Polyunsaturated Fatty Acid (PUFA) Changes in Serum and Liver of Undernourished Rats Given Dietary Vitamin B₆ Supplementation

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Summary The influence of vitamin B₆ on linoleic (LA), ω-linolenic (ALA), arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid content in serum and liver of rats fed with protein-energy deficient diets for 90 d, was studied. To estimate the effect of dietary supplementation with vitamin B₆ on the composition and level of fatty acids in the serum and liver of rats, two experiments were performed. In these experiments control rats were fed ad libitum semisynthetic isocaloric diets of 1,466.5kJ/100g (350kcal/100g) throughout 90 d while the examined rats were offered 50% and 30% of the previously determined daily intake of the diet consumed in the control group. The experimental diet was supplemented with vitamin B₆ to the level 4-times higher than in the control diet. A reduction to the half consumption of a standard diet supplemented with vitamin B₆ caused a significant decrease of LA and ALA in blood serum at 30 and 60 d. At 90 d of the experiment the value of LA was lower and the content of AA was higher in comparison to the control group. After 30 d of consumption of vitamin B₆ enriched diet in rats subjected to feed restriction to only 30% of the control intake, an increase of ALA and a decrease of AA, EPA and DHA were noticed in serum. At 60 d an increase of DHA was observed. Ninety days of feeding this diet caused a significant increase of AA level. Feeding animals for 90 d with a vitamin B₆ enriched diet, with limited consumption to 50%, caused a significant decrease of LA and ALA and an increase of EPA content.

Key Words polyunsaturated fatty acids, vitamin B₆, protein-energy malnutrition

It is known that caloric restriction increases longevity and retards age-associated diseases of laboratory rodents. Such restriction reduces oxidative stress and improves risk factors for cardiovascular disease not only in animals but also in humans. A study of Wang and coworkers (1) has shown independent influence of caloric restriction and body weight on longevity of rats. Energy restriction increased sympathetic nervous system activity which is partly responsible for the increase of basal metabolism and meal induced thermogenesis (2). It has a direct influence on lipid metabolism.

The influence of protein and energy deprivation on polyunsaturated fatty acid (PUFA) metabolism has been shown in experiments performed on humans and animals. Malnutrition is often accompanied by PUFA deficiency in serum and also other tissues. Results of many observations show a decrease of PUFA content among undernourished children (3–6). The same effect was described by Ascuiti-Moura et al. (7) among elderly people, by Bjerve et al. (8) among patients with upper alimentary tract surgery and by Stein et al. (9) among patients fed intragastrically. Pregnolato et al. (10) have shown a complex interaction among nutritional factors such as protein, essential fatty acids (EFAs) and vitamin B₆ with prevailing influence of dietary protein on hepatic lipid composition. Low dietary EPA as well as vitamin B₆ intake change PUFA metabolism in rats.

Cunnane et al. (11) have described a similar interaction between vitamin B₆ and EFAs in rats. The authors noticed that in experimental vitamin B₆ deficiency plasma, concentrations of LA and ω-LA are increased and that of AA is decreased. The reverse changes were observed after vitamin B₆ supplementation. However Tolonen et al. (12) could not find any significant correlation between human plasma fatty acids and any of the vitamin B₆ variables.

Vitamins B₆ as well as folic acid and B₁₂ are the source of coenzymes participating in one carbon residue metabolism. In the case of a deficiency of these vitamins a higher concentration of homocysteine appears in blood plasma, which is a risk factor for cardiovascular diseases, stroke and thrombosis (13). In an attempt to decrease homocysteine concentration in humans an oral dose of 25 mg of vitamin B₆ was used for 10 d. The results showed no change in methionine or homocysteine; however, a plasma folate decrease was observed.
The aim of this experiment was to study the effect of dietary vitamin B₆ supplementation on serum and liver PUFA content of rats given restricted amounts of a diet.

**MATERIALS AND METHODS**

Growing male Wistar breed rats initially weighting 150.1±6.2 g were kept in individual stainless steel cages in a room with controlled temperature (23±1°C), humidity (60±5%) and lighting (12 h day cycle). Two experiments were performed to estimate the effect of food restriction and concomitant supplementation with pyridoxine on the level of the chosen PUFA in serum and rat liver. In these experiments rats were fed ad libitum semisynthetic isocaloric diets of 1,466.5 kJ/100 g (350 kcal/100 g) throughout 90 d. In the standard diet 20% energy was provided from protein, 15% from fats, including 2% from EFAs (control diet—Table 1A). In experiments the feed restriction to 50% and 30% of previously determined daily consumption of the control diet was used. Diets delivered to animals in restricted amounts were supplemented with 4.5 mg vitamin B₆/100 g (pyridoxine hydrochloride), a level 4 times higher than in the control diet. Twenty rats were used per treatment.

Blood samples were collected at 30, 60 and 90 d from the tail vein of rats. Twelve hours prior to the end of the experiment food was withheld. At the end of the experiment rats were sacrificed by cervical displacement while unconscious (general anesthesia using ethyl ether) to obtain livers. Livers were washed in situ with ice cold 1.15% KCl solution, excised, blotted dry and weighed. Liver homogenates were prepared in 1.15% ice cold KCl solution in a polytronic homogenizer. Contents of LA (C18:2, n-6), ALA (C18:3, n-3), AA (C20:4, n-6), EPA (C20:5, n-3) and DHA (22:6, n-3) in blood serum were estimated at the 30-, 60- and 90d of the experiment. In the liver the same analysis was performed at 90 d of the experimental period. Samples were stored at −20°C until analysis.

Serum and hepatic lipids were extracted by Folch (15) method using mixture of chloroform and methanol in the v/v ratio 2:1. Extracted lipids were saponified and esterified by the method according to International Standard-ISO 5509; 2000 (16).

Separation of fatty acids methyl esters was performed using Hewlett Packard HP 6890 gas chromatograph with HP 23-cis/trans 100 m capillary column (0.20 mm inner diameter). The He flow was 20 cm³/s under pressure of 43.4 psi. GC/MS interface 250°C, split 1:100, separation temperature 220°C. The total analysis time was 65 min. The results were registered using HP Chemstation Integrator software. Identification of fatty acids was based on the comparison of standard retention times. Fatty acid standards supplied by Sigma were analyzed separately or in the mixture. C-15 fatty acid was used as an internal standard. Quantity evaluation was calculated based on received spike area of the analyzed fatty acid in the sample with the analogical standard and expressed as a% of total fatty acid content.

ANOVA and median test were used for statistical data (17). Differences of means at p≤0.05 (n=20) were considered statistically significant.

**RESULTS**

Results of estimated fatty acid composition of the diet used are presented in Table 1B. Monounsaturated FAs were the main component (45.95%) of FA estimated in the rats' diet because oleic

Table 1A. Diet composition.

| Diet component | 20% energy from protein |
|----------------|------------------------|
| Sunflower oil  | 3.6                    |
| Lard           | 54.9                   |
| Casein         | 189.7                  |
| Egg powder     | 16.1                   |
| Wheat flour    | 194.3                  |
| Wheat starch   | 300                    |
| Potato starch  | 91.4                   |
| Sugar          | 100                    |
| Mineral mix.¹  | 40                     |
| Vitamin mix.²  | 10                     |

¹1,000 g mineral mixture contains: KH₂PO₄, 322.0 g; CaCO₃, 300.0 g; NaCl, 167.0 g; MgSO₄, 102.0 g; CaHPO₄, 75.0 g; FeC₆H₅O₇, 27.5 g; MnSO₄, 5.1 g; KI, 0.8 g; CuSO₄, 0.3 g; ZnCl₂, 0.25 g; CoCl₂, 0.05 g, and powdered sucrose to make 1,000 g.

²1,000 g vitamin mixture contains: V.D₃, 545,000 IU; V. K, 1.0 g; V. B₁₂, 30 μg; choline chloride, 10.0 g; folic acid, 1.01 g; biotin, 0.03 μg; inositol, 10.0 g; PABA, 10.0 g; V. A, 125,000 IU; V. B₆, 1.5 g; V. E, 2.5 g; V. B₃, 5.0 g; V. C, 25 g; V. PP (niacin), 5.0 g; V. B₉, 2.5 g; calcium panthotenate, 25.0 g; and powdered sucrose to make 1,000 g.

Table 1B. Fatty acid composition of the experimental diet.

| Fatty acids     | Composition (%) | Σ (%) |
|-----------------|-----------------|-------|
| Saturated       | 12:0=0.11; 14:0=1.02; 15:0=0.13; 16:0=18.81 | 37.12 |
|                 | 17:0=0.44; 18:0=15.41; 20:0=0.54; 21:0=0.66 |       |
| Monounsaturated | 16:1=2.98; 17:1=0.46; 18:1=40.69; 20:1=1.82 | 45.95 |
| Polyunsaturated | 18:2=15.56; 18:3=0.96; 20:4=0.41 | 16.93 |

Each mean is an average of 3 estimations.
Table 2. Body weight of rats given control diet (20% of energy from protein) ad libitum or with restriction to 50 and 30% of intake with/without vitamin B6 supplementation. n = 20.

| Feed | Body weight of rats (g) |
|------|------------------------|
|      | Day 0   | Day 30  | Day 60  | Day 90   |
| 20% energy from protein | 151.1±8.6 | 323.4±27.8 | 420.6±38.4 | 491.7±35.9 |
| Intake restricted to 50% | 151.1±6.8 | 214.5±12.2 | 250.5±9.0 | 284.6±13.8 |
| Intake restricted to 50% + B6 | 148.6±8.6 | 219.7±6.9 | 272.1±6.6* | 320.0±6.1* |
| Intake restricted to 30% | 149.9±7.7 | 175.4±8.1 | 189.9±8.4 | 199.7±9.3 |
| Intake restricted to 30% + B6 | 149.8±6.5 | 176.9±9.0 | 192.3±9.6 | 205.1±8.0 |

Means±SD.

* Significant difference between rats receiving the same diet with/without B6, at p<0.05 level.

Table 3. Fatty acid composition in blood serum of rats fed a basal diet (proteins supplying 20% of energy) with or without vitamin B6 supplementation for 90 d. Part of the animals had the amount of feed restricted to 50% of control group. n = 20.

| Fatty acid | Day 30 | Day 60 | Day 90 |
|-----------|--------|--------|--------|
| LA 10.60± | 12.39± | 8.56±  |
| 0.91      | 1.49   | 0.93*  |
| 0.12±     | 0.12±  | 0.04±  |
| 0.02      | 0.06   | 0.02*  |
| AA 11.69± | 15.96± | 15.40± |
| 1.75      | 0.72±  | 1.25   |
| ALA 0.19± | 0.13±  | 0.16±  |
| 0.04      | 0.10   | 0.16   |
| 0.20      | 0.38   | 0.07   |
| DHA 1.31± | 0.91±  | 1.10±  |
| 0.20      | 0.38   | 0.07   |
| Σ Fatty acids 23.91± | 29.51± | 25.26± |
| 2.00      | 2.62±  | 3.55*  |
| 1.02      | 2.54±  | 1.81   |

* Differences between similar diets with and without vit. B6 supplementation, p<0.05.

acid (18:1) was found to be main the FA participant (40.69%).

Average daily feed consumption estimated in the experiment was 24.2±1.2 g of the adequate diet. Intake restrictions up to 50% and 30% of the adequate diet daily consumption (control group), were used as a model of malnutrition. This model of rats' alimentation caused not only energy deprivation, but also proportional limitation of all diet constituents: protein, vitamin and mineral elements.

Restiction of feed intake caused depression in rats' growth (Table 2). This retardation of growth was very pronounced in groups of animals receiving only 30% control diet intake. Dietary vitamin B6 supplementation caused significantly higher body mass in rats receiving 50% of the standard amount, without any effect in the group of animals receiving only 30% of the control intake.

Rats given only one half of the control diet intakes were seen to posses a significantly increased amount of some of the examined FAs (Table 3). After 1 mo of feeding, a significantly higher level of AA was observed and in 60 and 90 d contents of LA and AA were increased. It caused an increase in the value of the PUFA sum, too. Vitamin B6 addition caused a significant decrease of LA concentration lasting the whole experimental period andALA at 30th and 60th day in rats fed 50% of diet control amount. In serum samples collected on the 60th day of the experiment a higher content of DHA and on day 90 a further increase of AA were also observed compared with results obtained in the control group. The above significant changes in contents of
Table 4. Fatty acid composition in blood serum of rats fed a basal diet (proteins supplying 20% of energy) with or without vitamin B6 supplementation for 90 d. Part of the animals had the amount of feed restricted to 30% of the control group. n=20.

| Fatty acid | 20% energy from protein | Feed restricted to 30% | Feed restricted to 30% + B6 |
|------------|-------------------------|-----------------------|-----------------------------|
| LA         | 9.93 ± 1.19 ± 11.19 ± 11.19 ± | 11.19 ± | 11.19 ± |
| ALA        | 0.17 ± 0.09 ± 0.19 ± 0.19 ± | 0.19 ± | 0.19 ± |
| AA         | 7.8 ± 17.15 ± 11.95 ± 11.95 ± | 13.32 ± | 16.02 ± |
| EPA        | 0.21 ± 0.25 ± 0.06 ± 0.06 ± | 0.04 ± | 0.12 ± |
| DHA        | 1.19 ± 1.31 ± 0.88 ± 0.88 ± | 1.34 ± | 0.91 ± |
| Σ Fatty acids | 19.34 ± 29.99 ± 24.27 ± 24.27 ± | 24.21 ± | 29.35 ± |

* Differences between similar diets with and without B6 supplementation, p≤0.05.

Further restriction of feed to 30% of the control intake caused a significant increase of total serum FAs (Table 4). After 1 mo of feeding, compared with the control animals, a higher concentration of AA and a lower of ALA was observed. After 2 mo of the experiment such diet restriction caused an increase in LA and a drop of DHA. In rats fed 3 mo of such limited amount of diet a significantly lower concentration of ALA was observed while content of LA was increased.

The restriction of diet consumption to only 30% daily consumption, with vitamin B6 addition, caused significant differences in PUFA concentration in blood serum of rats. The results of analysis of blood samples collected after 30 d of the experiment showed that rats supplemented with vitamin B6 in comparison with the control animals, possessed higher levels of ALA, and lower levels of AA, EPA and DHA in serum. The sum of the examined FAs was decreased, too. The increase of DHA content was found on day 60 while a considerably decreased AA content was found on day 90 compared with the control group. On day 90 the value of total examined PUFAs was lower too.

Table 5A. Composition of fatty acids in the liver of rats fed a control diet or the given amount of this diet restricted to 50% with or without vitamin B6 supplementation for 90 d.

| Fatty acid | Control diet= 20% energy from protein | Feed restricted to 50% | Feed restricted to 50% + B6 |
|------------|-------------------------------------|-----------------------|-----------------------------|
| LA         | 7.81 ± 1.57 ± 7.66 ± 0.41 ± 8.31 ± 0.87 ± | 8.31 ± 0.87 ± | 8.31 ± 0.87 ± |
| ALA        | 0.11 ± 0.06 ± 0.03 ± 0.07 ± 0.15 ± 0.06 ± | 0.15 ± 0.06 ± | 0.15 ± 0.06 ± |
| AA         | 19.28 ± 2.23 ± 22.32 ± 0.98 ± 21.95 ± 1.70 ± | 21.95 ± 1.70 ± | 21.95 ± 1.70 ± |
| EPA        | 0.15 ± 0.04 ± 0.12 ± 0.13 ± 0.18 ± 0.04 ± | 0.18 ± 0.04 ± | 0.18 ± 0.04 ± |
| DHA        | 4.47 ± 0.60 ± 5.12 ± 0.43 ± 4.85 ± 0.43 ± | 4.85 ± 0.43 ± | 4.85 ± 0.43 ± |
| Σ F.A.     | 31.82 ± 2.80 ± 35.47 ± 1.20 ± 35.44 ± 1.97 ± | 35.44 ± 1.97 ± | 35.44 ± 1.97 ± |

* Differences between similar diets with and without B6 supplementation, p≤0.05.

The rats given only 50% of the diet amount for 90 d of the experiment were shown to have a higher sum of examined FAs in the liver (Table 5). A higher concentration of AA and DHA was noticed. Further limitation of feed amount to 30% caused an increase in LA and a decrease in EPA.

Feeding animals throughout 90 d with the vitamin B6 enriched diet, with its limited 50% consumption,
caused a significant increase of ALA content in the liver but had no effect on the content of other tested FAs (Table 5A). The limitation of this diet consumption to 30% caused a significant decrease of LA and ALA content and an increase of EPA content (Table 5B).

**DISCUSSION**

Results of many experiments show a close relation between vitamin B₆ content in the diet and PUFA metabolism (18, 19). For the first time it was reported by Witten and Holman (20) that vitamin B₆ deficiency influences transformation of LA to AA. They observed a decrease of AA concentration in tissues of rats fed a vitamin B₆-deficient diet.

Goswami and Coniglio (18) confirmed this result. In rats receiving a vitamin B₆-deficient diet and refed with a diet supplemented with this vitamin they noticed, during a period of B₆ deficiency, simultaneously a decrease of LA and an increase of another PUFA concentration in rats' testes. Bergami et al. (21) have found a decreased level of AA and an increased value of LA in serum of rats fed vitamin B₆-deficient diets with normal lipid content. In animals fed the above-mentioned diet where the source of fat was fish oil, there were no further variations.

However in experiments performed by Scheier and Williams (22) a significant increase of hepatic AA concentration, caused by vitamin B₆ supplementation, was observed. They also observed an increase of AA concentration in hepatic phospholipids of rats given a diet low in protein and fat supplemented with pyridoxine. This effect of vitamin addition was most pronounced at the beginning of vitamin B₆ supplementation. The study of She et al. (19) has shown that this vitamin influences the activity of enzymes taking part in the transformation of LA to its metabolite e-LA. Rats given a 5-wk high-protein but vitamin B₆-deficient diet were found to have a decreased concentration of AA in the liver and an increased content of its substrate-LA in the liver and serum lipids as well. Authors of the study have shown that vitamin B₆ deficiency leads to inhibition of Δ-6 desaturase activity, which seems to be the main factor of AA synthesis inhibition. Delta-6-desaturase activity is influenced by many nutritional and non-nutritional factors, among which the most important are vitamin B₆ and age. Bordoni et al. (23) observed a diminished Δ-6 desaturase activity in the liver microsomes of 20-mo-old rats fed a diet with subnormal level of vitamin B₆. These results suggest that the impairment of Δ-6 desaturase activity by vitamin B₆ deficiency might be an important factor in decreasing the synthesis of n-6 and n-3 PUFAs. This may be particularly important in aging, where Δ-6 desaturase activity is already impaired.

It was shown that vitamin B₆ deficiency leads to peroxidative stress in rat organs. When fish oil was used in the diet to increase n-3 PUFA intake and at the same time only a marginal level of vitamin B₆ was given, it was noticed that LA increased and AA decreased. In rats given such a diet an increased lipid peroxidation was observed in the heart, but not in the liver (24). A diet high in fish fat enhanced peroxisomal β-oxidation and increased plasma triacylglycerol but mitochondrial oxidation was slightly reduced (25). Genetic disorders of oxidative phosphorylation causing increased NADH/NAD(+) ratio were associated with impaired DHA synthesis and secondary carnitine deficiency (26).

Choi and Yu (27) have shown that a dietary restriction consisting of 60% of daily caloric allowance causes a significant increase of prostaglandin/thromboxane ratio in rats' serum. They also observed a modulating effect of dietary restriction on serum lipid composition. Prolonged undernutrition of reindeer calves in experimental or natural winter conditions also affected proportions of PUFAs in major serum lipids (28). The observed decrease of LA and ALA was reversed during refeeding. However in rats the obtained results suggest that prolonged feed restriction causes an increase of LA concentration and, temporarily similar changes of AA as well.

Supplementation with vitamin B₆ under restricted consumption to 50% of the diet led to decrease of rats' serum LA while the content of AA was increased on day 90 of the experiment. So these changes are contrary to those caused by reduction of food intake. Results of vitamin B₆ influence in agreement with the observations of Tsuge et al. carried out during vitamin B₆ deficiency (29). Decreased AA and increased ALA concentrations in serum of vitamin B₆-deficient animals were observed in rats given casein-based diet with perilla oil (63% of linolenic acid) for 5 wk. These PUFA changes were accompanied by lowering of Δ-6 desaturase and acyl-CoA oxidase. This Δ-6 desaturase is a regulatory enzyme activated by Fe and Zn and inhibited by Cu ions. Saareks and coworkers (30, 31) have found an increase in prostacyclin production and an inhibition of thromboxane and leukotriene synthesis, the final products of PUFA metabolism, by supplementing the human diet with pyridoxine. This might be beneficial in disorders where production of prostacyclin is decreased and the synthesis of thromboxane and leukotrienes is enhanced.

Further limitation of food consumption to 30% caused quite different changes in serum PUFA concentrations but vitamin B₆ addition had an opposite effect on the content of ALA and AA, especially in the 1st month of the experiment.

In the liver of undernourished rats the dietary vitamin B₆ supplementation had an opposite influence on the level of ALA in the group with consumption restricted to 50% and LA as well as EPA concentration in the group consuming a diet restricted to 30%.

Negative effects caused by malnutrition may be to some extent minimized by addition of vitamin B₆.

The results obtained in this study suggest that vitamin B₆ dietary supplementation not only in protein- and energy-adequate conditions but during long-lasting feeding of rats using a low protein-energy diet (intake restriction) stimulates the pathways of PUFA metabolism.
Footnotes
A part of results of this experiment was presented at the Essential Fatty Acids and Human Nutrition and Health International Conference in Shanghai (China), April 2002.

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