Short Communication

Dietary strategies for improving folate status in institutionalized elderly persons

Laura M. Bermejo[1]*, Aránzazu Aparicio[1], Elena Rodríguez-Rodríguez[1], Ana M. López-Sobaler[1], Pedro Andrés[2] and Rosa M. Ortega[1]

[1]*Departamento de Nutrición, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain*
[2]*Laboratorio de Técnicas Instrumentales, Sección Departamental de Química Analítica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain*

The aim of this work was to compare the efficacy of two strategies designed to improve folate status: increasing the intake of vegetables, and the consumption of a folic acid-fortified food. Residents (126) from three old people's homes in the Madrid region (Spain) were studied. To each centre a dietary intervention was assigned to be followed for 6 months: (1) the consumption of margarine fortified with 200 µg folic acid/10 g portion (centre M), (2) increasing the consumption of vegetables to three servings per day (centre V), (3) control (centre C). At the beginning and end of the intervention period the subjects' intakes, serum and erythrocyte concentrations of folate were measured. The use of fortified margarine (centre M) led to a significant increase in folate intake (260·9 µg/d), serum concentration (10·3 (SD 8·3) nmol/l) and erythrocyte concentration (638·4 nmol/l). At centre V the increase in total vegetable intake achieved was very poor; these foods met with very poor acceptance, although the intake of certain vegetables particularly rich in folate improved. Therefore, the intake of this vitamin increased a little (26·7 (SD 33·0) µg/d); erythrocyte folate concentration also increased somewhat (460·5 nmol/l), although less than centre M. The daily consumption of margarine fortified with folic acid was the more effective strategy for improving the folate status of the study subjects.

Folate: Dietary strategies: Vegetables: Fortified food: Elderly persons

Folate deficiency is one of the most common vitamin deficiencies in elderly people[1,2]. The most common problems associated with this deficiency are anaemia[3], cancer, CVD, depression and dementia[4–6]. Given the importance of this vitamin, the latest intake reference figures from the USA[7] and the recommended intake for the Spanish population[8] have been raised to 400 µg/d for men and women over 51 years of age.

The aim of the present work was to improve the folate status of elderly people either via the consumption of a fortified food or increasing the consumption of vegetables to three servings per day.

Subjects and methods

The study subjects were 126 elderly people (all aged over 65 years) who resided at three old people's homes in the Madrid region (Spain). All subjects were informed of the nature of the study and gave their written consent to be included. The study was approved by the Research Committee of the Faculty of Pharmacy.

Study design

Prior to the dietary interventions, a baseline study of the nutritional status of the subjects was made via dietetic, anthropometric, haematological and biochemical examinations. All initial data were collected between April and May 2001 and the intervention was designed to run from June to November 2001.

The strategies to improve the folate status were planned to involve either the consumption of a fortified food or increasing the consumption of vegetables. To decide upon which fortified food to use, information was sought on the food habits of elderly people[9,10]. Since fortified breakfast cereals are not normally consumed by Spanish elderly, a low-fat margarine was chosen to substitute that normally used at the centres at breakfast time. This avoided an intervention-induced increase in fat intake.

The 6-month interventions undertaken at the three centres were: (1) Centre M: supplementation of the diet with 10 g margarine fortified with 200 µg folic acid/d. Together with the supply from other dietary sources (a further 200 µg/d), this would have approximated the folic acid intake to the

* **Corresponding author:** Dr Laura M. Bermejo, fax +34 91 394 18 10, email mlbermej@farm.ucm.es

recommended level (400 μg). (2) Centre V: diet modified to include three servings of vegetables per day. Leafy vegetables were those mostly chosen since these are the richest in folic acid (spinach, swiss chard, peas, grean beans etc.). The subjects received a non-fortified margarine in place of that normally used. Menu options were planned to provide a daily intake of 400 μg folic acid. (3) Centre C: no dietary intervention (control); the only change was the provision of the non-fortified margarine in place of that normally used.

To ensure that the menus of centre V were modified as planned, the quantity of vegetables provided were inspected on 6 d during the study period (one per month of the study, randomly chosen).

In the last week of the intervention the nutritional status of the subjects was again examined. This involved new dietetic, anthropometric, haematological and biochemical studies.

Of the 153 initially included subjects, fourteen died during the study period and eleven abandoned the study. Further, no blood sample could be taken from two subjects at the end of the study. Therefore, the final sample included 126 subjects.

Methods

Baseline and final dietetic studies. All foods and drinks consumed by the subjects were recorded over a period of 7 d using the 'precise individual weighing' method[11]. Data were collected at all three centres under the same conditions: the same, trained team collected the data at each centre and did so in the same fashion, data collection began on the same day of the week, and the same measuring instruments were used.

The subjects also kept a 'food record' to provide information on foods and drinks taken between established meal times, e.g. food provided by their relatives or that which they bought at the centres' cafeterias or elsewhere.

The energy and nutrient contents of all foods and drinks consumed were calculated using the Food Composition Tables of the Instituto de Nutrición[12], complemented with those of Moreiras *et al.* [13]. These intakes were then compared with those recommended for the Spanish population[8].

Energy expenditure was calculated using equations proposed by the WHO[14], multiplying the basal energy expenditure by the corresponding activity coefficient. To determine these coefficients, all subjects completed a questionnaire that reflected the number of hours devoted to different activities during the day[15].

Baseline and final anthropometric studies. The subjects' weight was measured using a Seca Alpha digital electronic scale (range 0·1–150 kg) (Seca Alpha, Igmy, France; range: 0·1–150 kg). Knee–heel lengths were also measured. This measurement allows an estimate to be made of subject height using the formula of Chumlea *et al.* [16]. The results permitted the calculation of BMI.

All measurements were taken with subjects in bare feet and wearing only their underwear. Measurements were taken following WHO recommendations[17].

Baseline and final biochemical studies. Blood samples were taken first thing in the morning after a 12 h overnight fast and maintained at 4–6°C until analysis, which was always performed within 48 h.

Serum and erythrocyte folate were determined by RIA using the Vitamin B_{12}/Folate Dual Radioassay Kit (Diagnostic Product Corp., Los Angeles, CA, USA), and employing a model 1612 Gamma Counter (Nuclear Enterprises Ltd, Edinburgh, UK)[18] (CV = 4·5 and 4·9 %, respectively).

Hb level was determined using a Coulter S. Plus apparatus[19]. Homocysteine levels were determined by HPLC (CV = 6·5 %). Separation was achieved with an RP-18 column (Symta, Madrid, Spain) using an intelligent pump (Merck-Hitachi L-6200 A; Hitachi, Tokyo, Japan). Detection was performed by fluorescence spectrophotometry[20].

Baseline and final health study. Information was collected on the diseases/disorders suffered by the subjects and the medications they took.

Statistical analysis

Means and standard deviations of all variables were calculated. To detect differences between the participating centres, one-way ANOVA was used followed by the Newman–Keuls test. The Student t test for paired samples was used to analyse the differences between the mean baseline and final results within centres. Differences between population percentages associated with different variables were analysed after first transforming binomially distributed results to a normal distribution using continuity correction[21]. Significance was set at $P < 0.05$. Calculations were performed using the RSIGMA BABEL 2000 software package (Horus Hardward, Madrid, Spain).

Results

The percentage of men and women was similar (64 % men, 36 % women) between centres. The mean age of the subjects was 82·4 (SD 7·3) years.

V subjects had a baseline weight and BMI significantly greater than those of M and C. This difference was taken into account in the statistical treatment of the results in order to adjust for its possible effect.

Baseline and final serum vitamin B_{12} levels were measured on participants and there were no subjects with serum B_{12} below the conventional cut-off point (148 pmol/l)[22] and the levels did not change during the intervention period in any of the intervention groups.

Table 1 shows the subjects' baseline and final folic acid intakes and serum and erythrocyte folate concentrations. The percentage of subjects with intakes of < 67 % of folate recommended dairy intakes[8] was very high at all three centres (C, 97·8 %; M, 97·4 %; V, 100 %; NS).

Further, only in centre M the percentage of subjects with intakes of folate below 400 μg/d[8] diminished from 100 % at the start of the study to 43·2 % at the end ($P < 0.001$). The change in the percentage of subjects whose folate intake was less than two-thirds of that recommended was even more remarkable: 97·4 % at baseline and 0·0 % at the end of the intervention ($P < 0.001$).

On a day per month of the intervention, the quantity of vegetables offered on the menu at centre V was inspected. The final mean weight of vegetables offered was 526 g/d (3·01 servings/d). However, V subjects did not eat all the vegetables provided (Table 1); the real consumption rate was 291 g/d (1·66 (SD 0·36) servings/d). Although the overall intake of

Table 1. Changes in vegetable intake, folate intake, blood folate variables and Hb and homocysteine levels due to the interventions‖

(Mean values and standard deviations)

	Baseline		Final		Difference	
	Mean	SD	Mean	SD	Mean	SD
Vegetable consumption (g/d) (n 126)						
Centre C	264	86·8	262	58·6	− 2·2	66·8
Centre M	293	88·6	292	79·0	− 0·5	88·2
Centre V	295	71·8	291	63·7	− 4·1	78·5
ANOVA	NS		NS		NS	
Folate intake (μg/d) (n 126)						
Centre C	164†	60·0	174†§	51·0	8·9§	33·0
Centre M	160†	38·8	421*†‡	91·5	261†‡	95·2
Centre V	190*‡	40·2	219*‡§	33·4	26·7§	44·4
ANOVA	P< 0·01		P< 0·001		P< 0·001	
Serum folate (nmol/l) (n 107)						
Centre C	14·6	5·9	14·5§	5·3	− 0·5§	5·0
Centre M	16·6	6·1	27·1*†‡	9·4	10·3†‡	8·3
Centre V	14·0	5·8	13·7§	7·7	− 0·6§	5·6
ANOVA	NS		P< 0·001		P< 0·001	
Erythrocyte folate (nmol/l) (n 96)						
Centre C	588	416	902*§	175	330§	321
Centre M	748	260	1403*†‡	438	638†‡	344
Centre V	698	800	1030*§	422	460§	354
ANOVA	NS		P< 0·001		P< 0·01	
Hb (g/l) (n 117)						
Centre C	131	16	142*†§	14	11§†	3
Centre M	129	14	132‡	17	3‡	11
Centre V	131	16	130‡	20	− 1‡	9
ANOVA	NS		P< 0·01		P< 0·001	
Homocysteine (μmol/l) (n 104)						
Centre C	18·0	5·2	17·0	4·8	− 0·1	5·1
Centre M	16·1	5·4	14·9	4·4	− 1·3	3·0
Centre V	17·7	5·6	16·5	5·8	− 1·0	4·5
ANOVA	NS		NS		NS	

Centre C, control centre; Centre M, centre with a consumption of margarine fortified with 200 μg folic acid; Centre V, centre with the consumption of vegetables increased to three servings per day.

Mean values were significantly different from baseline assesment: *P< 0·001.

Mean values were significantly different from those of centre V: †P< 0·05.

Mean values were significantly different from those of centre C: ‡P< 0·05.

Mean values were significantly different from those of centre M: §P< 0·05.

‖ For details of subjects and procedures, see Subjects and methods.

vegetables did not increase after 6 months, the folate intake increased significantly (Table 1), as consequence of a specific increase in the consumption of vegetables, particularly rich in folate (Swiss chard 3·3 (SD 5·6) v. 14·4 (SD 7·7) g/d, P< 0·001; spinach 1·6 (SD 1·1) v. 6·7 (SD 3·2) g/d, P< 0·001; peas 6·6 (SD 3·0) v. 15·0 (SD 5·5) g/d, P< 0·001; broad beans 0·0 v. 1·9 (SD 1·2) g/d, P< 0·001).

At the end of the intervention, only in the centre M, the serum folate concentration was significantly higher than at baseline (Table 1). In addition, the percentage of M subjects with serum folate levels below the reference value (≤ 13·6 nmol/l) fell between baseline and the end of the intervention, from 36·8 to 6·1 % (P< 0·001).

The erythrocyte folate concentration of the subjects of all three centres increased significantly after the intervention compared to baseline (Table 1). However, the increase experienced by M subjects was significantly greater than that experienced by V and C subjects.

Hb values were measured on participants and did not change during the intervention period in any of the intervention groups (Table 1).

Homocysteine concentration decreased in M and V subjects (Table 1), although these variations were not significant.

In addition, a negative correlation was found with the folate intake variation and the homocysteine levels variation (r − 0·4591, P< 0·01) in M subjects.

Discussion

The strategy based on the use of the fortified margarine led to the greatest increase in folate intake, and best improved the coverage of the recommended intake. It also led to significant increases in serum and erythrocyte folate levels. This strategy therefore achieved a significant improvement in the folate status of M subjects. With this strategy, the intake of folate achieved at 6 months (422 μg/d) was similar to that reported by other authors who incorporated different fortified foods into the diets (460 μg folate/d[23] and 518 μg folate/d[24]) of other population subgroups.

Serum folate concentration is an indicator of the recent intake of this vitamin, while erythrocyte folate levels reflect intake over the previous months[25]. Since the study was performed between June and November, it might be argued that the final erythrocyte folate levels reflect a normal summer increase in vegetable consumption. However, this would have affected all three centres equally, and the final

concentrations recorded for the centre M subjects were higher than those of the subjects at the other centres. This shows the increase to have been caused by the intake of the fortified margarine.

Most of studies performed in different groups[23,24,26,27] report folate intake to increase when supplements, fortified foods or natural foods rich in folate are provided. However, increases in erythrocyte folate concentration are reported to be much greater when fortified foods are taken, because synthetic folic acid monoglutamate is more bioavailable than the natural polyglutamate in foods[28]. In addition, folic acid monoglutamate is more stable than its polyglutamate counterpart during storage, preparation and cooking[29].

The strategy based on increasing the number of servings of vegetables consumed did not have the impact desired. The final dietetic study showed that the consumption of vegetables by V subjects was smaller than that which their menus would have allowed; the subjects largely rejected these foods. Nonetheless, these menus were designed to provide vegetables, particularly rich in folate (Swiss chard, spinach, peas, broad beans and green beans), and while the overall intake of vegetables did not increase, the intake of these particular vegetables did. This may explain why V subjects showed an increase in folate intake (Table 1). However, despite this increase in folate intake, the percentage of V subjects with intakes $<67\%$ that recommended only decreased from 100 to 92·7%. Thus, this strategy was not able to allow the recommended intake of this vitamin to be achieved, nor did it increase the serum folate concentration (Table 1), although their increased erythrocyte folate concentration could reflect their increased intake of certain types of folate-rich vegetables over the study period.

Previous studies have been carried out for less than 4 months[23,24,27,30,31] and they have combined different foods: vegetables, fruits, pulses, nuts and breakfast cereals. Nevertheless, in the present study, the dietary modification strategy was focused on increasing only the consumption of vegetables since they are folate-rich. Along with cereals these were the foods whose consumption was most widely different to that recommended[2,23].

Some studies have shown that the elderly suffer certain physical barriers with respect to fruit and vegetable consumption and have deeply rooted food habits[32], making it difficult to change their long-term dietary patterns, as the present results show.

Some studies indicate that 4–5 months may be needed for changes to be seen in erythrocyte folate levels following supplementation with the vitamin or an increase in the consumption of vegetables[33]. For this reason a 6-month study period was chosen. Further, longer intervention times may improve the chances of having an impact on food habits; in the present study it was hoped that this would promote the consumption of vegetables among V subjects, although success was limited.

Other authors, according to the present results, have observed an improvement in homocysteine levels when the folate intake increases that could have important benefits on elderly health[23,24,27].

The results suggest that improving the folate status of elderly people may be easier to achieve via the use of fortified foods, although further studies will be necessary to confirm this. However, the need to promote the consumption of vegetables by this population should not be overlooked.

The consumption of vegetables is low among the elderly yet there is good evidence that vegetable intake is associated with important health benefits[34].

Acknowledgements

References

1. Lokk J (2003) News and views on folate and elderly persons. *J Gerontol A Biol Sci Med Sci* **58**, 354–361.
2. Ortega RM, López-Sobaler AM, Aranceta J, *et al.* (2004) Are there any nutritional deficiencies in the Mediterranean diet? *Arch Latinoam Nutr* **54**, Suppl. 1, S87–S91.
3. Wickramasinghe SN (2006) Diagnosis of megaloblastic anaemias. *Blood Rev* **20**, 299–318.
4. Rampersaud GC, Kauwell GP & Bailey LB (2003) Folate: a key to optimizing health and reducing disease risk in the elderly. *J Am Coll Nutr* **22**, 1–8.
5. Requejo AM, Ortega RM, Robles F, *et al.* (2003) Influence of nutrition on cognitive function in a group of elderly, independently living people. *Eur J Clin Nutr* **57**, Suppl. 1, S54–S57.
6. Campbell AK, Jagust WJ, Mungas DM, *et al.* (2005) Low erythrocyte folate, but not plasma vitamin B-12 or homocysteine, is associated with dementia in elderly Latinos. *J Nutr Health Aging* **9**, 39–43.
7. Institute of Medicine (2001) *Dietary Reference Intakes*. Washington, DC: Food and Nutrition Board, National Academy of Sciences, National Research Council.
8. Ortega RM, Requejo AM, Navia B, *et al.* (2004) *Recommended Diary Intakes of Energy and Nutrients for Spanish People*. Madrid: Complutense.
9. Ortega RM, Andrés P, Redondo MR, *et al.* (1995) Dietary assessment of a group of elderly Spanish people. *Int J Food Sci Nutr* **46**, 137–144.
10. Ortega RM, Redondo MR, Zamora MJ, *et al.* (1995) Eating behaviour and energy and nutrient intake in overweight/obese and normal-weight Spanish elderly. *Ann Nutr Metab* **39**, 371–378.
11. Maisey S, Loughridge J, Southon S & Fulcher R (1995) Variation in food group and nutrient intake day of the week in an elderly population. *Br J Nutr* **73**, 359–373.
12. Instituto de Nutrición CSIC (1994) *Food Composition Tables*. Madrid: Complutense.
13. Moreiras O, Carbajal A & Cabrera M (1998) *Food Composition Tables*. Madrid: Pirámide.
14. World Health Organization (1985) *Energy and Protein Requirements. Report of a Joint FAO/WHO/ONU Expert Consultation. WHO Technical Report Series* no. 724. Geneva: WHO.
15. Dallosso HM, Morgan K, Bassey EJ, *et al.* (1988) Levels of customary physical activity among the old and the very old living at home. *J Epidemiol Community Health* **42**, 121–127.
16. Chumlea WC, Roche AF & Steinbaugh ML (1985) Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriatr Soc* **33**, 116–120.

17. World Health Organization (1976) *Methodology of Nutritional Surveillance. Report of a Joint FAO/UNICEF/WHO Expert Consultation. WHO Technical Report Series* no. 53. Geneva: WHO.

18. Lindenbaum J (1983) Status of laboratory testing in the diagnosis of megaloblastic anemia. *Blood* **61**, 624–627.

19. Cox CJ, Haberman TM & Payne BA (1985) Evaluation of the Coulter Counter Model S-Plus IV. *Am J Clin Pathol* **84**, 297–306.

20. Turnell DC & Cooper JD (1982) Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography. *Clin Chem* **28**, 527–531.

21. Wonnacott HW & Wonnacott RJ (1977) *Introductory Statistics*. New York: John Wiley and Sons.

22. Morris MC, Evans DA, Bienias JL, *et al.* (2005) Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol Nov* **62**, 1785–1786.

23. Brouwer IA, van Dusseldorp M, West CE, *et al.* (1999) Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J Nutr* **129**, 1135–1139.

24. Riddell LJ, Chisholm A, Williams S, *et al.* (2000) Dietary strategies for lowering homocysteine concentrations. *Am J Clin Nutr* **71**, 1448–1454.

25. Stopler T (2004) Medical nutrition therapy for anemia. In *Krause's Food, Nutrition and Diet Therapy*, 11th ed., pp. 838–859 [LK Mahan and S Escott-Stump, editors]. Philadelphia: Saunders.

26. Cuskelly GJ, McNulty H & Scott JM (1996) Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. *Lancet* **9**, 657–659.

27. Silaste ML, Rantala M, Alfthan G, *et al.* (2003) Plasma homocysteine concentration is decreased by dietary intervention. *Br J Nutr* **89**, 295–301.

28. Gregory JF (1995) The bioavailability of folate. In *Folate in Health and Disease*, pp. 195–235 [LB Bailey, editor]. New York: Marcel Dekker.

29. Herbert V (1987) Recommended dietary intakes (RDI) of folate in humans. *Am J Clin Nutr* **45**, 661–670.

30. Venn BJ, Mann JI, Williams SM, *et al.* (2002) Dietary counseling to increase natural folate intake: a randomized, placebo-controlled trial in free-living subjects to assess effects on serum folate and plasma total homocysteine. *Am J Clin Nutr* **76**, 758–765.

31. Pinto X, Vilaseca MA, Balcells S, *et al.* (2005) A folate-rich diet is as effective as folic acid from supplements in decreasing plasma homocysteine concentrations. *Int J Med Sci* **2**, 58–63.

32. Sahyoun NR, Zhang XL & Serdula MK (2005) Barriers to the consumption of fruits and vegetables among older adults. *J Nutr Elder* **24**, 5–21.

33. Furne JK, Springfield JR, Ho SB, *et al.* (2003) Simplification of the end-alveolar carbon monoxide technique to assess erythrocyte survival. *J Lab Clin Med* **142**, 52–57.

34. Bermejo LM, Aparicio A, Andrés P, *et al.* (2007) The influence of fruit and vegetable intake on the nutritional status and plasma homocysteine levels of institutionalised elderly people. *Public Health Nutr* **10**, 266–272.