

## **EFFECT OF LACTO-OVO-VEGETARIAN DIET ON SERUM VITAMIN B12 CONCENTRATIONS – FIVE-YEAR PROSPECTIVE STUDY**

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**Background.** Avoiding of meat is the basic principle of vegetarian diet with several variants existing. In the present study we have focused on lacto-ovo-vegetarian (LOV) diet which includes dairy products and eggs. We have aimed to assess its long-term effect on vitamin B12 status and intended to verify the influence of natural and vitamin B12-fortified food.

**Material and methods.** The prospective study was carried out comprising 16 healthy omnivores (12 females, 4 males) who intentionally declared to follow strict LOV diet for at least 5 years. The studied group was divided into 2 subgroups. First subgroup followed LOV diet based entirely on natural products while the second consumed food fortified with B12 vitamin. Evaluation of vitamin B12 concentration was performed before and after 6, 12, 24 and 60 months from LOV diet implementation.

**Results.** Five-years of LOV diet resulted in significant decrease of serum vitamin B12 concentrations in the whole studied group ( $p < 0.05$ ). However, the significant drop of vitamin B 12 level was in fact limited to the subgroup consuming exclusively natural products ( $p < 0.05$ ). In none of the subjects abnormal serum vitamin B12 levels were observed.

**Conclusions.** Five-year lacto-ovo-vegetarian diet did not result in a risk of vitamin B12 deficiency in healthy subjects. However, the levels of serum vitamin B12 concentrations in a subgroup consuming natural diet tend to be significantly lower as compared with the group consuming fortified food. It confirms the need of follow-up (and potentially vitamin B12 fortification of food and/or its supplementation) in long-term lacto-ovo-vegetarians.

**Key words:** lacto-ovo-vegetarians, omnivores, nutrition, vitamin B12, fortified food

## INTRODUCTION

Vegetarianism is the practice of following a diet which excludes meat or meat and other animal-derived products. The diet is very often linked with specific life-style and usually adopted for ethical, health, environmental, culture or religious reasons. Most of vegetarians in the world do not eat meat for the economic situation [Asok 2003]. Regardless of the cause, the issue concerns huge part of the world's population and in the western countries is presently more popular than ever before.

The number of vegetarians is continuing to grow at an astonishing rate. In UK during the last ten years it has been more than doubled, reaching 7% of all British and 12% of adolescents and young adults. In other European countries vegetarian population varies from 0.3% in Portugal to 4.3% in Netherlands. Data from 2008 put the number of U.S. adult vegetarians at 7.3 million (3.2%), while in Poland more than 1% declare vegetarianism [Herrmann and Knapp 2002]. A 2006 survey in India found that 40% of the population (399 million people) are vegetarians [www.raw-food-health.net 2009].

There are many reports about beneficial effects of vegetarian diet on general health. People following modern vegetarian diet demonstrate lower risk of obesity, osteoporosis and cardiovascular diseases, decreased total cholesterol level and declined mortality due to some cancers [Messina and Burke 1997, Key et al. 2009, Newby et al. 2005, Obeid et al. 2002]. However, the elimination of animal products from the diet diminishes the intake of some essential nutrients, such as vitamin B<sub>12</sub>, calcium, iron, zinc or selected amino acids [Hronek and Kudlackova 2005, Janelle and Barr 1995, Suter and Moser 2005]. Lower plasma vitamin B<sub>12</sub> levels in vegetarians than in omnivores have been clearly demonstrated [Huang et al. 2003, Mezzano et al. 2000, Obeid et al. 2002]. Nevertheless, the importance of adequate vitamin B<sub>12</sub> status is often underestimated because the dietary intake of vitamin B<sub>12</sub> is usually far above dietary reference intakes in mixed Western diets and overt clinical manifestation of its deficiency has been so far a rare clinical condition in children and young adults. Vitamin B<sub>12</sub> (cobalamin) functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine and for a separate reaction that converts L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA. Its deficiency results in impairment of the above enzymes and may result in elevated levels, respectively of homocysteine and methylmalonic acid. Increased serum homocysteine level above 15 µmol/l is strongly related to the risk of ischemic heart disease, deep vein thrombosis and stroke [Wald et al. 2002].

Nowadays there are few long-term prospective studies assessing the impact of vegetarian diets on vitamin B<sub>12</sub> status. Avoiding of meat is the basic principle of all vegetarian diets but there are several variants [Abdulla et al. 1981]. In the present study we have focused on lacto-ovo-vegetarian diet that includes dairy products and eggs. We have aimed to assess its long-term effect on vitamin B<sub>12</sub> status and intended to verify the influence of natural and vitamin B<sub>12</sub>-fortified food.

## MATERIAL AND METHODS

The prospective study was carried out comprising 16 healthy omnivores (12 females, 4 males) who intentionally declare to follow strict lacto-ovo-vegetarian diet for

Table 1. Characteristics of the studied group

Parameter	Range	Mean $\pm$ SEM (median)
Age (years)	21-26	23.5 $\pm$ 0.4 (24.0)
BMI	18.5-26.6	21.6 $\pm$ 0.6 (21.2)

at least 5 years (Table 1). The studied group was divided into 2 subgroups. First subgroup followed lacto-ovo-vegetarian diet based entirely on natural products while the second consumed the food fortified in B12 vitamin.

In all subjects serum vitamin B12 levels were determined before and after 6, 12, 24 and 60 months from the implementation of the diet. Vitamin B12 was assessed with the use of chemiluminescence immunoassay. (Immunolite-Vitamin B12 test, Diagnostic Products Corporation; IMX Folate Calibration, Abbott). Values lower than 193 pg/ml were considered as abnormal.

The differences in B12 concentrations were analysed with the use of Friedman test (dependent samples). The level of statistical significance was set at  $p < 0.05$ . The study protocol was approved by Bioethics Committee of Poznań University of Medical Sciences.

## RESULTS

Five-year lacto-ovo-vegetarian diet resulted in a significant decrease of serum vitamin B12 concentrations in the whole studied group ( $p < 0.05$ ). However, the significant drop of vitamin levels was in fact limited to the subgroup consuming exclusively natural products ( $p < 0.05$ ). In none of the subjects abnormal serum vitamin B12 levels were observed (Table 2).

Table 2. Serum Vitamin B12 concentrations within 60 months period of the study

Type of diet (month of the observation)	Vitamin B12, pg/ml					
	whole group		subgroups			
			natural diet*		fortified products	
mean $\pm$ SEM (median)	range	mean $\pm$ SEM (median)	range	mean $\pm$ SEM (median)	range	
Omnivore (0)	396 $\pm$ 34 (372)	211-712	390 $\pm$ 48 (307)	211-712	403 $\pm$ 17 (402)	306-534
LOV (6)	372 $\pm$ 29 (344)	197-622	353 $\pm$ 35 (299)	197-622	391 $\pm$ 20 (377)	299-534
LOV (12)	346 $\pm$ 23 (345)	205-512	321 $\pm$ 27 (299)	205-512	371 $\pm$ 20 (387)	267-498
LOV (24)	342 $\pm$ 22 (328)	203-488	326 $\pm$ 26 (290)	203-488	358 $\pm$ 19 (361)	254-458
LOV (60)	345 $\pm$ 24 (330)	196-475	310 $\pm$ 27 (260)	196-455	380 $\pm$ 20 (384)	249-462

LOV – lacto-ovo-vegetarian.

## DISCUSSION

Despite existing evidence that well-planned vegetarian diets provide numerous health benefits and are appropriate for all stages of the life cycle it is also known that animal foods provide micronutrients that are nonexistent or available only in limited amounts in plant foods [Elmadfa and Singer 2009]. The above facts indicate the possibility of insufficient intake of some macro, microelements and vitamins in diet based on fruits and vegetables with elimination of meat. The problem concerns zinc, calcium, iron, iodine some amino acids, vitamins B12 and D3 [Larsson and Johansson 2005, Miller et al. 1991].

The only reliable unfortified sources of vitamin B12 in human diet are meat, dairy products and eggs [Linnel and Matthews 1984]. Thus different vegetarian diets could potentially exert varied effects. In a cross-sectional study Chabasińska et al. have demonstrated that type of vegetarian diet and length of its application influences vitamin B<sub>12</sub> body resources [Chabasińska et al. 2008]. Allen underlined that inadequate intake, due to low consumption of animal-source foods, is the main cause of low serum vitamin B12 in younger adults [Allen 2009]. Therefore, in the current study we have followed lacto-ovo-vegetarians subjects for 5 years. Although changes were statistically significant, in none of the subjects abnormal vitamin B12 concentrations were stated. Our finding confirms Allen's observations that low intakes of animal-source foods, such as the ones that occur in some lacto-ovo vegetarians may cause vitamin B12 depletion [Allen 2009]. Cobalamin can be stored in small amounts by humans. Total body store is about 2-5 mg in adults, around 80% of it is stored in the liver. Vitamin B12 is excreted in the bile and in healthy subjects is effectively reabsorbed (enterohepatic circulation). Therefore, diets with low content of vitamin B12 do not lead in a short period of time to its deficiency. This process may take even up to 20 years. On the contrary, malabsorption may create such a situation in 3 years time [Dwyer 1999, Linnel and Matthews 1984]. It should be thoroughly considered since some of vegetarians started their diet due to certain gastrointestinal complaints. Early noticeable symptoms of vitamin B<sub>12</sub> deficiency are nonspecific (unusual fatigue, digestion problems, frequent upper respiratory infections) and can be very easily ignored by patients as well as medical professionals.

In view of our results we agree that vegetarians must be advised to carefully plan their diets and to monitor their plasma vitamin B12 on a regular basis to facilitate early detection of low cobalamin status [Elmadfa and Singer 2009]. In the present study we proved that fortified foods potentially could in a significant degree influence vitamin B12 resources. Hoey et al documented that voluntary consumption of fortified food is associated with an increase in dietary intake of B12 vitamin and has potential beneficial effects on health. However, those who do not consume fortified foods regularly may have insufficient B12 vitamin status to achieve the known and potential health benefits [Hoey et al. 2007]. A wide range of B12 fortified foods are available. Fermented soya products, seaweeds, and algae have all been suggested as containing significant B12 contents. The other option is simple vitamin B12 supplementation [Hoey et al. 2007].

## CONCLUSIONS

Five-year lacto-ovo-vegetarian diet did not result in the risk of vitamin B12 deficiency in healthy subjects. Nevertheless, serum vitamin B12 concentrations in the sub-

group consuming natural diet were significantly lower as compared with the group consuming fortified food. It confirms the need of follow-up (and potentially vitamin B12 fortification of food and/or its supplementation) in long-term lacto-ovo-vegetarians.

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## WPLYW DIETY LAKTOOWOWEGETARIAŃSKIEJ NA STĘŻENIE WITAMINY B12 W SUROWICY KRWI – PIĘCIOLETNIE BADANIE PROSPEKTYWNE

**Wprowadzenie.** Unikanie spożywania mięsa jest podstawą diety wegetariańskiej. Można wyróżnić wiele jej odmian. Przeprowadzone badanie dotyczy diety laktoowo-wegetariańskiej dopuszczającej spożywanie produktów mlecznych i jaj. Celem badania była próba oceny wpływu jej długotrwałego stosowania na stężenia witaminy B12, jak również weryfikacji efektu spożywania produktów naturalnych i wzbogaconych w tę witaminę.

**Material i metody.** Badaniem prospektywnym objęto 16 osób (12 kobiet, 4 mężczyzn), które zadeklarowały chęć przestrzegania ścisłej diety laktoowo-wegetariańskiej przez co najmniej 5 lat. Badaną grupę podzielono na dwie podgrupy: pierwsza stosowała dietę składającą się wyłącznie z produktów naturalnych, podczas gdy druga sięgała po żywność wzbogaconą w witaminę B12. Ocenę stężeń witaminy B12 przeprowadzono przed wprowadzeniem diety oraz po 6, 12, 24 i 60 miesiącach jej stosowania.

**Wyniki.** Pięcioletnie stosowanie diety laktoowo-wegetariańskiej doprowadziło do znacznego obniżenia stężeń witaminy B12 w całej badanej grupie ( $p < 0,05$ ). Jednakże istotnie statystyczny spadek dotyczył *de facto* jedynie podgrupy stosującej dietę z produktami naturalnymi ( $p < 0,05$ ). U żadnej spośród osób biorących udział w badaniu nie odnotowano występowania nieprawidłowych stężeń B12.

**Wnioski.** Pięcioletnie stosowanie diety laktoowo-wegetariańskiej u zdrowych osób nie wiąże się z ryzykiem wystąpienia niedoboru witaminy B12. Jednakże stężenia witaminy B12 u osób spożywających wyłącznie produkty naturalne wydają się być niższe w porównaniu z grupą stosującą żywność wzbogaconą w witaminę B12. Potwierdza to potrzebę stałego nadzoru nad osobami długo stosującymi dietę wegetariańską oraz potencjalną potrzebę stosowania żywności wzbogaconej lub suplementacji witaminy B12.

**Słowa kluczowe:** laktoowo-wegetarianie, osoby odżywiające się tradycyjnie, żywienie, witamina B12, żywność wzbogacona

*Accepted for print – Zaakceptowano do druku: 20.10.2009*

*For citation – Do cytowania: Mądry E., Lisowska A., Chabasińska M., Przysławski J., Schlegel-Zawadzka M., Walkowiak J., 2009. Effect of lacto-ovo-vegetarian diet on serum vitamin B12 concentrations – five-year prospective study. Acta Sci. Pol., Technol. Aliment. 8(4), 71-76.*