

Original Research

Folate Status Worsens in Recently Institutionalized Elderly People without Evidence of Functional Deterioration

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Key words: Folate, homocysteine, vitamin B-6, dietary intake, biochemical status, blood counts, cognitive deterioration, hospitalized elderly people

Objective: To follow folate status, hematological and cognitive changes during the first year of institutionalization among elderly subjects.

Design: Prospective study.

Setting: Long-stay unit of the Dijon University Geriatric Hospital.

Subjects: Twenty women and four men older than 65 years admitted consecutively.

Main outcome measures: Folate and vitamin B-6 dietary intake was evaluated by a five-day record on admission (day 1 or d 1), at day 45 (d 45), day 90 (d 90), day 135 (d 135), day 180 (d 180), day 360 (d 360). Circulating levels of folate, vitamin B-6, total homocysteine (tHcy), blood counts and cognitive performance were determined in parallel.

Results: From d 1 to d 360, mean folate and vitamin B-6 intakes remained below the French RDA and mean folate intakes decreased significantly ($\Delta = -10.2\%$, $p < 0.05$). Mean plasma or erythrocyte folate decreased significantly ($\Delta = -33.7\%$, $p < 0.05$ and $\Delta = -30.2\%$, $p < 0.001$, respectively) from d 1 to d 360; no significant change was observed for the other blood parameters. The incidence of folate deficiency increased (8% vs. 37% for plasma folate < 6.8 nmol/L and 8% vs. 17% for erythrocyte folate < 340 nmol/L) from d 1 to d 360. Mean plasma pyridoxal 5'-phosphate (PLP) remained < 20 nmol/L during the one-year follow-up. There was no difference between genders for plasma tHcy. Although mean plasma tHcy was < 14 $\mu\text{mol/L}$, plasma tHcy was > 14 $\mu\text{mol/L}$ in about one-third of the subjects. At each period, 50% or more subjects were anemic (Hct $< 35\%$ in women and Hct $< 40\%$ in men), but the anemia was normocytic (MCV < 100 fL). Subjects had a moderate dementia at admission, and no change was observed during the study.

Conclusions: Subjects were already vitamin B-6 deficient at admission. Folate status was impaired during the study. Low vitamin intakes were the main cause of vitamin B-6 deficiency and folate status deterioration. Hematology and mental status capacity were not aggravated by folate status deterioration. Plasma tHcy didn't appear to be an earlier predictor of folate deficiency.

INTRODUCTION

Several dietary and biochemical studies have shown an elevated prevalence of folate, vitamin B-6 and vitamin B-12 deficiencies among elderly people [1,2]. These deficiencies are more frequent in institutionalized than in free-living elderly people [3–6]. Although such deficiencies may be implicated in pathologies which frequently occur in this population [7], few

studies have related vitamin B-6, vitamin B-12 and folate status to functional or clinical markers. Deficiency of either cobalamin or folate can cause hematological abnormalities that include megaloblastic anemia. In some studies, an association between low folate levels and dementia has been shown [8,9]. Paucity of studies relating functional and mental capacity to vitamin status may be due to many factors. The recognition and diagnosis of folate, vitamin B-6 and vitamin B-12 deficiency

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often present difficult and confusing problems. In view of the increasing interest in an evaluation of disease risks, development of functional markers of vitamin status is therefore urgently needed. From knowledge of the metabolic roles of vitamin B-6, vitamin B-12 and folate, it has been possible to predict which substrates will accumulate in vitamin B-6, vitamin B-12 and folate deficiencies. In recent years, two metabolites, methylmalonic acid (MMA) and tHcy, have received special attention and have been used for the primary diagnosis of vitamin B-12 and folate deficiency [10,11]. Although it is generally believed by geriatricians that folate deficiency actually develops in the elderly during the course of institutionalization, no prospective study has been undertaken to indicate whether low folate status is, in fact, institution-related or simply a reflection of the status of subjects upon admission. Therefore, it was the intent of this study to answer the following questions: 1) Does the folate and vitamin B-6 status of elderly subjects deteriorate progressively during the first year of institutionalization? 2) Can plasma tHcy level be used as an early predictor of vitamin B-6 or folate deficiency? To these ends, we estimated likelihood of vitamin B-6 and folate deficiency in 24 elderly subjects on admission to and throughout the first year in a long-stay unit of the Burgundy University Hospital. Comprehensive vitamin B-6 and folate status was obtained by combining dietary information with biochemical tests. Nutrient intakes were assessed from five-day food records. Vitamin B-6 and folate intakes calculated from food composition tables were compared with those from laboratory-analyzed foods consumed by the subjects. Biochemical vitamin status was examined by measuring blood (plasma and red blood cells) vitamin and metabolites (such as tHcy) levels. Cognitive function was evaluated using the Folstein Mini-Mental State Examination (MMS) [12] and hematological parameters were assessed.

MATERIALS AND METHODS

Subjects

From April 1993 to March 1995, 47 patients older than 65 years and consecutively admitted to the geriatric long-stay unit of the University Hospital of Burgundy (France) entered the study. They were selected after evaluation of their clinical characteristics and measurement of vitamin B-12 plasma level. A plasma vitamin B-12 value <74 pmol/L (100 pg/mL) was used to define deficiency. Subjects with vitamin B-12 deficiency were excluded because vitamin B-12 deficiency clinical effects are nearly identical to those of folate deficiency. Within 24 hours after admission in the geriatric unit, each subject's record was reviewed. The data included gender, diagnoses, with emphasis on any history of liver disease, renal disease, gastric or small-bowel surgery, inflammatory bowel disease, pancreatitis, and therapy. Physical examination of the subjects was performed during the study. Patients were excluded if they

were on a therapeutic diet or couldn't walk on their own and feed themselves or had a sensorimotor deficit, delirium or acute physical conditions. Subjects with malignant disease or an expected poor prognosis were not included in the study.

Table 1 contains the characteristics of the subjects studied. All subjects were measured for weight and height. Subjects dropped from the study for various reasons. Two subjects received a folate supplement, three subjects moved to another unit, and 18 subjects died before the end of the study. Therefore, results presented here corresponded to only 24 subjects (20 women and four men). Most of these subjects were affected by genitourinary system diseases ($n = 18$, 75%), musculoskeletal system diseases ($n = 15$, 62%), nervous system diseases ($n = 13$, 54%) and digestive system diseases (biliary colic, gastric ulcer, constipation, diarrhea, intestinal infection) ($n = 12$, 50%).

The protocol was approved by the Human Research Committee of the University of Burgundy. Informed consent was obtained from all the subjects studied before they joined this study.

General Procedure

Subjects were investigated at their admission in the long-stay unit (d 1), then 45 days (d 45), 90 days (d 90), 135 days (d 135), 180 days (d 180) and 360 days (d 360) after. At each period a physician examined each patient, and clinical observations were recorded together with medical history and medication profiles. Consecutively, subjects were administered the French-language version of the MMS to evaluate their cognitive functions. Points were awarded between 0 and 30 with 24 or more considered as normal. Food was weighed during five days. Food consumed by our subjects and considered as good sources of vitamin B-6 and folate were collected and analyzed for their vitamin B-6 and folate content. Blood samples were withdrawn and used for hematology, vitamin B-6 and folate status assessment and creatinine serum level determination. Folate status was assessed by measuring plasma and erythrocyte folate level and plasma tHcy level and vitamin B-6 status by measuring plasma pyridoxal 5'-phosphate (PLP) level.

Dietary Assessment

The intake of nutrients was estimated by a food record covering five consecutive weekdays. Weighing was done by a

Table 1. Anthropometric Profile of Subjects ($n = 24$) at Admission

	Women ($n = 20$)	Men ($n = 4$)
Age (years)	87.0 ± 6.8	84.5 ± 8.7
Weight (kg)	52.1 ± 10.1	64.5 ± 15.8
Height (m)	1.49 ± 0.06	$1.65 \pm 0.06^\ddagger$
BMI*	23.6 ± 4.4	23.4 ± 5.1

Values represent means \pm S.D.

*BMI = Body Mass Index. Represents the weight in kg divided by the square of the height in m^2 . $^\ddagger = p < 0.001$.

trained dietitian in the kitchen, so it is unlikely that consumption habits have been influenced by this procedure. No special attention was given to avoid the usual errors of the kitchen delivery system. The dietitian paid special attention to the possibility that subjects could have had access to foods through family members or other contacts. Foods and drinks that were habitually obtained in this way were recorded. All food consumption data were converted into figures of intake of nutrients with a computerized food composition database (Nutri, Faculté de Médecine, Dijon, France) which uses the most recent data on French food composition [13].

Folate and vitamin B-6 intake of each subject was also evaluated by measuring these two vitamins in duplicate portions. To extract folate from foods, 1 g of sample was homogenized in 9 volumes of 0.1 M phosphate buffer (pH = 6.3) containing 5 mg/mL of ascorbic acid. Homogenates were treated with folate hydrolase and diluted according to the presupposed folate concentration of the category of food. Aliquots were submitted to a *Lactobacillus rhamnosus* microassay and the final bacterial development measured by a titrimetric semi-automated method [14]. Vitamin B-6 was extracted from foods by autoclaving and measured by high performance liquid chromatography [15] coupled with fluorimetry. Folate and vitamin B-6 contents were analyzed in vegetables and legumes: raw and boiled carrots, raw and boiled fennel, boiled squash, boiled celery, boiled cauliflower, mashed potatoes, canned green beans, steamed spinach, leek and potato soup; in fruits: apple, banana, pear, orange, grapefruit, peach, kiwi fruit, mandarin orange, lemon and canned fruit salad; in meat and fish: roasted pork, braised beef, roasted beef, cooked ground beef, stewed steak, cooked veal, roasted veal fillet, roasted chicken, fresh cod and salmon; in hard-boiled eggs, omelet, half-fat UHT milk, French cheese, milk-chocolate, French bread and wholemeal bread.

The adequacy of nutrient intake was assessed by two approaches. First, mean nutrient intakes were compared with the French Recommended Dietary Allowance (FRDA) for older adults. Second, the proportions of the study population having an intake <1/2 FRDA, or an intake between 1/2 FRDA and 2/3 FRDA, or an intake between 2/3 FRDA and FRDA or an intake >FRDA were evaluated. This approach recognizes that the RDA overestimates the nutrient requirement of most individuals in the population. Half an RDA has frequently been used as an arbitrary cut-off point below which nutrient intake may be considered as low [16].

Collection of Clinical and Biochemical Data

Fasting blood samples were obtained from each subject by venipuncture between 0700 and 0730 hour. Less than 20 mL of blood was drawn from each subject into three Vacutainers (Becton Dickinson): one containing K₂-EDTA for complete blood counts (CBCs); one containing K₂-EDTA for folate, vitamin B-6 and B-12 status assessment; one dry Vacutainer for

serum creatinine measurement. All blood samples were immediately transported in an isolated box to the laboratory. The temperature during transport was ~4°C. The transport time was in the range of 0.1 to 0.2 hours, and the blood samples were immediately centrifuged on arrival if necessary. All aliquots of plasma used for PLP and tHcy analysis were stored at -70°C. Serum creatinine was determined by using an Ektachem 700 × RC analyzer. CBCs were carried out on the hospital Coulter counter calibrated by using 5C hematology reference controls (Coulter Diagnostics, France). Measurements performed on the blood samples included red blood cell (RBC) count, total lymphocyte (TLC) count, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), leukocyte count and platelet count. Plasma vitamin B-12 concentrations were measured by the Bio-Rad Quantaphase II assay. Plasma tHcy level, which included free and protein bound homocysteine (Hcy) and the homocysteine-cysteine mixed disulfide, was measured by using high performance liquid chromatography coupled with fluorometric detection [17]. The between day CV of the tHcy assay was <10%. Plasma and blood used for folate measurement were homogenized in water containing ascorbic acid at a concentration of 10 mg/ml of water. Ascorbic acid was used to protect labile reduced forms of folate. To check day-to-day reproducibility of the assay, folate levels of two independently pooled plasma samples were measured each time the assay was performed. We have found that the coefficient of variation for folate assay is approximately 10%. Erythrocyte 5-methyltetrahydrofolate polyglutamates must be deconjugated to the monoglutamate form before being assayed [18]. Hydrolysis was achieved by lysing cells in ascorbic acid at a pH suitable for the activity of endogenous plasma conjugase enzyme (γ -glutamyl hydrolase, EC 3.4.12.10). Red cell and plasma folate concentrations were measured with a semi-automated microbiological microassay using *Lactobacillus rhamnosus* [14]. Synthetic pteroyl monoglutamic acid (PGA) was used as a standard. Red cell folate concentrations were derived from whole-blood folate and serum folate concentrations by using the traditional Ht correction of Hoffbrand *et al.* [18] as follows: erythrocyte folate (nmol/L) = {whole blood folate - [serum folate (1-Ht)]}/Ht. Vitamin B-6 status was assessed by measuring plasma PLP level by using HPLC [19].

Anemia was defined as Hb <12 g/dL for women and <14 g/dL for men, and macrocytosis as a mean corpuscular volume (MCV)>100 fL. Creatinine clearance was estimated using the formula of Cockcroft and Gault [20]: for men, creatinine clearance (ml/minute) = wt (kg) × [140 - age (years)]/[72 × serum creatinine (mg/100 mL)]; for women, multiply by 0.85 [20]. An impaired renal function was arbitrarily defined as creatinine clearance <30 mL/min. Reference ranges for clinical chemistry and hematological values were from different authors: Ortega *et al.* [21] for Hb, RBC and MCHC, Savage *et al.* [10] for Ht, MCV, leukocyte and platelet count and Volkert *et al.* [22] for TLC. Reference ranges for tHcy were based on the mean ± 2 standard

deviations (SD) for 30 normal blood donors, ranging in age from 20 to 40 years. For the results reported here, the reference ranges were: tHcy <5.4 and >14.0 $\mu\text{mol/L}$. The reference ranges for folate, and vitamin B-6 concentrations were: very low plasma folate, <6.8 nmol/L; low, ≥ 6.8 and <11.3 nmol/L and normal, ≥ 11.3 nmol/L; low erythrocyte folate, <227 nmol/L; intermediate, ≥ 227 and <340 nmol/L and normal, ≥ 340 nmol/L; very low plasma PLP, <20 nmol/L; low, ≥ 20 and <30 nmol/L and normal, ≥ 30 nmol/L.

Statistical Analysis

Values are means \pm SD. For each parameter studied, every subject was investigated six times corresponding to d 1, d 45, d 90, d 135, d 180 and d 360. To assess longitudinal changes, repeated measures ANOVA with two fixed factors (gender and period) was used for each variable studied. The repeated measures ANOVA was followed by the Newman/Keul's range test. Analysis using one way ANOVA was done to compare mean values of each parameter at each period for women with plasma tHcy <14 $\mu\text{mol/L}$ at baseline vs. women with tHcy >14 $\mu\text{mol/L}$ at baseline. The Pearson r was used to assess correlation of change from baseline for all variables studied. A *p* value <0.05 was considered as significant.

RESULTS

Mean daily energy, protein and vitamin intakes are presented in Table 2. The percentage of subjects who had energy, protein or vitamin intakes <1/2 FRDA, between 1/2 FRDA and 2/3 FRDA, between 2/3 FRDA and FRDA, >FRDA is shown in Fig. 1. At each period, mean dietary intakes of energy, protein and vitamin B-12 were above the respective FRDA (1,500 kcal/d for energy, 60 g/d for protein and 3 $\mu\text{g/d}$ for vitamin B-12 intake). These means tended to decrease from d 1 to d 360, although this decrease was not statistically significant. From d 1 to d 360, vitamin B-6 intakes were rather low as shown by the average vitamin B-6 intake, which was below the FRDA (2 mg/d). Almost all the subjects had an intake below

this value during the one year follow-up study and one quarter of them consumed less than 1/2 FRDA. Mean vitamin B-6/protein ratios were under the normal value of 0.02 mg vitamin B-6 per g of protein consumed. Mean dietary intakes of folate tended to decrease from d 1 to d 360; however mean folate intake was only different for d 45 vs. d 180. Almost all the subjects had folate intake < FRDA (300 $\mu\text{g/d}$). For 1,000 kcal of overall intake the mean nutritional density of folate did not decrease significantly from d 1 to d 360.

There was no significant difference between analyzed vitamin B-6 intake and calculated vitamin B-6 intake by using a computerized food table (Fig. 2). Similar results were also shown for folate intake. Group 2 (meat, fish, eggs) and plant groups (group 3: vegetables; group 4: fruits and juice) contributed in equal part for about one third each one to the daily vitamin B-6 intake (Table 3). The contribution of the groups 2 and 4 increased between d 45 and d 180. Foods from plant sources contributed about 40% of the total folate intake at each period. Fruits and juice contribution to folate intake increased from d 1 to d 360. Animal food groups contributed about 30% of folate intake at each period.

In Table 4, mean biochemical and hematological parameters and mean MMS scores are given. Except for mean plasma and erythrocyte folate concentrations which decreased significantly from d 1 to d 360 ($\Delta = -33.7$ and $\Delta = -30.2\%$ for plasma and erythrocyte folate, respectively), no significant change was observed for the other parameters. Except at d 360, no subject had a value in the range of major folate deficiency (as indicated by an erythrocyte concentration below 227 nmol/L) (Fig. 3). Mean plasma folate concentrations were above the normal value (i.e. 11.3 nmol/L) from d 1 to d 90, whereas, from d 135 to d 360, they were below the normal value. At d 1 and d 360, two and nine subjects could be classified as deficient (plasma folate <6.8 nmol/L). We classified plasma PLP <20 nmol/L as deficient; by this standard, more than sixty percent of the subjects were judged deficient. During the current study, mean plasma PLP concentrations remained under the normal value (30 nmol/L). At d 1, mean plasma tHcy was rather high (12.20 ± 4.72 $\mu\text{mol/L}$ for women and 12.96 ± 4.15 $\mu\text{mol/L}$ for

Table 2. Mean (\pm SD) Daily Intake of Energy, Protein, Vitamin B-6, Folate and Vitamin B-12 of Subjects (n = 24)

	d 1	d 45	d 90	d 135	d 180	d 360	<i>p</i>
Energy (kcal)							
Women (n = 20)	1722 \pm 335	1642 \pm 332	1610 \pm 316	1565 \pm 385	1521 \pm 391	1527 \pm 391	ns
Men (n = 4)	1578 \pm 319	1743 \pm 313	1882 \pm 343	1797 \pm 333	1588 \pm 482	1897 \pm 402	ns
Protein (g)	65.31 \pm 14.61	62.22 \pm 14.33	65.12 \pm 15.07	62.73 \pm 18.41	58.59 \pm 16.73	58.98 \pm 17.70	ns
Vitamin B-6 (mg)	1.20 \pm 0.33	1.09 \pm 0.25	1.18 \pm 0.27	1.14 \pm 0.30	1.04 \pm 0.31	1.05 \pm 0.29	ns
(mg/g of protein)	0.0183 \pm 0.0023	0.0177 \pm 0.0028	0.0184 \pm 0.0032	0.0185 \pm 0.0026	0.0179 \pm 0.0027	0.0187 \pm 0.0057	ns
(mg/1,000 kcal)	0.70 \pm 0.12	0.66 \pm 0.12	0.71 \pm 0.08	0.71 \pm 0.11	0.67 \pm 0.08	0.66 \pm 0.09	ns
Folate (μg)	245 \pm 64 ^{ab}	250 \pm 68 ^a	248 \pm 59 ^{ab}	233 \pm 67 ^{ab}	209 \pm 59 ^b	220 \pm 57 ^{ab}	*
($\mu\text{g}/1,000$ kcal)	144 \pm 26	153 \pm 36	150 \pm 22	145 \pm 25	137 \pm 22	139 \pm 21	ns
Vitamin B-12 (μg)	4.14 \pm 1.63	4.04 \pm 1.57	4.27 \pm 1.40	3.66 \pm 1.25	3.51 \pm 1.39	3.59 \pm 1.27	ns
($\mu\text{g}/1,000$ kcal)	2.43 \pm 0.78	2.41 \pm 0.78	2.61 \pm 0.75	2.24 \pm 0.56	2.26 \pm 0.53	2.21 \pm 0.48	ns

p values are the probabilities of the Fisher's F. Common superscripts correspond to values that are not different at *p* <0.05. * = *p* <0.05. ns = not significant.

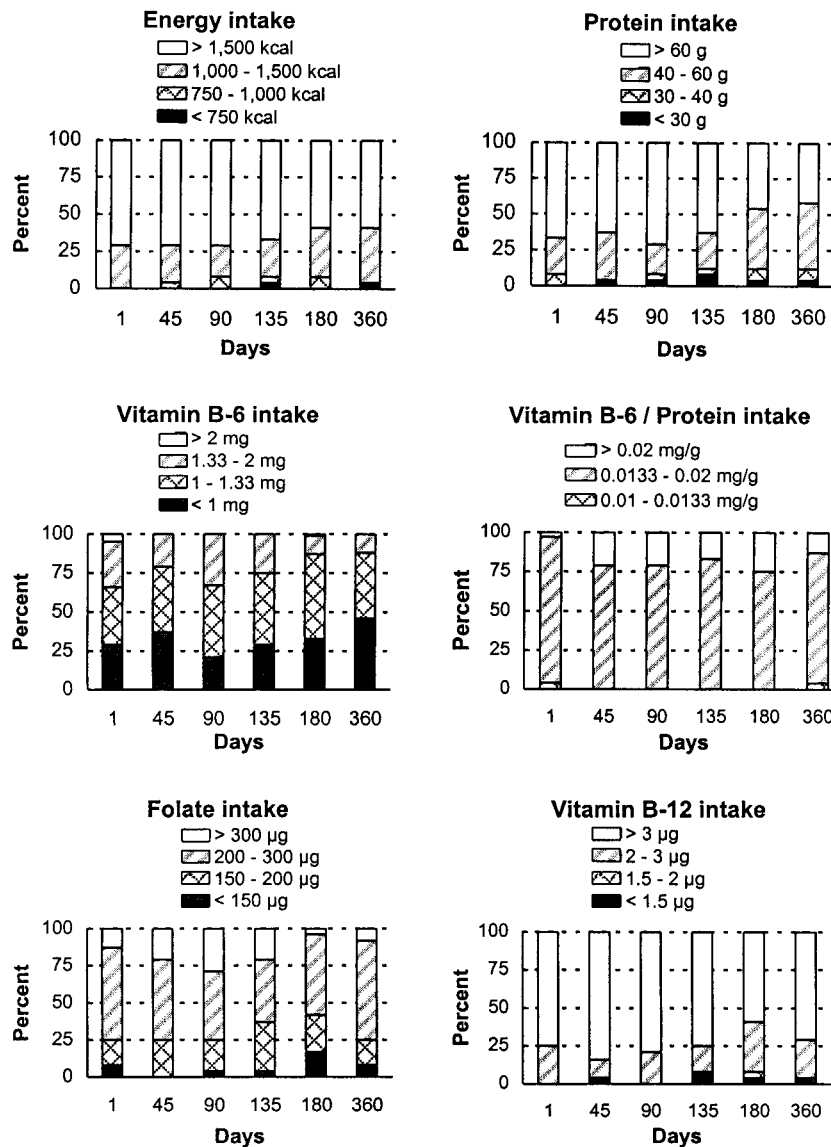


Fig. 1. Percentage of subjects with energy, protein, vitamin B-6, vitamin B-6/protein, folate, vitamin B-12 intakes >FRDA, <FRDA and >½ FRDA, <½ FRDA and >¼ FRDA, <¼ FRDA.

men; mean±SD); it increased from d 1 to d 360 but this increase was not significant. The average increase of plasma tHcy from d 1 to d 360 was 1.14 µmol/L (Δ = +9%) for women and 1.84 µmol/L (Δ = +14%) for men. The within-person change in tHcy level during this study ranged from 8.6 to 41.7% (mean±SD = 22.8±9.2). From d 1 to d 360, about one-third of the subjects in this study had high plasma tHcy concentrations (>14 µmol/L).

At each period, mean MCV, MCHC, TLC and leukocyte count of the subjects were above their normal respective values (defined as 80–100 fL for MCV, 32 g/dL for MCHC, 1.5×10⁹/L for TLC and 4×10⁹/L for leukocyte count) (data not shown). Mean Hb concentrations, mean Ht and mean RBC were under their respective normal values (defined as 12 g/dL

for Hb, 35% for Ht for women; 14 g/dL for Hb, 40% for Ht for men and 4×10¹²/L for RBC for both men and women) (data not shown). At each period, fifty percent or more subjects were under one of these normal values (Fig. 4). From d 1 to d 360, mean MMS scores remained under the score of 24 and three quarters or more of the subjects remained under this score.

Correlation of change from baseline (d 1) was calculated for all parameters studied. Strong relationships were observed between changes in plasma PLP level and changes in vitamin B-6 intake, between changes in plasma tHcy and changes in MCV, leukocyte count, protein intake, vitamin B-6 intake and creatinine clearance (Table 5). Significant relationships were observed between plasma PLP and vitamin B-6 intake (r = 0.47, p<0.001, n = 144) on the one hand and between plasma tHcy

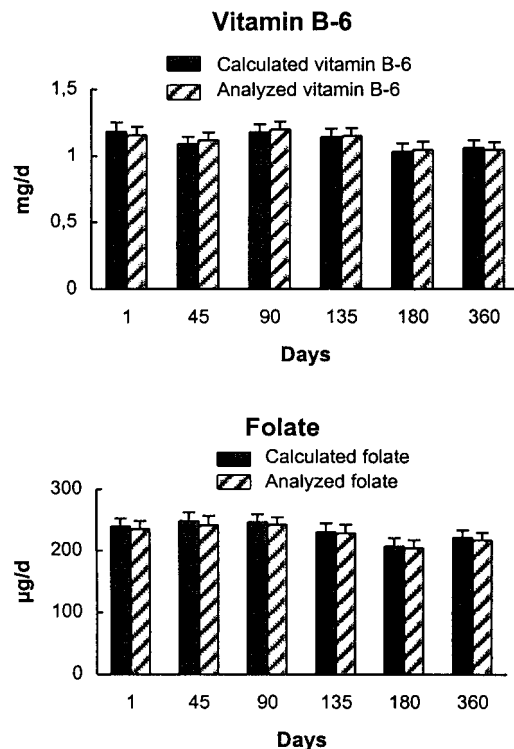


Fig. 2. Mean calculated vitamin intakes using a computerized food table compared to mean analyzed food vitamin intakes. *: $p < 0.05$.

and plasma folate ($r = -0.17$, $p < 0.05$, $n = 144$), Hb ($r = -0.30$, $p < 0.001$, $n = 144$), Ht ($r = -0.25$, $p < 0.001$, $n = 144$), MCV ($r = -0.21$, $p < 0.01$, $n = 144$), leukocyte count ($r = 0.20$, $p < 0.05$, $n = 144$) and creatinine clearance ($r = -0.22$, $p < 0.001$, $n = 144$) on the other hand (data not shown). A relationship was also shown between plasma folate and folate intake ($r = 0.19$, $p < 0.05$, $n = 144$).

Women were divided in two groups according to their plasma tHcy level at baseline (cut-off value = $14 \mu\text{mol/L}$). In each group, we looked at change from baseline for each variable studied (Table 6). No significant change was shown from d 1 to d 360 for all parameters in both groups. In the group with plasma tHcy $< 14 \mu\text{mol/L}$ at baseline, plasma tHcy increased from d 1 to d 360, although this increase was not statistically significant. Plasma folate decreased much more in the group with plasma tHcy $> 14 \mu\text{mol/L}$ at baseline than in the group with plasma tHcy $< 14 \mu\text{mol/L}$ at baseline, but this decrease was not significant. Erythrocyte folate decreased similarly in the two groups. Except at d 360, mean plasma tHcy was significantly lower in the group with plasma tHcy $< 14 \mu\text{mol/L}$ at baseline than in the group who had plasma tHcy $> 14 \mu\text{mol/L}$ at baseline. No significant difference was noticed between plasma PLP, folate or erythrocyte folate (Table 6) and all others parameters (data not shown) studied in women with plasma tHcy $< 14 \mu\text{mol/L}$ at baseline vs. those in women who had plasma tHcy $> 14 \mu\text{mol/L}$ at baseline.

DISCUSSION

The main purpose of this study was to assess folate and vitamin B-6 status of elderly subjects during the first year of institutionalization. Prevalence of deficiency of vitamin B-6, B-12 and folate is believed to be higher in institutionalized elderly than in their free-living counterparts [1,5]. However, evidence that institutionalization *per se* leads to deterioration of vitamin status is weak. Thus, by using a longitudinal approach, it was possible to evaluate to what extent vitamin status of recently institutionalized elderly subjects progressively deteriorates. Vitamin status of the institutionalized elderly may be adversely affected by the chronic nature of some conditions, including changes in dietary habits and decrease of nutrient intake induced by changes in quality and style of life, illness and multiple drug use. Deterioration of vitamin status among institutionalized elderly people can have functional implications of clinical importance. For example, Ortega *et al.* [21] showed that mental and functional deterioration which are major problems faced by the elderly may be caused or worsened by folate deficiency. Folate deficiency can also condition the appearance of macrocytic-megaloblastic anemia and immunological irregularities [23,24]. Based on these facts, we assessed folate status of elderly subjects longitudinally during the first year of institutionalization. Folate status was evaluated by measuring plasma and erythrocyte folate concentrations and plasma tHcy concentration, the latter being considered a sensitive indicator of folate and cobalamin deficiencies [25]. Research suggests that vitamin B-12 and vitamin B-6 deficiencies result in an increased concentration of tHcy [1]. Therefore, vitamin B-6 status was also evaluated, and subjects with low plasma vitamin B-12 concentration ($< 100 \text{ pmol/L}$) were not included in the study. The measurement of circulating levels of vitamins identifies risk potentials for various vitamins in the population; however, it does not allow one to state whether low circulating levels are due to inadequate dietary intakes or to an inability to absorb the vitamin. A more complete assessment can be performed by combining accurate dietary information with biochemical measurements. In the current study, vitamin B-6 and folate intake was calculated from the amount eaten using food table values and analytical values. The hypothesis that deterioration of folate status is a condition associated with cognitive deterioration or hematological alteration was also tested.

Mean folate intakes were lower than the FRDA ($300 \mu\text{g/d}$) throughout the study. About 75% of the subjects or more had an intake below this value. Although the mean intake of folate was similar to that reported in another study [26], the higher recommended amount in France ($300 \mu\text{g/d}$) as compared with the 1989 United States Recommended Dietary Allowances ($200 \mu\text{g/d}$) [27] accounted for the difference in reported adequacy of dietary intake. Intake of folate was the only nutrient examined that decreased significantly over time (Table 2). Changes in vitamin B-12 and vitamin B-6 intakes with time were not

Table 3. Evolution of the Contribution (in Percentage) of Food Categories to Vitamin B-6 and Folate Intakes during the Six Periods

	Food groups	d 1	d 45	d 90	d 135	d 180	d 360	<i>p</i>
Vitamin B-6	1	13.2	13.9	13.5	13.2	13.8	14.4	ns
	2	30.4 ^{ab}	24.1 ^a	27.9 ^{ab}	30.3 ^{ab}	32.2 ^b	27.6 ^{ab}	*
	3	29.5	32.1	29.1	31.6	27.3	28.1	ns
	4	7.9 ^{ab}	10 ^a	8.5 ^{ab}	6.8 ^{ab}	6.1 ^b	10.5 ^a	‡
	5	10.6	10.8	11.3	9.1	10	9.4	ns
	8	8.4	8.4	9.6	8.8	10	9.9	ns
	6+7	0.8	0.6	0.1	0.2	0.6	0.1	ns
	Folate	1	12.8	13.9	13.7	13.2	13.5	15
	2	15.4	15.8	15.9	17.1	15.5	16.1	ns
	3	35.4	34.2	33.7	37.5	33.6	31.2	ns
	4	7.1 ^a	6.1 ^a	5.2 ^a	5 ^a	4 ^a	12.2 ^b	‡
	5	9.1	8.5	8.9	7.6	8.3	7.5	ns
	8	19.3	20.5	21.8	18.5	24.1	17.2	ns
	6+7	0.9	1	0.8	1.1	1	0.8	ns

Group 1: milk, dairy products; Group 2: meat, fish, eggs; Group 3: potatoes, vegetables; Group 4: fruit, juice; Group 5: cereals, bread; Group 6: oils, fats; Group 7: sweets; Group 8: others. *p* values are the probabilities of the Fisher's F. Common superscripts correspond to values that are not different at *p*<0.05. * = *p* <0.05; † = *p*<0.01; ‡ = *p* <0.001. ns = not significant.

statistically significant. One conclusion that can be drawn from this longitudinal analysis was that there was a time effect on dietary intake among our elderly subjects. The diminution of folate intakes was real as mean plasma and erythrocyte folate concentrations of our subjects decreased. Moreover, plasma folate concentrations were correlated with folate intakes and plasma folate concentration is considered as a good reflector of recent folate intakes. Folate in the main foods has been chemically analyzed throughout the study, and no significant difference was observed between analyzed folate food content and calculated folate food content (Fig. 2). Thus, the decrease in folate intake was effectively related to the diet and not to an effect of food tables inaccuracy. Mean daily energy intake decrease averaged 100 kcal from d 1 to d 360. Therefore, reduction of energy intake may account for the decrease in folate, vitamin B-6 and vitamin-B12 intake, since nutrient density of these three vitamins didn't change from d 1 to d 360. A lower energy intake at higher age has been documented either for independently living populations or for institutionalized elderly people [28]. Physical inactivity and health status have been suggested to be important factors [5]. Since physical impairment is one reason for long-term institutionalization,

relatively low food consumption may be expected for elderly people living in an institution. There was no change in contribution of the main food contributors of folate intake throughout the study (Table 3). Thus the diet habits of these elderly subjects remained relatively unchanged during the study.

It is interesting to notice that, with an average folate intake remaining below 250 µg/d, reductions in both plasma and erythrocyte folate concentrations were significant and continuous through the one-year period. Furthermore, at the end of the study, mean plasma folate concentration was below the normal value (11.3 nmol/L). At admission in the study, few subjects (8%) had very low plasma (<6.8 nmol/L) and low erythrocyte (<340 nmol/L) folate concentrations. The incidence of deficiency increased with the length of stay. At d 360, 37 and 17% of subjects were <6.8 nmol/L and <340 nmol/L for plasma and erythrocyte folate concentrations, respectively (Fig. 3). Thus it appeared that this level of folate intake was not sufficient for maintenance of folate status in our institutionalized elderly subjects. Similarly, on diets estimated to contain 135 µg folate/d, all of 21 elderly men and women living at home sustained red cell folate >227 nmol/L and were hematologically normal; but nine had red cell folate between 227 and

Table 4. Means (±SD) of Biochemical Parameters, MMS Scores and Creatinine Clearance of Subjects (n = 24)

	d 1	d 45	d 90	d 135	d 180	d 360	<i>p</i>
Plasma PLP (nmol/L)	16.22 ± 6.96	16.04 ± 7.69	15.17 ± 7.52	15.94 ± 7.02	14.71 ± 7.37	13.96 ± 6.54	ns
Plasma folate (nmol/L)	12.93 ± 5.40 ^a	12.02 ± 8.2 ^{ab}	12.53 ± 5.82 ^{ab}	10.45 ± 5.96 ^{ab}	11.97 ± 5.74 ^{ab}	8.54 ± 6.67 ^b	*
Erythrocyte folate (nmol/L)	755 ± 282 ^a	725 ± 293 ^a	712 ± 27 ^a	590 ± 174 ^{ab}	617 ± 214 ^{ab}	527 ± 181 ^b	‡
Plasma total Hcy (µmol/L)							
(Women n = 20)	12.20 ± 4.72	12.66 ± 5.12	12.28 ± 4.86	12.23 ± 4.62	12.86 ± 6.13	13.34 ± 4.46	ns
(Men = 4)	12.96 ± 4.15	13.66 ± 2.79	15.36 ± 3.50	14.02 ± 2.61	14.52 ± 4.37	14.80 ± 6.16	ns
Creatinine clearance (mL/min)	40.72 ± 13.03	39.86 ± 11.73	38.66 ± 12.82	38.50 ± 12.65	38.97 ± 16.16	38.35 ± 14.93	ns
MMS score	19.0 ± 6.5	19.0 ± 5.5	17.1 ± 6.7	16.2 ± 7.1	16.8 ± 5.9	17.3 ± 7.9	ns

Hcy = homocysteine. *p* values are the probabilities of the Fisher's F. Common superscripts correspond to values that are not different at *p* <0.05. * = *p*<0.05; ‡ = *p* <0.001. ns = not significant.

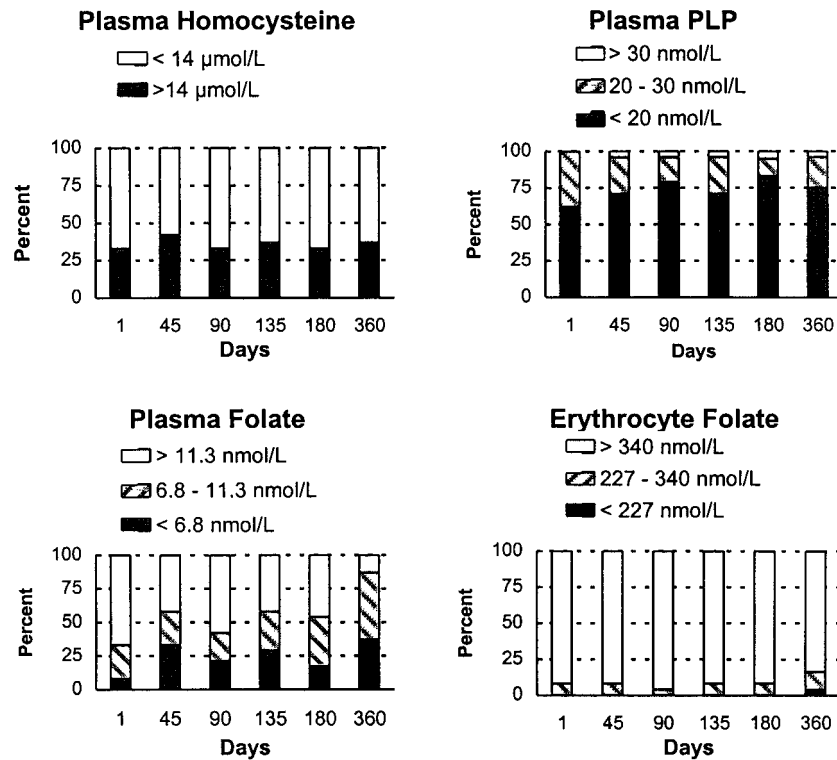


Fig. 3. Percentage of subjects with high, moderate or no risk of vitamin deficiency.

340 nmol/L [29]. Although our study was not designed to estimate folate requirements of elderly men and women, it can provide interesting information since data are still insufficient to conclude that folate requirements and allowances for the elderly population are different from those for younger age groups due to the physiological effects of aging. Our results suggested that 200 $\mu\text{g/d}$ of dietary folate was not sufficient to allow for the “margin of safety” inherent in RDA.

Folate status was also assessed by measuring plasma tHcy concentration, since plasma tHcy is considered a marker for individual or composite status of vitamin B-12, folate, and vitamin B-6 [30]. High plasma concentrations of tHcy can be largely attributed to inadequate plasma concentrations of B vitamins [31], and it has been suggested that measurement of tHcy is necessary to adequately identify persons with low concentrations of vitamin B-12 and folate who are actually deficient [32]. Although mean plasma and erythrocyte folate concentrations decreased significantly from d 1 to d 360, no significant change was shown for mean plasma tHcy concentrations during this period, even if plasma tHcy was inversely correlated with plasma folate as reported in others studies [1,2]. No relationship was found between changes in plasma tHcy and changes in erythrocyte folate concentrations and between changes in plasma tHcy and changes in plasma folate or erythrocyte folate (Table 5). Furthermore, wide variations in intra-individual plasma tHcy were shown. The mean within-person variance was 7.95, based on a mean change of +1.13 $\mu\text{mol/L}$

from d 1 to d 360. The individual tHcy levels appeared to fall and rise randomly from one period to another, and the change in tHcy concentrations ranged from 9% to 42%. These results are similar to those of Santhosh-Kumar *et al.* [33] who observed unpredictable intra-individual variations in serum Hcy levels among subjectively healthy adults receiving a folic acid supplement (1 mg folic acid/d). Although group values of serum Hcy fell during folic acid supplementation, intra-individual variation was so great that subjects with subclinical folate deficiency could not be identified. Jacob *et al.* [34,35] determined the Hcy response to folate depletion and repletion in two controlled metabolic unit studies of adult men and women. This format allowed for study of the plasma Hcy response to folate intake levels, while other factors of diet and lifestyle that might also have affected Hcy levels were held constant. In the first study of folate depletion in men [34], although plasma Hcy changed inversely with dietary folate intake and plasma levels, the time course and magnitude of Hcy changes differed substantially among the subjects. In the second study, plasma Hcy response to folate depletion was assessed among 10 postmenopausal women. As in the earlier study, each subject served as her own control. And as in the men’s study, plasma Hcy responded differently among individuals. The Hcy values for three subjects changed only within the normal range, five subjects showed mild elevations, one subject showed a decrease, while another one showed an unusually large Hcy increase upon folate depletion. Clarke *et al.* [36]

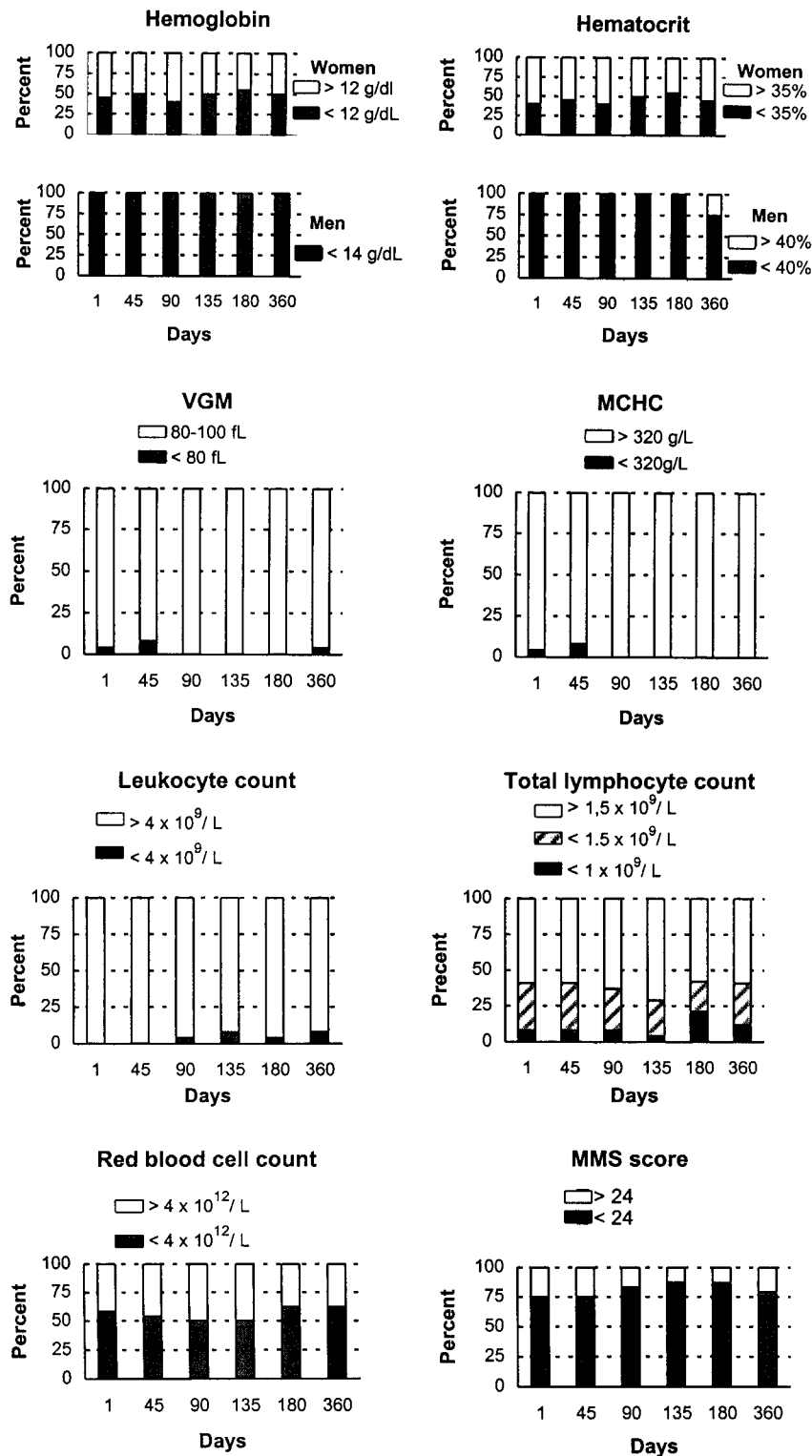


Fig. 4. Percentage of subjects with low or normal hematological parameters and low or normal MMS score.

examined the variability of plasma tHcy in 96 individuals over a one-year period. They showed that blood tHcy concentrations varied from 7.1 $\mu\text{mol/L}$ in the bottom quintile to 14.5 $\mu\text{mol/L}$

in the top quintile; the within-person SD was 0.93 $\mu\text{mol/L}$, which is lower than the value found in our study (2.82 $\mu\text{mol/L}$). Our results and those of Santhosh-Kumar *et al.* [33] and Jacob

Table 5. Pearson's Correlation Coefficient between Changes from Baseline of Plasma PLP, Folate, Total Homocysteine, Erythrocyte Folate Levels and Changes from Baseline of Other Blood Biochemical Indices and Dietary Intakes of the 24 Subjects (n = 120)

Parameters	r	p
Plasma PLP vs. vitamin B-6 intake	0.29	†
Plasma folate vs. folate intake	0.02	ns
Erythrocyte folate vs. folate intake	0.11	ns
Plasma homocysteine vs. plasma PLP	0.04	ns
vs. plasma folate	-0.16	ns
vs. erythrocyte folate	0.01	ns
vs. MMS score	0.04	ns
vs. hematocrit	0.16	ns
vs. hemoglobin	0.13	ns
vs. leucocyte count	0.30	‡
vs. lymphocyte count	0.04	ns
vs. MCV	0.33	‡
vs. MCHC	0.01	ns
vs. energy intake	-0.16	ns
vs. protein intake	-0.19	*
vs. vitamin B-6 intake	-0.19	*
vs. folate intake	-0.17	ns
vs. creatinine clearance	-0.19	*

ns = not significant. * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$.

et al. [34,35] suggest that single measurements of serum or plasma tHcy can hardly be used to assess folate status with confidence.

Prevalence of moderate hyperhomocysteinemia characterized by plasma tHcy of 14–30 $\mu\text{mol/L}$ did not increase from d 1 to d 360, although the number of subjects with a plasma folate concentration below 6.8 nmol/L increased from two to nine. In fact, moderate hyperhomocysteinemia may result from vitamin B-6 and early vitamin B-12 and folate deficiency, renal insufficiency [37,38], mutations in genes coding for enzymes involved in the methionine-metabolism pathway, such as 5,10-methylenetetrahydrofolate reductase (MTHFR) [39] and cystathionine β -synthase [40]. Of the B vitamins, folate nutrition generally seems to exert the greatest influence on Hcy concentrations, although vitamin B-12 may be more important for elderly who suffer from cobalamin malabsorption [41]. In the current study, all subjects had a plasma vitamin B-12 concentration above 74 pmol/L and were considered as being not deficient. However, a recent pilot study raises the possibility that measurement of holotranscobalamin II (TCII) in serum may be an earlier measure of developing vitamin B-12 deficiency than measurement of total cobalamin in serum [42]. Unfortunately, for technical reasons, we didn't measure TCII. In consequence, a cobalamin negative balance, occurring prior to fall in total cobalamin, may have resulted in a Hcy increase among some subjects. Vitamin B-6 status of our subjects was judged deficient. One-quarter and more subjects had intake below $\frac{1}{2}$ FRDA (1 mg/d) and more than 60% had plasma PLP concentrations < 20 nmol/L at each period. Mean plasma PLP

decreased from d 1 to d 360, but this decrease was not statistically significant, and a high and positive correlation was observed between changes from baseline of plasma PLP concentrations and changes from baseline of vitamin B-6 intakes. Impaired vitamin B-6 status in institutionalized elderly subjects has already been reported [3,4]. Total Hcy is either remethylated to methionine or metabolized to cysteine by the transsulfuration pathway, which is vitamin B-6 dependent. Thus vitamin B-6 deficiency may contribute to impaired transsulfuration [43]. In the current study, no relationship was found between changes from baseline in plasma tHcy and changes from baseline in plasma PLP concentrations. Furthermore, mean plasma PLP concentrations at d 1 to d 360 of women with baseline plasma tHcy < 14 $\mu\text{mol/L}$ did not differ from those of women with baseline plasma tHcy > 14 $\mu\text{mol/L}$ (Table 6). These results suggested that, although subjects were vitamin B-6 deficient, this state didn't greatly affect their plasma tHcy circulating concentration. They also confirmed that fasting plasma Hcy concentrations generally do not reflect vitamin B-6 status, unless tHcy is measured after a large oral dose of L-methionine [44]. In addition to its relation to folate, vitamin B-12 and vitamin B-6 status, plasma tHcy may also be related to protein intake. An inverse relationship was found between changes from baseline in plasma tHcy concentration and changes from baseline in protein intake. Therefore, in the present study, the moderate hyperhomocysteinemia observed could not be related to protein intake as a source of methionine as suggested by others [45]. Mean creatinine clearance, calculated from serum creatinine by using Cockcroft-Gault formula [20], did not vary from d 1 to d 360. Furthermore, mean creatinine clearance of women with baseline plasma tHcy < 14 $\mu\text{mol/L}$ did not differ from those of women with baseline plasma tHcy > 14 $\mu\text{mol/L}$ at the six periods studied (data not shown). Thus moderated hyperhomocysteinemia could not be related to an impaired kidney function. Nevertheless, it should be noticed that changes from baseline of plasma tHcy were inversely related to changes from baseline of creatinine clearance and an inverse relationship between plasma tHcy and creatinine clearance was shown.

The prevalence of folate-deficiency anemia was estimated. Cut-off values of < 6.8 nmol/L for serum folate, < 227 nmol/L for erythrocyte folate, ≥ 100 fL for MCV and < 120 g/L for Hb were used to define folate-deficiency anemia according to Herbert [46]. In our study, anemia (Ht $< 40\%$ in men and Ht $< 35\%$ in women) was present in 12 or more subjects (50% or more) from d 1 to d 360 (Fig. 4). The anemia was typically normocytic (MCV between 80–100 fL) throughout the study. Mean Hb concentrations were low, but did not change during the study. No relationship was observed between plasma or erythrocyte folate concentrations and Hb, Ht or MCV (data not shown). Thus, there is no clear indication that the lowering of folate values among our elderly subjects can lead to functional deficits. However, macrocytosis may be a nonsensitive indicator of folate deficiency [47]. Megaloblastosis occurs after about

Table 6. Means (\pm SD) of Plasma Total Hcy, Plasma PLP, Plasma Folate and Erythrocyte Folate of Women according to their Plasma Total Hcy at Baseline

	d 1	d 45	d 90	d 135	d 180	d 360	Δ %
Plasma total Hcy (μ mol/L) (n = 13) ^a	9.33 \pm 2.99	10.2 \pm 3.00	10.31 \pm 2.81	10.61 \pm 3.32	10.44 \pm 3.68	12.01 \pm 4.35	+28.7
Plasma total Hcy (μ mol/L) (n = 7) ^b	17.53 \pm 2.68 \ddagger	17.21 \pm 5.28 \ddagger	15.95 \pm 5.9 \ddagger	15.24 \pm 5.40*	17.34 \pm 7.47*	15.81 \pm 3.77	-9.8
Plasma PLP (nmol/L) (n = 13) ^a	14.4 \pm 6.7	14.1 \pm 7.4	14.8 \pm 7.6	16.3 \pm 5.2	12.5 \pm 6.4	13.2 \pm 5.2	-8.3
Plasma PLP (nmol/L) (n = 7) ^b	17.0 \pm 7.5	17.1 \pm 9.2	14.1 \pm 8.5	14.8 \pm 5.1	17.0 \pm 5.3	13.0 \pm 6.5	-23.5
Plasma folate (nmol/L) (n = 13) ^a	12.6 \pm 6.2	13.5 \pm 9.5	12.2 \pm 6.4	10.5 \pm 5.6	12.3 \pm 6.6	10.5 \pm 7.5	-16.6
Plasma folate (nmol/L) (n = 7) ^b	12.3 \pm 3.8	8.1 \pm 2.9	13.5 \pm 4.2	10.8 \pm 7.4	12.7 \pm 4.7	6.3 \pm 6.2	-48.8
Erythrocyte folate (nmol/L) (n = 13) ^a	724 \pm 259	787 \pm 310	742 \pm 373	593 \pm 178	575 \pm 184	544 \pm 211	-24.9
Erythrocyte folate (nmol/L) (n = 7) ^b	724 \pm 283	545 \pm 163	640 \pm 105	647 \pm 137	647 \pm 181	532 \pm 134	-26.5

Hcy = homocysteine. ^a total plasma Hcy <14 μ mol/L at baseline; ^b total plasma Hcy >14 μ mol/L at baseline. * = ^a significantly different from ^b at p <0.05; \ddagger = ^a significantly different from ^b at p <0.01; \ddagger = ^a significantly different from ^b at p <0.001; p values are the probabilities of the Fisher's F. Δ % = [(d 360 - d 1)/d 1] \times 100.

19 weeks on a folate deficient diet and may be masked by the presence of iron deficiency [48]. Hypersegmentation of polymorphonuclear cells appears after about seven weeks of depletion [48]. Unfortunately for technical reasons we didn't evaluate neutrophil hypersegmentation. Nevertheless, our results are consistent with the notion that folate deficiency that is relatively early or latent, i.e., not expressed hematologically as a megaloblastic anemia, is common in institutionalized elderly people [2,49].

Is there a relationship between folate levels and behavior in elderly people? In the present study, no relationship was found between the MMS scores and plasma or erythrocyte folate concentrations (data not shown) or between MMS scores and plasma tHcy concentrations. The relationship between folate status and cognitive function of our subjects was then rather uncertain. This was in agreement with some investigators [8,9,50,51] who found no relationship between folate status and cognitive state. This has not been a constant feature. On the other hand, Elwood *et al.* [52] and Sommer and Wolkowitz [53] showed a positive correlation between erythrocyte folate levels and MMS exam. Similarly, Riggs *et al.* [54] and Gottfries *et al.* [55] showed that serum Hcy concentration can be considered an early and sensitive marker for cognitive impairment. Lack of relationship between plasma tHcy concentrations and MMS scores in the current study may be related to the length of the follow-up period and to the small number of subjects studied.

In conclusion, most of our subjects were already vitamin B-6 deficient at admission in the long-stay unit and remained deficient during the follow-up study. Folate status of the subjects was impaired during the first year of institutionalization, as shown by an increase in the number of subjects with a low plasma folate level (<6.8 nmol/L). Low vitamin intake was the main cause of the vitamin B-6 deficiency and of the folate status deterioration. Subjects had not changed their diet habits, and the low vitamin B-6 and folate intakes were mainly related to a low food intake. The deterioration of folate status didn't result in hematological or cognitive status deterioration. The plasma tHcy appeared not to be an earlier predictor, nor a

marker of folate deficiency since it varied independently of folate status in our subjects. Increased plasma tHcy might be related in a part to a decrease in renal clearance, indicating a progressive loss of renal function.

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