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Effect of ispaghula husk on the faecal output of bile acids in healthy volunteers

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Abstract

Faecal bile acids are associated with both colorectal cancer and serum cholesterol levels. We investigate whether dosing with ispaghula husk affects the faecal bile acid weights and concentrations in healthy adults. Sixteen healthy volunteers consumed 7.0 g/day ispaghula husk, containing 5.88 g/day Englyst-determinable dietary fibre, for the middle 8 weeks of a 12-week period. Stool samples were collected, analysed for faecal bile acids and their form and dry weight determined. Correlations between the faecal bile acids, the stool parameters and the dietary intake were tested. Ispaghula husk treatment significantly lowers faecal lithocholic and isolithocholic acids and the weighted ratio of lithocholic acids to deoxycholic acid. These effects revert towards their initial states at the end of the treatment period. These changes in the faecal bile acid profiles indicate a reduction in the hydrophobicity of the bile acids in the enterohepatic circulation.

1. Introduction

Some soluble fibres are known to be efficacious in reducing total serum cholesterol [1–4]. One particular soluble fibre (ispaghula husk) is being used to treat primary hypercholesterolaemia. Its mechanism of action remains to be proven although it has been explained [5] mainly in terms of bile acid binding by the fibre resulting in increased excretion and synthesis from cholesterol. In addition, there may be possible contributions from the propionic acid produced by fermentation, inhibiting cholesterol synthesis and causing a reduced adsorption of cholesterol from the gut.

Dietary fibre is also thought to help prevent colorectal cancer, which caused the deaths of 55,000 people in the USA in 1995. Most evidence is epidemiological, however, and there is little direct evidence [6–9]. Ispaghula fibre has shown anticarcinogenic effects in rats [10]. It is thought that the fibre may attenuate its effects by reducing gut transit time, thereby causing a reduction bile acid metabolism by the gut microflora, dilution of the bile acids by stool bulking, alteration of microbial bile acid metabolism due to fibre fermentation, reduction of the pH and production of short chains fatty acids [11], or by direct binding to the bile acids and hence preventing their metabolism.

It has been promulgated that it is the secondary bile acids, deoxycholate and lithocholate, and in particular their ratio that is responsible for promoting this cancer [12]. Evidence of this is epidemiological, direct and indirect [13]. As examples, there is a correlation between colorectal cancer and faecal bile acid concentrations, bile acids are cocarcinogens in rat colon where they affect the mucosa, and colorectal cancers have binding sites for bile acids that are absent otherwise.

Ispaghula husk is the mainly soluble, Englyst-determinable non-starch polysaccharide from *Plantago ovata*, also known as psyllium. Analysis has shown that a variety of heterogeneous structures are possible, depending on the method of purification and nature of

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the source material [14–17]. The main feature of the structure is a β-1-4-xylopyranose backbone with about a third of its residues substituted with α-L-arabinofuranose side chains. It is known that peripheral residues are removed in the colon and fermented [10], whereas the remaining arabinoxylan core gives the stools form and extra water-holding capacity [17].

In this study, we aimed to investigate whether dosing with ispaghula husk affects the faecal bile acid weights and concentrations in healthy adults. A secondary objective was to investigate the relationship, if any, between the food intake, faecal form, bile acid excretion and ispaghula fibre.

2. Methods

The study, which was approved by the local ethics committee before recruitment commenced, was conducted in accordance with the Declaration of Helsinki and the EC Note for Guidance on Good Clinical Practice. All the volunteers were white Caucasians and gave informed written consent to the study protocol. All the volunteers were examined by the study Medical Investigator prior to the study and complied with a number of criteria, such as good health, not on special diets, alcohol consumption within government guidelines, non-smoking and compatible concurrent medication, to ensure that they could be considered to have normal bowel habit, diet and gut metabolism. They were allowed a free choice of their diet throughout this study.

2.1. Preliminary study

A preliminary study was undertaken in order to establish the natural variability of the bile acid output in healthy adults. Six volunteers were used (three male, three female, age range 23.3–27.1 years, mean 24.8 years, SD 1.37 years; body mass index (BMI) range 21.4–26.7 kg m^{-2}, mean 23.3 kg m^{-2}, SD 2.0 kg m^{-2}). The design required 24-h stool samples to be collected once a week from each volunteer for 8 weeks. Bowel habit diaries and diet diaries were kept. The stool samples were analysed for bile acids by HPLC and enzyme assay. Power calculations based on this study indicated that 16 volunteers would be sufficient to detect appropriate differences in bile acid weights between treatment and non-treatment periods.

2.2. Experimental subjects

The target structure for this study comprised eight male, eight female volunteers, aged between 20 and 30 years and with BMI in the range 18–28. In the event, 22 healthy volunteer subjects were recruited into the study, of whom two withdrew consent, one failed to fulfil the screening criteria and one was withdrawn for non-compliance. The remaining 18 volunteers completed the study and, as per protocol, the stools from the first 16 volunteers to complete (6 male, age range 23.7–30.7 years, mean 27.4 year, SD 2.8 year; BMI range 22.5–28.5 kg m^{-2}, mean 24.8 kg m^{-2}, SD 2.1 kg m^{-2} and 10 female, age range 22.4–36.4 years, mean 26.7 years, SD 4.1 years; BMI range 18.1–28.4 kg m^{-2}, mean 24.2 kg m^{-2}, SD 3.1 kg m^{-2}) were analysed.

2.3. Experimental design

This was a single Centre study over a single 12-week period consisting of a 2-week pre-treatment phase immediately followed by an 8-week treatment phase and a 2-week post-treatment phase. Eighteen 24-hour stool samples were taken for analysis from each volunteer (Tuesdays in Weeks 1, 2, 5–10 and 12; Sunday, Tuesday, Thursday and Saturday in Week 3; Tuesday and Friday in Week 4; and Monday, Wednesday and Friday in Week 11). If no stool was produced on a particular collection day, then the product of the next call to stool was utilised. This occurred 18% of the times; in 2% of the cases (6/288) the overlap with the next sample required meant that a sample was not available for analysis. Complete weighed (±1 g) dietary records were kept for the Sunday–Tuesday of each week and analysed using a validated dietary analysis program (Compeat 4) to estimate the week's intake [18]. Bowel habit diaries (frequency and form) were kept for all 84 days of the study period. Stools were scored when produced, by the pre-trained volunteers, on a 5-point scale (1, loose/watery; 2, mushy/heaped; 3, snake-like or sausage shape with smooth surface; 4, sausage shape with deep cracks; 5, fragmented/pellety). The average score for each call to stool was used. Stools were collected and sealed in a purpose-designed bag, stored immediately at −20°C and transported frozen to a −70°C freezer. Stool weights were corrected for the number of calls to stool each week, although this had no qualitative effect on the results. During the 8-week treatment phase (Weeks 3–10 inclusive) each volunteer was required to take 3.5 g of ispaghula husk (in the form of one sachet, of Fybogel® Orange, Reckitt & Colman Products, which contained 2.94 g of Englyst-determinable total dietary fibre of which 80% is classified as soluble) mixed in 150 ml of cold water, twice a day after breakfast and after the evening meal.

2.4. Analytical methods

The stool samples were analysed at a rate of 12 samples/week according to a coded randomised plan that ensured that (a) the samples produced in any week were stored before analysis, on average, for

about the same period as any other week, (b) the analytical programme was started as soon as possible after the first sample donations, minimising the storage time, (c) any effect of the co-analysis of samples had minimal effects on the grouped data and (d) there was no operator bias. The methodology was based on previous work [19–25] and was validated prior to use by using spiked faecal samples. The mean bile acid recovery determined from faecal samples ($n = 3 \times 2$) from an ispaghula treated volunteer, spiked with a mixture of bile acids, was 102% with a standard deviation of 20%. Repeated analyses showed a 7% average standard deviation of recovered bile acids ($n = 6$). Procedural losses were compensated by the use of the internal standard. The procedure was shown to produce a linear response with respect to the bile acid concentrations over the range encountered.

Stools from a particular collection day were combined and quantitatively transferred to a single Stomacher bag. A Stomacher 400 bag was used if the faeces weighed less than 200 g and a Stomacher 3500 bag was used if the faecal weight was more than 200 g. A weight of 10.0 mM HCl equal in weight to the transferred stools was added and the samples were thawed. Samples were homogenised, using the appropriate Stomacher. Weighed samples of faecal homogenate (about 2 ml) were transferred to labelled freeze-drier flasks. Two millilitres internal standard ((R)-12-hydroxy-Cis-9-octadecanoic acid (Sigma); 1.0 mg/ml in 50 mM NaOH) was added to each flask and lyophilised overnight. Samples were extracted with methanol/chloroform by (a) suspending the dry faeces in 24 ml (HPLC-ECD grade, Fisons) methanol with the aid of a triangular (disruptive) stirrer; (b) refluxing for 110 min, using a stirrer isomantle; (c) adding 6 ml pure water through top of condenser and continuing the reflux for 10 min; (d) cooling and transferring to a labelled centrifuge tube; (e) removing solids by centrifugation at 926 *g* for 10 min retaining the supernatant; (f) transferring the pellet back into the appropriate labelled reflux vessel; (g) adding 30 ml (HPLC grade, Fisons) chloroform/methanol (50 : 50 v/v) and resuming the reflux for 2 h; (h) removing solids by centrifugation at 926 *g* for 10 min retaining the supernatant. The two matched supernatants were combined and evaporated to dryness using a centrifugal evaporator.

The residue was dissolved in methanol and washed out into a graduated test tube to give 5.0 ml. Two 0.5-ml aliquots were evaporated to dryness using minimal heat and stored at −20°C for analysis using a 'bile acids' enzyme assay kit (Sigma) using cholic acid (Sigma) as the standard [24]. This assay is specific for 3-α-hydroxy-bile acids and gives equal molecular responses for these but no response for 3-keto- or 3-β-hydroxy-bile acids such as isolithocholic acid. The solutions used in this assay were also used as a measure of the extractable colour from the stools (A_{560nm} ml/g dry weight). To the remaining 4 ml methanol sample, 6.0 ml of 0.1 M sodium phosphate (HPLC-ECD grade, Fisons) pH 7.5 was added. Any precipitate present was removed by heating the stoppered tubes briefly to 60°C to dissolve any co-precipitated bile acids and centrifuging at 926 *g* for 20 min, or until clarified. Samples were cleaned up using methanol-preconditioned Sep-Pak C18 cartridges by washing with 5 ml of 40 : 60 v/v methanol : 0.1 M phosphate pH 4.5 buffer followed by 2 ml water and eluted with 2×2.0 ml HPLC grade methanol which were evaporated to dryness and refrigerated at −20°C until analysis. Bile acids were analysed by HPLC [25] after dissolving the evaporated sample in 0.4 ml of HPLC-ECD grade methanol, adding 0.02 ml of 50% NaOH (HPLC-ECD grade, Fisons) and 0.60 ml water, followed by heating the stoppered tubes briefly to 60°C. Bile acid solution (100 μl) was injected onto the column using a gradient mobile phase (4.5–20% acetonitrile (HPLC-ECD grade, Fisons), 0.7 M sodium acetate (HPLC-ECD grade, Fisons), 0.1 M NaOH (HPLC-ECD grade, Fisons), 0.8 ml min^{-1}) at 60°C. In two cases (out of the 251 analyses), where the peaks for lithocholic acid and isolithocholic acid were poorly resolved, the peak was split in the overall ratio found. The effect of this on the overall analysis was found to be negligble, but this was thought better than ignoring these points. Glycine and taurine conjugates were analysed but were present in such low, often zero, amounts that their variations were not statistically meaningful.

2.5. Compliance and validation

Adherence to consuming the ispaghula husk was checked by counting the returned sachets at the end of each week. Four volunteers consumed three sachets less than they should (109/112) have, and one volunteer consumed one extra sachet. Double manual data entry, computer validation and consistency checks were incorporated. Randomised quality control checks were made on a number (23.4%) of the bowel habit and food diaries. The bile acid data was independently audited.

2.6. Statistical methods

For statistical analysis, five treatment periods were identified: (1) *pre-treatment*, Weeks 1 and 2, containing two stool collections from the Tuesday of each week; (2) *begin treatment*, Week 3, containing three stool collections from the Tuesday, Thursday and Saturday; (3) *mid treatment*, Weeks 5, 6 and 7, containing three stool collections from the Tuesday of each week; (4) *end treatment*, Weeks 8, 9 and 10, containing three

stool collections from the Tuesday of each week; (5) *post-treatment*, Weeks 11 and 12, containing three stool collections from the Wednesday and Friday of Week 11 and the Tuesday of Week 12. Preliminary examination of the data using the one sample Kolmogorov–Smirnov test strongly suggested that the log transformation was appropriate for all bile acid measurements, including ratios. The bile acid data for cholic acid (3/220 zero values) and chenodeoxycholic acid (80/220 zero values) were transformed using the function Log_e (data $+\epsilon$) where ϵ was half the lowest determinable amount. A square-root transformation was appropriate for the wet and dry weight data. The remaining data, including the dietary data did not require transformation. All data was back-transformed after processing. Where databased values were shown to be statistical outliers, the sensitivity of the results to these data points was explored. In no case did this change the qualitative outcome of the analyses. Due to the number of parameters involved and since the effect of treatment is estimated within subjects, the relationship between food intake, bowel habit and faecal bile acids was investigated in a pairwise fashion by assessing within-subject changes using a stepwise multiple linear regression model with a significance level for entry of 0.10. Pooled within-subject (WS; 63df) and between-subject (BS; 14df) correlations were also investigated by use of the Pearson correlation using the combined and transformed data.

3. Results

There were no adverse events noted that could be attributed to the treatment.

3.1. Diet

Treatment had no significant effect on the energy intake or the protein, fat, carbohydrate, alcohol or water content of the diet (Table 1). There was a significant drop in non-ispaghula dietary fibre throughout the study ($p = 0.02$) with a 15% drop between the pre-treatment period and mid treatment ($p = 0.01$) and a 19% drop between the pre-treatment period and post-treatment ($p = 0.004$). The dietary NSP intake showed positive pooled within-subject correlations with the energy intake ($+0.35$, $p = 0.004$), protein ($+0.41$, $p = 0.0007$) and carbohydrate ($+0.48$, $p = 0.0001$) intake but no such between-subject correlations were evident.

3.2. Bowel habit parameters

Investigation of the covariate effect of the food intake parameters failed to show any effect on the bowel habit parameters except that water consumed influenced the % water in stool ($p = 0.01$) and dry weight ($p = 0.02$) but had no significant effect on wet weight ($p = 0.013$). However, adjustment for water consumed made no qualitative difference to the treatment effect. There were increases in the mean faecal wet (26 g/day, +21%) and dry weights (5 g/day, +18%), and the water in the stools (+1%), between pre- and end treatment but these were not found to be statistically significant (Table 2). There was a significant difference between treatment periods in the extractable colour ($A_{560\text{nm}}$ ml/g dry weight) in the stools (Table 2). Mean extractable colour levels were about 19% lower during treatment than at pre- and post-treatment and there was a statistically significant difference between mid- and post-treatment. Stool wet weight was revealed as a statistically significant covariate for the mean appearance score ($p = 0.03$), but this had no qualitative effect on the treatment effect following adjustment. There were no statistically significant changes in the time between calls to stool or faecal form during treatment. However, during the post-treatment period, the time between calls to stool was longest and the faecal form lowest (Table 2).

Faecal form showed negative correlations with stool

Table 1
Variation of the dietary intake with the study periods[a]

Period	*n*	Energy (Cal)	Protein (g)	Fat (g)	Carbohydrate (g)	Alcohol (g)	NSP (g)	Water (g)
Pre-treatment	32	2148	78	91	245	13	17	2302
95% CI		1875–2420	70–87	75–107	213–277	8–17	15–19	1956–2649
Begin treatment	16	2051	77	81	241	14	16	2272
95% CI		1738–2364	65–90	66–96	197–284	6–23	13–19	1690–2853
Mid-treatment	48	2182	81	93	248	11	14	2188
95% CI		1995–2369	75–88	83–104	226–269	7–15	13–16	1963–2413
End treatment	48	2028	76	81	237	14	14	2317
95% CI		1898–2157	70–82	74–88	221–253	10–18	13–15	2125–2510
Post-treatment	32	2102	72	97	229	9	14	2260
95% CI		1838–2365	65–79	76–119	207–252	5–13	12–15	2035–2485

[a] 95% confidence intervals (CI) are based on un-transformed data.

Table 2
Variation of stool parameters with study period

Group	*n*	Wet weight (g/day)	Dry weight (g/day)	Water in stool (%)	Extractable colour ($A_{560\,nm}$ ml/g)	Period for call to stool; weekly mean (*n*) (h)	Faecal form; daily mean (*n*)
Pre-treatment	32	121	29.7	73.5	1.38	21.8 (32)	3.40 (187)
95% CI[a]		93–153	24.2–35.8	71.1–76.0	1.19–1.58	18.8–25.4	3.26–3.55
Begin treatment	47	147	33.8	75.1	1.17	24.3 (16)	3.33 (93)
95% CI		115–182	27.7–40.4	73.2–77.0	1.00–1.35	19.4–30.5	3.15–3.51
Mid-treatment	48	136	32.7	74.4	1.12	22.0 (48)	3.48 (277)
95% CI		109–167	27.1–38.8	72.4–76.3	1.00–1.25	19.6–24.6	3.37–3.60
End treatment	48	147	34.9	74.4	1.17	21.8 (48)	3.30 (293)
95% CI		116–183	29.1–41.2	72.7–76.1	1.05–1.29	19.7–24.2	3.19–3.42
Post-treatment	45	126	31.9	72.6	1.46	24.8 (32)	3.26 (177)
95% CI		95–161	25.3–39.2	70.7–74.5	1.31–1.60	21.8–28.1	3.11–3.40
ANOVA (*p*)		ns[b]	ns	ns	0.02	0.06	0.06
p (Mid–Post)		–	–	–	0.005	0.02	0.008

[a] 95% CIs are based on transformed data where appropriate.
[b] ns: not significant ($p > 0.05$).

wet weight (BS −0.61, $p = 0.01$; WS −0.23, $p = 0.07$) and water content (BS −0.68, $p = 0.004$), these being much weaker with respect to dry weights (BS −0.47, $p = 0.06$; WS −0.21, $p = 0.09$). The interval between calls to stool showed borderline negative correlation with the 24-hr stool wet (BS −0.50, $p = 0.05$; WS −0.22, $p = 0.07$) and dry weights (WS −0.32, $p = 0.01$). The stool wet and dry weights showed statistically significant positive within-subject and between-subject correlations with the majority of the bile acid weight measurements (e.g. total bile acids vs. wet weight, BS +0.69, $p = 0.003$; WS +0.64, $p = 0.001$; total bile acids vs. dry weight, BS +0.70, $p = 0.002$; WS +0.63, $p = 0.001$). However, apart from between-subject correlations with cholic acid concentrations (e.g. wet weight vs. cholic acid concentration relative to dry weight, BS +0.77 $p = 0.0005$), no bile acid concentrations relative to wet or dry weight showed any significant correlation with stool wet or dry weight.

3.3. Faecal composition

Investigation of the covariate effect of food intake, bowel habit parameters and stool storage time revealed no statistically significant treatment covariates ($p > 0.05$) on the bile acid parameters, except water on cholic acid levels and NSP on chenodeoxycholic acid levels but these had no qualitative effect on the treatment effect following adjustment. Most of the faecal bile acid output consisted of deoxycholic acid, lithocholic acid and isolithocholic acid (Table 3). The enzyme assay analyses for total 3-α-hydroxy bile acids (these have not been tabulated) showed low response of about half that expected from the HPLC analysis. Analysis of bile acids using the enzyme assay correlated with analysis using HPLC (BS +0.69, $p = 0.003$; WS +0.40, $p = 0.001$), but they clearly measured different parameters due possibly to unknown materials interfering with the enzyme assay. In particular, it was observed that the enzyme assay values for the pre-treatment period were particularly low and the difference between periods was statistically significant when the data were adjusted for an apparent covariate effect of the protein consumed. This result could not be fully explained but indicated the presence of inhibiting substances in the stool samples.

The amount of bile acids determined by the enzyme assay positively correlated with the water content in the stool (BS +0.90, $p < 0.0001$; WS +0.39, $p = 0.001$). The recoveries, using the enzyme assay relative to HPLC, were higher during treatment, and appeared to show positive associations with cholic acid content and wet weight and negative associations with deoxycholic acid, faecal form and the times between the calls to stool. This may indicate that the unknown

Table 3
Variation of faecal bile acids with study period

Group	n	Total bile acid (T)	Cholic acid	Deoxycholic acid (D)	Chenodeoxy-cholic acid	Lithocholic acid (L)	Isolithocholic acid (I)	$T \times (I+L)/D$	L/D
		mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	
Pre-treatment	32	114	0.8	37	0.6	40	21	215	1.08[b]
95% CI[a]		76–170	0.4–1.6	22–62	0.3–1.2	28–58	14–32	129–356	0.78–1.51
Begin treatment	47	118	1.0	47	0.7	41	22	180	0.87
95% CI		87–160	0.6–1.6	34–66	0.4–1.2	31–55	16–30	134–243	0.77–1.00
Mid treatment	48	105	0.9	49	0.5	34	12	108	0.68
95% CI		77–144	0.5–1.4	35–71	0.2–0.9	25–45	9–16	79–146	0.57–0.82
End treatment	48	89	1.3	40	0.3	29	12	89	0.72
95% CI		67–119	0.8–1.9	29–57	0.2–0.6	22–38	9–15	66–120	0.60–0.86
Post-treatment	45	93	1.2	40	0.5	31	13	113	0.77
95% CI		70–123	0.8–1.9	29–56	0.3–0.9	23–41	10–17	84–153	0.64–0.92
ANOVA (p)		ns[c]	ns	ns	ns	ns	0.001	0.005	0.06[b]
p (Pre–Mid)		–	–	–	–	–	ns	ns	0.008[b]
p (Pre–End)		–	–	–	–	–	0.007	0.002	0.02[a]
		mg/g dry wt	mg/g dry wt	mg/g dry wt	mg/g dry wt	mg/g dry wt	mg/g dry wt	mg/g dry wt	
Pre-treatment	32	4.16	0.033	1.35	0.020	1.46	0.78	7.08	
95% CI		3.07–5.64	0.019–0.056	0.87–2.11	0.010–0.038	1.10–1.93	0.57–1.06	4.67–10.72	
Begin treatment	47	3.92	0.033	1.56	0.020	1.37	0.73	5.35	
95% CI		3.23–4.77	0.024–0.046	1.22–2.02	0.012–0.033	1.15–1.63	0.60–0.88	4.45–6.43	
Mid treatment	48	3.64	0.033	1.71	0.013	1.17	0.43	3.50	
95% CI		2.96–4.49	0.024–0.045	1.32–2.22	0.006–0.024	0.94–1.44	0.35–0.52	2.82–4.34	
End treatment	48	2.80	0.041	1.28	0.008	0.91	0.37	2.86	
95% CI		2.35–3.34	0.030–0.056	1.00–1.63	0.004–0.015	0.78–1.07	0.32–0.43	2.40–3.42	
Post-treatment	45	3.38	0.046	1.47	0.017	1.13	0.47	3.75	
95% CI		2.73–4.18	0.034–0.062	1.09–1.98	0.009–0.030	0.94–1.35	0.40–0.55	3.18–4.41	
ANOVA (p)		0.04	ns	ns	ns	0.02	< 0.0001	0.001	
p (Pre–Mid)		ns	–	–	–	ns	0.0004	0.004	
p (Pre–End)		0.007	–	–	–	0.004	< 0.0001	0.0003	
		mg/g wet wt	mg/g wet wt	mg/g wet wt	mg/g wet wt	mg/g wet wt	mg/g wet wt	mg/g wet wt	
Pre-treatment	32	1.06	0.008	0.35	0.005	0.37	0.20	1.81	
95% CI		0.81–1.40	0.005–0.013	0.23–0.52	0.002–0.009	0.28–0.49	0.15–0.27	1.17–2.78	
Begin treatment	47	0.94	0.008	0.38	0.005	0.33	0.18	1.29	
95% CI		0.78–1.15	0.006–0.011	0.29–0.48	0.003–0.008	0.28–0.39	0.14–0.21	1.06–1.56	
Mid treatment	48	0.90	0.008	0.43	0.003	0.29	0.11	0.87	
95% CI		0.73–1.11	0.006–0.011	0.33–0.55	0.001–0.006	0.23–0.36	0.09–0.13	0.69–1.09	
End treatment	48	0.70	0.010	0.32	0.002	0.23	0.09	0.71	
95% CI		0.58–0.84	0.008–0.013	0.25–0.41	0.001–0.003	0.19–0.27	0.08–0.11	0.59–0.86	
Post-treatment	45	0.89	0.012	0.39	0.004	0.30	0.12	0.99	
95% CI		0.74–1.09	0.010–0.015	0.29–0.52	0.002–0.008	0.25–0.35	0.11–0.14	0.85–1.16	
ANOVA (p)		0.03	ns	ns	ns	0.02	< 0.0001	0.001	
p (Pre–Mid)		ns	–	–	–	ns	< 0.0001	0.002	
p (Pre–End)		0.003	–	–	–	0.002	< 0.0001	0.0002	

[a] 95% CIs are based on log transformed data.
[b] These values are mainly influenced by two outliers in the pre-treatment period; ANOVA $p = 0.09$ if these are omitted and the comparison p values cannot be calculated.
[c] ns: not significant ($p > 0.05$).

inhibitory substance(s) are produced in greater amounts during longer transit times.

There were drops in the amounts and proportions of the lithocholic acids during treatment (Table 3). There were statistically significant decreases in the lithocholic acid and isolithocholic acid concentrations relative to wet (lithocholic acid, −39%, isolithocholic acid, −54%) and dry weights (lithocholic acid, −37%; isolithocholic acid, −53%) between pre-treatment and end treatment. These decreases appear to depend on the duration of the treatment.

Most bile acid parameters started to return to their initial values subsequent to treatment, with some changes being marginally significant between the end- and post-treatment periods (per wet weight, lithocholic acid, $p = 0.05$, isolithocholic acid, $p = 0.03$). The ratio of the total lithocholic acids to deoxycholic acid weighted by the total bile acid weight showed highly statistically significant differences between treatments ($p = 0.005$) with a significant 59% reduction between pre-treatment and end treatment ($p = 0.002$). All these ratios appear to start returning to their initial levels after treatment. Although there was a drop in the total amount of bile acids and an initial rise in the deoxycholic acid content, neither of these was statistically significant. There was however a significant drop in the concentration, relative to both wet and dry weight, of the total bile acids by the end of the treatment period, changes which again appeared to start to be reversed when treatment was terminated.

There was a negative pooled within-subject correlation between the lithocholic acid/deoxycholic acid ratio and the faecal cholic acid content (-0.26, $p = 0.04$) and wet and dry weight cholic acid concentrations (-0.36, $p = 0.003$ and -0.35, $p = 0.004$ respectively). There were strong positive correlations between the amounts of the bile acids, between their wet concentrations and between their dry concentrations.

4. Discussion

Analysis of the data in this study shows a number of compatible trends between the bile acids and stool parameters.

4.1. Food intake

There was a general downward trend in the non-ispaghula NSP intake by the volunteers throughout the study. This resulted in a maximum reduction of 2.5 g (−15%) during ispaghula husk treatment, with a reduction of 3.2 g in the post-treatment phase relative to the pre-treatment phase. It may be that the volunteers, who were not restricted in their diet by the protocol, partially compensated, consciously or subconsciously, for the increased dietary fibre in the ispaghula husk by reducing their intake of other dietary fibre; this effect on their diet continuing after the treatment phase had ended.

There was no significant variation in any of the other nutrient intakes, in general agreement with a published study [26] that found that a higher daily intake of ispaghula husk was necessary to reduce perceived hunger.

4.2. Bowel habit parameters

There was a slight effect of ispaghula husk treatment on the time between calls to stool and the mean score for the stool appearance. Although the overall differences were not statistically significant, the period between calls to stool increased by 14% subsequent to the end of the treatment phase. There was also a drop in the mean appearance score (looser stools) at that time. These changes may be expected due to the reduction in fibre intake reducing the structure and form of the stools whilst the gut biomass remains elevated. The lack of any major change in the form of the stools during treatment may be ascribed to the fibre tending to produce wet well-formed stools.

There were higher mean wet and dry stool weights during treatment with ispaghula. The wet weight dropped to close to its initial value after treatment but the dry weight only returned to a weight corresponding to about half of the increase. These changes, due to the partially fermentable nature of ispaghula husk [11], were not statistically significant but agree with previous work [11].

4.3. Faecal bile acids

The enzyme assay is designed to determine the total concentration of 3-α-hydroxysteroid bile acids but not 3-β-hydroxysteroid bile acids such as isolithocholic acid. Its inclusion in this study was in order to confirm any overall trends shown by the HPLC assay. This assay, which was originally developed for cleaner and more hydrophilic serum and bile analyses, unfortunately appears to consistently underestimate the total 3-α-hydroxysteroid bile acids in our faecal samples.

Lithocholic acid and isolithocholic acid are both reduced during the ispaghula husk treatment. The daily amount of lithocholic acid produced decreased throughout treatment eventually to a value of 73% of the starting values, then showed indications of returning to the initial level. Although this was not statistically significant, there were similar, but statistically significant, trends with the concentration of lithocholic acid relative to the stool wet and dry weights. The daily amount of isolithocholic acid produced decreased

during treatment to a value of 55% of the starting values, then showed indications of returning to the initial level. The differences were statistically significant and there were similar, statistically significant, trends relative to the stool wet and dry weights. The decreases in total bile acids and the ratio of lithocholic acid to deoxycholic acid during treatment are not surprising given the marked reductions in the lithocholic acids. It is clear that the simplistic argument that the lowering of serum cholesterol by ispaghula husk is due solely to the removal of bile acids in the faeces has not been sustained by this study. This is unsurprising as the control mechanisms involved are complex and, to some extent, compensatory [27].

The ratio of lithocholic acid to deoxycholic acid [28] and the product of this with the bile acid concentration [29] have both been put forward as risk factors in colorectal cancer. This study shows that the ratio of lithocholic acid to deoxycholic acid tends to be reduced during treatment with ispaghula husk and there are also statistically significant reductions in the ratio of total lithocholic acids to deoxycholic acid multiplied by the total bile acid output.

The mechanism for the effect of ispaghula husk treatment on the lithocholic acids can be explained in terms of their metabolism [27]. Ispaghula acts as a substrate for the colonic microflora. Fibre fermentation reduces the 7-α-dehydroxylase activity [12]. The reduction in the proportion of secondary bile acids (deoxycholic acid and lithocholic acid) being formed and reabsorbed, reduces the hydrophobicity of the bile acids feeding back to the liver. This alters the balance between two pathways [30,31], involving the hepatic 7-α-hydroxylation and blood vessel 27-hydroxylation of cholesterol, for the production of the primary bile acids (cholic acid and chenodeoxycholic acid) with a consequential decrease in the relative amounts of chenodeoxycholic acid to cholic acid being produced. When these bile acids are themselves 7-α-dehydroxylated in the colon, they produce a lower amount of lithocholic acid. As isolithocholic acid is made from lithocholic acid by the gut flora, any reduction in lithocholic acid is expected to also reduce isolithocholic acid. In addition, undigested dietary fibre increases stool bulk and its water-holding ability, thereby diluting the concentrations. The tendency of increased dietary fibre to produce large stools having a lower proportion of lithocholic acid relative to the level of deoxycholic acid has been reported previously [32].

The unfermented fibre forms a viscous network, which will reduce diffusion of the bile acids to the colonic surface. It will essentially hide the bile acids within the fibre and lower bile acid absorption. It is also likely that the fibre may bind bile acids and directly or by partition, or by reducing their ionisation and hence their solubility. Dietary fibre stimulates colonic motility by mechanical effects [11], thereby reducing whole gut transit time [33]. Quicker throughput lowers bile acid absorption and the consequential deoxycholic acid pool size [34]. It should, perhaps, be noted that the ispaghula husk is given as a dispersion rather than a solution; its description as 'soluble fibre' depending on the capability of 80% of it being dissolved under conditions (100°C, pH 7, 1 h) far harsher than those physiologically achieved.

The considerable effects that ispaghula husk treatment has on the levels of the lithocholic acids may have significant therapeutic benefits. There exists a considerable body of evidence linking lithocholic acid with colorectal cancer initiation and progression. Lithocholic acid strips the protective mucus layer [35] increases the proliferation of normally non-replicative mucosal surface cells [36] is comutagenic in rats [37] causes DNA damage in human cell lines [38] stimulates carcinogen production in the microbial flora, inhibits detoxifying enzymes [39] and reduces the normal defence mechanisms in the mucosa [40]. The level of lithocholic acid relative to deoxycholic acid has been found to be raised above unity in the faeces of patients with large adenomas [41] and colorectal cancers [42–44].

Dietary fibre from ispaghula husk lowers serum cholesterol in hypercholesterolemia [45,46]. As it has been reported that lithocholic acid elevates serum cholesterol [47], an interesting consequence of the reduction in faecal lithocholic acid is the subsequent reduction in serum cholesterol by the stimulating effect of the more hydrophilic bile acid pool on bile acid synthesis and the reduction of cholesterol ester export from the liver.

References

[1] J.W. Anderson, N. Zettwoch, T. Feldman, J. Tietyen-Clark, P. Oeltgen, C.W. Bishop, Cholesterol lowering effects of psyllium hydrophilic mucilloid for hypercholesterolemic men, Arch. Int. Med. 148 (1988) 292–296.

[2] W.L. Haskell, G.A. Spiller, C.D. Jensen, B.K. Ellis, J.E. Gates, Role of water-soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects, Am. J. Cardiol. 69 (1992) 433–439.

[3] S.R. Glore, D. van Treeck, A.W. Kuchans, M. Guild, Soluble fiber and serum lipids: a literature review, J. Am. Diet Assoc. 94 (1994) 425–436.

[4] K.W. Weingand, N.A. Le, B.R. Kuzmak, W.V. Brown, B.P. Daggy, T.A. Miettinen, B.V. Howard, W.J. Howard, Effects of psyllium on cholesterol and low-density lipoprotein metabolism in subjects with hypercholesterolemia, Endocrinology and Metabolism 4 (1997) 141–150.

[5] J. Scott, The role of fibre in lowering cholesterol, Gastroenterology in Practice 9 (2) (1994) 6–8.

[6] S.A. Bingham, Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer, Proc. Nutr. Soc. 49 (1990) 153–171.

[7] S.A. Bingham, Plant cell wall material and cancer protection, in: K.W. Waldron, I.T. Johnson, G.R. Fenwick (Eds.), Food and Cancer Prevention: Chemical and Biological aspects, Royal Society of Chemistry, Cambridge, 1993, pp. 339–347.
[8] S. Bingham, Colorectal cancer, Eur. J. Gastroent. Hepat. 5 (8) (1993) 574–577.
[9] D.S. Alberts, C. Rittenbaugh, J.A. Story, M. Aickin, S.R. McGee, M.K. Buller, J. Atwood, J. Phelps, P.S. Ramanujam, S. Bellapravalu, J. Patel, L. Bettinger, L. Clark, Randomized, double-blinded, placebo-controlled study of effect of wheat bran fiber and calcium on fecal bile acids in patients with resected adenomatous colon polyps, J. Nat. Cancer Inst. 88 (1996) 81–92.
[10] M. Wilpart, M. Roberfroid, Intestinal carcinogensis and dietary fibers: The influence of cellulose or Fybogel chronically given after exposure to DMH, Nutr. Cancer 10 (1987) 39–51.
[11] P. Marteau, B. Flourié, C. Cherbut, J-L. Corrèze, P. Pellier, J. Seylaz, J-C. Rambaud, Digestibility and bulking effect of ispaghula husks in healthy humans, Gut 35 (1994) 1747–1752.
[12] B.S. Reddy, Nutritional factors as modulators of colon carcinogensis, in: D.V. Parke, C. Ioannides, R. Walker (Eds.), Food, Nutrition and Chemical Toxicity, Smith-Gordon, London, 1993, pp. 325–336.
[13] R.W. Owen, M.H. Thompson, M.J. Hill, M. Wilpart, P. Mainguet, M. Robertfroid, The importance of the ratio of lithocholic to deoxycholic acid in large bowel carcinogensis, Nutr. Cancer 9 (1987) 67–71.
[14] J.F. Kennedy, J.S. Sandhu, D.A.T. Southgate, Structural data for the carbohydrate of Ispaghula husk ex, *Plantago ovata* Forsk. Carbohydr. Res. 75 (1979) 265–274.
[15] J.S. Sandhu, G.J. Hudson, J.F. Kennedy, The gel nature and structure of the carbohydrate of Ispaghula husk ex, *Plantago ovata* Forsk. Carbohydr. Res. 93 (1981) 247–259.
[16] E. Angelini, C. Boniglia, M. Mosca, G. Bellomonte, Detection and determination of plant gums by gas-chromatographic determination of their constituent monosaccharides, Rivista della Soc Ital di Scienza dell'Alimentatazione 13 (6) (1984) 479–484.
[17] J. Stevens, P.J. VanSoest, J.B. Robertson, D.A. Levitsky, Comparison of the effects of psyllium and wheat bran on gastrointestinal transit time and stool characteristics, J. Am. Diet. Assoc. 88 (3) (1988) 323–326.
[18] F.W. Chalmers, M.M. Clayton, L.O. Gates, R.E. Tucker, A.W. Wertz, C.M. Young, W.D. Foster, The dietary record — How many and which days?, J. Am. Diet. Ass. 28 (1952) 711–717.
[19] K.D.R. Setchell, A.M. Lawson, N. Tanida, J. Sjövall, General methods for the analysis of metabolic profiles of bile acids and related compounds in faeces, J. Lip. Res. 24 (1983) 1085–1100.
[20] M.C. Vescina, A. Mamianetti, N.M. Vizioli, S.E. Lucangioli, V. Rodriguez, A. Orden, D. Garrido, C.N. Carducci, Evaluation of faecal bile acid profiles by HPLC after using disposable solid-phase columns, J. Pharm. Biomed. Anal. 11 (11–12) (1993) 1331–1335.
[21] K.D.R. Setchell, J.A. Ives, G.C. Cashmore, A.M. Lawson, On the homogeneity of stools with respect to bile acid composition and normal day-to-day variations: a detailed qualitative and quantitative study using capillary column gas chromatography-mass spectrometry, Clinica Chimica Acta 162 (1987) 257–275.
[22] P. Eneroth, K. Hellstrom, J.A Sjövall, method for quantitative determination of bile acids in human faeces, Acta Chem. Scand. 22 (1968) 1729–1744.
[23] J.A. Story, E.J. Furumoto, Bile acid analysis: methods and problems, Eur. J. Cancer Prev. 1 (1991 Suppl 2) 29–33.
[24] H.U. Bergmeyer, J. Bergmeyer, M. Grassl, 3α-hydroxy bile acids: Colorimetric method for serum, gastric juice and faeces, in: Methods of Enzymatic Analysis, VIII, 3rd ed., Verlag Chemie, Weinheim, 1985, pp. 291–297.
[25] M.F. Chaplin, Analysis of bile acids and their conjugates using high pH anion exchange chromatography with pulsed amperometric detection, J. Chromatograph B: Biomed. Appl. 664 (1995) 431–434.
[26] K.A. Cybulski, J. Lachaussee, H.R Kissilett, The threshold for satiating effectiveness of psyllium in a nutrient base, Physiol. Behav. 51 (1) (1992) 89–93.
[27] M.F. Chaplin, Bile acids, fibre and colon cancer: the story unfolds, J. Royal Soc. Health 118 (1) (1998) 1–9.
[28] M.J. Hill, The ratio of lithocholic to deoxycholic acid in faeces: a risk factor in colorectal carcinogensis, Eur. J. Cancer Prevent. 1 (2) (1991) 75–78.
[29] R.W. Owen, P.J. Henly, D.W. Day, M.H. Thomson, M.J. Hill, Fecal steroids and colorectal cancer: Bile acid profiles in low and high risk groups, in: J.V. Joossens, M.J. Hill, J. Geboers (Eds.), Human Carcinogenesis, Elsevier, Amsterdam, 1985, pp. 165–170.
[30] C. Lee, K.O. Martin, N.B. Javitt, Bile acid synthesis: 7 alpha-hydroxylation of intermediates in the sterol 27-hydroxylase metabolic pathway, J. Lipid Res. 37 (6) (1996) 1356–1362.
[31] D. Kritchevsky, Bile acids: biosynthesis and functions, Eur. J. Cancer Prevent. 1 (2) (1991) 23–28.
[32] W.G. Brydon, M.A. Eastwood, R.A. Elton, The relationship between stool weight and the lithocholate/deoxycholate ratio in faeces, Br. J. Cancer 57 (1988) 635–636.
[33] D.P. Burkitt, Epidemiology of cancer of the colon and rectum, Cancer 28 (1) (1971) 3–13.
[34] I.P. Van Munster, F.M. Nagengast, The influence of dietary fibre on bile acid metabolism, Eur. J. Cancer Prevent. 1 (2) (1991) 35–46.
[35] L.L. Shekels, C.T. Lyftogt, S.B. Ho, Bile acid-induced alterations of mucin production in differentiated human colon cancer cell lines, Int. J. Biochem. Cell Biol. 28 (2) (1996) 193–201.
[36] G. Biasco, G.M. Paganelli, R.W. Owen, M.J. Hill, Faecal bile acids and colorectal cell proliferation, Eur. J. Cancer Prevent. 1 (2) (1991) 63–68.
[37] M. Wilpart, P. Mainguet, A. Maskens, M. Roberfroid, Mutagenicity of 1,2-dimethylhydrazine towards salmonella typhimurium, co-mutagenic effect of secondary biliary acids, Carcinogenesis 4 (1983) 45–48.
[38] B.L. Pool-Zobel, U. Leucht, Induction of DNA damage by risk factors of colon cancer in human colon cells derived from biopsies, Mutat. Res. 375 (2) (1997) 105–115.
[39] H. Schneider, H. Fiander, R.K. Latta, N.W. Ross, Bile acid inhibition of xenobiotic-metabolizing enzymes is a factor in the mechanism of colon carcinogenesis: tests of aspects of the concept with glucuronosyltransferase, Eur. J. Cancer Prev. 2 (2) (1993) 393–400.
[40] P. Arvind, E.D. Papavassiliou, G.J. Tsioulias, B.W. Duceman, C.I. Lovelace, W. Geng, L. Staiano-Coico, B. Rigas, Lithocholic acid inhibits the expression of HLA class 1 genes in colon adenocarcinoma cells, Differential effect on HLA-A, -B and -C loci. Mol. Immunol. 31 (8) (1994) 607–614.
[41] R.W. Owen, M. Dodo, M.H. Thompson, M.J. Hill, The faecal ratio of lithocholic acid to deoxycholic may be an important aetiological factor in colo-rectal cancer, Eur. J. Cancer Clin. Oncol. 19 (1983) 1307.
[42] R.W. Owen, P.J. Henly, M.H. Thompson, M.J. Hill, Steroids and cancer: Faecal bile acid screening for early detection of cancer risk, J. Steroid Biochem. 24 (1986) 391–394.
[43] R.W. Owen, M. Dodo, M.H. Thompson, M.J. Hill, The faecal ratio of lithocholic acid to deoxycholic acid may be an important aetiological factor in colorectal cancer, Biochem. Soc. Trans. 12 (1984) 861–863.
[44] C.H.E. Imray, S. Radley, A. Davis, G. Barker, C.W. Hendrickse, I.A. Donovan, A.M. Lawson, P.R. Baker, J.P.

Neoptolemos, Faecal unconjugated bile acids in patients with colorectal cancer or polyps. Gut 33 (1992) 1239–1245.
[45] J.W. Anderson, N. Zettwoch, T. Feldman, J. Tietyen-Clark, P. Oeltgen, C.W. Bishop, Cholesterol lowering effects of psyllium hydrophilic mucilloid for hypercholesterolemic men, Arch. Int. Med. 148 (1988) 292–296.
[46] W.L. Haskell, G.A. Spiller, C.D. Jensen, B.K. Ellis, J.E. Gates, Role of water soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects, Am. J. Cardiol. 69 (1992) 433–439.
[47] G. Loo, G. Kessie, E. Berlin, P.P. Nair, Effect of lithocholic acid feeding on plasma lipoproteins and binding of radioiodinated human lipoproteins to hepatic membranes in rats, Comp. Biochem. Physiol. Comp. Physiol. 102 (2) (1992) 379–383.