British Journal of Nutrition (2012), **108**, 1644–1651
doi:10.1017/S0007114511007070
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Evaluation of the effect of wheat aleurone-rich foods on markers of antioxidant status, inflammation and endothelial function in apparently healthy men and women

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(Submitted 11 April 2011 – Final revision received 23 November 2011 – Accepted 28 November 2011 – First published online 16 January 2012)

Abstract

Observational data show an inverse association between the consumption of wholegrain foods, and inflammation and related diseases. Although the underlying mechanisms are unclear, wholegrains, and in particular the aleurone layer, contain a wide range of components with putative antioxidant and anti-inflammatory effects. We evaluated the effects of a diet high in wheat aleurone on plasma antioxidants status, markers of inflammation and endothelial function. In this parallel, participant-blinded intervention, seventy-nine healthy, older, overweight participants (45–65 years, BMI $> 25\,kg/m^2$) incorporated either aleurone-rich cereal products (27 g aleurone/d), or control products balanced for fibre and macronutrients, into their habitual diets for 4 weeks. Fasting blood samples were taken at baseline and on day 29. Results showed that, compared to control, consumption of aleurone-rich products provided substantial amounts of micronutrients and phytochemicals which may function as antioxidants. Additionally, incorporating these products into a habitual diet resulted in significantly lower plasma concentrations of the inflammatory marker, C-reactive protein ($P=0{\cdot}035$), which is an independent risk factor for CVD. However, no changes were observed in other markers of inflammation, antioxidant status or endothelial function. These results provide a possible mechanism underlying the beneficial effects of longer-term wholegrain intake. However, it is unclear whether this effect is owing to a specific component, or a combination of components in wheat aleurone.

Key words: Aleurone: C-reactive protein: Inflammation: Wheat: Wholegrains

Observational studies have consistently reported inverse relationships between wholegrain intake and diseases with an inflammatory aetiology, such as CHD[1], diabetes[2], cancer[3] and respiratory system disorders[4]. It is unclear how these effects are mediated, but the consumption of wholegrain cereals may exert anti-inflammatory effects and reduce oxidative stress. This is supported by data from cross-sectional studies, which show that markers of inflammation, including C-reactive protein (CRP), TNF-α, plasminogen activator inhibitor-1 and IL-6, are inversely related to wholegrain intake[5–9].

Nutritional interventions using foods or diets high in antioxidants are reported to lower plasma concentrations of inflammatory proteins, such as CRP, monocyte chemoattractant protein and IL-1α, and to decrease markers of endothelial function[10,11]. Wholegrain products contain a wide array of antioxidants, and Fardet[12] has identified over thirty compounds, or groups of compounds, including micronutrients and phytochemicals, which are known to function as antioxidants, at least *in vitro*. For example, tocols are direct radical scavengers that are present in wholegrain cereals mainly in the form of α-tocopherol and β-tocotrienol, and in some wheat genotypes as γ-tocopherol[13]. Zn, Mn, Cu and Se are cofactors for antioxidant enzymes and are also present at relatively high concentrations in wholegrain cereals[14]. More recently, the anti-inflammatory properties of betaine have been proposed[15,16]. Phenolic phytochemicals, and in particular ferulic acid, which have been closely associated with the *in vitro* antioxidant activity of grain fractions[17] have direct free-radical-scavenging activities, as well as anticarcinogenic and anti-inflammatory properties[10,18]. In wheat grain, the most commonly consumed cereal in many countries, these components are found at the highest concentrations in the aleurone layer[19], which also displays the greatest antioxidant capacity[17]. Wheat aleurone has relatively recently become available as a food ingredient through novel milling techniques[19].

Thus, to evaluate wheat aleurone as a novel food ingredient, we carried out a controlled intervention study in

Abbreviations: CRP, C-reactive protein; FRAP, ferric-reducing antioxidant potential; RTE, ready-to-eat.

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apparently healthy men and women using products enriched with wheat aleurone to assess effects on plasma antioxidant status, inflammation and markers of endothelial function. However, despite the strong observational evidence for a relationship between wholegrain intake and inflammation-related diseases, intervention studies in this area are limited, and the findings are inconsistent[20–24]. Consequently, although the primary aim was to assess the effects of wheat aleurone, this exploratory study may also provide insights into components and mechanisms underlying the longer-term effects of the increased consumption of wholegrain wheat.

Methods

Participants

A total of eighty apparently healthy participants, who were older (45–65 years) and overweight (BMI > 25 kg/m^2), and thus at increased risk of the metabolic syndrome, were recruited from the Northern Ireland population using the following inclusion criteria: in general good health with no current or recent serious illness, no use of prescription medicine or vitamin or mineral supplements, non-smokers, without diagnosed diabetes, and not on a special diet. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the University of Ulster Research Ethics Committee. Written informed consent was obtained from all participants, and the study was registered on the Current Controlled Trials Register (ISRCTN93336504).

Study design

The study was a parallel, participant-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-week intervention, participants were asked to incorporate one portion of ready-to-eat (RTE) cereal and two bread roll portions per day into their habitual diet. 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. Fasting venous blood samples and weight, and waist/hip measurements were taken at baseline and post-intervention (4 weeks). Waist circumference was measured midway between the lowest rib and the top of the iliac crest; and hip circumference was measured as the maximal circumference about the buttocks. Dietary intakes were assessed with 4 d food diaries before (baseline) and during week 3 of the intervention; and energy, nutrient and fibre intakes estimated using dietary analysis software (Weighed Intake analysis Software Package for Windows, version 3.0; Tinuviel Software).

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 AG), resulting in a nominal dose of 27 g aleurone/d. The control bread and RTE cereal products were formulated with similar energy, macronutrient and fibre contents, and portion weights as their aleurone counterparts as previously described[25]. Products were analysed for Zn, Mn and Cu by inductively-coupled plasma atomic emission spectrometry[26], for Se by inductively-coupled plasma-MS[27], for betaine by NMR[28], and for phenolic acids by HPLC-MS[29] (Rothamsted Research). Tocols were measured using normal-phase HPLC with fluorescence detection[13] (University of Helsinki, Finland), and total antioxidant potential was assessed using a modification[30] of the ferric-reducing antioxidant potential (FRAP) assay[31].

Blood sampling and biochemical measurements

Fasting blood was collected into serum separator pre-evacuated blood tubes for high-sensitivity-CRP and cholesterol analyses, lithium heparin-containing blood tubes for FRAP and ferulic acid analyses, and EDTA-containing pre-evacuated blood tubes for other analyses. Erythrocytes were prepared from a single EDTA-tube by washing in PBS for three cycles (2500 ***g***; 15 min; 4°C). All other samples were kept on ice until centrifugation (3°C, 1000 ***g***, 15 min), within 4 h, and stored at −70°C until analysis.

Plasma antioxidant micronutrients

Plasma samples were analysed for α-tocopherol and γ-tocopherol by reversed-phase HPLC, as previously described by Thurnham *et al.*[32] with minor modifications. Briefly, lipids were extracted and samples reconstituted in amber vials with mobile phase (470 ml acetonitrile; 470 ml methanol; 120 ml dichloromethane; 0·1 g butylated hydroxytoluene) before analysis by HPLC using an isocratic pump (model 1515), an autosampler (model 717), a Sunfire C18 3·5 μm, 4·6 × 100 mm column and photodiode array detector (model 2996; Waters Limited). The flow rate was 1·5 ml/min, the run-time was 13 min, and peaks were detected at 292 nm. The method was validated using standard reference material (SRM 968c, NIST) and α-tocopherol acetate (Sigma-Aldrich) was the internal standard. Data acquisition and processing were with Empower 2™ software (Waters Corporation). Intra-assay CV were <7 % and <25 %, and inter-assay CV were <7 % and <18 % for α- and γ-tocopherol, respectively.

Plasma samples were analysed for Se, Cu and Zn, using inductively-coupled plasma-MS (XSeries I; Thermo Fisher Scientific), with collision cell technology mode for sample analysis; radio frequency power: 1340 W; nebuliser gas flow: 0·88–0·94 litres/min; auxiliary gas flow: 0·7 litres/min; cool gas flow: 13 litres/min, collision cell technology gas flow (8 % H_2/He): 4 ml/min; plasma source: 99·9 % argon (BOC Limited); sample uptake rate: 0·4 ml/min. Plasma samples were prepared with 2 % HNO_3 (1:50), and calibration curves were prepared daily using pooled fasting plasma samples and a mixed standard. The method was validated using a standard reference serum (UTAK Laboratories, Inc.) with gallium

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nitrate (Romil Limited) as the internal standard. PlasmaLab software (Thermo Fisher Scientific) was used for data acquisition and processing. Intra-assay CV were less than 8, 5 and 6%, and inter-assay CV were less than 5, 4 and 5% for Se, Cu and Zn, respectively, and regression coefficients of all standard curves were ≥ 0·999.

Plasma ferulic acid

Ferulic acid was analysed using a modification of the method of Matsui *et al.*[33]. Briefly, plasma samples (80 µl) were prepared with starting mobile phase (20 µl), followed by enzymatic deconjugation[34]. Samples were analysed by LC-MS/MS (HPLC system (model 1200, Agilent Technologies), coupled to a triple quadrupole mass spectrometer (API-4000, Applied Biosystems) with ion spray source, using a Zorbax Eclipse XDB-C18 2.1 30 mm × 3·5 µm column (Agilent Technologies) and mass detection by simultaneous ion monitoring mode with mass:charge ratio of parent and daughter ions 192·70 and 133·90 *m/z*, respectively). The mobile phase was water–acetic acid (100:0·01, v/v; solvent A) and acetonitrile (100%; solvent B) and were used in the LC gradient elution system based on the following gradient programme (v/v): 0 min, 85% solvent A; 2 min, 5% solvent A; 11 min, 85% solvent A; total run-time, 15 min. Calibration curves were prepared daily using pooled fasting plasma samples spiked with *trans*-ferulic acid (Sigma-Aldrich). Data acquisition and processing were performed with Analyst software (V1.4, Applied Biosystems). The mean recovery of ferulic acid was 92% and CV for the intra- and inter-batch plasma controls were 2·9 and 5·3%, respectively.

Plasma antioxidant activity

Total plasma antioxidant activity was measured using the FRAP assay[31] modified for the Ilab 650 (Clinical Chemistry System; Instrumentation Laboratory). The mean CV for the intra- and inter-batch plasma controls were 0·7 and 4·2%, respectively. Erythrocyte superoxide dismutase activity was analysed colorimetrically (Ransod; Randox Laboratories Limited) and expressed per g of erythrocyte Hb. The CV for the intra- and inter-batch controls were 3·3 and 7·8%, respectively.

Markers of inflammation and endothelial function

Plasma IL-1β, IL-6 and IL-10, TNF-α and monocyte chemoattractant protein 1 were measured with ELISA (R&D Systems Europe Limited). CV for the intra- and inter-batch plasma controls, respectively, were IL-1β, 17·1, 12·6%; IL-6, 23·8, 12·6%; IL-10, 11·9, 15·7%; TNF-α, 7·8, 17·1% and monocyte chemoattractant protein 1, 5·6, 8·6%. Serum CRP was measured by an ultra-sensitive colorimetric reaction (Quantex; Randox Laboratories Limited) modified for the Ilab 650, and the inter-batch CV for the plasma control was 2·6%. Plasma intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 were measured by ELISA (R&D Systems Europe Limited) and CV for the intra- and inter-batch plasma controls, respectively, were intracellular adhesion molecule-1, 1·8, 3·5%, and vascular cell adhesion molecule-1, 3·1, 6·2%.

Statistical analysis

Post-intervention data (4 weeks) were compared using ANCOVA with baseline data as the covariate. Data with skewed distributions were transformed logarithmically before analyses. Independent *t* tests were used to evaluate differences in reported compliance. All analyses were carried out using the Statistical Package for the Social Sciences 11.5 for Windows (SPSS, Inc.) and the data summarised using the means with their standard errors. Effects have been considered statistically significant when $P<0·05$.

Results

Participant characteristics and compliance

Of the eighty participants, one female dropped out for medical reasons not related to the study, and seventy-nine participants completed the intervention (forty male; thirty-nine female, age 51·6 (SE 0·5) years, BMI 28·9 (SE 0·4) kg/m^2; waist:hip ratio 0·91 (SE 0·01) cm). Reported compliance was high, with participants consuming 95·9 (SE 1·2)% of the aleurone products and 96·3 (SE 1·1)% of the control products ($P=0·774$ between groups). There was no effect of intervention on body weight or waist:hip ratio (data not presented).

Table 1. Characteristics of the cereal products (per portion)

	Aleurone products		Control products	
	RTE cereal	Bread	RTE cereal	Bread
Fresh weight (g)	40	67	39	67
Dry weight (g)	39	42	38	43
Energy				
kJ	518	578	517	606
kcal	124	138	124	145
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
Fibre* (g)	5·1	6·5	5·7	6·7
Total tocols (mg)	123	193	28	94
α-Tocopherol (mg)	0·16	0·71	0·14	0·57
γ-Tocopherol (mg)	0	0·15	0	0·10
Zn (mg)	1·65	1·80	0·38	0·58
Mn (mg)	1·39	1·48	0·30	0·40
Cu (mg)	0·27	0·29	0·07	0·10
Se (µg)	3·6	5·6	2·3	4·5
Betaine (mg)†	123	193	28	94
Total phenolics (mg)	44·6	50·0	2·7	3·4
Total ferulic acid (mg)	34·8	39·6	2·0	2·4
Free (mg)	0·17	0·68	0·04	0·13
Conjugated (mg)	0·78	1·48	0·12	0·14
Bound (mg)	33·8	37·4	1·85	2·09
Antioxidant potential‡ (µmol FRAP)	212	355	52	102

RTE, ready-to-eat; FRAP, ferric-reducing antioxidant potential.
* Englyst method.
† Reported in Price *et al.*[25].
‡ Ferric-reducing antioxidant potential.

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Analyses of cereal products and dietary intakes

The aleurone and control products were similar in macronutrient and fibre contents (Table 1). However, aleurone products were higher in antioxidant micronutrients and phytochemicals than their control counterparts (Table 1). The most abundant phenolic in the aleurone products was ferulic acid (approximately 79 % of total phenolics present), which was mainly in the bound form (approximately 96 % of total ferulic acid present). Total antioxidant activity, as measured by FRAP, was >3 times higher in the aleurone products compared to the control products. From the compliance data, we estimated that mean micronutrient intakes from these products per day, for the aleurone and control groups, respectively, were: total tocols, 488 *v.* 208 mg; Zn, 5·0 *v.* 1·5 mg; Cu, 0·8 *v.* 0·3 mg; Mn, 4·3 *v.* 1·1 mg; Se, 14·2 *v.* 10·9 μg; and total phenolic acids, 139 *v.* 9 mg (ferulic acid, 109 *v.* 7 mg).

Data from the 4 d food diaries at baseline and during the intervention are shown in Table 2. Overall, mean baseline daily intakes for vitamin E were 8·0 (SE 0·4; range 1·8–20·1) mg/d; Zn, 9·7 (SE 0·3; range 4·5–17·8) mg/d; Mn, 3·8 (SE 0·2; range 1·4–10·3) mg/d; Cu, 1·2 (SE 0·1; range 0·6–4·0) mg/d; Se, 44·6 (SE 2·2; range 15–121) μg/d; and during the intervention, these intakes were significantly higher in the aleurone group (Table 2). There were no significant differences in intakes of energy, fibre or macronutrients between the groups ($P>0·121$).

Plasma antioxidant status

The intervention had no significant effect on fasting plasma concentrations of tocols, Se, ferulic acid, FRAP and superoxide dismutase (Table 3). Furthermore, there were no significant effects on fasting plasma concentrations of Zn or Cu (data not shown).

Markers of inflammation and endothelial function

Baseline plasma CRP concentrations ranged from 0·2 to 11·4 mg/l, with thirteen participants classed as high risk for future cardiovascular events (>3 mg/l)[35]. Post-intervention, there was a significant difference in plasma CRP between the groups, with concentrations about 25 % lower in the aleurone group compared to the control group (effect size of intervention: −1·06 mg/l; Table 3). There were no significant differences between the aleurone and control groups for any of the other markers for inflammation or endothelial function that were measured (Table 3).

Discussion

Results from the present study showed that, compared to refined grain-based control products, aleurone-rich products provided substantially more micronutrients and phytochemical compounds which have antioxidant functions. Additionally, the incorporation of these products into a habitual diet was associated with a small, but significantly lower, fasting plasma concentration of the inflammatory marker CRP.

CRP is an independent risk factor for CHD in both the general population and in high-risk groups[36–38]. Furthermore, CRP is one of the most sensitive acute-phase reactants and, in the absence of new stimuli, concentrations remain stable over long periods, thus making CRP a stable, clinically relevant biomarker[39]. Thus, small changes in CRP, similar to the effect size found in the present intervention (−1·06 mg/l), may represent a lowering in the clinical risk of cardiovascular events. This is supported by data from epidemiological studies. For example, in the Reykjavik Study[40], differences between the lower and upper tertiles of CRP (<0·78 *v.* >2·0 mg/l) were associated with a 1·45-fold (95 % CI 1·25, 1·68) increased risk of CHD. Moreover, in the Women's Health Study[41], comparisons of the first (<1·5 mg/l) and

Table 2. Estimated daily intakes of energy and selected micronutrients at baseline and during intervention with aleurone and control cereal products*

(Mean values with their standard errors)

	Aleurone group (*n* 39)				Control group (*n* 40)				
	Baseline	SE	Intervention	SE	Baseline	SE	Intervention	SE	*P*† (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	12·1	240	10·4	249	11·4	246	9·5	0·92
Starch (g)	139	5·7	142	6·2	140	6·2	144	5·8	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
Fibre‡ (g)	14·0	0·6	26·8	0·7	15·5	0·8	29·4	0·8	0·12
Vitamin E (mg)	7·7	0·6	15·0	0·5	8·3	0·6	13·2	0·6	0·001
Zn (mg)	9·4	0·5	11·5	3·0	10·1	0·4	9·5	0·4	<0·001
Mn (mg)	3·81	0·26	6·38	0·13	3·74	0·19	3·81	0·21	<0·001
Cu§ (mg)	1·20	0·10	1·49	0·07	1·23	0·09	1·18	0·07	<0·001
Se (μg)	43·5	2·9	76·2	2·4	45·7	3·4	69·4	2·9	0·02

* Estimated from 4 d food records.
† Between-group effects during intervention using baseline data as a covariate (ANCOVA).
‡ Englyst method.
§ Data not normally distributed.

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Table 3. Plasma measurements at baseline and after 4-week intervention with aleurone-rich or control cereal products
(Mean values with their standard errors, *n* 79)

	Aleurone group (*n* 39)				Control group (*n* 40)				
	Baseline	SE	4 weeks	SE	Baseline	SE	4 weeks	SE	*P* (ANCOVA)*
Antioxidant status									
α-Tocopherol† (μmol/l)	29·1	1·6	28·9	1·7	29·3	1·3	29·4	1·1	0·64
γ-Tocopherol (μmol/l)	1·94	1·4	1·67	1·4	1·96	1·1	1·95	1·1	0·06
Se (μg/l)	76·8	1·9	79·0	1·7	82·5	1·4	83·4	1·5	0·70
Ferulic acid† (μmol/l)	98·0	4·3	97·2	3·0	101·4	6·0	106·3	7·4	0·52
FRAP (μmol/l)	1149	40	1121	36·2	1180	37	1174	36	0·27
SOD (U/g Hb)	1506	47	1499	42	1465	35	1450	32	0·68
Inflammatory markers									
IL-1β† (pg/ml)	0·13	0·01	0·13	0·01	0·12	0·01	0·12	0·01	0·12
IL-6† (pg/ml)	1·70	0·45	1·49	0·35	1·22	0·11	1·58	0·30	0·16
IL-10† (pg/ml)	1·17	0·04	1·21	0·06	1·08	0·03	1·05	0·04	0·16
CRP† (mg/l)	2·32	0·42	1·94	0·34	1·74	0·20	2·42	0·39	0·035
TNF-α† (pg/ml)	1·72	0·09	1·66	0·08	1·47	0·06	1·42	0·05	0·30
MCP-1† (pg/ml)	184	7	166	6	172	8	165	7	0·55
Endothelial function									
ICAM-1 (ng/ml)	210	10	210	9	217	7	221	7	0·29
VCAM-1 (ng/ml)	503	23	554	24	512	22	504	21	0·08

FRAP, ferric-reducing antioxidant potential; SOD, superoxide dismutase; CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein; ICAM-1, intra-cellular adhesion molecule; VCAM-1, vascular cell adhesion molecule.
* Comparison of post-intervention (4 weeks) data between groups, using baseline data as a covariate (ANCOVA).
† Data not normally distributed.

second (1·5–3·7 mg/l) quartiles of baseline CRP concentrations showed associated increases in relative risk for CHD (2·4-fold) and myocardial infarction or stroke (3·5-fold). The effect size of intervention for CRP in the present study is consistent with and of the same order of magnitude as wholegrain and wholegrain-rich diet interventions which used longer durations (−2·6 mg/l after 12 weeks[24]; −1·0 mg/l after 2 years[11]), and pharmacological studies using statins which have reported significant lowering of CRP (e.g. −1·22 and −1·43 mg/l after 12 weeks[42]).

Plasma CRP concentration is associated with obesity and is reported to be lowered following weight loss[43]. However, we did not find any change in body weight or waist circumference following the intervention, indicating that the decrease in plasma CRP concentration was not owing to weight loss. Changes in CRP may also reflect alterations in plasma lipids, and a meta-analysis of studies using cholesterol-lowering drugs concluded that a 1 mmol/l decrease in LDL-cholesterol was associated with a 0·89 mg/l decrease in CRP[44]. We have previously reported a significant decrease in LDL-cholesterol. However, the effect size of intervention on LDL-cholesterol was only 0·1 mmol/l[25], suggesting that the decrease in CRP (effect size of intervention −1·06 mg/l) was much larger than that which could be explained by a lowering in LDL-cholesterol alone.

In the present study, we observed a significant lowering in CRP but no change in other inflammatory markers after feeding an aleurone-rich diet for 4 weeks, findings similar to an earlier 12-week intervention using a wholegrain-enriched hypoenergetic diet[24]. Production and regulation of CRP takes place predominantly in the liver[45]. The change in CRP observed in the present study may therefore reflect alterations in hepatic metabolism resulting from consumption of the aleurone products. This hypothesis is suggested by previously reported data that showed an increase in plasma betaine, and a decrease in plasma homocysteine concentrations, which was attributed to alterations in the activity of the betaine–homocysteine methyltransferase enzyme, found mainly in the liver[25]. Plasma homocysteine concentrations have been positively associated with inflammation[46] and betaine, the methyl donor in the betaine–homocysteine methyltransferase pathway, has been hypothesised as an anti-inflammatory nutrient[16]. Moreover, betaine also functions as a lipotrope and may have a role as a therapeutic agent in non-alcoholic fatty liver disease[47] and increased CRP concentrations[48].

Although increased betaine status provides a plausible mechanism for the lowering of CRP, other components, such as alkylresorcinols, phytic acid, Zn, Mn, Cu or ferulic acid may have effects which underlie this anti-inflammatory response[12]. However, interactions with the phytic acid present in the aleurone[19] may have decreased the availability of Zn, Mn and Cu. Furthermore, previous studies indicate that the availability of ferulic acid may be primarily restricted to free ferulic acid. For example, an *in vitro* study using a model of the upper gastrointestinal tract found that the bioaccessibility of ferulic acid was less than 1 % of the total ferulic acid in wheat bran, wheat aleurone, and bread with 50 % wheat aleurone[49]. Furthermore, a study involving human subjects showed that the postprandial increase in plasma ferulic acid following consumption of 100 g wheat bran RTE cereal was chiefly owing to the absorption of free ferulic acid, which was less than 2 % of total ferulic acid[50]. Free ferulic acid also amounted to less than 2 % of the total in the aleurone products in the present study; this indicates that, despite the large increase in total ferulic acid intake, most of

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this was not available for absorption from the small intestine. However, Vitaglione *et al.*[51] have suggested that free ferulic acid is released by bacterial activity in the colon, and may be absorbed. Moreover, in contrast to the present results, consumption of 48 g/d of wholegrain RTE cereal, or wheat bran-based RTE cereals for 3 weeks, resulted in more than 2-fold increases in fasting plasma ferulic acid, which was attributed to the colonic absorption of ferulic acid released through microbial metabolism[52]. Taken together, these results indicate that the role of colonic events in the bioavailability of ferulic acid requires further study, and that it may be influenced by factors related to the food matrix, and/or the method of preparation.

Alternatively, it may be that no single anti-inflammatory component is responsible for the lowering of CRP, but rather that the effects are owing to the range of potentially bioactive components, provided by the aleurone products, and which may act independently, or by overlapping or interacting mechanisms. In support of this contention, Gaskins *et al.*[6] noted that the inverse association between wholegrain intake and CRP was not affected by adjustment for any specific wholegrain nutrient. Moreover, a strong inverse association has been shown between the total antioxidant capacity of the diet and CRP, but not with any single antioxidant compound[53].

However, although our results are scientifically plausible, and in keeping with other work, our study was an exploratory analysis with multiple endpoint measures, and thus, in line with a number of similar studies[20,22,23,54,55], Bonferroni corrections were not applied in the data analysis. When a Bonferroni correction is applied ($P<0{\cdot}002$), the effect on CRP concentration is no longer significant, and thus it is possible that there is a type 1 error.

In conclusion, this 4-week intervention has shown that the incorporation of aleurone-enriched cereal products into habitual diets results in significantly lower circulating concentrations of the inflammatory marker CRP, an independent risk factor for CVD. However, no changes were found in other markers of inflammation, antioxidant status or endothelial function. These results suggest a putative mechanism underlying the epidemiological evidence for the beneficial effects of wholegrain intake. However, whether the amelioration of CRP levels is attributable to one, a number, or a combination of components in aleurone, or whether the potential anti-inflammatory effect contributes to the observed beneficial effects of diets rich in wholegrain, requires further study.

Acknowledgements

The present study was financially supported by the European Commission in the Communities 6th Framework Programme, project HEALTHGRAIN (FOOD-CT-2005-514008). The authors are solely responsible for the work described in this article, and their opinions are not necessarily those of the European Union. The contributions of the authors were as follows: R. K. P., J. M. W. W., L. L. H., E. M. K., J. J. S. and R. W. W. designed the research; R. K. P., L. L. H. and E. M. K. conducted the research; R. K. P., L. L. H. and E. M. K. analysed the data; R. K. P., J. M. W. W., L. L. H., E. M. K., J. J. S. and R. W. W. contributed to interpretation of the data; M. J. P. provided statistical advice; R. K. P., J. M. W. W., L. L. H., E. M. K., J. J. S. and R. W. W. wrote the paper; R. K. P. had primary responsibility for the final content. All authors read and approved the final manuscript. There were no conflicts of interest. The authors thank Mr Walter von Reding and Ms Cäcilia Spoerndli, Bühler AG, Switzerland, for the provision of the aleurone and RTE cereals used in this study, Ms Michela Petronio and Mr Giancarlo Riboldi, Barilla G.e R. Fratelli, Italy for the production of the bread products, and Rettenmaier, Germany and Syral, Belgium for the provision of wheat fibre, starch and protein. The authors also thank Professor Peter Shewry and Dr Jane Ward (Centre for Crop Genetic Improvement, Rothamsted Research, UK) for the micronutrient and phenolic analyses of the foods, Professor Vieno Piironen (Department of Food and Environmental Services, University of Helsinki) for tocol analyses of foods, Dr Stephen McClean for his advice and assistance with LC/MS-MS, and Miss Danielle Graham and Mrs Ramandeep Garg for help in carrying out the intervention.

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