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Bioavailability and bioefficacy of folate and folic acid in man

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Folic acid is important because supplementation around the time of conception has been proven to lower the risk of having offspring with a neural-tube defect. Furthermore, both dietary folate and folic acid decrease plasma total homocysteine concentrations. Elevated plasma homocysteine concentrations are considered to be an independent risk factor for cardiovascular disease. The aim of the present review is to give an overview of factors influencing bioavailability and bioefficacy (the proportion of ingested nutrient converted to its active form) of food folate and folic acid, and to discuss the functional bioefficacy of folate and folic acid in decreasing plasma homocysteine concentrations. We use the mnemonic SLAMENGHI to group factors influencing bioavailability and bioefficacy: Species of folate; Linkage at molecular level; Amount of folate and folic acid consumed; Matrix; Effect modifiers; Nutrient status; Genetic factors; Host-related factors; mathematical Interactions between the various factors. Bioefficacy of folate from some foods is <50 % that of folic acid. This factor is most probably explained by the matrix factors, encapsulation and binding. However, often such effects cannot be distinguished from factors such as species, chain length of folate in food, effect modifiers and the amount of folate consumed in a meal. Folic acid provided as a supplement is well absorbed. However, the homocysteine-lowering capacity of doses of folic acid >500 μg is limited. It is unclear whether unmetabolised folic acid poses health risks. This factor is of importance, because food fortification is now implemented in many countries and folic acid supplements are freely available. In particular circumstances host-related factors, such as gastrointestinal illness and pH of the jejunum, can influence

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bioavailability. Genetic factors also deserve attention for future research, because polymorphisms may influence folate bioavailability.

Folate: Folic acid: Bioavailability: Bioefficacy: Homocysteine

Introduction

Folate is a B vitamin that serves as a methyl group donor in C_1 metabolism. The term folate refers to all derivatives with the biological activity of pteroylmonoglutamic acid (folic acid). Folic acid is the synthetic fully-oxidised form of pteroylglutamic acid monoglutamate; it is not present in significant quantities in nature but is synthesised commercially. In nature various reduced forms of folate with one or more glutamate moieties occur.

During the last decade folic acid has received much attention because new functions, apart from those related to the classical treatment of megaloblastic anaemia, have been discovered. Folic acid supplementation around the time of conception has been proven to make a contribution to the prevention of neural-tube defects (Medical Research Council Vitamin Study Research Group, 1991; Czeizel & Dudás, 1992). The Federal Government of the USA responded to these findings by introducing mandatory fortification of grain products with folic acid. This fortification programme stresses the importance of understanding the factors that affect the bioavailability (the proportion of the ingested amount available for metabolic processes) of folic acid added to foods.

Furthermore, dietary folate and folic acid both decrease total homocysteine levels in plasma effectively (Homocysteine Lowering Trialists' Collaboration, 1998; Brouwer *et al.* 1999). This factor is important because elevated plasma homocysteine concentrations have been identified as an independent risk factor for cardiovascular disease (Boushey *et al.* 1995; Graham *et al.* 1997). Plasma homocysteine concentrations can be regarded as a functional indicator of folate status (Jacob *et al.* 1995).

The present review examines the factors influencing bioavailability and bioefficacy of natural food folate and folic acid from fortified food products. (Bioefficacy is the proportion of the ingested nutrient converted to an active form of the nutrient; here the proportion of folate or folic acid converted to 5-methyltetrahydrofolate. Bioefficacy is a function of bioavailability and is often referred to as bioconversion.) The functional bioefficacy of folate and folic acid in decreasing plasma homocysteine levels is also discussed.

Intestinal absorption

Dietary folates are a mixture of various mono- and pteroylpolyglutamates (with two to seven glutamate moieties). Before absorption in the jejunum, dietary polyglutamyl folates must first be deconjugated by the enzyme pteroylpolyglutamate hydrolase (folate conjugase) to a monoglutamyl form.

Before the fully-oxidised monoglutamyl form of the vitamin, folic acid, enters the portal circulation through the mucosal cells of the jejunum it is reduced to tetrahydrofolate and is either methylated or formylated (Perry & Chanarin, 1973; Selhub *et al.* 1973, 1983; Strum, 1979). However, when a single dose of more than 250 μg folic acid is fed, unmetabolised folic acid has been shown to be present in serum (Kelly *et al.* 1997).

Definition of bioavailability

In pharmacokinetics, bioavailability is described as the area under the curve derived from an oral dose:the area under the curve derived from an intravenous reference dose (Rowland & Tozer, 1989). However, this definition is not applicable with respect to folate bioavailability because it assumes that clearance is independent of the route of administration. This is not the case for folates (Gregory *et al.* 1992). As a result of the reduction and either methylation or formylation that takes place in the jejunal mucosa during absorption, it is not possible to determine absolute bioavailability, but only bioavailability relative to the bioavailability of the fully-oxidised monoglutamate (folic acid; Rogers *et al.* 1997).

Within our group, the definitions of bioavailability, bioconversion and bioefficacy have developed over the years and reflect our current thinking (van Lieshout *et al*. 2001). However, these definitions do not include activity of ingested nutrients carrying out metabolic functions. Thus, we have introduced the term 'functional bioefficacy' which is the proportion of an ingested nutrient which carries out a certain metabolic function. Since plasma total homocysteine is a functional index of folate status, changes in plasma total homocysteine concentration in response to a given intake of folate or folic acid can be used as a measure of functional bioefficacy according to this definition. Changes in plasma folate or erythrocyte folate can be regarded as measurements of bioefficacy.

Factors influencing folate and folic acid bioavailability

de Pee & West (1996) published a review on dietary carotenoids and their role in combating vitamin A deficiency. They introduced the mnemonic 'SLAMANGHI' to order the factors influencing the bioavailability of carotenoids (de Pee & West, 1996), and the word was subsequently modified to SLAMENGHI (Castenmiller & West, 1998). The SLAMENGHI factors are not specific for carotenoid bioavailability, but can also be applied to the bioavailability and bioefficacy of other nutrients. In the present review we will discuss the factors influencing bioavailability and bioefficacy of folate with reference to SLAMENGHI: Species of folate; Linkage at molecular level; Amount of folate and folic acid consumed; Matrix; Effect modifiers; Nutrient status; Genetic factors; Host-related factors; mathematical Interactions between the various factors.

Species of folate

In this section the effects of different species of folate, particularly on bioefficacy, will be discussed. Folate occurs in many different forms. As discussed earlier (p. 268), folic acid (the major synthetic compound, which exists only in small amounts in nature) is the fully-oxidised monoglutamate form of the vitamin and does not have moieties that can be transferred as C_1 units. The more reduced forms of folate, dihydrofolate and tetrahydrofolate can be substituted with such moieties (Wagner, 1995). These reduced forms, e.g. 5-methyltetrahydrofolate and formyltetrahydrofolates, are much more common in nature.

The bioefficacy of oxidised and reduced folates with or without various C_1 units has been investigated in a series of intervention studies with human subjects (Table 1). Findings from these studies are not consistent. Perry & Chanarin (1970) found a greater increase in serum folate levels after ingestion of reduced folates than after ingestion of folic acid. However, urinary excretion of folic acid was higher than that of the other monoglutamyl forms of folate

Table 1. Effect of species of folate on folate bioefficacy (human intervention studies)

Authors	Design	Results	Conclusions and comments
Perry & Chanarin (1970)	Subjects: sixty-nine medical students (five groups; eleven to sixteen per group) Design: parallel Previous loading: 20 mg $PteGlu_1$/d for 3 d Treatment: Single dose of 10 μg/kg (20 μg/kg for $H_2PteGlu_1$) Folate species: $PteGlu_1$, $H_2PteGlu_1$, $H_4PteGlu_1$, 5-formyl$H_4PteGlu_1$, 5-methyl$H_4PteGlu_1$ Blood collection at 0, 1, 2 and 3 h + 6 h urine collection Measurement: plasma concentration and urinary excretion of folate	Bioefficacy relative to $PteGlu_1$ was for both measurements highest for 5-methyl$H_4PteGlu_1$, followed by 5-formyl$H_4PteGlu_1$ and lowest for $H_4PteGlu_1$. However, bioefficacy of all reduced forms was higher compared with $PteGlu_1$ when measured by 2 h change in serum folate, whereas it was lower when measured by urinary excretion	This study suggests that differences exist in bioefficacy between several monoglutamyl forms of folate. However, the short duration of the measurement period (6 h urine collection and 3 h blood collection) might not be long enough to measure true differences
Brown *et al.* (1973)	Subjects: twenty-one medical students and hospital staff volunteers (fasting) Design: cross-over, with 1 week between tests (parallel for pteroate, 5-formimino$H_4PteGlu_1$ and 5,10-methylene-$H_4PteGlu_1$; seven subjects per group) Previous loading: 10 mg $PteGlu_1$ dose orally 1 week before the first test. Thereafter, 5 mg loading doses after each test Treatment: oral administration of 0·68 μmol (300 μg) Folate species: pteroate, 5-formimino$H_4PteGlu_1$, 5,10-methylene$H_4PteGlu_1$, $H_4PteGlu_1$, $PteGlu_1$, 5-formyl$H_4PteGlu_1$, 5-methyl$H_4PteGlu_1$, 5,10-methenyl-$H_4PteGlu_1$, $H_2PteGlu_1$, 10-formyl$H_4PteGlu_1$ Blood collection after 1 and 2 h Measurement: increase in serum folate concentration	Pteroate, $H_4PteGlu_1$ and 5,10-methylene$H_4PteGlu_1$ increased by <3 ng/ml. $PteGlu_1$, 5-formyl$H_4PteGlu_1$, 5-formimino$H_4PteGlu_1$ and 5-methylH_4-$PteGlu_1$ increased by 6–9 ng/ml. 5,10-methenyl$H_4PteGlu_1$, $H_2PteGlu_1$ and 10-formyl$H_4PteGlu_1$ increased by >10 ng/ml	This study suggests that the various monoglutamyl forms of folate differ in bioefficacy. However, the short duration of the serum collection might also be due to differences in absorption time
Tamura & Stokstad (1973)	Subjects: six healthy males (fasting) Design: within-person comparison Previous loading with $PteGlu_1$: 2 × 10 mg on first day, 5 mg on second day, 2 mg on third day. Then 2 mg every other day Treatment: oral dose of 0·5 mg (equivalent to $PteGlu_1$) $H_4PteGlu_1$ and 5-methyl$H_4PteGlu_1$, a dose of 0.75 mg for 5-formyl$H_4PteGlu_1$ Folate species: $H_4PteGlu_1$, 5-methyl$H_4PteGlu_1$, 5-formyl-$H_4PteGlu_1$ Measurement: 24 h urinary excretion of folate	Folate bioefficacy compared with $PteGlu_1$: $H_4PteGlu_1$ 104·7 (range 40–136) % (*n* 6) 5-methyl$H_4PteGlu_1$ 120·8 (82–152) % (*n* 6) 5-formyl$H_4PteGlu_1$ 70·0 (64–76) % (*n* 2)	No significant difference in relative bioefficacy between $H_4PteGlu_1$, 5-formyl$H_4PteGlu_1$ and 5-methyl$H_4PteGlu_1$ compared with $PteGlu_1$. There was a wide variation in response between subjects

Pietrzik & Remer (1989)	Subjects: twelve healthy adults Design: cross-over with 1 week between tests No previous loading Treatment: single dose of 1 mg 5-formylH_4PteGlu_1 or PteGlu_1 Folate species: 5-formylH_4PteGlu_1 *v.* PteGlu_1 Measurement: AUC of serum folate concentrations (3 d)	The value of AUC for PteGlu_1:5-formyl-H_4PteGlu_1 was 1·02	This study suggests that bioefficacy of PteGlu_1 and 5-formylH_4PteGlu_1 in aqueous solution are similar
Gregory *et al.* (1992)	Subjects: seven adult males (fasting) Design: within-subject comparisons (3 week intervals) Previous saturation with 2 mg folic acid/d for 7 d Treatment: the different species were given orally in the 3′5′–$^2H_2$–labelled ($^2H_2$) form (50 % $^2H_2$, 41 % $^2H_1$, 9 % $^2H_0$), and [glu-$^2H_4$] PteGlu_1 ($^2H_4$; 88 % $^2H_4$, 12 % $^2H_2$, 0% $^2H_0$) was given intravenously as control Folate species: PteGlu_1, 10-formylH_4PteGlu_1, 5-methylH_4-PteGlu_1, 5-formylH_4PteGlu_1, H_4PteGlu_1 Measurement: urinary excretion of $^2H_2$ and $^2H_4$ for 48 h	Urinary $^2H_2$: $^2H_4$ excretion values: PteGlu_1 1·53, 10-formylH_4PteGlu_1 1·02, 5-methylH_4PteGlu_1 0·99, 5-formylH_4PteGlu_1 1·13, H_4PteGlu_1 0·71	This study indicates that bioefficacy of monoglutamyl folates differs. Bioefficacy of folic acid appears to be better than that of the reduced forms

PteGlu_1, pteroylmonoglutamate (folic acid); H_2PteGlu_1, dihydrofolate; H_4PteGlu_1, tetrahydrofolate; AUC, area under the curve.

(Perry & Chanarin, 1970). Brown *et al*. (1973) found that the bioefficacy of other monoglutamate forms was greater than that of folic acid, except that the bioefficacy of 5-formyltetrahydrofolate was similar and that of tetrahydrofolate was less (Brown *et al.* 1973). On the basis of a study using urinary excretion of orally-administered folates labelled with $^{2}H_{2}$:intravenously-administered folic acid labelled with $^{2}H_{4}$, Gregory *et al*. (1992) concluded that folic acid was more bioavailable than the reduced forms of the vitamin. Other studies have found no differences in bioefficacy between folic acid and the reduced forms (Tamura & Stokstad, 1973; Pietrzik & Remer, 1989; Bhandari & Gregory, 1992).

One problem with most studies investigating folate bioefficacy is that the variation in response between subjects can be quite substantial. Another problem is that it is not possible to determine whether these differences are caused by differences in absorption (bioavailability) or in post-absorption processes (bioconversion). In all studies, except that of Pietrzik & Remer (1989), subjects received one or more doses of folic acid for periods up to 7 d in order to saturate the tissues with folic acid.

To our knowledge there are no studies published investigating the effect of different species of folate on plasma total homocysteine concentrations, i.e. on functional bioefficacy.

Linkage at molecular level

Folate not only occurs as different species as discussed earlier, but also with more than one glutamate moiety. In this section the bioefficacy in human volunteers of folate with different numbers (one to seven) of glutamate moieties in the side chain will be discussed (Table 2).

As stated earlier, pteroylpolyglutamates are the major forms of folate in foods, and first have to be hydrolysed to monoglutamates before absorption in the small intestine can take place. A conjugase present in the jejunum is responsible for removing glutamate moieties from pteroylpolyglutamates (Reisenauer *et al.* 1977). Under normal circumstances, the activity of this folate conjugase enzyme is not rate limiting in the absorption process (Reisenauer & Halsted, 1987). This finding is in line with those from earlier studies using ^{3}H-labelled folate. The heptaglutamate is absorbed nearly as well as the monoglutamate (Rosenberg & Godwin, 1971; Godwin & Rosenberg, 1975). Two studies using 24 h urinary excretion of folate and the area under the curve of serum folate concentrations also found no significant differences in bioavailability of mono-, tri- and heptaglutamates (Tamura & Stokstad, 1973; Bailey *et al.* 1988). However, a well-designed study using labelled folates suggested that the bioavailability of hexaglutamate is less than that of the monoglutamate (Gregory *et al.* 1991). Earlier studies also suggested that less of the monoglutamate disappeared from the jejunum than the heptaglutamate (Halsted *et al.* 1975, 1978). Although the results of the studies are not unequivocal, absorption of the polyglutamates is often found to be less than that of the monoglutamate. This may imply that bioavailability of polyglutamates is less than that of monoglutamates. However, it cannot be excluded that uptake of polyglutamates takes longer, and that the net effect in the long term is similar to that of monoglutamates.

Amount of folate and folic acid

Bioavailability of folate or folic acid is likely to be influenced by the amount ingested. For absorption, there are two different transport systems. In the first transport system folates are bound to membrane-associated folate-binding proteins and transported across the brush-border

Table 2. Effect of linkage at molecular level, pteroylglutamate chain length, on folate bioavailability and bioefficacy (human intervention studies)

Authors	Design	Results	Conclusions and comments
Tamura & Stokstad (1973)	Subjects: six healthy males (fasting) Design: within-person comparison Previous loading with $PteGlu_1$: 2 × 10 mg on first day, 5 mg on second day, 2 mg on third day. Then 2 mg every other day for the duration of the study Treatment: oral dose of 0·75–2·0 mg equivalent to $PteGlu_1$ Chain length: $PteGlu_3$, $PteGlu_7$ *v.* $PteGlu_1$ Measurement: 24 h urinary excretion of folate	Folate availability compared with $PteGlu_1$: $PteGlu_3$ 85·2 (range 27–144) % (*n* 6) $PteGlu_7$ 90·4 (13–140) % (*n* 14)	There were no significant differences in relative bioavailability among $PteGlu_3$, $PteGlu_7$ and $PteGlu_1$. There was wide variation in response between the subjects
Godwin & Rosenberg, (1975)	Subjects: eleven healthy fasting volunteers (four men, seven women) Design: cross-over with 3 or 4 d interval No previous loading Treatment: oral dose of 300 ml water with either 0·6 μmol [^{3}H]$PteGlu_1$ or 0·6 μmol [^{3}H]$PteGlu_7$. After 4 h a flushing dose of 15 mg unlabelled folic acid was given Chain length: $PteGlu_1$ *v.* $PteGlu_7$ Measurement: urinary excretion of folate over 48 h	Folate urinary excretion (%): $PteGlu_1$ 70·8 (SD 13·0) $PteGlu_7$ 56·1 (SD 11·2)	This study shows that physiological doses of both $PteGlu_1$ and $PteGlu_7$ are absorbed. $PteGlu_1$ seems to be slightly better absorbed than $PteGlu_7$
Halsted *et al.* (1975)	Subjects: five healthy volunteers Design: jejunal perfusion of [^{3}H]$PteGlu_1$ and [^{14}C]$PteGlu_7$ No previous loading Treatment: equimolar solutions of [^{3}H]$PteGlu_1$ and [^{14}C]-$PteGlu_7$ were provided Chain length: $PteGlu_7$ *v.* $PteGlu_1$ Measurement: luminal disappearance	Percentage luminal disappearance: $PteGlu_1$, 74·7 $PteGlu_7$, 52·6	$PteGlu_1$ and $PteGlu_7$ were both taken up by the jejunum. Uptake of $PteGlu_1$ appeared to be higher than that of $PteGlu_7$
Halsted *et al.* (1978)	Subjects: six healthy adults, four patients with coeliac sprue Design: single 48 h experiment No previous loading Treatment: jejunal perfusion with solution containing 2 μmol/l each of [^{3}H]$PteGlu_1$ and [^{14}C]$PteGlu_7$ Chain length: $PteGlu_7$ *v.* $PteGlu_1$ Measurement: urinary isotopic recovery after jejunal folate perfusion	Urinary recovery of $PteGlu_7$ was lower than that of $PteGlu_1$	Bioavailability of the monoglutamate appeared to be greater than that of the polyglutamate

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Table 2. *Continued*

Authors	Design	Results	Conclusions and comments
Bailey *et al.* (1984)	Subjects: thirteen healthy male subjects in the age categories 20-29 years and 65-83 years Design: single 48 h experiment Treatment: jejunal perfusion with solution containing 3 μmol/l each of [^{3}H]$PteGlu_1$ and [^{14}C]$PteGlu_7$ Chain length: $PteGlu_7$ *v.* $PteGlu_1$ Measurement: urinary isotopic recovery after jejunal folate perfusion and luminal disappearance	Both urinary recovery and luminal disappearance were higher for $PteGlu_1$ than for $PteGlu_7$ in subjects in both age categories	This study suggests that bioavailability of $PteGlu_1$ is higher than that of $PteGlu_7$
Bailey *et al.* (1988)	Subjects: nine healthy males (fasting) Design: cross-over with 2 weeks interval No previous loading Treatment: single dose of 750 μg $PteGlu_1$ or equivalent amount of $PteGlu_7$ given in solution or with bran or spinach Chain length: $PteGlu_7$ *v.* $PteGlu_1$ Measurement: AUC of serum folate concentrations for 8 h	AUC of $PteGlu_7$ and $PteGlu_1$ were not different when ingested as solution or with spinach. AUC for $PteGlu_7$ ingested with bran was lower than $PteGlu_1$ ingested with bran	This study suggests no effect of chain length on bioefficacy when folates are ingested in solution, but a greater inhibitory effect of dietary fibre on bioefficacy of $PteGlu_7$ compared with that on bioefficacy of $PteGlu_1$
Keagy *et al.* (1988)	Subjects: seven healthy young men Design: all subjects received all treatments Constant loading with 500 μg folic acid/d Treatment: folate absorption tests were conducted during the last 4 d of a 9 d period in which subjects received $PteGlu_1$ and $PteGlu_7$ in a formula on alternate days Chain length: $PteGlu_1$ and $PteGlu_7$ Measurement: serum folate concentration after 1 and 2 h and urinary excretion of folate (24 h)	$PteGlu_7$ produced a lower rise in serum folate and in urinary excretion. $PteGlu_7$ excretion was 63 % of the $PteGlu_1$ excretion	This study suggests that bioefficacy of $PteGlu_7$ is either lower, or that absorption takes longer compared with $PteGlu_1$

Gregory *et al.* (1991)	Subjects: seven adult males (fasting) Design: within-person comparison (3-week interval) Previous loading: 2 mg $PteGlu_1$ for 7 d Treatment: oral dose of 677 nmol $[^2H_2]$folate followed by intravenous dose of 502 nmol $[^2H_4]PteGlu_1$ Chain length: $PteGlu_6$ *v.* $PteGlu_1$ Urine was collected for 48 h Measurement: excretion value of urinary folates ($[^2H_2]$folate dose (%):$[^2H_4]$folate dose %)	Urinary excretion values: $PteGlu_1$ 1·45 (SEM 0·10) $PteGlu_6$ 0·67 (SEM 0·04)	This study suggests that $PteGlu_6$ is less bioavailable than $PteGlu_1$
Wei *et al.* (1996)	Subjects: seven adult males (fasting) Design: cross-over with 3-week intervals Previous loading: 10 mg $PteGlu_1$ for 1 week starting 3 weeks before experiment, followed by 2 mg/d until the end of the experiment Treatment: oral dose of 677 nmol $[^2H_2]PteGlu_6$ and 677 nmol $[^2H_4]PteGlu_1$ given in combination with water, orange juice, tomato homogenate, lima bean homogenate, or with citric acid Chain length: $PteGlu_6$ *v.* $PteGlu_1$ Measurement: excretion value of urinary $[^2H_2]PteGlu_6$: $[^2H_4]PteGlu_1$ (48 h urine collection)	The $^2H_2$:$^2H_4$ excretion value for tomato, lima beans and citrate buffer was similar to that of the control (1·00 (SD 0·17)). The $^2H_2$:$^2H_4$ value for orange juice was significantly lower ($P < 0·05$) (about 66 % relative to that in the control trials)	These findings indicate that orange juice affects the absorption of ingested polyglutamate, which would imply that the effect occurs at the level of the intestinal deconjugation process by pteroylpolyglutamate hydrolase

$PteGlu_1$, pteroylmonoglutamate (folic acid); $PteGlu_6$, pteroylhexaglutamate; $PteGlu_7$, pteroylheptaglutamate; AUC, area under the curve.

membrane by a carrier-mediated mechanism. However, at high intraluminal concentration of folate (>10 μmol/l) a second non-saturable diffusion-mediated transport system plays a major role in folate absorption (Mason, 1990). The effect of the amount ingested is most likely to be of significance if the saturable transport system is saturated. At physiological concentrations (<5 μmol/l) of folate in the lumen, transport occurs mainly via the saturable transport system (Mason, 1990). A level of intake that causes saturation of this transport system is unlikely to be reached with normal intakes of natural folate from food, but could easily be reached with synthetic folic acid.

Many studies have investigated effects of the amount of synthetic folic acid (pteroylmonoglutamate) on bioefficacy (Table 3). Heseker & Schmitt (1987) showed that plasma folate concentrations reached a steady-state after 4 weeks of supplementation with 1 mg folic acid/d. Levels in erythrocytes increased over the total intervention period of 17 weeks. This pattern synchronises with the lifetime of the erythrocyte, which is known to incorporate folate only during erythropoiesis (Shane, 1995). Truswell & Kounnavong (1997) provided subjects with folic acid supplements, containing 100, 500 or 1000 μg folic acid/d, for 3 weeks in addition to the regular diet. The greatest relative increase in plasma folate was provided by the 100 μg folic acid dose, while the greatest absolute increase was established by the 1000 μg dose. The study does not make clear whether the same level of serum folate can be reached in the long term (Truswell & Kounnavong, 1997). Malinow *et al*. (1998) also showed in a cross-over study that a dose of 127 μg folic acid/d for 5 weeks was relatively more effective in raising plasma folate (30·8 %) than doses of 499 (64·8 %) and 665 (105·7 %)μg/d. This observation suggests that low doses of folic acid increase plasma folate concentrations more effectively than do higher doses. However, the effect on raising plasma folate concentrations may be slightly underestimated in the groups receiving the higher doses, because the wash-out period between the intervention periods was only 5 weeks (Malinow *et al.* 1998). This period is probably too short to avoid carry-over effects (Brouwer *et al*. 1999).

As discussed earlier, the effect of folic acid on plasma total homocysteine concentrations can be described as functional bioefficacy. Many authors have investigated the effect of different amounts of folic acid on plasma total homocysteine concentrations. In a meta-analysis (Homocysteine Lowering Trialists' Collaboration, 1998) Clarke compared most of these studies. This meta-analysis showed similar homocysteine-lowering effects for doses between 0.5 and 5 mg folic acid. Thus, it it would appear that doses of folic acid >500 μg folic acid/d have no additional homocysteine-lowering effect. In addition to the studies included in the meta-analysis, a few other studies have examined the effect of lower doses of folic acid on plasma total homocysteine concentrations. Ward *et al*. (1997) showed that 200 μg folic acid/d had a similar effect to that of 400 μg folic acid/d. However, 6 weeks of supplementation with 100 μg folic acid/d was not sufficient to reach a similar level of plasma total homocysteine (Ward *et al.* 1997). The latter study does not exclude the possibility that supplementation with 100 μg folic acid for a longer period would have resulted in lower concentrations of plasma total homocysteine. Malinow *et al*. (1998) found no significant decrease in plasma total homocysteine concentrations after 5 weeks of supplementation with 127 μg folic acid/d. However, the short wash-out period between the experimental and placebo periods makes it difficult to interpret their results.

Kelly *et al*. (1997) found unmetabolised fully-oxidised folic acid (pteroylmonoglutamate) in serum of subjects receiving >266 μg folic acid/d. They suggest that the excess of folic acid cannot be used for lowering plasma total homocysteine. This observation is in line with other studies that show no additional homocysteine-lowering effect of doses of >500 μg folic acid/d, or even >200 μg folic acid/d (Ward *et al.* 1997; Bonnette *et al.* 1998; Homocysteine Lowering

Table 3. Effect of amount of folic acid consumed on folic acid bioefficacy and functional bioefficacy (intervention studies with human subjects)

Authors	Design	Results	Conclusions and comments
Heseker & Schmitt (1987)	Subjects: six healthy subjects (four men, two women) Design: parallel No previous loading Treatment: 500 μg folic acid was administered twice daily for 17 weeks in addition to their regular diet Measurement: plasma and erythrocyte folate concentrations	Mean folate concentration in plasma reached a steady-state after about 4 weeks. This level was maintained by continuous doses of folic acid. Levels in erythrocytes increased continuously during 17 weeks	A daily dose of 1 mg was sufficient to reach a steady-state for plasma folate after 4 weeks. Erythrocytes only incorporate folate during erythropoiesis. Lifetime of the erythrocyte is approximately 120 d. Thus, it will take approximately 120 d to reach a steady-state in erythrocytes
Ward *et al.* (1997)	Subjects: thirty healthy males Design: sequential design No previous loading Treatment: folic acid was administered daily at doses increasing from 100 μg (6 weeks), to 200 μg (6 weeks), to 400 μg (14 weeks) in addition to the regular diet Measurement: fasting total plasma homocysteine, serum folate and erythrocyte folate (measured at start and end of study)	Both 100 and 200 μg folic acid/d significantly decreased total plasma homocysteine, 400 μg/d had no further decreasing effect. Serum folate increased gradually with all three doses. Erythrocyte folate increased over the study period	A dose of 200 μg folic acid/d seems as effective as 400 μg/d in lowering total plasma homocysteine. It is not clear whether 100 μg/d in the long term could be as effective as 200 μg/d
Truswell & Kounnavong (1997)	Subjects: Expt 1 *n* 13; Expt 2 *n* 16; Expt 3 *n* 6; Completed all three experiments *n* 6 Design: parallel, consecutive experiments (28 d between experiments) No previous loading Treatment: subjects received folic acid supplements (in aqueous solution) in addition to their regular diet (/d): Expt 1 100 μg for 3 weeks, then 1000 μg for 3 weeks Expt 2 500 μg for 3 weeks, then 1500 μg for 3 weeks Expt 3 1000 μg for 3 weeks, then 2000 μg for 3 weeks Measurement: response in serum folate after 2 and 3 weeks	The relative greatest increase was for the first 100 μg folic acid. Serum folates appeared to take longer to reach the highest possible level after small doses of folic acid than after doses of 1000 μg/d or more	The wash-out period between the experiments was only 4 weeks. Thus, the starting value of serum folate increased from Expt 1 to Expt 3

Continued

Table 3. *Continued*

Authors	Design	Results	Conclusions and comments
Kelly *et al.* (1997)	Subjects: Expt 1 *n* 14; Expt. 2 *n* 6 (+5); Expt 3 *n* 30, elderly; Expt 4 *n* 16, elderly Design: parallel Treatment: Expt 1 subjects received folic acid-fortified foods for 5 d (amount of folic acid ranging from 90 to 1200 μg/d) Expt 2 each subject received 400, 300 and 200 μg folic acid/d (in isotonic saline; 9 g NaCl/l), separated by 2-week intervals Expt 3 after pretreatment with 400 μg folic acid/d for 18 d, each subject (geriatric patients) was given a constant dose of 150–600 μg/d in bread for three consecutive days Expt 4 elderly patients routinely received fortified milk and cereals (172–190 μg/d). Each subject was given low-fat milk (200 ml) fortified with folic acid (200 μg/d) Measurements: fasting and postprandial (2·25 h after meal) blood samples were taken. Total folate and folic acid were determined in serum	Unchanged folic acid was found in subjects consuming more than 266 μg folic acid/d	The implication of having unchanged folic acid in serum is not clear
Caudill *et al.* (1997, 1998)	Subjects: twelve pregnant and twelve non-pregnant women No previous loading Design: parallel Treatment: women received 120 μg folate/d and either 330 or 730 μg folic acid/d for 12 weeks Measurement (1997): serum folate, erythrocyte folate and urinary 5-methylfolate excretion Measurement (1998): excretion of folate catabolites, para-aminobenzoylglutamate and acetamidobenzoylglutamate	Less excretion of folate catabolites in the group receiving 450 μg folate/d than in the group receiving 850 μg folate/d	450 μg of folate/d appeared to be used more efficiently than 850 μg/d
Homocysteine Lowering Trialists' Collaboration, (1998)	Meta-analysis of randomised trials Subjects: individual data on 1114 subjects included in twelve trials Design: meta-analysis of effects of folic acid-based supplements on total plasma homocysteine concentrations. Folic acid was provided in various doses (0·4–5 mg/d) in addition to the diet Measurement: total plasma homocysteine	There was no evidence for differences in homocysteine-lowering effects between daily doses of <1 mg (mean dose 0·5 mg), of 1–3 mg, or of >3 mg folic acid	Doses of 0.5 mg folic acid appeared to be as effective in lowering total plasma homocysteine as doses above that level. No studies investigating doses <0·4 mg folic acid were included in the meta-analysis

Malinow *et al.* (1998)	Subjects: seventy-five subjects with coronary artery disease (twenty-five per treatment) No previous loading Design: cross-over between treatment and placebo Treatment: subjects received 30 g cereal supplemented with 127, 499 or 665 μg folic acid/d for 5 weeks. Wash-out period was 5 weeks Measurement: plasma folate and total homocysteine concentrations	All doses significantly increased plasma folate levels. A dose of 499 μg and 665 μg folic acid decreased total plasma homocysteine significantly by 11 and 14 % respectively. A dose of 127 μg folic acid resulted in a non-significant decrease of 3·7 %	A wash-out period of 5 weeks is too short for total plasma homocysteine concentrations to return to baseline. It is not clear whether subjects were already taking folic acid-fortified foods before the trial started and even during the trial
Schorah *et al.* (1998)	Subjects: ninety-four healthy volunteers No previous loading Design: parallel Treatment: subjects received either unfortified cereals, or cereals fortified with 200 μg folic acid/d, or cereals fortified with 200 μg folic acid/d and other vitamins Measurement: total homocysteine, cysteine and vitamin B_{12} in plasma, serum folate and erythrocyte folate at weeks 0, 4, 8 and 24	Folic acid supplementation significantly increased serum folate (66 %) and erythrocyte folate (24 %) and decreased homocysteine (10 %). There was no additional effect of the other vitamins in the fortified cereals	A dose of 200 μg folic acid in addition to the regular diet was sufficient to significantly decrease total plasma homocysteine concentrations and increase serum and erythrocyte folate
Bonnette *et al.* (1998)	Dietary controlled trial Subjects: twelve pregnant women (weeks 14–26) and twelve non-pregnant women Design: 2 × 2 factorial No previous loading Treatment: all subjects received 120 μg food folate/d and either 330 or 730 μg supplemental folic acid/d for 12 weeks Measurements: plasma total homocysteine concentration weekly	Homocysteine concentrations of pregnant women (5·4 (SD 1·4) μmol/l) were lower than those of non-pregnant women (8·7 (SD 1·7) μmol/l). Within the groups of pregnant and non-pregnant women no significant difference between the doses was shown	No significant differences between the doses were shown. However, the power of the study was such that only differences among the groups of more than 3 μmol total homocysteine concentration in plasma could be detected

Trialists' Collaboration, 1998; Schorah *et al.* 1998). Thus, although bioefficacy of the excess folate can be high, its functional bioefficacy is low.

Matrix

Matrix effects on bioavailability involve both encapsulation and binding. Natural food folate can be encapsulated in plant cells or subcellular components. Generally for folic acid added to food, binding is more important, although in food preparation encapsulation may occur. Comparison of folate bioefficacy among different foods (Table 4) involves not only matrix effects, but also effects of molecular linkage, species and effect modifiers. However, studies comparing folate bioefficacy among foods cannot distinguish these factors.

Retief (1969) was one of the first researchers to study the effects of different foods on folate bioefficacy. His study is not included in Table 4 because it involved only one subject. Studies investigating the effect of food matrix of single foods on the bioefficacy of dietary folate have shown that folate is absorbed to some extent (Retief, 1969; Tamura & Stokstad, 1973; Babu & Srikantia, 1976; Sauberlich *et al.* 1987; Keagy *et al.* 1988). However, the bioefficacy of food folate relative to folic acid differed enormously between products (Retief, 1969; Tamura & Stokstad, 1973; Babu & Srikantia, 1976; Sauberlich *et al.* 1987; Keagy *et al.* 1988).

Few studies have investigated the bioefficacy of folate from mixed diets. Sauberlich *et al.* (1987) estimated from a strictly-controlled trial that the bioefficacy of folate in a mixed diet would be no higher than 50 %. Our group found that the bioefficacy of folate from vegetables and citrus fruit was 60–98 % relative to that of folic acid, depending on the end point chosen. The fact that the folic acid tablets were taken every other day may have overestimated the effect of food folate slightly (Brouwer *et al.* 1999). Cuskelly *et al.* (1996) provided women on average 400 μg folate/d in foods in addition to their normal diets. Since dietary intake was not strictly supervised as in controlled dietary intervention studies, and because of the small number of subjects, the power may not have been sufficient to observe a significant effect (Cuskelly *et al.* 1996). Riddell *et al.* (2000) performed a study in a non-controlled setting. They showed that intake of additional folic acid supplements and fortified cereals significantly decreased total plasma homocysteine concentrations ($P < 0.001$) and improved serum folate concentrations. Although advising subjects to increase intake of dietary folate improved their folate status and decreased total plasma homocysteine concentrations, it only significantly increased serum folate concentrations ($P < 0.001$). The study suggested that bioefficacy was less than 50 % for dietary folate compared with folic acid. As the intake of dietary folate was not controlled and the subjects were provided with a list of products high in dietary folate, they may have overestimated their intake. This approach may have led to the lack of effect. The folate-rich products on the list came from several food groups. It is likely that the bioefficacy of the folate from the products ranged from good to poor (Riddell *et al.* 2000). A controlled dietary study carried out by Appel *et al.* (2000) compared diets with a modified fat content and increased intake of fruits and vegetables. The study showed that the most pronounced effects on plasma total homocysteine concentrations were seen in the group with the highest dietary folate intake. Unfortunately, it was not possible to calculate bioefficacy of folate from this study (Appel *et al.* 2000). Thus, folate from a mixed diet might be absorbed by more than 50 %. Bioefficacy would seem to be strongly dependent on the products consumed. It is difficult to predict the proportion of folate from a mixed diet that is absorbed because folate occurs in many different food products. Bioefficacy of folate from a mixed diet may also be expected to depend on factors other than the matrix.

Table 4. Effect of the food matrix of single foods and mixed diets on the bioavailability and bioefficacy of folate and folic acid

Authors	Design	Results	Conclusions and comments
Tamura & Stokstad (1973)	Human intervention study Subjects: healthy males (*n* 6) Design: cross-over Previous loading with folic acid: 2 × 10 mg on first day, 5 mg on second day, 2 mg on third day, then 2 mg every other day Treatment: each subject received different foods i.e. orange juice, romaine lettuce, romaine-lettuce extract, egg yolk, banana, lima beans (dry and frozen), liver, brewer's yeast, brewer's yeast extract, cabbage (cooked and raw), defatted soyabean meal, wheat germ Measurement: urinary folate excretion (24 h)	Bioefficacy of food relative to folic acid (%): Food — Mean — Range Orange juice — 31 — 17–40 Romaine lettuce — 25 — 12–37 Romaine-lettuce extract — 48 — 38–52 Egg yolk — 59 — 23–129 Banana — 82 — 0–148 Lima beans (dry, cooked) — 70 — 0–138 Lima beans (frozen, cooked) — 96 — 48–181 Liver (cooked) — 50 — 22–103 Brewer's yeast — 60 — 55–67 Brewer's yeast extract — 63 — 59–69 Cabbage (cooked) — 47 — 0–127 Cabbage (raw) — 47 — 0–93 Defatted soyabean meal — 46 — 6–83 Wheat germ — 30 — 0–64	The large amounts of food, e.g. 500–700 g cabbage and lettuce may have adversely affected the bioefficacy. Bioefficacy of folate varies considerably between products. For all products the mean relative bioefficacy is lower than that for folic acid
Colman *et al.* (1975)	Human intervention study Design: parallel (intervention period on average 30 d) Subjects: women in late-stage pregnancy No previous loading Treatment groups provided with a single dose daily: Subjects received 1000 (*n* 20), 500 (*n* 27), or 300 μg folic acid in maize meal (*n* 23) or 300 μg folic acid in tablet form (*n* 34). Control subjects received no folic acid (*n* 18) Measurement: concentration of folate in erythrocytes and serum at delivery	Erythrocyte folate: The slope of the regression lines for the group receiving 500 μg folic acid in maize meal was similar to that of the group receiving 300 μg folic acid in tablet form Serum folate: The slope of the regression lines for all four intervention groups was significantly greater than that for the control group. The slopes were similar for 300 μg folic acid in maize, 300 μg folic acid in a supplement and for 500 μg folic acid in maize	This study suggests that the bioefficacy of 500 μg folic acid in maize meal was equal to that of 300 μg folic acid in a supplement
Colman *et al.* (1975)	Human intervention study Subjects: healthy adults (*n* 7) Design: cross-over Previous loading with 15 mg folic acid/d for 3 d Treatment: subjects received 1 mg folic acid in either an aqueous solution, or in maize, in bread, or rice for four consecutive days Measurement: sum of increases in serum folate 1 and 2 h after ingesting the folic acid on day 4	Relative bioefficacy (%) of folic acid in fortified products compared with folic acid in aqueous solution: Maize — 53 — (range 32–77) Rice — 58 — (range 42–84) Bread — 38 — (range 18–68)	This study suggests that folic acid provided in combination with maize, rice or bread either takes longer to be absorbed or that the bioefficacy is less than that of folic acid in solution. Folic acid was added to the products before preparation and this procedure may have resulted in some loss of folic acid

Continued

Table 4. *Continued*

Authors	Design	Results	Conclusions and comments
Margo *et al.* (1975)	Human intervention study Design: one group compared with Colman *et al.* (1975) Subjects: women in late-stage pregnancy (*n* 15) No previous loading Treatment: subjects received 900 μg folic acid/d in bread Measurement: Erythrocyte folate concentrations	The increase in erythrocyte folate concentrations was similar to the increase observed in the groups receiving 300 μg folic acid as a supplement or 500 μg folic acid in maize meal (Colman *et al.* 1975)	It is not clear whether the amount of folic acid in the bread was measured before or after processing of the bread
Babu & Srikantia (1976)	Human intervention study Subjects: healthy males (*n* 10) Design: cross-over Previous loading with 5 mg/d for 6 d, followed by 2 mg every other day + 400 μg folic acid was provided with each food Treatment: a single meal of the following foods was provided which contained (μg folate): bengal gram 282 green gram 314 tomato 300 spinach 310 banana 192–252 egg 210–350 goat liver 315 brewer's yeast 300 Measurement: 24 h urinary excretion (dose–response curve)	Bioavailability (%) as measured by dose–response curves: bengal gram 69 green gram 55 tomato 37 spinach 63 banana 46 egg 72 liver 70 yeast 10	There was considerable variation in bioavailability between the subjects
Sauberlich *et al.* (1987)	Human intervention study Subjects: healthy nonpregnant women (*n* 10) Design: parallel and consecutive No previous loading with folic acid Treatment: after a depletion period of 28 d subjects received increasing amounts of food folate or synthetic folic acid Measurement: plasma and erythrocyte folate concentrations, lymphocyte deoxyuridine suppression, neutrophil segmentation and urinary folate excretion	Plasma folate concentrations decreased 60 % during the depletion period and continued to decrease until subjects received 200 μg dietary folate/d. An amount of 300 μg dietary folate was sufficient to increase plasma folate slightly. Erythrocyte folate concentrations still continued to decrease. Dietary folates seemed to be no more than 50 % bioavailable compared with synthetic folic acid	This strictly controlled study revealed that bioefficacy of dietary folate from a mixed diet was no more than 50 % compared with that of folic acid

Keagy *et al.* (1988)	Human intervention study Subjects: healthy young men (*n* 7) Design: all subjects received all treatments Constant loading with 500 μg folic acid/d Treatment: four folate absorption tests were conducted during the last 4 d of each 9 d period in which subjects received either a formula, or wheat bran, or white beans. Folic acid and $PteGlu_7$ were given on alternate days during the absorption tests Measurement: increase in serum folate after 1 and 2 h and 24 h urine excretion	Adding wheat bran to the formula diet increased the AUC for folic acid. $PteGlu_7$ was not affected by wheat bran. Wheat bran increased the absorption of folic acid relative to $PteGlu_7$ whereas beans minimised the difference between the AUC	The apparent enhancing effect of wheat bran on folic acid bioefficacy could be due to endogenous folate in the wheat bran or to interaction of bran with inhibitors of folate absorption
Bailey *et al.* (1988)	Human intervention study Subjects: nine healthy males (fasting) Design: cross-over with 2 week interval No previous loading Treatment: single dose of 750 μg $PteGlu_1$ and equivalent amount of $PteGlu_7$ given in solution or with bran or spinach Measurement: AUC of serum folate concentrations for 8 h	AUCs of $PteGlu_7$ and $PteGlu_1$ were not different when ingested as solution or with spinach. AUC for $PteGlu_7$ ingested with bran was lower than $PteGlu_1$ ingested with bran	This study suggests that glutamate chain length has no effect on bioefficacy when folates are ingested in solution, but that dietary fibre reduces bioefficacy of $PteGlu_7$ compared with that of $PteGlu_1$
Cuskelly *et al.* (1996)	Human intervention study Design: parallel (3 months) Subjects: forty-one women (five groups) No previous loading Treatment: subjects received either folic acid supplements (400 μg/d) or folic acid-fortified food (additional 400 μg/d), or dietary folate, or dietary advice or nothing (control) for 3 months Measurement: erythrocyte folate concentrations	Folic acid supplements and foods fortified with folic acid both effectively increased erythrocyte folate concentrations, while food folate had no significant effect on erythrocyte folate concentrations	The study is based on small numbers of women in each group and the food and supplement intake was not controlled. However, intake of dietary folate was assessed by a validated dietary assessment method
Pfeiffer *et al.* (1997)	Human intervention study Design: cross-over Subjects: fourteen adults No previous loading Treatment: 1. subjects received [$^{13}C_5$]folic acid in white bread, whole-wheat bread, rice or pasta or in solution concurrently with [$^{2}H_2$]folic acid intravenously 2. subjects received [$^{13}C_5$]folic acid with or without a light breakfast Measurement: urinary excretion of [$^{13}C_5$]folate:urinary excretion of [$^{2}H_2$]folate	The bioefficacy of [$^{13}C_5$]folic acid in the fortified products appeared to be lower than that of folic acid in solution, but these differences were not significant (P =0·607). The absorption of folic acid seemed slightly lower when consumed after breakfast than without food but the difference was also not significant (P =0·085)	The bioefficacy of [$^{13}C_5$]folic acid in fortified cereal grains was high. Between-subject variation in this study was high in comparison with studies using previous loading with folic acid. The sensitivity of this protocol was such that only urinary excretion ratios <50 % of the control could be detected

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Table 4. *Continued*

Authors	Design	Results	Conclusions and comments
Brouwer *et al.* (1999)	Human intervention study Design: parallel dietary-controlled study (4 weeks) Subjects: sixty-six subjects (twenty-two per group) No previous loading Treatment: subjects received either a diet with a normal folate content plus placebo tablets daily (placebo group), or a diet with the same folate content plus 500 μg folic acid and placebo tablets every other day (folic acid group), or a diet high in folate (extra 350 μg/d; dietary folate group) Measurement: change in concentrations of folate in plasma and erythrocytes, and plasma concentrations of total homocysteine	Compared with folic acid the relative bioefficacy (%) was dependent on the end point: plasma folate 78 erythrocyte folate 98 plasma homocysteine 60	Folate from vegetables and fruit decreased total plasma homocysteine concentrations and improved the folate status. The effects are probably slightly overestimated because folic acid was provided as a supplement every other day instead of each day
Riddell *et al.* (2000)	Human intervention study Design: parallel (12 weeks) Subjects: sixty-five subjects (four groups) No previous loading Treatment: all subjects were advised to consume a fat-modified diet. Subjects in the dietary folate group were advised to increase their folate intake to about 600 μg/d, the cereal group was asked to consume 350–400 μg folic acid from fortified cereals (total folate + folic acid about 600 μg/d), the supplement group was instructed to take 450 μg folic acid/d from supplements, and the control consumed just the fat-modified diet Measurement: change in concentrations of folate in plasma and erythrocytes, and plasma concentrations of total homocysteine and vitamin B_{12}.	Relative change in total homocysteine compared with the control group (%): Folic acid supplements (actual intake 437 μg folic acid/d) –21 Folic acid fortification (actual intake 298 μg folic acid/d) –24 Folate-rich diet (418 μg increase in dietary folate) –9 Change in serum folate (nmol/l) compared with the control group: Folic acid supplements 27 Folic acid fortification 21 Folate-rich food 7	Folic acid supplements and intake of products fortified with folic acid appear to be most effective in decreasing total homocysteine and increasing serum folate levels. The subjects were free-living. Dietary intake was not controlled. Subjects completed a 4 d diet record. Subjects may have overestimated their actual intake of folate-rich products as they had a list of folate-rich products and therefore knew what to record
Appel *et al.* (2000)	Human intervention study Design: parallel, dietary controlled study (8 weeks), 3 weeks run-in Subjects: 118 subjects (three groups) No previous loading Treatment: Subjects received either a control diet, low in fruits, vegetables and dairy products, with a fat content typical of US consumption, or a diet rich in fruits and vegetables, or a combination diet rich in fruits, vegetables and low in fat content Measurement: change in total plasma homocysteine concentration	Change in total homocysteine (μmol/l): control diet +0·46 fruits and vegetables +0·21 combination diet –0·34 The change in total plasma homocysteine was inversely correlated with the change in serum folate levels	Modification of the diet can influence total plasma homocysteine concentrations. The change appeared to be influenced mainly by the intake of folate in the diet

$PteGlu^1$, pteroylmonoglutamate; $PteGlu_7$, pteroylheptaglutamate; AUC, area under the curve.

The US Federal Government introduced mandatory fortification of flour products with folic acid as from 1 January 1998. This action was taken in order to increase the folic acid intake of women in the fertile age-group, because an increased intake would be expected to lower the risk of having offspring with a neural-tube defect (US Department of Health and Human Services, Food and Drug Administration, 1996). Thus, it is important to know the bioefficacy of folic acid added as fortificant to flour, and the effect of other foods that may be eaten at the same time. It is now well established that the introduction of fortified flour products in the USA has improved the folate status of the population substantially. This improvement was shown in measurements made in middle-aged and older adults in the Framingham Offspring Study cohort (Jacques *et al*. 1999) and in California (Lawrence *et al*. 1999) since the fortification was introduced.

Several studies from a research group in Johannesburg (Colman *et al.* 1975; Margo *et al.* 1975; Colman, 1982) investigated the effects of maize meal, rice and bread on the bioefficacy of folic acid (Table 4). Bioefficacy of folic acid consumed with bread was found to be 58 (range 42–84) % of that when it was consumed with water (Colman *et al.* 1975). In contrast, wheat bran has been found to stimulate rather than inhibit the serum folate response to ingested folic acid (Keagy *et al*. 1988). The enhancing effect of bran might be caused by endogenous folate in wheat bran, but interaction of bran with folate inhibitors cannot be excluded. Bailey *et al*. (1988) also showed no inhibitory effect of bran on the absorption of folic acid, although bran decreased absorption of pteroylheptaglutamate. Pfeiffer *et al*. (1997) used a dual-label stable-isotope protocol to determine absorption of folic acid from fortified cereal-grain products. No significant differences were found between absorption of folic acid added to white bread, whole wheat bread, rice, pasta or water. The fact that the between-subject variation was high in this study may have considerably affected the interpretation of the results (Pfeiffer *et al.* 1997).

Effect modifiers

Effect modifiers are components in foods that influence nutrient bioavailability and bioefficacy. The effect of folate antagonists and other drugs will not be discussed in the present review.

Since the intestinal brush-border conjugase is Zn dependent, Zn intake and Zn status (see p. 286) can be expected to affect folate absorption. Supplementation with 3·5 or 14·5 mg Zn/d in combination with folic acid for 25 d was shown to have no effect on the concentration of folate in serum, erythrocytes and urine. This finding suggests that absorption of folic acid is not influenced by Zn intake (Kauwell *et al.* 1995). However, we are not aware of studies investigating the effect of Zn supplementation on the bioefficacy of dietary folate.

Certain components in the food may have the ability to inhibit the activity of the folate conjugase enzyme and thereby decrease the bioavailability of pteroylpolyglutamate. Tomatoes and orange juice inhibit the pteroylglutamate hydrolase (folate conjugase) activity in the human intestine (Bhandari & Gregory, 1990). Furthermore, citrate, and to a lesser extent malate and formate, have been shown to affect intestinal brush-border conjugase activity *in vitro* (Wei & Gregory, 1998). This finding suggests that organic acids affect the absorption of dietary polyglutamate folate by interfering with the intestinal deconjugation of the glutamate chain.

Alcohol could be another effect modifier. Folate deficiency is prevalent among chronic alcoholic patients whose dietary intake of minerals and vitamins is often inadequate. However, alcohol may also affect folate absorption (Halsted, 1995). In ethanol-fed pigs hydrolysis of pteroylpolyglutamates appears to be disturbed (Naughton *et al.* 1989; Reisenauer *et al.* 1989). This observation has not been confirmed in studies with human subjects, although ethanol

ingestion in five chronic alcoholic patients increased urinary excretion of folic acid (Russell *et al.* 1983). In combination with a diet deficient in folate, intake of ethanol decreased the uptake of folic acid in alcoholic subjects (Halsted *et al.* 1971). In normal non-alcoholic subjects ingestion of ethanol also decreased plasma folate concentrations (Eichner & Hillman, 1973). Thus, alcohol seems to affect folate bioefficacy.

Nutrient status

Status of the host with respect to folate, vitamin B_{12} and Zn may influence folate bioefficacy. Only a few studies have investigated the effect of folate status on folate bioavailability. Babu & Lakshmaiah (1987) showed no effect of folate deficiency on jejunal conjugase activity in rats. To our knowledge, there are no studies comparing folate bioefficacy in folate-deplete and folate replete subjects. However, the study by Bower *et al*. (1993) showed that the increase in serum folate concentration after a pteroylpolyglutamate load (4·5 mg pteroylheptaglutamate) was higher in subjects with higher serum folate levels compared with subjects with lower baseline serum folate levels. This finding could be explained by a longer circulation time of folate in serum of replete subjects, implying that in depleted subjects folate is transferred rapidly from serum to tissues (Bower *et al.* 1993).

Distribution of folate over the tissues changes during folate deficiency. Liver of folate-deficient rats contains more polyglutamates of higher chain length than do those of folate-replete rats (Cassady *et al.* 1980; Ward & Nixon, 1990; Varela-Moreiras & Selhub, 1992). Folate concentrations decrease and chain length increases in liver, spleen and kidney in folate-deficient rats, but both concentration and chain length are similar in brain of folate-deficient and folate-replete rats (Richardson *et al.* 1979). Thus, folate status affects folate distribution over the tissues, but it is not clear whether it also affects folate bioefficacy.

Vitamin B_{12} can influence folate bioefficacy, as its function is interrelated with that of folate. Methylcobalamin serves as a cofactor for methionine synthase, the enzyme responsible for the remethylation of homocysteine into methionine. In the same reaction 5-methyltetrahydrofolate is demethylated to provide tetrahydrofolate (Savage & Lindenbaum, 1995). In cobalamin deficiency, 5-methyltetrahydrofolate cannot be converted to tetrahydrofolate. This lack of formation of tetrahydrofolate, referred to as the 'methyl folate trap', has consequences for the formation of other folate coenzymes (Herbert & Zalusky, 1962). Although this theory has been criticised and many variations of the theory have been put forward (Savage & Lindenbaum, 1995), all these variations suggest that vitamin B_{12} deficiency influences folate bioefficacy because it changes the distribution of the various folate forms. This process also influences the overall folate status, because tetrahydrofolate is a much better substrate than 5-methyltetrahydrofolate for the enzyme folate polyglutamate synthetase. This enzyme is required for the synthesis of polyglutamates (Cichowicz & Shane, 1987*a*,*b*). Polyglutamyl folates are retained better in cells and are more effective coenzymes than are monoglutamyl folates (Lowe *et al.* 1993).

Adequate Zn status is known to be important in folate bioefficacy. Tamura *et al*. (1978) showed that Zn depletion reduced the increase in serum folate concentration after supplementation with pteroylheptaglutamate by 53 %, while absorption of the monoglutamate form seemed to be unaffected (Tamura *et al.* 1978). This finding suggested that intestinal pteroylpolyglutamate hydrolase is Zn dependent and that Zn depletion inhibits hydrolysis of polyglutamates. Chandler *et al*. (1986) confirmed the Zn dependency of the brush-border folate hydrolase. Tamura (1995) reviewed the literature concerning the nutrient interaction of folate and Zn. He

concluded that although folate conjugase is Zn dependent its clinical significance is not clear (Tamura, 1995).

Genetic factors

Some genetic mutations are known to influence folate metabolism. This section will discuss some commonly-occurring genetic factors influencing folate bioavailability and bioefficacy.

In mice expression of the reduced folate carrier *RFC-1* gene regulates the pH-dependent folate absorption in the small intestine (Chiao *et al.* 1997). The organisation and structure of the human *RFC-1* gene encoding for a folate transporter has also been determined (Tolner *et al.* 1998). However, the significance of this gene for folate absorption needs further investigation.

Another gene that is linked to folate status is the gene encoding for methylenetetrahydrofolate reductase. A variant of methylenetetrahydrofolate reductase was found to have lower specific activity and higher sensitivity to heat (Kang *et al.* 1988). This thermolabile variant is caused by an alanine-to-valine missense mutation (Goyette *et al.* 1994). Jacques *et al.* (1996) demonstrated that individuals homozygous for this mutation with plasma folate concentrations <15·4 nmol/l had 24 % higher fasting plasma total homocysteine concentrations than individuals with the normal genotype and similar plasma folate concentrations. No difference between genotypes was seen among individuals with plasma folate concentrations ⩾15·4 nmol/l. They suggested that individuals homozygous for this polymorphism need more folate to regulate their plasma homocysteine concentrations (Jacques *et al.* 1996). This observation implies that the functional bioefficacy of folate is diminished by this polymorphism when folate status is not optimal. However, high intakes of folate or of folic acid would seem to be able to overcome the negative effects of the polymorphism.

Methionine synthase is the enzyme involved in the remethylation reaction from homocysteine to methionine. To our knowledge, no polymorphisms in the gene encoding for this enzyme have been shown to influence folate status or functional bioefficacy.

Host-related factors

Host-related factors are factors of the host other than nutrient status and genetic factors that could influence bioavailability or bioefficacy. Examples of such factors are age, pregnancy, illness and malabsorption.

Bailey *et al.* (1984) investigated the absorption of pteroylpolyglutamates and pteroylmonoglutamates in different age-groups. They found that neither absorption nor activity of folate conjugase was affected by age.

Pregnancy increases the demand for folate. This higher demand may be explained by accelerated folate breakdown (Kownacki Brown *et al.* 1993; McPartlin *et al.* 1993). However, Caudill *et al.* (1997) found no differences between pregnant and non-pregnant women with respect to increase in serum folate or erythrocyte folate concentrations or in urinary excretion of 5-methyltetrahydrofolate after supplementation with 450 and 850 μg folate/d. Although the same research group suggested, from results of a controlled dietary trial, that pregnant women made more efficient use of 450 μg folic acid than of 850 μg folic acid, they found no significant difference in catabolism between pregnant and non-pregnant women (Caudill *et al.* 1998). Thus, it is not clear what causes the higher demand for folate during pregnancy.

Two randomised trials have shown that folic acid supplementation in the periconceptional

period reduces the risk of having offspring with neural-tube defects (Medical Research Council Vitamin Study Research Group, 1991; Czeizel & Dudás, 1992). Decreased capacity to absorb folate by the mother has been suggested as a cause for the folate-related cases of neural-tube defects. However, Bower *et al*. (1993) showed that intestinal hydrolysis of pteroylpolyglutamates was not impaired in women who had previously had a child with a neural-tube defect. Moreover, Davis *et al*. (1995) found no difference in the absorption of folic acid between those mothers with and those without a history of bearing a child with a neural-tube defect (Davis *et al*. 1995). In contrast, Neuhouser *et al*. (1998) found that women who had previously given birth to a child with a neural-tube defect required a larger dose of folic acid or folate to elicit a plasma response equivalent to that of the general population. Thus, diminished maternal bioavailability of folate may lead to neural-tube defects in their offspring.

Halsted (1990) summarised studies from his group investigating the effect of gastrointestinal diseases on the absorption of ^{3}H-labelled folate and ^{14}C-labelled pteroylheptaglutamate. Absorption of folate and pteroylheptaglutamate was not affected by ulcerative colitis, but was diminished by tropical and coeliac sprue (Halsted, 1990). The saturable folate transport system in the jejunum, and thus folate bioavailability, is pH dependent, with an acidic pH optimum (Halsted, 1979; Mason, 1990).

Mathematical interactions

Mathematical interactions arise when the combined effect of two or more factors is different from that of the sum of separate effects of the factors. To our knowledge there are no reports in which this complicated problem has been addressed.

Conclusions

Various factors can influence bioavailability and bioefficacy of nutrients. Of the factors influencing bioavailability and bioefficacy of folate and folic acid, two stand out: the effect of the food matrix and the amount of folic acid consumed. Bioavailability of folate from some foods is less than 50 % that of folic acid. The most likely explanation for this difference would be matrix factors: encapsulation and binding. However, often matrix effects cannot be distinguished from other factors, such as the form and chain length of folate in food. Food folate can be substituted with various C_1 groups and with one to seven glutamate moieties. Although some studies suggest that C_1 substitution of folate affects bioavailability, this effect seems to be only a minor factor. There is evidence that chain length affects bioavailability; studies in the present review suggest that polyglutamates are less bioavailable than monoglutamates. However, we think that differences in chain length can explain at most half the difference in bioefficacy between food folate and folic acid. Bioavailability and bioefficacy might also be influenced by other factors in food, such as organic acids. Indeed, organic acids have been shown in *in vitro* studies to inhibit the conjugase responsible for the removal of glutamate residues from polyglutamates to provide monoglutamates. Such a role for organic acids in decreasing the bioavailability of folate needs to be confirmed in *in vivo* studies. On the basis of the studies in the present review we conclude that matrix is the main factor influencing bioavailability and bioefficacy.

The amount of folic acid consumed also appears to be a very important factor. The bioavailability of folic acid provided in supplements is good. However, the homocysteine-

lowering capacity (functional bioefficacy) of doses of folic acid >500 μg is limited, and it is not clear whether unmetabolised folic acid poses health risks. This factor is important, because now food fortification is implemented in many countries and folic acid supplements are freely available.

In particular circumstances host-related factors, such as illness and pH of the jejunum can influence bioavailability and bioefficacy. Genetic factors also deserve our attention in future research. Mutations of certain genes may influence folate bioavailability and bioefficacy. In this respect, we should not only search for mutations, but also investigate the clinical implication and possible therapies to overcome the negative impact of such mutations.

The techniques presently available for measuring bioavailability and bioefficacy make quantification of the effect of the various factors very difficult. The accuracy of most techniques depends on reaching a steady-state situation in the body. To establish such a state, most studies have used a folic acid preloading scheme to saturate tissues with folic acid. Saturation of the tissues reduces the intra-individual variation in response to treatment. However, it is not clear how this factor affects bioavailability and bioefficacy. Thus, further development of techniques such as stable-isotope techniques is needed. A major disadvantage of stable-isotope techniques is the availability, and hence the price, of a range of labelled compounds. Thus, it is difficult to obtain sufficient amounts of these compounds for experiments with sufficient subjects and/or of sufficient duration. This factor explains why no such intervention studies with appropriately large intervention groups have been carried out up until now. Stable-isotope studies could be improved by developing more sensitive methods for measuring isotopic enrichment of folate in plasma. This factor would enable studies to be carried out with limited perturbation of the steady-state and at lower cost. In conclusion, food matrix and the amount of folic acid consumed are the major factors influencing bioavailability and bioefficacy in healthy individuals. Food manufacturers can play an important role in increasing the bioavailability. Development of new methods of food preparation could modify the food matrix in such a way that folate will become more bioavailable. It is clear that processing and storage of foods can have negative effects on the amount of folate in food (Witthöft *et al*. 1999). Thus, future research should also focus on improving storage and processing techniques, so that more folate will be retained in the food until consumption. Optimum techniques for processing food at the household level to retain folate and to increase bioavailability should also be determined. Better bioavailability of food folate would make it easier for individuals to reach adequate folate status. Many individuals do not consume sufficient folate (Brussaard *et al*. 1995). Folate status can also be improved by increasing intake of folic acid. This increase could be achieved by consumption of foods fortified with folic acid and by using folic acid supplements. However, intakes of folic acid >500 μg/d seem to have no additional functional bioefficacy, at least in healthy individuals without a genetic polymorphism that influences folate bioefficacy. Moreover, it is unclear whether such doses pose health risks. Thus, high intakes of folic acid by the general population should be avoided.

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References

Appel LJ, Miller ER III, Jee SH, Stolzenberg-Solomon R, Lin P-H, Erlinger T, Nadeau MR & Selhub J (2000) Effect of dietary patterns on serum homocysteine. Results of a randomized, controlled feeding study. *Circulation* **102**, 852–857.

Babu S & Lakshmaiah N (1987) Availability of food folate by liver folate repletion in rats. *Nutrition Reports International* **35**, 831–836.

Babu S & Srikantia SG (1976) Availability of folates from some foods. *American Journal of Clinical Nutrition* **29**, 376–379.

Bailey LB, Barton LE, Hillier SE & Cerda JJ (1988) Bioavailability of mono and polyglutamyl folate in human subjects. *Nutrition Reports International* **38**, 509–518.

Bailey LB, Cerda JJ, Bloch BS, Busby MJ, Vargas L, Chandler CJ & Halsted CH (1984) Effect of age on poly- and monoglutamyl folacin absorption in human subjects. *Journal of Nutrition* **114**, 1770–1776.

Bhandari SD & Gregory JF (1990) Inhibition by selected food components of human and porcine intestinal pteroylpolyglutamate hydrolase activity. *American Journal of Clinical Nutrition* **51**, 87–94.

Bhandari SD & Gregory JF (1992) Folic acid, 5-methyl-tetrahydrofolate and 5-formyl-tetrahydrofolate exhibit equivalent intestinal absorption, metabolism and in vivo kinetics in rats. *Journal of Nutrition* **122**, 1847–1854.

Bonnette RE, Caudill MA, Boddie AM, Hutson AD, Kauwell GPA & Bailey LB (1998) Plasma homocyst(e)ine concentrations in pregnant and nonpregnant women with controlled folate intake. *Obstetrics and Gynecology* **92**, 167–170.

Boushey CJ, Beresford SA, Omenn, GS & Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *Journal of the American Medical Association* **274**, 1049–1057.

Bower C, Stanley FJ, Croft M, de Klerk N, Davis RE & Nicol DJ (1993) Absorption of pteroylpolyglutamates in mothers of infants with neural-tube defects. *British Journal of Nutrition* **69**, 827–834.

Brouwer IA, van Dusseldorp M, Thomas CMG, Duran M, Hautvast JGAJ, Eskes TKAB & Steegers Theunissen RPM (1999) Low-dose folic acid supplementation decreases plasma homocysteine: a randomized trial. *American Journal of Clinical Nutrition* **69**, 99–104.

Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CMG, Duran M, van het Hof KH, Eskes TKAB, Hautvast JGAJ & Steegers Theunissen RPM (1999) Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled study. *Journal of Nutrition* **129**, 1135–1139.

Brown JP, Scott JM, Foster FG & Weir DG (1973) Ingestion and absorption of naturally occurring pteroylmonoglutamates (folates) in man. *Gastroenterology* **64**, 223–232.

Brussaard JH, van der Berg H, Brants HAM, van Loon CJAM & Löwik MRH (1995) *Folate Intake and Status among Adults in The Netherlands (Dutch Nutrition Surveillance System). Descriptive Statistics*. Zeist, The Netherlands: TNO Nutrition.

Cassady IA, Budge MM, Healy MJ & Nixon PF (1980) An inverse relationship of rat liver folate polyglutamate chain length to nutritional folate sufficiency. *Biochimica et Biophysica Acta* **633**, 258–268.

Castenmiller JJM & West CE (1998) Bioavailability and conversion of carotenoids. *Annual Review of Nutrition* **18**, 19–38.

Caudill MA, Cruz AC, Gregory JF, Hutson AD & Bailey LB (1997) Folate status response to controlled folate intake in pregnant women. *Journal of Nutrition* **127**, 2363–2370.

Caudill MA, Gregory JF, Hutson AD & Bailey LB (1998) Folate catabolism in pregnant and nonpregnant women with controlled folate intakes. *Journal of Nutrition* **128**, 204–208.

Chandler CJ, Wang XL & Halsted CH (1986) Pteroylpolyglutamate hydrolase from human jejunal brush borders; purification and characterization. *Journal of Biological Chemistry* **261**, 928–933.

Chiao JH, Roy K, Tolner B, Yang C-H & Sirotnak FM (1997) *RFC-1* gene expression regulates folate absorption in mouse small intestine. *Journal of Biological Chemistry* **272**, 11165–11170.

Cichowicz DJ & Shane B (1987*a*) Mammalian folylpoly-gamma-glutamate synthetase. 1. Purification and general properties of the hog liver enzyme. *Biochemistry* **26**, 504–512.

Cichowicz DJ & Shane B (1987*b*). Mammalian folylpoly-gamma-glutamate synthetase. 2. Substrate specificity and kinetic properties. *Biochemistry* **26**, 513–521.

Colman N (1982) Addition of folic acid to staple foods as a selective nutrition intervention strategy. *Nutrition Reviews* **40**, 225–233.

Colman N, Green R & Metz J (1975) Prevention of folate deficiency by food fortification. II. Absorption of folic acid from fortified staple foods. *American Journal of Clinical Nutrition* **28**, 459–464.

Cuskelly GJ, McNulty H & Scott JM (1996) Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. *Lancet* **347**, 657–659.

Czeizel AE & Dudás I (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine* **327**, 1832–1835.

Davis BA, Bailey LB, Gregory JF, Toth JP, Dean J & Stevenson RE (1995) Folic acid absorption in women with a history of pregnancy with neural tube defect. *American Journal of Clinical Nutrition* **62**, 782–784.

de Pee S & West CE (1996) Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. *European Journal of Clinical Nutrition* **50**, Suppl 3, S38–S53.

Eichner ER & Hillman RS (1973) Effect of alcohol on serum folate level. *Journal of Clinical Investigation* **52**, 584–591.
Godwin HA & Rosenberg IH (1975) Comparative studies of the intestinal absorption of [3H]pteroylmonoglutamate and [3H]pteroylheptaglutamate in man. *Gastroenterology* **69**, 364–373.
Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG & Rozen R (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nature Genetics* **7**, 195–200, 551.
Graham IM, Daly LE, Refsum HM *et al*. (1997) Plasma homocysteine as a risk factor for cardiovascular disease. *Journal of the American Medical Association* **277**, 1775–1781.
Gregory JF, Bhandari SD, Bailey LB, Toth JP, Baumgartner TG & Cerda, JJ (1991) Relative bioavailability of deuterium-labeled monoglutamyl and hexaglutamyl folates in human subjects. *American Journal of Clinical Nutrition* **53**, 736–740.
Gregory JF, Bhandari SD, Bailey LB, Toth JP, Baumgartner TG & Cerda, JJ (1992). Relative bioavailability of deuterium-labeled monoglutamyl tetrahydrofolates and folic acid in human subjects. *American Journal of Clinical Nutrition* **55**, 1147–1153.
Halsted CH (1979) The intestinal absorption of folates. *American Journal of Clinical Nutrition* **32**, 846–855.
Halsted CH (1990) Intestinal absorption of dietary folates. In *Folic Acid Metabolism in Health and Disease*, 1st ed., pp. 23–45 [MF Picciano, ELR Stokstad and JF Gregory III, editors]. New York: Wiley-Liss.
Halsted CH (1995) Alcohol and folate interactions: Clinical implications. In *Folate in Health and Disease*, pp. 313–327 [LB Bailey, editor]. New York: Marcel Dekker.
Halsted CH, Baugh CM & Butterworth CE (1975) Jejunal perfusion of simple and conjugated folates in man. *Gastroenterology* **68**, 261–269.
Halsted CH, Reisenauer AM, Shane B & Tamura T (1978) Availability of monoglutamyl and polyglutamyl folates in normal subjects and in patients with coeliac sprue. *Gut* **19**, 886–891.
Halsted CH, Robles EA & Mezey E (1971) Decreased jejunal uptake of labeled folic acid (3H-PGA) in alcoholic patients: roles of alcohol and malnutrition. *New England Journal of Medicine* **285**, 701–706.
Herbert V & Zalusky R (1962) Interrelations of vitamin B12 and folic acid metabolism: folic acid clearance studies. *Journal of Clinical Investigation* **41**, 1263–1276.
Heseker H & Schmitt G (1987) Effect of long-term supplementation of folate on folate status in plasma and erythrocytes. *Journal of Nutritional Sciences and Vitaminology* **33**, 163–168.
Homocysteine Lowering Trialists' Collaboration (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *British Medical Journal* **316**, 894–898.
Jacob RA, Pianalto FS, Henning SM, Zhang JZ & Swendseid ME (1995) In vivo methylation capacity is not impaired in healthy men during short-term dietary folate and methyl group restriction. *Journal of Nutrition* **125**, 1495–1502.
Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J & Rozen R (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* **93**, 7–9.
Jacques PF, Selhub J, Bostom AG, Wilson PWF & Rosenberg IH (1999) The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *New England Journal of Medicine* **340**, 1449–1454.
Kang S-S, Zhou J, Wong PWK, Kowalysin J & Strokosch G (1988) Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *American Journal of Human Genetics* **43**, 414–421.
Kauwell GPA, Bailey LB, Gregory JF, Bowling DW & Cousins RJ (1995) Zinc status is not adversely affected by folic acid supplementation and zinc does not impair folate utilization in human subjects. *Journal of Nutrition* **125**, 66–72.
Keagy PM, Shane B & Oace SM (1988) Folate bioavailability in humans: effects of wheat bran and beans. *American Journal of Clinical Nutrition* **47**, 80–88.
Kelly P, McPartlin JM, Goggins M, Weir DG & Scott JM (1997) Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. *American Journal of Clinical Nutrition* **65**, 1790–1795.
Kownacki Brown PA, Wang C, Bailey LB, Toth JP & Gregory JF (1993) Urinary excretion of deuterium-labeled folate and the metabolite *p*-aminobenzoylglutamate in humans. *Journal of Nutrition* **123**, 1101–1108.
Lawrence JM, Petiti DB, Watkins M & Umekubo MA (1999) Trends in serum folate after food fortification. *Lancet* **354**, 915–916.
Lowe KE, Osborne CB, Lin BF, Kim JS, Hsu JC & Shane B (1993) Regulation of folate and one-carbon metabolism in mammalian cells. II. Effect of folylpoly-gamma-glutamate synthetase substrate specificity and level on folate metabolism and folylpoly-gamma-glutamate specificity of metabolic cycles of one-carbon metabolism. *Journal of Biological Chemistry* **268**, 21665–21673.
McPartlin JM, Halligan A, Scott JM, Darling MD & Weir DG (1993) Accelerated folate breakdown in pregnancy. *Lancet* **341**, 148–149.
Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, Gluckman RA, Block PC & Upson BM (1998) Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. *New England Journal of Medicine* **338**, 1009–1015.
Margo G, Barker M, Fernandez-Costa F, Colman N, Green R & Metz J (1975) Prevention of folate deficiency by food fortification. VII. The use of bread as a vehicle for folate supplementation. *American Journal of Clinical Nutrition* **28**, 761–763.
Mason JB (1990) Intestinal transport of monoglutamyl folates in mammalian systems. In *Folic Acid Metabolism in*

Health and Disease, 1st ed, pp. 47–64 [MF Picciano, ELR Stokstad and JF Gregory III, editors]. New York: Wiley-Liss.
Medical Research Council Vitamin Study Research Group (1991) Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131–137.
Naughton CA, Chandler CJ, Duplantier RB & Halsted CH (1989) Folate absorption in alcoholic pigs: in vitro hydrolysis and transport at the intestinal brush border membrane. *American Journal of Clinical Nutrition* **50**, 1436–1441.
Neuhouser ML, Beresford SAA, Hickok DE & Monsen ER (1998) Absorption of dietary folate and supplemental folate in women with prior pregnancies with neural tube defects and controls. *Journal of the American College of Nutrition* **6**, 625–630.
Perry J & Chanarin I (1970) Intestinal absorption of reduced folate compounds in man. *British Journal of Haematology* **18**, 329–339.
Perry J & Chanarin I (1973) Formylation of folates as a step in physiological folate absorption. *British Medical Journal* **ii**, 58–59.
Pfeiffer CM, Rogers LM, Bailey LB & Gregory JF (1997) Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. *American Journal of Clinical Nutrition* **66**, 1388–1397.
Pietrzik K & Remer T (1989) Zur Bioverfugbarkeitsprufung von Mikronahrstoffen (Bioavailability study of micronutrients). *Zeitschrift für Ernahrungswissenschaft* **28**, 130–141.
Reisenauer A & Halsted C (1987) Human folate requirements. *Journal of Nutrition* **117**, 600–602.
Reisenauer AM, Buffington CAT, Villanueva JA & Halsted CH (1989) Folate absorption in alcoholic pigs: in vivo intestinal perfusion studies. *American Journal of Clinical Nutrition* **50**, 1429–1435.
Reisenauer AM, Krumdieck CL & Halsted CH (1977) Folate conjugase: two separate activities in human jejunum. *Science* **198**, 196–197.
Retief FP (1969) Urinary folate excretion after ingestion of pteroylmonoglutamic acid and food folate. *American Journal of Clinical Nutrition* **22**, 352–355.
Richardson RE, Healy MJ & Nixon PF (1979) Folates of rat tissue. Bioassay of tissue folylpolyglutamates and a relationship of liver folylpolyglutamates to nutritional folate sufficiency. *Biochimica et Biophysica Acta* **585**, 128–133.
Riddell LJ, Chisholm A, Williams S & Mann JI (2000) Dietary strategies for lowering homocysteine concentrations. *American Journal of Clinical Nutrition* **71**, 1448–1454.
Rogers LM, Pfeiffer CM, Bailey LB & Gregory JF (1997) A dual-label stable-isotopic protocol is suitable for determination of folate bioavailability in humans: evaluation of urinary excretion and plasma folate kinetics of intravenous and oral doses of [13C5] and [2H2]folic acid. *Journal of Nutrition* **127**, 2321–2327.
Rosenberg IH & Godwin HA (1971) The digestion and absorption of dietary folate. *Gastroenterology* **60**, 445–463.
Rowland M & Tozer TN (1989) *Clinical Pharmacokinetics: Concepts and Applications*. Philadelphia, PA: Lea and Febiger.
Russell RM, Rosenberg IH, Wilson PD, Iber FL, Oaks EB, Giovetti AC, Otradovec CL, Karwoski PA & Press AW (1983) Increased urinary excretion and prolonged turnover time of folic acid during ethanol ingestion. *American Journal of Clinical Nutrition* **38**, 64–70.
Sauberlich HE, Kretsch MJ, Skala JH., Johnson HL & Taylor PC (1987) Folate requirement and metabolism in nonpregnant women. *American Journal of Clinical Nutrition* **46**, 1016–1028.
Savage DG & Lindenbaum JL (1995) Folate-cobalamin interactions. In *Folate in Health and Disease*, pp. 237–285 [LB Bailey, editor]. New York, Marcel Dekker.
Schorah CJ, Devitt H, Lucock M & Dowell AC (1998) The responsiveness of plasma homocysteine to small increases in dietary folic acid: a primary care study. *European Journal of Clinical Nutrition* **52**, 407–411.
Selhub J, Brin H & Grossowicz N (1973) Uptake and reduction of radioactive folate by everted sacs of rat small intestine. *European Journal of Biochemistry* **33**, 433–438.
Selhub J, Dhar GJ & Rosenberg IH (1983) Gastrointestinal absorption of folates and antifolates. *Pharmacology and Therapeutics* **20**, 397–418.
Shane B (1995) Folate chemistry and metabolism. In *Folate in Health and Disease*, pp. 1–22 [LB Bailey, editor]. New York: Marcel Dekker.
Strum WB (1979) Enzymatic reduction and methylation of folate following pH-dependent carrier-mediated transport in rat jejunum. *Biochimica et Biophysica Acta* **554**, 249–257.
Tamura T (1995) Nutrient interaction of folate and zinc. In *Folate in Health and Disease*, pp. 287–312 [LB Bailey, editor]. New York: Marcel Dekker.
Tamura T, Shane B, Baer MT, King JC, Margen S & Stokstad ELR (1978) Absorption of mono- and polyglutamyl folates in zinc-depleted man. *American Journal of Clinical Nutrition* **31**, 1984–1987.
Tamura T & Stokstad ELR (1973) The availability of food folate in man. *British Journal of Haematology* **25**, 513–532.
Tolner B, Roy K & Sirotnak FM (1998) Structural analysis of the human RFC-1 gene encoding a folate transporter reveals multiple promotors and alternatively spliced transcrips with 5′ end heterogeneity. *Gene* **211**, 331–341.
Truswell AS & Kounnavong S (1997) Quantitative responses of serum folate to increasing intakes of folic acid in healthy women. *European Journal of Clinical Nutrition* **51**, 839–845.
US Department of Health and Human Services, Food and Drug Administration (1996) Food standards: amendment of the standards of identity for enriched grain products to require addition of folic acid. *Federal Register* **61**, 8781–8807.

Van Lieshout M, West CE, Muhilal, Permaesih D, Wang Y, Xu X, Van Breemen RB, Creemers AFL, Verhoeven MA & Lugtenburg J (2001) Bioefficacy of beta-carotene dissolved in oil studied in children in Indonesia. *American Journal of Clinical Nutrition* **73**, 949–958.

Varela-Moreiras G & Selhub J (1992) Long-term folate deficiency alters folate content and distribution differentially in rat tissues. *Journal of Nutrition* **122**, 986–991.

Wagner C (1995) Biochemical role of folate in cellular metabolism. In *Folate in Health and Disease*, pp. 23–42 [LB Bailey, editor]. New York: Marcel Dekker.

Ward GJ & Nixon PF (1990) Modulation of pteroylpolyglutamate concentration and length in response to altered folate nutrition in a comprehensive range of rat tissues. *Journal of Nutrition* **120**, 476–484.

Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG & Scott JM (1997) Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiological doses of folic acid. *Quarterly Journal of Medicine* **90**, 519–524.

Wei MM, Bailey LB, Toth JP & Gregory JF (1996) Bioavilability for humans of deuterium-labeled monoglutamyl and polyglutamyl folates is affected by selected foods. *Journal of Nutrition* **126**, 3100–3108.

Wei MM & Gregory JF (1998) Organic acids in selected foods inhibit intestinal brush border pteroylpolyglutamate hydrolase in vitro: potential mechanism affecting the bioavailability of dietary polyglutamyl folate. *Journal of Agricultural and Food Chemistry* **46**, 211–219.

Witthöft CM, Forssén K, Johannesson L & Jägerstad M (1999) Folates –food sources, analyses, retention and bioavailability. *Scandinavian Journal of Nutrition/Närinsforskning* **43**, 138–146.