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Changes in Tissue Fatty Acid Composition Due to a Fat Free Diet

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Summary

Linoleic (C18:2, n-6) and linolenic (C18:3, n-3) acids cannot be synthesized in the organism and are therefore essential components of the diet. Fat free diets (FFD), which contain less than 1 % of total energy content as fats, are used as therapy in some gastrointestinal diseases and lipid metabolism disorders. Because of the lack of the essential fatty acids (EFA), fat free diets can induce symptoms of essential fatty acids deficiency.

In this study, we have investigated changes in fatty acid composition of total lipids of brain, heart and liver, as well as of erythrocyte membranes, induced by FFD fed to rats for two weeks. Control and FFD were isoenergetic, with the lipid portion substituted by carbohydrates. The most pronounced differences were found in liver tissue, while in other tissues modifications were of a lesser degree, but still evident.

As a consequence of a FFD, total lipids contained less linoleic acid, as well as other long chain polyunsaturated fatty acids (arachidonic, C20:4,n-6; eicosapentaenoic, C20:5,n-3; docosahexaenoic, C22:6,n-3), with the consecutive increase of endogenously synthesized monoenoic acids (palmitoleic, C16:1,n-9 and oleic, C18:1,n-9). In this way, requested ratio of saturated to unsaturated fatty acids is preserved, what is of special importance for the composition of phospholipids, responsible for the structure and function of cell membranes. The results express the ability of cells to compensate the lack of EFA in the diet by endogenous synthesis, »tendering« the effect of insufficient ingestion of fats and EFA, respectively.

Key words: fat free diet, essential fatty acid, brain, heart, liver, erythrocyte, rat

Introduction

Nutrition is the process of food utilization by living organisms. Optimal nutrition should fulfill daily energetic requirements, as well as essential components.

Despite the ability of human organism to synthesize fatty acids, linoleic (C18:2, n-6) and linolenic (C18:3, n-3) acids have been found to be essential, as originally reported by Burr and Burr (*1*) and later by Holman *et al.*(*2*). These essential fatty acids (EFA) are needed for maintaining the function and integrity of membrane structure, for fat metabolism and transport, and for the synthesis of prostaglandins. The most characteristic symptom of EFA deficiency is a scaly dermatitis. Although EFA deficiency is very rare, it is being seen primarily in low-birth-weight infants on artificial formulas lacking EFA and in hospitalized patients maintained on total parenteral nutrition for long periods of time.

On the other hand, recent studies suggest that high-fat intakes are associated with increased risk of colon, breast and prostate cancer (*3*). Although it is not yet certain whether the cancer risk is associated with fat intake *per se* or with the excess calories associated with a high-fat diet, there is a growing number of persons who avoid fat consumption. If not controlled, such conditions can easily result in EFA deficiency.

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As demonstrated by Wene *et al.* (*4*), the analysis of fatty acid composition in blood serum of persons on fat free diet (FFD) showed changes as soon as after ten days from the introduction of the diet. In such cases, Judd *et al.* (*5*) found a decrease of EFA content, as well as other long chain polyunsaturated fatty acids (PUFA). Investigating the fraction of fatty acids in animal tissues, a decrease of EFA was followed by the increase in eicosatrienoic acid (C20:3, n-9), as published by Bourre *et al.* (*6*).

Studies carried out in our laboratory by Popović *et al.* (*7*) and Steiner-Biočić *et al.* (*8*) with animals fed a short term (2 weeks) on fat free diet confirmed such effects. Total lipids of liver and lungs of rats fed FFD contained more palmitoleic (C16:1, n-9) and oleic (C18:1, n-9) acids relative to control animals.

We have extended these investigations to other tissues with the aim to compare fatty acid compositions of total lipids (TL) from brain, heart, liver and erythrocytes of the animals on FFD and control diet (CD).

Materials and Methods

Animals

Male Wistar rats, two months old, mean body weight 230 g, were divided into two groups, six animals each. They were fed *ad libitum* either with a fat free diet (FFD) prepared in our Laboratory according to the model of Iritani and Narita (*9*), or standard chow diet (Pliva, Zagreb). Detailed diet compositions are presented in both Table 1 and 2. After two weeks animals were sacrificed by abdominal vein or heart puncture in Ketalar anesthesia. Brain, heart and liver were removed, rinsed with cold saline, weighed and frozen. Blood samples were collected with EDTA and centrifuged. Plasma with white blood cells and platelets was removed and erythrocytes were washed with cold saline and hemolyzed under hypotonic conditions in order to obtain pure membranes.

Table 1. Overall composition of control (CD) and fat free diet (FFD) expressed in %

Main ingredients	CD	FFD
Total proteins	19.37	11.94
Total fats	3.67	0.66
Total carbohydrates	64.82	75.33
Total nitrogen	3.10	1.91
Dry weight	90.96	89.84

Table 2. Energetic composition of control (CD) and fat free diet (FFD) expressed in kJ/g

	CD	FFD
Proteins	3.29	2.03
Fats	1.43	0.26
Carbohydrates	11.02	12.81
Total	15.74	15.10

Methods

Frozen tissues were homogenized by means of Ultra-Turax at 2000 r.p.m. in cold. In homogenates soluble proteins were determined according to the method of Lowry *et al.* (*10*) and the rest was freeze-dried in Univapo 100 H evaporator coupled to Unicryo MC 2L (Uniequip, Martinsried, Germany) cold station. Total lipids were extracted using the method by Folch *et al.* (*11*) and in the extract total lipid phosphorus was determined according to the method of Parker and Peterson (*12*). Fatty acid methyl esters were prepared by hydrolysis in methanolic HCl and analyzed by Perkin Elmer gas-liquid chromatograph (GLC), model Sigma 2, provided by a flame ionization detector and a capillary column AT WAX (Alltech GmbH, Germany), length 30 m, i.d. 0.25 mm and film thickness 0.25 μm, with nitrogen as a carrier gas. Oven temperature was ranged from 150 to 250 °C at rate of 5 °C/min, while the final temperature was kept constant for 20 minutes. Injector and detector temperatures were 250 and 260 °C, respectively. Fatty acids were identified in comparison to known reference material (Supelco Inc., Bellefonte, PA). Results of fatty acid analysis were expressed as median values and were compared by nonparameter Mann-Whitney U – Wilcoxon Rank Sum W Test.

Results

After 14 days of the experiment, body mass gain for the animals on FFD was 8 %, as compared to 4 % for the animals on CD. There were no visible changes in the behavior of animals or anatomic changes of investigated organs in either group. Table 3 contains data of the organ mean mass, as well as their protein and total lipid phosphorus mass fraction.

Figs. 1 and 2 show typical GL chromatograms of fatty acid methyl esters from the brain total lipids of the rats on control and fat free diet, respectively. Fatty acid composition is expressed as the percentage of particular fatty acid relative to total fatty acid content. Data obtained for each animal in the group were compared and presented as median ± max.

Changes in fatty acid composition of brain lipids as a consequence of a fat free diet are illustrated in Fig. 3. As expected, the predominant fatty acids were palmitic, stearic and oleic acid. The statistical analysis pointed out the significant decrease of docosahexaenoic acid ($p < 0.05$), and the less pronounced, but still important, increase of oleic and decrease of palmitoleic acid ($0.05 < p < 0.10$).

Comparative results for fatty acid composition of heart tissue of control and treated animals are shown in Fig. 4. In this tissue much higher fraction of linoleic and arachidonic acid (C20:4, n-6) was present than in brain tissue, while docosahexaenoic acid was absent at all. Significant differences ($p < 0.05$) between fatty acid profiles for animals fed CD and FFD were found in the content of palmitoleic and oleic acid. Relative amount of both acids was increased in rat heart tissue of animals on FFD.

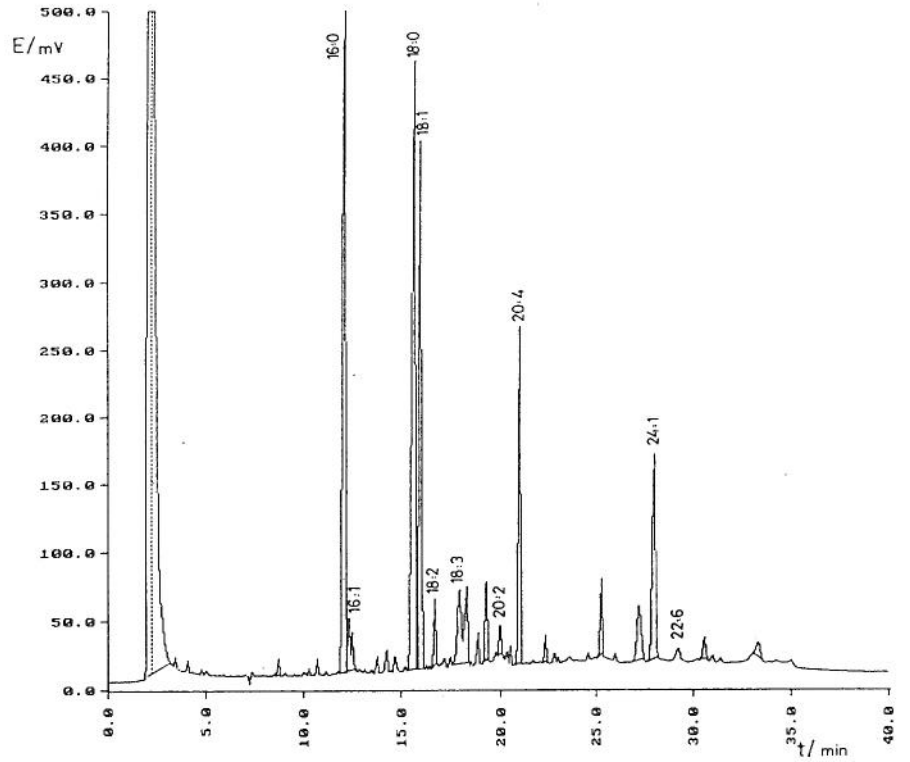

Fig. 1. GLC pattern of fatty acid methyl esters from brain total lipids of rats on control diet. Preparation of methyl esters and GLC conditions were as described in **Materials and Methods**. Fatty acids are designated by the number of carbon atoms : the number of double bonds

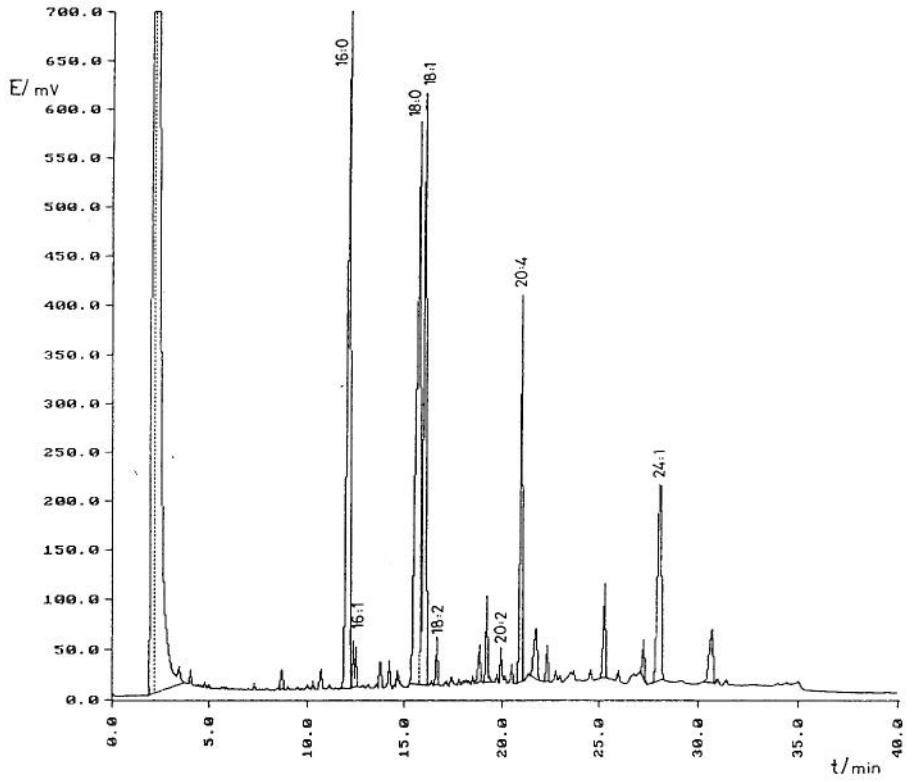

Fig. 2. GLC pattern of fatty acid methyl esters from brain total lipids of rats on fat free diet. Preparation of methyl esters and GLC conditions were as described in **Materials and Methods**. Fatty acids are designated by the number of carbon atoms : the number of double bonds

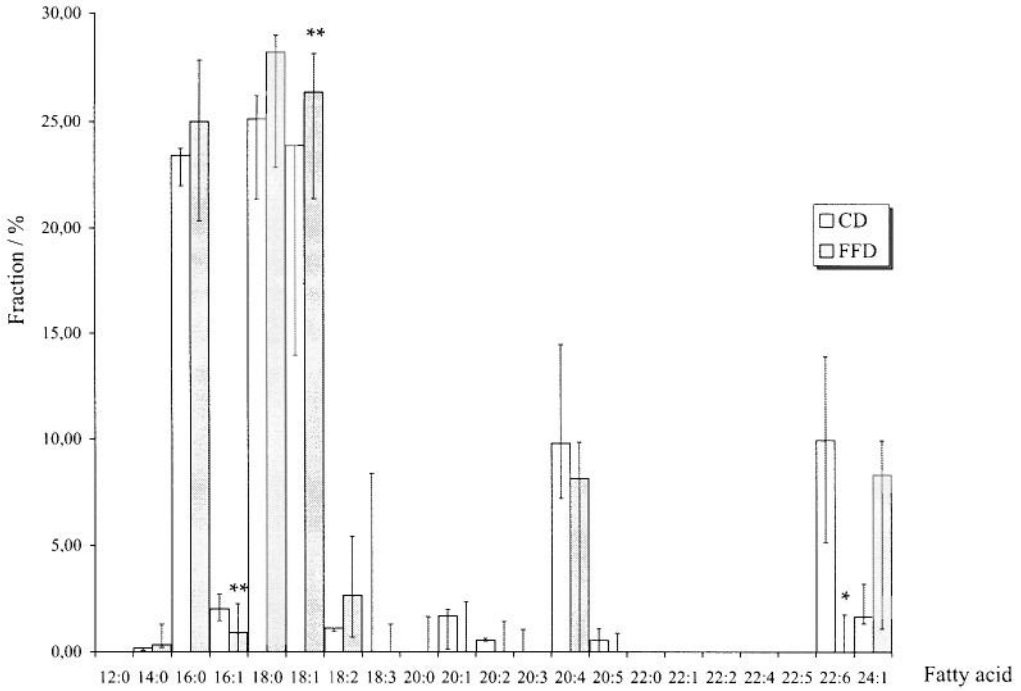

Fig. 3. Fatty acid fraction of total brain lipids of rats on control (CD) and fat free diet (FFD) for two weeks. Results are expressed as median ± max, *p < 0.05, ** 0.05 < p < 0.10

Changes in fatty acid profile induced by FFD were much more pronounced in liver tissue relative to both brain and heart. As shown in Fig. 5, significant decrease ($p < 0.05$) of a series of fatty acids (C18:0, C18:2, C20:3, C20:4, C20:5, C22:6 and C24:1) was found. In contrast, the relative amount of palmitic acid was increased, although the statistical analysis did not reveal its significance.

Fatty acids of lipids from erythrocyte membranes were analyzed too, and the results are shown in Fig. 6. Administering of FFD caused a significant fall ($p < 0.05$) of stearic acid, accompanied with a simultaneous increase of C14:0 and C22:0 acids. Moreover, the reduction of the presence of linoleic and C22:0 acids in the range $0.05 < p < 0.10$, was found, too.

Discussion

According to the data from American Heart Association (AHA) and other health organizations, elevated levels of plasma lipids, particularly LDL cholesterol, and obesity, are among specific risk factors associated with heart and blood vessel diseases. In order to prevent risks development, the AHA recommends a reduction of dietary fat intake (*13*) and in accordance with this suggestion, low fat diets (LFD) were developed.

In some cases, fat free diet, which contains less than 1 % of fats is used, but in addition to reduced risk from coronary heart disease, FFD is accompanied with the risk of developing essential fatty acid deficiency (EFAD). Since linoleic and linolenic acids had been recognized as essential, the effects of their absence from nutrition have been followed.

In this paper, changes caused in particular rat organs and erythrocyte membranes by feeding a fat free diet for two weeks, are presented. At the first sight the fact that a mass gain in animals fed FFD was 8 % might be negligible, or even paradoxical, in comparison with 4 % mass gain in the control group, but one must bear in mind that two diets were isoenergetic. That means that fats depleted from the diet had to be substituted with the adequate amount of carbohydrates, causing probably the effect of elevated blood glucose concentration, resulting in induced lipogenesis. However, according to the values for the ratio of total lipid phosphorus *vs.* tissue proteins, such conditions did not significantly affect the content of phospholipids in investigated tissues (Table 3).

Table 3. Mean organ masses, mass fraction of proteins in wet tissue and fraction of total lipid phosphorus *vs.* tissue proteins (mean ± st. dev.)

ORGAN	mass / g		*w*(protein)/ (mg/g wet tissue)		*w*(P)/ (μg /mg protein)	
	CD	FFD	CD	FFD	CD	FFD
Brain	1.30 ± 0.14	1.23 ± 0.09	36.29 ± 7.33	28.93 ± 7.55	101.2 ± 27.4	103.8 ± 11.8
Heart	0.77 ± 0.05	0.69 ± 0.08	46.68 ± 5.56	49.95 ± 2.44	24.4 ± 4.1	27.4 ± 2.5
Liver	10.01 ± 1.14	8.35 ± 0.81	76.40 ± 9.05	96.56 ± 17.59	13.6 ± 5.7	11.6 ± 2.4

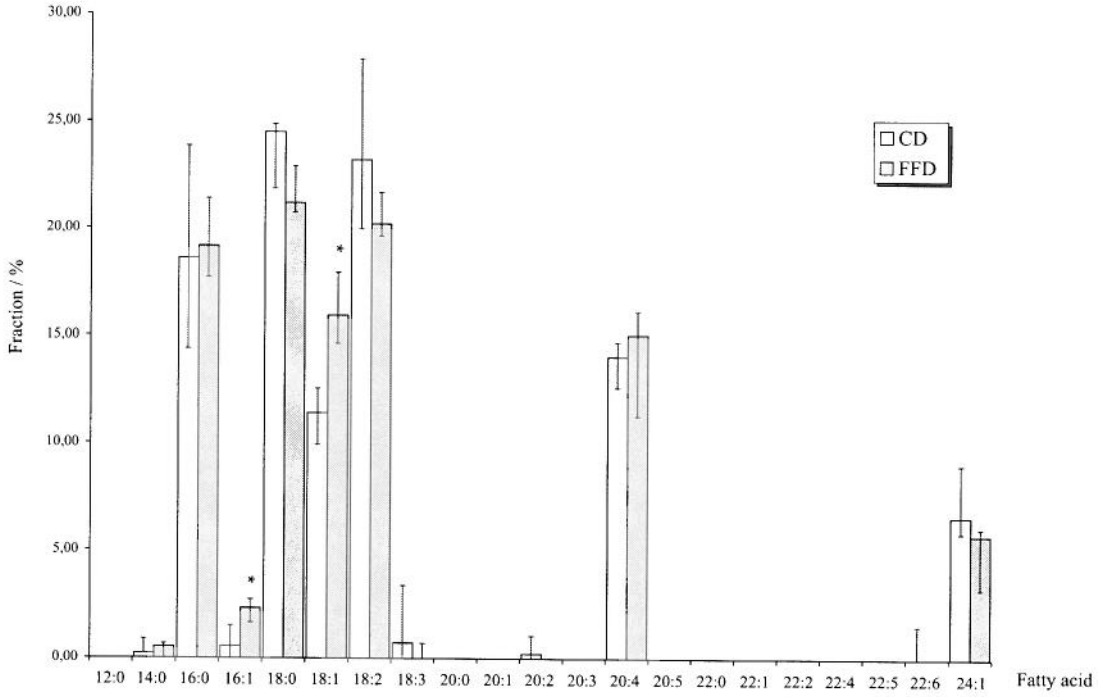

Fig. 4. Fatty acid fraction of total heart lipids of rats on control (CD) and fat free diet (FFD) for two weeks. Data are expressed as median ± max, *p < 0.05

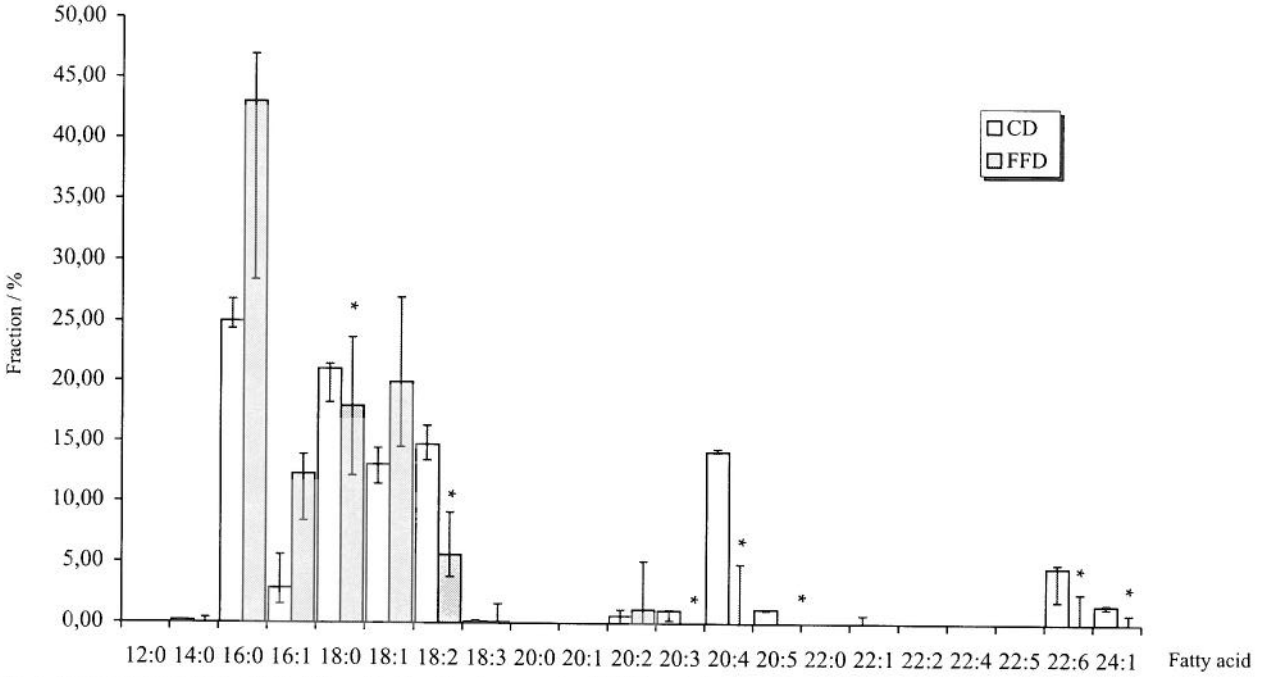

Fig. 5. Fatty acid fraction of total liver lipids of rats on control (CD) and fat free diet (FFD) for two weeks. Results are expressed as median ± max, *p < 0.05

Data obtained for fatty acid composition of investigated tissues show more or less expected changes. The most pronounced differences were found in liver. Besides linoleic acid, the content of stearic and of almost all the long chain polyunsaturated fatty acids was also decreased. This might be the result of intensive liver metabolism and the attempts to insure necessary amounts of lacking metabolites to other tissues. When both, linoleic and linolenic acid are present in the diet in sufficient amounts, they will follow a series of elongation and desaturation steps, which enables the syntheses of arachidonic, eicosapentaenoic (C20:5, n-3) and docosahexaenoic (C22:6, n-3) acid, respectively. Since they are missing in the FFD, stearic and oleic acid, which can be synthesized endogenously, take their place as substrates for desaturases and elongases, resulting in elevated

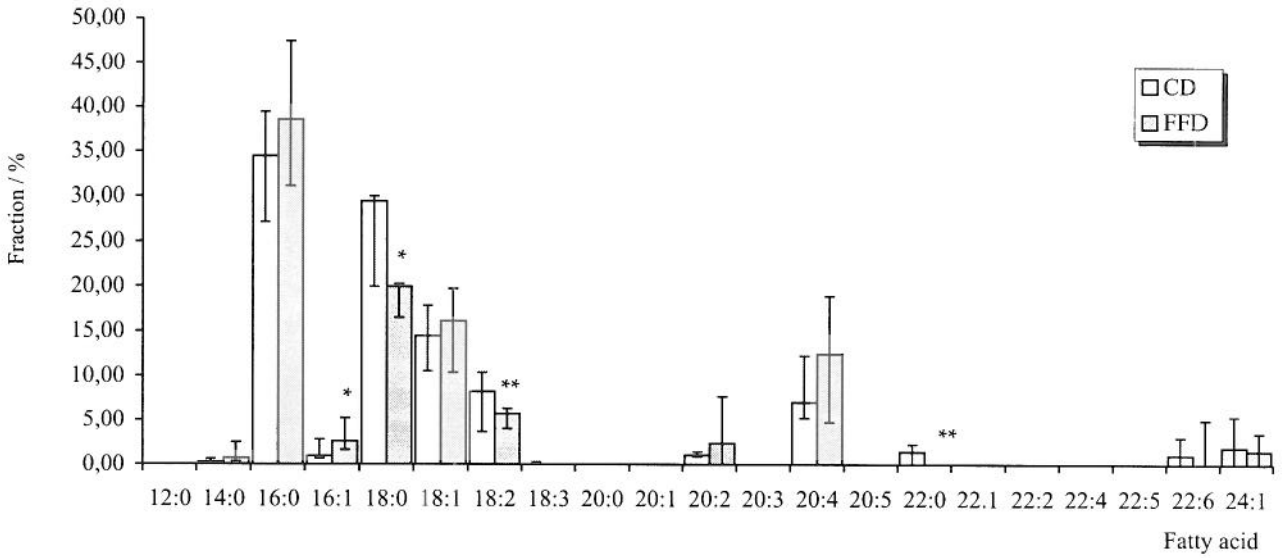

Fig. 6. Fatty acid fraction of total lipids of erythrocyte membranes of rats on control (CD) and fat free diet (FFD) for two weeks. Results are expressed as median ± max, *p < 0.05, ** 0.05 < p < 0.10

amounts of eicosatrienoic acid. Such effects have been confirmed by many authors (*5,6,14*). Changed fatty acid composition of lipids, especially phospholipids, as a consequence of EFAD, has been shown to disturb lipoprotein production as well as their release from liver and enterocytes (*15–17*).

In contrast to liver, the pattern of fatty acids in brain was much more preserved. Having in mind that the blood – brain barrier is selective for fatty acids, it seems reasonable that dietary fatty acid composition influences brain lipids to a lesser extent (*18*). However, some changes were observed, *e.g.* the inhibited synthesis of docosahexaenoic acid. Although statistically not significant, the reduction in arachidonic acid content is still evident. This effect has been pronounced by many authors, who are stressing the importance of PUFA in the process of brain development (*19–21*). Numerous experiments confirmed beneficial effects on formation and function of nervous system during the growth of infants fed on artificial diets, when DPA and DHA were added to the diet (*22–24*).

Similar fatty acid compositions were found in total lipids of heart and of erythrocyte membranes, revealing the significant increase in palmitoleic and oleic acid content. The data obtained in these experiments are in a good correlation with our previous results (*7,8*), and suit well the idea of »tendering effect«, which enables the organism to compensate dietary variances. It can be concluded, that while feeding with the FFD, the metabolism increases the endogenous syntheses of monounsaturated fatty acids and their elongation and desaturation products, respectively, in order to maintain the optimal ratio of saturated *vs.* polyunsaturated fatty acids. This ratio is of special importance for membrane phospholipids, since their influence on the membrane fluidity, as well as on the function of membrane proteins, is the well-known fact (*25,26*). Further experiments are in progress with the aim to reveal the effects of such FFD on the composition of fatty acids of phospholipids.

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Promjene sastava masnih kiselina tkiva kao posljedica bezmasne prehrane

Sažetak

Linolna (C18:2, n-6) i linolenska (C18:3, n-3) kiselina ne mogu se sintetizirati u organizmu, te su stoga esencijalni sastojak hrane. Bezmasne se dijete, s manje od 1 % ukupne energetske vrijednosti u obliku masti, primjenjuju ponekad pri oštećenju gastrointestinalnog sustava i poremećaja metabolizma masti. Takve dijete predstavljaju rizik za razvoj simptoma nedostatka esencijalnih masnih kiselina.

U ovom su radu ispitivane promjene u sastavu masnih kiselina ukupnih lipida mozga, srca, jetre i membrana eritrocita štakora uzrokovane bezmasnom dijetom tijekom dva tjedna. Kontrolna i bezmasna dijeta bile su izoenergetske, pri čemu je udjel lipida supstituiran ugljikohidratima. Najuočljivije su promjene u sastavu masnih kiselina lipida jetre, dok su u ostalim tkivima promjene manjeg intenziteta, ali primjetne. Očituju se smanjenjem udjela linolne kiseline, a i drugih dugolančanih polinezasićenih masnih kiselina (arahidonska, C20:4, n-6; eikozapentaenska, C20:5, n-3; dokozaheksaenska, C22:6, n-3), uz kompenzacijsko povećanje endogeno sintetiziranih mononezasićenih masnih kiselina (palmitoleinska, C16:1, n-9 i oleinska, C18:1, n-9). Tako se nastoji održati odgovarajući omjer zasićenih kiselina prema nezasićenim masnim kiselinama, koji je od posebne važnosti u sastavu fosfolipida, odgovornih za strukturu i funkciju staničnih membrana. Rezultati upućuju na tendenciju organizma da nedostatak esencijalnih masnih kiselina supstituira endogenom sintezom, »prigušujući« tako učinak nedostatnog unosa masti, odnosno esencijalnih masnih kiselina.