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EVALUATION, IN AN ANIMAL MODEL STUDY, OF THE EFFECT OF DIET COMPOSITION CHANGE AND DIET SUPPLEMENTATION WITH B-GROUP VITAMINS ON THE LIVER FATTY ACID PROFILE*

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ABSTRACT

Introduction. Contemporary diet of men is characterised by a significant contribution of processed and purified products impoverished by technological processing in, e.g., B-group vitamins taking part in the synthesis of fatty acids. One of the means to prevent their insufficient intake is supplementation of food products with those components. Hence, an animal model study was undertaken in order to determine whether modification of diet composition in which whole-grain components (whole grains of wheat and maize) are isocalorically substituted with white flour and saccharose, and its complementary supplementation with B-group vitamins may trigger changes in the profile of fatty acids synthesized in liver of rats.

Material and methods. The study was conducted on 30 male rats aged 5 months. Group I was receiving the basal feed mixture (Labofed B), which contained among other things whole grains of wheat and maize. Groups II-III, in free access, were administered modified feed mixture in which 83.5% of wheat present in the basal diet was substituted with wheat flour, and 50% of maize – with saccharose. Contents of vitamin B_1, B_2, B_6 and PP and basic chemical composition in the feed mixture are determined by HPLC method and the fatty acid profile with the modified Folch method using gas chromatography. Groups I and II were receiving water to drink, whereas the animals from group III were administered 25 ml of an aqueous solution of vitamins in the following doses: B_1 – 0.94 mg, B_2 – 0.48 mg, B_6 – 0.5 mg, PP – 1.9 mg. In group III – to supplement differences in contents of those vitamins between feed mixtures resulting from the exchange of components, which to some extent simulated the mode of supplementation in humans. Concentration of glucose was determined in blood serum and the amount of fat was determined with Soxhlet method in the dissected animals liver and the fatty acid profile with the modified Folch method using gas chromatography.

Results. While analysing the achieved results it was stated that the animals, despite comparable quantities of ingested feed, differed in their body weight gains reached in the course of the experiment. In contrast, both the liver mass and its fat content did not differ significantly between the analysed groups of animals. Furthermore, livers of the animals fed the modified diet were characterised by a decreased sum of monoenoic fatty acids and an increased sum of polyenoic fatty acids, compared to the animals receiving the basal diet. The fatty acid composition determined in livers of the animals fed the modified diet and supplemented with B_1, B_2, B_6 and PP vitamins did not differ significantly from that assayed in livers of the rats fed the modified diet.

Conclusions. Modification of diet composition, consisting of substituting whole grains of cereals with wheat flour and saccharose, and its complementary supplementation with B-group vitamins caused significantly higher body weight gains of the examined animals. Modification of diet composition had a significant effect on a decreased level of monoenoic and an increased level of polyenoic fatty acids in rat livers.

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Supplementation with B-group vitamins did not correct the changes in the fatty acid profile in rat livers caused by diet modification.

Key words: vitamin B-group, male rats, liver, fatty acid profile

LIST OF ABBREVIATIONS

AA – arachidonic acid C20:4, n-6
ALA – α-linolenic acid C18:3, n-3
DGLA – dihomo-γ-linolenic acid C20:3, n-6
DHA – docosahexaenoic acid C22:6, n-3
DPA – docosapentaenoic acid C22:5, n-3
EDA – eicosadienoic acid C20:2, n-6
EPA – eicosapentaenoic acid C20:5, n-3
GLA – γ-linolenic acid C18:3, n-6
LA – linoleic acid C18:2, n-6
LC PUFA – long chain PUFA
MUFA – monounsaturated fatty acids
OA – oleic acid C18:1, n-9
PUFA – polyunsaturated fatty acids
SFA – saturated fatty acids

INTRODUCTION

It is common knowledge that polyunsaturated fatty acids (PUFAs) occur in the body among other things as constituents of phospholipids of cellular membranes and intracellular organelles, and that they affect their structure and permeability. Among these acids, significant are those belonging to n-6 and n-3 families, where the digit denotes the location of the first unsaturated bond counting from the methyl terminal of a carbon chain. The PUFAs include LA (C18:2, n-6) and ALA (C18:3, n-3), which are not synthesized in the body and therefore should be supplied with diet.

In the processes of elongation and desaturation ongoing in endoplasmic reticulum, these acids are transformed into LC PUFA being of a great biological significance: AA (C20:4, n-6), EPA (C20:5, n-3), DHA (C22:6, n-3). Once released from phospholipids of cellular membranes, they are becoming precursors of the synthesis of tissue hormones that exhibit pro- and anti-inflammatory effects [Tapiero et al. 2002].

As the contemporary diet of man is characterised by a significant contribution of processed and purified products impoverished by the technological processing in, e.g., B-group vitamins taking part in the synthesis of fatty acids, one of the means to prevent their insufficient intake is food products supplementation with those components. In fact, however, the supplemented quantities declared by producers on food products' labels often differ from real values determined analytically [Jantarska et al. 2007]. The conducted study demonstrates that in order to prevent nutritional deficiencies, diet supplementation with, e.g. B-group vitamins, is applied by 25 to 75% of the population. And what is more, the supplementation is often done according to someone's assumption, without any medical recommendation, in the quantities exceeding recommended by few to even several dozen times [Pietruszka and Brzozowska 1999].

Hence, an animal model study was undertaken in order to determine whether modification of diet composition in which whole-grain components (whole grains of wheat and maize) are isocalorically substituted with white flour and saccharose, and its complementary supplementation with B-group vitamins may trigger changes in the profile of fatty acids synthesized in liver of rats.

MATERIAL AND METHODS

Upon the approval of the Local Ethical Commission (Approval no 2/2010), the study was conducted on 30 male rats of WISTAR strain, aged 5 months and with the initial body weight of 429.3 g ±54.0 g. Rats were obtained from the animal husbandry of Chair and Department of Toxicology, Poznań University of Medical Sciences, Poland. After one-week conditioning under vivarium conditions (temp. 21-22°C, relative air humidity 55-60%, light/dark cycle 12/12 h), the animals were divided into three equal groups (n = 10) that were fed *ad libitum* with pelleted feed mixtures produced from the same components, except for the differentiating ones, by the Feed Mixtures and Concentrates

Production Plant in Kcynia. Group I was receiving the basal feed mixture (Labofed B), that met the requirements stipulated for Reeves et al. [1993] feed mixture and contained among others whole grains of wheat and maize. Groups II-III were administered modified feed mixture in which 83.5% of wheat present in the basal diet was substituted with wheat flour, and 50% of maize – with saccharose (Table 1). Contribution of other components in feed mixtures was identical.

Table 1. Component composition of compound feeding stuffs used in the experiment

Item	Basic feed	Modified feed
Ingredients, %		
Wheat	36.4	6
Corn grain	20	10
Wheat bran	20	20
Dry whey	3.0	3.0
Fodder salt	0.3	0.3
Soy-bean grain 48%	17	17
Fodder chalk	2	2
Phosphate 1-CA	0.3	0.3
Premix LRM	0.8	1
Wheat flour (type 500)	–	30.4
Saccharose	–	10
Nutrient composition, %		
Total protein	19.7	18.6
Crude fat	2.0	3.3
Carbohydrates	62.3	63.4
Crude fiber %	2.91	2.73
Dry matter	90.6	91.4
Total ash	6.6	6.2
Brutto energy		
kcal·g^{-1}	3.89	3.99
kJ·g^{-1}	16.3	16.7
Metabolic energy		
kcal·g^{-1}	3.45	3.57
kJ·g^{-1}	14.4	14.9
Zn, mg·100 g^{-1}	7.29	7.01

Substitution of dietary components made while composing the modified feed mixture was aimed at mirroring, to some extent, contemporarily observed nutritional mistakes, i.e. increasing contribution of saccharose in the calorific value of food ration and increased contents of refined carbohydrates in diet. The prepared feed mixtures were subjected to a chemical analysis (AOAC 2003) in order to determine the contents of: total nitrogen (with Kjeldahl method) expressed per protein content, crude fat (with Soxhlet method), dry matter (with the gravimetric method), and ash (with the gravimetric method). The content of carbohydrates was computed from the difference between dry matter and the sum of the other solid dietary components. The gross and metabolic energy contents were calculated with the generally applied energy equivalents. The feeds mixtures were prepared maintaining the isocaloric and isoprotein balance (Table 1). The contents of vitamin B_1, B_2, B_6 and PP in the feeds were extracted by ammonium in dimethylsulphoxide solution and acetic acid and they were determined by HPLC method (on Agilent 1200SL with diode array detector) at the Research Institute of Animal Production National Laboratory for Feeding Stuffs in Szczecin, Poland (Table 2). Analyses were conducted with the use of a Luna C18 (2) column 5 µm (250 × 4.6 mm), under the following chromatographic conditions: mobile phase: gradient B – acetonitrile + A – potassium acetate; flow Rate: 1.5 mL/min and wavelength: 273 nm.

Table 2. Contents of selected vitamins in mg/100 g diet

Trait	Basic feed	Modified feed	Vitamin content difference	
				%
Thiamine B_1	2.5	0.62	1.88	75.2
Riboflavin B_2	2.1	1.14	0.96	45.7
Pyridoxine B_6	2.35	1.35	1	42.5
Niacin PP	8.6	4.8	3.8	44.2

The content of Zn in the feeds was assayed by wet digestion in concentrated HNO_3 in microwave oven MDS 2000 and determined by using atomic emission spectrometry induction coupled plasma (ICP-AES) in the apparatus Jobin Yvan JY-24 type. In addition, at the Department of Animal Nutrition, University of Agriculture in Krakow, Poland, the diets administered to the rats were determined for the fatty acid profile (Table 3) with the modified Folch method using a 450-GC gas chromatography by Varian Company with an FID detector. Analyses were conducted (in 2 replicates) with the use of a CP-SIL 88 column (100 m × 0.25 mm), under the following chromatographic conditions: carrier gas – helium, injector's temperature – 200°C, detector's temperature – 240°C, and column's temperature – programmed from 60°C to 220°C.

Groups I and II were receiving water to drink, whereas the animals from group III – in the time of intensified activity – were administered daily in free access 25 ml of an aqueous solution of vitamins in the following doses: B_1 (*Thiamini hydrochloridum*) – 0.94 mg, B_2 (*Riboflavinum*) – 0.48 mg, B_6 (*Pyridoxinum hydrochloricum*) – 0.5 mg, PP (*Nicotinamidum*) – 1.9 mg. Once the solution of minerals was administered, the animals were provided with pure, settled tap water. The vitamins originated from commercially available pharmaceutical preparations. In group III – to supplement differences in contents of those vitamins between feed mixtures resulting from the exchange of components which to some extent simulated the mode of supplementation in humans. Once the solution of vitamins was administered, the animals were provided with pure, tap water. After one-week conditioning period, the experiment spanned for 6 weeks, during which feed intake in all groups as well as vitamins intake in the supplemented group were monitored systematically. Body weight of the animals was measured once a week.

The animals were fasted 12 h before the end of the experiment. Next, they were anaesthetized with Ketanest (Pfizer Ireland Pharmaceuticals) and blood samples were collected from their hearts. Having centrifuged the coagulate, the resultant blood serum was determined for concentration of glucose determined with the enzymatic colorimetric technique of Trinder [1969] by using Bio Systems bio tests onto a Marcel Media Bio spectrophotometer. The amount of fat in the dissected animals' livers was determined with Soxhlet's method according to AOAC [2003] on a Soxtec HT6 apparatus by Foss Tecator. The livers of rats were determined for the fatty acid profile with the modified Folch method using a 450-GC gas

Table 3. Content of fatty acids in feed male rat

Fatty acid %	Basic feed	Modified feed (Mf)
Saturated fatty acids		
Myristic acid C14	0.39	0.25
Palmitic acid C16	21.7	16.2
Stearic acid C18	3.69	2.23
Monounsaturated fatty acids		
Oleopalmitic acid C16:1, n-7	0.82	0.57
Oleic acid C18:1, n-9	24.33	35.56
Eicosanoic acid C20:1, n-9	0.24	0.32
Erucic acid C22:1, n-9	0.04	0.09
Polyunsaturated fatty acids		
α-linolenic acid C18:3, n-3	4.20	4.64
Eicosapentaenoic acid C20:5, n-3	0	0
Docosapentaenoic acid C22:5, n-3	0	0
Docosahexaenoic acid C22:6, n-3	0.04	0.09
Total, n-3	4.24	4.73
Linoleic acid C18:2, n-6	42.47	38.95
Eicosadienoic acid C20:2, n-6	0	0
Dihomo-γ-linolenic acid C20:3, n-6	0	0
Arachidonic acid C20:4, n-6	0.28	0.09
Total, n-6	42.75	39.04
Unidentified fatty acids	1.75	1.09
Saturated fatty acids SFA	25.78	18.68
Monounsaturated fatty acids MUFA	25.43	36.54
Polyunsaturated fatty acids PUFA	46.99	48.42
n-6/n-3	10.1	8.4

Each value is a mean of 2 replicates.

chromatograph by Varian Company with an FID detector at the Department of Animal Nutrition, University of Agriculture in Krakow, Poland.

Results are shown as means ±standard deviation. Statistical significance was done by one-way analysis of variance (ANOVA) using the Statistica 9.0. When the ANOVA indicates significant difference among the means, the differences were further evaluated using the Duncan's multiple range tests. The difference was considered significant when $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

It has been ascertained during the course of analysis of the achieved results that irrespective of isocaloric feed and comparable amount of its consumption by the animals under research, the higher percentage of fat in the modified feed could, after all, influence the body mass gain achieved in the course of experiment (Table 4). The greatest body weight gains, both the absolute ones and these expressed per 100 g of ingested feed, were noted for the animals fed the modified diet and supplemented with B-group vitamins. In contrast, both the mass of liver and its fat content did not differ significantly between the analysed groups of animals.

No significant effect of the factors applied in the study was either observed in respect of glucose concentration in blood plasma of the rats (Table 4).

It was found, however, that diet modification caused a significant increase in the levels of stearic (C18), and linoleic acids (C18:2, n-6) and a significant decrease in the levels of: oleopalmitic (C16:1), oleic (C18:1, n-9), eicosanoic (C20:1, n-9), eicosadienoic (C20:2, n-6) and dihomo-γ-linolenic acids (C20:3, n-6) in rats' livers (Table 5).

Taking into consideration groups of fatty acids, a significantly higher content was noted for the n-6 fatty acids in liver of rats fed the modified diet compared to the animals administered the basal diet. In contrast, the applied modification of diet composition did not affect the content of n-3 fatty acids. The computed n-6/n-3 ratio also did not differ significantly between the analysed groups of animals.

Furthermore, livers of the animals fed the modified diet were characterised by a decreased sum of monoenoic fatty acids and an increased sum of polyenoic fatty acids, compared to the animals receiving the basal diet (Table 5).

The fatty acid composition determined in livers of the animals fed the modified diet and supplemented

Table 4. Effects of diet and vitamins B group supplementation on feed consumption, body weight gain at male rats ($\bar{x}$ ±SD, n = 30)

Trait	Basic feed (a)	Modified feed (Mf) (b)	Mf + complementary supplementation (c)	Statistical significant
Feed intake, g	872.6 ±78.9	881.3 ±64.8	863.7 ±67.3	–
Feed intake, g·100 g^{-1} of metabolic body weight	192.2 ±17.0	187.0 ±18.1	182.9 ±10.5	–
Body weight, g	29.0 ±12.4	42.1 ±9.5	47.2 ±17.3	a-b* a-c**
Body weight gain, g·100 g^{-1} of feed	3.39 ±1.5	4.76 ±0.9	5.47 ±2.0	a-c**
Liver, g	12.2 ±6.0	11.9 ±1.9	12.9 ±1.4	–
Liver relative weight, g·100 g^{-1} of body weight	2.62 ±0.3	2.53 ±0.6	2.66 ±0.4	–
Hepatic fat, %	2.76 ±0.2	2.61 ±0.2	2.85 ±0.4	–
Glucose, mmol·l^{-1}	6.22 ±0.7	6.62 ±1.3	6.33 ±0.6	–

*Statistically significant difference $p \leq 0.05$.
**Statistically significant difference $p \leq 0.01$.

Table 5. Content of fatty acids in liver in male rat depending on diet and supplementation B group vitamins ($\bar{x}$ ±SD, n = 30)

Fatty acid %	Basic feed (a)	Modified feed (Mf) (b)	Mf + complementary supplementation (c)	Statistical significant
Saturated fatty acids				
Myristic acid C14	1.06 ±0.85	0.56 ±0.46	0.56 ±0.61	–
Palmitic acid C16	25.8 ±1.1	25.2 ±1.6	26.0 ±0.7	–
Stearic acid C18	17.6 ±3.4	20.9 ±3.5	19.5 ±2.2	a-b*
Monounsaturated fatty acids				
Oleopalmitic acid C16:1, n-7	2.89 ±1.11	1.85 ±0.63	2.01 ±0.6	a-b** a-c*
Oleic acid C18:1, n-9	19.3 ±6.2	14.5 ±4.5	15.8 ±3.6	a-b*
Eicosanoic acid C20:1, n-9	0.41 ±0.2	0.21 ±0.05	0.27 ±0.11	a-b** a-c*
Polyunsaturated fatty acids				
α-linolenic acid C18:3, n-3	0.79 ±0.40	0.59 ±0.25	0.60 ±0.21	–
Eicosapentaenoic acid C20:5, n-3	0.16 ±0.08	0.14 ±0.04	0.16 ±0.04	–
Docosapentaenoic acid C22:5, n-3	0.34 ±0.14	0.35 ±0.11	0.38 ±0.11	–
Docosahexaenoic acid C22:6, n-3	1.89 ±0.63	2.30 ±0.35	2.15 ±0.36	–
Total, n-3	3.17 ±0.5	3.38 ±0.3	3.29 ±0.2	–
Linoleic acid C18:2, n-6	14.0 ±0.8	15.2 ±1.1	15.2 ±1.6	a-b* a-c*
Eicosadienoic acid C20:2, n-6	0.20 ±0.04	0.15 ±0.04	0.14 ±0.02	a-b** a-c**
Dihomo-γ-linolenic acid C20:3, n-6	0.29 ±0.05	0.24 ±0.04	0.23 ±0.03	a-b* a-c**
Arachidonic acid C20:4, n-6	14.3 ±4.5	17.3 ±2.7	16.3 ±2.6	–
Total, n-6	28.0 ±6.5	32.8 ±2.9	31.9 ±3.7	a-b*
Unidentified fatty acids	0.96 ±0.18	0.63 ±0.13	0.73 ±0.37	a-b** a-c*
Saturated fatty acids	44.5 ±2.6	46.6 ±2.8	46.0 ±1.5	–
Monounsaturated fatty acids	22.6 ±7.5	16.5 ±5.2	18.1 ±4.3	a-b*
Polyunsaturated fatty acids	31.9 ±5.4	36.2 ±3.0	35.2 ±3.9	a-b*
n-6/n-3	8.8 ±1.6	9.7 ±0.9	9.7 ±0.7	–

*Statistically significant difference $p \leq 0.05$.
**Statistically significant difference $p \leq 0.01$.
Each value is a mean of 2 replicates.

with B_1, B_2, B_6 and PP vitamins (Table 5) did not differ significantly from that assayed in livers of the rats fed the modified diet.

DISCUSSION

In the conducted experiment, likewise in studies by other authors [Debski et al. 2007, França and Vianna 2010], it was stated that both diet modification and its supplementation with B-group vitamins had no significant effect on feed intake by the examined animals.

Correspondingly to a research by Toida et al. [1996], there were no statistically significant body weight gains in the animals receiving a modified diet with saccharose, compared to the group fed the basal diet, despite the known effect of such a diet on increasing body weight gains in animals [Bruckdorfer et al. 1972]. This may be due to the fact that the synthesis of fatty acids from glucose and fructose, that provide carbon atoms and NADPH, requires appropriate quantities of B-group vitamins being co-factors in metabolic transformations [Clayton 2006, Mooney et al. 2009], whose content in the modified diet was impoverished in the range of 42-75%. In turn, the supplementation with B-group vitamins was observed to exert a significant effect on increasing body weight gains of the animals when expressed in absolute values and per 100 g of ingested feed.

The applied diet modification and vitamin supplementation had no significant effect on glucose concentration in blood plasma of the animals, which points to the introduction of glucose and fructose originating from feed digestion into metabolic pathways, among other things, into fatty acids synthesis.

In rats, the major (42%) site of fatty acids synthesis is liver [Gandemer et al. 1983], and precursors of their synthesis may include both dietary fats [Cawthorne and Cornish 1979], and carbohydrates [Romsos and Leveille 1974]. A diet being poor in saturated fatty acids inhibits activities of acetyl-CoA carboxylase [Volpe and Vagelos 1974] and fatty acids synthase [Knoche et al. 1973] whereas the polyunsaturated fatty acids are regulating the quantity of lipogenic enzymes in liver [Musch et al. 1974, Toussant et al. 1981]. Investigations have shown [De Schrijver and Privett 1983, Romsos and Leveille 1974, Volpe and Vagelos 1974], that the type of dietary carbohydrates exerts an inducing effect on the synthesis and profile of fatty acids in liver. Bruckdorfer et al. [1972] were observing the most enhanced fat synthesis in liver while feeding rats a diet containing fructose and saccharose, and the least synthesis when administering animals a diet with glucose and starch. In the conducted experiment, the modified diet containing saccharose was expected to affect metabolic transformations of both glucose – through the cycle of citric acid and the pentose phosphate pathway providing carbon atoms and NADPH for fatty acids synthesis, and of fructose phosphorylated under the influence of hexokinase to fructoso-6-phosphate which is further metabolized to acetyl-CoA being a precursor of fatty acids synthesis. However, the modified diet in which part of the whole grains of cereals was isocalorically substituted with flour and saccharose, although having a higher by 1.3% fat content than the basal diet, had no significant effect on fat synthesis in liver of the animals examined, and the complementary supplementation of this diet with B-group vitamins did not change anything in this respect.

The applied diet modification did, however, affect a change in fatty acid profile in rat livers, because analyses demonstrated a decreased content of monoenoic and an increased content of polyenoic fatty acids. This may be acknowledged as an adverse effect, since those acids are more susceptible to peroxidation in the body [Saito and Kubo 2003], especially at thiamine [Sushko and Lukienko 1981] and pyridoxine [Cabrni et al. 1998, Taysi 2005] deficiency in diet. The diminished content of monoenoic fatty acids in livers of this group of animals may result from the deficiency of PP vitamin in the modified diet, as this vitamin is synthesized from saturated fatty acids with the share of the enzymatic system of Δ_9-desaturase which in the endoplasmic reticulum catalyzes the transformation of palmitoyl-CoA into palmitoleyl-CoA or stearoyl-CoA into oleyl-CoA, and this reaction requires oxygen, as well as NADH or NADPH [Jeffcoat and James 1977].

Desaturases, participating in the synthesis of PUFA metabolites, compete for PUFAs of the n-6 and n-3 families present in diet, but still Δ_4 and Δ_6 desaturases are observed to utilize the n-3 PUFAs than the n-6 PUFAs [Connor et al. 1993] more willingly. In the reported experiment, the change in diet composition did not have significant effect on the profile of the n-3

fatty acids, but affected an increase in the synthesis of the n-6 family acids.

Taking into account that in rats the synthesis of EPA and DHA from ALA is several times more efficient than in man or other mammals [Anderson and Ma 2009, Brenna et al. 2009] and that ALA concentration in the diet was higher by 3.5%, the expected outcome of administering this diet to rats should be enhanced synthesis of EPA and DHA, yet such a dependency was not observed in the study. This may result from a lower content of B_6 vitamin in the modified diet, because deficiency of this vitamin leads to the inhibition of Δ_6-desaturase activity [Bordoni et al. 1998, Tsuge et al. 2000], as well as from a lower content of zinc in diet, because its deficiency facilitates enhanced β-oxidation of ALA [Clouet et al. 1989, Cunnane 2003, Eder and Kirchgessner 1996], and thus contributes to a decreasing pool of this acid in the body, which in turn disturbs conversion of its metabolites.

Linoleic acid (LA) supplied with feed is indispensable for the synthesis of AA in animal tissues in elongation and desaturation processes by GLA and DGLA [Okey et al. 1961, Witten and Holman 1952]. In the conducted experiment, a significant increase was observed in LA synthesis in livers of rats fed the modified diet, which could be due to a higher content of this acid in the diet as well as to a low supply of B_2 vitamin [Olpin and Baters 1982]. In contrast, the modified diet was observed to affect a decrease in the content of DGLA, which may result from a suppressed activity of Δ_6-desaturase of the microsomal fraction of liver hepatocytes which is influenced by the presence of carbohydrates in diet [Bolton-Smith et al. 1997, Jeffcoat and James 1977], and by deficiency of pyridoxine [She et al. 1994]. The increased content of AA in livers of rats administered the modified diet, affecting – though insignificantly – the summary content of n-6 fatty acids, may be due to the enhanced activity of Δ_5-desaturase as a result of a higher fat content in the diet [Jeffcoat and James 1977]. In addition, the increased pool of arachidonic acid might be ascribed to the conversion of oleic acid (C18:1, n-9), the content of which in the modified diet was higher by 11% compared to the basal diet [Jeffcoat and James 1977, Lands et al. 1990]. Such a phenomenon was observed by De Schrijver and Privett [1983] while applying the saccharose-containing diet. The changes observed in the synthesis of n-6 fatty acids may be of significance to the modulation of cells functions [Horrobin 1991].

Considering the fact that the B-group vitamins participate in fatty acids synthesis and that the saccharose-rich modified diet was poor in those components, complementary supplementation was applied in the experiment, which to some extent imitated nutritional patterns of the contemporary society. In liver, thiamine (as a thiamine diphosphate) serves the function of a co-factor of transketolase (EC 2.2.1.1) in the pentose cycle as a catabolic pathway of glucose obtained from digestion of the modified feed, where the resultant NADPH participates in fatty acids synthesis [Eitenmiller and Landen 1999, Thornalley 2005, Zhao and Zhong 2009]. Riboflavin, being an active coenzyme, functions as a carrier of hydrogen in oxidation-reduction reactions. Pyridoxine stimulates the elongation and denaturation of PUFAs [McCormick et al. 1988, Powers 2003]. Niacin as an amide of nicotinic acid is a substrate indispensable for the synthesis of NAD^+ and its phosphorylated form: $NADP^+$ – a coenzyme taking part in fatty acids synthesis [Garza 1998, Maiese et al. 2009]. The expected outcome of feeding the animals the modified diet supplemented with B-group vitamin was the fatty acid profile in their livers comparable to that determined in the group of animals fed the basal diet. However, such an effect was not achieved, because both fat content of liver and contents of individual fatty acids in that group of animals were comparable to the respective values noted in the group of animals receiving the modified diet, which means that the applied supplementation with B-group vitamins did not yield any improvement in this respect.

While analysing the entirety of changes in fatty acids contents of livers of the investigated animals, which occurred upon diet modification and its complementary supplementation with B-group vitamins and consisted in the decreased level of monoenoic and the increased level of polyenoic fatty acids, it may be speculated that those changes may increase the susceptibility to oxidative processes in the body, while through modifying the profile of n-6 family fatty acids they may affect the permeability of cellular membranes and the possibility of synthesizing pro-inflammatory tissue hormones.

CONCLUSIONS

The analysis of the results achieved demonstrated that:

1. Modification of diet composition, consisting of substituting whole grains of cereals with wheat flour and saccharose, and its complementary supplementation with B-group vitamins caused significantly higher body weight gains of the examined animals.

2. Modification of diet composition had a significant effect on the decreased level of monoenoic and the increased level of polyenoic fatty acids in rat livers.

3. B-group vitamins supplementation did not correct the changes in the fatty acid profile in rat livers caused by diet modification.

REFERENCES

Anderson B.M., Ma D.W., 2009. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis. 8, 33-53.

AOAC 2003. Official Methods of Analysis. Association of Official Analytical and Chemists, Gaithersburg, USA.

Bolton-Smith C., Woodward M., Tavendale R., 1997. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. Eur. Clin. Chem. Nutr. 51, 619-624.

Bordoni A., Hrelia S., Lorenzini A., Bergami R., Cabrini L., Biagi P.L., Tolomelli B., 1998. Dual influence of aging and vitamin B6 deficiency on delta-6-desaturation of essential fatty acids in rat liver. Prostagland. Leukot. Essent. Fatty Acids 58, 417-420.

Brenna J.T., Salem N., Sinclair A.J., Cunnane S.C., 2009. Alphalinolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostagland. Leucot. Essent. Fatty Acids 80, 85-91.

Bruckdorfer K.R., Khan I.H., Yudkin J., 1972. Fatty acid synthetase activity in the liver and adipose tissues of rats fed with various carbohydrates. Biochem. J. 129, 439-446.

Cabrini L., Bergami R., Fiorentini D., Marchetti M., Landi L., Tolomelli B., 1998. Vitamin B6 deficiency affects antioxidant defences in rat liver and heart. Biochem. Mol. Biol. Int. 46, 689-697.

Cawthorne M.A., Cornish S., 1979. Lipogenesis in vivo in lean and genetically obese (ob/ob) mice fed on diets with a high fat content. Int. J. Obes. 3, 83-90.

Clayton P., 2006. B6-responsive disorders: A model of vitamin dependency. J. Inherit. Metab. Dis. 29, 317-326.

Clouet P., Niot I., Bézard J., 1989. Pathway of α-linolenic acid through the mitochondrial outer membrane in the rat liver and influence on the rate of oxidation. Biochem. J. 263, 867-873.

Connor W.E., DeFrancesco C.A., Connor S.L., 1993. N-3 fatty acids from fish oil. Ann. NY Acad. Sci. 683, 16-34.

Cunnane S.C., 2003. The contribution of α-linolenic acid in flaxseed to human health. In: Flax – The Genus Linum. Eds A. Muir, N. Westcott. Taylor and Francis London, 150-180.

De Schrijver R., Privett O.S., 1983. Hepatic fatty acids and acyl desaturases in rats: Effects of dietary carbohydrate and essential fatty acids. J. Nutr. 113, 2217-2222.

Debski B., Bertrandt J., Klos A., Gralak M., 2007. The influence of folic acid, vitamins B2 and B6 supplementation on feed intake, body and organs weight, and liver fatty acids composition of rats subjected to 3 months moderate protein deprivation. Vet. Med. A, 54, 57-61.

Eder K., Kirchgessner M., 1996. Zinc deficiency and the desaturation of linoleic acid in rats force-fed fat-free diets. Biol. Trace Elem. Res. 54, 173-183.

Eitenmiller R.R., Landen W.O., 1999. Vitamin analyses for the health and food sciences. CRC Press Boca Raton, 271-276.

França C.F., Vienna L.M., 2010. The response of young and adult rats to the riboflavin supplementation. Braz. Arch. Biol. Technol. 53, 855-860.

Gandemer G., Durand G., Pascal G., 1983. Relative contribution of the main tissues and organs to body fatty acid synthesis in the rat. Lipids 18, 223-228.

Garza C., 1998. Niacin. In: Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B-6, vitamin B-12, pantothenic acid, biotin, and choline. Ed. R.M. Pitkin. National Academy Press Washington DC, 123-149.

Horrobin D.F., 1991. Interactions between n-3 and n-6 essential fatty acids (EFAs) in regulation of cardiovascular disorders and inflammation. Prostagland. Leukot. Essent. Fatty Acids 44, 127-131.

Jantarska D., Ratkowska B., Kunachowicz H., 2007. Wzbogacanie żywności – wartości deklarowane a rzeczywiste [Enrichment of food-declared and actual value]. Przem. Spoż. 61, 24-27 [in Polish].

Jeffcoat R., James A.T., 1977. Interrelationship between the dietary regulation of fatty acid synthesis and the fatty acyl-CoA desaturase. Lipids 12, 469-474.

Knoche H., Esders T.W., Koths K., Bloch K., 1973. Palmityl coenzyme A inhibition of fatty acid synthesis. Relief by bovine serum albumin and mycobacterial polysaccharides. J. Biol. Chem. 248, 2317-2322.

Lands W.E., Morris A., Libelt B., 1990. Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. Lipids 25, 505-516.

Maiese K., Chong Z.Z., Hou J., Shang Y.C., 2009. The vitamin nicotinamide, translating nutrition into clinical care. Molecules 14, 3446-3485.

McCormick D.B., Innis W.S.A., Merrill A.H. Jr., Bowers--Komro D.M., Oka M., Chastain J.L., 1988. An update on flavin metabolism in rats and humans. In: Flavin and flavoproteins. Eds D.E. Edmondson, D.B. McCormick. Walter de Gruyter New York, 459-471.

Mooney S., Leuendorf J.E., Hendrickson C., Hellmann H., 2009. Vitamin B6: A long known compound of surprising complexity. Molecules 14, 329-351.

Musch K., Ojakian M.A., Williams M.A., 1974. Comparison of alphalinolenate and oleate in lowering activity of lipogenic enzymes in rat liver: evidence for a greater effect of dietary linoleinate independent of food and carbohydrate intake. Biochem. Biophys. Acta 337, 343-348.

Okey R., Shannon A., Tinoco J., Ostwald R., Miljanich P., 1961. Fatty acid components of rat liver lipids: Effect of composition of the diet and of restricted access to food. J. Nutr. 75, 51-60.

Olpin S.E., Bates C.J., 1982. Lipid metabolism in riboflavin-deficient rats I. Effect of dietary lipids on riboflavin status and fatty acid profiles. Br. J. Nutr. 47, 577-588.

Pietruszka B., Brzozowska A., 1999. Vitamins and mineral supplement among adults in Central and Eastern Poland. Nutr. Res. 19, 817-826.

Powers H.J., 2003. Riboflavin (vitamin B-2) and health. Am. J. Clin. Nutr. 77, 1352-1360.

Reeves P.G., Nielsen F.H., Fahey G.C., 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76 rodent diet. J. Nutr. 123, 1939-1951.

Romsos D.R., Leveille G.A., 1974. Effect of dietary fructose on in vitro and in vivo fatty acid synthesis in the rat. Biochim. Biophys. Acta 360, 1-11.

Saito M., Kubo K., 2003. Relationship between tissue lipid peroxidation and peroxidizability index after α-linolenic, eicosapentaenoic, or docosahexaenoic acid intake in rats. Br. J. Nutr. 89, 19-28.

She Q.B., Hayakawa T., Tsuge H., 1994. Effect of vitamin B6 deficiency on linoleic acid desaturation in the arachidonic acid biosynthesis of rat liver microsomes. Biosci. Biotechnol. Biochem. 58, 459-463.

Sushko L.I., Lukienko P.I., 1981. Effect of vitamin B1 deficiency on xenobiotic hydroxylation and lipid peroxidation in rat liver microsomes. Farmakol. Toksikol. 2, 102-104.

Tapiero H., Ba G.N., Couvreur P., Tew K.D., 2002. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathogies. Biomed. Pharmacother. 56, 215-222.

Taysi S., 2005. Oxidant/antioxidant status in liver tissue of vitamin B6 deficient rats. Clin. Nutr. 24, 385-389.

Thornalley P.J., 2005. The potential role of thiamine (vitamin B1) in diabetic complications. Curr. Diabetes. Rev. 1, 287-298.

Toida S., Takahashi M., Shimizu H., Sato N., Shimomura Y., Kobayashi I., 1996. Effect of high sucrose feeding on fat accumulation in the male Wistar rat. Obes. Res. 4, 561-568.

Toussant M.J., Wilson M.D., Clarke S.D., 1981. Coordinate suppression of liver acetyl-CoA carboxylase and fatty acid synthetase by polyunsaturated fat. J. Nutr. 111, 146-153.

Trinder P., 1969. Quantitative determination of glucose using the GOP-PAP method. Clin. Biochem. 6, 24-27.

Tsuge H., Hotta N., Hayakawa T., 2000. Effects of vitamin B-6 on (n-3) polyunsaturated fatty acid metabolism. J. Nutr. 130, 333S-334S.

Volpe J., Vagelos P.R., 1974. Regulation of mammalian fatty acid synthetase. The roles of carbohydrate and insulin. Proc. Nati. Acad. Sci. USA 71, 889-893.

Witten P.W., Holman R.T., 1952. Polyethenoid fatty acid metabolism VI. Effect of pyridoxine on essential fatty acid conversions. Arch. Biochem. Biophys. 41, 266-273.

Zhao J., Zhong C.J., 2009. A review on research progress of transketolase. Neurosci. Bull. 25, 94-99.

OCENA, NA MODELU ZWIERZĘCYM, WPŁYWU ZMIANY SKŁADU DIETY I JEJ SUPLEMENTACJI WITAMINAMI Z GRUPY B NA PROFIL KWASÓW TŁUSZCZOWYCH W WĄTROBIE

STRESZCZENIE

Wstęp. Współczesna dieta ludzi charakteryzuje się znaczną ilością produktów przetworzonych i oczyszczonych, zubożonych poprzez zastosowane procesy technologiczne, m.in. w witaminy z grupy B biorące udział w syntezie kwasów tłuszczowych. Jednym ze sposobów zapobiegania ich niedostatecznemu spożyciu jest wzbogacanie nimi produktów spożywczych. Dlatego postanowiono zbadać, na modelu zwierzęcym, czy może dochodzić u szczurów do zmiany profilu kwasów tłuszczowych syntetyzowanych w wątrobie pod wpływem zmiany składu diety, w której składniki całościowe (pełne ziarna pszenicy i kukurydzy) zastąpiono izokalorycznie białą mąką i sacharozą oraz zastosowano suplementację uzupełniającą wybranymi witaminami z grupy B.

Materiał i metody. Badania przeprowadzono na 30 samcach szczura w wieku 5 miesięcy. Grupa I otrzymywała paszę podstawową (Labofed B), grupy II i III – paszę zmodyfikowaną, w której 83,5% pszenicy obecnej w paszy podstawowej zostało zastąpione mąką pszenną (typ 500), a 50% kukurydzy – sacharozą. W paszy oznaczono podstawowy skład chemiczny, zawartość witamin B_1, B_2, B_6 i PP metodą HPLC oraz profil kwasów tłuszczowych zmodyfikowaną metodą Folcha z użyciem chromatografu gazowego. Grupy I i II do picia otrzymywały w wolnym dostępie wodę, zwierzęta grupy III otrzymywały 25 ml wodnego roztworu witamin w ilości: B_1 – 0,94 mg, B_2 – 0,48 mg, B_6 – 0,5 mg, PP – 1,9 mg. W grupie III uzupełniono różnice w zawartości tych witamin pomiędzy paszami powstałe po zamianie składników, co do pewnego stopnia imitowało sposób suplementacji u ludzi. W surowicy krwi oznaczono stężenie glukozy, a w wypreparowanych wątrobach zwierząt określono zawartość tłuszczu metodą Soxhleta oraz profil kwasów tłuszczowych zmodyfikowaną metodą Folcha z użyciem chromatografu gazowego.

Wyniki. Zwierzęta, pomimo porównywalnych ilości spożywanej paszy, różniły się przyrostami masy ciała osiąganymi w czasie doświadczenia. Zarówno masa wątroby, jak i zawartego w niej tłuszczu nie różniły się istotnie między badanymi grupami zwierząt. Sumarycznie stwierdzono w wątrobach grupy zwierząt żywionych paszą zmodyfikowaną zmniejszenie zawartości monoenowych, a wzrost zawartości polienowych kwasów tłuszczowych w stosunku do zwierząt żywionych paszą podstawową. Oznaczony profil kwasów tłuszczowych w wątrobach zwierząt, w żywieniu których zastosowano dietę zmodyfikowaną oraz suplementację witaminami B_1, B_2, B_6 oraz PP, nie różnił się istotnie od profilu kwasów tłuszczowych oznaczonego w wątrobach szczurów żywionych paszą zmodyfikowaną.

Wnioski. Zmiana składu diety – polegająca na zastąpieniu pełnych ziaren zbóż mąką pszenną i sacharozą oraz jej uzupełniającej suplementacji wybranymi witaminami z grupy B – spowodowała istotnie większe przyrosty masy ciała badanych zwierząt. Zmiana składu diety wpłynęła istotnie na zmniejszenie zawartości monoenowych, a wzrost polienowych kwasów tłuszczowych w wątrobach szczurów. Zastosowana suplementacja witaminami z grupy B nie korygowała zmiany profilu kwasów tłuszczowych w wątrobach zwierząt wynikającej z modyfikacji diety.

Słowa kluczowe: witaminy z grupy B, samce szczura, wątroba, profil kwasów tłuszczowych

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