Eur J Nutr (2009) 48:365–371
DOI 10.1007/s00394-009-0022-1

ORIGINAL CONTRIBUTION

[6S]-5-Methyltetrahydrofolate enhances folate status in rats fed growing-up milk

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Received: 27 January 2009 / Accepted: 2 April 2009 / Published online: 23 April 2009

Abstract
Background and aims To investigate the efficiency of [6S]-5-methyltetrahydrofolate or Metafolin® ([6S]-5-CH_3-H_4folate) on the recovery of folate status, we conducted a depletion–repletion rat model study using a growing-up milk as the folate carrier.
Methods The effect of [6S]-5-CH_3-H_4folate was compared to that of folic acid (PGA or Pte-Glu), by feeding two groups of folate-depleted rats a diet of fortified growing-up milk containing either 1,000 μg/l (2.2655 μmol/l) of Pte-Glu or 1,041.91 μg/l (2.2655 μmol/l) of [6S]-5-CH_3-H_4folate over a 4-week period. At the end of the study, the folate concentration in plasma, erythrocytes and liver was measured to establish the folate status of the animals. The folate content was determined in the plasma and erythrocytes by a time-resolved fluoroimmunoassay method and in the liver by a HPLC method.
Results Plasma, erythrocyte and liver folate concentrations were significantly ($P < 0.001$) lower after a depletion period in rats fed the folate-deficient diet compared to rats fed a control diet. The folate form used significantly influenced the folate concentration in erythrocytes and liver, but not in plasma, after the rats' body folate reserves were replenished by consuming the fortified growing-up milk. Thus, rats fed [6S]-5-CH_3-H_4folate-fortified growing-up milk showed significantly higher folate content in erythrocytes and liver (1,100.37 ng/ml and 4.22 μg/g, respectively), than did those fed Pte-Glu-fortified growing-up milk (827.71 ng/ml and 3.04 μg/g, respectively, in erythrocytes and liver).
Conclusion We conclude that the natural diastereomer [6S]-5-CH_3-H_4folate may adequately serve as an alternative to folic acid for the folate fortification of infant foods.

Keywords [6S]-5-Methyltetrahydrofolate · Folic acid · TR-FIA assay · Growing-up milk · Weanling rats

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Introduction

Folate is a generic term for the various biochemical moieties of the B vitamin. Important sources of folate are fruits, cereals, vegetables, dairy products and liver products [17]. Folates are essential cofactors involved in the acquisition, transport and enzymatic reactions of one-carbon units and are particularly important in metabolism and metabolic regulation of amino acids and nucleic acids [2]. Low-folate status can cause megaloblastic anemia [5], can increase the risk of neural-tube defects [3] and is associated with increased risk of some cancers [15]. Furthermore, inadequate folate intake can increase plasma homocysteine concentration, which in turn is associated with increased risk for cardiovascular disease [34]. Folic acid, or pteroyl-γ-L-glutamic acid (Pte-Glu), the fully oxidized and stable form of this vitamin, is used extensively in dietary supplements and food fortification. However, in many European countries, Pte-Glu food fortification is not mandatory, and the folate intake of the population is usually lower than recommended [4]. Thus, although there is much debate over the exact folate requirements for optimal health and function, increasing the folate intake of populations to recommended levels is

desirable [20]. Folate intake can be increased by various strategies, including (1) supplementation, (2) food fortification and (3) adequate consumption of naturally folate-rich food products. Currently, Pte-Glu is used in food fortification because of its high bioavailability in humans and high stability during processing. However, Pte-Glu fortification is still under discussion, as a high intake (>1 mg per day) can mask a vitamin B_{12} deficiency. Therefore, the use of other natural folate forms is an area that requires further investigation [13].

The most abundant natural folate in human plasma is the diastereomer [6S] of 5-methyltetrahydrofolate ([6S]-5-CH_3-H_4folate). Theoretically, the use of [6S]-5-CH_3-H_4folate is less likely to mask the anemia of vitamin B_{12} deficiency, as 5-CH_3-H_4folate (but not Pte-Glu) requires the cobalamin-dependent methionine synthase reaction for conversion into tetrahydrofolate (H_4folate) [31]. In the presence of cobalamin deficiency, [6S]-5-CH_3-H_4folate is unable to stimulate erythropoiesis, because vitamin B_{12} is necessary for conversion of 5-MTHF to THF, which is the precursor of the folate forms that support hematopoiesis. Pte-Glu, on the other hand, is converted to dihydrofolate (H_2folate) and THF [29].

Milk and milk products are widely consumed worldwide. They can serve as a potential source for Pte-Glu fortification because of the presence of folate-binding proteins that seem to be involved in folate bioavailability. In addition, both Pte-Glu and 5-MTHF appear to be highly bioaccessible from the milk matrix [36]. Therefore, folate fortification of dairy products could help prevent the aforementioned defects caused by folate deficiency.

The EU Standing Committee on the Food Chain and Animal Health has recently included [6S]-5-CH_3-H_4folate in its positive list of vitamins [1]. Folate's bioavailability has been extensively studied in healthy adults in previous studies. However, there is still a lack of information concerning this new compound in terms of bioavailability with regard to infant nutrition. Infants, due to their fast growth rate, have higher requirements of essential compounds than adults. Cell division is increased during this period of life, and the synthesis of proteins and nucleic acids becomes a determining factor. Folates are involved as carbon donors in purine and pyrimidine synthesis (RNA and DNA), and they also play a role in serine and glycine metabolism, methionine biosynthesis and DNA methylation [2]. These are key compounds for adequate and healthy infant growth. Folate deficiency results in impaired cell division due to reduced purine and pyrimidine synthesis, resulting in megaloblastic anemia. Therefore, folate fortification of infant formulas and growing-up milk might be recommended.

The objective of the present study was to compare the folate status of folate-depleted weanling rats after consuming [6S]-5-CH_3-H_4folate-fortified growing-up milk versus after consuming Pte-Glu-fortified growing-up milk. Growing-up milks are partially modified follow-on formulas destined for children from 12 months onwards, serving as an intermediate step between the follow-on formula and cow's milk, and they are suitable for nutrient evaluation purposes. A depletion–repletion rat model was used, and the folate concentration in plasma, erythrocytes and liver served as indicators of the folate status.

Materials and methods

Materials

All chemicals, unless otherwise indicated, were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemical (St. Louis, MO, USA).

Depletion phase

This experiment was conducted in accordance with the guidelines approved for animal experimental procedures for the Spanish Ethical Committee of University of Murcia. Weanling male Sprague-Dawley rats (Central Animal Care, University of Murcia, Murcia, Spain; 45–55 g) were individually housed in wire-bottom stainless steel cages in an environmentally controlled room with a 12 h light/dark cycle at 21 °C. Rats were randomly divided into two groups of equal mean weight. One group of 30 rats were fed a folate-deficient diet (AIN-93G purified rodent diet containing 1% of succinylsulfathiazole) [11] for 28 days. The folate content of this diet was negligible, as any residual amount contained in the diet was under the detection limit of the HPLC method. A second group of six rats were fed the same diet, but supplemented with 1 mg of Pte-Glu/kg (control diet) [23]. Both diets were prepared by Harland Interfauna Ibérica S.L. (Barcelona, Spain). Rats were given free access to food and water, weighed weekly and examined daily for general condition and any symptoms associated with folate deficiency.

Repletion phase

In the last day of the depletion phase, six rats from the folate-supplemented group and six rats from the folate-depleted group were selected at random, fasted overnight, weighed and euthanized, as indicated below, in order to establish a baseline for biochemical values. The remaining rats from the folate-depleted group were divided into two groups of 12. Each of the groups received a growing-up milk as the sole food source, fortified with either 1,000 μg of Pte-Glu/l (2.2655 μmol Pte-Glu/l) or 1,041.91 μg of

[6S]-5-CH_3-H_4folate/l (2.2655 μmol/l) for 4 weeks. The growing-up milk, is a liquid milk designed for infants from 1–3 years old. It was kindly provided by Hero Spain, S.A, Murcia, Spain. Due to the liquid form of the milk, no preparation was necessary after opening each bottle and it was directly given to the rats. The growing-up milk comprised (g/100 g): 9.96 cow's skim milk, 86.62 water, 3.27 lactose, 0.10 vitamin mix, 0.04 mineral mix, 0.01 aroma. The initial weight of rats at the beginning of the repletion period was 175 ± 6.75 g and 178 ± 6.36 g for the groups fed with Pte-Glu and [6S]-5-CH_3-H_4folate, respectively. The folate content in the growing-up milk was periodically verified by HPLC following the procedure described below. Both forms of folate were found to be stable over the period of the study (982 ± 9.03 μg of Pte-Glu/l for the Pte-Glu-fortified growing-up milk and 1,005 ± 7.61 μg of of 5-CH_3-H_4folate/l for the [6S]-5-CH_3-H_4folate-fortified growing-up milk).

Sampling procedure

At the end of the repletion phase, the rats were fasted overnight, weighed and anaesthetized with isoflurane. Then, the rats were killed by exsanguination through the collection of blood, via direct cardiac puncture, into evacuated tubes containing sodium heparin. The tubes containing the blood samples were placed immediately on ice. Plasma was separated from erythrocytes via centrifugation at 1,000 g for 20 min at 4 °C and was transferred to 1.5 ml polypropylene tubes. In addition, the livers were removed and rinsed in ice-cold 0.9% saline solution. Plasma and liver samples were stored at −80 °C until analyzed.

Time-resolved fluoroimmunoassay method

Plasma and erythrocyte folate content was measured using the AutoDELFIA Folate time-resolved fluoroimmunoassay method (TR-FIA) (Perkin Elmer Wallac, Turku, Finland) at the Veterinary Clinical Hospital, Campus de Espinardo (Murcia, Spain). Folate was measured in plasma or whole-blood hemolysate with a competitive reaction between europium-labelled pteroylglutamic acid and the sample folate, for a limited amount of folate-binding protein. The assay involved a chemical pretreatment step to release folate from carrier proteins and to convert it into a stable, measurable form. For the erythrocyte folate measurement, whole EDTA-treated blood was hemolysed with 0.5% ascorbic acid for 90 min before analysis. After measurement, the folate concentration was divided by the hematocrit value to obtain the erythrocyte folate concentration.

Table 1 Intra and inter-assay precision of the time-resolved immunofluorometric assay for rat folate

Sample	Mean folate concentration (nmol/L)	Intra-assay CV (%) ($n = 6$)	Inter-assay CV (%) ($n = 6$)
1	102.856	2.19	9.99
2	102.286	2.15	11.93
3	2.353	10.69	16.11
4	1.570	3.15	16.41

Evaluation of the fluoroimmunoassay

Precision

The intra-assay variation was expressed as the coefficient of variation (CV%) and was determined by measuring four rat serum samples with different amounts of folate (low and high contents of folate) six times in the same analytical series. The inter-assay variation was assessed by measuring the same samples on three different days (Table 1).

Accuracy

Accuracy was evaluated by linearity under dilution. Two rat serum samples with high folate concentration were serially diluted (1:2, 1:4, 1:8, 1:16, 1:32) in DELFIA® Assay buffer. Afterward, curves representing the measured folate concentration versus the expected folate concentration were constructed (Fig. 1).

Limit of detection

The analytical limit of detection was defined as the lowest concentration to deliver a signal equal to the mean response

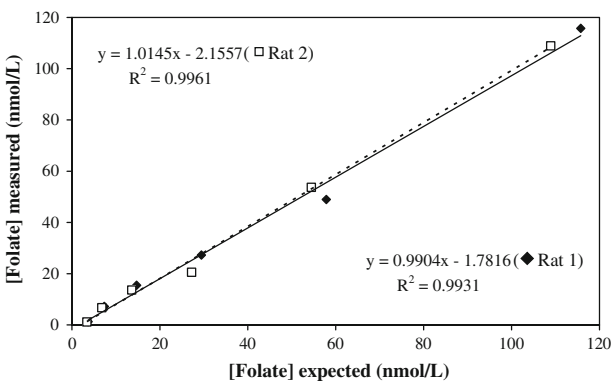

Fig. 1 Accuracy of folate concentrations assessed by dilution of serum samples with high folate content from two rats

of the zero calibrator diluent (assay buffer) plus three standard deviations. The limit of detection was 0.0428 nmol/l.

HPLC analysis

Folates from the growing-up milk, diet and liver were extracted, purified and determined following the methodology described by Konings [16], Pfeiffer et al. [26] and Vahteristo et al. [32]. Briefly, folates from samples (10 ml or 1–2 g) were heat-extracted using 50 mM Ches/Hepes extraction buffer (pH 7.85) containing 2% sodium ascorbate and 10 mM 2-mercaptoethanol under nitrogen atmosphere. Then, pH was adjusted to 4.9 with 6 M HCl (only in liver samples) and samples were made up to a final volume of 50 ml. After that, an aliquot of 5 ml was incubated for 3 h at 37 °C under nitrogen atmosphere with 1 ml of hog kidney conjugase prepared from fresh pig's kidneys, as previously described by Gregory et al. [8]. The samples were filtered through 0.45-μm pore size and 25-mm Ø nylon disposable filters (Whatman, Florham Park, NJ, USA) and purified in strong anion-exchange (SAX) cartridges (3 ml/500 mg of quaternary amine N^+, counter ion Cl^-, no. 52664-U, Bellefonte, PA, USA) connected to a Supelco 12-port vacuum manifold (Supelco, Bellefonte, PA, USA). The extraction, deconjugation and purification procedures were carried out under subdued light to prevent photodegradation of folates. Folates were determined using a HPLC Merck-Hitachi 7000 (Merck, Darmstadt, Germany) equipped with a fluorescence detector (LaChrom, Merck-Hitachi, model 7485) and a UV detector (LaChrom, Merck-Hitachi, model 7400). A LiChrosphere® 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany), protected with a guard column (LiChroCART® 4-4, Merck, Darmstadt, Germany), was used to separate the folate compounds. Gradient elution was performed with acetonitrile and 30 mM phosphate buffer pH 2.2 to separate folates at a flow of 0.9 ml/min. The gradient was started at 6% acetonitrile, which was maintained isocratically for the first 6 min, and then the acetonitrile concentration was increased to 25% over 24 min and decreased back to 6% after 5 min. The running time was 30 min, and the time between injections was 40 min. Fluorescence absorbance, at excitation and emission wavelengths of 280 and 350 nm, respectively, was used to detect and quantify the elution of H_4folate, 5-CH_3-H_4folate, [6S]-5-CH_3-H_4folate and 5-HCO-H_4folate, whereas UV absorbance at 290 nm was used to detect and quantify the elution of the synthetic form Pte-Glu. Peak identification was based on the retention time compared with standards, and spiking (addition of standard compounds into the purified sample extract) was used to confirm peaks for any samples in which identification using the retention time was inaccurate. Pte-Glu, H_4folate (calcium salt), 5-CH_3-H_4folate (sodium salt) and 5-HCO-H_4folate (sodium salt) were obtained from Dr. Schirck's Laboratories (Switzerland), while [6S]-5-CH_3-H_4folate (calcium salt), or Metafolin®, was purchased from Merck KGaA (Darmstadt, Germany).

Statistical analysis

The rats' weight, milk intake and folate concentration in plasma, erythrocyte and liver were analyzed by analysis of variance, and when significant differences were found ($P < 0.05$) within data, multiple comparisons between means were carried out using Tukey's test and Dunnett's T3 test (SPSS version 15.0; SPSS Inc., Chicago, IL, USA).

Results

Folate content during the depletion period

After the depletion period, no differences were observed in the mean body weight of the two groups of rats ($P = 0.95$; data not shown). Table 2 shows that the weight gain (86.10–102.66 g) and final body weight (259.76–283.03 g) of both groups was not significantly different ($P = 0.32$) at the end of the depletion period. In addition, it can be observed that plasma, erythrocyte and liver folate concentrations were sensitive indicators of the folate status.

At the end of the depletion period, rats fed a folate-deficient diet had significantly lower ($P < 0.001$) plasma, erythrocyte and liver folate concentrations than those fed the control diet. Plasma and erythrocyte folate concentrations of folate-depleted rats (0.91 and 369.94 ng/ml, respectively), were significantly lower ($P < 0.001$) than

Table 2 Weight gain, final weight, and plasma, erythrocyte and liver folate concentrations of rats fed either a folate-deficient diet or a control diet during a 28-day depletion period

	Folate-deficient diet		Control diet	
	Mean	SD	Mean	SD
Weight gain (g)	86.10	10.88	102.66	18.13
Final weight (g)	259.76	13.45	283.03	18.23
Plasma folate (ng/ml)	0.91***	0.17	30.85	6.50
Erythrocyte folate (ng/ml)	369.94***	67.99	751.64	80.08
Liver H_4folate (μg/g)	0.21***	0.02	1.51	0.21
Liver 5-CH_3-H_4folate (μg/g)	0.21***	0.02	1.67	0.12
Liver 5-HCO-H-H_4folate (μg/g)	ND	ND	ND	ND

Asterisks within the same row (mean values, $n = 6$) show significant differences in the folate-deficient diet group with respect to the control group

H_4folate tetrahydrofolate, *5-CH_3-H_4folate* 5-methyltetrahydrofolate, *5-HCO-H-H_4folate* 5-formyltetrahydrofolate, *ND* non detected

*** $P < 0.001$

those of the control group (30.85 and 751.64 ng/ml, respectively). Liver folate forms were also detected in significantly lower ($P < 0.001$) concentrations in rats fed the folate-deficient diet (0.21 μg/g of H_4folate and 0.21 μg/g of 5-CH_3-H_4folate), with respect to the group fed the control diet (1.51 μg/g of H_4folate and 1.67 μg/g of 5-CH_3-H_4folate). 5-HCO-H-H_4folate was not detected in the liver in either group.

Folate content during the repletion period

Table 3 summarizes the intake of folate-fortified growing-up milk and the plasma, erythrocyte and liver folate concentrations in repleted rats fed growing-up milk fortified with either Pte-Glu or [6S]-5-CH_3-H_4folate. Both groups of rats consumed a similar amount of milk (621.67–637.42 ml) during the repletion period. No significant differences ($P < 0.05$) were found in the final weight or weight gain of the rats due to the source of folate (data not shown). Mean plasma folate concentration was not significantly different ($P = 0.42$) between groups fed Pte-Glu- or [6S]-5-CH_3-H_4folate-fortified growing-up milk (36.65 and 40.58 ng/ml, respectively). However, the type of folate compound administered did affect the erythrocyte folate concentration, which was significantly ($P = 0.003$) higher when [6S]-5-CH_3-H_4folate (1,100.37 ng/ml) was added to the milk than when Pte-Glu (827.71 ng/ml) was used as a fortificant during the repletion phase. Both administered folate forms increased the rats' hepatic folate stores, but the group fed [6S]-5-CH_3-H_4folate-fortified growing-up milk (4.22 μg/g) showed significantly higher ($P < 0.001$) values than the Pte-Glu group (3.04 μg/g) for 5-CH_3-H_4folate.

Table 3 Intake of growing-up milk and plasma, erythrocyte and liver concentrations of folate in folate-depleted rats fed growing-up milk fortified with either pteroyl-γ-L-glutamic acid (Pte-Glu) or [6S]-5-methyltetrahydrofolate ([6S]-5-CH_3-H_4folate)

	Pte-Glu		[6S]-5-CH_3-H_4folate	
	Mean	SD	Mean	SD
Intake of growing-up milk (ml)	621.67	89.30	637.42	117.93
Plasma folate (ng/ml)	36.65	3.27	40.58	1.56
Erythrocyte folate (ng/ml)	827.71*	132.90	1100.37	143.87
Liver H_4folate (μg/g)	3.69	0.24	2.32	0.42
Liver 5-CH_3-H_4folate (μg/g)	3.04*	0.40	4.22	0.51
Liver 5-HCO-H-H_4folate (μg/g)	ND	ND	ND	ND

Asterisks within the same row (mean values, $n = 6$) show significant differences in the Pte-Glu-fed group with respect to the [6S]-5-CH_3-H_4folate-fed group

H_4folate tetrahydrofolate, *5-CH_3-H_4folate* 5-methyltetrahydrofolate, *5-HCO-H-H_4folate* 5-formyltetrahydrofolate, *ND* non detected

* $P < 0.05$

Based on these results, a positive correlation between erythrocyte and liver folate stores was established, for the Pte-Glu group ($r = 0.91$, $P < 0.001$) and for the [6S]-5-CH_3-H_4folate group ($r = 0.93$, $P < 0.001$). Liver 5-HCO-H-H_4folate content was not detected by HPLC in this study.

Discussion

In the present study, we evaluated the effect of [6S]-5-CH_3-H_4folate added to a growing-up milk compared with Pte-Glu in the recovery of folate status in folate-depleted weanling rats over a 4-week period. The folate status of the rats was determined by the concentration of folates in plasma, erythrocytes and liver. Studies on human babies are very limited because of ethical and methodological reasons. Therefore, in vitro tests that mimic physiological conditions of the organism under study are very useful. An alternative is in vivo studies using pup rats as an animal infant model for the evaluation of folates availability in infant formulas, because of their presumably more similar gastrointestinal physiology to that of human infants. Moreover, the high sensitivity of a folate depletion/repletion model, the rapid turnover of folates in the rat and the opportunity to quantify folate availability using body tissue concentrations in addition to plasma concentrations, make the rat a useful model in the assessment of folate bioavailability [24].

The use of HPLC allowed us to determine the different folate forms found in the liver after both the depletion and the repletion periods. While H_4folate and 5-CH_3-H_4folate were quantified in the liver samples, the form 5-HCO-H_4folate was not detected.

The rats were first fed a folate-deficient diet during 28 days to drop their folate reserves. The folate status of the rats was compared with that of a group of rats fed a control diet. The mean plasma folate concentration of folate-depleted rats in our study was significantly lower than that observed previously [12], but it was in agreement with other studies where plasma folate concentration ranged from 0.35 to 0.84 ng/ml [11]. This dissimilarity in the results could be due to the different analytical techniques employed. Previous studies used either a microbiological assay or a radioimmunoassay to determinate plasma folate content. However, we used the plasma folate assay, a solid time-resolved fluoroimmunoassay (TR-FIA) based on the competitive reaction between the europium (Eu^{3+})-labelled PGA and the folate sample. TR-FIA has been reported as an immunoassay technique with higher sensitivity than methods based on tritiated tracers or enzyme-conjugated tracers.

In other studies carried out in humans, the administration of folates is preceded by a saturation regimen of the subjects in order to minimize within-subject variation of

baseline plasma folate concentration [25, 27, 35]. In the present investigation as well as in previous studies [11, 12] a folate-depleted rat model was used to minimize inter-individual differences in baseline plasma folate concentrations before starting the treatment. Thus, all the rats start the treatment having a similar folate plasma concentration (2.07 ± 0.39 nmol/l) and the extent of recovery of the folate plasma concentration is readily observed and comparable between groups.

Regarding folate concentration in blood, it is known that folate concentration is higher in erythrocytes than in plasma and that practically all folate found in the blood is in the form of 5-MTHF [30]. The erythrocyte folate concentration found in folate-depleted rats (369.94 ± 67.99 ng/ml) did not differ from values obtained by Huang et al. [12] (275.4 ± 40.92 ng/ml). Liver 5-CH_3-H_4folate concentrations in depleted rats (0.21 ± 0.02 μg/g) were in good agreement with those reported by House et al. [11] (0.27–0.60 μg/g), despite the different techniques used. In that study, liver folate concentration was analyzed by a microbiological assay. Despite several drawbacks, this assay is the officially recognised AOAC method for analysis of folate in vitamin preparations and infant formulas (AOAC International 992.05 and 944.12) [14]. In our opinion, the microbiological assay may overestimate the total folate content as other compounds present in the samples may also stimulate the growth of the microorganism, *Lactobacillus casei* subspecies *rhamnosus*, used in the assay. In addition, other methodologies such as HPLC are able to separate and quantify the different forms of folates, which is important due to these compounds' differing degrees of bioavailability depending on their one-carbon substituents [7].

The similar plasma folate response after the ingestion of Pte-Glu or [6S]-5-CH_3-H_4folate observed in our study is consistent with findings reported by other researchers that indicate that the intestinal transport process does not show a particular preference for any of the folate forms [22]. However, 5-CH_3-H_4folate concentration in the erythrocytes and the liver was significantly ($P < 0.05$) higher in the group fed the [6S]-5-CH_3-H_4folate-fortified growing-up milk, compared with the group fed the Pte-Glu-fortified growing-up milk. Previous studies in humans also have shown that the administration of [6S]-5-CH_3-H_4folate is equally or more effective, compared with Pte-Glu, at increasing plasma folate and red-cell folate concentration in women of childbearing age [19, 33] and during lactation [10].

Although the intestinal folate-transport system has a similar avidity for Pte-Glu and 5-CH_3-H_4folate, as mentioned above, the [6S]-5-CH_3-H_4folate absorbed by enterocytes needs only to be transported to portal circulation and to pass through the liver to appear in systemic circulation. On the other hand, the Pte-Glu that reaches portal circulation must undergo reduction and one-carbon substitution in the liver, but only if unbound to folate-binding proteins [9]. Moreover, the higher concentration of 5-CH_3-H_4folate found in the erythrocytes of rats fed the [6S]-5-CH_3-H_4folate-fortified growing-up milk could be due to differences in the relative affinity for diverse forms of folate of the influx and efflux transporters of cells in the erythroid lineage, as previously reported by Houghton et al. [10]. The facts given above could explain at least in part, if not completely, the significantly higher concentration of 5-CH_3-H_4folate found in both the erythrocytes and the liver in the group of rats that consumed [6S]-5-CH_3-H_4folate compared with the group fed Pte-Glu.

Aside from differences in absorption, [6S]-5-CH_3-H_4folate has the advantage over Pte-Glu in that it is less likely to mask the anemia of vitamin B_{12} deficiency. Although this deficiency mainly occurs in the elderly [28], it has a higher incidence in infants from underdeveloped countries. It has been recently reported that 11% of infants and children from Venezuela are deficient in this nutrient [6]. Vitamin B_{12} deficiency in infancy may cause failure to thrive, megaloblastic anemia and severe neurological disorders [21]. Possible causes of vitamin B_{12} deficiency in childhood include decreased intake, abnormal absorption and defects in vitamin B_{12} transport and metabolism. Furthermore, this deficiency has been observed in exclusively breast-fed infants of strictly vegetarian mothers [18].

In summary, the present study used the depletion–repletion model to investigate folate status in rats fed two forms of folate incorporated into a growing-up milk. The results confirmed that a better folate status was shown in folate-depleted rats when [6S]-5-CH_3-H_4folate was used compared with Pte-Glu, based especially on 5-CH_3-H_4folate concentrations in the erythrocytes and the liver. [6S]-5-CH_3-H_4folate is a synthetic folate form that is chemically similar to the natural folate form 5-CH_3-H_4folate, which is the folate form that is absorbed by the jejunal mucosal cells. To the best of our knowledge, this is the first study showing a better response to [6S]-5-CH_3-H_4folate as compared to Pte-Glu when incorporated into a growing-up milk in weanling rats. The results from this study, along with the unlikelihood of [6S]-5-CH_3-H_4folate masking the hematological symptoms of vitamin B_{12} deficiency, place this synthetic compound as an adequate alternative to Pte-Glu for maintaining or increasing folate status during infancy, when necessary.

Acknowledgments The authors are grateful to Hero España S.A. (Murcia, Spain) for providing the samples used in this work and to the Ministry of Education and Science of the Spanish Government for Dr. D. Pérez-Conesa's contract (Programa Juan de la Cierva). This work was financially supported by the Ministry of Education and Science of Spain (AGL-2003-3758).

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