

## Short communication

# Wheat bran supplementation does not affect biochemical markers of bone turnover in young adult women with recommended calcium intake

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We investigated the effect of wheat bran on biochemical indicators of Ca and bone metabolism in nineteen healthy women, aged 25.5 (SE 0.9) years. Subjects received six wheat bran biscuits or six white flour biscuits per day for a period of 4 weeks (crossover). Wheat bran consumption increased fibre intake from 17.7 (SE 1.3) to 29.6 (SE 1.3) g/d (7 d food record) and enhanced P intake from 1225 (SE 59) mg/d to 1663 (SE 65) mg/d;  $P < 0.001$ . Mean daily Ca intake during wheat bran consumption (1110 (SE 82) mg/d) significantly ( $P = 0.008$ ) exceeded Ca ingestion during the white flour period (955 (SE 67) mg/d). Wheat bran increased the number of defecations per week from 7.9 (SE 0.8) to 12.2 (SE 1.4) ( $P = 0.0018$ ). Urinary Ca excretion over 24 h significantly ( $P = 0.021$ ) decreased from 473 (SE 53)  $\mu\text{mol}/\text{mmol}$  creatinine (control period) to 339 (SE 37)  $\mu\text{mol}/\text{mmol}$  creatinine (wheat bran period). Serum 25-hydroxyvitamin D, 2 h fasting urinary Ca/creatinine excretions and 24 h urinary P excretion remained constant. No differences in serum levels of carboxy-terminal propeptide of type I procollagen (biomarker of bone formation) or in 2 h fasting urinary hydroxyproline/creatinine excretions (biomarker of bone resorption) were observed at the end of the two cycles of dietary supplementation. We conclude that a high fibre intake of approximately 30 g/d has no significant adverse effects on bone turnover in subjects with Ca intakes above 1000 mg/d and that the reduction in 24 h urinary Ca excretion is most probably the result of an adaptation process, induced by a decrease in net absorbed Ca.

### Wheat bran: Calcium: Bone turnover

Recommendations for dietary fibre intake differ between European countries. Diets should contain an average of 18 g fibre/d in Great Britain (Department of Health, 1991). The German Society for Nutrition has recommended a minimum intake of 30 g fibre/d (Deutsche Gesellschaft für Ernährung, 1991), while mean fibre intake of female adults is 23 g/d in Germany (Adolf *et al.* 1994). Since cereals are high in fibre content, cereal products can be used to meet an intake of 30 g fibre/d.

Fibre concentrates like bran increase stool weight and are useful in preventing or treating constipation (Wisker *et al.* 1988). Moreover, cereal fibre may protect against large-bowel cancer (McIntyre *et al.* 1993). However, diets high in cereal fibre may also adversely affect Ca retention (Spiller *et al.* 1986). It has been demonstrated in several studies that

wheat bran and high-fibre diets decrease Ca absorption (Balasubramanian *et al.* 1987; Knox *et al.* 1991; Weaver *et al.* 1991). This effect may be the result of the high Ca-binding capacity of wheat bran (Weaver *et al.* 1996). Moreover, there is evidence that the vitamin D-mediated mechanism for active absorption of dietary Ca may be diminished by dietary fibre: in subjects receiving an additional 20 g dietary fibre/d from wheat bran, the plasma half-life of tritium-labelled 25-hydroxyvitamin D (25-OH-D) was reduced by 30% (Batchelor & Compston, 1983). The plasma or serum level of 25-OH-D is an important predictor of Ca absorption efficiency, as recently demonstrated in premenopausal women (Zittermann *et al.* 1998). Moreover, the occurrence of rickets and osteomalacia in Asian immigrants living in Great Britain was associated with a

**Abbreviations:** Cr, creatinine; 25-OH-D, 25-hydroxyvitamin D; OHP, hydroxyproline; PICP, carboxy-terminal propeptide of type I procollagen.

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high intake of fibre-rich chapatties (unleavened bread) (Ford *et al.* 1972).

An impairment of Ca balance and bone metabolism may also enhance the risk of osteoporosis by increasing bone loss in elderly subjects or by reducing peak skeletal mass in young subjects.

Reliable biochemical variables like serum levels of carboxy-terminal propeptide of type I procollagen (PICP) and renal hydroxyproline (OHP<sub>r</sub>) are available for the measurement of bone formation and resorption processes (Nordin *et al.* 1976; Ericksen *et al.* 1993). Thus, it was the aim of the present study to investigate the effect of a high fibre intake in the form of wheat bran on Ca metabolism and biomarkers of bone turnover in young female subjects.

## Materials and methods

### Subjects

Twenty healthy women with a BMI > 18 kg/m<sup>2</sup> and < 25 kg/m<sup>2</sup> were enrolled in the study. Participants had a mean age of 25.7 (SE 1.0) years. Height was 1.69 (SE 0.02) m and body weight was 58.4 (SE 1.4) kg. Subjects gave written informed consent to the investigations, in accordance with the Helsinki declaration.

### Study protocol

The study was performed during wintertime (latitude: 51°N). Each dietary period lasted 28 d. Subjects were randomly assigned to two groups of ten subjects. By using a crossover design (Fig. 1), participants received a dietary supplement of six white wheat flour biscuits per day (period A) or six wheat bran biscuits per day (period B), while they remained on their habitual diet. The biscuits were made of eggs, butter, salt, sucrose, deionized water and white wheat flour or a commercial wheat bran product (Dr. Kousa Weizenkleie, Germany) as ingredients. Six bran biscuits contained 14.2 g fibre (12.8 g insoluble and 1.4 g soluble fibre) and six white flour biscuits contained 0.6 g fibre, as calculated from food tables (Souci *et al.* 1994). Each subject had to complete prospective 7 d food records during periods

A and B on days 8–15 and 35–42 respectively. A standardized protocol was used as described elsewhere (Zittermann *et al.* 1998). Subjects had to register the number of defecations on the protocol sheet each day.

Urine specimens (24 h) were collected before the study began (day 0) and at the end of each period on days 28 and 56 of the study. Samples were collected from 07.00 hours until 07.00 hours of the following day. On that following morning (days 1, 29 and 57) blood was withdrawn from the vena cubitalis after an overnight fast (serum monovettes). A 2 h fasting urine sample (second spontaneous urine) was collected at 09.00 hours (before breakfast). Portions of samples were frozen consecutively at –20° until analysis.

### Analytical procedure

All samples were measured in duplicate during the same assay sequence. Serum 25-OH-D was analysed using a radioimmunoassay. The method is based on an antibody with specificity to 25-OH-D (Hollis *et al.* 1993). The intra-assay CV was 6.7%. Serum PICP levels were measured by means of a sandwich ELISA supplied by Hermann Biermann GmbH (Bad Nauheim, Germany). Briefly, standards and samples were incubated on a microtitre plate coated with monoclonal mouse anti-PICP, followed by a rabbit anti-PICP and an alkaline phosphatase-conjugated anti-rabbit immunoglobulin G. After addition of substrate solution (*p*-nitrophenylphosphate) absorbance was read at 405 nm. The CV was 7.2%. Alkaline phosphatase (*EC* 3.1.3.1) activity was determined using a kinetic test (Rick, 1977) with an imprecision of 4%. Ca was measured by atomic absorption spectrometry (Model 420; Perkin Elmer & Co. GmbH, Ueberlingen, Germany) at 422.7 nm after sample dilution (1 : 101) with a SrCl<sub>2</sub> solution (Paschen, 1970). The CV was 2.2%. P was analysed after acidification of urine samples with 68.5 mM-HCl by a colorimetric assay at 690 nm (Goldenberg & Fernandez, 1966). The CV was 2.8%. Fasting urinary OHP<sub>r</sub> was measured by a colorimetric reaction with dimethylaminobenzaldehyde after chloramine T oxidation and after 16 h of resin-catalysed hydrolysis of peptide-bound OHP<sub>r</sub> (Goverde & Veenkamp, 1972). The CV was 6.2%. Urinary creatinine (Cr) was measured by the Jaffé

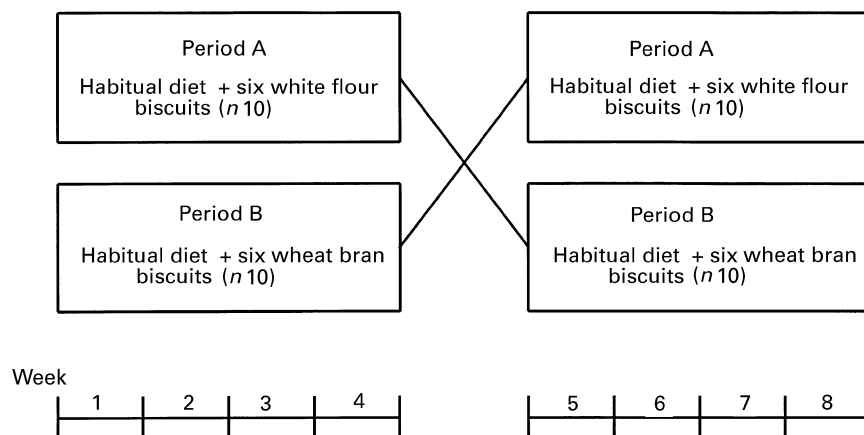


Fig. 1. Study design.

reaction. Results for urinary Ca, P and OHP<sub>r</sub> were expressed in relation to urinary Cr excretion.

### Statistics

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS/PC+, version 8.0; SPSS Inc., Chicago, IL, USA). Data were tested for homogeneity of variance using the Kolmogorov-Smirnov test. Two-tailed Student's *t* test for paired values was used for the comparison of two groups with normally distributed values. Otherwise, the Wilcoxon test was performed. *P* values below 0.05 (two-tailed test) were considered as significant. The statistical power ( $\alpha$  0.05;  $\beta$  0.80) was sufficient to detect differences of 22 % in 25-OH-D, 6.5 % in alkaline phosphatase, 11.8 % in PICP, 14.7 % in OHP<sub>r</sub>, 15 % in 24 h renal Ca/Cr excretion and 31 % in fasting renal Ca/Cr excretion. Data are presented as means with their standard errors.

### Results

One subject was excluded from the study, since she reported skin irritations during intake of the bran biscuits due to an allergic reaction. The remaining subjects had a mean increase in cereal fibre intake of 11.9 g/d during wheat bran consumption (Table 1). P intake significantly increased during period B. Higher intake was associated with the ingestion of phytic acid from the wheat bran biscuits. Ca intake during period B was slightly higher than during period A. Energy and vitamin D intakes were similar during the two recording periods.

Wheat bran consumption resulted in a 28 % decrease in

24 h urinary Ca concentrations (Table 1) with a reduction in the mean daily Ca excretion (24 h Ca/Cr  $\times$  24 h Cr excretion) of 1.28 mmol (51.3 mg). The 2 h fasting urinary Ca levels, and 24 h urinary P concentrations remained constant. Serum levels of 25-OH-D and Ca were similar during both dietary periods. No changes in alkaline phosphatase, PICP or OHP<sub>r</sub>/Cr concentrations occurred.

The mean number of defecations increased significantly from 7.9 (SE 0.8) per week (period A) to 12.2 (SE 1.4) per week (period B) ( $P < 0.0018$ ).

### Discussion

Recommended dietary allowances should guarantee optimal functioning of those physiological reactions of the human body which depend on the specific nutrient, without putting the subject at risk of undesirable side-effects due to that nutrient or food constituent. In regard to dietary fibre the optimal intake level is still questionable. As a consequence, there are different recommendations in European countries. For this reason, the habitual fibre intake of the study group (Table 1) was adequate compared with British recommendations (Department of Health, 1991) but was low in regard to German recommendations (Deutsche Gesellschaft für Ernährung, 1991).

The increase in total fibre intake to approximately 30 g/d during supplementation of the diet with bran (Table 1) enhanced the number of defecations and was, thus, effective in preventing constipation. However, high cereal fibre intake also affected Ca metabolism, as shown by a reduction in 24 h Ca excretion (Table 1). Since fasting urinary Ca excretion, as an indicator of Ca loss from bone (Nordin *et al.* 1976), did not change significantly (Table 1), results for

**Table 1.** Nutrient intakes and biochemical markers of calcium and bone metabolism in subjects consuming six white wheat flour biscuits (period A) or six wheat bran biscuits (period B) per day  
(Mean values with their standard errors for nineteen subjects)

	Period A		Period B		Statistical significance of difference between means: <i>P</i> =
	Mean	SE	Mean	SE	
<b>Energy and nutrient intakes</b>					
Energy (kJ/d)	7358	278	7576	405	0.62
Calcium (mg/d)	955	67	1110	82	0.008
Phosphorus (mg/d)	1225	59	1663	65	< 0.001
Vitamin D ( $\mu$ g/d)	2.0	0.4	1.7	0.16	0.65
Fibre, total (g/d)	17.7	1.3	29.6	1.3	< 0.001
Phytic acid (mg/d)	378	31	1368	47	< 0.001
<b>Serum variables</b>					
25-OH-D (nmol/l)	52.3 (87.5)*	6.8	56.8 (95.0)	8.8	0.40
Calcium (mmol/l)	2.31 (101.0)	0.03	2.27 (99.1)	0.05	0.31
AP (U/l)	78.9 (98.3)	4.4	81.6 (101.6)	4.7	0.58
PICP (ng/ml)	97.4 (98.7)	8.6	95.7 (97.0)	5.8	0.88
<b>Urine variables</b>					
24 h volume (litres)	1.77 (98.3)	0.22	1.86 (103.3)	0.21	0.44
24 h Cr (mmol/l)	7.20 (105.7)	0.7	7.52 (110.4)	0.8	0.67
24 h Ca/Cr ( $\mu$ mol/mmol)	473 (141.2)	53	339 (101.2)	37	0.021
24 h P/Cr (mmol/mmol)	2.77 (95.5)	0.14	2.82 (97.3)	0.24	0.87
2 h fasting Ca/Cr ( $\mu$ mol/mmol)	219 (136)	40	171 (106.2)	26	0.26
2 h fasting OHP <sub>r</sub> /Cr ( $\mu$ mol/mmol)	10.7 (84.3)	0.9	10.5 (82.7)	1.2	0.86

25-OH-D, 25-hydroxyvitamin D; AP, alkaline phosphatase; PICP, carboxy-terminal propeptide of type 1 procollagen; Cr, creatinine.

\* Values in parentheses represent the mean value as a percentage of the baseline value.

Ca metabolism indicate a reduced excretion of net absorbed Ca. The slightly higher Ca intake during the period of bran consumption (Table 1) led us to assume that a reduction in the amount of ingested Ca was not responsible for the observed effect. Another explanation may be that additional amounts of absorbed P can decrease renal Ca output via mild secondary hyperparathyroidism and can increase endogenous faecal Ca loss (Draper & Bell, 1979). However, the unchanged renal P excretion during wheat bran consumption (Table 1) indicates that the extra amount of consumed P was not absorbed. This low bioavailability of P may be due to that fact that most of the P content in cereals is present as part of phytic acid. As a constituent of phytic acid, unabsorbed P can form insoluble Ca salts, which result in low bioavailability of Ca: 1 g wheat bran can bind approximately 0.45 mmol Ca at pH 8.0 and this effect has been attributed to the phytic acid content of the bran (Heynck *et al.* 1995). Thus, the markedly enhanced intake of phytic acid during bran consumption (Table 1) may have decreased net absorbed Ca.

The unchanged 25-OH-D serum levels (Table 1) indicate that the intrinsic ability to absorb Ca did not change. Data confirm results on 25-OH-D serum levels obtained in young male subjects after supplementation of the diet with wheat bran (O'Brien *et al.* 1993). In that study, wheat bran did not change fractional Ca absorption, measured in the fasting state by a radiocalcium test. However, apparent absorption, as calculated by the difference between daily Ca intake and daily faecal excretion, was reduced by 23.5% during the high-fibre diet in that study (O'Brien *et al.* 1993) supporting the assumption that the lower Ca bioavailability is the result of gastrointestinal Ca binding by food constituents.

It may well be that the reduction in plasma half-life of 25-OH-D, which has been observed after cereal fibre consumption (Batchelor & Compston, 1983), can be compensated by an enhanced 25-hydroxylation of the parent vitamin D. Vitamin D is stored in significant amounts in adipose tissue (Holmes & Kummerow, 1983). It may also be that a high fibre intake *per se* does not have an effect on 25-OH-D metabolism. There is evidence that, through a poorly understood mechanism, a low gastrointestinal Ca uptake is the primary stimulus for a depletion of 25-hydroxyvitamin D (Vieth *et al.* 1987), while serum 25-OH-D levels are enhanced after 6–7 weeks of daily supplementation with 2 g Ca (Berlin & Björkhem, 1988). Probably the supplementation period of our investigation was too short to detect a change in serum 25-OH-D levels or the adverse effect of wheat bran consumption on Ca metabolism was too small to significantly influence serum 25-OH-D levels.

The unchanged concentrations of renal OHPr and serum PICP indicate that wheat bran had no significant effect on bone turnover. We cannot rule out the possibility that the statistical power of our measurements was not sufficient to detect small changes in bone metabolism due to an effect of wheat bran on Ca metabolism.

Improving Ca supply by Ca supplementation can decrease fasting renal OHPr by 15–35% (Horowitz *et al.* 1988; Fardollone *et al.* 1998). This effect on renal OHPr can occur within 12 h and may persist for several months (Horowitz *et al.* 1988). However, supplementation of the

diet with Ca decreases fasting OHPr concentrations only in those subjects with low Ca intakes (566 (SE 131) mg/d) but not in subjects with high habitual Ca intake (mean: 1062 (SE 278) mg/d) (Fardollone *et al.* 1998) indicating that renal OHPr is a sensitive marker of nutritional Ca supply.

Previous studies have shown that Ca is a threshold nutrient (Matkovic & Heaney, 1992). When a specific plateau of Ca absorption is reached, a higher amount of absorbed Ca does not cause further gain in Ca retention. As determined by Ca-balance studies, maximal Ca retention occurs at a mean intake of 957 mg Ca/d in young adults (Matkovic & Heaney, 1992). Ca intake during the period of bran consumption was above this level (Table 1) thereby covering actual recommendations of 900–1000 mg/d (Deutsche Gesellschaft für Ernährung, 1991). Thus, we hypothesize that on an adequate Ca intake, the wheat bran-induced reduction in 24 h urinary Ca excretion is a consequence of the decrease in net absorbed Ca without changing Ca retention. This assumption is supported by investigations on Ca balance performed in young adults. In young men, mixed food fibre had little effect on Ca balance, when Ca intakes were high ( $\geq 1.5$  g/d) (Haack *et al.* 1998). In young women, an intake of 30 g fibre/d from whole cereal products was associated with an increase in dietary Ca intake of 58 mg and a rise in faecal Ca of 100 mg/d (Wisker *et al.* 1987). As in our present study, intake of phytic acid increased by approximately 1000 mg/d and urinary Ca excretion decreased by 57 mg/d (1.42 mmol) compared with a control diet. Overall Ca balance of the subjects, who were on a Ca intake of 1260 mg/d, was not affected. Consequently, the decrease in urinary Ca excretion seems to be the result of an adaptation process, induced by the reduction in intestinal Ca uptake.

Nevertheless, other possible adverse effects of dietary fibre have to be considered. High fibre intakes of approximately 39 g/d can impair Zn retention and can decrease Fe availability in young women (Wisker *et al.* 1991). Thus, it has to be clarified whether or not an enhanced intake of these trace elements can compensate for these adverse effects of high fibre intakes.

In conclusion, high wheat bran intake, equivalent to 14 g dietary fibre/d, has no significant adverse effect on bone turnover in young female adults when Ca intake is high ( $> 1$  g/d) and total fibre intake is 30 g/d.

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