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# Effect of Olive Oil on Brain's Lipid and Calcium Content after Partial Hepatectomy in Mice

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## ABSTRACT

*The aim of this study was to investigate the influence of olive oil (OO) enriched diet on the lipid content of mice brain during the early phase of liver regeneration and to test a relationship of these changes with calcium content. C57Bl mice were fed over 21 days with diet enriched with olive oil, containing predominantly oleic acid (18:1n-9). The animals were one-third partially hepatectomised (pHx) under aether anaesthesia. Total lipids were extracted from tissue samples with a chloroform-methanol (2:1, v/v) mixture according to Folch et al. Mineral concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after microwave brain tissue digestion. The diet containing OO increased both total lipid content and the calcium concentration in brain during the early phase of liver regeneration (12hrs post pHx), suggesting that monounsaturated oleic acid might interact with some metal-dependent activities that control changes in the brain during liver regeneration.*

**Key words:** partial hepatectomy, diet, olive oil, total lipids, calcium, brain

## Introduction

Liver regeneration that follows after partial hepatectomy (pHx) is a commonly used model for investigation of growth processes, during which the liver architecture remains intact and hepatocyte replication occurs in normal parenchyma<sup>1-3</sup>. The process of regeneration lasts from 5 to 7 days<sup>4</sup>.

The synaptic plasma membrane is composed of proteins, cholesterol and glycerolipids, as is the plasma membrane of all cells. However, the synaptic plasma membrane is unique in that it contains phospholipids (PL) highly enriched in polyunsaturated fatty acids (PUFA), particularly arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3). The synaptic plasma membrane participates in frequent exocytotic events, and PUFA may facilitate these processes by conferring a high degree of membrane fluidity<sup>5</sup>. The lipids of excitable membranes are metabolically active. Alterations in the content and composition of lipids may modulate the release, uptake, and postsynaptic effects of neurotransmitters<sup>5</sup>. AA and its oxygenated metabolites may act as regulators of neurotransmitter release and second messengers for the postsynaptic actions of neurotransmitters<sup>5,6</sup>.

Stress can cause selective alterations in brain lipids metabolism, resulting in an accumulation of free fatty acids (FFA), diacylglycerols, and the oxygenated products of AA, prostaglandins, and leukotrienes. Early and reversible alterations in lipid metabolism may reflect an overstimulation of the metabolic pathways that normally operate during neurotransmission, such as the remodeling of the fatty acid (FA) composition of membrane glycerolipids and the production from membrane lipids of second messengers, like diacylglycerols, inositol-1,4,5-tris-phosphate, and eicosanoids. Subsequent pathological changes in membrane lipids are likely due to the generalized breakdown of cell membranes, perhaps triggered by an overstimulation of the normal metabolic pathways or by the production of highly reactive free radicals<sup>5</sup>.

Nerve cell death from oxidative stress has been implicated in a variety of pathologies, including stroke, trauma, and diseases such as Alzheimer's and Parkinson's disease. An understanding of the changes in brain lipid metabolism after partial hepatectomy, will provide us with important insight into the role membrane lipids play in the fundamental operation of the central nervous system.

Evidence indicates that feeding diets differing in FA composition can induce physiological changes in the membrane function, involving the activity of enzymes, hormone-activated functions and the expression of activity in the nucleus<sup>7</sup>. Despite the ability of the human body to synthesize FA necessary for cell structures, some FA, i.e. linoleic (LA, 18:2n-6), and linolenic (LNA, 18:3n-3), are essential<sup>8</sup>. Subsequently, many studies revealed that these FAs function as constitutive elements, as well as precursors for other long chain PUFA and their derivatives. Broadhurst and Cunnane<sup>9</sup> even set out the idea that food rich in DHA provides brain-specific nutrition and plays a significant role in human brain evolution. Furthermore, Cunnane<sup>10</sup> suggests the reconsideration of the term »essential fatty acids«, originally used for LA and LNA, into the term conditionally-indispensable, with the aim to improve the understanding of the function and metabolism of polyunsaturated long-chain FA and their dietary essentiality throughout the whole life<sup>11,12</sup>.

Monounsaturated oil (MUFA-diet) such as virgin olive oil (OO) and polyunsaturated oil (PUFA-diet) such as corn oil are suggested to have selective physiological effects on membrane in tissues. Changes in the lipids composition of the liver, kidney and submandibular gland of mice, fed with oils of various content, and the role of vegetable oils in the processes of regeneration and reparation of tissue, were already determined with our previous research<sup>12–14</sup>. Also, the mineral content (zinc, iron, magnesium, and calcium) in the liver, spleen, thymus, and submandibular gland of mice fed with vegetable oils enriched diet was analysed<sup>12,15</sup>.

Trace elements such as zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe), and copper (Cu), are essential oligo-elements and play an important role in biological processes and enzyme reactions, including cell proliferation<sup>16</sup>. They also interfere with the metabolism of lipids, specifically fatty acids.

Mounting evidences support the idea that endogenous biometals, such as copper, iron, zinc and exogenous ones such as aluminium, can be involved as factors or cofactors in the etiopathogenesis of a variety of neurodegenerative diseases<sup>17</sup>. Metal ions, as well as proteins, lipids, nucleic acids, carbohydrates and vitamins, are essential to life<sup>18</sup>.

Calcium ions are essential for a wide variety of extracellular processes as well as intracellular processes<sup>19</sup>. In all types of cells, the calcium ion ( $\text{Ca}^{2+}$ ) is an important second messenger for intracellular signal transduction, while a high concentration of free  $\text{Ca}^{2+}$  can also be a cellular toxin as has been demonstrated in ischemic brain damage. The intracellular concentration of free  $\text{Ca}^{2+}$  is, therefore, precisely controlled at a low level, and each cell/tissue/organ can express its function such as secretion and contraction, leading to memory, movement, pain, etc<sup>20</sup>. Many enzymes require calcium for their activity<sup>16</sup>. In human platelets, oleic acid and PUFA linolenic acid stimulate  $\text{Ca}^{2+}$  release from intracellular stores<sup>21</sup>.

The aim of this study was to examine the influence of OO enriched diet and 1/3 pHx on tissue total lipid con-

centration and calcium concentration in the brain from mice during the early phase of liver regeneration.

## Material and Methods

### Animals

Wild type C57Bl male mice aged 8–10 weeks were used in the experiment. They were housed in groups of six to eight animals, kept under standard conditions and exposed to a natural day-night cycle. Animals were bred and maintained according to the »Guide for Care and Use of Laboratory Animals« (NIH, 1996). The study was approved by the School of Medicine, University of Rijeka Ethic's committee.

### Study design/Partial hepatectomy

Under ether anesthesia, mice were subjected to one-third partial hepatectomy (pHx) by removal of the median liver lobe modified according to Higgins and Anderson<sup>22</sup>. To avoid possible diurnal variability, all operations were performed between 8:00–9:00 a.m. Animals were sacrificed by bleeding on hours 6<sup>th</sup>, 12<sup>th</sup>, 24<sup>th</sup> and 48<sup>th</sup> after surgery (pHx) (Figure 1). The brain of mice sacrificed by exsanguinations was carefully removed using plastic instrumentation, washed with saline solution to remove blood, weighed, frozen in liquid nitrogen and stored at  $-75^{\circ}\text{C}$  for metal and lipid analysis.

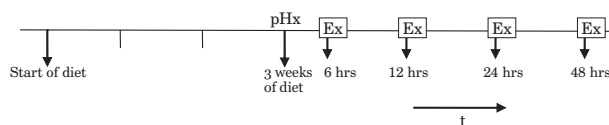


Fig. 1. Time scale of the experiment. pHx – partial hepatectomy; Ex – sacrifice.

### Feeding procedure

The animals were divided into two treatment groups, different due to types of diet. During the three research weeks animals were fed standard laboratory pellet (FSP, Control) or diets enriched with OO (5 g addition to 100 g of standard pellet), containing predominantly oleic acid (18:1n-9) (FOO). All diets were stored at  $20^{\circ}\text{C}$  and prepared weekly. Animals continued to receive the same diets until they were sacrificed. Body weight was recorded daily. Distribution of animals to experimental groups is shown in Table 1.

The extra virgin OO sample (acidity < 1%) was collected from an individual producer from the Island of Krk, Croatia and stored at  $4^{\circ}\text{C}$  in the dark until analysis. The fatty acid composition of the OO is determined by gas chromatography (GC). The fatty acid composition of the OO is shown in Table 2.

### Lipid analysis

Total lipids were extracted from the brain tissue samples by the modified method of Folch et al.<sup>23</sup> Samples

**TABLE 1**  
DISTRIBUTION OF ANIMALS TO EXPERIMENTAL GROUPS

Group	Control INT – FSP	Control INT – FOO	Experimental pHx – FSP	Experimental pHx – FOO
Treatment	Standard diet	Standard diet + olive oil	Standard diet, sacrificed 6,12,24, 48 hours after pHx	Standard diet + olive oil, sacrificed 6,12,24, 48 hours after pHx

N=6 per group; INT – intact liver; pHx – partial hepatectomy; FSP – fed with standard pellet; FOO – fed with standard pellet + 5% olive oil

**TABLE 2**  
FATTY ACID COMPOSITION OF OLIVE OIL

Fatty acid w/%	
Saturated	14,81
16:0	11,47
17:0	0,13
18:0	2,26
20:0	0,93
22:0	0,33
24:0	trace
Unsaturated	85,19
16:1 n-9	1,61
18:1 n-9	72,74
18:2 n-6	9,60
18:3 n-3	0,61
20:1	0,31
22:1	trace
Total	100,00

were weighed (150–200 mg) and homogenized in a chloroform-methanol (2:1, v/v) mixture (Kinematica AG, Polytron PT 1600, three times for 3 min at 5000 rpm). During the extraction procedure, lipids were protected against oxidation by adding 50 mg/L the antioxidant butylated hydroxytoluene (BHT) to the solvents. Homogenates were left two hours at 4°C. After filtration 0.034% MgCl<sub>2</sub> aqueous solution was added. The mixture was well mixed and left at 4°C overnight. Water and organic phases were separated and the lower, organic phase was evaporated to dryness under N<sub>2</sub> gas. Total lipid extract was weighed and dissolved in 1 mL of the chloroform-methanol (2:1, v/v) mixture. All reagents were from Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia) and were of the highest purity available.

#### Mineral determination

Trace elements concentrations were determined in the brain by inductively coupled plasma atomic emission spectroscopy (ICP-AES)<sup>24</sup>, after microwave brain tissue digestion<sup>25</sup> by HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> according to the method a previously described<sup>26</sup>. Measurements were performed using a ICP-AES Spectrometer (Prodigy Leeman Labs. Inc.), at a fixed wavelength of 315.887 nm for calcium. The microwave digestion was performed in the MLS 1200 Mega Microwave Digestion System (Milestone, Italy) with MDR technology.

#### Statistical analysis

Statistical analysis was performed using Statistica software. The nonparametric Mann-Whitney U-test was used to assess significant differences between the groups and diets. Statistical significance was assessed at  $p < 0.05$  and data are reported as  $X \pm SD$ .

#### Results

The FA composition of the OO is shown in Table 2. Analysis of the dietary extra virgin OO shows that it mainly contains oleic acid, 18:1n-9 (72.74%).

The body weight, brain weight and total lipids weight, isolated from the brain tissue, have been measured.

#### Body and brain weights

Body weight of the mice has been recorded daily. Mice in all experimental groups had similar food intake. Animals fed different experimental diets increased comparable amounts of weight during the experimental period, but there were no statistical differences in weight between the experimental groups (data not shown).

The brain of the animals fed the standard diet (FSP) and ones enriched with extra virgin olive oil diet (FOO) have been weighted. Figure 2. shows changes in wet weight of the brain in partially hepatectomised mice fed different diets for 3 weeks before pHx.

The weight of the brain in mice regenerating liver model showed a significant decrease 12 h after pHx when compared to the control group (FSP) but later it increased again to the earlier value (Figure 2a).

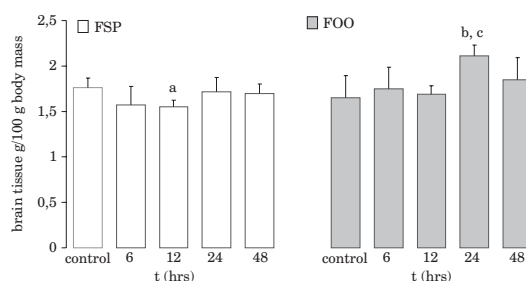


Fig. 2. Changes in wet weight of the brain in partially hepatectomized (pHx) mice. Data are  $X \pm SD$  of 6 mice. <sup>a</sup> $p < 0.05$ : significantly different from control group (FSP); <sup>b</sup> $p < 0.05$ : significantly different from control group (FOO); <sup>c</sup> $p < 0.05$ : 24hrs after pHx-FOO group, significantly different from 24hrs after pHx-FSP group. pHx – partial hepatectomy; FSP – standard pellet; FOO – diet enriched with 5% olive oil.

The weight of the brains in mice fed with food enriched OO showed significant increase 24 h after pHx when compared to the control group (FOO), but 48h after pHx it decreased again (Figure 2b).

The results showed significant differences in weight of the brain between the groups FOO and FSP 24 h after pHx (Figure 2c). The weight of the brain was significantly higher in mice fed with olive oil-enriched diet 24 h after pHx, in comparison with brain of mice fed with standard diet (laboratory pellet).

### Changes in tissue lipid content in the brain induced by pHx and by diet

Figure 3. shows the changes in total lipid content in the brain of mice fed with standard laboratory pellet induced by liver regeneration. The total lipid level in brain was significantly higher 6h and 48h after pHx, and significantly lower 12h after pHx, in comparison with the control group (INT-FSP).

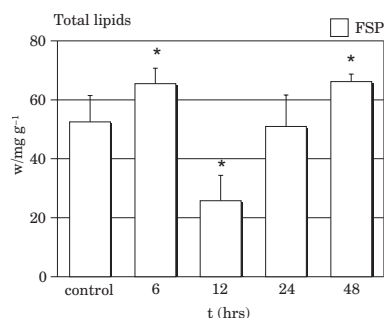


Fig. 3. Changes in tissue lipid content in the brain induced by liver regeneration and standard diet. Each column represents  $X \pm SD$ ;  $N=6$ . \*  $p < 0.05$ : significantly different from control-FSP group. TL – total lipids; pHx – partial hepatectomy.

Figure 4. shows the changes in tissue lipid content in the brain induced by diet. The total lipid level in the brain of mice fed with olive oil-enriched diet were significantly higher 6h and 48h after pHx, in comparison with the control group (INT-FOO).

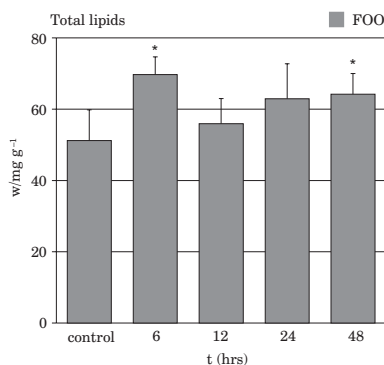


Fig. 4. Changes in tissue lipid content in the brain induced by olive oil enriched diet and pHx. Each column represents  $X \pm SD$ ;  $N=6$ . \*  $p < 0.05$ : significantly different from control-FOO group. TL – total lipids; pHx – partial hepatectomy.

Significant differences were found in the total lipid content between the FOO and FSP groups 12h after pHx (Figure 5). The total lipid levels were significantly higher 12h after pHx in mice fed olive oil-enriched diet (FOO-group) than in the FSP (mice fed with standard diet).

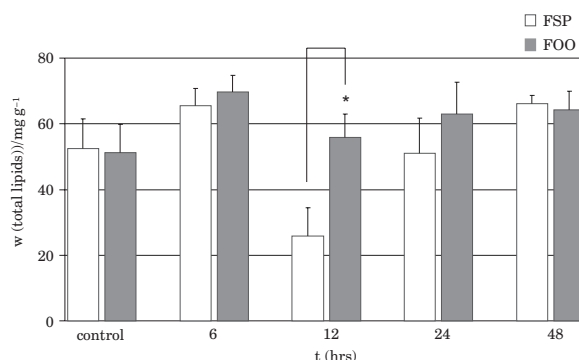


Fig. 5. Changes in tissue lipid content in the brain induced by pHx and by diet: the effect of olive oil enriched diet. Data are  $X \pm SD$  of 6 mice; \*  $p < 0.05$ : comparison between the FOO and FSP fed groups; significantly different between pHx12 hour groups. TL – total lipids; pHx – partial hepatectomy; FSP – standard pellet; FOO – diet enriched with olive oil (5% addition to standard pellet).

### Changes in tissue dynamics of calcium in the brain induced by pHx and by diets

Calcium content in the brain tissue of mice fed the standard diet (FSP) or diets enriched with olive oil (FOO) for 3 weeks before pHx, is shown in Figure 6. In mice fed with standard diet the concentration of  $Ca^{2+}$  decreased (after 24 h) and accumulated (after 48 h) in the brain during the early phase of liver regeneration, compared to the control group. These changes were, however, significantly less expressed in mice fed OO enriched diets, where there is no significant change in the concentration of  $Ca^{2+}$  during liver regeneration. We also found no significant differences in concentration of  $Ca^{2+}$ , between the research groups FOO and FSP.

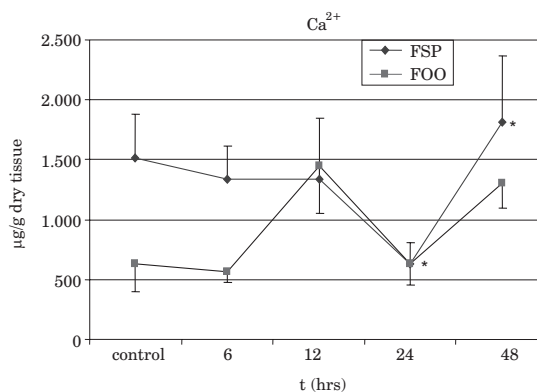


Fig. 6. Concentration of  $Ca^{2+}$  in the brain tissue of pHx mice fed the standard diet or diet enriched with 5% olive oil. Data are  $X \pm SD$ . \*  $p < 0.05$  (compared to the control-FSP group).

## Discussion and Conclusions

Lipid metabolism is very complex and can be affected by many parameters such as diet, oxidative stress, drugs, etc. PL are major structural components of cells and intracellular membranes in all living organisms. Dietary induced changes in membranes are recognized as functionally important for a specific role of membrane lipids in modulating membrane function. Nutritional balance between different FA is a very important objective, but this balance is difficult to achieve because FAs are numerous and their physiological functions are multiple and complex. Imbalanced essential FA ratio has led to an increase of various metabolic disorders affected by diets or diseases.

Weber and co-workers have made the modulation of brain lipids of rats by various dietary oils: sunflower, high-oleic sunflower, olive, rapeseed and coriander oil<sup>27</sup>. The data presented by Weber indicate that rapeseed oil and OO may have beneficial effects on the prevention of stroke by increasing the proportions of DHA in the membrane lipids of brain. Coriander oil, on the other hand, might be useful for modulating the level of AA in lipids of cerebral membranes in specific conditions of health and disease<sup>27</sup>.

Popović and co-workers have been investigating changes in the FA composition of different lipid classes induced by specific diets<sup>28,29</sup>. The most commonly advised dietary intervention for protection against cardiovascular disease is a low or modified fat diet. However, such interventions may have a variety of effects, both positive and negative, on other specific risk factors<sup>30</sup>. Delaš and co-workers have evaluated the impact of fat-free diet (FFD) on the FA composition of PL in the brain and liver of rats, and their results showed that short-term FFD caused minor changes in the FA composition of brain PL<sup>30</sup>. As expected, the FA composition of brain PL was even more resistant to disturbances in dietary fats because of the blood-brain barrier. However, a developing brain is much more vulnerable under nutritional insufficiency. It is well known that mother's nutrition during pregnancy strongly influences the brain PL composition and thus brain function<sup>31,32</sup>. However, a number of studies indicate that some neurodegenerative diseases are accompanied by disturbances in FA composition, mostly in glycerophosphatides. Fenton et al. reviewed clinical research on abnormalities in membrane FA composition and therapeutic trials of FA in schizophrenia<sup>33</sup>. Very interesting observations come from epidemiological studies, which underline the strong correlation between cholesterol lowering diets and increased incidence of suicides, murders and accidents<sup>34</sup>. According to Hibbeln and Salem Jr., this could be the result of n-3 PUFA deficiency as a consequence of low fat/cholesterol lowering diets, applied in prevention of coronary heart disease<sup>35</sup>. This correlation between dietary n-3 FA deficiency and psychiatry is nowadays generally accepted and well documented<sup>36,37</sup>.

In our previous work we have investigated the PL fatty acid profile and dietary FA effect in the liver, lung, spleen, and submandibular gland tissues in mice<sup>13,38</sup>. Presented data support the hypothesis that corn oil diet induced changes increase LA, and OO increases the oleic acid (18:1n-9) content in total PL fatty acid composition in the majority of the examined tissues. Liver tissue was most affected by the applied diet<sup>13</sup>. The tissue FA profile plays an important role before and after induced oxidative stress<sup>13</sup>. Diet intervention modified this profile, and in this way reduced dysfunctions in the eicosanoid metabolism and disorders such as cardiovascular and gastrointestinal diseases as well as in incidence of cancer<sup>13</sup>.

Many authors agree with the fact that the OO is the main source of fat in the Mediterranean regions<sup>39,40</sup>. The alleged beneficial effects of extra virgin olive oil have been linked to both its MUFA (oleic acid) and its antioxidant components, e.g., hydroxytyrosol and oleuropein, most of which are phenolic in nature<sup>41</sup>. OO is known for its many positive effects on the human health. The results of Visioli's research show that OO phenolics exert in vitro and in vivo antioxidant and potentially cardioprotective activities<sup>42–44</sup>. It has been suggested that OO has a positive effect on the prevention of a variety of chemically induced tumors<sup>45</sup>.

Our present study was designed to investigate the effect of dietary lipids (OO) on lipid content and calcium concentration in the brain of mice regenerating liver.

Marked changes in mineral tissue dynamics can be observed in regenerating liver but also in the thymus, spleen and submandibular gland (SMG). This points to regulatory effect of minerals in the highly interconnected network of cytokine and growth factor-regulated pathways activated during liver compensatory growth. The calcium concentration is influenced by both olive oil diet and partial hepatectomy, as we have previously described in our group<sup>38,12</sup>. Mice fed olive oil diet had higher calcium concentration in liver, spleen and submandibular gland. Furthermore, after corn oil diet, calcium concentration was higher in liver probably via arachidonic acid and its lipooxygenase formed metabolites which can facilitate Ca influx through calcium channels<sup>46</sup>. In the same set of experiments, spleen calcium concentration was higher after olive oil diet which is consistent with increased lymphocyte calcium uptake as found by Peck et al.<sup>47</sup>. The increased spleen calcium concentration in the early phase of liver regeneration suggests its key role in neuro-immunoregulatory network. On the other hand, in this set of experiments, the brain calcium concentration changed significantly despite the existence of fine mechanism preserving the concentration of calcium outside the brain in much higher range than inside of the brain so that the cells do not suffer from possible chronic activation. Mice fed with olive oil had lower calcium concentration in the brain in comparison to control group with standard mice diet, which differed from the liver<sup>38</sup>. After partial hepatectomy both brain's calcium and total lipid content increased in early phase of hepatectomy showing the possible protective role of olive oil in light of

liver regeneration process. Even though the liver regeneration process lasts for seven days, we have found that the brain calcium concentration did return to its initial values 48 hours after pHx. Therefore, we have expected the changes in the brain calcium content only in early period of liver regeneration.

In conclusion, the obtained results indicate that food enriched with olive oil influences the lipid content in the brain of mice regenerating liver.

Partial hepatectomy influences the concentration of calcium in the brain and it changes significantly during the early phase of liver compensatory growth.

Our results suggest that some specific signals transmitted during the liver regenerative process have induced alterations in the lipid content and changes in calcium concentration in the brain, which can be modified by the diet.

The brain tissue phospholipids' content can be affected by different diet, oxidative stress, drugs, etc. Chan-

ges in lipid content are of special importance because of possibility for a membrane function modulation. Further experiments are needed to assess these changes and to obtain better insight to complex neuro-immunoregulatory network.

Our future studies will be aimed to determine the brain tissue-specific phospholipid profile and the effect of the dietary lipid source on possible modification in the total phospholipid fatty acid content.

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## **UTJECAJ PREHRANE MASLINOVIM ULJEM NA SADRŽAJ LIPIDA I KALCIJA MOZGA U MIŠEVA NAKON PARCIJALNE HEPATEKTOMIJE**

### **S A Ž E T A K**

Cilj ovoga istraživanja bio je ispitati utjecaj prehrane uz dodatak maslinovoga ulja na lipide mozga u miševa podvrnutih parcijalnoj hepatektomiji, te ga povezati s promjenama u koncentraciji kalcija. C57/Bl miševi hranjeni su 21 dan hranom obogaćenom maslinovim uljem (5%, w/w) u kojem prevladava oleinska kiselina (18:1n-9). Eksperimentalne životinje anestezirane su eterom te podvrgnute 1/3 parcijalnoj hepatektomiji (pHx). Ukupni lipidi ekstrahirani su iz tkiva mozga u sustavu otapala kloroform-metanol (2:1 v/v) prema modificiranoj Folchovoj metodi. Koncentracija metala u mozgu određena je primjenom atomske emisijske spektroskopije uz induktivno spregnutu plazmu (ICP-AES), nakon što je tkivo razčinjeno mikrovalnom digestijom. Utvrđeno je da hrana obogaćena maslinovim uljem povećava udio ukupnih lipida, te koncentraciju kalcija u mozgu tijekom rane faze regeneracije jetre što upućuje na interakciju mononezasićene oleinske kiseline s metabolizmom metala u procesu regeneracije.