±0.10	0.58±0.07[c]	0.00*	0.51±0.06	0.63±0.15[b]	0.65±0.12[c]
Σ MUFA	**13.64±0.60***	**15.96±2.13**	**15.75±0.98**	**18.11±2.65[c]**	**14.02±0.68**	**13.18±0.93**	**12.47±0.65**	**12.42±1.60**	**18.35±2.19***	**17.50±0.40**	**22.32±2.34**	**16.29±2.66[f]**

Values are area per cent (mean±SD of 6-8 mice/group); *significant difference during PHx among same diet using *Kruskal-Wallis* Anova by Ranks test; [a]significant difference between the control and st day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [b]significant difference between the control and 2nd day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [c]significant difference between the control and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [d]significant difference between the 1st and 2nd day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [e]significant difference between the 1st day and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$); [f]significant difference between the 2nd and 7th day PHx using *Kruskal-Wallis* test: multiple comparisons of mean ranks for all groups ($P<0.05$).

contrast a high-fat diet rich in SFAs barely affected the liver where it exhibited a mixed response compared to a low-fat diet.

Soni et al. [15] investigated the effect of dietary n-3 FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the immune response regulation in the spleen. They suggested that EPA and DHA down-regulated the splenic immune response induced by a high-fat diet based on corn oil as well as that the spleen is a target organ for the anti-inflammatory effects of these n-3 fatty acids.

Fatty acid ratios in different tissues or lipid fractions are useful for the determination of the direction of a specific enzyme and thus their activity. A 20:4/22:6 ratio greater than 18:2/20:4 on the 7th day achieved higher AA in the spleen as shown in Tables 2 and S1. Similarly, changes in the n-3/n-6 ratio indicate an unbalanced metabolism of unsaturated FAs, therefore a lower n-3/n-6 ratio can be associated with the higher 20:4n-6, as found on day 7 in the spleen. In addition, a significant negative correlation was found between the n-9/n-6 ratio and the n-3 PUFAs in the spleen for the standard control diet group (R=-0.57) and the FCO group (R=-0.82).

The homeostasis of PUFA was under control of diet intake and hepatic metabolism. The key enzymes in the PUFA synthesis are the delta-5 desaturase (D5D, FADS1), delta-6 desaturases (D6D, FADS2), fatty acid elongases-2 (Elovl2) and 5 (Elovl5). Fatty acid synthase (FASN), stearoyl CoA desaturase 1 (SCD1) and fatty acid elongase-6 (Elovl6) catalysed *de novo* lipogenesis and MUFA synthesis [54] (see FAs pathways in Figure 1). Fatty acid synthase (FASN) is the central enzyme of the *de novo* fatty acid biosynthesis pathway.

The Elovl6 and SCD1 ratios significantly correlated in the spleen in the FCO and FOO groups (R=0.83 and R=0.72, respectively), as in the liver [29]. Analogous to this, the Elovl6 activity correlated with n-3 and n-6 PL FAs in the spleen only in the group FCO (R=0.83, R=0.70, respectively), while in the liver, the positive significant correlation found in the group fed a standard diet was between Elovl6 and n-3 PL FAs and Elovl6 and n-6 PL FAs in the group FCO.

The activity of desaturases D5D and SCD1 was also higher in the spleen than in the liver in the FCO group and the FOO group. As shown Figures 3A and 3C, the FCO and FOO diets induced significant changes in spleen desaturase and elongase activity. In the spleen and the liver, the activities of D5D and D6D increased during PHx. Thus, an FCO-enriched diet activated more D5D and inhibited D6D in the spleen on the 2nd day after PHx. In comparison to the aforementioned results, hepatic D6D decreased in the liver on the 1st day. Then, splenic D5D decreased more in the group fed FOO diets on the 2nd day after PHx, while D6D remained almost unchanged. Similarly, the hepatic D6D was inhibited on the 1st day.

It is necessary to point out that it the highest activity of Elovl6 was in the liver in the group fed with standard diet (Figure 3D) where the Elovl6 ratio was more than 3-fold higher than that of the FCO and FOO groups. As opposed to the liver, no significant changes were found in the activity of Elovl6 in the spleen in the group fed on a standard diet (Figure 3C). However, significant changes were found in the activity of Elovl6 during PHx in the spleen in the groups FCO and FOO (Figure 3C).

The SCD1 activity decreased in the spleen during PHx in the group fed an FCO diet. In comparison to this, an olive oil-enriched diet increased the activity of SCD1: in the spleen peaked on the 2nd day and in the liver on the day 7 after PHx (Figure 3C). In all administered diets, SCD1 showed maximal activity on the 7th day after PHx in the liver (Figure 3D).

Recently, nutritional and tissue-specific regulation of elongase expression has been examined [55]. Chen et al. [56] observed significantly high transcript levels of bovine Elovl6 in adipose tissue and intestines and moderate levels in the lungs, stomach, spleen, kidney, liver and heart, and the lowest levels in the skeletal muscles among the tissues investigated. Some reports have demonstrated that Elovl6 is highly expressed in brown adipose tissue (BAT). Since BAT has the highest mitochondrial density of all tissues, it was proposed that Elov6 is a regulator of thermogenesis in the BAT [57]. Tan et al. [58] connected with the lipid composition of the adipose tissue upon development of obesity and the metabolic dysfunction of obesity, while the mechanism of incorporation of the majority of FAs into adipose tissue in the fed state was examined via the Elovl6, SCD1 and DNL indices. The oleate rich diet strongly suppressed fatty acid synthase (FAS), Elovl6 and SCD1 in the liver. These authors concluded that the oleate-rich diets reduced the expression of the mRNA expression of Elovl6 and SCD1 in the liver [58]. Elongase-5 (Elovl5) plays a key role in MUFA and PUFA synthesis and regulates hepatic triglyceride catabolism in obese C57BL/6J mice as demonstrated by Tripathy et al. [59]. Authors suggested Elovl5 as the target for the treatment of diet-induced hyperglycemia [60].

Our results confirm the transcriptomic results reported as showing a higher expression of Elovl6 in the liver as compared to that of the spleen.

If SCD1 is overexpressed, then it can increase intracellular MUFA and can lead to lipid accumulation in the liver. However, a suppressed SCD1 should decrease oleic acid and affect triacylglycerol (TAG) accumulation. That could suggest SCD1 as a target for cell stress response and induced apoptosis. A significant positive correlation was found here between SCD1 ratio and MUFA ($R_{Control}$=0.89, R_{FCO}=0.96, R_{FOO}=0.81), and SCD1 ratio and 18:1n-9 ($R_{Control}$=0.86, R_{FCO}=0.69, R_{FOO}=0.75) in the spleen in all diet groups.

In the study by Wang et al. [61], authors proposed the mechanism underlying FADS1 and its polymorphisms in modulating hepatic lipid deposition by altering gene transcription and controlling lipid composition in human livers. This work highlights a multilevel integrated omics (systems biology) approach to better understand causal mechanisms behind the genotype-phenotype associations as a perspective in improving or preventing hepatic fat accumulation and/or other metabolic comorbidities.

During ageing, the membrane fatty acid profile changes with increased PUFAs. The liver and spleen in rodents are long-lived organs with a high degree of unsaturation (approximately 50% and 60%, respectively) and a different contribution of PUFA. The spleen has a lower peroxidability index (PI) and double bond index (DBI) compared with the liver.

Both administered oil-enriched diets significantly changed unsaturation and peroxidizability during PHx in the spleen as determined *via* the DBI and PI indices (Table S1).With the intake of the FCO enriched diet, DBI and PI decreased during PHx, however, intake of an FOO diet decreased DBI and PI on the 1st and 2nd days after PHx and then increased on the 7th day. These parameters significantly correlated with n-3 PUFAs (R=0.86, R=0.88, R=0.93, respectively) in the spleen in the group fed on a standard diet, in the FCO group (R=0.96, R=0.97, R=0.97, respectively) and in the group FOO (R=0.90, R=0.94, R=0.96, respectively) (Tables S1 and S2). As reported previously, the liver showed a lower correlation between n-3 PUFAs and ACL, DBI and PI in all diet groups (Table S3) [29].

The membrane FAs profile is a criterion of the resistance to lipid peroxidation in the prediction of their longevities. The study Arranz et al. [62] used female BALB/c mice which were divided into three groups: adult (28 weeks), old (76 weeks) and exceptionally old (128 weeks). Membrane fatty acids composition were analyzed and the results showed significantly lower PI and lipoxidation-derived protein damage in the brain and the spleen in the group of exceptionally old animals as compared with old animals and adult animals. However, the adaptation of membrane unsaturation of each cell, tissue and organ and organism to ageing, depending on surgical damage, diseases, recovery, possible dietary intervention, etc., will be opened with new research.

Conclusion

The lipid metabolism is fundamental to the understanding of a number of human diseases and conditions. Therefore, fatty acid metabolism is an inevitable participant in a network of biological processes available for cellular metabolism. An integrative approach involving transcript identification, protein and lipid analysis in order to the betterment of understanding the physiological role of lipid metabolism in mammals as a biochemical system.

Here, we focused on fatty acid composition in polar lipids in the spleen and its response a) to stress induced by PHx, b) to the involvement of dietary fats in the modulation of phospholipid fatty acids.

The most abundant PL FA in the mice spleen was palmitic acid (16:0) as SFA, arachidonic acid (20:4n-6) as n-6 PUFA, docosahexaenoic acid (22:6n-3) as n-3 PUFA and oleic acid (18:1n-9) as n-9 MUFA, but only 22:6n-3 and 18:1n-9 was changed in the spleen during PHx.

A diet supplemented with 5% corn oil, rich in 18:2n-6 FA reduced 18:2n-6, pro-inflammatory abundant 20:4n-6 and increased endogenous palmitic acid in the spleen. In addition, a diet supplemented with 5% olive oil and rich in 18:1n-9, enhanced 20:4n-6 and reduced 18:1n-9. If the amount of dietary 18:2n-6 is low, desaturation is directed to 18:1n-9 (due to the high amount in the diet with olive oil).

These results confirmed the importance of diet in stress conditions such as liver damage, not only in the liver but also in others organs such as the spleen. In conclusion, despite the beneficial effect of diet, lipid homeostasis was regulated more by liver regeneration than a n-6 and n-9 diet after one-third PHx.

The one-third PHx serves as a model for studying the regulation of PL FAs not only in the liver but also in the spleen.

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