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## The impact of high non-starch polysaccharide intake on serum micronutrient concentrations in a cohort of women

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### Abstract

**Objective:** Many public health campaigns encourage increased fibre consumption, but short-term studies suggest that various components of dietary fibre inhibit the absorption of certain micronutrients including carotenoids. These do not take into account long-term adaptation to nutrient intake levels. We aimed to investigate the effect of non-starch polysaccharide (NSP) fibre on plasma micronutrient concentrations in a large free-living population consuming their usual diet.

**Design:** Prospective cohort study. Semi-weighed 4-day food diaries were analysed for micronutrient and NSP fibre intakes. Blood samples were taken and analysed for carotenoids, vitamin A, vitamin E, thiamine, riboflavin, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, folic acid, vitamin C and trace metals.

**Setting:** Participants in a large national cohort study who lived within 30 miles of Leeds.

**Subjects:** Two hundred and eighty-three middle-aged women.

**Results:** The association between NSP intake and plasma nutrient concentrations was assessed taking into account nutrient intakes and other dietary and lifestyle factors. Higher levels of NSP were not associated with lower plasma concentrations of the micronutrients measured, even allowing for the higher nutrient levels generally found in high-fibre foods.

**Conclusions:** Amongst middle-aged women we have shown that current guidelines for increasing the population's NSP consumption can be safely applied. Such guidelines are unlikely to reduce serum micronutrient concentrations, although other, more vulnerable population groups may benefit from further investigation.

**Keywords**  
Dietary fibre  
Micronutrients  
Non-starch polysaccharides

Major publications such as *The Nutritional Aspects of Cardiovascular Disease* report<sup>1</sup>, the *Nutritional Aspects of the Development of Cancer* report<sup>2</sup> and the Health of the Nation dietary targets<sup>3</sup> recommend an increase in the intake of complex carbohydrates by increasing the consumption of fruit, vegetables, bread and potatoes. This will inevitably lead to an increase in the intake of non-starch polysaccharides (NSP) and of some micronutrients. However, there are concerns that because certain micronutrients are less well absorbed from a high-NSP diet, micronutrient status might be adversely affected by such an increase of NSP intakes. This may impact on a range of nutrients<sup>4</sup> including trace minerals<sup>5,6</sup>, folate<sup>7,8</sup> and fat-soluble antioxidants including carotenoids<sup>7,9–12</sup>. This is important because there is some evidence that levels of micronutrient intake, even when above those required to prevent classical deficiency diseases, are involved in the

prevention of major diseases including atherosclerosis, cancer and neural tube defects<sup>1,2,13,14</sup>. To meet these concerns, we have investigated the impact of NSP intake on plasma micronutrient concentrations in the general population.

Many of the studies suggesting that NSP intake influences the absorption of micronutrients have been undertaken on animals or as small, short-term intervention studies in humans that have been unphysiological in terms of the quantity of NSP used and the diets provided<sup>6</sup>. The applicability of these findings to the general population is therefore limited because the effects found are influenced by a number of dietary factors, including variations in animal and plant food intakes, levels of dietary protein and other enhancers of absorption<sup>9,15</sup>. In addition, absorption or metabolism altered by the use of drugs or smoking may complicate these associations. Furthermore, previous

studies have been too short in duration to take into account the potential adaptation to very low or high micronutrient intakes<sup>6</sup>.

Using participants in the UK Women's Cohort Study (UKWCS), a national cohort study funded by the World Cancer Research Fund (WCRF), we aimed to complement the existing research with an epidemiological investigation of the association between NSP intake and plasma micronutrients in a free-living population consuming their usual dietary intake. We aimed to take into account lifestyle factors that may also influence plasma micronutrient concentrations. Owing to the characteristics of the cohort, which has a high proportion of vegetarian participants, we anticipated a wide range of NSP intakes. This permits an assessment of the impact of high NSP consumption and therefore provides grounds for commenting on the effect of recommendations to increase intake of NSP in the diet.

## Subjects and methods

### Subjects

The UKWCS is a national cohort of 35 000 women aged 35–69 years at recruitment. Funded by the WCRF, it was set up to investigate associations between diet and cancer. Participants in the cohort were responders to a direct mail survey of potential supporters of the WCRF. Sampling was stratified to give approximately equal proportions of self-defined vegetarians (including vegans) and age-matched red-meat eaters<sup>16</sup>. All meat eaters not eating red meat were also included. In this way the cohort is optimised for investigations requiring a wide range of fruit and vegetable intakes. This also ensures a wide range of NSP intake, so that the physiological effects of higher NSP consumption on micronutrients could be identified.

To investigate the effect of NSP on plasma nutrient concentrations, participants were restricted to those living within a 30 mile radius of Leeds, or one hour's drive, to facilitate collection of blood samples. A nurse contacted the women to arrange collection of the blood sample. Subjects were also sent a 4-day food diary and additional questionnaires to complete and return to the nurse at the time of blood sampling.

The number of women required for the study was based on 80% power to detect a statistically significant difference ( $P < 0.05$ ) between two groups, e.g. high and low NSP consumers. If the difference in blood nutrient concentrations is one-third of a standard deviation, i.e. approximately a 15% difference in plasma micronutrient concentrations between the high and low NSP consumers<sup>5–8,10</sup>, then 150 women are needed in each of the two intake groups. The tests used in this paper treating NSP as a continuous variable and tests for trends over several groups will be at least as powerful.

### Questionnaire and food diary

All women in the UKWCS completed baseline data collection including details of lifestyle habits including physical exercise, supplement use, current illnesses, smoking and drug use. At the time of arranging the blood collection, between 3 and 5 years after baseline data collection, women were also asked to complete a semi-weighted 4-day food diary (specifically including dietary supplement use), from which was calculated their intakes of macronutrients,  $\alpha$ -carotene,  $\beta$ -carotene equivalents, vitamin A, vitamin E, thiamine, riboflavin, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, folic acid, vitamin C, trace metals and both Englyst (NSP) and Southgate fibre, using COMP-EAT<sup>17</sup>. Intakes of micronutrients derived from supplement use were also calculated and incorporated in the analysis. Standard food composition tables were too incomplete to accurately assess intakes of different fibre fractions and lycopene, lutein and cryptoxanthin because of missing data in the tables<sup>18</sup>.

### Blood analysis

Blood was collected at home after an overnight fast. Samples were collected into lithium heparin (8 ml) for carotenoids ( $\alpha$ - and  $\beta$ -carotene, lycopene, cryptoxanthin, lutein), ascorbic acid, total vitamin C,  $\alpha$ -tocopherol and trace metal (iron, copper, zinc) analysis, and into ethylenediaminetetraacetic acid (5 ml) for plasma and red-cell folate. Samples were kept cool, and separated and prepared for storage at  $-70^{\circ}\text{C}$  within 2 h of collection. All blood analyses were undertaken in the Division of Pathological Sciences, Department of Clinical Medicine, at the University of Leeds. Antioxidant vitamins were analysed by high-performance liquid chromatography<sup>19,20</sup>, folic acid by competitive binding, and trace metals by atomic absorption analysis<sup>21</sup>.

### Statistical analysis

Statistical analyses were carried out using SPSS version 9<sup>22</sup> and Stata version 6<sup>23</sup>. To investigate baseline demographic characteristics, NSP intake was grouped into the lower quarter (below the lower quartile), the middle half (between the lower and upper quartiles) and the upper quarter (above the upper quartile). To test for linear trends across NSP groups, analysis of variance and Cuzick's non-parametric test for ordered groups<sup>24</sup> were used for continuous data, as appropriate, and chi-squared tests for trend were used for categorical data. All  $P$ -values presented are two-sided.

Simple linear regression was used to investigate relationships between blood nutrient concentrations and NSP intake prior to adjustment for potential confounders. All micronutrient concentrations were log-transformed to ensure statistical assumptions were valid and to aid interpretation. In this analysis NSP was kept as a continuous variable and dietary intakes as measured by the 4-day food diary were used throughout.

Multiple regression was used to examine the same associations with adjustment for dietary and supplementary micronutrient intakes, age, body mass index (BMI), current smoking status, blood cholesterol, total energy intake including alcohol, current chronic disease and socio-economic status. For iron, drinking tea with a meal was also taken into account. In this way, the association between NSP intake and plasma micronutrients was estimated taking account of micronutrient intake and lifestyle. NSP was kept as a continuous variable, apart from when potential threshold effects were investigated by treating NSP intake as a categorical variable in the regression models and calculating separate effect estimates for each level of NSP intake.

To investigate the effect of dietary underreporting, the ratio of reported energy intake to estimated basal metabolic rate was calculated<sup>25</sup>. Results for women above the median of this ratio were compared with results for women below the median, and formally tested by adding to the model the interaction between this dichotomised variable and NSP intake.

The study was given approval by the local research ethics committee.

## Results

Altogether, 634 of the UKWCS subjects were mailed and asked to take part in this project. Three hundred and ninety-one (62%) subjects responded to the contact by the fieldworker; of these, 78 (20%) refused and for 30 (8%) a convenient appointment could not be found. Two hundred and eighty-three subjects returned food diaries and were included in the final analysis; 274 subjects had blood samples analysed.

Table 1 describes the basic demographic characteristics of the sample. The cohort women were on average of a relatively high socio-economic status, were well educated,

and had a low proportion of smokers. Higher NSP intake was associated with higher socio-economic status ( $P = 0.02$ ), higher educational qualifications ( $P = 0.009$ ) and lower prevalence of smoking ( $P = 0.002$ ), despite an overall low proportion of smokers in the whole sample. About half of the high NSP fibre group were vegetarians, with 15% of the low NSP fibre group declaring themselves to be vegetarian ( $P < 0.001$ ). The most common fibre supplement was bran. Higher supplemental intake was associated with higher NSP intake from all sources.

Mean nutrient intakes estimated by the 4-day food diary are shown in Table 2. Women in the lowest NSP fibre quarter had a mean intake of NSP of 9.6 g (standard deviation (SD) 2.1), which is about half of what might be expected for a group of high social class<sup>25</sup>. The mean NSP intake for subjects in the top quarter was 24.8 g (SD 4.4). Subjects in the top quarter of NSP intake had the highest intakes for all nutrients except vitamin B<sub>12</sub> and retinol. A large proportion of total nutrient intake was from supplements, and this was taken into account in the statistical analysis: for vitamin A, 26% was from supplements; vitamin E, 78%; vitamin B<sub>12</sub>, 33%; folate, 17%; vitamin C, 45%; iron, 26%; zinc, 20%; copper, 65%. Supplemental sources here exclude fortified foods.

There was a wide range of blood concentrations for most of the nutrients, particularly the carotenoids, with plasma  $\beta$ -carotene concentrations ranging from 42 to 6100 nmol l<sup>-1</sup>. Exploring the sample according to the quartiles of NSP intake showed increasing blood measures of  $\beta$ -carotene, folate and vitamin C across the NSP groups. There was no difference in the trace metal concentrations in the blood according to NSP intake. These findings persisted in a multiple regression analysis adjusting for total energy intake, dietary micronutrient intake, supplement use and other lifestyle factors (Table 3). Taking into account these confounding factors that may explain the relationship, a doubling in NSP intake was associated

**Table 1** Basic demographic description of the sample by NSP fibre intake

	NSP intake			Total sample ( <i>n</i> = 283)	<i>P</i> -value
	Lowest quarter ( <i>n</i> = 70)	Middle half ( <i>n</i> = 142)	Upper quarter ( <i>n</i> = 71)		
Age (years), mean (SD)	52 (8)	52 (9)	55 (8)	53 (9)	0.08
BMI (kg m <sup>-2</sup> ), mean (SD)	25.0 (5)	24.5 (4)	24.1 (4)	24.5 (4)	0.2
Minutes of vigorous physical exercise per week, median (IQR)	30 (0–120)	60 (0–150)	60 (0–120)	60 (0–120)	0.7
Socio-economic status, median (IQR)	4 (2–6)	3 (2–4)	3 (2–4)	3 (2–4)	0.02
Qualified to 'A'-level or above, <i>n</i> (%)	22 (31)	66 (47)	38 (54)	126 (45)	0.009
Current smoker, <i>n</i> (%)	10 (14)	5 (4)	1 (1)	16 (6)	0.002
Vegetarian (self-reported), <i>n</i> (%)	9 (14)	53 (39)	32 (48)	94 (35)	<0.001
Supplement use					
Vitamins, <i>n</i> (%)	38 (56)	76 (58)	43 (63)	154 (59)	0.4
Fish liver oil, <i>n</i> (%)	26 (38)	43 (33)	22 (33)	91 (34)	0.6
Fibre (inc. bran), <i>n</i> (%)	7 (10)	20 (16)	14 (22)	41 (16)	0.08

NSP – non-starch polysaccharides; SD – standard deviation; BMI – body mass index; IQR – interquartile range.

Numbers shown are mean (SD), median (IQR) or number of women (%) as appropriate, with test for linear trend. Where numbers do not sum to total, this is because of small quantities of missing data. All *P*-values are two-sided.

**Table 2** Nutrient intakes from 4-day food diary by NSP fibre intake and overall

	NSP intake			<i>P</i> -value	Total sample Mean (SD)
	Lower quarter	Middle half	Upper quarter		
	Mean (SD)	Mean (SD)	Mean (SD)		
Total energy (MJ)	7.3 (3.9)	7.8 (1.7)	8.6 (1.9)	0.001	7.9 (2.5)
Protein (g)	66 (31)	64 (16)	74 (18)	0.02	67 (22)
Fat (g)	65 (24)	69 (22)	72 (25)	0.1	69 (23)
Carbohydrate (g)	200 (91)	241 (52)	280 (80)	<0.001	241 (76)
Sugar (g)	98 (85)	117 (36)	139 (65)	<0.001	118 (61)
Starch (g)	98 (29)	119 (34)	132 (38)	<0.001	117 (36)
Southgate fibre (g)	14.1 (3.2)	20.9 (3.9)	30.8 (8.0)	<0.001	21.7 (7.8)
Englyst fibre (NSP) (g)	9.6 (2.1)	15.9 (2.0)	24.8 (4.4)	<0.001	16.6 (6.1)
Vitamin A (µg)	737 (890)	876 (719)	1089 (616)	0.005	896 (750)
Retinol (µg)	450 (836)	434 (656)	412 (528)	0.7	432 (676)
β-Carotene equivalents (µg)	1126 (886)	1898 (1110)	3122 (2092)	<0.001	2019 (1556)
Vitamin E (mg)	5.5 (2.6)	7.7 (3.6)	9.4 (3.7)	<0.001	7.6 (3.6)
Vitamin B <sub>12</sub> (µg)	3.9 (4.0)	4.0 (3.2)	3.7 (3.1)	0.8	3.9 (3.4)
Folate (µg)	219 (100)	278 (84)	364 (93)	<0.001	285 (104)
Vitamin C (mg)	76 (51)	130 (84)	170 (119)	<0.001	127 (93)
Iron (mg)	9.5 (3.2)	11.9 (2.9)	15.5 (4.1)	<0.001	12.2 (3.9)
Zinc (mg)	7.3 (4.0)	7.5 (2.1)	9.5 (2.0)	<0.001	8.0 (2.8)
Copper (mg)	1.1 (0.9)	1.3 (0.6)	1.7 (0.7)	<0.001	1.4 (0.7)

NSP – non-starch polysaccharides; SD – standard deviation.  
All *P*-values are two-sided.

with a 31% (95% confidence interval: 12–53%) increase in β-carotene concentration in the blood, and similar increases in α-carotene, lutein, cryptoxanthin and lycopene. There was no evidence of a threshold effect on any of these relationships as we had postulated, other than a borderline small effect for folate (*P* = 0.05) where women in the highest third of NSP intake had serum folate concentrations similar to those in the lowest third.

Neither was there any evidence of dietary under-reporting significantly influencing results. When results from women with the lowest ratios of reported energy intake to estimated basal metabolic rate were compared to

those from women with the highest ratios, there were no significant differences.

## Discussion

Our results suggest that increased NSP intakes are associated with increased concentrations of β-carotene, lutein, cryptoxanthin, plasma folate and possibly ascorbic acid in the blood. There is no evidence of a clear threshold effect in these relationships, other than for folate, which increases up to a point. These results are in line with other smaller studies that have found high-NSP diets to have

**Table 3** Fasting blood nutrient concentrations by NSP intake and the percentage increase in plasma concentration of each nutrient for a doubling in NSP intake

	NSP intake			% increase in plasma nutrient associated with doubling of NSP intake (95% CI)	<i>P</i> -value
	Lower quarter	Middle half	Upper quarter		
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)		
Vitamin A (µmol l <sup>-1</sup> )	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)	-1 (-5, 4)	0.8
α-Carotene (nmol l <sup>-1</sup> )	116 (102, 131)	144 (135, 154)	174 (155, 195)	34 (17, 54)	<0.001
β-Carotene (nmol l <sup>-1</sup> )	448 (391, 514)	548 (510, 588)	650 (573, 737)	31 (12, 53)	<0.001
Lutein (nmol l <sup>-1</sup> )	479 (439, 524)	567 (540, 596)	655 (603, 710)	25 (13, 38)	<0.001
Cryptoxanthin (nmol l <sup>-1</sup> )	236 (206, 269)	293 (272, 315)	352 (312, 398)	34 (15, 56)	<0.001
Lycopene (nmol l <sup>-1</sup> )	422 (375, 476)	496 (464, 530)	569 (509, 636)	24 (8, 42)	<0.001
Vitamin E (µmol l <sup>-1</sup> )	34.6 (32.2, 35.1)	34.0 (33.3, 34.8)	34.4 (33.0, 35.8)	2 (-3, 7)	0.5
Vitamin B <sub>12</sub> (ng l <sup>-1</sup> )	345 (309, 384)	344 (323, 366)	343 (309, 381)	0 (-12, 13)	0.95
Plasma folate (µg l <sup>-1</sup> )	8.4 (7.4, 9.4)	9.4 (8.7, 10.2)	8.5 (7.6, 9.6)	4 (-9, 18)	0.6
Vitamin C (µg ml <sup>-1</sup> )	13.0 (12.1, 13.9)	13.1 (12.6, 13.6)	13.2 (12.4, 14.2)	2 (-7, 10)	0.7
Ascorbic acid (µg ml <sup>-1</sup> )	10.4 (9.5, 11.4)	11.2 (10.7, 11.8)	11.9 (10.9, 13.0)	10 (-1, 23)	0.09
Iron (µmol l <sup>-1</sup> )	17.0 (14.9, 19.3)	15.4 (14.4, 16.4)	14.2 (12.6, 16.0)	-12 (-24, 2)	0.1
Zinc (µmol l <sup>-1</sup> )	12.4 (12.0, 12.7)	12.3 (12.2, 12.5)	12.3 (12.0, 12.7)	0 (-3, 3)	0.9
Copper (µmol l <sup>-1</sup> )	17.8 (16.9, 18.7)	17.4 (16.9, 17.9)	17.2 (16.3, 18.0)	-3 (-8, 4)	0.9

NSP – non-starch polysaccharide; CI – confidence interval.

Figures from multiple regression showing the increase in blood nutrients associated with a doubling in NSP intake, and geometric means, are adjusted for dietary and supplementary micronutrient intakes, age, body mass index, drinking tea with meal, current smoking status, blood cholesterol, total energy intake including alcohol, current chronic disease and socio-economic status. All *P*-values are two-sided.

positive nutritional consequences for the whole diet<sup>26,27</sup>. However, in our study these associations are independent of the amount of nutrients in the diet, supplement use, age, BMI, amount of tea drunk with meals, current smoking status, plasma cholesterol concentration, total energy intake and general health. Therefore the cause of the increases is unlikely to be attributable to any of these factors, although the possibility of some residual confounding remains. The lack of association between NSP intake and vitamin A, vitamin B<sub>12</sub>, iron, copper and zinc may be because plasma concentrations of these micronutrients are largely controlled by binding proteins, and much less by the diet. There was a slight non-significant but negative trend for iron.

These findings are in contrast to previous research under more controlled conditions, which suggested that various fibre fractions reduced the utilisation of a variety of vitamins and minerals<sup>5-12</sup>. While these previous studies were tightly controlled, they generally involved only a small number of subjects, were short-term and used high dosages of nutrients<sup>6</sup>. By being tightly controlled, they did not allow for the variety found between people's normal diets. By being short-term in nature, sometimes with wash-in periods of low-nutrient meals, they do not allow for the long-term adaptation of the body to regular dietary patterns<sup>6</sup>. The findings from our study in a free-living sample of women are therefore more readily applicable to the general population than previous studies.

The women in this study have been drawn from responders to the UK Women's Cohort Study, which was designed to contain a high proportion of vegetarians. Although these women tend to be above average social class and have an interest in their diet and health, the participants nevertheless consume a very wide range of dietary intakes including many with low NSP intakes as well as high intakes. For example, women in the lowest NSP fibre quarter had mean intakes of about half of what might be expected for a group of high social class<sup>25</sup> and had a mean BMI bordering the overweight category (25.0 kg m<sup>-2</sup>). A small study from a more typical population sample would have insufficient power to investigate the effects of high NSP consumption.

The advantage of epidemiological studies like this is that participants are much closer to eating their usual intakes of NSP fibre and nutrients as part of their usual food consumption patterns than in highly controlled experiments. It has therefore been possible to investigate whether or not intake of NSP influences availability of nutrients in practice, in a free-living population. There has been a call for such studies for some time<sup>6</sup>. Another advantage is the ability to study a large number of subjects, as we have in this study.

It has been suggested that dietary pectin could affect lipid metabolism by interacting with bile acids, leading to increased faecal excretion of bile acids and a resulting decrease in the absorption of fats and fat-soluble

substances such as carotenoids<sup>28</sup>. Carotenoids are passively absorbed by the mucosa of the small intestine and require mixing in the intestine with lipid (bile salt) micelles<sup>11</sup>. Our results suggest that this effect of NSP fibre is not important for subjects eating their usual diet. There are many other factors that could influence the amount of a nutrient which is absorbed. These include the efficiency of digestion, adaptation to previous sustained levels of intake of the nutrient, current nutrient status, total transit time, storage, the presence of other foods eaten at the same time, and other factors associated with the physical form of the food source such as degree of processing<sup>28</sup>.

The many advantages of this study being able to answer a practical question by using a free-living population eating their usual diets are balanced by the limitations of possible residual confounding and the inability to investigate potential mechanisms within the same study. Mechanisms may well depend on the micronutrient in question. It is possible that the more NSP there is, the looser the matrix, possibly encouraging micronutrients to be in closer contact with the gut wall and more available for absorption, at least for levels found in a typical diet. It is also possible that the effects we report are independent of gut physiology, but are caused by residual confounding. Additionally, although different methods of cooking were taken into account in analysing the food diaries, the effects we report could arise if women who eat diets higher in NSP use substantially fresher products or cook for a significantly shorter time. This would lead to decreased loss of micronutrients during food preparation and storage compared with those reflected by the UK composition of foods. The food diary analysis would then underestimate the habitual intake of micronutrients in these women. However, these potential biases are unlikely to be so large that they would turn a strong negative influence of NSP intake into the strong positive influence that we have found.

The finding that high NSP intakes are not associated with poorer micronutrient status within the broad range of intakes observed in this free-living population is relevant to current health education advice. Our findings mean that current guidelines on healthy eating remain valid. Concern that advice to consume more NSP conflicts with attempts to improve micronutrient concentrations, by leading to reduced serum micronutrient concentrations, is unfounded. We expect these findings to be of use to nutritionists, dietitians, healthcare professionals, caterers and food manufacturers as they seek to improve the public's diet.

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