

EFFECTS OF DIET SUPPLEMENTATION WITH B-COMPLEX VITAMINS ON FATTY TISSUE ACCUMULATION IN RATS

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The experiment described in the paper focused on the effects of complementary and excess diet supplementation on feed consumption, body weight changes, and fat tissue accumulation in rats. Untreated wheat, corn grains, and barley grits in the original diet were isocalorically substituted with wheat flour, sucrose, and B-complex vitamins.

Diet supplementation was found to significantly modify the level of feed intake. The lowest amount of feed (per unit body weight) was consumed by the rats fed the excess supplementation diet. In addition, males of that group showed the lowest (per unit feed weight) body weight gains (2.84 ± 0.75 g) and the highest (0.430 ± 0.100 g) accumulation of pericardial fat, significantly higher than that in the non-supplemented (0.053 ± 0.012) and complementarily supplemented (0.086 ± 0.029 g) groups; moreover, the accumulation of perivisceral fat (5.74 ± 1.42 g) was significantly higher than in the non-supplemented group (8.789 ± 1.832 g).

On the other hand, the females on the excess supplementation diet showed slightly lower (per 100 g food consumed) body weight gains (2.01 ± 0.77 g), compared to the females fed the modified, non-supplemented diet (2.23 ± 0.80), their body weight gains being significantly higher than those recorded in the females on the complementary supplementation diet (1.15 ± 0.70 g). The higher body weight gains in that group were accompanied by a significantly higher accumulation of pericardial (0.663 ± 0.176 g) and perivisceral (12.79 ± 2.40 g) fatty tissue as well as by an increased muscle tissue fat content (10.5%), compared to the females fed modified non-supplemented diet (0.057 ± 0.018 g; 5.644 ± 0.986 g; 6.30%, respectively) and the complementary supplementation diet (0.048 ± 0.011 g; 5.958 ± 1.174 g; 5.24%, respectively).

INTRODUCTION

Recent Polish publications on dietary habits of people of different social groups and various age classes have revealed numerous irregularities, including substantial B-complex vitamin deficiencies [Friedrich, 1999; Ziemiański & Wartanowicz, 1999]. The situation is enhanced by an increased consumption not only of highly refined products, but also those that are a source of a substantial amounts of protein and fats. For this reason, the daily diet not only fails to meet the basic demand for B-complex vitamins, but also, due to its composition, requires their higher amount to maintain appropriate metabolic equilibrium [Okada *et al.*, 1998; 1999; Pregnolato *et al.*, 1994].

On the other hand, the increasing nutrition-related health awareness, aggressive advertising, and fashion have resulted in vitamin supplementation of a daily diet, practiced more and more frequently by healthy individuals of various age classes [Brzozowska *et al.*, 1994; Pietruszka & Brzozowska, 1995, 1999]. Due to their availability and, frequently, moderate price, the kind, composition, and dosage of vitamin-rich dietary supplements are seldom consulted with the doctor and are rarely adjusted to actual needs [Pietruszka & Brzozowska, 1999].

As B-complex vitamins play an important part in, *e.g.* carbohydrate-lipid metabolism, it was thought necessary to explore the effects of complementary and excess supple-

mentation of a diet in which the original whole wheat, corn, and barley grains were isocalorically substituted with wheat flour, sucrose, and B-complex vitamins active in carbohydrate-lipid metabolism, on feed consumption, body weight changes, and fatty tissue accumulation in rats.

MATERIALS AND METHODS

With the consent of the Local Ethics Commission, the experiment was carried out on 360 Wistar breed rats (males and females) aged 8 months; the females used had already produced their last brood of litter. The animals were randomly assigned to four groups of 90 individuals (45 males and 45 females), their mean body weights were as follows: males: group I – 543.1 ± 66.8 g, group II – 537.1 ± 63.2 g, group III – 545.6 ± 65.2 g, group IV – 539.2 ± 59.2 g; females: group I – 313.0 ± 37.2 g, group II – 320.2 ± 35.2 g, group III – 318.6 ± 34.8 g and group IV – 316.4 ± 33.7 g. Rats were fed *ad libitum* pelleted feed mixes manufactured by GRSP Miłosław. Group I received the basic mix, while Groups II, III, and IV were fed modified mixes. The modification involved substitution of the whole wheat grains with wheat flour of the basic mix; 50% of corn and 30% of barley grits in the original mix were substituted with sucrose. The composition of feeds used in the experiment is given in Table 1a.

To determine the actual chemical composition of the feed mixes, they were analysed – following the methodolo-

TABLE 1a. Composition (%) of feeds used in the experiment.

Component	Basic feed	Modified feed
Wheat	20	-
Wheat flour (type 500)	-	20
Corn grains	20	10
Barley grits	15	10.5
Sucrose	-	14.5
Wheat bran	10	10
Milk substitute	15	15
Meat-bone meal	8	8
Soybean 44%	5	5
Dried green forage	5	5
Fodder chalk	1	1
Polfamix "F"	1	1
Total	100%	100%

TABLE 1b. Chemical composition of feeds used in the experiment.

Component	Basic feed	Modified feed
Protein (%)	13.4	13.1
Fat (%)	6.0	6.0
Carbohydrates (%)	66.8	67.1
Dry matter (%)	93.0	93.3
Ash (%)	6.9	7.0
Dietary fibre (%)		
Hemicellulose	10.31	5.69
Cellulose	6.09	5.67
Lignin	1.42	1.31
Gross energy (kJ/g)	17.2	17.2
Metab. energy (kJ/g)	15.7	15.7

gy described by Gawęcki & Jeszka [1995] – for the content of crude protein, fat, dry matter, and ash. The carbohydrate contents were calculated from the differences between dry matter contents and the sum of the remaining components. The content of dietary fibre was determined as well, in an ANKOM 220 apparatus (Table 1b). The gross and metabolic energy contents were calculated by means of the commonly applied energy equivalents.

Group I and II had access to tap water left to stand, the drinking vessels being refilled as the water was used. The rats of group III (complementary supplementation) were offered, at the time of increased activity, 50 mL of aqueous solution of vitamins B1, B2, B6 and nicotinic acid per unit feed weight consumed. The amounts of vitamins supplemented, *i.e.* 1.083 mg thiamine, 0.361 mg riboflavin, 0.898 mg pyridoxine, and 9.58 mg nicotinic acid amide per 1 kg feed, were calculated from the differences in their contents in the basic diet and in the modified one from which

they were partly removed by component substitution. Animals from Group IV (excess supplementation) received, at the time of increased activity, 50 mL of aqueous solution of vitamins obtained from the over-the-counter preparation Vitaminum B-compositum (Pliva Kraków). Vitamins were administered at the following doses: 29.4 mg of thiamine, 43.4 mg of riboflavin, 44.8 mg of pyridoxine, 364.0 mg of nicotinic acid amide per kg of feed. The amounts of vitamins consumed by animals exceeded several times the difference between their contents in the basic diet and the modified one: 14 x for thiamine, 10 x for riboflavin, 9 x for pyridoxine, and 17 x for nicotinic acid amide. Having drunk the vitamin solution, the rats drank pure water. When calculating the vitamins doses to be administered in aqueous solutions the animals's demand for vitamins has not been considered. It was taken for granted that the vitamin dose in the basic diet was fully satisfactory.

The experiment took 6 weeks during which the amount of feed consumed was controlled by weighing the rats once a week. On termination of the experiment, the rats were put to sleep with an anaesthetic and the amount of pericardial, peri-intestinal, and intramuscular fat was determined.

Pericardial and peri-intestinal fat was dissected out immediately after sacrificing the rats, and weighed to exact 0.001 g.

Intramuscular fat was obtained from the shoulder and thigh muscles. The samples were used to determine, following the methodology described by Gawęcki & Jeszka [1995], the percentage of crude fat. The assays were performed with the Soxhlet technique in a Soxtec HT6 apparatus (Foss Tecator).

The data obtained were subjected to statistical treatment, using the computer software Statistica®; the least significant difference (LSD) test and the two-way analysis of variance (diet x sex) for independent variables were applied.

RESULTS

The changed diet and the type of diet supplementation were found to significantly alter the amount of feed ingested, both in absolute terms and per 100 g body weight. The lowest food ration (per unit body weight) was consumed by those males and females whose diet was supplemented in excess (Table 2).

Analysis of the effects of the diets applied revealed the diet in which whole grains were substituted with flour and sucrose to enhance body weight gains in both sexes; however, neither did it affect the accumulation of perivisceral fatty tissue or intramuscular fat increase (Table 3). Both these effects were noticeable in dietary regimes involving excess supplementation. The lowest body weight gains were accompanied by the highest, and significantly higher than in

TABLE 2. Feed consumption in rats ($\bar{x} \pm SD$; n=360).

Feed consumption	Sex	Basic feed	Modified feed (Mf)	Mf+complementary supplementation	Mf+redundant supplementation
Total (g)	Male	1224.8±103.4 ^b	1099.0±67.3 ^a	1186.1±68.6 ^b	1179.9±71.7 ^b
	Female	826.9±75.8 ^a	898.7±119.3 ^{ab}	825.5±136.9 ^a	949.8±77.7 ^b
Feed consumption g/100g body weight	Male	231.6±19.8 ^c	223.5±20.5 ^{bc}	215.4±8.9 ^b	200.3±11.4 ^a
	Female	253.9±19.1 ^b	271.0±17.1 ^c	255.0±25.5 ^b	218.9±13.5 ^a

a, b, c – means denoted with the same letters in a row are not significantly different ($p \leq 0.05$)

TABLE 3. Diet type and supplementation effects on body weight gains and amounts of perivisceral and intramuscular fatty tissue in rats ($\bar{x} \pm SD$, n=360).

Trait	Feed Sex	Basic feed	Modified feed (Mf)	Mf+complementary supplementation	Mf+redundant supplementation	Effect		
						Diet	Sex	Interaction
Body weight gains (g/100 g feed)	Male	3.08±0.65 ^{ab}	3.55±0.67 ^b	3.14±0.72 ^{ab}	2.84±0.75 ^a	**	**	*
	Female	1.32±0.47 ^a	2.23±0.80 ^b	1.15±0.70 ^a	2.01±0.77 ^b			
Pericardial fat (g)	Male	0.071±0.024 ^a	0.053±0.012 ^a	0.086±0.029 ^a	0.430±0.100 ^b	**	**	**
	Female	0.072±0.023 ^a	0.057±0.018 ^a	0.048±0.011 ^a	0.663±0.176 ^b			
Pericardial fat (g/100 g body weight)	Male	0.013±0.004 ^a	0.011±0.003 ^a	0.015±0.005 ^a	0.073±0.016 ^b	**	**	**
	Female	0.023±0.006 ^a	0.017±0.006 ^a	0.015±0.005 ^a	0.153±0.039 ^b			
Pericardial fat (g/100 g feed)	Male	0.006±0.002 ^a	0.005±0.001 ^a	0.007±0.002 ^a	0.036±0.008 ^b	**	**	**
	Female	0.009±0.002 ^a	0.006±0.002 ^a	0.006±0.002 ^a	0.070±0.007 ^b			
Peri-intestinal fat (g)	Male	6.114±1.109 ^a	5.742±1.417 ^a	7.741±1.382 ^b	8.789±1.832 ^b	**	*	**
	Female	6.226±0.885 ^a	5.644±0.986 ^a	5.958 ± 1.174 ^a	12.792±2.405 ^b			
Peri-intestinal fat (g/100 g body weight)	Male	1.160±0.231 ^a	1.155±0.227 ^a	1.414±0.291 ^b	1.480±0.241 ^b	**	**	**
	Female	1.928±0.366 ^a	1.715±0.291 ^a	1.859±0.406 ^a	2.698±0.636 ^b			
Peri-intestinal fat (g/100 g feed)	Male	0.503±0.103 ^a	0.521±0.114 ^a	0.658±0.141 ^b	0.744±0.144 ^b	**	**	**
	Female	0.759±0.138 ^b	0.631±0.093 ^a	0.728±0.120 ^{ab}	1.349±0.239 ^c			
Intramuscular fat (%)	Male	4.47±0.19 ^b	4.14±0.20 ^a	6.59±0.11 ^d	5.42±0.10 ^c	*	**	*
	Female	6.32±0.08 ^b	6.30±0.08 ^b	5.24±0.06 ^a	10.48±0.09 ^c			

a, b, c, d – means denoted with the same letters are not significantly different at $p \leq 0.05$; * difference significant at $p \leq 0.05$; ** $p \leq 0.01$

the non-supplemented animals, accumulation of peri-intestinal fat. An analysis of intramuscular fat content revealed it to be the highest and significantly higher in muscular tissue of males fed the complementary diet, and the lowest and significantly lower in the male rats on the modified non-supplemented diet.

The females treated with excess vitamin supplementation were found to put on slightly less weight, per 100 g feed consumed, than those on the modified non-supplemented diet; their body weight gains, however, were significantly higher than those in the females fed both the basic diet and the complementary diet. Yet, in this group of females the highest and significantly higher deposition of perivisceral fatty tissue, accompanied by an increase in fat content of the muscular tissue, were noted when compared to the other female test groups (Table 3).

The changes observed were found to be significantly sex-specific; the sex-diet interaction proved significant with respect to all the parameters studied (Table 3).

DISCUSSION

Analysis of the effects of diet supplementation on feed consumption revealed the excess vitamin B supplementation to result in a significantly lower feed intake.

Blood glucose level and the rate of intracellular glucose transformations belong to the major metabolic factors controlling physiological mechanisms of hunger and satiation. The feed used in the experiment contained white flour and sucrose which, easily digested and rapidly releasing high glucose amounts to the blood, should speed up the appearance of the sense of satiation. Why was this effect absent in the animals on the modified non-supplemented diet? The observations could explain the results reported by Kabir *et al.* [1998] who demonstrated the glycaemic index of diet in rats to have no significant effect on the amount of feed intake. On the other hand, the reduced feed intake observed in the animals supplemented in excess could have

been related, by diet supplementation with vitamins (thiamine, riboflavin, pyridoxine, niacin) that are involved in glucose metabolism, with increased intracellular glucose transformations. As shown by Trigazis *et al.* [1998], this is a stronger feed intake inhibitor than the absolute blood glucose level. The amount of perivisceral fatty tissue, increased in that group of rats, could have also induced an inhibitory effect on feed consumption. The perivisceral fat was not only a source of triacylglycerols rapidly secreted to the blood, but also sent direct information on energy content of the system [Archer *et al.*, 2002].

An analysis of the effect of diet supplementation type on body weight gains showed both types to result in lower body weight gains than those observed in the rats on the modified non-supplemented diet.

In the living organism lipogenesis involves primarily the transformation of glucose and components of indirect metabolism, such as pyrogronate, lactate, and acetyl-CoA, into fat. In rats, the pathway mentioned is exceptionally active in the fatty tissue and in the liver. The entire lipogenesis is controlled by nutrition and diet composition. In the present experiment, the diet contained easily accessible starch and sucrose which, by fast digestion and release of increased amounts of glucose, forced the release of insulin activating acetyl-CoA carboxylase responsible for carbohydrate-acetyl-coA transformation. Why, however, were the newly synthesised lipids deposited within the body (a highly disadvantageous condition) instead of being accumulated peripherally (as in the animals on the modified non-supplemented diet)? It is known at present that metabolic activity of fatty tissue, particularly that accumulated in the abdominal cavity, is very high, its triacylglycerols constantly releasing fatty acids affecting numerous functions of the body [Shimomura *et al.*, 1996; Atzmon *et al.*, 2002]. It has also been demonstrated that metabolic implications of increased amounts of intra-abdominal fat can be important for the development of insulin resistance, type 2 diabetes, and/or functional disorders in the cardiovascular system [Kim *et al.*,

2000; Noguchi & Tanaka, 1995; Shimomura *et al.*, 1996]. In their study on men of various age classes, Walton *et al.* [1995] demonstrated android obesity to be directly correlated with the blood triacylglycerol content. They also showed the accumulation of visceral fat tissue to be more responsible for unfavourable changes in lipid blood components than the absolute amount of fat in the body.

Fatty tissue accumulation control is a complex problem. As shown by physiological and clinical studies, it occurs at the cellular and molecular level. The deposition site is physiologically determined by sex hormones; effects of testosterone and estrogen on adipocyte metabolism are similar, but these two hormone groups target at different fatty tissue regions [Bjorntorp, 1997]. However, excessive accumulation of perivisceral fatty tissue may be related to disorders cortisol secretion, the hormone being instrumental in stimulating lipid accumulation pathways and inhibiting triacylglycerol retention, and responsible for fat redistribution to the perivisceral tissue [Bjorntorp, 2000]. This has been confirmed by the observations reported by Friedrich [1997] who found normalisation of the blood insulin and cortisol levels in obese women to be more conducive to waist/hip ratio (WHR) reduction than the absolute body weight reduction.

In the experiment discussed, the increased content of the perivisceral fatty tissue both in the females and males supplemented could have been, however, related to the excess thiamine administered. By participating in glucose transformation along the pentose-phosphate pathway, thiamine contributes to an increase in the content of NADPH utilised in reducing syntheses, including those of fatty acids and steroids. The changes observed must have also been intensified by the uptake of the excess pantothenic acid and biotin [Wahlberg *et al.*, 1992]. Perivisceral fatty tissue accumulation could have also been enhanced by the consumption of smaller amounts of feed, which reduced the uptake of not only the basic nutrients, but also the regulatory ones. Masini *et al.* [1994] are of the opinion that an increased accumulation of perivisceral fat tissue is frequently correlated with dietary iron deficiency and weakens oxidative metabolism; once iron is supplemented, the mitochondrial oxidative phosphorylation rate increases and the amount of perivisceral fatty tissue is reduced. A similar relationship was described by Zemel *et al.* [2002] who demonstrated dietary calcium deficiency to stimulate the release of calcitriol, the compound increasing calcium ion flux to cells, lipogenesis rate, and triacylglycerol accumulation not only in the perivisceral fatty tissue, but also in the muscles. Intramuscular fat accumulation, at dietary calcium deficiency, observed, in this experiment especially in the females consuming the lowest amounts of feed, was also supported by the results reported by Shi *et al.* [2001], and Parikh and Yanowski [2003].

Although the experimental females had given birth and retained their reproductive function, no protective effect of estrogens was observed with respect to fatty tissue redistribution [Bjorntorp, 1997]. Considering the range of changes observed, the females studied can be regarded as even more sensitive to excess vitamins than males.

CONCLUSIONS

1. An analysis of the results allowed concluding that diet supplementation, particularly the excess one, with B-com-

plex vitamins caused reduced feed consumption, body weight gains disproportionate to feed energy content, perivisceral fatty tissue accumulation, and an increase in fat content of muscular tissue.

2. Females proved more sensitive to the supplementation applied than males.

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WPLYW SUPLEMENTACJI DIETY WITAMINAMI Z GRUPY B NA GROMADZENIE TKANKI TŁUSZCZOWEJ U SZCZURA

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W przeprowadzonym doświadczeniu badano wpływ uzupełniającej i nadmiarowej suplementacji witaminami z grupy B diety, w której pełne ziarna pszenicy, kukurydzy oraz kaszę jęczmienną izokalorycznie zastąpiono mąką pszenną i sacharozą, na pobieranie paszy, zmiany masy ciała i gromadzenie tkanki tłuszczowej u szczura.

Stwierdzono, że suplementacja diety istotnie modyfikowała wielkość pobieranej przez zwierzęta paszy. Najmniej paszy, w przeliczeniu na jednostkę masy ciała, spożywały zwierzęta, których dieta była suplementowana w sposób nadmiarowy (tab. 2). U samców towarzyszyły temu najmniejsze przyrosty masy ciała i istotnie większe w porównaniu z grupą niesuplementowaną i suplementowaną w sposób uzupełniający, gromadzenie tłuszczu okołosercowego oraz istotnie większe w porównaniu ze zwierzętami niesuplementowanymi, gromadzenie tłuszczu okołojelitowego (tab. 3). Natomiast samice suplementowane w sposób nadmiarowy przyrastały nieco mniej niż samice na diecie zmodyfikowanej niesuplementowanej, ale istotnie więcej od samic na diecie suplementowanej w sposób uzupełniający. Większym przyrostom masy ciała samic tej grupy towarzyszyło istotnie większe odkładanie okołonarządowej tkanki tłuszczowej oraz wzrost zawartości tłuszczu w tkance mięśniowej w porównaniu z samicami na diecie zmodyfikowanej niesuplementowanej i suplementowanej w sposób uzupełniający (tab. 3).