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Functionality of endogenous folates from rye and orange juice using human *in vivo* model

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Summary *Background* Cereals contribute about a quarter of the daily folate intake from a typical diet in several European countries. However, studies on bioavailability of endogenous folates, in particular of cereal sources, are scarce. *Aim of the study* We aimed to study how well natural folates from rye (different rye breads and muesli made of malted rye) and orange juice function in improving folate status of human volunteers compared to a diet containing folic acid fortified wheat bread. *Methods* Healthy human volunteers aged 20–66 y took part in a four-week intervention trial in which bread, breakfast cereal and juice were provided. The study had a parallel design with two groups, 1) rye and orange juice group (33 volunteers) and 2) fortified wheat bread and apple juice group (31 volunteers). The test foods provided on average 184 µg and 188 µg folate per day in rye and wheat groups, respectively. Test foods were consumed as part of the subjects' normal diet. *Results* In both groups statistically significant increases in serum and red cell folates were observed after the intervention period. The serum folate increased 26 % and 31 %, and red cell folate levels increased 17 % and 15 % in rye and orange juice and wheat and apple juice groups, respectively. The effects did not differ between the rye and wheat groups. Increases in serum and red cell folate were more profound among subjects with low starting folate levels. Decrease in the plasma homocysteine concentrations was observed only in the highest tertile of both groups but not in the group means. *Conclusions* Endogenous folates incorporated into a healthy diet, even in moderate amounts, is an efficient way to improve folate status among healthy adults. Folates from different rye products and orange juice showed good bioavailability that was similar to folic acid from fortified white bread.

Key words folate – rye – bioavailability – endogenous – clinical study

Introduction

It is commonly known and well documented that sufficient intake of folate is necessary in preventing megaloblastic anemia and neural tube defects. This has led to folic acid fortification – either mandatory or voluntary – in several countries throughout the world. Folate functionality has been of interest to researchers since diet rich in folates can also lower plasma homocysteine concentrations [1–3], a phenomenon not only applicable to synthetic folic acid. Elevated plasma homocysteine has been shown to be an independent risk factor for cardiovascular disease in several studies [4]. In addition to

homocysteine lowering capacity, increased intake of folate can have a positive impact on public health for several other reasons as discussed elsewhere (e.g. [5–8]).

In Finland where fortification is not practised except for a few speciality products, cereals contribute about a quarter of the daily folate intake from a typical diet [9]. This proportion is very similar to several European countries [6]. The consumption of rye in Finland in the year 2000 was 15.3 kg per person [10]. Based on calculated intake this contributes about 11–12 % of the average daily folate intake [11], which makes rye the best single source of folates. Folate content of whole rye grain is about 70 μg/100 g fresh weight [12], which is considerably higher than that of wheat grain (10–22 μg/100 g wheat flour) [13].

Rye in the diet is used mainly as bread traditionally produced using sour dough fermentation although in recent years several other applications have been developed. Effects of fermentation on the folate content of rye are not well known. Bakeries often use starter cultures – different lactic acid bacteria and yeasts – of their own and the use of commercial starters is still not very common in Finland. It has been suggested that yeast can synthesise folates during bread making [14, 15]. Also some lactic acid bacteria strains have been shown to produce and secrete folates into the medium [16]. Malting of rye can also significantly increase folate content of rye (Vahteristo & Kariluoto, unpublished).

Folate bioavailability from natural folate sources is not well understood. However, the bioavailability of endogenous folates appears to be frequently incomplete, even low compared to folic acid administered in tablet form [17]. In part, this can be explained by the presence of folates in foods mainly in the polyglutamate form. Moreover, the food matrix is likely to have a significant effect on the bioavailability of folates. However, considering the importance of cereals to the daily intake of folates, studies on bioavailability of natural cereal folates are scarce. Fenech et al. [18] showed a good short-term (7 h) bioavailability of folate from novel wheat aleurone flour in a human intervention study.

In order to evaluate bioavailability of natural folates we studied to what extent endogenous folates in rye and orange juice are able to improve folate status of human volunteers compared to a diet containing folic acid fortified wheat bread.

Subjects and methods

Volunteers

Healthy volunteers aged 20–66 y were recruited through advertising at the University of Helsinki, in local newspaper and by distribution of leaflets. Volunteers were excluded if they used folic acid, multivitamin tablets containing B-vitamins or iron. Exclusion criteria for participation were also use of any medication which might interfere with folate metabolism (such as antiepileptics or methrotrexate). After recruitment and screening two volunteers withdrew before the diet period started and three withdrew during the study. The final study group consisted of 64 volunteers; 46 females and 18 men. All subjects signed a written consent form in which the objective and procedures of the study were explained. The study was approved by the Ethical Committee of the Faculty of Agriculture and Forestry of the University of Helsinki.

After recruitment screening of the volunteers for elevated blood glucose and abnormal haematology or low haemoglobin concentration was carried out. No volunteers were excluded based on their screening results. Plasma total homocysteine was also determined to allow stratified randomisation of the study groups according to homocysteine levels. The study started with a 3–4 week run-in period during which subjects continued their normal diet but were instructed to avoid large amounts of foods rich in folate (separate list provided). Subjects were requested to comply with the same instructions throughout the whole study period. During the run-in period a one-day dietary record was kept by each volunteer (one completed it after the study period) to assess the intake of major nutrients and micronutrients including folate at the group level (MicroNutrica, Kela). A leaflet demonstrating different portion sizes was provided with the diary. The conversions and calculations were conducted by a nutritionist. In addition, all subjects completed a general questionnaire before the run-in period, in which frequency of exercise, sports and alcohol consumption, as well as smoking and other subject characteristics were questioned. All the subjects were in a good health status and no significant differences were observed between the two study groups (Table 1).

Study design

The study was conducted using a parallel design with two groups. The run-in period described above was followed by a 4-week intervention period in March-April 2001. The subjects were randomly assigned, stratified with homocysteine level at the screening, to 1) rye with orange juice group or 2) fortified wheat bread with apple juice group. For seven subjects no screening results were available on time and therefore they were randomly allocated to the two groups. The average length of the diet period was 27 days (range 23–32 days). Serum and red cell folate, vitamin B_{12}, plasma total homocysteine and serum total cholesterol, HDL and LDL cholesterol together with blood count including haemoglobin were measured at the beginning and at the end of the

Table 1 Characteristics of subjects at the time of entry to the study[1]

	Subjects in the rye and orange juice group[2] (n = 33)	Subjects in the fortified wheat bread and apple juice group[2] (n = 31)
Age	35.8±13.3	36.1±13.3
BMI (kg/m^2)	20.8±8.7	23.0±6.2
Plasma homocysteine (μmol/L)	8.6±3.0	7.9±2.5
Serum folate[3] (nmol/L)	9.9±2.9	11.9±5.3
Red cell folate[3] (nmol/L)	561±190	606±224
Vitamin B_{12}[3] (pmol/L)	311±123	325±89
Serum total cholesterol[3] (mmol/L)	5.5±1.2	5.4±1.1
Folate intake[4] (μg/d)	277±96	273±94
Bread intake[4] (g/d)	127±76	132±73
Fibre intake[4] (g/d)	25±9	25±9
Energy intake (MJ/d)	8.1±2.2	8.8±2.2
Protein intake[4] (% of energy)	16.5±3.8	15.9±3.9
Fat intake[4] (% of energy)	30.9±8.8	33.0±8.8
Carbohydrate intake[4] (% of energy)	52.0±8.9	49.6±8.1

[1] n = 64 subjects aged 20–66, 18 men and 46 women; [2] x ± sd; [3] As measured at the beginning (time zero) of the intervention period; [4] 24-h dietary records were obtained from each subject during the run-in period

diet period after over-night fast. The weight and blood pressure of the subjects were also measured at the start and end of the study period.

Diet

The test foods – bread, breakfast cereal and juice – provided the subjects with the same amount of folate and energy. During the diet period customarily used breads and breakfast cereals were to be replaced with the products provided; the other part of the diet was to be kept unchanged. The rye and orange juice group received foods rich in natural folates, whereas wheat group foods low in natural folates but the white bread fortified with folic acid. Test foods were distributed to the subjects twice a week. Additional use of bread, if necessary, was advised to be from the same group; rye bread for the rye and orange juice group and unfortified white bread (wheat) for the wheat group. Other cereal foods (e. g. pasta, rice, pastry) were advised to be consumed as usually eaten by the subject. Deviations from the diet, in particular in the amount of bread, breakfast cereals or juice consumed, were to be recorded on a food record together with possible medications or sickness during the diet period.

Test foods

The rye and orange juice diet contained rye muesli high in folates, produced particularly for the study, fresh rye breads (mostly sour dough fermented), dry rye breads (knäckebröd and Finn crisps) and orange juice. The wheat and apple juice diet contained fortified white wheat bread (sliced loaf bread), low folate breakfast cereal (wheat or oats/rice based) and apple juice. The determined folate contents are presented in Table 2. In the rye and orange juice group the highest folate content of the test foods was in the rye muesli (188 μg/100 g), in other rye products the folate content ranged 21–72 μg/100 g. In the wheat group the fortified wheat bread was the major source of folate from the test foods. Orange juice was used to easily increase the folate content of the diet rich in endogenous folates to the same level as the diet with fortified wheat bread. Average portions consumed throughout the study provided 184 and 188 μg of folate for the volunteers in rye and wheat group, respectively.

The rye muesli was provided by a company specialised in malted products (Laihian Mallas Ltd, Laihia, Finland). The main ingredient, malted rye grain flakes, were manufactured by malting rye, thus increasing the original folate content considerably. Freeze-dried strawberry pieces and powdered sugar were added to the carefully dried product. The muesli was packed in sealed bags ready for consumption. Fresh and dry rye breads for the study were provided by Vaasan & Vaasan bakery (Vaasan & Vaasan Ltd, Helsinki, Finland). Variability in bread selection was offered by using five different rye breads during the intervention period. The amount of bread provided was calculated on dry matter basis to provide the same amount of cereal each week. The fortified wheat bread was obtained from Lapinjärvi Bakery (Unibake Finland, Lapinjärvi). It was baked using food grade folic acid from Eprova AG (Schaffhausen, Switzerland). Folic acid was first dissolved to about one litre of

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Table 2 The composition, folate contents and intake of test foods during the intervention period[1]

Food	Folate content µg/100 g (range in parenthesis)	Daily portion[x]	Amount of folate provided (µg)
Rye muesli with strawberries	188±29	29.5±6.6 g	55±12
Fresh rye bread, different varieties[2]	30 (21–43)	107±43 g	32±13
Knäckebröd (dry rye bread)[3]	66 (62–71)	15.3±2.7 g	10±2
Finn crisp (dry rye bread)	72	22.8±2.4 g	16±2
Orange juice	25	2.8±0.3 dl	70±7
Sum of folate in rye and orange juice group			**184±24**
Fortified white wheat bread	102±2	177±5.7 g	180±6
Breakfast cereal or muesli (wheat/oats/rice)[3]	26 (18–34)	27±0.8 g	7±0.2
Apple juice	0.3	2.5 dl	1±0
Sum of folate in wheat and apple juice group			**188±8**

[1] Test food used for feeding the volunteers. For single products a mean of analysis results of folate content is given, if a range of products was used the range for different products is given. Results are expressed per 100 g fresh weight.
[2] Number of different varieties used during the study was five.
[3] Number of different varieties used during the study was two.
[x] Mean and standard deviation for the whole group calculated from laboratory records (foods provided) subtracting or adding possible deviations from food records kept by each subject.

lukewarm water and then added with stirring to the rest of the water for the bread dough. Fortification level was aimed to be 75 µg/100 g fresh bread. Total folate content, determined by microbiological folate assay (see below), of the white bread was 102 ± 2 µg/100 g. Folic acid levels between breads or different parts of bread were found to be similar. The sliced bread was freshly frozen and stored at –20 °C until distribution to the subjects. The slices were packed in a sealed plastic bag with a daily portion in each bag. The breakfast cereals for the wheat group were purchased from the supermarket and repacked in the laboratory to daily portions. The juices for the study (pasteurised and aseptically packed juice made from concentrates and marketed in cool chain) were provided by the manufacturer (Valio Ltd, Helsinki, Finland).

Laboratory measurements

Folate in food

Samples (1–3 g) were extracted with 30 ml of extraction buffer (50 mM Ches, 50 mM Hepes, containing 2% ascorbate and 10 mM 2-mercaptoethanol, pH 7.85) under a nitrogen atmosphere [19]. Tubes were capped and placed for 10 min in a boiling water bath, cooled on ice and homogenised for 30 s at 13,500 rpm using an Ultra Turrax T25 homogeniser (IKA, Staufen). Samples and standards were protected from daylight and oxygen at all stages of analysis whenever possible.

Trienzyme treatment (conjugase in combination with α-amylase and protease) was performed according to Kariluoto et al. [12]. Samples were incubated at pH 4.9 with 1 ml of hog kidney conjugase and 20 mg of α-amylase (EC 3.2.1.1, A-6211 Sigma, St. Louis, MO, USA) for 3 h at 37 °C. After that, pH was brought to 7.0 with potassium hydroxide and samples were incubated with 4 mg of protease (EC 3.4.24.31, P-5147, Sigma) for 1 h at 37 °C. To inactivate the enzymes, samples were boiled for 5 min and then cooled on ice. Samples were filled to exact volume (50 ml) with extraction buffer.

Samples were analysed for total folates by a microbiological method on 96-well microtiter plates (Tissue culture treated; Costar Corporation, Cambridge, MA) using *Lactobacillus rhamnosus* (ATCC 7469) as the growth organism. Assay medium (Folic acid casei medium, Difco Laboratories, MI, USA) was used as 75% strength of the amount recommended by the manufacturer [20]. Standard for microbiological analysis, (6S)-5-formyltetrahydrofolate (sodium salt; Eprova AG, Switzerland) was dissolved as described by van den Berg et al. [21] and purity was calculated using molar absorptivity coefficient at pH 7.0 [22]. Nine levels of standard (0–100 pg 5-formyltetrahydrofolate/well) were placed into each microtiter plate which were incubated 21 h at 37 °C. Certified reference material (CRM 121 wholemeal flour) was used as a quality control sample. The optical density of the wells was measured with a microplate reader (iEMS Reader MF; Labsystems, Helsinki, Finland) at 595 nm after 10 s mixing at 1150 rpm. For the verification of fortification level of white bread, HPLC

analysis following purification with affinity chromatography utilising bovine folate binding protein was used (detailed for cereal samples by Kariluoto et al. [12]). Recovery rate for folic acid analysis was 112.3 ± 6.79 %, and results for food analysis were corrected for recovery. Food samples were determined for their folate contents in duplicate.

Blood samples

All samples of venous blood were collected the morning after the subjects had fasted overnight (10 h). For the plasma total homocysteine (tHcy) determination, the blood samples were drawn into EDTA-containing evacuated tubes (Venoject, Terumo Europe N. V., Leuven, Belgium), cooled in the refrigerator and centrifuged within 45 min at 2000 rpm for 10 min at 4 °C. For serum samples (vitamin B_{12}, folate and cholesterol) the blood was allowed to clot for 30 min at room temperature and centrifuged at 3000 rpm for 10 minutes. Whole blood samples for the blood count and red cell folate measurements were drawn into EDTA tubes. The tubes were protected from light and taken to the Helsinki University Hospital Diagnostics Department for analysis, except for cholesterol for which the serum samples were frozen at –20 °C until analysis at the department.

Folate in serum samples was measured using time-resolved fluoro-immunoassay (AutoDELFIA™ Folate kit, Wallack Ltd, Turku, Finland). For the measurement of red cell folate the hematocrit was recorded, and the same assay was used as for the serum folate (between assay variation 7–9.6 %, within assay variation about 5 %). Hemolysis for this assay was achieved by incubating whole blood with 0.5 % ascorbic acid at room temperature for 90 min. The final dilution was 1:23.

The assay for tHcy was performed by HPLC with fluorescence detection (excitation at 385 nm, emission at 515 nm). To 100 µl of plasma 10 µl of internal standard (10 mg/l 2-mercaptoethylamine) and 100 µl of reducing agent (10 g/l TCEP-HCl) were added and the mixture was incubated at room temperature for 5 minutes. Proteins were precipitated by addition of 100 µl perchloric acid (0.6 M containing 1 mM EDTA). To derivatise the thiols, 50 µl of thiol-specific reagent, ammonium-7-fluoro-1,2,3,-benzoxadiazole-4-sulfonate (SBD-F), 1 g/l in borate buffer, was added to 50 µl of clear supernatant. This mixture was heated for 1 hour at 60 °C. A Lichrospher 100 (RP-18, 5 µm, 250 x 4.6 mm) column with a mobile phase of 5 % acetonitrile in 0.1 M phosphate buffer (pH 2.3) at a flow rate of 1 ml/min, was used for the separation. The detection limit of the assay was 1 µmol/l and the inter-assay coefficient of variation was 5–8 % at 9–35 µmol/l.

The assay for vitamin B_{12} in serum samples was performed using the intrinsic factor binding assay [23]. Determinations of total, HDL and LDL cholesterol were made by enzymatic colourimetric methods using Konelab 20-analyser (Thermo Clinical Labsystems, Espoo, Finland).

Statistical analysis

Most data are reported as means ± SDs. All the reported or recorded dietary intakes were used in calculating the average intakes of test foods.

The effect of the intervention on the level of the serum and red cell folate and plasma total homocysteine was tested using paired t-test. Wilcoxon signed rank test (SAS software) was used as a sensitivity analysis. The significance of the observed difference between treatments (rye vs wheat group) was tested using analysis of covariance for serum and red cell folate. The initial value before intervention was used as covariate.

Results

In both groups the subjects were on average 36 years of age the range being from 20 to 66 years. Both sexes were equally represented in the two groups: 9 males in the rye group out of 33 subjects and 9 males out of 31 subjects in the fortified wheat group. No weight gain was observed in either of the groups. The average intake of folate in the rye and orange juice group during the run-in period was 277 ± 96 µg per day and 273 ± 94 µg per day in the wheat group. Serum and red cell folate levels measured at the start of the intervention period were normal. Average for the two groups, rye and wheat, were 9.9 and 11.9 nmol/L for serum folate and 561 and 606 nmol/L for red cell folate (Table 1). Reference values of the laboratory for serum and red cell folate were 6.2–40 nmol/L and 340–990 nmol/L. Three subjects, one in rye group and two in wheat group, had a slightly low serum folate (5.2–5.5 nmol/L) but as red cell folate did not show any signs of folate deficiency they were not excluded from the study. One subject had a low B12 status and was excluded from the tHcy analysis. The average total serum cholesterol was slightly higher than the current recommendation in Finland (below 5 mmol/L [23]) in both groups and neither of the diets lowered total, HDL or LDL cholesterol.

In both groups a statistically significant increase in serum and red cell folates was observed (Table 3) after the intervention period. Increase in serum folate appeared slightly higher in the group receiving folic acid from the fortified bread (31 % increase) than in group receiving natural folates from rye products and orange juice (26 % increase). The difference was not, however, statistically significant. Increase in the red cell folate was of the same magnitude (15.3 % and 17.3 %) in both groups.

Table 3 Plasma and red blood cell folate concentrations before and after high natural folate diet with rye and orange juice or after diet with fortified white bread and apple juice

	Natural folates from rye and orange juice	Folic acid from fortified white bread
Plasma folate (nmol/L)		
Beginning	9.9 ± 2.9	11.9 ± 5.3
End of diet	12.5 ± 3.6*	15.6 ± 5.8*
Difference	2.6 ± 3.2	3.7 ± 3.3
Increase (%)	26.1 %	31.2 %
Red cell folate (nmol/L)		
Beginning	561 ± 190	606 ± 224
End of diet	658 ± 227*	697 ± 250*
Difference	97 ± 92	91 ± 79
Increase (%)	17.3 %	15.3 %

* = statistically significant increase from the beginning ($p < 0.0001$)

Increase in serum folate was more profound among volunteers with low starting serum folate levels in both groups (Fig. 1). The average increase in serum folate for those having the lowest serum folate at the beginning of the study was as high as 59–68 % (increase of 3.9 nmol/L in the rye group and 5.0 nmol/L in the wheat group). The average serum folate concentrations in the lowest tertile at the beginning of the study were 6.3 ± 1.3 nmol/L and 7.4 ± 1.0 nmol/L in the rye and wheat groups, respectively. For those having the highest folate level at the start (average 13.3 ± 0.9 nmol/L and 18.0 ± 5.0 nmol/L for rye and wheat groups, respectively) the increase after the intervention was only 12–14 % (equal to an increase of 1.6 nmol/L and 2.5 nmol/L in rye and wheat groups).

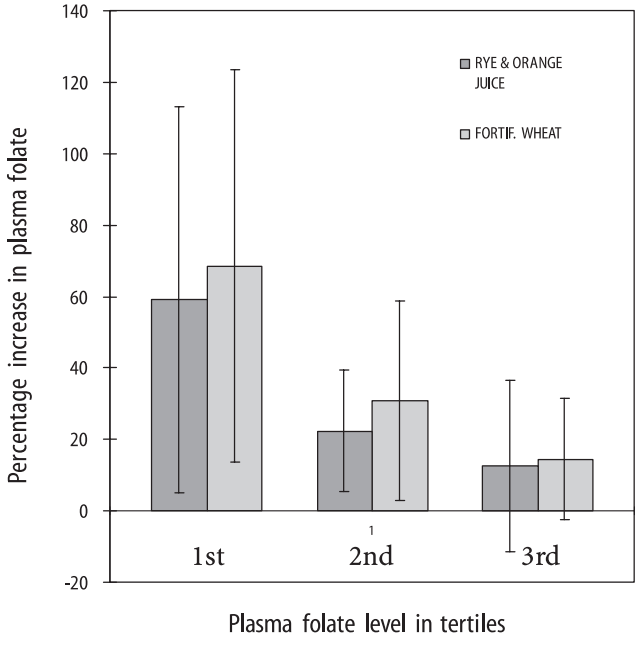

Fig. 1 Percentage increase (± sd) in plasma folate after a 4 week rye and orange juice or fortified wheat diet in tertiles of starting plasma folate levels

The effect of dietary intervention on red cell folate level was also significant in both groups (15 and 17 % increases, Table 3) but the effect was not as consistent and dependent on the starting level of red cell folate as in the case of serum folate. However, those with the lowest folate status (measured as red cell folate concentration) had the largest relative increase in the red cell folate.

Decrease in the plasma homocysteine concentration was observed only in the highest tertile of groups but not in the group means. In the wheat group the average plasma tHcy was 7.8 ± 2.4 μmol/L at the start and at the end of the diet period (7.8 ± 1.8 μmol/L). In the rye and orange juice group the figures were 8.5 ± 3.2 μmol/L (at the start) and 8.8 ± 2.5 μmol/L (at the end). In the rye group the highest tertile of tHcy decreased from 12.0 to 11.4 μmol/L and in the wheat group from 10.4 to 9.2 μmol/L, neither of the decreases being statistically significant. Volunteers with a tHcy value above 10 μmol/L had an average decrease of 0.7 μmol/L in the rye and orange juice group (n = 7) and a decrease of 1.9 μmol/L in the fortified wheat group (n = 4).

During this relatively short intervention the compliance of the subjects was good based on the dietary records of the volunteers but also on the personal contacts with the volunteers and the regularity with which the volunteers fetched their test foods. The deviations from the diet revealed in the dietary records were minor.

Discussion

The results clearly demonstrate that diet rich in rye and orange juice increased serum and red cell folate in healthy volunteers, with a magnitude comparable to that of folic acid fortified wheat bread. The findings of Brouwer et al. [1] from a controlled dietary intervention, also with natural folates from vegetables and citrus juice, are well in line with our findings except for homocysteine. In their study a higher amount of additional natural folate (350 μg/d) in the diets was used which might partly explain the difference. The starting tHcy in plasma was also higher (11 μmol/L vs. 8 μmol/L in this study) providing another reason for the observed difference as it is well known that the effect of folate on tHcy is very dependent on its starting level.

In another interventional study homocysteine lowering was examined in response to low-dose folic acid (100–400 μg/d over 26 weeks) [24]. When the results were expressed as tertiles of baseline plasma tHcy a significant decrease in homocysteine was found only in the two highest tertiles. No significant response to folic acid treatment was observed in the lowest tertile, in which the mean baseline tHcy concentration was 7.07 ± 0.84 μmol/L. Their results suggest that baseline folate status

(red cell folate) in this group was already optimal in the beginning, and therefore no significant increase in response to folic acid treatment was observed.

The results of Schorah et al. [25] further confirm the characteristics of Hcy lowering. They reported a 10 % decrease in plasma homocysteine after 24 weeks of daily consumption of folic acid fortified cereals (extra 200 µg/d). The decrease was more profound in subjects initially having a low serum folate or a high homocysteine. Moreover, the small decrease observed after four weeks – corresponding to the length of our study – was not significant. Differences in diet responsiveness to increased dietary folate intake, as well as responsiveness of plasma homocysteine can also be attributed to different genotypes related to homocysteine metabolism [3, 26].

Wald et al. [8] reported a change of 2.1 nmol/L in serum folate levels for each 0.1 mg increase of folic acid administered as tablets (0–1 mg range). In our study 2.6 nmol increase in serum folate in the rye and orange juice group and 3.7 nmol/L increase in the fortified wheat bread group was observed when the estimated increase in daily intake of folate (endogenous or fortified) was only 120–130 µg.

The observation that the lower the plasma folate level is at the beginning of the intervention the higher the increase indicates the need of careful interpretation or extrapolation of one's results to another place or group. Coincidentally selecting volunteers with insufficient or marginal intake of a particular nutrient may easily lead to an overestimation of the effects of a particular treatment. Most probably, since the duration of the treatment in this study was not very long, and varied between subjects (3 to 4 weeks), the effect on red cell folate was not as constant and dependent on the starting level as in the case of serum folate.

There are plenty of studies suggesting reduced bioavailability of natural folates compared to folic acid administered in tablets or fortified products (e.g. 27). However, one study on cereals made from aleurone flour, exceptionally rich in folates, clearly showed good bioavailability of natural, mostly monoglutamated folates [18]. In this study the observed increase in plasma folate was the same as that from folic acid tablet administered together with extruded wheat bran cereal. In our study the improvement of the folate status among subjects in rye and orange juice group was higher than anticipated. That might result not only from good bioavailability of endogenous folates *per se* but also from the promotion of intestinal biosynthesis of folate induced by the increase in non-starch polysaccharide intake as suggested by Houghton et al. [28]. Houghton and her colleagues showed that non-starch polysaccharide intakes of female adolescents (14–19 y) were positively associated with serum folate concentrations. In contrast, insoluble fibre intakes were unrelated to serum folate concentrations. As discussed in their paper there is a great deal of evidence suggesting that net microbial biosynthesis of folate is related to diet. Some of the rye polysaccharides as well have been suggested to promote, for example bifidobacteria growth in the human colon.

Recent findings related to functionality of several compounds in rye have enhanced the popularity of rye among consumers. The results of this study will further strengthen the image of rye as a healthy dietary choice.

Conclusions

Based on our results the incorporation of natural folates into a healthy diet in even moderate amounts is an efficient way to improve folate status (plasma & red cell folate) among healthy adults. A moderate or small increase in folate intake via consumption of either folate fortified wheat bread or folate rich foods (rye products and orange juice) daily over a 4-week period increases both plasma and red cell folate. Typically, the lower the folate status at the beginning of the intervention, the higher the observed increase. Low starting Hcy levels (as group means) could not be lowered with modest fortification or increased consumption of foods rich in folate. Endogenous folates from different rye products and orange juice showed good bioavailability that was similar to folic acid from fortified white bread.

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