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Consumption of Wheat Aleurone-Rich Foods Increases Fasting Plasma Betaine and Modestly Decreases Fasting Homocysteine and LDL-Cholesterol in Adults[1–4]

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Abstract

There is strong evidence that whole-grain foods protect against heart disease. Although underlying mechanisms and components are unclear, betaine, found at high levels in wheat aleurone, may play a role. We evaluated the effects of a diet high in wheat aleurone on plasma betaine and related measures. In a parallel, single-blinded intervention study, 79 healthy participants (aged 45–65 y, BMI $\geq$ 25 kg/m^2) incorporated either aleurone-rich cereal products (27 g/d aleurone) or control products balanced for fiber and macronutrients into their habitual diets for 4 wk. Fasting blood samples were taken at baseline and postintervention (4 wk) from participants. Compared with the control, the aleurone products provided an additional 279 mg/d betaine and resulted in higher plasma betaine ($P < 0.001$; intervention effect size: 5.2 μmol/L) and lower plasma total homocysteine (tHcy) ($P = 0.010$; -0.7 μmol/L). Plasma dimethylglycine and methionine, which are products of betaine-mediated homocysteine remethylation, were also higher ($P < 0.001$; $P = 0.027$) relative to control. There were no significant effects on plasma choline or B vitamins (folate, riboflavin, and vitamin B-6). However, LDL cholesterol was lower than in the control group ($P = 0.037$). We conclude that incorporating aleurone-rich products into the habitual diet for 4 wk significantly increases plasma betaine concentrations and lowers tHcy, which is attributable to enhanced betaine-homocysteine methyltransferase-mediated remethylation of homocysteine. Although this supports a role for betaine in the protective effects of whole grains, concomitant decreases in LDL suggest more than one component or mechanism may be responsible. J. Nutr. 140: 2153–2157, 2010.

Introduction

Epidemiological studies indicate that diets high in whole grains are associated with decreased incidence of chronic diseases (1,2). However, the components and mechanisms underlying these beneficial effects are poorly understood. Betaine is a component found at relatively high concentrations in wheat grain, particularly in the bran and aleurone fractions (3,4), and although its potential role in the health benefits of whole grains has been proposed (5), it largely has been neglected. Betaine is an osmolyte (6), but it also plays a role in remethylating total homocysteine (tHcy)[8] (7), a risk factor for cardiovascular disease and particularly stroke (8). Recent studies have shown that betaine supplementation decreases plasma tHcy (9–12) and that betaine status or intake is inversely associated with plasma tHcy (13–15). Betaine can also act as a lipotrope (6) and may have a role as a therapeutic agent for nonalcoholic fatty liver disease (16–18), a condition linked to metabolic syndrome (19,20).

Previous work using explorative metabolomic approaches showed the effects of a whole-grain diet on plasma and hepatic betaine in animals (21,22). Additionally, work from our group has shown significant postprandial increases in plasma betaine following the consumption of wheat bran and to a greater extent wheat aleurone (23). However, there do not appear to be any

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[3] This trial was registered at the Current Controlled Trials register (ISRCTN93336504).
[4] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
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[8] Abbreviations used: DMG; dimethylglycine; PLP; pyridoxal 5′-phosphate, RTE; ready-to-eat; tHcy; total homocysteine.

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previous reports on the effect of chronic consumption of whole-grain fractions on betaine status and related biomarkers. We therefore carried out a 4-wk intervention study in apparently healthy, older, overweight men and women at risk for metabolic syndrome by using cereal products enriched with wheat aleurone to evaluate the effect on plasma betaine and related biomarkers.

Materials and Methods

Participants. Eighty healthy participants were recruited from the Northern Ireland population. The inclusion criteria were: aged 45–65 y, BMI ≥ 25 kg/m^2, in general good health with no current or recent serious illness, no use of prescription medicine or vitamin or mineral supplements, nonsmoker, without diagnosed diabetes, and not consuming a special diet. The study was approved by the University of Ulster Research Ethics Committee and all participants gave written informed consent.

Study design. The study was a parallel, single-blinded, placebo-controlled intervention trial. Participants were stratified by sex and age and randomly assigned to receive either aleurone-rich cereal products (aleurone group) or control cereal products (control group). During the 4-wk intervention, participants were asked to incorporate 1 portion of ready-to-eat (RTE) cereal and 2 bread roll portions/d into their habitual diets. Fasting venous blood samples and weight measurements were taken at baseline and postintervention (4 wk). Cereal products were supplied weekly and compliance was monitored by self-reported records and by the collection of unused or empty packets at the end of each week. Dietary intakes were assessed with 4-d food diaries before (baseline) and during wk 3 of the intervention. Energy, nutrient, and fiber intakes were estimated by using dietary analysis software (Weighed Intake analysis Software Package for Windows, version 3.0, Tinuviel Software) to which choline and betaine values were added (3). Reported energy intakes from the dietary records were compared against estimated energy requirements calculated from published sex- and age-specific equations (24) using a corresponding activity level of "low active."

Cereal products. Aleurone-enriched bread (in the form of rolls) and extruded RTE cereal products were developed for use in this study. Each cereal portion contained 9 g of aleurone (Bühler), resulting in a nominal dose of 27 g/d aleurone. The control bread and RTE cereal products were formulated with similar energy, macronutrient, and fiber contents and portion weights as their aleurone counterparts, using wheat starch (Meritena 200, Syral), wheat gluten (AG110, Syral), wheat fiber (Vitacel), and palm oil. The major ingredient in all products was refined (white) wheat flour. The RTE cereal (Bühler) was portioned into individual packages for distribution to participants. Bread (Barilla) was packaged and stored at −20°C until required. Products were analyzed for folate by a microbiological assay (25) (University of Helsinki), betaine and choline by NMR (26) (Rothamsted Research), and riboflavin and vitamin B-6 by HPLC using Comité Européen de Normalisation standard reference methods (Eurofins Steins Laboratorium).

Blood sampling and biochemical measurements. Fasting blood samples were collected into serum separator preevacuated blood tubes for lipid analyses and EDTA-containing preevacuated blood tubes for other analyses. Samples were held on ice until centrifugation (3°C, 1000 × *g* for 15 min) within 4 h and stored at −70°C until analysis. Plasma choline, betaine, dimethylglycine (DMG), tHcy, methionine, riboflavin, and pyridoxal 5'-phosphate (PLP), the main active derivative of vitamin B-6, were determined by liquid chromatography-tandem MS (27,28) and plasma folate by a microbiological assay (29). These analyses were carried out by Bevital AS. Serum total cholesterol, HDL cholesterol, and triacylglycerol were measured by standard kits (Randox) using an automatic centrifugal clinical chemistry system (Ilab 650 Clinical Chemistry System, Instrumentation Laboratory); LDL cholesterol was calculated (30).

Statistics. Sample size calculations based on Fenech et al. (31) indicated that 36 participants in each treatment group would give sufficient power ($P < 0.05$; 80% power) to detect significant effects on plasma tHcy. We increased the participant numbers to 40 to allow for drop-outs. Postintervention data (4 wk) were compared by ANCOVA using the General Linear Model with baseline data as a covariate. Data with skewed distribution were transformed logarithmically prior to analyses. Simple and multiple linear regression were used to determine relationships between variables and independent t-tests to evaluate differences in reported compliance between groups. SPSS 11.5 for Windows was used for all statistical analyses. Results are expressed as mean ± SEM and differences were considered significant at $P < 0.05$.

Results

Compliance and baseline profile. One female dropped out for medical reasons not related to the study, and 79 participants completed the intervention (**Supplemental Fig. 1**; **Table 1**). Reported compliance was good and participants consumed 95.9 ± 1.2% of the aleurone products and 96.3 ± 1.1% of the control products ($P = 0.77$).

Analyses of cereal products. Analysis of the cereal products indicated that the aleurone and control products were similar in macronutrient and fiber contents (**Table 2**). From the compliance data, we estimated that mean micronutrient intakes from these products per day for the aleurone and control groups, respectively, were: choline, 74 vs. 35 mg; betaine, 487 vs. 208 mg; folate, 99 vs. 61 μg; riboflavin, 0.08 vs. 0.04 mg; and vitamin B-6, 0.32 vs. 0 mg.

Dietary intakes and changes in body weight. From the 4-d food diaries, participants reported consuming 80.2 ± 2.4% of their estimated energy requirements at baseline. Overall mean baseline daily intakes for betaine and folate were 127 ± 6 mg/d (range 40–347) and 265 ± 10 μg/d (range 4–41), respectively, and 16 (20%) participants reported consuming less than the recommended 200 μg/d folate (32). During the intervention, intakes of betaine, choline, folate, and vitamin B-6 were significantly higher in the aleurone group compared with control (**Table 3**) and betaine intake in the aleurone group was almost double the control. There were no significant differences in energy, fiber, or macronutrient intakes between groups and the intervention did not affect body weight (change from baseline data −0.15 ± 0.15 kg and −0.16 ± 0.18 kg for the aleurone and control group, respectively).

Plasma betaine, choline, and B vitamins. The overall mean baseline plasma betaine and choline concentrations were 33.9 ± 0.8 μmol/L and 8.8 ± 0.2 μmol/L, respectively. Plasma riboflavin and vitamin B-6 were within normal ranges and no participants had riboflavin < 5.0 nmol/L or PLP < 20 nmol/L (33). The overall mean baseline plasma folate concentration was 13.5 ± 0.8 nmol/L and folate deficiency [plasma folate < 7 nmol/L (32)] was evident in 10 participants. Postintervention plasma betaine and DMG concentrations were significantly

TABLE 1 Participant characteristics

	Aleurone group	Control group
n	39	40
Gender, *% male*	51	50
Age, *y*	51.5 ± 0.8	51.8 ± 0.8
BMI, *kg/m^2*	28.7 ± 0.6	29.0 ± 0.5

[1] Values are means ± SEM.

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TABLE 2 Weights and energy, macronutrient, fiber, and micronutrient contents of cereal products

	Aleurone products		Control products	
	RTE cereal	Bread	RTE cereal	Bread
	per portion			
Fresh weight, *g*	40	67	39	67
Dry weight, *g*	39	42	38	43
Energy, *kcal*	124	138	124	145
kJ	518	578	517	606
Protein, *g*	5.3	7.3	5.1	7.4
Carbohydrate, *g*	26.6	22.6	26.5	24.4
Starch, *g*	24.8	18.6	24.8	20.4
Sugars, *g*	1.8	4.0	1.7	4.0
Fat, *g*	0.3	2.7	0.4	2.7
Fiber,[1] *g*	5.1	6.5	5.7	6.7
Betaine, *mg*	123	193	28	94
Choline, *mg*	11	33	6	15
Folate, *μg*	20	41	6	29
Riboflavin, *mg*	0.01	0.04	0	0.02
Vitamin B-6, *mg*	0.13	0.10	0	0

[1] Englyst method.

higher in the aleurone group compared with control (**Table 4**), and there was a significant positive correlation between changes in plasma betaine and DMG ($P < 0.001$; R^2=0.34). Plasma concentrations of the other tHcy-related micronutrients, choline, folate, riboflavin, and PLP did not differ between groups.

Plasma tHcy and methionine. The overall mean baseline plasma tHcy concentrations were 10.1 ± 0.3 μmol/L (range 6.0–20.2) and 2 of the participants were hyperhomocysteinemic at baseline (i.e. tHcy >15 μmol/L) (34). Compared with the control group, the aleurone group had significantly lower postintervention plasma tHcy and higher methionine concentrations (Table 4) and the overall effect size of the intervention on tHcy was −0.7 μmol/L. A median split of participants in the aleurone group into lower and higher baseline plasma folate indicated a mean change in tHcy concentration of −0.9 μmol/L (n = 19) and 0.1 μmol/L (n = 20) in the lower and higher plasma folate groups, respectively.

TABLE 3 Estimated daily intakes at baseline and during a 4-wk intervention with aleurone-rich or control cereal products[1,2]

	Aleurone group, n = 39		Control group, n = 40		P[3]
	Baseline	Intervention	Baseline	Intervention	(ANCOVA)
Energy, *kcal*	2036 ± 83	2044 ± 78	2122 ± 97	2074 ± 92	0.51
kJ	8520 ± 347	8551 ± 327	8878 ± 408	8678 ± 386	
Carbohydrate, *g*	243 ± 12	240 ± 10	249 ± 11	246 ± 9	0.92
Starch, *g*	139 ± 6	142 ± 6	140 ± 6	144 ± 6	0.90
Fat, *g*	80.4 ± 4.4	79.5 ± 3.9	85.2 ± 4.5	75.7 ± 4.9	0.13
Protein, *g*	83.3 ± 3.8	90.6 ± 3.4	87.3 ± 4.0	92.0 ± 3.6	0.82
Fiber,[4] *g*	14.0 ± 0.6	26.8 ± 0.7	15.5 ± 0.8	29.4 ± 0.8	0.12
Betaine, *mg*	127 ± 9	551 ± 10	128 ± 10	289 ± 12	<0.001
Choline, *mg*	284 ± 13	363 ± 19	303 ± 17	310 ± 19	0.002
Folate, *μg*	258 ± 12	281 ± 11	273 ± 16	257 ± 11	0.005
Riboflavin, *mg*	1.76 ± 0.08	1.53 ± 0.09	1.81 ± 0.11	1.52 ± 0.07	0.77
Vitamin B-6, *mg*	2.15 ± 0.09	2.09 ± 0.09	2.29 ± 0.13	1.88 ± 0.10	0.019

[1] Values are means ± SEM.
[2] Data from self-reported 4-d food diaries collected prior to (baseline) and during wk 3 of the intervention.
[3] Comparison of intervention data between groups.
[4] Englyst method.

Serum lipids. Serum total, LDL, and HDL cholesterol and triacylglycerol concentrations were within normal ranges. Postintervention serum LDL-cholesterol was significantly lower in the aleurone group than in the control group, but the intervention did not affect other lipids (Table 4).

Discussion

These results show that incorporating products enriched with the aleurone fraction of wheat into a habitual diet significantly increases both intake and plasma concentrations of betaine. These results support preliminary findings from our postprandial work (23) and indicate that wheat aleurone may be a good source of dietary betaine. Baseline betaine intakes and plasma betaine concentrations were comparable to other studies, which reported mean intakes of 100–314 mg/d (35–37) and plasma concentrations of 31.4–40.7 μmol/L (12,38,39). However, unlike other studies with supplements that used much larger doses of betaine (1–6 g/d) to give significant increases in plasma betaine concentrations (>20 μmol/L) (9,12), we found a significant increase in plasma betaine (5.2 μmol/L) with a difference in betaine intake of 279 mg/d betaine. Furthermore, to our knowledge, the only other study that has resulted in elevated plasma betaine without the use of supplements was a 2-wk intervention that compared food-derived betaine (~0.59 g/d additional betaine) and betaine supplementation (1 g/d) and showed similar increases in plasma betaine (~20 μmol/L increase from baseline) irrespective of the source of betaine (40).

The increases in betaine intake and plasma betaine were accompanied by a significant decrease in plasma tHcy and significant increases in plasma methionine and DMG. Homocysteine metabolism can occur through 3 pathways: the betaine-homocysteine methyltransferase pathway, requiring betaine or betaine derived from choline and yielding DMG and methionine; the 5-methyltetrahydrofolate pathway, which requires vitamin B-12 and folate and yields methionine; or the vitamin B-6–dependent transsulfuration pathway, which yields cysteine. The lack of changes in plasma B vitamins and choline and the significant increase in DMG suggest that the main pathway by which tHcy has been lowered in this study is via the betaine-homocysteine methyltransferase pathway, predominately as a result of the higher betaine intake. The magnitude of the intervention effect size on tHcy was modest (−0.7 μmol/L). However, this effect was more pronounced in participants with lower baseline plasma folate (−0.9 μmol/L); this is consistent with previous work that suggests that the relationship between plasma betaine and tHcy is stronger when folate status is low (7,14). It is notable that a significant lowering of tHcy occurred over a relatively short time and in a small cohort where raised tHcy was not an inclusion criterion. Furthermore, the change in tHcy concentration was of a similar order of magnitude to those reported in previous interventions that used larger doses of betaine in participants with higher tHcy (tHcy effect sizes of intervention: −1.3 and −1.8 μmol/L for 1.5 (10) and 6 g/d (11) betaine, respectively for 6 wk). Additionally, Fenech et al. (31) included participants with elevated plasma tHcy and low plasma folate concentrations and observed that, following consumption of 67 g/d aleurone for 4 wk (2.5 times the level in the present study), the intervention effect size on tHcy was −0.8 μmol/L,

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TABLE 4 Plasma betaine and related compounds, and serum lipids at baseline and after a 4-wk intervention with aleurone-rich or control cereal products[1]

	Aleurone group, $n = 39$		Control group, $n = 40$		
	Baseline	4 wk	Baseline	4 wk	P^2 (ANCOVA)
Betaine, *μmol/L*	33.7 ± 1.3	38.4 ± 1.4	34.1 ± 1.0	33.6 ± 1.0	<0.001
Choline, *μmol/L*	9.0 ± 0.3	9.1 ± 0.3	8.6 ± 0.3	8.7 ± 0.3	0.53
DMG, *μmol/L*	3.5 ± 0.2	4.0 ± 0.2	3.5 ± 0.1	3.3 ± 0.1	<0.001
Folate,[3] *nmol/L*	13.1 ± 1.1	11.5 ± 0.8	13.9 ± 1.2	13.4 ± 1.4	0.69
Riboflavin, *nmol/L*	20.7 ± 6.6	18.9 ± 5.7	17.7 ± 2.8	16.8 ± 2.2	0.64
PLP,[3] *nmol/L*	64.1 ± 5.1	65.1 ± 4.7	64.3 ± 4.2	63.7 ± 5.3	0.20
Methionine, *μmol/L*	28.8 ± 0.8	29.5 ± 0.7	29.1 ± 0.7	28.3 ± 0.6	0.027
tHcy, *μmol/L*	9.9 ± 0.5	9.5 ± 0.4	10.3 ± 0.4	10.6 ± 0.4	0.010
Total cholesterol, *mmol/L*	5.09 ± 0.16	5.04 ± 0.16	5.45 ± 0.18	5.38 ± 0.15	0.57
HDL cholesterol, *mmol/L*	1.45 ± 0.05	1.46 ± 0.06	1.49 ± 0.05	1.46 ± 0.05	0.43
LDL cholesterol, *mmol/L*	2.94 ± 0.12	2.87 ± 0.12	3.25 ± 0.14	3.28 ± 0.11	0.037
Triacylglycerol,[3] *mmol/L*	1.53 ± 0.18	1.57 ± 0.26	1.58 ± 0.15	1.42 ± 0.09	0.58

[1] Values are means ± SEM.
[2] Comparison of postintervention (4 wk) data between groups.
[3] Data not normally distributed.

with further decreases after 8 wk. These authors attributed the lowering in plasma tHcy to the high-folate content of aleurone, which provided ~615 μg/d folate but did not consider a potential role for betaine as a contributing factor (31). At present, there are no reports of intervention studies evaluating the impact of whole grains on tHcy; however, a cross-sectional study showed a strong inverse association between tHcy concentrations and whole grain intake ($P < 0.01$), an association not affected by adjustment for folate and other B vitamin intake (41).

Betaine supplementation has also been shown to increase plasma total and LDL cholesterol, possibly through the increased synthesis and availability of phosphatidycholine, which promotes VLDL production, leading to clearance of triacylglycerol from the liver (42). However, LDL cholesterol significantly decreased in the present study. The factors underlying this effect are unclear, but it may be a result of the lower dose of betaine used here compared with the doses used in supplementation studies. This suggestion is supported by an intervention study that showed a small decrease in LDL cholesterol on a high-betaine diet (0.59 g/d) but no change with a higher dose betaine supplement (1 g/d) (40). Also, a population-based study showed that plasma betaine concentrations were inversely associated with non-HDL cholesterol (43). Furthermore, our results are consistent with both cross-sectional and intervention studies that suggest that consumption of whole grain-rich diets are associated with lower concentrations of LDL cholesterol (41,44,45). However, it is possible that other components present in the aleurone, such as the phenolic acids, underlie the effects on LDL cholesterol (46).

In conclusion, this 4-wk intervention has shown that the incorporation of aleurone-enriched cereal products into habitual diets significantly increased plasma betaine and modestly lowered plasma tHcy and LDL cholesterol. This suggests that aleurone, and in particular the betaine component of aleurone, may play a role in the health benefits of whole grains. However, there is need for further longer term interventions using aleurone-rich or whole-grain foods.

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Supplemental Material can be found at: