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THE EFFECT OF DIETARY SUPPLEMENTS WITH FOLIC ACID AND VITAMIN B_{12} ON PLASMA HOMOCYSTEINE LEVEL IN AN EGYPTIAN ELDERLY GROUP

Hussein, Mona M.; Sorya T. El-Damhougy; Abeer A. Afify; Madiha M. Abd El-Kader and Seham S. Kasam

Food Science and Nutrition Department, National Research Center, Dokki, Cairo, Egypt

ABSTRACT

Elevated plasma total homocysteine (tHcy) concentrations have been confirmed as a risk factor for ischemic heart disease and other vascular disorders. High plasma homocysteine concentrations can be largely attributed to inadequate intake of folic acid and vitamin B_{12}. On the other hand, data from several studies indicate that plasma homocysteine increases with age, independent of vitamin status.

56 subjects (30 males and 26 females) similar in socio-economic class (upper-middle), with an age range of 60– 70 years completed the study course. All were healthy working professionals. Clinical, nutritional and anthropometric evaluations were carried out. Biochemical analysis for blood homocysteine, vitamin B_{12} and folic acid were performed. All the evaluated parameters were performed at the beginning of the study and after four months of supplementation with biscuits and dietary intake rich in vitamin B_{12} and folic acid. The results of study show that before the supplementation, the plasma homocysteine level was 13.8 ± 2.2 µmol^{-1} this level was significantly high, which indicating a state of mild hyperhomocysteinemia, then returned to its normal values after four months of supplementation with increased dietary intake of vitamin B_{12} and folic acid. Significant decrease in plasma tHcy and increase in vitamin B_{12} and folic acid levels is recorded after the four months of supplementation P (< 0.01).

In conclusion, the daily consumption of B_{12} and folic acid supplements appears to be the most effective factor for reducing tHcy concentration.

As a result vitamin B_{12} and folic acid supplementation for elderly is recommended

Keywords: Homocysteine, Supplementation, folic acid, Vitamin B_{12}

INTRODUCTION

Homocysteine is an intermediate compound formed during the metabolism of the essential sulfur containing amino-acid methionine.

The possible mechanism of hyperhomocysteinemia induced vascular damage remains obscure but includes adverse effect on endothelium platelets and clotting factors. Its relationships to folate, vitamins B_{12} and B_6 result from the cofactor requirements for methionine formation from homocysteine and the alternative pathway of homocysteine disposition, via cystathionine (Bates *et al.,* 1997).

Elevated total homocysteine concentrations have been confirmed as a risk factor for ischemic heart disease (IHD) and other vascular disorders (Lynnette *et al.,* 2000).

The major acquired causes of increased homocysteine levels are likely to be absolute or relative deficiencies of folate, vitamin B_{12} or B_6. Folic acid administration, either alone or with vitamins B_{12} or B_6 decreases homocysteine concentrations in both older and younger people.

It was observed that tHcy increases steadily with age and that tHcy is higher in men than women (Rasmussen *et al.,* 2000 and De Bree *et al.,* 2001).

The aim of this study was to investigate the effect of vitamin B_{12} and folic acid supplement in the form of biscuits, in addition to increase the intake rich sources of folic acid and B_{12} on plasma tHcy level.

MATERIALS AND METHODS

1- Materials

This study initially recruited 80 healthy elderly men and women who freely consented to participate. The protocol was approved by ethical committee of National research Center (NRC).

The medical history of each subject was taken before enrolment. Subjects who had history of cancer or cardiovascular diseases were not included. Subjects were excluded also if they were smokers, had a gastrointestinal disorder, or has used any of the following preparations during the four month preceding the trial i.e vitamins, minerals, yeast, antiepileptic drugs or thyroid hormones because these drugs can interfere with plasma tHcy levels (Lashers *et al.,* 2003).

56 subjects (30 males and 26 females similar in socio-economic (upper-middle level) with an age range of 60 – 70 years old completed the study course.

All of the participants were mentally and physically capable of participating in the study and all of them gave their informed consent. The researcher explained the idea of the work and its aim, before starting this work.

2- Methods

During the four months of the study, the intake of 50% of daily requirements of vitamin B_{12} and folic acid was achieved by consuming for biscuits (Hamed *et al.,* 2005) for every one of the volunteers daily. Content of folic acid and vitamin B_{12} in the biscuits are shown in table (I). In addition a list of food items rich in folic acid and vitamin B_{12} was advised to be taken in excess to increase the total intake of folic acid and vitamin B_{12} (included in table II).

We re-evaluated the biochemical levels to investigate whether an average daily intake of 400 µg supplement folic acid and 6 mg B_{12} could be an option to decrease plasma tHcy in healthy elderly (women and men) after four months.

All cases were subjected to the following plan of work at the beginning of the study:

1- Three days of dietary intake was recorded.
2- Anthropometric measurements were taken.
3- Blood samples were obtained for the determination of biochemical parameters.

1- Food intake; data on dietary intake were calculated from the three day's menus served in their homes. To estimate the individual food intake, menu items, portion size as well as the amount of food left for each subjects were recorded. Any snaks taken between meals were also recorded. The total dietary intake expressed as the means of the three day's intakes, was coded and analyzed using the world food dietary assessment computer program. Thus converting the food intake into nutrients, the adequacy of diets with regard to energy and nutrients was evaluated using the RDA of FAO/WHO (1998), included in table 3.

2- The anthropometric examination measurements of height and body weight were taken to calculate body mass index (BMI) = Wt/HL^2 (Blanchard *et al.,* 1990).

-Blood pressure was measured one time at each arm with a standard mercury sphygmomanometer in subjects who had been lying down for 10 min.

3- Biochemical measurements: the biochemical examination included the determination of plasma tHcy homocysteine, vitamin B_{12} and folic acid.

Laboratory procedures:

Venous blood samples were collected after subjects had fasted overnight, at the beginning of the experiment and after the supplementation period (4month). Total plasma homocysteine, plasma folate and vitamin B_{12} concentration were determined in all blood samples.

Blood samples were drawn into EDTA and kept in a refrigerator (–4°C) within 15– 30 min of collection. Plasma was separated within 1 – 3 hr., and samples were stored at -35°C for folate and vitamin B_{12} and at -80°C for plasma total homocysteine determination.

Plasma tHcy concentration was measured by the Axis Homocysteine Enzyme Immunoassay (EIA) technique distributed by IBL-Humurg, Germany (IBL Cat. No.: Ax51301). Samples for folate and vitamin B_{12} measurements were performed by DPC's solid phase No. Boil Dualcount simultaneous assay of vitamin in $B_{12}$and folic acid. Evaluation is accomplished by means of master tracer with two isotopes, cobalt-57 and iodine -125, which are then separated by dual-channel gamma counter.

Study design and statistic:

The study was designed to compare results and data before and after four months, after the increased intake of vitamin B_{12} and folic acid, on the same experimental group. Statistical analysis of the result was performed using SPSS computer program

The arithmetic mean, standard division paired t-test was calculated.

RESULTS AND DISCUSSION

Table (1) shows the vitamin B_{12} and folate content in every 100 g of each component forming the supplemented biscuits.

Table 1: The composition of biscuits which contains vitamin B_{12} and folate content in every 100 g of different components (Hamed *et al.,* 2005)

Nutritional item	Vitamin B_{12}/100g	Folate/100g
Whey protein	4 µg	200 µg
Germinated wheat	0.0	218 µg
Brower's yeast	12.5 µg	785 µg
Soya bean	0.0	375 µg

Table (2) includes the recommended item to be eaten in excess $B_{12}$and folic acid.

Table 2: Item recommended for intake for their high content of vitamin B_{12} and folic acid

Folic acid	Vitamin B_{12}
Fortified cereals	Cheese
Leafy green veggies	Eggs
Spinach	Fish and shellfish
Legumes	Fortified cereals
Chickpeas	Meat
Garbanzo beans	Milk
Kidney beans	Poultry
Lentils	
Orange juice	
Tomato juice	

Table (3) shows the energy and nutrients daily intake as means ±S.D. for the studied group. It was noticed that the intake of vitamin B_{12} is 4.8 ± 1.7 µg day^{-1} for men and 4.1 ± 1.5 µg day^{-1} for women, which is less than the recommended daily allowances (RDA) which is 6 µg day^{-1} for both men and women. As for folate intake, it was 180 ± 65 µg day^{-1} for men and 168 ± 67 µg day^{-1} for women, which is very low compared with the (RDA), which is 400 µg day^{-1} for both men and women.

Table 3: Energy and nutrients from daily intake (mean ± SE) of elderly women and men

Variable	Elderly		RDA FAO/WHO	
	Women (n=26)	Men (n =30)	Women	Men
Energy *(Kcal)*	1804.4 ±110	1760.7 ±120	1800	2400
Protein *(g)*	79.8 ±12	74 ±13	50	63
Fat *(g)*	46.8 ±7.3	38.3 ±5.4	60	80
Carbohydrate *(g)*	266 ±37	280 ±29	265	357
Vitamin B_{12} *(µg)*	4.8 ±1.7	4.1 ±1.5	6	6
Folate *(µg)*	180 ±65	168 ±67	400	400
Vitamin A *(RE)*	374 ±110	247 ±90	800	1000
Vitamin E *(TE)*	1.67±0.2	2.1 ±0.2	8	10
Vitamin C *(mg)*	37.9 ±5	43 ±4.6	60	60
Thiamin *(mg)*	1.1 ±0.1	1.4 ±0.1	1.1	1.2
Riboflavin *(mg)*	1.1 ±0.1	1.2 ±0.1	1.1	1.3
Niacin *(mg)*	11.5 ±1.5	13.2 ±1.2	14	16
Calcium *(mg)*	634 ±63	576 ±53	1200	1200
Phosphorus *(mg)*	1267 ±250	1489 ±260	700	700
Magnesium *(mg)*	283 ±87	393 ±69	320	420
Potassium *(mg)*	2201 ±360	2.134 ±290		
Iron *(mg)*	9.9 ±2	10.5 ±1.9	10	10
Zinc *(mg)*	10.6 ±1.9	10.7 ±1.4	12	15
Selenium *(µg)*	37 ±4	48 ±6.5	55	70

Table (4) shows the anthropometric measurements and blood pressure of elderly women and men (mean ± S.E.). Values are within the normal range for age within Egyptian population.

Table 4: Anthropometric measurements and blood pressure of elderly women and men (mean ± SE)

Variable	Women (n=26)	Men (n =30)
Age (year)	63.25 ± 3.33	68 ± 2.06
Body height (cm)	165 ± 7.1	175 ± 5.8
Body weight (kg)	68.4 ± 13	81.5 ± 12.5
Body mass index (kg m^{-2})	24.2 ± 3	26.6 ± 7
Systolic B.P.	150 ± 24	145 ± 19.5
Diastolic B.P.	90 ± 10	90 ± 13

Table (5) shows the biochemical parameters as (means ± S.E.) before and after intake. From this table, the first finding is that plasma homocysteine level was significantly reduced as the intake of folic acid and B_{12} increased during the four months period of the study.

Table 5: Biochemical parameters (mean ± SE) before and after the supplementation *t*-test of significance to compare values before and after the supplementation.

Parameters	Before	After	P-value
Homocysteine μmol l^{-1}	13.8 ± 2.2	9.5 ± 1.8	<0.01
Vitamin B_{12} pg ml^{-1}	242.5 ± 57.4	368.9 ± 115.5	<0.01
Folic acid ng ml^{-1}	4.3 ± 2.5	7 ± 2.57	<0.01

Note: *t*-test is significant at *p*<0.01.

The basal plasma homocysteine level was 13.8 ± 2.2 μmol l^{-1} before intake the supplementation and foods rich in folic acid and vitamin B_{12}. Then this level decreased to 9.5± 1.8 μmol l^{-1} after the intake. Before the intake; the level of tHcy was significantly high indicating a state of hyperhomocysteinemia, then returned to within normal values with the increased intaking. The magnitude of reduction on tHcy that was observed with folic acid plus vitamin B_{12} supplementation and (foods rich in folic acid and vitamin B_{12}) was considered large and significant (3.7 μmol l^{-1}). The similar reasons for the high level of reduction in tHcy concentration seen in this study were attributed to the older age of our subjects and the poor folate and vitamin B_{12} status at study entry. Both of these factors are associated with elevated tHcy concentrations as shown at the beginning of the study. Previous studies reported that blood tHcy concentration ranged from 7.1 to 14.5 μmol l^{-1} in elderly population(Clarke and Ulvik 1998; Elinson *et al.,* 2004 and Gonzalez-Gross *et al.,* 2007). There is some differences among scientists about factors an elevated homocysteine level. Some researchers designated the upper quartile of homocysteine levels of the healthy volunteers as 13.3 μmol l^{-1} or higher – as an elevated level (Hankey and Eikelboom, 1999). Other studies defined hyperhomocysteine (tHcy > 12 μmoll^{-1}) (Clarke and and Ulvik, 1998). Previous studies reported the same finding and confirmed that folic acid and vitamin B_{12} reduce blood homocysteine (Köseoglu and karaman, 2007), which agreed with the obtained results.

Serum tHcy is an independent risk factor for vascular disease, including myocardial infraction, vascular dementia and stroke. Several biological mechanisms have been proposed for these association, including an enhanced tendency for thrombosis mediated via increased endothelial disturbance, platelet activation, reduced cell expression of thrombomodulin and inhibition of activated protein C (Parnetti *et al.,* 1997; Zhou *et al.,* 2003).

Homocysteine (tHcy) is a sulfhydryl amino acid. Its precursor, methionine, is an essential amino acid derived from dietary protein. The enzymes responsible for metabolisming tHcy are cystathionine synthase, methionine synthase, and 5,10-methylenetetrahydrofolate reductase. The activity of these enzymes is dependent on four micronutrients: folic acid, vitamin B_{12}, riboflavin (vitamin B_2) and vitamin B_6 (Pyridoxal 6-phosphate), deficiencies of which cause elevation in plasma tHcy. Folic acid and vitamin B_{12} supplementation reduces tHyc across a wide range of reactions, (Bates *et al.,* 1997; Duthie *et al.,* 2002). Plasma homocysteine may be considered a functional indicator of B vitamin status since high plasma homocysteine concentrations can be largely attributed to inadequate status of these vitamins (Elinson *et al.,* 2004).

On the other hand, data from several studies indicate that plasma homocysteine increases with age independent of vitamin status, and that hyperhomocysteinemia is highly prevalent in the elderly (Almeida *et al.,* 2004). Ageing is associated with elevated tHcy concentration and a reduced activity of cystathionine synthase, one of the key tHcy metabolizing enzymes. Therefore, older patients may be at a particular risk of tHcy mediated disease.

Homocysteine plasma levels elevation is associated with sex, more in males, increase with age, coffee and tea consumption, elevated blood pressure and cigarette smoking (Kalmijin *et al.,* 1999, Ottar *et al.,* 1997).

Important finding of this study is that the dietary intake of folic acid and vitamin B_{12} among our tested elderly group is extremely inadequate. As expected, blood level of folic acid and vitamin B_{12} was low. Vitamin B_{12} plasma level before supplementation was 242.5 ± 57.4 pg ml^{-1} versus 368.9 ± 115.5 pg ml^{-1} after supplementation. At the beginning of the study, vitamin B_{12} blood level was low. After four months of intake, highly significant increase was recorded, $P < 0.01$. The same case was for folic acid plasma level, before supplementation was 4.3 ± 2.5 ng ml which considered very low and became 7.0 ± 2.57 ng ml after supplementation, significant increase was recorded, $P < 0.01$.

Data of this study revealed that the intake of vitamin B_{12} was 4.8 ± 1.7 µg day^{-1} for men and 4.1 ± 1.5 µg day^{-1} for women, which is less than the RDA, (6µg day^{-1}) for both. As for folate intake, it was 180 ± 65 µg day^{-1} for men and 168 ± 67 µg day^{-1} for women, which are very low compared to the RDA, (400 µg day^{-1}) in both. In the present study, supplementation by biscuits and foods rich in vitamin B_{12} and folic acid increased vitamin levels and improved vitamin status as expected. This was reported by several studies (Fernstrom, 2000, Jacques *et al.,* 1999, Lashers *et al.,* 2003, Nourhashémi *et al.,* 2000). In addition, the poor intake of vitamins, caused the physiologic consequences of atrophic gastritis accompanying aging include changes in gastric emptying and decreased secretion of intrinsic factor in severe cases of gastric atrophy which could contributed to their plasma levels. Nevertheless, atrophic gastritis has been reported to limit the bioavailability of vitamin B_{12} through impaired release of vitamin B_{12} from food proteins and peptides due to impaired acid secretion and reduced digestion by pepsin (Duthie *et al.,* 2002; Lynnette *et al.,* 2000).

Conclusion:

In conclusion, the increase daily consumption of B_{12} and folic acid supplements appears to be the most effective in reducing tHcy concentration.

Therefore, it is appropriate to recommend that elderly people and particularly those with higher risk of vascular disease take vitamin B_{12} and folic acid either through supplementation or through diet rich in these elements or both.

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تــأثير الــدعم الغــذائي بحــامض الفوليــك وفيتــامين B12 علــي مســتوي بلازماالهيموسيستين لمجموعة من كبار السن المصريين.

منـى محمـد حسين، ثريـا طـاهر الـدمهوجي، عبيـر أمـين عفيفـي، مديحـة عبـد القـادر و سهام سيد قاسم
قسم علوم الأطعمة والتغذية – المركز القومي للبحوث – الدقي – القاهرة

يعتبر أرتفاع تركيز الهيموسيستين في بلازما الدم من العوامل المسببة للعديد من الأمراض منها أمراض القلب – عدم كفاءة وظائف المخ – اختلال في الأوعية الدموية

ويرجع الأرتفاع في تركيز مستوى الهيموسيستين في بلازما الدم على عدم كفاية المتناول من فيتامين ب12 وحامض الفوليك فيقل نسبتهما في الدم.

وقد أجريت هذه الدراسة لمعرفة تأثير الدعم الغذائي بفيتامين ب12 وحامض الفوليك على مستوى تركيز الهيموسيستين في بلازما الدم وقد أجريت هذه الدراسة على 65 شخص من كبار السن وقد تم أختيارهم بعد استبعاد من لديهم أي مشاكل صحية وتاريخ مرضي بالأمراض السرطانية والزهيمر وقد تم اختيارهم في مستوى أجتماعي واحد (فوق المتوسط) وأعمارهم تتراوح ما بين 60 – 70 عام.

وقد تم أجراء البحث على النحو التالي:-

1. عمل استمارة استبيان للوقوف على الحالة الصحية واخذ القياسات الأنثروبمترية.
2. عمل استمارة استبيان للوجبات الغذائية المتناولة وما يتناوله الفرد ما بين الوجبات لمدة ثلاث أيام.
3. تم أخذ عينات دم للمجموعة المختارة للوقوف على مستوى تركيز الهيموسيستين في بلازما الدم وكذلك تركيز مستوى فيتامين ب12 وحامض الفوليك.
4. تم أعطاء كل فرد اربعة بسكويتات (في اليوم) من البسكويت الذي تم أعداده من عناصر ذات كفاءة غذائية عالية مع النصح بتناول أغذية غنية بفيتامين ب12 ، وحامض الفوليك وذلك لمدة أربعة أشهر متتالية.
5. ثم بعد تلك المدة تم أخذ عينات دم لمعرفة مدى تأثير الدعم الغذائي على مستويات تركيز الهيموسيستين ، فيتامين ب12 ، حامض الفوليك في بلازما الدم

وقد دلت النتائج على أرتفاع مستوى تركيز الهيموسيستين في بلازما الدم وأنخفاض كلاً من تركيز فيتامين ب12 وحامض الفوليك في بداية الدراسة. بعد تناولهم البسكويت المدعم وتناولهم أغذية غنية في محتواها من فيتامين ب12 وحامض الفوليك وجد أنخفاض ملحوظ في مستوى تركيز الهيموسيستين في بلازما الدم وأيضاً أرتفاع ملحوظ في مستويات كلاً من فيتامين ب12 وحامض الفوليك في بلازما الدم

ومن هنا ننصح كبار السن الأكثار من تناول الأغذية الغنية بفيتامين ب12 وكذلك حامض الفوليك حتى لا يتعرضوا لأرتفاع مستوى تركيز الهيموسيستين في بلازما الدم مما يؤدي إلى مشاكل في وظائف القلب ووظائف المخ وقصر في عمل الأوعية الدموية.

قام بتحكيم البحث

أ.د / صفاء محمد على حسن	**كلية الزراعة – جامعة المنصورة**
أ.د / سلوى احمد مصطفى الشبينى	**المركز القومى للبحوث**