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ASSESSMENT OF EFFECTS OF DIET COMPOSITION AND VITAMIN B SUPPLEMENTATION ON FREE RADICAL-RELATED PROCESSES IN THE BODY. CONTENTS OF NON-ENZYMATIC COMPONENTS OF ANTIOXIDATION DEFENCE AND LIPID PEROXIDATION PRODUCTS IN RAT TISSUES

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The experiment described was aimed at following effects of diet alteration involving replacement of whole cereal grains with wheat flour and sucrose, and supplementation of such a diet with selected B-group vitamins on contents of non-enzymatic components of antioxidation defence as well as on contents of malonic dialdehyde (MDA) regarded as a marker of free radical-related processes in the blood and liver of female and male rats.

In female rats, diet alteration resulted in a significant decrease in the GSH content both in the blood and in the liver (from 0.42±0.005 to 0.21±0.06 μmol/g Hb and from 2.77± 0.73 to 1.99±0.60 μmol/g protein, respectively); diet supplementation was found to intensify the process. The -SH contents in the blood and liver were significantly affected by diet composition only (reduction from 1.72±0.15 to 1.14±0.29 μmol/g Hb and from 10.64±1.94 to 8.22±1.84 μmol/g protein, respectively). While the blood MDA concentration was significantly affected by the supplementation applied and increased from 1.64±0.38 to 3.45±0.07 and to 3.73±0.53 μmol/g Hb, the liver MDA concentration was significantly dependent on the modified diet and increased from 0.08±0.03 to 0.12±0.05 μmol/g protein.

In males, too, diet alteration resulted in a reduction of both the GSH content and the –SH group content in the blood and liver; excess supplementation intensified the reduction in the liver. The blood MDA content in males was significantly affected by diet composition and increased, excess supplementation exerting a significant effect on the MDA content in the liver.

Analyses demonstrated a significant effect of sex and of diet x sex interaction on all the parameters analysed.

INTRODUCTION

The growing awareness of incorrect dietary habits, suggestive advertising campaigns, and current fashion persuade more and more healthy people of all age classes to use vitamin formulas as supplements of an everyday diet. As the vitamin preparations are widely available and often only moderately expensive, their type, composition, and amount applied are rarely brought to the attention of a physician who would advise whether as to the real need of supplementation [Pietruszka & Brzozowska, 1999].

Therefore, it was decided to study, on an animal model, effects of diet alteration involving replacement of whole cereal grains with wheat flour and sucrose, and of supplementation of the altered diet with selected B-group vitamins, applied in amounts compensating, or exceeding, the losses brought about by the change of diet, on contents of non-enzymatic antioxidation defence components and on a marker of free-radical-related lipid peroxidation processes in the blood and liver.

MATERIALS AND METHODS

Following the consent of the Local Ethical Commission, the experiment was carried out in the vivarium of the Department of Human Nutrition Physiology, Agricultural University of Szczecin. The experiment involved males (60) and females (60) of SPRD strain of laboratory rats aged 6-8 months. The initial individual body weights of females and males were 225±25 and 325±25 g, respectively.

The rats were kept in metal cages places in an air-conditioned room at 21±1°C, at the light/dark cycle of 12 h/12 h.

Prior to the experiment, the rats were adapted to the vivarium conditions for a week. They were drinking pure water that had been left to stand some time before, and were fed a standard feed. Subsequently, they were divided into 4 treatment groups 15 individuals each: group I – fed the standard feed; group II – fed the modified feed; group III – fed the modified feed supplemented with complementary amount of B-group vitamins; and group IV – fed the modified feed supplemented with vitamins B in the amount exceeding (3-5 times) the deficiency brought about by the diet change.

The rats were fed *ad libitum* with pelleted, isocaloric, and isoproteinic feed mixes manufactured with identical components by Wytwórnia Pasz i Koncentratów (Feed and Concentrate Manufacturers) in Kcynia, Poland, following implementation of Procedure 5.14.5. (Cleaning of Machinery and Equipment). The standard feed contained, *i.a.*, whole cereal grains.

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In the modified feed, whole grains were replaced with Type 500 wheat flour, 50% of corn grains being replaced by sucrose. Other feed components were left unchanged. The composition of the feeds used in the experiment is shown in Table 1.

To determine the actual chemical composition of the feeds, they were subjected to chemical assays performed as recommended by the appropriate Polish Standards [1994, 1996, 2000, 2002, 2005]. Prior to each experiment, feed samples were collected to be analysed for dry weight and contents of total lipids, total protein, and ash.

Dry weight was determined by drying a sample for 12 h at 100°C. Total lipids and protein were determined with the Soxhlet and Kjeldahl methods, respectively; the ash content was calculated from the weight loss on combustion carried out for 10 h at 550°C. Carbohydrate contents were calculated from differences between the dry weight and the sum of the remaining components, as recommended by Gawęcki & Jeszka [1986]. The proximal chemical composition of the feeds used in the experiment is shown in Table 2.

The total gross energy content of the feeds used was calculated by physical energy equivalents: 4.15 kcal/g (17.4 kJ/g) for carbohydrates; 5.65 kcal/g (23.6 kJ/g) for protein; and 9.45 kcal/g (39.6 kJ/g) for lipids. The metabolic energy content of the feeds was calculated using Atwater's equivalents of 4.0 kcal/g (16.7 kJ/g) for carbohydrates; 4.0 kcal/g (16.7 kJ/g) for protein; and 9.0 kcal/g (37.6 kJ/g) for lipids.

TABLE 1. Diet composition (%).

Components	Basic diet (%)	Modified diet (%)
Wheat	36.4	6.0
Corn grains	20.0	10.0
Wheat bran	20.0	20.0
Soya-bruised grain	17.0	17.0
Dry milk whey	3.0	3.0
Fhosphate 2-CA	0.8	0.8
Fodder chalk	1.5	1.5
Fodder salt	0.3	0.3
Premix LRM	1.0	1.0
Wheat flour (type 500)	—	30.4
Sucrose	—	10.0

TABLE 2. Chemical composition of diet used in the experiment.

Components	Basic diet	Modified diet
Protein (%)	19.16	18.48
Fat (%)	2.81	2.33
Carbohydrates (%)	63.76	65.46
Dry matter (%)	91.84	92.26
Ash (%)	6.09	5.98
Gross energy		
(kcal/g)	3.99	3.98
(kJ/g)	16.73	16.67
Metab. energy		
(kcal/g)	3.57	3.56
(kJ/g)	14.95	14.94

The contents of vitamins B_1, B_2, and B_6 in the feeds applied (as determined HPLC methods at the Biotechnology Institute of the Agricultural and Feed Industry) as well as the contents of vitamin PP, as calculated from relevant tables, are shown in Table 3.

The amount of feed consumed by rats was calculated from the difference between the weight of the ration offered, the amount removed from the feeder, and the amount that fell down on the cage floor.

The group I and II rats were drinking pure water left to stand for a period of time beforehand. The group III (complementary supplementation) were offered, at the time of intensified activity, 30 mL of aqueous vitamin solution, kept in dark bottles until used; the solution was prepared from the generally available vitamin formulas containing vitamin B_1 in the form of thiamine nitrate, B_2 in the form of riboflavin, B_6 in the form of pyridoxine chloride, and PP and nicotine amide, per unit feed weight consumed. The amounts of vitamin supplemented *i.e.*, 1.33 mg thiamine, 0.38 mg riboflavin, 1.06 mg pyridoxine, and 11.22 mg nicotine amide per 1 kg feed were calculated from differences of their contents in the basic diet and in the modified one from which they were partly removed by component substitution. Group IV animals (excess supplementation) were offered, at the time of intensified activity, 30 mL of aqueous vitamin solution, in the amount exceeding (3-5 times) the deficiency brought about by diet change, per unit feed weight consumed.

When calculating vitamin doses to be administered in aqueous solution the animals' demand for vitamins has not been considered. It was assumed that the vitamin dose in the basic diet was fully satisfactory. In addition to vitamin solution, the rats of both groups were drinking pure, stand water.

On each day of the experiment, the amount of vitamin solution drunk and the amount of feed consumed were calculated; and the rats were weighed once a week. After 6 weeks, the rats were put to sleep with the anaesthetic Ketanest, following which the blood was drawn from the heart and the liver, and visceral fat was removed for the assays.

Reduced glutathione (GSH) was assayed with the modified Ellman's technique; concentration of total sulphhydryl groups (-SH) was determined with Ellman's technique, in haemolysed blood; whereas products of lipid peroxidation (MDA) were assayed in the blood plasma using Rice-Evans' technique. According to the protocols used, blood contents of non-enzymatic antioxidants were referred to 1 g haemoglobin, the content of which was determined from reaction with Drabkin's reagent.

Contents of non-enzymatic antioxidants and MDA in liver homogenates were determined using methods and reagents

TABLE 3. Contents of selected vitamins in 100 g of diet.

Vitamin	Basic diet (mg)	Modified diet (mg)	Vitamin content difference (%)
Thiamin (B_1)	0.225	0.092	59.1
Riboflavin (B_2)	0.080	0.042	47.4
Pyridoxine (B_6)	0.156	0.050	68.0
Nicotinate PP)	1.684	0.562	66.6

identical to those applied in the blood assays; the amounts obtained were referred to 1 g protein, the content of which was determined in the homogenates with the Lowry's technique.

Visceral fat (from the *duodenum* to a *colon*) was removed on termination of the experiment, once the blood and the liver were collected; the fat was weighed to 0.001 g.

The data obtained were checked for normality of distribution and subjected to statistical treatment with the Statistica® 6 software for Windows. The statistical treatment involved Duncan's multiple range test and 2-way analysis of variance (sex x diet).

RESULTS

No significant effects in the amount of food consumed by females were observed after diet modification. However, supplementation did affect the food uptake. Those females that received complementary supplementation consumed the significantly lowest amount of food, both in absolute terms and per 100 g body weight. The significantly highest food uptake was recorded in the females receiving excess supplementation (Table 4).

Notwithstanding the lack of significant effect of diet composition and supplementation on female body weight increments, both in absolute terms and per unit feed consumed, the two factors were found to significantly affect the amount of visceral fat. The change in diet composition promoted an increase in visceral fat deposition, both per 100 g body weight and per 100 g feed consumed. Complementary supplementation, meant to compensate for the deficiencies resulting from diet modification, intensified the process. It was only when excess supplementation was applied that the amount of the visceral adipose tissue reverted to the level observed in the females fed the standard feed.

In males, food consumption – but only when referred to 100 g body weight – was significantly affected by the change in diet composition (Table 5). As opposed to females, differences in feed composition produced a significant effect on body weight increments. The significantly highest increments, both in absolute terms and per 100 g feed consumed, were observed in those males receiving the modified feed. Supplementation, particularly in complementary amounts, contributed to the return of the increments to the levels observed in the males fed the standard feed.

The males examined showed no effect of diet modification on visceral fact accumulation. On the other hand, vitamin supplementation, particularly the excess type, significantly increased the process, both per unit body weight and per unit feed consumed.

Changes in all the parameters studied were found to be significantly sex-dependent; moreover, interaction between diet (D) and sex (S) proved significant in feed consumption,

TABLE 4. Effects of diet type and supplementation on diet intake, body weight increments and amounts of perivisceral fat tissue in female rats, $\bar{X}$ ±SD, n=60.

Examined trait	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences
Diet intake (g)	689±50	654±40	619±49	706± 27	a-c**, b-d*, c-d**
Diet intake (g/100 g body weight)	265±9	259±10	234±18	267±15	a-c**, b-c**, c-d**
Body weight (g)	9.6±7.7	7.1±4.3	10.4±9.9	12.0±5.8	–
Body weight (g/100 g fodder)	1.41±1.20	1.08±0.68	2.13±1.28	1.68±0.77	–
Peri-intestinal fat (g/100 g body weight)	0.913±0.228	1.142±0.151	1.227±0.168	1.111±0.226	a-b*, a-c**
Peri-intestinal fat (g/100 g fodder)	0.910±0.175	1.156±0.121	1.232±0.131	1.124±0.227	a-b,c**

differences significant at: *p=0.05 and **p=0.01.

TABLE 5. Effects of diet type and supplementation on diet intake, body weight increments and amounts of perivisceral fat tissue in male rats, $\bar{X}$ ±SD, n=60.

Examined trait	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences
Diet intake (g)	980±51	1021±63	973±57	1006±59	–
Diet intake (g/100 g body weight)	257±15	246±7	244±8	247±11	a-b,c,d*
Body weight (g)	43.5±13.6	63.2±13.2	48.5±13.5	53.0±15.4	a-b**, b-c*
Body weight (g/100 g fodder)	4.46±1.44	6.18±1.19	4.49±1.37	5.36±1.71	a-b*
Peri-intestinal fat (g/100 g body weight)	0.591±0.100	0.635±0.122	0.845±0.246	0.812±0.117	a-c,d**, b-c,d**
Peri-intestinal fat (g/100 g fodder)	0.224±0.042	0.257±0.048	0.344±0.097	0.328±0.048	a-c,d**, b-c,d**

differences significant at: *p=0.05 and **p=0.01

body weight increments, and visceral lipid accumulation per unit body weight (Table 6).

Analysis of the results obtained showed the change of diet composition to produce a significant reduction in the GSH content in females, both in the blood and in the liver; the supplementation applied intensified the reduction (Table 7). On the other hand, the blood and liver –SH concentrations were affected by diet composition only. The blood MDA contents were found to be significantly affected by the supplementation only; on the other hand, the liver MDA content was significantly depended on diet composition.

In males, too, the change in diet composition resulted in reduced contents of GSH and –SH groups in the blood and the liver, supplementation intensifying the process in the liver (Table 8). On the other hand, the male blood MDA content was significantly affected by diet composition, supplementation significantly affecting the liver MDA content.

Analysis of changes produced by diet composition and supplementation in the contents of reduced glutathione, sulphhydryl groups, and malonic dialdehyde in the blood and liver of the experimental rats showed a significant effect of sex and diet x sex interaction in all the parameters studied (Table 9).

DISCUSSION

An analysis of feed consumption showed the rat males only to have responded to the change in diet composition; the response was manifested as a lower uptake of the altered feed.

TABLE 6. Effects of diet type (D) on diet intake, body weight increments and amounts of perivisceral fat tissue in rats, depending on sex (P), $\bar{X}$ ±SD, n=120.

Examined trait	Diet Sex	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences		
						Diet (D)	Sex (P)	DxP
Diet intake (g)	Female	689±50	654±40	619±49	706±27			
	Male	980±51	1021±63	973±57	1006±59	**	**	*
	$\bar{X}$	857±155	858±194	788±188	864±156			
Diet intake (g/100 g body weight)	Female	265±9	259±10	234±18	267±15			
	Male	257±15	246±7	244±8	247±11	**	*	**
	$\bar{X}$	260±13	251±10	239±15	256±16			
Body weight (g)	Female	9.6±7.7	7.1±4.3	10.4±9.9	12.0±5.8			
	Male	43.5±13.6	63.2±13.2	48.5±13.5	53.0±15.4	_	**	*
	$\bar{X}$	29.2±20.6	38.3±30.4	31.8±21.1	34.3±24.0			
Body weight (g/100 g fodder)	Female	1.41±1.20	1.08±0.68	2.13±1.28	1.68±0.77			
	Male	4.46±1.44	6.18±1.19	4.49±1.37	5.36±1.71	_	**	*
	$\bar{X}$	3.18±2.02	3.92±2.78	3.63±1.95	3.68±2.30			
Peri-intestinal fat (g/100 g body weight)	Female	0.913±0.228	1.142±0.151	1.227±0.168	1.111±0.226			
	Male	0.591±0.100	0.635±0.122	0.845±0.246	0.812±0.117	**	**	*
	$\bar{X}$	0.743±0.210	0.875±0.297	0.983±0.268	0.939±0.247			
Peri-intestinal fat (g/100 g fodder)	Female	0.910±0.175	1.156±0.121	1.232±0.131	1.124±0.227			
	Male	0.224±0.042	0.257±0.048	0.344±0.097	0.328±0.048	**	**	_
	$\bar{X}$	0.548±0.386	0.672±0.468	0.660±0.453	0.687±0.439			

differences significant at: * p=0.05 and **p=0.01.

TABLE 7. Effects of diet type and supplementation on concentrations of glutathione reductions (GSH), sulfhydryl total group (-SH) and MDA in blood and liver of female rats, $\bar{X}$ ±SD, n=60.

Examined trait	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences
Blood GSH (μmol/g Hb)	0.42±0.05	0.21±0.06	0.20±0.02	0.17±0.08	a-b,c,d**
-SH (μmol/g Hb)	1.72±0.15	1.14±0.29	0.95±0.17	0.99±0.19	a-b,c,d**
MDA (μmol/g Hb)	1.26±0.17	1.64±0.38	3.45±0.07	3.73±0.53	a-c,d**, b-c,d**
Liver GSH (μmol/g protein)	2.77±0.73	1.99±0.60	1.54±0.38	1.47±0.76	a-b,c,d**, b-d*
-SH (μmol/g protein)	10.64±1.94	8.22±1.84	7.53±1.31	8.21±1.67	a-b,c,d**
MDA (μmol/g protein)	0.08±0.03	0.12±0.05	0.13±0.02	0.14±0.02	a-b,c,d**

differences significant at: * p=0.05 and **p=0.01.

TABLE 8. Effects of diet type and supplementation on concentrations of glutathione reductions (GSH), sulfhydryl total group (-SH) and MDA in blood and liver of male rats, $\bar{X} \pm SD$, n=60.

Examined trait	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences
Blood GSH (μmol/g Hb)	0.23±0.04	0.11±0.02	0.12±0.02	0.10±0.02	a-b,c,d**
-SH (μmol/g Hb)	0.54±0.15	0.34±0.08	0.31±0.05	0.31±0.04	a-b,c,d**
MDA (μmol/g Hb)	0.98±0.09	1.79±0.38	1.77±0.21	1.82±0.20	a-b,c,d**
Liver GSH (μmol/g protein)	1.91±0.43	1.06±0.19	0.99±0.23	0.93±0.17	a-b,c,d**
-SH (μmol/g protein)	6.70±0.69	4.07±0.94	3.56±0.57	3.09±0.45	a-b,c,d**, b-d**
MDA (μmol/g protein)	0.53±0.28	1.02±0.69	0.70±0.23	2.88±1.69	a-d**, b-d**, c-d**

differences significant at: * p=0.05 and **p=0.01.

TABLE 9. Effects of diet type (D) on diet intake on concentrations ofglutathione reductions (GSH), sulfhydryl total group (-SH) and MDA in blood and liver of rats, depending on sex (P), $\bar{X} \pm SD$, n=120.

Examined trait	Diet Sex	Basic diet (a)	Modified diet (b)	Modified diet + complementary supplementation (c)	Modified diet + redundant supplementation (d)	Significant differences		
						Diet (D)	Sex (P)	DxP
Blood GSH (μmol/g Hb)	Females	0.42±0.05	0.21±0.06	0.20±0.02	0.17±0.08			
	Males	0.23±0.04	0.11±0.02	0.12±0.02	0.10±0.02	**	**	**
	$\bar{X}$	0.31±0.10	0.15±0.07	0.16±0.05	0.13±0.06			
-SH (μmol/g Hb)	Females	1.72±0.15	1.14±0.29	0.95±0.17	0.99±0.19			
	Males	0.54±0.15	0.34±0.08	0.31±0.05	0.31±0.04	**	**	**
	$\bar{X}$	1.07±0.62	0.69±0.45	0.61±0.35	0.59±0.36			
MDA (μmol/g Hb)	Females	1.26±0.17	1.64±0.38	3.45±0.07	3.73±0.53			
	Males	0.98±0.09	1.79±0.38	1.77±0.21	1.82±0.20	**	**	**
	$\bar{X}$	1.12±0.19	1.72±0.37	2.49±0.93	2.64±1.01			
Liver GSH (μmol/g protein)	Females	2.77±0.73	1.99±0.60	1.54±0.38	1.47±0.76			
	Males	1.91±0.43	1.06±0.19	0.99±0.23	0.93±0.17	**	**	**
	$\bar{X}$	2.29±0.74	1.48±0.63	1.27±0.41	1.16±0.52			
-SH (μmol/g protein)	Females	10.64±1.94	8.22±1.84	7.53±1.31	8.21±1.67			
	Males	6.70±0.69	4.07±0.94	3.56±0.57	3.09±0.45	**	**	**
	$\bar{X}$	8.55±2.45	6.02±2.54	5.66±2.24	5.24±2.85			
MDA (μmol/g protein)	Females	0.08±0.03	0.12±0.05	0.13±0.02	0.14±0.02			
	Males	0.53±0.28	1.02±0.69	0.70±0.23	2.88±1.69	**	**	**
	$\bar{X}$	0.36±0.28	0.58±0.72	0.44±0.35	1.48±1.79			

differences significant at: * p=0.05 and **p=0.01.

Generally, consumption of food containing easily digestible carbohydrates is accompanied by an increased blood glucose concentration and the associated insulin release, in both humans and rats. However, in view of results reported by Kabir *et al*. [1998], who showed the glycaemic index of the diet to be uncorrelated with food uptake, the effect observed seems to be related more to the intensity of intracellular glucose transformations which equally strongly modify the function of food centres in the hypothalamus [Schwartz *et al*., 1999].

On the other hand, considering effects of supplementation on feed consumption, the supplementation applied was found to significantly modify the feed uptake in females only. Complementary supplementation significantly reduced the amount of feed consumption, which may be explained by the role the vitamins involved play in carbohydrate metabolism [Depeint *et al*., 2006], by their involvement in key reaction of the citric acid cycle, and by their control of the rate of those reactions [van Berkel *et al*., 2006]. On the other hand, the fact that excess supplementation reverted the feed uptake to the control level could have been produced by the rate of glucose transformations that caused reduction in the blood glucose concentration, as observed in rats by Friedrich & Sadowska [2005].

Intensification of triacylglycerol synthesis processes, manifested as accumulation of, *i.a.,* visceral adipose tissue, could have been a significant factor that affected feed uptake by the rats used in the experiment described. In this context, effects of adipose tissue hormones cannot be ruled out. As shown by Peyron-Caso *et al*. [2002], consumption, by rats, of a sucrose-containing diet that promotes fat tissue accumulation is accompanied by increased concentrations of leptin and adiponectin. A similar effect in humans was observed by Westphal *et al*. [2007] at pyridoxine supplementation.

A comparison of feed uptake by males and females showed the latter to consume more feed per unit body weight. The increased uptake was not, however, translated to body weight increments, which were significantly lower than those in males. The lower female weight increments were accompanied by a significantly higher deposition of visceral fat tissue, stimulated by the diet composition. Accumulation of visceral fat in males was intensified by the supplementation applied.

It seems that the increased visceral fat accumulation in females could have been caused by, *i.a.,* a change in the visceral adipose tissue fatty acid profile, observed in an earlier study [Friedrich & Sadowska, 2005], triggered by the diet applied. As shown by Gaiva *et al*. [2003], the observed increase in polyunsaturated fatty acids promotes, in rats, accumulation of visceral fat tissue. On the other hand, supplementation-induced intensification of visceral fat accumulation in rat males could have resulted from the role of the vitamins applied, particularly thiamine and niacin, in carbohydrate-lipid metabolism. Pyridoxine, too, could have a stimulating effect, by enhancing delta-6-saturase activity and increasing absorption of magnesium, important in lipid metabolism. Lipid accumulation could have been also enhanced by reduced uptake of other regulatory components, *e.g.,* calcium. Zemel *et al*. [2005] found dietary calcium deficiency to stimulate lipogenesis gene expression and to inhibit lipolysis.

Diet x sex interaction proved significant in feed uptake, body weight increments, and fat tissue accumulation.

It is, however, difficult to unequivocally identify a direct cause of the effects observed. Should the cause be sought in differences in feed uptake and, consequently, in different amounts of supplemented vitamins and other regulatory components present in the diet? Or, perhaps, important was the effect of sex hormones, or lipid metabolism, somewhat different in males and females?

When analysing effects of diet composition and supplementation with selected B-group vitaminson antioxidation indicators in the blood and tissues of the rats studied, it should be remembered that the modified feed used in the experiment was impoverished not only in vitamins B, but also in numerous other compounds. The missing compounds include those naturally occurring in whole, unrefined products. Those compounds affect different metabolic pathways and serve as dietary antioxidants that strengthen the antioxidation defence of the body. The array of such compounds includes, *i.a.,* vitamin E, phenolic acids, amides, flavonoids, and sterols present in whole wheat grains [Yu & Zhou, 2004], glycogen, lutein, zeaxanthin, xanthin, and carotenoids present in corn [Batifoulier *et al*., 2006] as well as anthocyanes and polyphenols [Del Pozo-Insfran *et al*., 2006].

An analysis of diet composition effects on the reduced glutathione concentration in the blood and liver showed the effect to be significant in both sexes. Although females showed higher concentrations of reduced glutathione in both tissue types assayed than males, the observed per cent reduction of glutathione concentration was similar, particularly in the blood. The supplementation applied produced a significant effect only when applied in excess; the effect was observed in the liver of the female rats studied.

Glutathione is the basic non-protein thiol component of cells and extracellular fluids. It has highly reactive–SH and –COOH groups. Glutathione concentration is usually reduced due to glutathione ability to directly react with hydroxy and organic radicals and hydrogen peroxide that appear in the medium. It should be, however, remembered that GSH is also a substrate for the antioxidation enzyme glutathione peroxidase (GPx), a hydrogen donor for numerous enzymes and low molecular weight oxidants, and a thiol group donor in protein glutathionylation processes.

The reduction of GSH content, recorded in the experiment, could have then resulted from the direct involvement of glutathione in reactions with free radicals and in reactions that caused reduction of oxidised biological molecules, which was associated with the reduced, in the modified diet, amounts of the compounds serving as natural antioxidants and of B-group vitamins.

The role of B-group vitamins is most pronounced in regeneration and *de novo* synthesis of reduced glutathione in cells. Therefore it is intriguing why the vitamin B supplementation applied produced no increase in the GSH concentration, and why, in females, it augmented the GSH reduction caused by diet alteration. An explanation may be sought in results of a study described by Sawicki *et al.* [2005], who demonstrated inhibitory effect of vitamins B on the activity of glutathione S-transferase in the laboratory animals they studied, which could have led to accumulation of toxic organic hyperoxides. It seems that this could have been the case in the present study, particularly at excess supplementation.

The study demonstrated a significant effect of sex, and a significant diet x sex interaction, in the reduced glutathione content in the tissues analysed.

Effects of sex on reduced GSH retention in tissues are visible in rats as early as at the embryonic stage [Kochhar *et al*., 2001]. Similarly, higher tissue GSH contents were reported also from women [Lavoie & Chessex, 1997], as a result of, *i.a*., effects of female sex hormones on GSH metabolism gene expression [Rinn & Snyder, 2005].

The experiment revealed also a significant, diet modification-induced, reduction in the content of –SH groups present in glutathione and other biological molecules; the reduction was intensified in males by the excess supplementation. The effect seems to be related to, *i.a*., the observed reduction in the GSH content, the thiol groups intercepting the role of molecule reduction and their oxidation to thiyl radicals (R-S') and sulphenic (R-SOH), sulphinic ($S-SO_2H$) and sulphonic acids ($S-SO_3H$), and S-nitrose thiols (SNT). An increasing concentration of low molecular weight disulphides induces S-thiylation and glutathionylation, whereby an organism modifies biological activity of proteins [Biswas *et al*., 2006]. It should be borne in mind that the reduced –SH group concentration is accompanied by an increase in the content of protein-bound cystein, which additionally reduces its pool necessary for glutathione biosynthesis and effective protection of proteins.

Like the GSH contents, those of –SH groups were found to be significantly affected by both sex and diet x sex interaction. The effect was a result of the relationship, presented earlier, between the GSH and –SH contents in tissues.

The changes in non-enzymatic antioxidation defence of the experimental rats may be indicative of intensified free radical-related processes brought about both by the diet that lacked natural antioxidants and by the supplementation applied. To find out how efficient were other mechanisms of defence against oxidation reactions, the tissues studied were assayed for the content of malonic dialdehyde (MDA), a marker of oxidation-produced lipid damage.

The change in diet composition was found to increase the blood MDA content, the process being additionally intensified by the supplementation applied. On the other hand, as far as the liver is concerned, a significant increase in MDA content in females was caused by the change in diet composition, excess supplementation causing a significant increase in the liver MDA content in males.

Intensified lipid peroxidation, observed in the rats kept on the modified diet, could have been related to elimination of numerous natural antioxidants from the modified diet [Vijaya *et al.*, 2005]. The increased lipogenesis observed in the experimental rats, intensified by the supplementation applied, could have caused oxidative stress. Numerous studies have demonstrated that an increased amount of adipose tissue, including the visceral fat, is an independent causative factor in lipid peroxidation increase [Olusi, 2002]. In addition, the composition of the sucrose-containing diet, too, could have promoted an increase of the MDA content in the tissues studied [Busserolles *et al*., 2002].

As shown by the experiment, diet supplementation with the vitamins regarded as important in strengthening the antioxidation system not only did not prevent peroxidation processes, but intensified them.

The relevant literature data are not unequivocal. Arun *et al.* [1999] demonstrated an inhibitory effect of niacin on lipid oxidation processes. On the other hand, *in vitro* studies of Higashi-Okai *et al.* [2006] showed a strong prooxidative effect of vitamins B_1, B_2, and niacin. In this context, the results obtained in this study, carried out *in vivo* as well and indicating a prooxidative potential of vitamin B supplementation effect on membrane lipids, are interesting.

The experiment described revealed a significant effect of sex and a diet x sex interaction on the concentration of malonic dialdehyde both in the blood and in the liver. The underlying causes included significantly higher concentrations of non-enzymatic antioxidants (GSH and –SH) in females and hence their lower susceptibility to lipid peroxidation processes.

CONCLUSIONS

1. Modification of the diet, whereby whole cereal grains were replaced by wheat flour and sucrose, was found to disturb the prooxidation-antioxidation equilibrium not only because the diet was impoverished in numerous natural regulative or antioxidative substances, but also because the modified diet contributed to an increase in lipid metabolism intensity, manifested as accumulation of visceral adipose tissue, which is an independent factor inducing free radical-related processes.

2. Compensation of the resultant deficiencies by diet supplementation with selected synthetic vitamins B did not reverse the adverse effects in the antioxidation defence system; on the contrary, those effects were intensified, which was reflected in increased concentrations of lipid peroxidation products.

3. Even a small excess supplementation was found to unfavourable affect antioxidation defence status of the body, manifested as a significant reduction in concentration of the non-enzymatic antioxidants and an increase in contents of lipid peroxidation products in the tissues studied.

4. The response of the body to diet modification and supplementation applied was significantly sex-dependent.

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