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ORIGINAL PAPER

ANIMAL-MODEL ASSESSMENT OF THE IMPACT OF DIET MODIFICATION AND VITAMIN B SUPPLEMENTATION ON THE PERICARDIAL FATTY TISSUE AND ITS FATTY ACID PROFILE*

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Abstract

The objective of this research is to examine, on an animal model, the consequences of a diet modification where ingredients such as full wheat and maize grain have been isocalorically replaced with white flour and sucrose. This research also examines how dietary supplementation (adequate and excessive one) with synthetic B vitamins affects the amount of pericardial fatty tissue and its fatty acid profile. 48 male Wistar rats were divided into 4 groups: group I was fed Basic Diet (BD), while groups II-IV were given Modified Diet (MD), in which 83.5% of wheat was replaced with wheat flour and 50% of maize was replaced with sucrose. Animals from groups I-II received only water to drink, while group III was given an aqueous solution of vitamins supplementing the deficiency created by the dietary modification (MD+Adequate Supplementation); group IV received a solution in order to supplement both the deficiency resulting from the dietary modification and the recommended prophylactic dose of vitamins (MD+Excessive Supplementation). Subsequently, the blood serum was examined to determine the glucose level. The heart and pericardial fats were dissected and their content of fatty acids was determined by the GC method. It has been ascertained that MD led to a significant increase in feed and energy consumption but did not influence the increase in body mass, heart mass, pericardial fatty tissue, or glucose concentration in the blood of the animals. However, it did cause changes in its fatty acid profile, lowering the content of MUFA and increasing PUFA. The MD+AS supplementation did not yield the same effect. The MD+ES supplementation combined with the lowered consumption of feed caused an increase in glycaemia and changes in the profile of pericardial fatty tissue, similar to those observed in the control group.

Keywords: rats, thiamine, riboflavin, pyridoxine, niacin, fatty acids profile.

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INTRODUCTION

In 2015, the consumption of sugar in Poland was 1.09 kg a month per one inhabitant of a household, which is 6 teaspoons per day (Gus 2016). A high level of sucrose consumption leads not only to the accumulation of fatty tissue, but also to metabolic disorders such as glucose tolerance disorder, insulin resistance and dyslipidemia (Bilek et al. 2014). In Poland and elsewhere in the world, the recommended consumption of sucrose is below 10% of the energy value of a diet (Jarosz 2012). The research conducted among various subpopulation in Poland showed that the energy coming from sucrose in a diet constituted from 11 to 18% (Terlikowska et al. 2012, Charzewska et al. 2013, Włodarek, Głąbska 2014). A diet containing a significant amount of processed and purified products, impoverished by technological processes applied in their production, is poor in vitamins B1, B2, B6 and niacin, which (in the form of co-enzymes) are essential for controlling metabolic changes, including the metabolism of carbohydrates. Therefore, to avoid subclinical and clinical symptoms of vitamin shortage, the World Food Conference (Codex Alimentarius 1994) recommends that a food intervention in such cases should include enrichment of a diet with the absent nutrients and supplementation, the latter only periodically and in some situations. However, a large share of the human population uses supplementation, often on their own authority and without medical approval, exceeding Recommended Dietary Allowances (RDA) several or even dozens of times.

The research conducted on model animals testing a diet which included both 10% of sucrose as well as its supplementation with synthetic B group vitamins showed an increase in periorganic fat content (Friedrich, Goluch -Koniuszy 2009). Trapping significant amounts of free fatty acids from blood, pericardial tissue takes part in the process of lipogenesis and constitutes a local energy source for the cardiac muscle; it is characterized by a rapid rate of lipolysis and it also protects the cardiac muscle from exposure to extreme concentrations of fatty acids. An increased amount of pericardial fatty tissue may lead to a higher synthesis of proinflammatory factors and these factors which can change the activity of blood vessels and the cardiac muscle (Iacobellis 2009).

Therefore, this research has aimed to verify, using an animal model, if a modification of the dietary components and dietary supplementation (adequate and excessive one) with synthetic group B vitamins might lead to a change in the amount of pericardial fatty tissue and its profile of fatty acids.

MATERIAL AND METHODS

Study material

The design of the study is shown in Figure 1. Having received an approval from the Local Commission of Ethics in Szczecin (approval no 8/2015), we conducted the research on 48 male Wistar rats, aged 5 months, with the

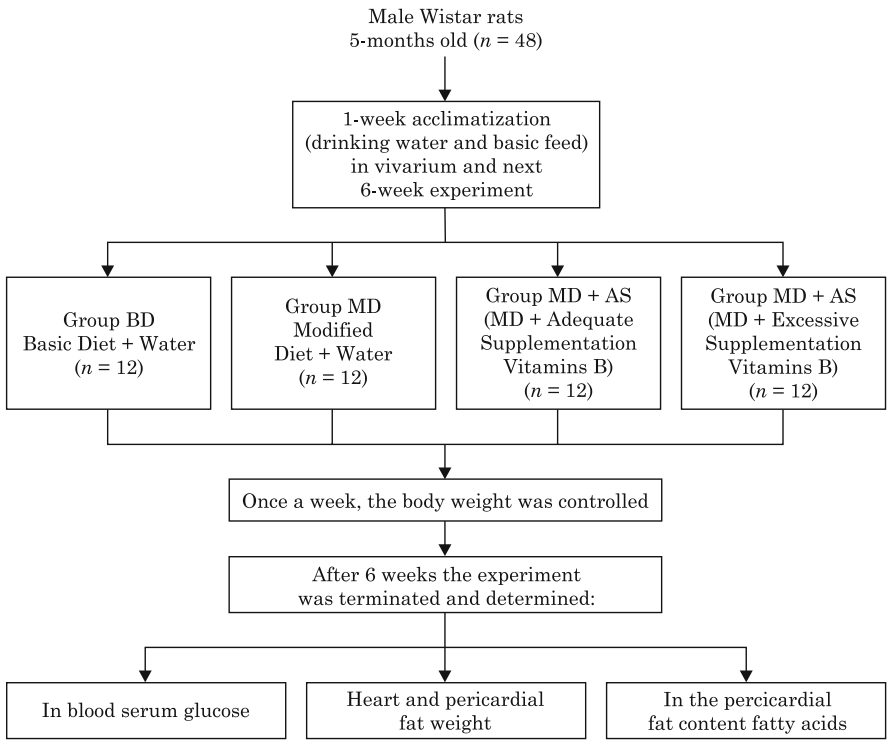

Fig. 1. Study design

initial body mass of 393.1 ± 25.4 g, which were placed in individual cages. Animals came from a stock maintained at the Department and Plant of Toxicology of Medical University in Poznań. After a week of acclimatization (drinking water and basic feed BD) in vivarium conditions (temp. 21-22°C, relative air humidity 55-60%, cycle of light and dark 12/12 h), animals were divided into four equal nutrition groups (n = 12), which were fed *ad libitum* with granulated feed produced by the Morawski Feed and Concentrate Production in Kcynia (Poland).

The basic feed (BD) (Labofed B) corresponded to the feed requirements AIN-93M (REEVS et al. 2003) and included full wheat and maize grain. In the modified feed (MD), 83.5% of wheat grain from the basic feed was replaced by wheat flour (type 500) and 50% of maize grain was replaced by sucrose (Table 1).

Table 1

Ingredients and the chemical composition of feeds used in the experiment

Specification	Basic Diet (BD)	Modified Diet (MD)
	Component	
Wheat grain (g)	36.4	6.0
Maize grain (g)	20.0	10.0
Wheat bran (g)	20.0	20.0
Dry whey (g)	3.0	3.0
NaCl (g)	0.27	0.27
Soyabean meal 48%* (g)	17.0	17.0
Fodder chalk** (g)	2.0	2.0
Phosphate 1-Ca*** (g)	0.27	0.27
Mineral and vitamin mix# (g)	0.8	0.8
Wheat flour type 500 (g)	-	30.4
Sucrose (g)	-	10.0
	Chemical composition	
Total protein (g)	19.1	17.8
Crude fat (g)	3.08	4.18
Carbohydrates (g)	62.3	63.8
Crude fibre (g)	2.91	2.73
Dry weight (g)	91.4	91.2
Total ash (g)	6.94	6.47
Thiamine (mg)	2.5	0.62
Riboflavin (mg)	2.1	1.14
Pyridoxine (mg)	2.35	1.35
Niacin (mg)	8.6	4.8
Gross energy (kj g^{-1})	16.6	16.9
Metabolizable energy (kj g^{-1})	14.8	15.2

Explanations:
* soyabean meal 48%: extracted, content 48% crude protein and 7% fibre;
** fodder chalk: content per kg: Ca 350 g, Mg 3.20 g; Na 10.00 mg, P 15.00 mg;
*** phosphate 1-CA: additive contains min, 22% P and 15% Ca;
# vitamin and mineral Premix LRM contents per kg: [IU] A 1 500 000, vit, D_3 100 000; mg: vit, E 8000; vit, K 300, vit, B_1 1200, vit, B_2 1200, vit, B_6 1000, vit, B_{12} 8, Se 100, Fe 16 000, Mn 4500, Zn 6000, Cu 1300, I 100, Co 200

The prepared feed mixtures were subjected to chemical analysis (AOAC 2012) in order to determine the content of the following: total nitrogen (with the Kjeldahl method) converted into an amount of protein; crude fat (with the Soxhlet method); dry matter (with the gravimetric method), and ash (with the gravimetric method). The National Research Institute of Animal

Production in Szczecin established the content of vitamins B_1, B_2, B_6 and niacin (by HPLC on an Agilent 1200SL apparatus) (Table 1) and the content of raw fibre (with the weighing method PB-02/PS on an apparatus ANKOM 220). The content of carbohydrates was evaluated from the difference between dry mass and the sum of the other solid ingredients. The content of gross energy and metabolic energy was evaluated with the use of commonly applied energy equivalents (FAO 2003).

The amount of fatty acids in the feed (Table 2) was determined with the method of gas chromatography (GC). To achieve this, first the fat was extracted with the BLIGH and DYER (1959) method and next the methyl esters of

Table 2

Content of fatty acids in fed male rat (% in total fatty acids)

Fatty acids	BD	MD	Difference
SFA			
Myristic acid	0.22	0.29	0.07
Palmitic acid	29.6	25.5	4.09
Stearic acid	5.92	3.67	2.25
MUFA			
Oleopalmitic acid	0	0	0
Oleic acid	28.1	24.8	3.31
Eicosanoic acid	0.28	0.24	0.04
Erucic acid	0.52	1.09	0.57
PUFA			
α-Linolenic acid ALA	2.26	2.44	0.18
Eicosapentaenoic acid EPA	0	0	0
Docosapentaenoic acid DPA	0	0	0
Docosahexaenoic acid DHA	0	0	0
Total *n*-3	2.26	2.44	0.18
Linoleic acid LA	31.9	41.3	9.45
Eicosadienoic acid	0	0	0
Dihomo-γ-linolenic acid DGLA	0	0	0
Arachidonic acid AA	0	0	0
Total *n*-6	31.9	41.3	9.45
SFA	35.7	29.5	6.27
MUFA	28.9	26.1	2.78
PUFA	34.1	43.7	9.63
n-6/n-3	14.1	16.9	2.83

Each value is a mean of 2 replicates.
BD – Basic Diet, MD – Modified Diet, MD+AS – Modified Diet + Adequate Supplementation, MD+ES – Modified Diet + Excessive Supplementation

fatty acids (FAME) were prepared according to PN-EN ISO 12966-2; 2011. The FAMEs were separated on a gas chromatographer (Agilent 7890A), coupled with a mass spectrometer (Agilent 7975C) and equipped with a split/split less type injector. Conditions of the FAMEs separation were as follows: SPTM 2560 column, 100 x 0.25 mm ID, 0.20 μm film, catalogue no. 24056, carrier gas helium at a constant flow rate of 1.2 cm^3 min^{-1}, split 1:50, injector temp. 220°C; detector temp. 220°C; programmed furnace temperature: 140°C (5 min) increased to 240°C at a rate of 4°C min^{-1}, analysis time: 45 min. The qualitative interpretation of chromatograms was based on the comparison of retention times and mass spectra of the particular FAME of a sample with standards by Sigma company analogous to FAME (Lipid Standard). Analytical grade solvents (methanol, chloroform) were purchased from POCH S.A. (Poland). The reagents 14% BF3 in MeOH came from Supelco (Bellefonte, USA).

During the experiment, group I received the basic feed (BD), while groups II-IV received the modified feed (MD). Animals from groups I – II received tap water, which had been resting for some time, while animals from groups III and IV received 25 ml of water solution with active ingredients of vitamins B1 (*Thiamini hydrochloridum*), B2 (*Riboflavinum*), B6 (*Pyridoxinum hydrochloricum*) and niacin (*Nicotinamidum*), which had come from commonly available pharmaceuticals. Animals from group III (MD + Adequate Supplementation) received 25 ml of water solution with the following vitamins: B1 – 0.94 mg, B2 – 0.48 mg, B6 – 0.5 mg, niacin – 1.9 mg. Animals from group IV (MD + Excessive Supplementation) received: B1 – 3.1 mg, B2 – 2.3 mg, B6 – 2.4 mg, niacin – 6.65 mg. Vitamins were given in amounts calculated according to the method of supplementation, so that in the third group (MD+AS) the differences which occurred after dietary modifications were complemented (imitating diet enrichment). In the fourth group (MD+ES) both the differences between amounts of vitamins between BD and MD were taken into account, as well as the amounts which exceeded the RDA norms for people 2 to 4 times, calculated into amounts appropriate for a rat, which imitated dietary supplementation with prophylactic amounts of these vitamins by people. The complete amount of vitamins given to this group exceeded the recommended daily dose, depending on the type of vitamins, in the following way: B_1 – 2.3 times, B_2 – 3.8 times, B_6 – 3.8 times, niacin – 2.5 times, which to some extent imitates supplementation used by people.

The research, after one week of an acclimatization period, took 6 weeks, during which the amounts of consumed feed and drank fluids were evaluated daily. Once a week the body mass was controlled. Twelve hours before finishing the experiment, animals were stopped to be fed. Next, they were put to sleep with the anaesthetic Ketanest (*Pfizer Ireland Pharmaceuticals*) by intramuscularly injecting a dose of 10 mg kg^{-1} of body mass, and blood was taken from their hearts. Immediately after euthanizing the animals, the heart and pericardial fat (epicardial fat between the heart muscle and the visceral layer of pericardium, and paracardial fat outside the pericar-

dium) were dissected and weighed to an accuracy of 0.001 g. In the fat, the content of fatty acids was determined with the GC method. FAME was prepared and separated with the same method as in the case of fat from the feed.

Chemical analyses

After centrifuging the clot (at a temp. of 4°C, at 3500 r.p.m., for 20 min) in an MPW-350R centrifuge, the serum was obtained, in which the concentration of glucose was determined with the colorimetric method (Trinder 1969) by using biotests made by BioSystems (catalogue no 11803).

Calculations and statistical analysis

The Feed Conversion Ratio (FCR) was evaluated from the formula: FCR = total consumption of feed (g) per final body mass (g) (Johnson, Gee 1986).

The results, after testing the normality of distribution with the Shapiro-Wilk test and the variation of homogeneity with the Laven's test, were calculated logarithmically and evaluated statistically. An Anova analysis of variation with the Tukey's test was applied, estimating the differences between the parameters under research at the levels of significance $P \leq 0.05$ and $P \leq 0.01$, with the use of Statistica®12.0 statistical program.

RESULTS AND DISCUSSION

It was ascertained that significantly ($P \leq 0.01$) the highest consumption of feed was characteristic for animals fed MD (Table 3), which had been similarly ascertained by other authors (De Castro et al. 2008). It had been shown previously (Spadaro et al. 2015) that the consumption of monosaccharides influenced the regulation of appetite in the hypothalamus by introducing changes to the signalization in the hunger and satiety centre, and had a stimulating impact on serotonin gene expression (*Sert*). Both the adequate (MD+AS) and excessive supplementation (MD+ES) in the modified diet had a significant impact by decreasing the feed consumption among animals, which declined to amounts observed in the group fed BD, thereby indicating the regulating influence of vitamins in this respect.

Values of the Feed Conversion Ratio (FCR) showed that, significantly ($P \leq 0.01$), animals from the MD feeding group utilized the feed for gaining the final body mass the best (2.49%), while the worst feed utilization occurred among animals from group MD+ES (2.10%). The highest energy consumption was ascertained in the group fed MD (4208 kJ) and the lowest – in the control group fed BD (3664 kJ).

Table 3

Effects of the type of diet and B-group vitamin supplementation on the feed intake, body weight gain and pericardial fat content in male Wistar rats ($\bar{x}$ ± SD, n = 48)

Parameter	BD (n = 12)	MD (n = 12)	MD+AS (n = 12)	MD+ES (n = 12)	P value
Initial body weight (g)	394.4 ± 30.9	396.2 ± 29.1	391.4 ± 28.4	389.3 ± 19.7	0.939
Final body weight (g)	416.6 ± 32.4	423.4 ± 36.3	418.0 ± 31.5	413.9 ± 15.3	0.910
Feed intake (g)	915.9^{B} ± 39.8	1049.2^{Aa} ± 44.1	994.0^{Ab}±60.9	910.6^{B}±32.8	<0.001
FCR (%)	2.21^{Bb} ± 0.1	2.49^{A} ± 0.2	2.38^{a} ± 0.1	2.10^{Bb} ± 0.1	<0.001
Energy intake (kJ 100 g^{-1} body weight)	3664.7^{B} ± 238	4208.6^{A} ± 302	4027.6^{Aa} ± 214	3721.1^{Bb} ± 156	<0.001
Body weight gain (g 100 g^{-1} feed)	2.61 ± 1.5	2.68 ± 1.8	2.69 ± 0.9	3.1 ± 0.6	0.326
Liquids intake (ml per day)	27.4^{Bb} ± 2.2	29.0 ± 2.6	30.5^{a} ± 2.5	31.0^{A} ± 2.1	0.002
Liquids intake (ml 100 g^{-1} body weight)	270.0^{Aa} ± 24.5	282.0 ± 23.2	301.2^{b} ± 34.7	306.5^{B} ± 16.8	0.003
Thiamine intake (g 100 g^{-1} body weight)	5.5^{C} ± 0.4	1.54^{D} ± 0.1	10.8^{B} ± 0.8	32.1^{A} ± 1.2	<0.001
Riboflavin intake (g 100 g^{-1} body weight)	4.6^{C} ± 0.3	2.8^{D} ± 0.2	7.4^{B} ± 0.5	25.3^{A} ± 0.9	<0.001
Pyridoxine intake (g 100 g^{-1} body weight)	5.2^{C} ± 0.3	3.4^{D} ± 0.2	8.1^{B} ± 0.5	26.8^{A} ± 1.0	<0.001
Niacin intake (g 100 g^{-1} body weight)	19.0^{C} ± 1.2	12.0^{D} ± 0.9	30.2^{B} ± 1.9	76.6^{A} ± 2.8	<0.001
Heart relative weight (g 100 g^{-1} body weight)	0.283^{a} ± 0.02	0.263^{b} ± 0.02	0.262^{b} ± 0.02	0.259^{b} ± 0.01	0.001
Pericardial fat (g 100 g^{-1} feed)	0.045 ± 0.01	0.039 ± 0.01	0.042 ± 0.01	0.040 ± 0.01	0.465
Pericardial fat (g 100 g^{-1} body weight)	0.099 ± 0.02	0.097 ± 0.02	0.099 ± 0.02	0.089 ± 0.03	0.443
Pericardial fat (g 100 g^{-1} heart weight)	35.2 ± 6.7	36.8 ± 7.8	35.6 ± 11.9	34.4 ± 11.3	0.942
Glucose (mmol l^{-1})	6.22^{b} ± 0.7	6.62 ± 1.3	6.33 ± 0.6	7.49^{a} ± 1.3	0.038

BD – Basic Diet, MD – Modified Diet, MD+AS – Modified Diet + Adequate Supplementation; MD+ES – Modified Diet + Excessive Supplementation; FCR – Feed Conversion Ratio;
^{aAbBcC} – within rows means assigned different superscripts differ significantly at $P < 0.05$ (small letters) or $P < 0.01$ (capitals)

Despite significant differences in the feed and energy consumption between the groups of animals under research, an almost equal final body mass among all the groups was observed. A similar result has been reported by other researchers (De Castro et al. 2008, Sheludiakova et al. 2012).

The vitamin supplementation applied did not influence the final body mass significantly and similar results were achieved by França and Vianna (2010).

Despite the known influence of a diet containing sucrose on the increase in body mass (Kendig et al. 2014), no such effect was found in our research in animals fed MD compared to the group fed BD. This might have been the result of the fact that proper amounts of group B vitamins are crucial for the synthesis of fatty acids from glucose and fructose, which supply carbon elements and NADPH, as the said vitamins are cofactors in these changes, and it is exactly the content of these that was reduced by 42 - 75% in the MD feed. Neither did the applied supplementation have a significant impact on the body mass gain in animals under research, which can be explained by their significantly worse utilization of feed (lower levels of the FCR indicator). Significantly highest ($P \leq 0.05$) consumption of liquids was ascertained in animals supplemented (MD+AS, MD+ES), in comparison to animals fed BD, which was probably induced by the osmolality of plasma being raised by the consumed sucrose.

In our research, significantly the highest heart weight was characteristic for animals fed the basic feed (BD) – Table 3. The applied dietary modification as well as supplementation significantly decreased the heart weight. It was ascertained that the glucose concentration in blood serum of the animals was significantly ($P \leq 0.05$) the highest in animals fed MD+ES in comparison to the group fed BD. An increase in the glucose concentration in this group of animals could have resulted from the higher supply of niacin in comparison to other groups. An excessive amount of this vitamin in a diet causes an increase in the glucose concentration in blood, even if simultaneous supplementation with thiamine, known for normalizing the metabolism of carbohydrates, is applied (Thornalley 2005).

Pericardial fatty tissue, capturing significant amounts of free fatty acids from blood, participates not only in the process of lipogenesis, but also in lipolysis, down to the determined physiological minimum (Bjørndal et al. 2011). However, the MD feed did not significantly affect the synthesis of pericardial fat in the examined animals (Table 3), which may be a positive finding because in pathological conditions this tissue produces numerous factors tied to insulin resistance, inflammation and proatherogenic mediators (Aslanabadi et al. 2014). The supplementation did not change anything in this respect, despite the fact that increased glycemia providing glycerol as substrate to triacyloglycerols synthesis was found in the group fed MD+ES, which could lead to elevated pericardial fatty tissue synthesis. No such result was observed, which indicates a modifying role of the applied vitamins in the synthesis of fatty acids.

It was ascertained that the MD group animals consumed significantly more ($P \leq 0.01$) of myristic, erucic, and linoleic acids, and less of stearic acid with their feed, compared to animals fed BD (Table 4). In total, the MD group animals consumed significantly less of SFA and significantly more of

Table 4

Influence of a diet's content and its supplementation with chosen group B vitamins on the amount of consumed fatty acids by male rats ($\overline{x}$ ± SD, n = 48)

Fatty acids (g 100 g^{-1} feed)	BD (n = 12)	MD (n = 12)	MD+AS (n = 12)	MD+ES (n = 12)	P *value*
	SFA				
Myristic acid	$0.47^{A} \pm 0.03$	$0.71^{B} \pm 0.05$	$0.68^{B} \pm 0.04$	$0.63^{C} \pm 0.03$	<0.001
Palmitic acid	$65.4^{Aa} \pm 4.25$	$63.5^{A} \pm 4.56$	$60.8^{b} \pm 3.23$	$56.2^{Bc} \pm 2.35$	<0.001
Stearic acid	$13.1^{A} \pm 0.85$	$9.1^{B} \pm 0.66$	$8.73^{Ba} \pm 0.5$	$8.07^{Cb} \pm 0.34$	<0.001
	MUFA				
Oleopalmitic acid	0	0	0	0	-
Oleic acid	$62.0^{A} \pm 4.04$	$61.7^{A} \pm 4.43$	$59.1^{a} \pm 3.14$	$54.6^{Bb} \pm 2.28$	<0.001
Eicosanoic acid	$0.61^{A} \pm 0.04$	$0.59^{AC} \pm 0.04$	$0.56^{BCa} \pm 0.03$	$0.52^{Bb} \pm 0.02$	0.001
Erucic acid	$1.15^{C} \pm 0.07$	$2.70^{A} \pm 0.19$	$2.59^{ABa} \pm 0.14$	$2.39^{Bb} \pm 0.10$	<0.001
	PUFA				
α-Linolenic acid	$4.99^{C} \pm 0.32$	$6.08^{A} \pm 0.44$	$5.81^{ABa} \pm 0.31$	$5.37^{Bb} \pm 0.22$	<0.001
Eicosapentaenoic acid	0	0	0	0	-
Docosapentaenoic acid	0	0	0	0	-
Docosahexaenoic acid	0	0	0	0	-
Total n-3	$4.99^{C} \pm 0.32$	$6.08^{A} \pm 0.44$	$5.81^{ABa} \pm 0.31$	$5.37^{Bb} \pm 0.22$	<0.001
Linoleic acid	$70.3^{C} \pm 4.57$	$103^{A} \pm 7.38$	$98.4^{ABa} \pm 5.23$	$90.9^{Bb} \pm 3.81$	<0.001
Eicosadienoic acid	0	0	0	0	-
Dihomo-γ-linolenic acid	0	0	0	0	-
Arachidonic acid	0	0	0	0	-
Total n-6	$70.3^{C} \pm 4.57$	$103^{A} \pm 7.38$	$98.4^{ABa} \pm 5.23$	$90.9^{Bb} \pm 3.81$	<0.001
SFA	$78.9^{Aa} \pm 5.13$	$73.4^{ACb} \pm 5.27$	$70.2^{BCb} \pm 3.73$	$64.9^{Bc} \pm 2.72$	<0.001
MUFA	$63.8^{A} \pm 4.15$	$65.0^{A} \pm 4.67$	$62.2^{a} \pm 3.31$	$57.5^{Bb} \pm 2.41$	<0.001
PUFA	$4.99^{C} \pm 0.32$	$6.08^{A} \pm 0.44$	$5.81^{ABa} \pm 0.31$	$5.37^{Bb} \pm 0.22$	<0.001
Total unsaturated	$68.8^{A} \pm 4.47$	$71.1^{A} \pm 5.11$	$68.1^{a} \pm 3.62$	$62.9^{Bb} \pm 2.63$	<0.001
n-6/n-3	14.09	16.92	16.92	16.92	

Each value is a mean of 2 replicates.
BD – Basic Diet, MD – Modified Diet, MD+AS – Modified Diet + Adequate Supplementation; MD+ES – Modified Diet + Excessive Supplementation,
$^{\alpha AbBcC}$ – within rows means assigned different superscripts differ significantly at $P < 0.05$ (small letters) or $P < 0.01$ (capitals)

PUFA compared to animals from group BD, which resulted from the modification of dietary ingredients and partial replacement of full grain in BD with flour and sucrose in MD (Table 2).

The adequate supplementation with synthetic vitamins (MD+AS) did not significantly change the structure of the consumed fatty acids. However, the

excessive supplementation of the modified feed (MD+ES) significantly ($P \leq 0.01$) contributed to a lower consumption of myristic acid, palmitic, stearic, oleic, eicosanoic, erucic, and α-linoleic acids and a lower consumption of linoleic acid in comparison to the animals from the control group (BD). Overall, the animals from this group consumed significantly ($P \leq 0.01$) less SFA, MUFA and PUFA acids than the ones fed MD. The observed consumption of acids in this group of animals indicated some normalizing influence of the excessive supplementation with B vitamins, as its consumption was similar in the amount to that observed in the control group BD. The amendments to the composition of the diet and its supplementation caused an increase in the value of the n-6/n-3 acid ratio.

Our analysis of the profile of fatty acids in the pericardial fatty tissue (Table 5) showed a significant ($P < 0.01$) decrease in the myristic, palmitoleic and oleic acid content under the influence of MD. In general, a decrease in the MUFA acid content and an increase in the PUFA content were observed in this group of animals. A decrease in the MUFA content in pericardial fatty tissue might have been a consequence of the deficiency of niacin in MD, because these acids are synthesized from SFA acids with the participation of the enzyme system Δ9-desaturasis, and oxygen and NADH or NADPH are necessary for these reactions. An increase in the PUFA content results from its higher content in the feed and its higher consumption, which is not beneficial because these acids are more susceptible to peroxidation in an organism, especially at some deficiency of thiamin and pyridoxine in a diet. In the present experiment, the MD diet did not significantly change the fatty acid profile from the n-3 acid series, but it effected a significant ($P \leq 0.01$) increase of the synthesis of n-6 acids.

In rats, in which the synthesis of EPA and DHA from ALA acid is several times more efficient than in human beings and other mammals (Anadreson, Ma 2009), no such effect was observed as its content was higher by only 0.18% in the MD feed. This may have resulted from a smaller content of vitamin B6 in the MD feed, and deficiency of this vitamin leads to the suppression of Δ_6-desaturases activity. LA acid supplied with feed is necessary for the synthesis of arachidonic acid AA in animal tissue. Despite the higher content of LA acid in the feed (by 9.45%) and its significantly ($P \leq 0.01$) higher consumption with the MD feed, no significantly increased synthesis of LA and AA acids in pericardial fatty tissue in this group of animals was observed compared to the control group BD. This is positive because free AA acid is the synthesis precursor with the use of cyclooxygenases and lipogenases of eicosanoids, which have a wide range of biological roles, for instance they have inflammatory properties.

The expected result of feeding MD+AS to animals was to observe the fatty acid profile in pericardial tissue that would be comparable to that observed in the animals fed MD. However, the applied excessive supplementation (MD+ES) resulted in a significant ($P < 0.01$) rise in DPA acid, all n-3 acids and eicosanoic acid compared to animals fed the modified feed (MD).

Table 5

Content of fatty acids in pericardial fat in Wistar male rat depending on a diet and supplementation B group vitamins ($\bar{x} \pm SD$, $n = 48$)

Fatty acids (%)	BD ($n = 12$)	MD ($n = 12$)	MD+AS ($n = 12$)	MD+ES ($n = 12$)	P value
SFA					
Lauric acid	$0.03^{A} \pm 0.01$	0.02 ± 0.01	$0.02^{B} \pm 0.01$	$0.02^{B} \pm 0.01$	<0.001
Myristic acid	$1.31^{A} \pm 0.27$	$0.89^{B} \pm 0.25$	$0.73^{B} \pm 0.29$	$0.75^{B} \pm 0.20$	<0.001
Pentadecanoic acid	0.14 ± 0.04	0.15 ± 0.03	0.15 ± 0.02	0.17 ± 0.05	0.218
Palmitic acid	$26.0^{a} \pm 1.72$	25.1 ± 2.21	24.4 ± 2.05	$23.7^{b} \pm 1.32$	0.027
Stearic acid	8.3 ± 2.03	10.0 ± 3.11	11.3 ± 2.62	10.9 ± 3.43	0.066
MUFA					
Oleopalmitic acid	$3.04^{A} \pm 0.78$	$1.35^{B} \pm 0.65$	$1.10^{B} \pm 0.34$	$1.30^{B} \pm 0.62$	<0.001
Oleic acid	$27.8^{A} \pm 2.39$	$23.1^{B} \pm 2.26$	$21.7^{B} \pm 2.00$	$22.8^{B} \pm 3.47$	<0.001
Eicosanoic acid	0.48 ± 0.12	0.47 ± 0.10	$0.39^{b} \pm 0.10$	$0.54^{a} \pm 0.13$	0.015
Nervonic acid	$0.37^{b} \pm 0.16$	0.50 ± 0.42	$0.85^{a} \pm 0.52$	$0.84^{a} \pm 0.45$	<0.001
PUFA					
α -linolenic acid	$1.04^{b} \pm 0.23$	1.17 ± 0.27	1.26 ± 0.17	$1.37^{a} \pm 0.27$	0.010
Docosapentaenoic acid	$0.07^{b} \pm 0.03$	$0.09^{b} \pm 0.08$	0.16 ± 0.11	$0.21^{a} \pm 0.10$	0.001
Total n-3	$1.11^{Bb} \pm 0.23$	$1.27^{b} \pm 0.26$	$1.41^{a} \pm 0.20$	$1.59^{Aa} \pm 0.26$	<0.001
Linoleic acid	26.8 ± 3.40	30.5 ± 7.49	28.2 ± 6.01	28.2 ± 5.81	0.495
Eicosadienoic acid	$0.30^{b} \pm 0.10$	0.45 ± 0.27	$0.67^{a} \pm 0.41$	$0.63^{a} \pm 0.28$	0.006
Dihomo-γ–linolenic acid	0.13 ± 0.04	0.28 ± 0.47	0.27 ± 0.14	0.28 ± 0.14	0.422
Arachidonic acid	$4.27^{b} \pm 1,97$	6.03 ± 4.41	$8.78^{a} \pm 4.61$	8.38 ± 4.55	0.029
Total n-6	$31.5^{B} \pm 2.58$	$37.3^{A} \pm 3.58$	$37.9^{A} \pm 2.22$	$37.5^{A} \pm 1.79$	<0.001
SFA	35.8 ± 3.49	36.1 ± 4.54	36.7 ± 3.08	35.5 ± 4.16	0.907
MUFA	$31.6^{A} \pm 2.94$	$25.4^{B} \pm 2.58$	$24.0^{B} \pm 2.03$	$25.4^{B} \pm 3.68$	<0.001
PUFA	$32.6^{A} \pm 2.76$	$38.5^{B} \pm 3.71$	$39.3^{B} \pm 2.29$	$39.1^{B} \pm 1.80$	<0.001
n-6/n-3	$29.2^{a} \pm 4.58$	$30.3^{a} \pm 5.66$	27.3 ± 3.98	$24.2^{b} \pm 3.88$	0.012

Each value is a mean of 2 replicates.
BD – Basic Diet, MD – Modified Diet, MD+AS – Modified Diet + Adequate Supplementation, MD+ES – Modified Diet + Excessive Supplementation,
^{aAbBcC} – within rows means assigned different superscripts differ significantly at $P < 0.05$ (small letters) or $P < 0.01$ (capitals)

A significant increase of the DPA acid synthesis resulted from the elevated synthesis of ALA acids in this group of animals, despite significantly lower consumption of this acid with the MD feed. In total, the applied experimental factors in this group of animals significantly increased the content of n-3 acids and simultaneously lowered the n-6/n-3 ratio in comparison to the animals fed MD. The changes observed in this group of animals point to the

normalizing impact of excessive vitamin supplementation on the fatty acid profile in pericardial fatty tissue, similarly to that observed in the control group of animals (BD).

In conclusion, the applied modification of a diet and dietary supplementation with selected B vitamins did not significantly influence the amount of pericardial adipose tissue, but it influened its fatty acid profile.

CONCLUSIONS

1. The diet modification which consisted of replacement of full grain with wheat flour and sucrose, with an increased consumption of the feed, did not significantly affect the glucose concentration in blood of the animals nor did it influence the amount of pericardial fatty tissue, but it altered its fatty acid profile.

2. Supplementing the modified diet with synthetic vitamins B_1, B_2, B_6 and niacin, in amounts which complemented the modification resulting from the replacement of certain elements of the diet, did not correct the influence of the diet modification.

3. The excessive dietary supplementation with the aforementioned vitamins, in amounts exceeding 2 to 4 times the recommended daily consumption for a human being, calculated into a dose appropriate for a rat, with a decreased consumption of the feed, resulted in a rise in glycaemia and changes in the profile of pericardial fatty tissue, similar to those observed in the control group.

REFERENCES

Anderson B.M., Ma D.W. 2009. *Are all n-3 polyunsaturated fatty acids created equal*? Lipids Health Dis., 8, 33-53. DOI: 10.1186/1476-511X-8-33

AOAC. 2012. *Official methods of analysis of AOAC international (OMA)*, 19th ed. Gaithersburg, USA, ISBN 0-935584-83-8.

Aslanabadi N., Salehi R., Javadrashid A., Tarzamni M., Khodadad B., Enamzadeh E., Montazerghaem H. 2014. *Epicardial and pericardial fat volume correlate with the severity of coronary artery stenosis*. J. Cardiovasc. Thorac. Res., 6(4): 235-239. DOI: 10.15171/jcvtr.2014.018

Bilek M., Stawarczyk K., Pasternakiewicz A. 2014. *Content of glucose, fructose and sucrose in selected soft drinks*. Probl. Hig. Epidemiol., 95(2): 438-444.

Bjørndal B., Burri L., Staalesen V., Skorve J., Berge R.K. 2011. *Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents*. J. Obes., 2011: 490650. DOI: 10.1155/2011/490650

Bligh E.G., Dyer W.J. 1959. *A rapid method of total lipid extraction and purification.* Can. J. Biochem. Physiol., 37(8): 911-917. https://doi.org/10.1139/o59-099

Charzewska J., Wajszczyk B., Chwojnowska Z., Chabros E. 2013. *Saccharose intake and the primary choices of foods by children and adolescents*. Żyw. Człow. Metab., 40(3): 181-191. (in Polish)

Codex Alimentarius. 1994. *Foods for special dietary uses, food and including food for infants and children*. Rome, Food & Agriculture Org., ISBN 9251035105.

de Castro G.S.F., de Almeida L.P, Vannucchi H., Portari G.V., Jordão A.A. 2008. *Effects of diets containing different types of carbohydrates on hepatic metabolism*. Scand. J. Lab. Anim. Sci., 35(4): 321-328.

FAO. 2003. *Methods of food. Analysis.* In: *Food energy – methods of analysis and con-version factors*. Food Nutrit. Paper, 77, Rome, FAO, ISBN 92-5-105014-7.

França C.F., Vianna L.M. 2010. *The response of young and adult rats to the riboflavin supplementation*. Braz. Arch. Biol. Technol., 53(4): 855-860. http://dx.doi.org/10.1590/S1516--89132010000400015

Friedrich M., Goluch-Koniuszy Z. 2009. *Evaluation of the influence of diet content and its supplementation with chosen group of B vitamins on lipids and lipoproteins concentration in female rat serum*. Rocz. Panstw. Zakl. Hig., 60(1): 91-95. (in Polish)

GUS. 2016. *Statistical Yearbook of the Republic of Poland*. Warszawa, ISSN1506-0632. (in Polish)

Iacobellis G. 2009. *Epicardial and pericardial fat: close, but very different*. Obesity (Silver Spring)., 17(4): 625. DOI: 10.1038/oby.2008.575

Jarosz M. 2012. *Nutrition Standards for the Polish Population – An Amendment*. Wyd. IŻŻ, Warszawa. (in Polish)

Johnson I.T., Gee J.M. 1986. *Gastrointestinal adaptation in response to soluble non-available polysaccharides in the rat*. Br. J. Nutr., 55(3): 497-505.

Kendig M.D., Rooney K.B., Corbit L.H., Boakes R.A. 2014. *Persisting adiposity following chronic consumption of 10% sucrose solution: Strain differences and behavioural effects*. Physiol. Behav., 130: 54-65. DOI: 10.1016/j.physbeh.2014.03.021

PN-EN ISO 12966-2:2011. *Animal and vegetable fats and oils — gas chromatography of fatty methyl esters*. Part 2. *Preparation of methyl esters of fatty acids*. Polish Committee for Standardization, Warsaw, Poland.

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(11): 1939-1951.

Sheludiakova A., Rooney K., Boakes R.A. 2012. *Metabolic and behavioral effects of sucrose and fructose/glucose drinks in the rat*. Eur. J. Nutr., 51(4), 445-454. DOI: 10.1007/s00394-011--0228-x

Spadaro P.A., Naug H.L., Du Toit E.F., Donner D., Colson N.J. 2015. *A refined high carbohydrate diet is associated with changes in the serotonin pathway and visceral obesity*. Genet. Res., 97: e23. DOI: 10.1017/S0016672315000233

Terlikowska K.M., Dobrzycka B., Witkowska A., Zujko M.E. 2012. *The diet and risk of cardiovascular disease in perimenopausal women*. Part I. *Basic nutrients, saccharose, fiber*. Bromat.Chem. Toksykol., 45(3): 669-674. (in Polish)

Thornalley P.J. 2005. *The potential role of thiamine (vitamin B1) in diabetic complications*. Curr. Diabetes Rev., 1(3): 287-298.

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

WHO. 2015. *Sugars intake for adult and children guideline*. WHO, Geneva, ISBN: 978 92 4 1549028.

Włodarek W., Głąbska D. 2014. *Analysis of the satisfaction of nutritional needs of elderly women depending on malnutrition risk*. Probl. Hig. Epidemiol., 95(2): 331-338. (in Polish)