

Bioavailability of heptaglutamyl relative to monoglutamyl folic acid in healthy adults¹⁻³

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ABSTRACT

Background: The bioavailability of dietary folate has been estimated to be ≈50% of that of synthetic folic acid. Folate in the diet is linked to a polyglutamate chain that may restrict folate absorption.

Objective: Our goal was to quantify the bioavailability and bioefficacy of low doses of polyglutamyl folic acid relative to that of monoglutamyl folic acid.

Design: In total, 180 men and women aged 50–75 y ingested capsules containing 323 nmol heptaglutamyl folic acid/d or 262 nmol monoglutamyl folic acid/d or placebo in a randomized parallel trial. Serum and erythrocyte folate and plasma homocysteine concentrations were measured after an overnight fast at baseline and after 12 wk of intervention.

Results: Mean serum and erythrocyte folate concentrations increased less in the polyglutamyl folic acid group [6.1 (95% CI: 5.3, 7.0) and 155 (122, 188) nmol/L, respectively] than in the monoglutamyl folic acid group [11.8 (10.3, 13.3) and 282 (246, 318) nmol/L, respectively]. Differences remained statistically significant ($P < 0.05$) after correction for the difference in the amount of folic acid administered. The decrease in plasma homocysteine concentrations did not differ significantly between treatment groups [polyglutamyl: -12.1% (-14.8% , -9.3%); monoglutamyl: -14.1% (-16.3% , -11.9%)]. The relative bioavailability of polyglutamyl folic acid was 64% (52%, 75%) on the basis of serum folate and was 68% (51%, 84%) on the basis of erythrocyte folate concentrations. Bioefficacy, determined by changes in plasma homocysteine concentrations, was 106% (77%, 134%).

Conclusion: The polyglutamate chain of folates in the diet reduces their bioavailability. *Am J Clin Nutr* 2004;79:424–9.

KEY WORDS Polyglutamyl folic acid, monoglutamyl folic acid, folic acid, bioavailability, bioefficacy, serum folate, erythrocyte folate, plasma homocysteine

INTRODUCTION

Folate is an essential B vitamin in the human diet. Low folate intake causes mild hyperhomocysteinemia, which is a potential risk factor for cardiovascular disease (1, 2). Supplementation with folic acid leads to a significant reduction in plasma homocysteine concentrations in healthy subjects, even at low doses (3–5). An increased intake of dietary folate from vegetables and fruit also lowers plasma homocysteine concentrations (6).

The term *folic acid* refers to the synthetic form of the vitamin, whereas *folate* refers to the natural forms, such as those present in foods. The bioavailability and bioefficacy of dietary folate

appear to be less than those of folic acid (7). *Bioavailability* is defined as the fraction of folate that is absorbed and can be used for metabolic processes or storage as measured by changes in folate status, whereas *bioefficacy*, or more correctly *functional bioefficacy*, is defined as the fraction that has a positive effect on a functional parameter, for instance, the lowering of homocysteine concentrations (8).

One of the most important determinants of dietary folate bioavailability, and thus of bioefficacy, could be the linkage to a polyglutamate chain. In the Netherlands, where no food fortification with folic acid is allowed, two-thirds of dietary folate intake is ingested in the polyglutamate form, mainly derived from vegetables, bread, and fruit (9). Before the absorption of polyglutamated folates takes place, the enzyme folylpoly γ -glutamyl carboxypeptidase, which is present in the brush border of the jejunum, cleaves glutamate moieties from the folate molecule. Subsequently, folate is absorbed mainly in the monoglutamate form. Limitation or inhibition of this enzymatic process might decrease the absorption of folate from polyglutamate sources.

Until now, many studies on the bioavailability of polyglutamyl compared with monoglutamyl folic acid have been carried out (10–18). However, results have been equivocal and have resulted in estimates of the bioavailability of polyglutamyl relative to monoglutamyl folic acid that vary from 50% to 100% depending on the study design and outcome parameters used. In our view, it is not yet clear to what extent the lower bioavailability of dietary folate relative to that of folic acid can be attributed to the polyglutamate chain. In addition, the bioefficacy of polyglutamyl folic acid relative to monoglutamyl folic acid for reduction of plasma homocysteine concentrations has not been studied previously. Knowledge of the extent to which linkage to a poly-

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glutamate chain decreases folate bioavailability and bioefficacy would indicate which products are better folate sources and whether it is necessary to find methods to improve the bioavailability of dietary folate. The present study was carried out to quantify the bioavailability and bioefficacy of low doses of polyglutamyl relative to monoglutamyl folic acid in a long-term study in persons aged 50–70 y.

SUBJECTS AND METHODS

Recruitment of subjects

Subjects were recruited from a database of volunteers maintained at the Division of Human Nutrition, Wageningen University, Netherlands. A letter explaining the research and a medical questionnaire were sent to 957 men and women aged 50–70 y. The questionnaire provided information on the health criteria that subjects had to fulfill for inclusion in the study, and subjects were asked to return the questionnaire only when they believed all criteria were met. Of the 957 questionnaires, 387 were completed and returned. Exclusion criteria were as follows: hematologic diseases and chronic diseases including cancer, renal insufficiency, liver disease, and diagnosed gastrointestinal disorders; use of antiinflammatory, antacid, or anticonvulsant drugs; and chronic consumption of aspirin or B vitamin supplements or of other drugs or dietary supplements that interfere with folate or homocysteine metabolism. Women had to be postmenopausal. Subjects with low serum vitamin B-12 concentrations (<160 pmol/L), high serum creatinine concentrations (>125 μ mol/L), or high plasma homocysteine concentrations (>26 μ mol/L) were also excluded from participation. On the basis of the questionnaire data, 290 subjects were eligible for admission to the study. Forty-one persons withdrew from participation before the start of the study for logistic, medical, or personal reasons. Because the aim was to recruit ≈ 180 subjects, we excluded 61 persons randomly. Thus, the population invited for biochemical screening consisted of 188 apparently healthy adults aged 50–70 y. The research protocol was approved by the Medical Ethical Committee of Wageningen University. All subjects gave written informed consent.

Study design

The study comprised a screening visit, a 5-wk run-in period, a baseline visit, a 12-wk intervention period, and 2 follow-up visits after 2 and 12 wk of intervention. The follow-up visit after 2 wk was applied because we wanted to explore whether the bioavailability of polyglutamyl folic acid could be delayed compared with that of the monoglutamyl form (16). At the screening visit, a fasting blood sample was taken to determine serum vitamin B-12, serum creatinine, and plasma homocysteine concentrations. Furthermore, weight and height were measured. All subjects took part in a 5-wk run-in period during which they ingested placebo capsules. In the meantime, biochemical analyses were conducted. The serum vitamin B-12 concentration was too low in one subject and the plasma homocysteine concentration was too high in another subject. These subjects, therefore, were excluded from further participation. An additional 4 subjects reported taking a medication that is known to interfere with folate or homocysteine metabolism between the screening and first baseline visit and were therefore excluded. The remaining 182 subjects entered the intervention phase of the study, which had a random-

ized, double-blind, parallel design with 3 groups and lasted 12 wk. To guarantee similar distributions of homocysteine concentrations in all groups at baseline, randomized blocks based on screening homocysteine concentrations were used for the assignment of subjects to the 3 groups. Subjects sharing one household (21 couples) were allocated to the same intervention group to minimize the possibility of the wrong capsules being taken.

The 3 intervention groups received 323 nmol monoglutamyl folic acid (≈ 145 μ g), 262 nmol heptaglutamyl folic acid (≈ 320 μ g), or placebo in the form of one capsule per day. Throughout the study, subjects were asked not to consume liver because of the high folate content. In addition, consumption of liver products, such as pâté and liver paste, was not allowed for 3 d before blood was drawn. Compliance was checked by counts of capsules that were returned after the study. Furthermore, subjects kept a diary for monitoring compliance, illnesses, use of drugs or dietary supplements, and departure from restriction of consumption of liver and liver products.

After the run-in period, blood was drawn from all subjects at 3 time points: baseline and after 2 and 12 wk of follow-up. At each time point, blood was collected twice from all subjects on 2 separate days, each 3 d apart. Measurements in the blood samples taken 3 d apart were averaged to improve the statistical power of the study (19). Blood was drawn at the Division of Human Nutrition after subjects had fasted overnight. For the measurement of serum vitamin B-12, creatinine, and folate concentrations, 5 mL blood was collected in a serum separator tube and, after standing for 30 min at room temperature, it was centrifuged for 10 min at $2600 \times g$. For the measurement of folate concentrations in whole blood, 4 mL blood was collected in EDTA-coated tubes and placed on a roller bank for 10 min to ensure thorough mixing. From this sample, 0.4 mL was diluted with 1.6 mL ascorbic acid (0.1%, wt:vol) and homogenized on a vortex. For the measurement of plasma homocysteine concentrations, 5-mL blood samples were collected into EDTA-coated tubes, which were immediately placed on ice water and centrifuged within 30 min for 10 min at $2600 \times g$. Serum, plasma, and whole-blood samples were stored at -80 °C until analyzed.

Capsules

Monoglutamyl folic acid was obtained from Merck & Co (Whitehouse Station, NJ) and heptaglutamyl folic acid was obtained from Schircks (Jona, Switzerland) as the ammonium salt (chemical purities: $>98\%$). Identical capsules containing monoglutamyl folic acid or polyglutamyl folic acid, with a target amount of 450 nmol per capsule, and placebo capsules were produced manually at the pharmacy of the Gelderse Vallei Hospital, Ede, Netherlands. The folic acid content of the capsules was determined by HPLC with ultraviolet detection (20). Polyglutamyl folic acid was hydrolyzed to monoglutamyl folic acid by incubation with rat plasma before injection to the HPLC. Six batches of each type of capsule were analyzed with each batch, which consisted of 20 randomly chosen capsules. The folic acid contents of the capsules, expressed as nmol per capsule, were as follows: placebo capsules, 0 (range: 0–0); monoglutamyl folic acid capsules, 323 (range: 219–373); and polyglutamyl folic acid capsules, 262 (range: 249–297). Thus, the actual content of the monoglutamyl folic acid capsules was 71% and of the polyglutamyl folic acid capsules was 58% of the targeted dose (450 nmol per capsule). CVs of the folic acid content in the capsule batches did not exceed 6%.

TABLE 1Characteristics of the study population on admission to the study¹

	Placebo (<i>n</i> = 60)	Monoglutamyl folic acid (<i>n</i> = 59)	Polyglutamyl folic acid (<i>n</i> = 61)
Age (y)	61 ± 5	60 ± 6	59 ± 5
BMI (kg/m ²)	26.7 ± 3.9	25.4 ± 3.0	26.2 ± 2.9
Sex (%)			
Men	62	53	61
Women	38	47	39
Smokers (%)	14	17	18
Plasma homocysteine (μmol/L)	10.3 ± 2.0	10.4 ± 2.2	10.5 ± 2.4
Serum vitamin B-12 (pmol/L)	321 ± 94	315 ± 83	307 ± 89
Serum creatinine (μmol/L)	85 ± 13	81 ± 12	85 ± 12

¹ $\bar{x} \pm SD$. There were no statistically significant differences between groups.**Biochemical analyses**

The concentrations of serum vitamin B-12, serum folate, and whole-blood folate were measured with a commercial chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Company, Los Angeles). For the measurement of folate in whole blood, samples were diluted further with a concentrated human protein-based matrix (Immulite 2000 diluent) before measurement. Folate concentrations were measured at the Central Clinical Chemistry Laboratory of the University Medical Centre, Nijmegen, Netherlands (intraassay variation: <5% for serum folate and <14% for whole-blood folate). Creatinine concentrations were measured with a kit (Dimension; DuPont, Wilmington, DE) based on the kinetic Jaffé reaction. Concentrations of vitamin B-12 and of creatinine were assessed at the General Practitioner's Laboratory at Larenstein (Velp, Netherlands). Plasma total homocysteine concentrations were measured by HPLC with fluorimetric detection at the Division of Human Nutrition and Epidemiology, Wageningen University (21, 22); the intra- and interassay CVs were 2% and 7%, respectively. Serum folate, whole-blood folate, and plasma homocysteine concentrations were measured in all samples from the same run collected at weeks 0, 2, and 12 to eliminate interassay variation.

Calculations and statistics

Power calculations were based on expected changes in the concentrations of folate in serum and of homocysteine in plasma. We expected an increase of 9.5 nmol/L in serum folate concentrations in the group receiving monoglutamyl folic acid and, assuming a relative bioavailability of polyglutamyl folic acid of 75%, an increase of 7.1 nmol/L in the group receiving polyglutamyl folic acid. Likewise, for plasma homocysteine concentrations, we expected a decrease of 2.5 μmol/L (20%) in the group receiving monoglutamyl folic acid and of 1.9 μmol/L (16%) in the group receiving polyglutamyl folic acid. Thus, 54 subjects per group would be required to detect a difference in the effect of the 2 forms of folic acid on serum folate concentrations and 45 per group would be required to detect a difference in the effect of the 2 forms of folic acid on plasma homocysteine concentrations with 80% power and $\alpha = 0.05$.

Erythrocyte folate concentrations were calculated by using the following formula:

$$\text{Erythrocyte folate} = W - [S \times (100 - H)/100] \times (100/H) \quad (1)$$

where *W* is the whole-blood folate concentration, *S* is the serum

folate concentration, and *H* is hematocrit. Mean changes in serum folate, erythrocyte folate, and plasma homocysteine concentrations in the groups receiving either monoglutamyl or polyglutamyl folic acid were computed and corrected for changes in the placebo group. Differences between the folic acid groups were tested by analysis of variance (SAS; SAS Institute Inc, Cary, NC).

$$\text{Bioavailability} = (\Delta[C]_{\text{poly}} - \Delta[C]_{\text{placebo}}/\Delta[C]_{\text{mono}} - \Delta[C]_{\text{placebo}}) \times \text{dosage}_{\text{mono}}/\text{dosage}_{\text{poly}} \quad (2)$$

where $\Delta[C]$ is the change in serum or erythrocyte folate concentrations over time in the monoglutamyl folic acid, polyglutamyl folic acid, or placebo group. Likewise, bioefficacy was calculated by using the decrease in plasma homocysteine concentrations expressed as a percentage.

RESULTS

Data from 2 subjects were excluded from the analyses because they had taken a drug that was on the exclusion list. Therefore, all analyses were based on data from 180 subjects. During the intervention period, one person dropped out just before the final measurements because of illness (lung cancer). The average compliance with intervention, based on counts of returned capsules, was 99% (>80% for all subjects). Study groups did not differ significantly in age, body mass index, sex, smoking behavior, or blood concentrations of homocysteine, vitamin B-12, and creatinine at screening. The characteristics of the study population at baseline are shown in **Table 1**.

The changes in concentrations of serum and erythrocyte folate and in plasma homocysteine are shown in **Table 2**. Serum folate concentrations in the group that received monoglutamyl folic acid increased by 81% at 12 wk of intervention after correction for changes in the placebo group. In the group that received polyglutamyl folic acid, the serum folate concentration increased by 46% after 12 wk. Erythrocyte folate concentrations increased by 41% in the monoglutamyl folic acid group and by 21% in the polyglutamyl folic acid group after 12 wk of intervention. Correction for the difference in capsule content did not alter the statistically significant differences in folate concentrations between groups. Decreases in plasma homocysteine concentrations were not significantly different between the 2 folic acid groups, namely 14% in the monoglutamyl folic acid group and 12% in the polyglutamyl folic acid group after 12 wk. The decreases were

TABLE 2

Serum folate, erythrocyte folate, and homocysteine concentrations during the intervention and changes from baseline at 2 and 12 wk of intervention

	Placebo (n = 60)	Monoglutamyl folic acid (n = 59) ¹	Polyglutamyl folic acid (n = 60)	Difference between folic acid groups
Serum folate (nmol/L)				
Week 0	14.2 ± 3.9 ²	15.2 ± 4.1	14.4 ± 5.4	
Week 2	13.6 ± 3.7	18.8 ± 4.7	15.8 ± 4.6	
Week 12	12.9 ± 3.8	25.7 ± 7.4	19.3 ± 5.6	
Change at week 12 (nmol/L)		11.8 (10.3, 13.3) ³	6.1 (5.3, 7.0)	5.6 (3.9, 7.4) ⁴
Erythrocyte folate (nmol/L)				
Week 0	685 ± 208	737 ± 211	800 ± 278	
Week 12	636 ± 215	969 ± 254	905 ± 311	
Change at week 12 (nmol/L)		282 (246, 318)	155 (122, 188)	127 (78, 176) ⁴
Plasma homocysteine (μmol/L)				
Week 0	10.4 ± 1.7	10.4 ± 2.1	11.0 ± 2.6	
Week 2	10.5 ± 1.8	10.0 ± 1.9	10.3 ± 2.3	
Week 12	10.6 ± 1.9	9.0 ± 1.7	9.7 ± 2.0	
Change at week 12 (%) ⁵		-14.1 (-16.3, -11.9)	-12.1 (-14.8, -9.3)	2.1 (-1.5, 5.6)

¹ In week 2, values for one subject were missing (n = 58).² $\bar{x} \pm$ SD.³ \bar{x} ; 95% CI in parentheses. Values were corrected for the change in the placebo group.⁴ P < 0.001.⁵ Values are expressed as percentages to correct for the differences in baseline values and because absolute changes were not normally distributed.

not significantly different after correction for the difference in molar folic acid content in the capsules.

The bioavailability of polyglutamyl folic acid relative to monoglutamyl folic acid was not different when calculated on the basis of changes in serum folate (64%; 95% CI: 52%, 75%) or on the basis of changes in erythrocyte folate (68%; 95% CI: 51%, 84%) after 12 wk of intervention. On the basis of plasma homocysteine concentrations, the bioefficacy of polyglutamyl folic acid relative to that of monoglutamyl folic acid was 106% (95% CI: 77%, 134%) after 12 wk of intervention (**Table 3**).

DISCUSSION

In the present study we found that the bioavailability of polyglutamyl folic acid was 66% of that of the monoglutamyl form of the vitamin on the basis of both serum folate and erythrocyte folate concentrations after 12 wk of intervention. No difference

in bioefficacy between the 2 forms of the vitamin was observed on the basis of plasma homocysteine concentrations.

Many previous studies have found that the bioavailability of polyglutamyl folic acid compared with monoglutamyl folic acid is of the same order of magnitude as we found (11, 12, 16). However, a similar number of studies showed no difference between the bioavailability of polyglutamyl and monoglutamyl folic acid (10, 15, 18). Studies with protocols that involved the use of single doses encountered the problem that relatively high doses or a high preload dose were needed to gain a measurable folate response in blood. Such protocols may not be appropriate for measuring the bioavailability of folate at intakes of folate within the range normally consumed. In addition, short-term studies in which folate polyglutamates are administered may underestimate the bioavailability of such polyglutamates because there is no time for up-regulation of folylpoly γ -glutamyl carboxypeptidase activity.

The present intervention study was the first in which low doses of polyglutamyl and monoglutamyl folic acid were administered daily long term to quantify the bioavailability of polyglutamyl folic acid relative to that of monoglutamyl folic acid. Folate intake was restricted to the habitual diet and to the supplements supplied during the study. Because food fortification with folic acid is not allowed in the Netherlands and because the subjects did not use any supplements containing B vitamins, no other sources of folic acid could have interfered with the results of the study. Furthermore, the subject groups were well randomized and sufficiently large to quantify bioavailability within narrow confidence limits.

Analysis by HPLC showed that the folate content of the monoglutamyl folic acid and heptaglutamyl folic acid capsules was lower than expected (71% and 58%, respectively). Reanalysis with the use of a microbiological assay led to similar results. For the heptaglutamyl folic acid capsules, the lower than expected folate content may have been due in part to impurity of the raw

TABLE 3Bioavailability and bioefficacy of heptaglutamyl folic acid relative to those of monoglutamyl folic acid¹

	Based on 2 wk of intervention	Based on 12 wk of intervention
Bioavailability		
Serum folate	57 (30, 83)	64 (52, 75)
Erythrocyte folate	—	68 (51, 84)
Bioefficacy		
Plasma homocysteine	183 (64, 302)	106 (77, 134)

¹ \bar{x} ; 95% CI in parentheses. Values were calculated as $(\Delta[C]_{\text{poly}} - \Delta[C]_{\text{placebo}}) / (\Delta[C]_{\text{mono}} - \Delta[C]_{\text{placebo}}) \times \text{dosage}_{\text{mono}} / \text{dosage}_{\text{poly}}$, where $\Delta[C]$ is the change in serum or erythrocyte folate concentrations over time in either the group receiving monoglutamyl folic acid (mono), polyglutamyl folic acid (poly), or placebo. Likewise, the bioefficacy was calculated on the basis of the decrease in plasma homocysteine concentrations expressed as a percentage.

material because HPLC analysis showed that only 70% was polyglutamyl folic acid. Moreover, the moisture content of the preparations used was higher than expected, although this would only explain the lower folate content to a minor extent. On the basis of the pharmacy report, we had no reason to believe that the encapsulation procedure was not carried out correctly, and we were confident that analytic error was kept to a minimum. The differences in doses of monoglutamyl and polyglutamyl folic acid capsules were taken into account when the bioavailability and bioefficacy were calculated; this correction did not change the results.


On the basis of our results, we estimated the extent to which the bioavailability of dietary folate is affected by the polyglutamyl chain. We found that, relative to monoglutamyl folic acid, only 66% of polyglutamyl folic acid becomes available for use and storage in the body. In the Netherlands, about two-thirds of dietary folate is provided by polyglutamates and one-third is provided by monoglutamates, on the basis of a cross-sectional study in 1275 men and 1160 women aged 20–65 y (9). The bioavailability of folate from a mixed diet would then be calculated as $(\frac{2}{3} \times 0.66 + \frac{1}{3}) \times 100\% = 77\%$, compared with a diet in which all folate would be present in the monoglutamate form. Other factors, such as the food matrix, may attenuate bioavailability even further.

We did not give any instructions to our subjects concerning whether the capsules should be consumed with a meal. Although we cannot exclude some modulating effect of food, we do not believe that it was of major importance. First, the folic acid that we provided was not incorporated into food. Therefore, any modulating effects from the food matrix would have been negligible. Second, any modulating effects from food that may have affected the bioavailability of both monoglutamyl acid and polyglutamyl folic acid would have been the same in both intervention groups. Any specific food factors that affect the bioavailability of the polyglutamyl form only, such as inhibition of deconjugation by organic acids, may have affected the results. Third, not all subjects would have ingested the capsules with food. In summary, food effects may have occurred but only to a minor extent.

In the present study, we attempted to measure the bioefficacy of polyglutamyl folic acid relative to that of monoglutamyl folic acid on the basis of decreases in plasma homocysteine concentrations. The estimated relative bioefficacy of polyglutamyl folic acid after 12 wk of intervention was found to be 106%, although the 95% CIs were wide (Table 3). This is somewhat surprising because the mean estimate of the relative bioavailability of polyglutamyl folic acid, based on changes in the concentrations of folate in serum and erythrocytes over 12 wk, was 66%. Because folate status is an important determinant of plasma homocysteine concentrations, one would also expect a lower relative bioefficacy of polyglutamyl folic acid. It should be realized that bioefficacy probably depends on the administered dose. Recently, we reported the results of a dose-finding study in which we studied the effect of the dose of folic acid on homocysteine concentrations in subjects who were comparable with subjects in the present study and found that doses of $\geq 400 \mu\text{g}/\text{d}$ are needed to reach stable plasma homocysteine concentrations (23). At lower doses, the dose-response relation of folic acid with homocysteine is almost linear. This implies that, at high doses, estimates of bioavailability would approach 100%. It is highly unlikely, however, that the maximum decrease in homocysteine concentra-

tions was reached in our study because we used doses far lower than $400 \mu\text{g}$.

Several other factors complicated the interpretation of our bioefficacy data. First, the observed reductions in homocysteine concentrations—and hence the statistical power to detect a difference between the groups ingesting monoglutamyl and polyglutamyl folic acid—was lower than anticipated, mainly because of the lower folic acid content of the capsules. Second, we were confronted with a somewhat higher mean baseline homocysteine concentration in the polyglutamyl folic acid group than in the monoglutamyl folic acid group, despite the careful randomization at screening. Because of the phenomenon of regression to the mean—ie, when concentrations are high when initially measured, subsequent measurements are generally lower—the reduction in homocysteine concentrations in the polyglutamyl folic acid group may have been slightly exaggerated. Nevertheless, when we excluded 2 subjects from the polyglutamyl folic acid group who had high baseline homocysteine concentrations, which resulted in a mean baseline homocysteine concentration close to that of the monoglutamyl folic acid group, the estimate of bioefficacy was virtually unchanged.

In conclusion, the relative bioavailability of polyglutamyl folic acid is 66% of that of monoglutamyl folic acid on the basis of serum and erythrocyte folate concentrations. Because about two-thirds of dietary folate is in the polyglutamate form, the maximum bioavailability of folate from a mixed diet would be 77%. Thus, the best way to improve the folate status of the population is to increase the amount of monoglutamate folate in the food supply. This would best be achieved by fortifying foods with monoglutamyl folic acid. Other food-based approaches include the consumption of foods with a high monoglutamate folate content (24) and an increase in the amount of bioavailable folate in food via specific food-processing techniques (25), plant breeding, or genetic engineering. 

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