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Influence of folic acid-fortified foods on folate status in a folate depletion–repletion rat model

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An increasing number of foods fortified with varying levels of folic acid are appearing in the market place, targeted either at the general population or at specific consumer groups. Although it is assumed that the folate in these products should be highly bioavailable, there is a need to carry out studies to ascertain that this is, in fact, the case. The present study investigated the ability of selected folic acid-fortified foods (targeted at different types of consumer) to increase the folate status of folate-deficient rats. Forty-two weanling male rats (Wistar strain) were fed a folate-deficient diet containing 1 % succinyl sulfathiazole (w/w) for 28 d. Following depletion, seven rats were randomly assigned to each of five repletion diets containing folic acid, Complan®, Slim Fast®, Opti-Fuel2® or Cola Coa® calculated to provide 200 μg folate/kg of each diet. Calculations were based on folate information from the product labels. After a further 28 d, plasma, liver and kidney folate concentrations were determined by microbiological assay. Plasma homocysteine was measured by HPLC as a functional indicator of folate status. The folate content of the foods was measured by tri-enzyme extraction followed by microbiological assay. Our analyses suggest that there may be considerable inaccuracies on the part of the manufacturers in relation to the folate declarations on the product labels. Despite this, the four foods evaluated were highly effective in elevating plasma, liver and kidney folate and lowering plasma homocysteine concentrations in rats. These results lend support to the policy of food fortification with folic acid as a means of raising the folate status of the population, and in particular to the fortification of specific foods which may target areas of the population where increased folate status is most needed.

Folic acid: Fortified foods: Rat folate status

Food fortification has been used historically to improve the nutritional quality of much of the world's food supply, in the process virtually eliminating serious diseases such as goitre, pellagra and rickets where it has been carried out. More recently, guidelines for food fortification have evolved, with the emphasis being shifted from deficiency prevention to optimization of nutritional health. The recent research achievements regarding folic acid and its role in preventing neural tube defects provide a rare opportunity to engineer a major preventive impact on a devastating condition by means of an effective fortification programme (Willett, 1992). Additionally, there is evidence of an association between elevated plasma homocysteine concentrations and cardiovascular disease (Boushey *et al.* 1995), suggesting a possible role for folic acid in the prevention of widespread heart disease.

Despite this urgent need to increase folate intake in target populations (i.e. women of childbearing age and those at risk of developing occlusive vascular diseases), concern arises regarding the safety of individuals outside the target populations who may be at risk of consuming more than the current recommended safe upper limit of 1 mg folate/d. The major safety concern revolves around the ability of increased folic acid intake to mask the development of vitamin B_{12} deficiency by correcting the anaemia associated with the disease while allowing the related neurological complications to develop undetected. In the USA, the Food and Drug Administration overcame this quandary of balancing underconsumption in one group with overconsumption in another by mandating the fortification of a wide range of cereal grain products with 140 μg folic acid/100 g grain, fortification of ready-to-eat cereals at 100 μg folic acid/serving, and retaining the limitations for use of folic acid in supplements to 400 μg folic acid/d (Yetley & Rader, 1996). This was considered the appropriate fortification level to ensure that folate intake by the target population increases to recommended dietary levels while maintaining safe levels of intake for all

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consumers. Meanwhile, in Europe, the situation regarding fortification of the food supply with folic acid is still evolving. Current regulations allow the fortification of folic acid to virtually any food. Manufacturers lack the necessary guidelines as regards which foods are appropriate for fortification and at what levels of folic acid. The result is that an increasing number of foods fortified with varying levels of folic acid are appearing in the market place, targeted either at the general population or at specific consumer groups. Although it is assumed that the folate in these products should be highly bioavailable, there is a need to carry out studies to ascertain that this is, in fact, the case.

The objective of the present study was to investigate the ability of selected folic acid-fortified foods (targeted at different types of consumer) to increase the folate status of folate-deficient rats. Rats were depleted of folate for 28 d and subsequently repleted with folic acid or folic acid-fortified foods incorporated into the basal folate-free diet for a further 28 d. Folate status was determined by measuring plasma, liver and kidney folate and plasma homocysteine concentrations.

Materials and methods

Depletion phase

Forty-nine weanling male rats (Wistar strain) were obtained from the Biological Services Unit, University College, Cork, Republic of Ireland. Throughout the experiment, rats were housed individually in wire-bottom stainless-steel cages in an environmentally controlled room with a 12 h light – 12 h dark cycle at 21 ± 1°C. Rats were randomly divided into two groups of equal mean weight. One group of forty-two rats was depleted of folate for 28 d by being fed a folate-free diet containing 1 % succinylsulfathiazole (w/w; Walzem & Clifford, 1988). The other group of seven rats was fed the same diet supplemented with 200 μg folic acid/kg diet also containing 1 % succinylsulfathiazole (w/w). Rats were given free access to food and water, weighed weekly and examined daily for general condition and symptoms associated with folate deficiency. At the end of this 28 d depletion phase, the group of seven folate-supplemented rats and seven of the group of forty-two folate-depleted rats were fasted overnight and their body weights recorded. Rats were anaesthetized with diethyl ether and bled to death by cardiac puncture. The blood was transferred into heparinized tubes and cooled on ice, and the plasma was separated by centrifugation (1000 ***g***). The plasma was transferred to 1·5 ml polypropylene tubes and stored at −20°C. The liver and kidneys were excised, immediately frozen in liquid N_2 to preserve labile folates and subsequently stored at −20°C.

Repletion phase

Following the depletion phase the remaining thirty-five rats were fed one of five repletion diets for a further 28 d. For the first diet, folic acid (Sigma Chemical Co., Poole, Dorset) was incorporated into the basal folate-free diet at 200 μg/kg diet. For the other four diets, fortified foods (Complan® HJ Heinz (Ireland) Ltd, Blackrock, Co. Dublin, Republic of Ireland, SlimFast® GB/NI Sun Nutritional Inc., Berkshire, UK, Opti-Fuel2® Twin Laboratories Inc., Ronkonkuma, NY, USA and Cola Coa® Nutrexpa, Barcelona, Spain) were purchased locally and appropriate amounts (118, 123, 50 and 67 g/kg respectively) were added to the basal diet to adjust the folate content to 200 μg folate/kg. These amounts were derived using information on the folate content of the products taken from the product labels. Diets were identical as regards energy, protein, carbohydrate, fat and fibre content. At the end of this repletion phase rats were fasted overnight, weighed, anaesthetized and bled to death, and blood and tissue samples were collected as previously described.

Extraction of folate from tissues

Frozen liver or kidney (approximately 0·5 g) was added to nine volumes of 50 mM phosphate buffer (pH 6·1), homogenized for 1 min, autoclaved for 10 min at 121°C, mixed and cooled in an ice-bath. Chilled homogenates were centrifuged for 15 min at 2000 ***g***. The clear supernatant was transferred to plastic vials and stored at −20°C.

Deconjugation of folate extracts

The frozen extracts of liver and kidney were thawed, and 250 μl aliquots were transferred to tubes containing 4·33 ml phosphate buffer (50 mM, pH 6·1), 200 μl hog kidney conjugase preparation (Martin, 1999) plus 100 μl mercaptoethanol (2-mercaptoethanol obtained from Merck, Munich, Germany; 1 ml mercaptoethanal/10 ml phosphate buffer) and mixed. The mixture was incubated in a shaking water-bath for 6 h to allow polyglutamates to be converted to monoglutamates, and stored at −20°C until required for folate analysis.

Folate analysis of experimental foods

The folate content of the experimental foods was determined by tri-enzyme extraction (Martin *et al.* 1990), followed by microbiological assay (Scott *et al.* 1974; Wilson & Horne, 1982). Five samples of each food were purchased and five extractions were made on each sample. Separate batch numbers were purchased where possible; however, in all cases at least two different batch numbers were used. Subsequently, microbiological determination was carried out in triplicate.

Folate analysis of plasma and tissues

The folate contents of plasma and conjugase-treated liver and kidney extracts were analysed by microbiological assay (Scott *et al.* 1974; Wilson & Horne, 1982).

Homocysteine analysis of plasma

The determination of homocysteine in plasma was based on the method of Araki & Sako (1987). Homocysteine concentration was determined by HPLC using a Shimadzu LC-6A® (Shimadzu Corporation, Kyoto, Japan) system

Table 1. Weight gain, plasma, liver and kidney folate concentrations and plasma homocysteine concentrations of rats fed either a folate-deficient or a control diet during a 28 d period†

(Means and standard errors of the means for seven rats)

	Depletion phase		Repletion phase	
	Mean	SE	Mean	SE
Weight gain (g/week)	45·9	4·37	47·6	3·75
Final weight (g)	221	18·1	232	13·4
Plasma folate (ng/ml)	3·29*	0·39	11·4	1·45
Liver folate (μg/g)	0·45*	0·05	0·75	0·07
Kidney folate (μg/g)	0·29*	0·04	0·54	0·09
Plasma homocysteine (μg/ml)	26·6*	4·08	9·93	1·02

Mean values were significantly different from those at the end of the repletion phase (ANOVA): *$P < 0·05$.

† During the depletion phase the rats were fed 0 μg folate/kg diet and during the repletion phase the rats were fed 200 μg folate/kg diet. For details of diets and procedures, see p. 442.

with one pump. Samples were introduced with a Rheodyne® (Rheodyne Inc., Cotati, CA, USA) injection valve fitted with a 20 μl sample loop. Separation was carried out at ambient temperature with a Nucleosil 5C18® (Macherey-Nagel GmbH 7 Co., KG, Düren, Germany) analytical column. The mobile phase was 0·1 M acetate buffer (pH 4·0, prepared from 0·1 M acetic acid and 0·1 M sodium acetate) containing 2 % methanol (v/v). The buffer was filtered through a water-based filter, mixed with methanol, and degassed immediately before use. Fluorescence intensities of homocysteine standards and samples were measured with excitation at 385 nm and emission at 515 nm, using a Shimadzu RF-535® (Shimadzu Corporation) fluorescence spectrophotometer. The detector signal was recorded and the peak area was quantified with a Spectra-Physics SP 4290® integrator (Spectra-Physics, San Jose, CA, USA).

Statistical analysis

The folate content of the experimental foods are presented as means with their standard errors. Plasma, liver and kidney folate levels and plasma homocysteine levels were analysed by ANOVA and, where significant differences were found ($P < 0·05$) within the data, multiple comparisons between means were carried out using Duncan's new multiple range test (SPSS version 8.0; SPSS Inc., Chicago, IL, USA).

Results

During depletion, food consumption of rats fed a folate-deficient diet was similar to that of rats fed a control diet. Both groups continued to gain weight and there was no significant difference in body weights at the end of the depletion phase (Table 1). Physical signs of folate deficiency were observed during the last week of the depletion phase, i.e. lethargy and severe lesions of the skin in some of the animals.

Rats fed the folate-deficient diet had significantly lower ($P < 0·05$) plasma, liver and kidney folate concentrations at the end of the depletion phase than did controls. The mean plasma, liver and kidney folate concentrations of the folate-depleted rats were only 29, 60 and 54 % respectively of those of rats fed the control diet. Also, the mean plasma homocysteine concentrations of rats fed the folate-deficient diet were significantly higher ($P < 0·05$), by approximately 168 %, than those of rats fed the control diet at the end of the depletion phase.

Plasma, liver and kidney folate and plasma homocysteine concentrations of folate-depleted rats fed a diet containing either folic acid or selected folate-fortified foods are summarized in Table 2. Mean plasma folate concentrations of rats fed the diets containing Complan®, Slim Fast® and Cola Coa® were significantly ($P < 0·05$) higher than those of rats fed the folic acid-containing diet. Mean plasma folate concentrations of rats fed the Opti-Fuel2®-containing diet were similar to those of rats fed the folic acid-containing diet.

The mean liver folate concentrations of rats fed diets containing folate-fortified foods were similar to those of rats fed the folic acid-containing diet, with the exception of the Cola Coa®-containing diet, which yielded values significantly ($P < 0·05$) higher than those of rats fed the folic acid-containing diet.

The mean kidney folate concentrations of rats fed diets containing folate-fortified foods were again similar to those of rats fed the folic acid-containing diet, with the exception of the Complan®-containing diet, the values for which were significantly ($P < 0·05$) higher than those for rats fed the folic acid-containing diet.

The mean plasma homocysteine concentrations of rats fed the diets containing Complan®, Slim Fast® and Cola Coa® were significantly ($P < 0·05$) lower than those of rats fed the folic acid-containing diet. The mean plasma homocysteine concentrations of rats fed the Opti-Fuel2®-containing diet were similar to those of rats fed the folic

Table 2. Plasma, liver and kidney folate concentrations and plasma homocysteine concentrations of folate-depleted rats fed a diet containing folic acid or foods as sources of folate incorporated into the basal diet at 200 μg folic acid/kg diet*

(Means and standard errors of the means for seven rats)

	Folic acid		Complan®		Slim Fast®		Opti-Fuel2®		Cola Coa®	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma folate (ng/ml)	7·2^{a}	0·45	10·6^{c}	0·76	9·3^{bc}	0·49	7·9^{ab}	0·50	14·4^{d}	1·0
Liver folate (μg/g)	1·0^{a}	0·11	1·7^{ab}	0·24	1·1^{a}	0·25	1·7^{ab}	0·21	1·9^{b}	0·35
Kidney folate (μg/g)	0·34^{a}	0·04	0·56^{b}	0·06	0·35^{a}	0·05	0·31^{a}	0·05	0·42^{ab}	0·06
Plasma homocysteine (μmol/l)	25·0^{d}	0·80	8·9^{a}	0·60	14·3^{b}	2·4	22·9^{cd}	0·70	21·1^{c}	0·20

^{a,b,c,d}Mean values within a row not sharing a common superscript letter were significantly different ANOVA; ($P < 0·05$).

* For details of food sources of folate, diets and procedures, see Table 3 and p. 442.

Table 3. Folate content of experimental foods (μg folate/100 g food) fed to folate-deficient rats

(Means and standard errors of the means for twenty-five determinations i.e. five inter-batch determinations of five samples of each food)

	Declared folate content†	Actual folate content	
Food	Mean	Mean	SE
Complan‡	170	204	10.5
Slim Fast§	163	213	9.1
Opti-Fuel2‖	400	298	13.0
Cola Coa¶	300	475	19.1*

Differences between batch samples were significant (ANOVA): $^*P < 0{\cdot}05$.
† Values based on manufacturer's nutritional labelling.
‡ HJ Heinz (Ireland) Ltd, Blackrock, Co. Dublin, Republic of Ireland.
§ GB/NI Sun Nutritional Inc., Berkshire, UK.
‖ Twin Laboratories Inc., Ronkonkuma, NY, USA.
¶ Nutrexpa, Barcelona, Spain.

acid-containing diet. Rats continued to gain weight during repletion and there was no significant difference in body weights at the end of the repletion phase.

The folate-fortified foods were incorporated into the diets on the basis of their declared folate content, i.e. the folate content given on the product label by the manufacturer. However, we subsequently analysed these foods to determine actual folate content, and these results are summarized in Table 3. Complan®, Slim Fast® and Cola Coa® yielded folate concentrations of 20, 31 and 58 % respectively, higher than their declared folate contents, while Opti-Fuel2® had 26 % less folate than its declared value. Thus, the quantity of Complan®, Slim Fast®, Opti-Fuel2® and Cola Coa®, which had previously been used to adjust the folate content of the repletion diets to 200 μg folate/kg diet would have actually supplied the diets with 241, 263, 148 and 318 μg folate/kg diet respectively. There were no significant differences between batches of Complan®, Slim Fast® and Opti-Fuel2®. However, there was a significant difference ($P < 0{\cdot}05$) between two batches of Cola Coa®.

Discussion

Naturally occurring folates in foods exist predominantly in the form of polyglutamates. Prior to absorption, these polyglutamates require deconjugation to monoglutamates by the enzyme conjugase. In man and pigs, this deconjugation takes place in the jejunal brush-border membrane, while in rats, conjugase is secreted mainly in pancreatic juice. Brush border and pancreatic forms of conjugase differ markedly in physical and catalytic properties including pH optimum, and for this reason much controversy has surrounded the appropriateness of rat models for studying folate bioavailability from foods. However, the synthetic form of folic acid (that which is used for fortification) does not require prior deconjugation and since both the rat and man have identical mechanisms of absorption of monoglutamyl folates, rat models appear to be fully valid and useful for studies involving the assessment of the bioavailability of folic acid added during food fortification (Gregory, 1997). In addition, the rapid turnover of folate in rats, the high sensitivity of a depletion–repletion protocol and the potential to quantify folate availability based not only on plasma folate concentrations but also on various body tissue folate concentrations make the rat a useful model in assessing the effect of folic acid-fortified foods on folate status.

Table 4. Composition of basal folate-free diet fed to rats for 28 d to induce moderate folate deficiency prepared according to Walzem & Clifford (1988)

Ingredient	Concentration (g/100 g diet)
Casein	10
Gelatin	4
L-Methionine	0·3
Succinylsulfathiazole	1
Cellulose	5
Maize oil	10
Choline chloride	0·2
Dextrin–sucrose (2:1, w/w)	64
Vitamin mix*	0·5
Mineral mix†	5

* Supplying per kg feed: 6 mg thiamin hydrochloride, 6 mg riboflavin, 7 mg pyridoxine hydrochloride, 30 mg nicotinic acid, 16 mg calcium D-pantothenate, 10 mg β-biotin (2 %, w/w), 50 mg cyanocobalamin (0·1 %, w/w), 50 mg DL-α-tocopheryl acetate, 600 μg retinyl palmitate, 12·5 μg cholecalciferol, 50 mg menadione sodium bisulphate.
† Supplying per kg feed: 14·6 g $CaCO_3$, 162·8 mg $CaHPO_4$, 17·16 g KH_2PO_4, 12·37 g NaCl, 4·99 g $MgSO_4{\cdot}7H_20$, 263·5 mg $Fe(C_6H_5O_7){\cdot}3H_20$, 121·8 mg $CuSO_4{\cdot}5H_2O$, 182 mg $MnSO_4{\cdot}H_2O$, 63 mg $ZnSO_4{\cdot}7H_2O$, 0·25 mg KI; 1·25 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0·48 mg Na_2SeO_3, 19·25 mg $CrK(SO_4)_2{\cdot}12H_2O$.

The first phase of this experiment involved the induction of moderate folate deficiency by feeding rats a folate-free diet containing 1 % succinylsulfathiazole (w/w) (Walzem & Clifford, 1988) for 28 d. Composition of the basal folate-free diet is given in Table 4. Following this depletion phase and in agreement with previous investigators (Keagy & Oace, 1982; Babu & Lakshmaiah, 1987, Clifford *et al.* 1990*b*; Miller *et al.* 1994*a*,*b*; Martin, 1999) the response variables measured were sensitive enough to distinguish between folate-depleted and folate-replete animals. Rats fed the folate-deficient diet showed significant decreases ($P < 0{\cdot}05$) in plasma, liver and kidney folate concentrations and a significant increase in plasma homocysteine compared with those fed a control diet supplemented with 200 μg folic acid/kg diet. The mean plasma folate concentrations of the depleted rats (3·29 ng/ml) were in good agreement with the values obtained by Martinez & Roe (1977) (6·8 ng/ml) and Swiatlo *et al.* (1990) (4·5 ng/ml), although it should be noted that Swiatlo *et al.* (1990) used a 12-week depletion phase. In contrast, Clifford *et al.* (1990*a*) and Martin, (1999) reported lower mean serum folate concentrations of 0·88 and 0·68 ng/ml respectively. The mean liver folate concentrations of depleted rats (0·45 μg/g) were lower than those reported by Martinez & Roe (1977) (4·1 μg/g) and Swiatlo *et al.* (1990) (5·7 μg/g), but broadly similar to those found by Clifford *et al.* (1990*a*) (0·73 μg/g), Miller *et al.* (1994*b*) (0·23 μg/g) and Martin, (1999) (0·14 μg/g). The mean kidney folate concentrations of depleted rats (0·29 μg/g) were again similar to those found by Clifford *et al.* (1990*a*) (0·39 μg/g) and Martin, (1999) (0·23 μg/g), but were lower than those reported by Martinez & Roe (1977) (2·1 μg/g). The mean plasma homocysteine concentrations of depleted rats (26·6 μmol/l) were somewhat lower than those reported by Martin, (1999) (42·8 μmol/l) and Miller *et al.* (1994*a*) (89·9 μmol/l), but

were still significantly higher than values for the control group.

Interestingly, moderate folate deficiency induced during the depletion phase resulted in the appearance of skin lesions on many of the folate-depleted rats during the last week of depletion. This physical or pathological deficiency did not, however, affect food intake, a phenomenon also observed by previous investigators (Martin, 1999). The essential role of folate in C_1 transfers, and consequently cell division, probably explains the occurrence of lesions. Once folate repletion had begun, the skin lesions quickly disappeared.

Most previous investigators have used larger doses of folic acid in the control (folate-replete) diet. However, Martin (1999) also fed their control rats 200 μg folic acid/kg diet during a 4-week depletion phase and reported mean values for serum folate (8·03 ng/ml), liver folate (0·88 μg/g), kidney folate (0·76 μg/g) and serum homocysteine (16·87 μmol/l) concentrations which were in good agreement with the plasma folate (11·39 ng/ml), liver folate (0·75 μg/g), kidney folate (0·54 μg/g) and plasma homocysteine (9·93 μmol/l) concentrations of control rats in the present study.

The importance of these results from the depletion phase are twofold. First, moderate folate deficiency ensured prompt recovery of the rats during the repletion phase, thereby minimizing the potentially confounding effects of severe depletion. Second, despite the fact that the control group were supplemented with only 200 μg folic acid/kg diet, all four response variables (plasma folate, liver folate, kidney folate and plasma homocysteine) successfully distinguished between folate-depleted and folate-replete rats. Hence, all were considered appropriate to use as measures of even fairly subtle changes in the folate status of rats during the repletion phase.

The objective of the repletion phase was to determine the effectiveness of various folic acid-fortified foods in raising plasma, liver and kidney folate and lowering plasma homocysteine concentrations of folate-depleted rats. The foods chosen represent a small segment of currently available foods which target specific and diverse population groups: Complan® targets the elderly and also those recovering from illness, Slim Fast® targets women of childbearing age, Opti-Fuel2® targets the athlete and Cola Coa® targets children. To ensure that the folic acid-fortified foods were incorporated into the diets at levels that required fairly minimal changes to the basal folate-free diet, a fortification rate of 200 μg folic acid/kg diet was considered appropriate. However, subsequent folate analysis of the folic acid-fortified foods revealed that the actual folate concentrations of the diets containing Complan®, Slim Fast®, Opti-Fuel2® and Cola Coa® would have been 241, 263, 148 and 318 μg/kg respectively. Despite this deviation in folate content, the effectiveness of all four products as vehicles for folate fortification is clearly discernible (Table 2), and their addition to the basal folate-free diet succeeded in increasing the folate status of folate-depleted rats at least as efficiently as did the addition of folic acid itself. Although the Opti-Fuel2® diet had a lower folate content than the folic acid diet (148 μg folate/kg diet compared with 200 μg folate/kg diet), it yielded higher plasma, liver and kidney folate concentrations and a lower plasma homocysteine concentration. Despite the fact that no significant differences were seen, questions regarding differences in bioavailability arise.

Our analyses of the folate content of the fortified foods used in the experimental diets (Table 3) suggest that there may be considerable inaccuracies on the part of manufacturers in relation to the folate declarations on the product labels. The accuracy of food folate analysis is highly dependent on the merits of the preparative methods employed. Traditionally, extraction of food folates prior to microbiological assay involved simple deconjugation of polyglutamates by conjugase, thereby making folate available to *Lactobacillus casei* in the mono- and diglutamate form. However, in addition to polyglutamates, folate exists in a bound form in many natural materials; both protein and starch have been found to be powerful binders of folates in food (Yamada, 1979; Cerna & Kas, 1983). Protease treatment in combination with the traditional folate conjugase treatment was first introduced by Yamada (1979). She reported that treatment with both enzymes resulted in significantly higher ($P < 0·05$) values for the folate content of breast milk, hog liver and cod compared with those obtained using conjugase alone. Similarly, Cerna & Kas (1983) and Pedersen (1988) reported that the use of α-amylase in addition to folate conjugase was necessary to obtain proper food folate values in certain food types. More recently, several researchers have reported that tri-enzyme extraction (α-amylase, protease and folate conjugase) is essential for the accurate determination of folate in foods (De Souza & Eitenmiller, 1990; Martin *et al.* 1990; Pfeiffer *et al.* 1997; Tamura *et al.* 1997). The tri-enzyme extraction method used in the present study, and evaluated intensively by the investigators mentioned earlier, has been found to yield more complete recovery and measurement of folates than traditional methods of extraction. In addition, the foods used here were subjected to twenty-five determinations of folate content in an effort to establish reliable food folate values. In the cases of Complan®, Slim Fast® and Cola Coa®, the labels understated the folate content of the products by 20, 31 and 58 % respectively. It is unclear whether these higher values were due to manufacturers' averages or reflect the contribution of endogenous folate in addition to the folic acid added as a fortificant. In contrast, Opti-Fuel2® yielded an actual folate content 26 % lower than that stated on its label. Considering the fact that the macronutrient composition of the products was similar, food matrix cannot be considered to be responsible for the lower-than-expected folate content of the Opti-Fuel2® product. It is, therefore, more likely that the label overstated the folate content. The significance of these results for consumers is worth highlighting. On the one hand, overstating the folate content of fortified foods is misleading, particularly for women contemplating pregnancy who may choose to use fortified foods (as opposed to a folic acid supplement) to meet the recommendations for folate. Inaccurate labels could put these women at increased risk of a pregnancy complicated by neural tube defects. On the other hand, understating the folate content of fortified foods is potentially dangerous for those

individuals at risk of consuming more than the considered safe upper limit of 1 mg folate/d. While most patterns of food consumption in the USA suggest that the implementation of flour fortification with 140 μg folic acid/100 g grain is unlikely to result in daily folate intakes greater than 1 mg/d, there is some risk of overconsumption by individuals who consume large amounts of folic acid-fortified foods. A recent study by Kloeblen (1999) reported that, of 251 low-income women interviewed, 20 % consumed more than 1000 μg folate/d exclusively from fortified-grain products. In addition, when the Food and Drug Administration were proposing potential fortification food vehicles, Yetley & Rader (1996) showed that fortification of foods such as fruit juices and dairy products, in addition to cereal-grain products, with low levels of folic acid (70 μg/100 g) resulted in daily folate intakes by high consumers in many groups in excess of 1 mg. This discovery is particularly important for individuals in European and other countries where, to date, no regulations regarding folic acid fortification have been implemented.

In summary, the present study investigated the bioavailabilty of folate from fortified foods targeted at different consumer groups using a folate-depletion–repletion rat model. The four foods evaluated were highly effective in elevating plasma, liver and kidney folate and lowering plasma homocysteine concentrations in rats. These results lend support to the policy of food fortification with folic acid as a means of raising the folate status of the population, and in particular to the fortification of specific foods which may target areas of the population where increased folate status is most needed. However, considerable differences between declared and measured folate concentrations in some of these foods were observed which, were this to apply across the whole spectrum of folate-fortified foods, could have important implications for folate intake by consumers.

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