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The effect of vitamin B_6 supplementation of protein deficiency diet on hematological parameters in the blood of rats subjected/non subjected to physical exertion – a pilot study

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Abstract

Deficient nutrition is associated with geographical/cultural reasons as well as deliberate malnutrition. Deprivation of proteins in diet results in reduction of muscle growth, decrease in metabolic rate and physical activity. An important nutritional factor that could protect against the effects of protein deficiency in patients undergoing physical exertion may be the B vitamins, especially vitamin B_6. The aim of the study was to evaluate the effect of vitamin B_6 supplementation on hematological parameters of rats fed 30 days a protein deficiency diet with or without physical exertion. B_6 supplementation significantly increased body mass and feed consumption in protein deficient rats. It also improved MCV and HGB levels. These results suggested that B_6 supplementation may protect bloodstream against effect of protein malnutrition in diets.

Key words: *rats, protein deficiency diet, vitamin B_6, training, hematologic parameters.*

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Introduction

Knowledge of the body's response to nutrient deficiencies in the diet is the basis for effective methods to reduce and prevent the effects of inadequate nutrition. This applies both to individuals who for geographical/cultural reasons consume the diets lacking in certain components, as well as people who consciously choose deficient diet (i.e. during weight loss or illness). One of these is a diet with reduced protein content, which is often used in kidney diseases [1, 2]. Protein deficiency leads to a reduction of muscle growth with a simultaneous decrease in metabolic rate and physical activity. It increases blood pressure which may be associated with endothelial dysfunction and increased sympathetic activity [3]. They also result in disorders in metabolism of lipids and carbohydrates. In rats fed a diet with protein deficiency, changes in plasma level of omega-3 and -6 fatty acids family, and liver steatosis was observed [4, 5]. In addition young rats fed this diet exhibited a lower vascularization in the pancreas, reduced number of islets and β cells, decreased insulin and glucagon level [6-8].

Special groups, in the case of protein malnutrition, are people's undergone large physical exertion (athletes or physically workers). Physical activity improves the body's blood circulation, reduces blood pressure, reduces fat excess and improves glucose metabolism [9, 10]. Unfortunately, long-term physical activity is destructive to the body and may increase the effects of undernutrition. An important nutritional factor that can protect against the effects of protein deficiency in patients undergoing physical exertion may be the B vitamins, especially vitamin B_6. It has been shown that the vitamin is involved in the metabolism of amino acids, carbohydrates and fats, is responsible of the proper action of nervous and hematopoietic system. A diet

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enriched in vitamin B_6 prevents alcohol-induced pressure and reduces blood pressure in spontaneously hypertensive rats [11, 12]. In addition, an increased dose of vitamin B_6 reduces the recovery period in rats with induced injuries [13]. Therefore, in this study we decided to determinate the effect of vitamin B_6 on hematological parameters of rats fed a protein deficiency diet with or without physical exertion.

Material and methods

Animals

The experiment lasted 30 days. The study was performed on 70 rats, male Wistar race, with an initial body weight about 127 ±7 g (without statistical differences between groups). The experimental protocol was approved by the IV Local Ethic Committee for Animals Studies in Warsaw. Animals were cultured in metal cages (5 units per cage) in air-conditioned room with constant temperature of 23°C and 12-hour light cycle. Rats were fed ad libitum semisynthetic isocaloric diet (energy value 350 kcal/100 g) for 30 days. Rats were divided into 6 groups: control (I, 10 rats), control + training (II, 10 rats), protein deficient (III, 10 rats), protein deficient + training (IV, 10 rats), protein deficient with B_6 supplementation (V, 15 rats) and protein deficient with B_6 supplementation + training (VI, 15 rats). Training groups were subjected to physical exercise for 5 days a week. Training consisted of whole 1 hour running on a treadmill belt at a speed of 20 m/min. Body weight of rats and feed consumption were examined two or three times a week. Blood samples (about 300 µl) from the tail after 30 days of experiment were collected.

Diets

In experiment three types of diet was used (Table 1):
- control diet – containing 20% energy from protein,
- experimental diet – containing 4.5% energy from protein,
- experimental diet – containing 4.5% of energy from protein with vitamin B_6 supplementation (300% of norm).

In the control diet 20% of energy coming from protein and 15% from fat of which approximately 2% of the essential fatty acids (EFAs). Diets were supplemented with mineral salts [14] and vitamins [15], in accordance with the guidelines for the rat. In experimental diet 4.5% of energy came from protein, and the remaining energy parts were supplemented with carbohydrates.

Hematological analysis

Blood samples were collected on 0.05% EDTA in PBS, and then hematologic analysis on Sysmex F-820 machine were performed (according to the Sysmex manufacturer procedure). Additionally to evaluate morphological parameters (FSC/SSC) flow cytometry analysis were performed (FACScalibur). Typical diagram of cytometric analysis, with an indication of WBC subpopulations were shown at Fig. 1.

Statistical analysis

Results were presented as means ± SEM. In the case of normal distribution of values parametric one-way ANOVA verified by Tukey post-test was done. With non-parametric distribution Kruskal-Wallis test were used (GraphPad Prism software). In every analysis values of $p < 0.05$ were considered significant.

Table 1. Composition of the diets

Diet component	20% energy from protein		4.5% energy from protein	
Unit	**g/100 g**	**kcal**	**g/100 g**	**kcal**
Sunflower oil	0.41	3.69	0.55	4.95
Lard	5.45	48.91	5.31	47.57
Casein	18.97	60.70	4.56	14.59
Eggs powder	1.61	9.33	0.20	1.16
Wheat flour	19.43	67.61	19.43	67.61
Wheat starch	30.00	120.00	30.00	120.00
Potato starch	9.14	–	11.36	–
Sugar	10.00	39.90	23.59	94.12
Mineral mixture*	4.00	–	4.00	–
Vitamin mixture**	1.00	–	1.00	–

**100 g mineral mixture contains: 32.2 g $KHPO_4$, 30.0 g $CaCO_3$, 16.7 g NaCl, 10.2 g $MgSO_4$, 7.5 g $CaHPO_4$, 2.75 g $FeC_6P_5O_7$, 0.51 $MnSO_4$, 0.08 g KJ, 0.03 g $CuSO_4$, 0.025 g $ZnCl_2$, 0.005 g $CoCl_2$.*

***100 g vitamin mixture contains: 54 500 IU vit. D_3, 0.1 g vit. K, 3.0 µg vit. B_{12}, 1.0 g choline chloride, 0.101 g folic acid, 0.003 g biotin, 1.0 g inositol, 1.0 g PABA, 125 000 IU vit. A, 0.15 g vit. B_6, 0.25 g vit. E, 0.5 g vit. B_1, 2.5 g vit. C, 0.5 g vit. PP, 0.25 g vit. B_2, 2.5 g calcium panthotenate.*

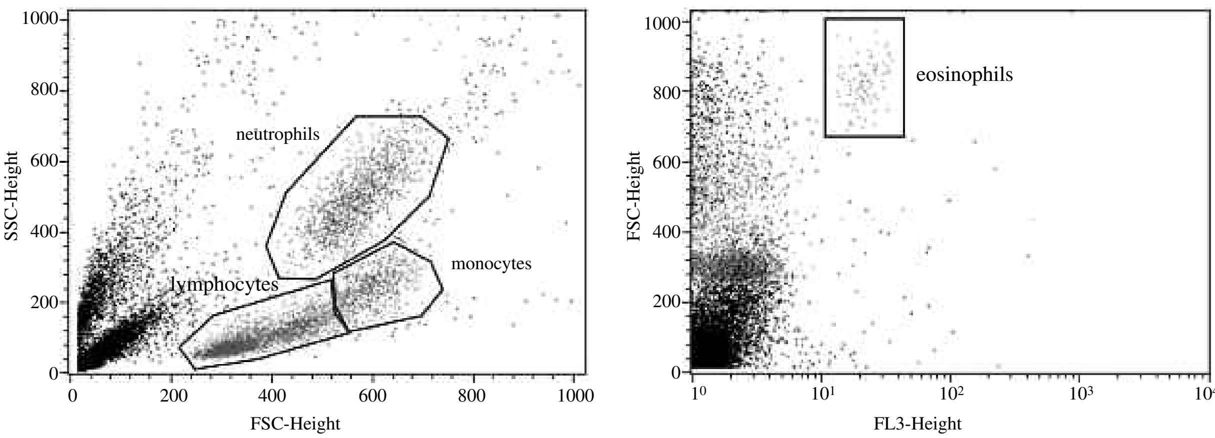

Fig. 1. Typical dot-plot of flow cytometry analysis of rat blood, with an indication of WBC subpopulations

Results

Body weight and diet consumption

It was not found statistical differences of body weight and diet consumption among trained and non-trained groups feed the same diet. Protein deficiency diet (4.5% of protein) caused significant reduction of average rat body mass beginning from first week, which were increased with time, in comparison to rats consuming control diet. After 30 days of experiment weight of control was about 2.5 times higher than in protein deficiency group. This observation was correlated with diet consumption. Protein deficiency rats consumed significantly less feed than those from control group throughout the experiment.

B_6 supplementation of protein deficiency diets resulted in statistical increase of mean body mass started from third week and after 30 day of experiment the difference amounted to 1.5 times in comparison to non-supplemented group. Further we didn't observed statistical changes in feed consumption in B_6 supplemented and non-supplemented groups. Described results were shown in Table 2 and 3.

Hematological analysis

It was not found significant changes in red blood cells (RBC) and platelets (PLT) concentration between rats feed control or protein deficiency diet. However, lower level of hemoglobin (HGB) and decreased of MCV and MCH parameters in protein deficiency rats was observed. B_6 supplementation didn't affect and MCH parameters but improves MCV and HGB level of rats feed protein deficiency diet. B_6 treated rats had also higher percent of hematocrit (HCT) comparable with control rats. Physical training changes some parameters within groups. It increased MCV (20% and 4.5% group) and decreased MCH parameters (20% and 4.5% group). Interestingly, training in B_6 supplementation diet didn't affect those parameters.

Either protein deficient as well as B_6 supplementation diets had no effect on total concentration, and specific subpopulations of white blood cells. We noticed only few significant differences, all between trained groups. B_6 supplementation, in both subjected and non-subjected to physical exertion rats, increased lymphocytes and monocytes and decreased neutrophils levels in comparison to protein deplet-

Table 2. Weekly measured body weight of rats fed control (20% of protein) and experimental diet (4.5% of protein, with or without B_6 supplementation) subjected or not subjected to physical exertion. Results were presented as means ± SEM. Significant differences ($p < 0.05$) were specified in comparison to: ^{a} – I group; ^{b} – II group; ^{c} – III group; ^{d} – IV group; ^{e} – V group

Body weight (g)	**20% of protein (I)**	**20% of protein + training (II)**	**4.5% of protein (III)**	**4.5% of protein + training (IV)**	**4.5% of protein + B_6 (V)**	**4.5% of protein + B_6 + training (VI)**
beginning	125.7 ±0.9	126.9 ±0.9	126.7 ±1.5	126.0 ±1.6	127.7 ±1.0	126.9 ±1.2
1st week	191.9 ±0.7	182.8 ±9.3	141.6 ±5.2^{a,b}	149.1 ±3.9^{a,b}	163.8 ±5.6^{a,c}	163.1 ±2.1^{a,c}
2nd week	247.3 ±3.0	245.2 ±13.1	146.8 ±7.2^{a,b}	163.9 ±5.6^{a,b}	184.9 ±7.7^{a,b,c}	175.9 ±3.5^{a,b}
3rd week	321.0 ±4.4	310.2 ±17.8	151.4 ±6.6^{a,b}	157.2 ±4.9^{a,b}	211.3 ±10.0^{a,b,c,d}	199.6 ±4.4^{a,b,c,d}
4th week	351.6 ±7.7	350.2 ±19.6	141.7 ±5.8^{a,b}	149.4 ±4.5^{a,b}	223.8 ±10.5^{a,b,c,d}	209.9 ±4.9^{a,b,c,d}
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 15$	$n = 15$

Table 3. Diet consumption of rats fed control (20% of protein) and experimental diet (4.5% of protein, with or without B_6 supplementation) subjected or not subjected to physical exertion. Results were presented as means ± SEM. Significant differences ($p < 0.05$) were specified in comparison to: [a] – I group; [b] – II group; [c] – III group; [d] – IV group; [e] – V group

Feed consuption (g/day/rat)	20% of protein (I)	20% of protein + training (II)	4.5% of protein (III)	4.5% of protein + training (IV)	4.5% of protein + B_6 (V)	4.5% of protein+ B_6 + training (VI)
1st week	17.6 ±0.3	17.3 ±0.6	12.1 ±0.8[a,b]	14.1 ±1.2	15.4 ±0.9	15.5 ±0.3
2nd week	21.6 ±0.9	20.0 ±0.7	13.6 ±1.0[a]	15.8 ±1.2	17.8 ±0.9	14.8 ±0.8[a]
3th week	26.8 ±0.6	22.4 ±0.7	14.0 ±1.2[a,b]	15.8 ±0.8[a]	20.0 ±0.4	18.2 ±1.0
4th week	25.7 ±0.9	23.1 ±0.2	12.2 ±1.3[a,b]	15.7 ±1.3[a]	18.8 ±0.4	18.3 ±0.5
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 15$	$n = 15$

Table 4. Hematological parameters of rats fed control (20% of protein) and experimental diet (4.5% of protein, with or without B_6 supplementation) subjected or not subjected to physical exertion after 30 days. Results were presented as means ± SEM. Significant differences ($p < 0.05$) were specified in comparison to: [a] – I group; [b] – II group; [c] – III group; [d] – IV group; [e] – V group

Hematological parameters	20% of protein (I)	20% of protein + training (II)	4.5% of protein (III)	4.5% of protein + training (IV)	4.5% of protein + B_6 (V)	4.5% of protein+ B_6 + training (VI)
RBC (10^6/µl)	8.2 ±0.2	7.1 ±0.3	8.0 ±0.3	8.6 ±0.4	8.8 ±0.3[b]	8.8 ±0.2[b]
HGB (g/l)	125.7 ±1.7	114.7 ±5.0	106.6 ±5.3[a]	112.7 ±4.2	119.5 ±3.1	120.4 ±2.1
HCT (%)	35.9 ±0.7	36.5 ±1.7	30.7 ±1.3	40.5 ±4.7[c]	35.6 ±1.2	36.4 ±1.2[c]
MCV (fl)	43.6 ±0.5	51.1 ±0.4[a]	38.4 ±0.6[a,b]	43.0 ±0.9[b,c]	40.3 ±0.6[a,b]	41.9 ±0.8[b,c]
MCH (pg)	15.3 ±0.3	16.1 ±0.5	13.4 ±0.3[a,b]	14.2 ±0.2	13.6 ±0.3[a,b]	13.7 ±0.3[a,b]
MCHC (g/l)	350.9 ±5.2	314.6 ±8.9[a]	348.3 ±5.5[b]	304.5 ±16.5	337.7 ±6.4	334.2 ±7.9
PLT (10^3/µl)	863.9 ±58.3	1063.0 ±90.5	859.3 ±57.7	739.2 ±95.7	922.0 ±54.6	939.6 ±75.4
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 15$	$n = 15$

Table 5. White blood cells (WBC) parameters of rats fed control (20% of protein) and experimental diet (4.5% of protein, with or without B_6 supplementation) subjected or not subjected to physical exertion after 30 days. Results were presented as means ± SEM. Significant differences ($p < 0.05$) were specified in comparison to: [a] – I group; [b] – II group; [c] – III group; [d] – IV group; [e] – V group

WBC parameters	20% of protein (I)	20% of protein + training (II)	4.5% of protein (III)	4.5% of protein + training (IV)	4.5% of protein + B_6 (V)	4.5% of protein+ B_6 + training (VI)
WBC (10^3/µl)	9.5 ±0.9	8.8 ±0.8	7.1 ±1.1	10.8 ±1.4	9.0 ±0.6	10.4 ±0.9
lymphocytes (%)	51.5 ±4.5	64.8 ±2.9	56.2 ±4.8	43.7 ±5.6	62.4 ±3.1[d]	62.6 ±3.1[d]
neutrophils (%)	42.2 ±4.1	28.3 ±1.4	37.3 ±4.8	52.0 ±6.1[b]	31.9 ±3.3[d]	30.2 ±3.0[d]
eosinophils (%)	2.6 ±0.4	3.8 ±0.8	2.8 ±0.9	1.6 ±0.3	1.6 ±0.3	2.0 ±0.3
monocytes (%)	3.7 ±0.4	3.1 ±0.3	3.7 ±0.3	2.8 ±0.6	4.1 ±0.5	5.2 ±0.5[b,d]
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 15$	$n = 15$

ed trained group. We also observed higher level of neutrophils (protein deficient trained rats) and monocytes (B_6 supplemented protein deficient trained rats) in comparison to control rats subjected to physical exertion.

Results of white blood cells (WBC) analysis were presented in Table 5 and the rest hematological parameters in Table 4.

Discussion

Protein malnutrition is the most common cause of early stunting and later chronic diseases. Long-term protein malnutrition, especially connected with energy deprivation, lead to marasmus and kwashiorkor disease, which often result in dead. That's why scientists searching for nutritional or chemical factors which may protect organism against protein deficiency effect. In presented article we decide to evaluate effect of B_6 supplementation of protein deficient diet after 30 day of deprivation on hematological parameters in Wistar rats. Additionally we want to check whether physical exercise improves or exacerbate the condition of organism fed protein deficient diet supplemented or non-supplemented with B_6 vitamin.

It has been shown that protein deficient diets significantly decreased body and organ weight and length of rats compared with control animals [16, 17]. We also observed these relations. Body mass of rats feed diet with protein malnutrition after 30 day of experiment were 2.5 times lower than in control group and was associated with feed consumption. What is interesting, significant differences between groups were observed already after the first week of study. Vitamin B_6 supplementation increased 1.5 times body weight of rats fed protein deficient diet in 30-th day of the experiment. There are a several evidences that B_6 vitamin deficiency decreased body mass in rats [18, 19]. In addition, B_6 supplementation improves status of overweight or obese woman [20]. Unfortunately, we didn't find in world literature articles dedicated to the effect of vitamin B_6 supplementation on gain weight. In our opinion, the effects of vitamin B_6 to increased body weight correlated with increased feed intake may be associated with an increased appetite in rats or a diet enriched in vitamin B_6 is tastier for rats. Several authors revealed changes in B_6 concentration and metabolism induced physical exercise [21, 22]. However, they also indicated that this change does not increase the vitamin B_6 requirement of growing rats [23]. In this study, we didn't notice statistically significant differences of body mass and feed consumption between trained and non-trained rats among the same dietary group. This data also indicate that prolonged exercise over 30 day of protein malnutrition does not affect body mass of rats fed protein deficient diet. Perhaps, short term of the study limits the possibility of finding that relations.

Protein malnutrition impairs protein, lipid and carbohydrates metabolism, insulin secretion and pancreas function [6, 8, 24]. It also affects cardiovascular and immunology system due to endothelial dysfunction, hypertension and anemia syndrome or disturb growth and development of thymus and spleen [3, 17, 25]. Protect role against protein deficiency in cardiovascular and immune system can play vitamin B_6. It has been shown, that B_6 lowers cholesterol, prevents atherosclerosis, regulates blood pressure and inhibits the release of histamine and participates in antibodies and red blood cells synthesis [26, 27]. The data from hematological analysis of rats fed control diet, presented in this article, were consistent with results obtained for Wistar rats [28]. Generally, we didn't observed significant changes in number of red blood cells (RBC) platelets (PLT) and between rats fed different diets or undergo physical exercise. Protein deficient diet cause decreased of hemoglobin (HGB), MCV and MCH parameters in rat blood which is origins of anemic state. B_6 supplementation not affected MCH but improves MCV and HGB level of protein deprived rats. Perhaps, long period of the study may reveal more differences among studied group.

These results suggest that B_6 supplementation may protect morphotic blood elements against negative effect of protein deficient diets. However, to fully explain the protective role of vitamin B_6 on hematological parameters in protein deprivation it is necessary to conduct further studies.

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