Aleurone Flour Is a Rich Source of Bioavailable Folate in Humans

Michael Fenech, Manny Noakes, Peter Clifton and David Topping
Commonwealth Scientific and Industrial Research Organisation, Human Nutrition, Adelaide, SA, Australia 5000

ABSTRACT With the use of novel milling technology, it has become commercially viable to isolate the aleurone layer of cells from wheat grain and to prepare a novel flour from this fraction that has a natural folate concentration of ∼500 µg/100 g. The aim of this study was to determine the relative bioavailability of natural folate from aleurone flour when ingested as a cereal. Using a series of randomized, short-term intervention trials with a cross-over involving eight men and eight women aged between 29 and 50 y, we compared the increment of plasma folate following ingestion of 1) 100 g wheat bran cereal (low folate control), 2) 100 g aleurone cereal, and 3) a tablet containing 500 µg folic acid taken together with 100 g wheat bran cereal (high folate control). Folate absorption was measured by estimating the area under the plasma folate concentration versus time curve. The extent of increase in plasma folate over the 7-hour period following ingestion of aleurone cereal was more than fourfold greater than that observed following the wheat bran cereal (P < 0.0001) and not different from that observed following the 500 µg folic acid tablet taken with wheat bran cereal. Differences were significant when data for males and females were analyzed separately (P < 0.001). This study has shown that cereal made from wheat aleurone flour is a good source of bioavailable, natural folate. J. Nutr. 129: 1114–1119, 1999.

KEY WORDS: • aleurone flour • folic acid • bioavailability • wheat bran • humans

Wheat aleurone flour is a novel food product that has the potential to make an important contribution to the intake of natural folate. The aleurone cells, together with the germ, contain the wheat grain’s essential nutrients required for the growth and development of the embryo (Clysedale 1994, Saxelby and Venn-Brown 1980). The phytochemicals, vitamins, and minerals in aleurone cells may be lost when wheat grain is refined. A unique and commercially viable milling process that enables the isolation of the aleurone cell layer and at the same time splits the cell walls to release the contents of these cells has recently been developed (by Goodman Fielder Pty. Ltd., Australia) (Stenvert 1995 and 1997). A schematic representation of the isolation of aleurone is shown in Fig. 1. The sheared aleurone cells together with a small amount of wheat germ was formulated into a novel aleurone flour (ALF). The aleurone flour described in this study was available commercially in Australia and internationally for more than 12 months and is sold widely as a major ingredient of bread and pasta. One of the most notable features of the composition of this product is the high level of folate that is present, a concentration between 400 and 600 µg per 100 g wet ALF. This natural level of folate is higher than that observed in wheat bran, fruits, and vegetables (usually between 20 and 200 µg/100 g wet) (Bailey 1995, Subar et al. 1989) and is comparable to folate/folic acid levels in fortified flour and cereal that provide 50% Recommended Dietary Intake (RDI) per serve (assuming an RDI of 400 µg and a serving size of 40 g wet weight) (Crane et al. 1995).

Folate is now recognized to play an important role in the prevention of neural tube defects in the fetus (Czeizel and Dudas 1992, Medical Research Council Vitamin Study Research Group 1991). There is also increasing evidence that an above average intake of folate may help reduce plasma homocysteine, a risk factor for cardiovascular disease (Boushey et al. 1995, Kang et al. 1992), and DNA damage, a risk factor for cancer (Blount et al. 1997, Fenech 1996, Ma et al. 1997). There is some concern that eating foods that are naturally rich in folate may not provide for a large enough and reliable intake of folate required to prevent spina bifida (Cuskelly et al. 1996). Therefore, it is important to identify novel, naturally rich sources of folate and to test that dietary strategies based on such foods may be effective for the optimization of tissue folate in the general population.

To assess the potential of ALF as a source of folate, it is necessary to measure how much folate actually appears in the blood after ingesting foods rich in this ingredient. To achieve this we performed a randomised, controlled intervention trial to compare the change in plasma folate after consumption of 1) a cereal made from ALF, 2) a cereal made from wheat bran (WB), and 3) a tablet containing 0.5 mg folic acid that was taken together with WB cereal.

MATERIALS AND METHODS

Sixteen healthy volunteers, eight males and eight females, aged between 20 and 50 y, were recruited for this study, which was approved by the Human Ethics Committee of Commonwealth Scientific and Industrial Research Organisation Human Nutrition and
Further blood samples were collected at 1, 2, 4, and 7 h after acid tablet was taken while the WB cereal was being ingested. Milk (containing 1.5 g/100 g fat) over a period of 30 min. The folic acid in each tablet was determined using nonparametric, repeated measures ANOVA (Friedman test). The area under the plasma folate concentration versus time curve (area under the curve (AUC)) was measured for each individual and for each group at each intervention round using plasma folate measured at 0 h as the baseline value. The significance of differences in the AUC values was also estimated using nonparametric, repeated measures ANOVA and Dunn's multiple comparison test. All statistical analyses including measurements of AUC were performed using PRISM software (GraphPad, San Diego, CA). All quoted p-values are for two-tailed tests, unless otherwise indicated. Differences were considered significant if P < 0.05.

RESULTS

Analyses. Proximate analyses of the aleurone and wheat bran flour indicated a higher starch and protein content and a lower fiber content in aleurone flour when compared to wheat bran flour (Table 1). The total folate level per 100 g in the WB cereal and the ALF cereals was 94 ± 4 μg (n = 2) and 515 ± 7 μg (n = 2), respectively; the folic acid in each tablet was 526 ± 24 μg (n = 3). The proportion of folate in the tablet, ALF cereal, and WB cereal that could be detected without prior treatment with folate conjugase was 100, 81, and 32%, respectively.

Intervention. None of the volunteers were folate deficient (plasma folate < 3.4 nmol/L). All volunteers completed the intervention successfully. The results for plasma folate at each time-point for each intervention round for males and females are shown in Table 2, and the mean values (±SEM) for the combined results for males and females are shown graphically in Fig. 2. There was a significant, positive correlation between individual base-line data from each intervention round (R = 0.69–0.78, P < 0.001).
The plasma folate data during the WB cereal intervention round clearly showed only a minimal increment in the vitamin level during the course of the intervention; the increment was significant in males only. In contrast, plasma folate levels following ingestion of the 0.5 mg folic acid tablet with WB cereal increased significantly in both males and females (P = 0.003 and P = 0.002, respectively); the combined results showed a sharp increase in plasma folate during the first 2 h, from a mean base-line level of 13.4 nmol/L to a peak at 2 h of 23.0 nmol/L, and a subsequent steady decline down to baseline during the next 5 h (Fig. 2). The increase in plasma folate following consumption of ALF cereal was also significant in both males and females (P = 0.0001) and appeared to be of the same magnitude as that observed after the ingestion of 0.5 mg folic acid supplement with WB cereal, with a steady increase in plasma folate during the first 2 h from a base-line of 13.9 nmol/L to a peak at 2 h of 23.5 nmol/L and a decline down to baseline by 7 h (Fig. 2). However, the time response following ALF cereal showed significant increments in plasma folate at 2 and 4 h after ingestion of the cereal. This suggested a slower rate of appearance of folate into the plasma compared to the results for the folic acid tablet with WB cereal, which showed significant increments in plasma folate at 1 and 2 h following ingestion (Table 2). Analysis of the combined male and female data showed that the observed increments in plasma folate following intake of ALF cereal or folic acid tablet with WB cereal were significant (P < 0.0001), but there was no change following ingestion of the WB cereal.

To assess the extent of net folate appearance in the blood, we also measured the area under the plasma folate concentration versus time curve for each individual for each intervention round. The folate AUC measured in the blood of all subjects did not differ following ingestion of 100 g ALF cereal [41.8 ± 6.2 (nmol/L)h] or ingestion of the 0.5 mg folic acid tablet with 100 g WB cereal [42.9 ± 7.0 (nmol/L)h]; these results were more than four times greater than the AUC measured following the WB cereal intake [6.8 ± 2.4 (nmol/L)h] (Fig. 3). The same conclusions were reached when the data for male (n = 8, P < 0.01) and female (n = 8, P < 0.05) volunteers are analyzed separately; no significant effect of gender on the AUC was detected for any treatment (Table 2).

To obtain a relative estimate of folate bioavailability, we calculated the AUC/ingested folate ratios for the WB cereal.

### TABLE 1

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Wheat bran flour</th>
<th>Aleurone flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>21.6</td>
<td>36.5</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>31.6</td>
<td>15.4</td>
</tr>
<tr>
<td>Fat</td>
<td>5.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Protein</td>
<td>17.8</td>
<td>23.6</td>
</tr>
<tr>
<td>Free sugars</td>
<td>6.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Ash</td>
<td>3.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Sum</td>
<td>96.3</td>
<td>98.4</td>
</tr>
</tbody>
</table>

1 Values are means of duplicate analyses.

### FIGURE 2

Change in plasma folate of men and women following ingestion of wheat bran (WB) cereal, aleurone flour (ALF) cereal, and 0.5 mg folic acid with WB cereal. Results represent the mean ± SEM; n = 16, males and females combined. The ANOVA P-values for the change in plasma folate with time for the WB cereal, ALF cereal, and 0.5 mg folic acid with WB cereal were 0.1139, < 0.0001, and < 0.0001, respectively.

### TABLE 2

<table>
<thead>
<tr>
<th>Time, h</th>
<th>WB cereal</th>
<th>ALF cereal</th>
<th>0.5 mg folic acid + WB cereal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.9 ± 1.1</td>
<td>15.4 ± 2.6</td>
<td>14.4 ± 2.1</td>
</tr>
<tr>
<td>1</td>
<td>16.1 ± 1.5</td>
<td>15.7 ± 3.1</td>
<td>19.0 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>15.1 ± 1.5</td>
<td>16.7 ± 3.2</td>
<td>23.4 ± 3.4**</td>
</tr>
<tr>
<td>4</td>
<td>14.8 ± 1.6</td>
<td>16.1 ± 3.3</td>
<td>21.2 ± 2.9**</td>
</tr>
<tr>
<td>7</td>
<td>14.2 ± 1.4</td>
<td>15.2 ± 2.6</td>
<td>16.5 ± 2.1</td>
</tr>
<tr>
<td>ANOVA P</td>
<td>0.029</td>
<td>0.680</td>
<td>0.0001</td>
</tr>
<tr>
<td>AUC (nmol/L)h</td>
<td>5.7 ± 3.2</td>
<td>8.0 ± 3.8</td>
<td>38.1 ± 6.8†</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; n = 8.
2 ANOVA P-values refer to time effect; *P < 0.05; **P < 0.01 for comparison to means at time 0 h.
3 †P < 0.05; ‡P < 0.01; significantly different from AUC after WB cereal consumption.
the ALF cereal, and the folic acid tablet for each subject. The bioavailability of folic acid from the tablet was calculated after subtracting the AUC value for WB cereal from the AUC value for the folic acid with WB cereal and then dividing by the folic acid intake from the tablet only. The AUC/ingested folate ratios for WB cereal, ALF cereal, and the folic acid tablet were 0.073 ± 0.025, 0.081 ± 0.012, and 0.068 ± 0.012, respectively, did not differ.

The individual AUC following ingestion of the ALF cereal were not correlated with those observed after intake of the tablet supplement with WB cereal, but they were positively correlated with the AUC observed following intake of the WB cereal (R = 0.41, P = 0.055, one-tailed test).

Significant positive correlations were observed between an individual’s mean baseline value of plasma folate measured before each intervention round and the AUC following ingestion of the WB cereal or the ALF cereal (R = 0.53, P = 0.03) (Fig. 4). However, this particular relationship was not significant for AUC data from the folic acid tablet intervention even though the correlation factor was positive (R = 0.33, P = 0.212). When the data for the ALF cereal intervention were combined with the data for the tablet intervention, the correlation coefficient was 0.427 (P = 0.014).

Plasma vitamin B-12 concentrations remained constant during the intervention (data not shown).

**DISCUSSION**

The lack of comprehensive and reliable information on the concentration and bioavailability of folate from natural foods has tended to favor the concept that fortification of staple foods with synthetic folic acid is a more reliable strategy for increasing tissue folate in the general population. It has recently been shown, in a 3-mo intervention trial involving 41 women, that significant increments in red cell folate from an initial mean value of 351 µg/L to a postintervention value of 492 µg/L were observed in those taking a supplement of 400 µg/d synthetic folic acid, but there were no significant changes in red cell folate in those who increased their intake of foods rich in folate from a level that provided an estimated 209 µg/d to a level that was estimated to provide 410 µg/d natural folate (Cuskelly et al. 1996). In contrast, a randomized, placebo-controlled trial designed to identify the minimum effective dose for food fortification to prevent neural tube defects found that a supplement of 200 µg/d synthetic folic acid was sufficient to raise red blood cell folate level above the 400 µg/L threshold (measured by microbiological bioassay) required for minimization of spina bifida risk (Daly et al. 1997). In a similar study, it was shown that 200 µg/d of synthetic folic acid appeared to be as effective as 400 µg/d with regard to lowering plasma homocysteine in apparently normal subjects (Ward et al. 1997). An increase of 100 µg/d of synthetic folic acid intake was shown by both these studies to be relatively ineffective in terms of adequately reducing folate-related risk factors for spina bifida and cardiovascular disease.

The results from our study on cereal made from aleurone flour show quite clearly that this natural source of folate can make a significant difference in blood folate concentration. Of main interest was 1) the much greater capacity for ALF cereal, relative to WB cereal, to increase plasma levels of folate and 2) that the increase in plasma folate following ingestion of 100 g of ALF cereal was the same as that observed following intake of 500 µg synthetic folic acid with 100 g WB cereal. Our data indicate that it is the higher content of aleurone flour rather than increased bioavailability of folate from this product that gives it value as a folate source in the diet.

Bailey et al. (1988) compared the bioavailability of monoglutamyl folate and polyglutamyl folate when ingested with bran cereal and found that monoglutamyl folate bioavailability is unaffected by dietary fiber, but wheat bran fiber appeared to marginally inhibit the uptake of polyglutamyl folate. Using a dual-label, stable isotope protocol, Pfeiffer et al. (1997) showed that consuming folic acid with a light breakfast meal only produced a small reduction in folic acid absorption (15%, P > 0.05) relative to a control without food. Thus the comparison, in our study, between the AUC estimates following ingestion of ALF cereal and folic acid in a tablet taken with wheat bran cereal may have been influenced only to a limited extent by fiber because 1) the tablet contained monoglutamyl folate, and wheat bran only contributed a small percentage of folate; and 2) the folate in the ALF cereal appeared to be in a...
relatively unconjugated form, and the level of fiber in the ALF cereal was almost half that of the wheat bran cereal (Table 1). The short-term, nonisotopic type of bioavailability study used is very similar to that reported by Keagy et al. (1988) and Bailey et al. (1988). In accordance with the recommendations from these studies, multiple samples were taken over a 7-h period to avoid errors in bioavailability estimation occurring because of different rates of absorption. Bailey et al. (1988), who measured plasma folate by microbiological assay, also reported that with such a protocol it was only possible to obtain consistent responses with doses of folic acid above 250 μg—the folic acid/folate levels in the tablet with WB cereal and the ALF cereal were well above this level. The WB cereal, which contained only 92 μg folate, produced a significant increment in plasma folate only in males, suggesting that such a level of ingested folate produces a change in plasma folate that is at the limit of detection of the system used. Consequently, the AUC measurement with the WB cereal may, therefore, be considered less accurate than those obtained for the ALF cereal and the folic acid tablet with WB cereal.

The apparent relative bioavailability of monoglutamyl and polyglutamyl folates varies according to the protocol used (Gregory 1995). For example, protocols with nonlabeled folates quantified by urinary excretion suggest a mean bioavailability of 85–90% for tri- and hepta-glutamyl folate relative to folic acid (Tamura and Stokstad 1973). Protocols with nonlabeled folate quantified by AUC of plasma folate concentration found equivalent bioavailability for 750 μg folic acid and the molar equivalent of heptaglutamyl folate, but the bioavailability of the latter was reduced when given with bran cereal but not spinach (Bailey et al. 1988). It is possible that in certain cases the reported lower bioavailability of polyglutamyl folate could have been caused by specific conjugase inhibitors and not the extent of folate polyglutamation (Rosenberg and Godwin 1971). Our results, obtained with similar techniques, for wheat bran and aleurone flour, suggest no difference between the bioavailability of folate in these products and that for folic acid in a tablet. Bioavailability of folate in the WB and ALF flours may have been overestimated if the folate level measured in the flours was underestimated by the single-enzyme (conjugase) method used. However, it was shown that for ready-to-eat cereals including wheat bran cereal, the level of folate measured using the single-enzyme (conjugase) method is the same as that measured by the tri-enzyme method involving α-amylase, folate conjugase, and protease (Rader et al. 1998). For other foods the tri-enzyme method yields a folate value that may be up to 30% higher than that determined by using conjugase alone (Rader et al. 1988).

The microbiological analyses in our study have shown that, unlike folate in wheat bran, most of the folate in aleurone flour could be detected without pretreatment with folate deconjugase enzyme. The reason for this difference is not known, but it is possible that in the process of shearing aleurone cells endogenous folate deconjugases are released and activated. The shearing of aleurone cells may increase the bioavailability of folate from this natural ingredient, particularly in those individuals who have difficulty in digesting the thick cell walls of aleurone cells. The apparent deconjugation of polyglutamate folate may make folate more available to people who have limited deconjugase activity in the small intestine, possibly because of suboptimal pH levels, which may occur in conditions such as atrophic gastritis, resulting in reduced gastric acid secretion (Gregory 1995). Our estimates based on the ratio of folate increments in the blood and ingested level of folate suggest, however, that bioavailability of folate from ALF cereal was not significantly greater than the bioavailability of folate from WB cereal or synthetic folic acid from a tablet.

The results from this study give some indication that individuals with low baseline plasma folate seem to have lower AUC for plasma folate than individuals with higher initial plasma levels. The correlation factors suggest that between 9 and 25% of the observed variation in the AUC measurements could be explained by baseline levels of plasma folate. The low plasma folate and AUC levels may indicate either reduced gut absorption or increased tissue uptake of folate from plasma. Suboptimal jejunal pH may explain inefficient absorption of folate in some cases (Gregory 1995, Russel et al. 1986). It may, therefore, be useful in future studies to verify that blood folate levels can be optimized by different strategies in those subjects with an impaired capacity to absorb folate.

The time-related increment in plasma folate during the initial 2 h occurred more rapidly following the ingestion of the folic acid tablet with WB cereal than it did following ingestion of ALF cereal. A conceivable explanation for the slower uptake of folate from ALF cereal relative to folic acid from the tablet is the necessity of folate deconjugation for the aleurone source of folate. An alternative explanation could be that folate from the ALF cereal was mainly absorbed by the jejunal, pH-dependent, and saturable transport process, while absorption of folic acid from the tablet may have partly involved an nonsaturable mechanism, such as passive diffusion (Gregory 1995, Halsted 1990, Mason 1990, Shoda et al. 1993, Strum 1979). It was reported that the latter mechanism may operate when a bolus of synthetic folic acid of between 400 and 800 μg is ingested by humans and could result in substantial amounts of unmetabolized folic acid appearing in plasma and urine (Gregory 1995, Kelly et al. 1997, Lucock et al. 1989). In contrast, physiological uptake by the saturable mechanism into jejunal mucosal cells ensures that monoglutamyl folate is reduced and methylated to form 5-methyltetrahydrofolate, the major cytosolic folate in mammalian tissues, before transport into the blood (Shane 1995). Therefore, it may be worthwhile in future studies to compare the ratio of unmetabolized folic acid and 5-methyltetrahydrofolate in plasma following ingestion of ALF cereal and a bolus of synthetic folic acid possibly using established HPLC methods (Kelly et al. 1997, Lucock et al. 1995).

Although the results from this study indicate that ALF cereal is an important source of folate, long-term studies are required to establish the extent to which folate from aleurone flour may reduce plasma homocysteine and increase the levels of red cell folate, which is considered to be a reliable biomarker of tissue folate stores. Such studies are currently underway in our laboratory. Longer-term studies are also important because aleurone flour increases the rate of fermentation of bacteria in the large bowel (Cheng et al. 1987). If ALF cereal is favorable to the increase of folate-producing bacteria such as Bifidobacteria (Krause et al. 1996), then there may be an additional folate contribution via this route because recent studies with rats and organ-cultured biopsies of human colon suggest that folate can be absorbed across the large bowel epithelium (Rong et al. 1991, Zimmerman 1990). These series of potential events may explain the apparent positive association between fiber intake and blood folate (Houghton et al. 1997). The significant contribution of folate from aleurone cells may also explain in part why an increased intake of whole-grain foods lowers low risk for various digestive tract cancers (Jacobs et al. 1998).

In conclusion, this study has shown that cereal made from wheat aleurone flour is a significant source of natural, bioavailable folate that can make an effective contribution to increas-
ing folate concentration. This effect is of a similar magnitude to that observed following ingestion of 500 µg synthetic folic acid given with wheat bran cereal. These results suggest that inclusion of foods made from wheat aleurone flour in the diet can be considered as an alternative, important strategy for increasing folate intake in the general population.

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LITERATURE CITED


