Intestinal absorption of oestrogen: the effect of altering transit-time

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Objective **The mechanism by which a high fibre diet may reduce serum oestrogens is unknown. We hypothesized that time is a rate-limiting factor in oestrogen absorption from the colon so that changes in colonic transit-rate affect the proportion of oestrogen that is deconjugated and/or absorbed.**

Aim **To determine if alteration of intestinal transit rate would influence the absorption of an oral dose of oestradiol glucuronide.**

Participants **Twenty healthy postmenopausal women recruited by advertisement.**

Setting **Department of Medicine, Bristol Royal Infirmary.**

Methods **Volunteers consumed, in turn, wheat bran, senna, loperamide and bran shaped plastic flakes, each for 10 days with a minimum 2 week washout period between study periods, dietary intake being unchanged. Before and in the last 4 days of each intervention whole-gut transit-time, defecation frequency, stool form, stool β-glucuronidase activity, stool pH and the absorption of a 1.5 mg dose of oestradiol glucuronide were measured.**

Results **Wheat bran, senna and plastic flakes led to the intended reduction in whole-gut transit-time, increase in defecatory frequency and increase in stool form score. Loperamide caused the opposite effect. The length of time the absorbed oestrogen was detectable in the serum fell with wheat bran and senna, although this was only significant for oestradiol. Oestrone, but not oestradiol, was detectable for a longer time with loperamide. Plastic flakes had no effect on either oestrogen. Areas under the curve did not change significantly but tended to fall with the three transit-accelerating agents and to rise with loperamide.**

Conclusion **Our data indicate there is likely to be an effect of intestinal transit on the absorption of oestrogens but more refined techniques are needed to characterize this properly.**

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Introduction

Several studies have shown low-fibre intake, with or without high fat intake, to be associated with increased risk of breast cancer [1–5]. How these diets exert this influence has not been established, but one possibility is by an effect on oestrogen metabolism. There is much evidence that oestrogens are important in the promotion and aetiology of carcinoma of the breast [6]. A high fibre diet [7], or addition of wheat bran to the diet of healthy women [8], was reported to reduce serum oestrogen levels. The mechanism by which a high fibre diet may reduce serum oestrogens is unknown.

Oestrogens are excreted into the urine and bile; those reaching the duodenum are almost exclusively in conjugated forms, which are biologically inactive. In the bile, oestrone occurs principally as a glucuronide but also as a sulphate; oestradiol mainly as a sulphoglucuronide [9,10]. These oestrogens have a variable need for bacterial β-glucuronidase to enable deconjugation and reabsorption from the gut; they are then reconjugated and recirculated, eventually to be excreted again. This enterohepatic circulation serves to conserve oestrogens and maintain plasma levels. Deconjugation is believed to occur in the distal small bowel and especially the colon, where bacteria containing β-glucuronidase abound. To a lesser extent, oestrogens are also desulphated by bacterial sulphatases and mucosal enzymes [11,12]. Small amounts of conjugated oestrogens are absorbed intact [13–15], but absorption is almost certainly facilitated by deconjugation. Reduction of the gut's bacterial flora with antibiotics increases faecal excretion of both conjugated and unconjugated oestrogens and reduces urinary and serum oestrogens [16–18], while surgical removal of the colon leads to a marked reduction in serum and urinary oestrogens [19]. These facts emphasize the role of the colon in the conservation (enterohepatic circulation) of oestrogens.

Changes in bowel transit are likely to influence the bacterial metabolism and reabsorption of oestrogens via changes in the bacterial flora. We have previously demonstrated a fall in serum oestrone and oestrone sulphate in premenopausal women after 2 months of treatment with senna in doses sufficient to shorten whole-gut transit-time [20]. Most forms of dietary fibre are laxative and speed up colonic transit [21]. If faster colonic transit does indeed lead to a reduction in oestrogen absorption, this could explain how wheat bran and a high fibre diet lower serum oestrogens. A laxative might also act indirectly by reducing the absorption of short chain fatty acids (produced by fermentation of unabsorbed starch and other carbohydrates, including non-starch polysaccharide) and so acidifying the colon; because a lower pH inhibits β-glucuronidase activity [22], this would also tend to decrease reabsorption.

We hypothesized that time is a rate-limiting factor in oestrogen absorption from the colon so that changes in colonic transit-rate affect the proportion of oestrogen that is deconjugated and/or absorbed. The primary aim of this study was to determine if alteration of intestinal transit rate would influence the absorption of an oral dose of oestradiol glucuronide. This is the conjugated oestrogen that is most dependent on bacterial β-glucuronidases for deconjugation [16,23]. To speed up intestinal transit, we used senna, wheat bran and bran-like plastic flakes made of polytetrafluroethylene (which has previously been shown to have similar effects to wheat bran on colonic function [24] but, being inert, would not bind to oestrogen in the intestine), and to slow it down we used loperamide. An additional aim was to assess whether alterations in colonic transit rate lead to changes in faecal pH and β-glucuronidase activity.

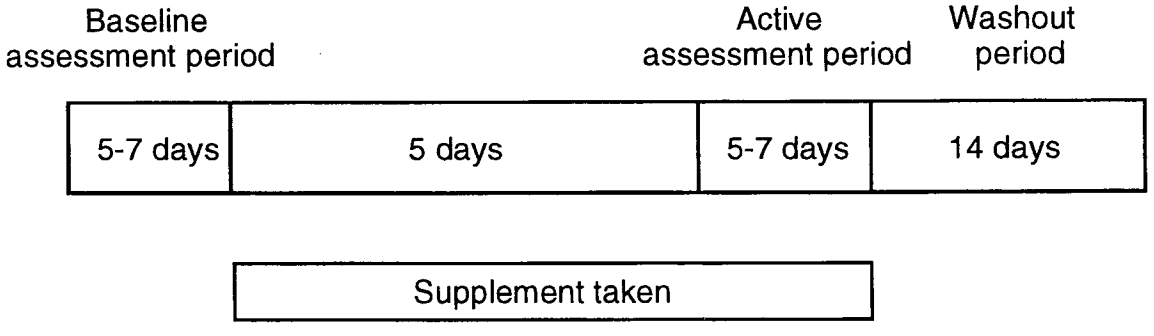
Methods

Healthy, omnivorous, post-menopausal women were recruited from advertisements placed in local hospitals. None were obese or had a significant medical history or a history of familial breast disease, and none had taken hormone replacement therapy or antibiotics within the last 3 months. By chance, all were non-smokers. Volunteers were confirmed as being post-menopausal by analysis of their serum luteinizing hormone (>30 IU l^{-1}) and follicular stimulating hormone (>30 IU l^{-1}) concentration.

Raw wheat bran (Prewett's, sieved to contain only particles 1.4–3.0 mm diameter), senna tablets (Senokot, Reckitt & Coleman) and bran-shaped plastic flakes, were used to accelerate intestinal transit. Loperamide capsules (Imodium, Janssen Pharmaceuticals) were used to slow down transit. Each agent was taken for 10–12 days and there were 2–4 week washout periods between agents to obviate any carry-over effects (Fig. 1). The interventions were studied in a set order (wheat bran, senna, loperamide and plastic flakes) because randomization would have required an impracticably large number of volunteers to ensure a balanced distribution between the 18 possible orders.

Volunteers' observations of their stool form were used to assess the effectiveness of the supplements in altering bowel transit speed. Volunteers were given a booklet to record when they defecated and the 'form' of each stool [using the seven point Bristol stool form scale [25,26], (Fig. 2)] throughout the study. The form or appearance of the stool depends on the rate of intestinal transit [27] and our scale ran from the hard discrete lumps of slow transit (type 1) to the non-cohesive (type 6) and liquid stools (type 7) of rapid transit (Fig. 2). The amounts of wheat bran (taken in portions through the day), senna and loperamide (both taken at night) were adjusted by the volunteers under our guidance to achieve a desired change in stool form. Bowel habit data in each of the four baseline assessment periods were compared to look for any carry-over effect on colonic function from the previous intervention.

Fig. 1



Experimental protocol

Fig. 2

Type 1	Separate hard lumps, like nuts.
Type 2	Sausage shaped but lumpy.
Type 3	Like a sausage or snake but with cracks on its surface.
Type 4	Like a sausage or snake, smooth and soft.
Type 5	Soft blobs with clear cut edges.
Type 6	Fluffy pieces with ragged edges, a mushy stool.
Type 7	Watery, no solid pieces.

Bristol stool form scale

Wheat bran, senna and loperamide were taken in the maximum tolerated dose, 24 g of plastic flakes were taken each day. The plastic particles were made from sintered (heated to 280 °C, which removes any contaminants) polytetrafluroethylene sheeting (600 mm x 35 m x 0.15 mm), the thickness being the same as the mean thickness of 200 randomly selected wheat bran particles. The sheeting was then repeatedly passed through a paper shredder (Fordishred 13), the resulting chaff being sieved to between 1.4 mm and 3.0 mm diameter. Wheat bran and plastic flakes were taken in divided doses with meals. Compliance was assessed by weighing the returned wheat bran and counting the tablets at the end of the study.

After an overnight fast (which continued for 1 h after the oestrogen had been taken) blood was taken before 9 a.m. Volunteers were then given 1.5 mg of oestradiol glucuronide orally. This dose was chosen after a review of published work suggested that this would be the lowest amount to provide easily detectable blood levels. Over the next 5 days venous blood was taken at approximately 0, 4, 8, 14, 24, 32, 48, 72, 96 and 120 h and the serum stored at –70 °C. The oestradiol glucuronide (Sigma UK) was >99% pure, as analysed by thin layer chromatography and high performance liquid chromatography. It was packaged with lactose in size 0 gelatine capsule which were then coated with a 100 μm (range 75–120 μm) layer of resin containing polymethacrylic acid polymer (Eudragit-L™ Röhm Pharma GMBH), designed to dissolve promptly at a pH above 6, thus releasing the capsules contents in the proximal small bowel (in order to mimic the arrival of oestrogen in the intestine via the bile).

Before and at the end of each intervention period, volunteers were weighed and asked to complete a 4 day dietary record (two week days and two weekend days) using scales or household measures. Where insufficient information was given the volunteers were contacted by phone for a fuller description of the items. Consumption of alcohol was also recorded. Volunteers were encouraged to keep their diets, alcohol intake and exercise patterns constant during the entire experiment. The records were analysed for dietary fibre (Southgate), non-starch polysaccharide, total fat, saturated fat, polyunsaturated fat, protein, carbohydrate, extrinsic sugar and alcohol using a computer programme based on McCance and Widdowson's *The Composition of Foods* [28] and using published values for non-starch polysaccharide [29,30].

Before and at the end of each intervention period, whole-gut transit-time was measured as a proxy for colonic transit using radio-opaque marker pellets swallowed on four consecutive days. Twenty-four hours after ingestion of the last set of markers the next two stools passed were collected, flattened and X-rayed [31].

On passing their stools into a container, volunteers immediately put them in a fridge. Within 12 h, the stools were weighed and the second stool was liquidized, tested for pH, then frozen at –20 °C for later β-glucuronidase activity analysis by modification of an established method [32]. Stool output per week was calculated as the mean weight of the two stools multiplied by the stated number of defecations per week.

Interventions were commenced after initial assessment of diet, whole-gut transit-time, serum oestrogen concentrations, stool pH and stool β-glucuronidase activity. The supplements were continued until the same data had been collected at the end of the experimental phase.

The study was approved by the Research Ethics Committee of the United Bristol Healthcare Trust.

Serum analysis

Oestradiol

Oestradiol-17β was extracted from serum (100 μl) in the presence of sodium hydroxide (10 μl, 2 mol l^{-1}). Radioimmunoassay was performed on the residue after evaporation of the ether, using antiserum raised to 17β-oestradiol-6-(O-carboxymethyl oxime)–bovine serum albumin and [^{125}I]-labelled oestradiol-17β. Only oestrone was found to cross react (approximately 10%) with this assay. Coefficient of variation assessed by repeated assay of a store of serum was 13% at a concentration of 167 pmol l^{-1} and 14% at a concentration of 335 pmol l^{-1}. Accuracy was 87–97%.

Oestrone

Oestrone was extracted from serum (100 μl) with hexane/diethyl ether (4:1 v/v, 1 ml × 2). Radioimmunoassay was performed on the residue, after evaporation of the solvent, using an antiserum to oestrone-6-(O-carboxymethyl oxime)–ovalbumin and [^{3}H]oestrone. Coefficients of variation were 8% at a concentration of 273 pmol l^{-1} and 13% at a concentration of 593 pmol l^{-1}. The accuracy was greater than 95%. Interference from other steroids could not be demonstrated.

Calculations

Oestrogen absorption curves were excluded as being uninterpretable if the oestrogen concentration failed to rise above the baseline concentration and/or to fall to the baseline concentration, usually because of a high baseline concentration. Baseline data were assessed as parametrically or nonparametrically distributed using histograms and Ryan Joiner tests. Oestrogen absorption was assessed either by measuring the time during which serum oestrogen was detectably higher than the baseline level, or by calculating the area under the serum concentration curves above the baseline value. Stool β-glucuronidase activity was analysed as $\log_{10}$ transformed data, with results expressed as geometric means and 95% confidence interval of the ratio of the geometric means. The differences between baseline and post intervention readings were then analysed using a two-tailed Student's *t*-test, or a Mann–Whitney test as appropriate. Because of the nonparametrically distributed nature of most of the data, correlations were calculated using Spearman's coefficients.

Results

Of the 20 women (Table 1) who were recruited to the study, only one failed to complete the protocol, being unable to tolerate the plastic particles.

No changes were seen in dietary intakes between the baseline periods and the intervention periods. The median baseline intakes of fibre (Southgate) were 15.5 g/day [interquartile range (IQ) 13.3, 20.0] and of non-starch polysaccharide 11.0 g/day (IQ range 8.0, 12.0). A median of 23.0 g/day (IQ range 19.3, 29.5) of wheat bran was consumed as a supplement (median Southgate dietary fibre 10.6 g/day or 8.2 g/day of non-starch polysaccharide).

Table 1 Demographic data and anthropomorphic measurements at entry into the study (n = 20)

	Median	Interquartile range
Age (years)	54	(50, 56)
Menarche, age (years)	13.0	(12.3, 14.0)
Menopause, age (years)	48.0	(43.0, 52.8)
Number of children	2	(2, 3)
Alcohol (units per week)	2.0	(0, 4.8)
Height (cm)	162.0	(157.9, 166.0)
Weight (kg)	67.5	(60.8, 74.8)
Body mass index (kg m^{-2})	24.9	(23.8, 30.5)

There were no differences between any of the various baseline measurements. Wheat bran, senna and plastic flakes led to the intended reduction in whole-gut transit-time, increase in defecatory frequency and increase in stool form score (Table 2). Loperamide caused the opposite effect. With senna and plastic flakes the stools became more acid while loperamide had the reverse effect, but no change in stool pH was seen with wheat bran (Table 3). Stool β-glucuronidase activity fell with plastic flakes from 43 to 27 mmol g^{-1} h^{-1} (95% CI of the ratio of the geometric means –2, –1, $P < 0.001$) and showed a trend to a reduction with senna and wheat bran (data not shown). No change in activity was seen with loperamide.

Figure 3 shows an example of absorption curves for one volunteer during a baseline assessment period. Table 4 shows the influence of each intervention on the time oestrogens were detectable at concentrations greater than the baseline, and Table 5 shows the influence of each intervention on the area under the oestrogen concentration curves. With senna and bran, oestradiol was elevated for a shorter time, and with loperamide, oestrone was

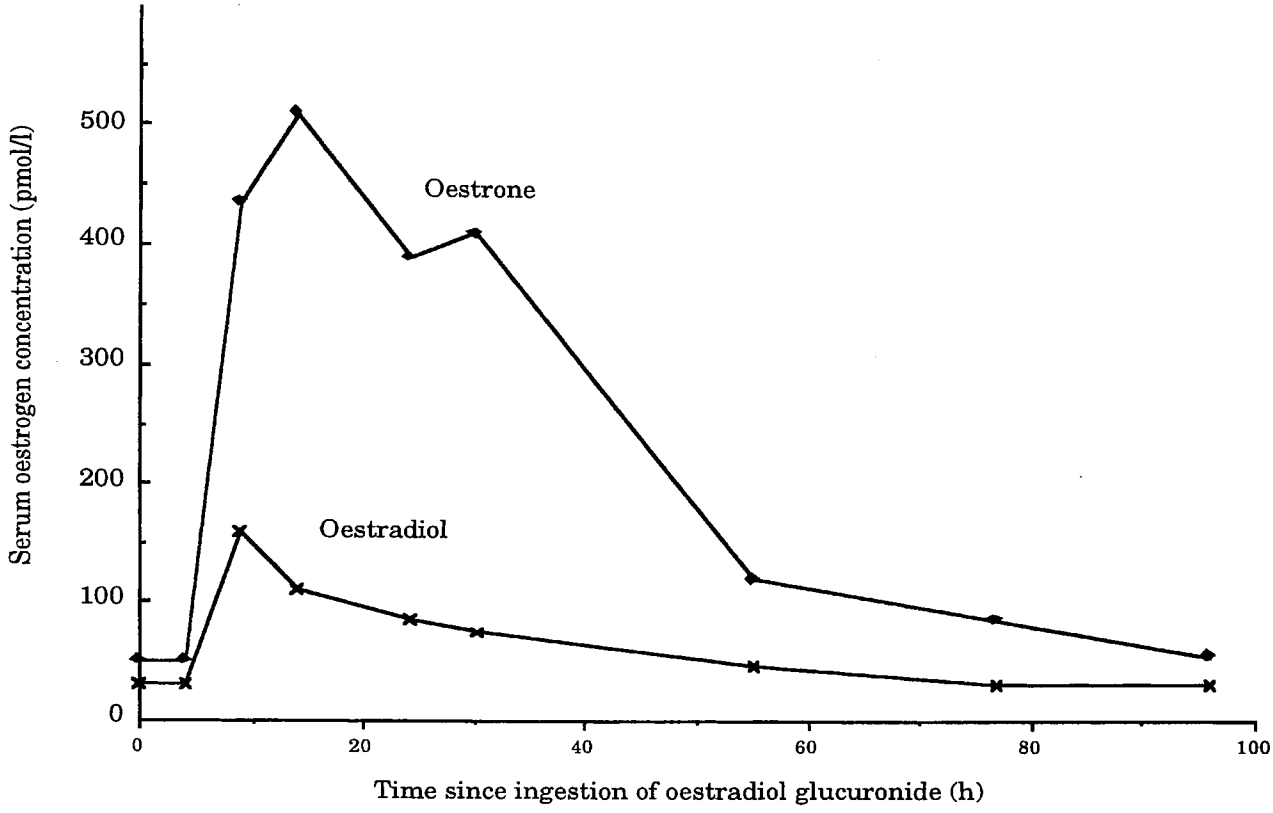
Table 2 Changes in transit-time, defecatory frequency measurements (median, interquartile range) and stool form score (mean, SD) with each intervention

Intervention	Before	End	Difference	95% CI	*P* value
Whole-gut transit-time					
Wheat bran	57.8 (35.1, 72.4)	37.4 (27.4, 62.5)	−10.1	−20.1, −2.8	0.015
Senna	53.5 (38.5, 71.2)	34.8 (26.2, 47.7)	−18.0	−24.3, −11.8	< 0.001
Loperamide	48.9 (37.4, 55.8)	72.4 (52.1, 85.5)	18.8	7.1, 31.5	0.008
Plastic flakes	48.5 (42.6, 62.3)	34.9 (28.5, 46.6)	−12.5	−21.0, −6.0	0.001
Defecations per week					
Wheat bran	8.0 (7.0, 11.8)	9.3 (8.1, 14.9)	2.2	(0.0, 4.7)	0.029
Senna	8.0 (7.0, 9.7)	9.6 (8.1, 14.0)	2.6	(1.1, 4.7)	0.001
Loperamide	8.2 (7.0, 11.8)	5.6 (4.2, 7.0)	−2.8	(−5.1,−2.0)	< 0.001
Plastic flakes	7.0 (7.0, 7.0)	10.5 (8.4, 15.7)	3.0	(2.1, 5.3)	< 0.001
Stool form score					
Wheat bran	3.83 (1.04)	4.73 (0.76)	0.90	(0.45, 1.35)	< 0.001
Senna	3.62 (0.84)	4.86 (0.79)	1.24	(0.92, 1.57)	< 0.001
Loperamide	3.97 (0.81)	2.82 (1.26)	−1.15	(−1.66, −0.63)	< 0.001
Plastic flakes	3.82 (0.52)	4.63 (0.82)	0.81	(0.46, 1.17)	< 0.001

Table 3 Stool pH (mean, SD, 95% CI of the difference and *P* value)

Intervention	Baseline	Active	95% CI	*P* value
Wheat bran	6.92 (0.43)	7.06 (0.56)	−0.14 (0.43)	0.310
Senna	7.15 (0.70)	6.58 (0.60)	−0.98 (−0.15)	0.010
Loperamide	6.95 (0.43)	7.40 (0.34)	0.22 (0.67)	< 0.001
Plastic flakes	7.10 (0.53)	6.71 (0.61)	−0.70 (−0.09)	0.014

Fig. 3



Example of the changes in serum oestrogens with time after swallowing capsule of oestradiol glucuronide under baseline conditions

Table 4 Time oestrogens elevated in the serum from onset of absorption to peak concentration, from peak back to baseline level and total time detectable during the four interventions (median, interquartile range)

		Time to peak		Time from peak		Total time	
Intervention	*n*	Before	During	Before	During	Before	During
Oestradiol							
Wheat bran	18	7.6 (5, 12)	7.3 (6, 9)	61.3 (42, 85)	48.0[b] (35, 66)	72.5 (57, 93)	53.7[a] (47, 76)
Senna	18	6.0 (5, 9)	6.8 (5, 12)	65.0 (48, 84)	40.5[d] (29, 61)	70.5 (54, 91)	48.0[e] (39, 68)
Loperamide	18	8.0 (6, 12)	7.5 (5, 14)	61.8 (35, 69)	58.3 (41, 72)	72.3 (47, 81)	70.0 (44, 87)
Plastic flakes	18	9.5 (5, 14)	8.6 (4, 13)	63.8 (43, 78)	61.9 (38, 78)	72.0 (52, 84)	70.3 (46, 88)
Oestrone							
Wheat bran	18	10.0 (8, 13)	10.0 (7, 13)	59.3 (46, 67)	57.5 (41, 63)	72.3 (56, 82)	67.4 (53, 76)
Senna	18	10.0 (8, 13)	13.5 (11, 20)	68.8 (48, 84)	64.6 (29, 61)	82.5 (69, 94)	77.0 (68, 94)
Loperamide	18	12.0 (9, 14)	9.8 (7, 14)	59.5 (58, 68)	83.5[c] (62, 93)	72.0 (65, 80)	96.8[f] (68, 104)
Plastic flakes	18	12.0 (9,14)	8.3 (6, 11)	66.0 (58, 81)	64.6 (44, 82)	77.0 (67, 96)	73.0 (50, 96)

[a]$P = 0.001$; [b]$P = 0.006$; [c]$P = 0.018$; [d]$P = 0.023$; [e]$P = 0.028$; [f]$P = 0.033$.

Table 5 Median area under the serum oestrogen concentration curves before the four interventions and change with each intervention (median, interquartile range and median with 95% CI)

Intervention	Before		Change	95% CI	*P* value
Oestradiol					
Wheat bran	1639	(791, 2641)	−215	−475 (13)	0.06
Senna	1846	(843, 2715)	−187	−1203 (309)	0.19
Loperamide	1435	(639, 2200)	+211	−135 (480)	0.13
Plastic flakes	1925	(937, 2690)	−328	−1271 (329)	0.42
Oestrone					
Wheat bran	7251	(5731, 13495)	−783	−2505 (442)	0.21
Senna	8135	(6302, 11525)	−175	−1654 (2412)	0.83
Loperamide	7000	(4483, 10872)	+1786	−519 (3011)	0.12
Plastic	8257	(6481, 15635)	−711	−4477 (455)	0.16

elevated for a longer time. Areas under the curve did not change significantly but tended to fall with the three transit-accelerating agents and to rise with loperamide. The fall with bran and oestradiol was very nearly significant (Table 5).

The lag phase from ingestion of the oestradiol glucuronide to the appearance of oestradiol in the serum for the combined baseline measurements was a mean of 3.3 h (SD 5.0) and 2.8 h (SD 4.4) for oestrone. The difference was not significant. There was no significant change in the lag phase with any intervention. Peak oestrogen concentrations reached during each of the four interventions did not correlate with the total area, area post-peak under the absorption curves or the length of time the absorbed oestrogen was detectable in the serum (data not given).

Discussion

The volunteers were reasonably representative of the population in that their baseline whole-gut transit-time (median 51 h) was similar to that (58 h) of a random sample of healthy post-menopausal women [33]. The intended changes in bowel function occurred with all four supplements. Stool pH fell with senna and plastic flakes, and rose with loperamide. Other than the lack of change in pH with wheat bran, the findings are in keeping with the known relationship between intestinal transit rate and stool pH [34]. Similarly, stool β-glucuronidase activity tended to fall with increased intestinal transit rate, but only significantly so with plastic flakes. The lack of difference between the serial baseline measurements of each volunteer suggests that the washout periods were adequate.

The length of time the absorbed oestrogen was detectable in the serum fell with wheat bran and senna in the case of oestrone and increased with loperamide in the case of oestradiol. This could imply that the faster intestinal transit led to greater excretion of oestrogen in the stool reducing the body's oestrogen load. These changes in raised oestrogen levels that were detected were limited to the post-peak period. This suggests that alteration of intestinal transit did not alter the absorption of the initial dose of oestradiol glucuronide but rather that of recycled oestrogen. However, the findings were not consistent because loperamide had no effect on oestradiol and plastic had no effect on either oestrogen.

While wheat bran, senna and plastic flakes tended to reduce and loperamide to increase the area under the oestrogen absorption curves for both oestradiol and oestrone, none of these changes reached statistical significance.

The inconsistency of our results suggests that either our hypothesis was wrong or that oestrogen absorption is indeed dependent on transit-time but our methodology for measuring this absorption was defective. It is possible that our sampling times were not frequent enough to detect peak serum concentrations. It can be argued, for example, that we were unable to distinguish recycled oestrogens from the originally absorbed oestrogen. Possibly, by using a smaller dose of oestradiol glucuronide differences in absorption may have been more apparent. Allowing a greater time for the dietary supplements before giving the oestrogen may have led to further changes in intestinal pH and enzyme activity, which might have altered the oestrogen's metabolism.

The lag phase between taking the dose of oestradiol and its appearance in the blood was shorter than expected. Considering that this period includes the time spent by the capsule in the stomach, this rapid appearance of oestrogen in the blood suggests absorption of the oestradiol occurred in the small bowel. This, in turn, implies that either oestradiol glucuronide is not as dependent on deconjugation for absorption as previously thought or that significant β-glucuronidase activity is present in the small bowel. If significant absorption of the oestradiol glucuronide occurred in the ileum, this would possibly reduce the influence of alterations in colonic transit. This is relevant because senna acts wholly on large bowel transit [35] while the other three agents may well affect large bowel more than small bowel function.

In conclusion, our data indicate that there is likely to be an effect of intestinal transit on the absorption of oestrogens but more refined techniques are required to characterize this properly.

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