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Faecal pH, bile acid and sterol concentrations in premenopausal Indian and white vegetarians compared with white omnivores

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Faecal bulk, pH, water content, the concentrations of neutral sterols and bile acids and dietary intakes were measured in twenty-two Indian vegetarian, twenty-two white omnivorous and eighteen white vegetarian premenopausal women. Faecal bulk and water content were greater and pH lower in the Indian vegetarians. Total faecal animal sterol and coprostanol concentrations expressed on a dry-weight basis were lower in the vegetarians compared with the omnivores. The faecal sterol concentrations were correlated with dietary cholesterol intake. Primary bile acids were detected in six Indian vegetarians, two white vegetarians and two white omnivores; secondary bile acids were detected in all the white omnivore and vegetarian subjects but not in two of the Indian vegetarians. Total faecal free bile acid and conjugated bile acid concentrations were lower in the white vegetarians compared with the omnivores. Faecal lithocholic acid concentrations were lower in both Indian and white vegetarians. The lithocholic : deoxycholic acid ratio and coprostanol : total animal sterols ratio were significantly lower in the Indian vegetarians compared with the omnivores. Both ratios were positively correlated with faecal pH. Stepwise multiple regression analyses were undertaken in order to identify which nutrients influenced faecal pH, lithocholic and deoxycholic acid concentrations. The intakes of starch and dietary fibre were negatively associated with faecal concentrations of lithocholic and deoxycholic acid. Starch intake alone was negatively associated with faecal pH. The results of this study confirm that diets high in dietary fibre decrease faecal bile acid concentrations and suggest that the complex carbohydrates present in Indian vegetarian diets influence faecal pH and inhibit the degradation of faecal steroids.

Bile: Lithocholic acid: Deoxycholic acid: Vegetarians

Colorectal cancer is less prevalent in nations where the intakes of meat and fat are low and that of starchy carbohydrates is high (Doll & Peto, 1981). The incidence of colorectal cancer has increased in recent years in Japan (Wilmink, 1997) where the diet has changed from being one low in fat and high in starchy carbohydrates. Cohort studies of vegetarians (Jensen, 1983; Phillips & Snowdon, 1985; Frentzel-Beyme & Chang-Claude, 1994; Thorogood *et al.* 1994) suggest that cancer incidence, including colorectal cancer is lower among vegetarians compared with the general population who follow an omnivorous diet. Some prospective cohort studies have found risk of colorectal cancer to be positively associated with meat and fat intake (Willett *et al.* 1990). On the other hand, consumption of fruit and vegetables is associated with a decreased risk (Willet *et al.* 1990; Block, 1992; Key *et al.* 1996). Lower rates of colorectal cancer have also been reported in the British South Asian ethnic minority, a high proportion of whom follow a vegetarian diet (Marmot, 1984).

One mechanism whereby dietary intake may influence risk of colorectal cancer is by influencing the secretion of bile acids and their degradation in the colon by bacteria. Secondary bile acids are formed after enzymic deconjugation and dehydroxylation of primary bile acids in the large bowel by anaerobic bacteria. Lower faecal concentrations of total and secondary bile acids have been found in low-risk populations (Thompson, 1985). However, case–control studies in populations where colorectal cancer is prevalent only show a weak relationship between faecal bile acids and risk (Nagengast *et al.* 1995; Radley *et al.* 1996). It has also been argued that coprostanol, a degradation product of cholesterol may also be associated with increased risk of colorectal cancer (Peuchant *et al.* 1987). Faecal steroid concentrations in vegans are similar to those observed in

Abbreviations: DCA, deoxycholic acid; LCA, lithocholic acid.

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people living in low-risk countries (Aries *et al.* 1971), but less striking differences have been observed in lacto-vegetarians (Nair *et al.* 1984; Turjiman *et al.* 1984; van Faassan *et al.* 1993). High colonic pH is believed to increase risk of colorectal cancer (Thornton *et al.* 1981) and both ecological and case–control studies have shown an association between high faecal pH and risk of colorectal cancer (Mortensen & Clausen, 1995). The fermentation of carbohydrate to short-chain fatty acids in the large bowel leads to a reduction of faecal pH (Mortensen & Clausen, 1995) and low faecal pH inhibits the degradation of sterols in the large bowel (van Munster *et al.* 1994). Furthermore, faecal pH was decreased by a change from an omnivorous diet to a vegan diet (Van Dokkum *et al.* 1983), but not by a change to a lacto-vegetarian diet (Allinger *et al.* 1989; van Faassen *et al.* 1993). However, South Asians of Indian origin who are known to be at lower risk of colorectal cancer are predominantly lacto-vegetarian. The aim of the present study was to compare faecal pH, neutral steroid and bile acid profiles in South Asian vegetarian, white vegetarian and white omnivorous premenopausal women and to see if any observed differences could be related to their dietary intakes of starch, dietary fibre, fat and cholesterol.

Methods

Vegetarian women of Indian descent and white subjects consuming mixed diets were recruited by random sampling from general practice lists in north London. White vegetarian subjects were recruited through the Vegetarian Society and by placing advertisements in *The Vegetarian* magazine. Subjects were visited at home and completed a 7 d weighed food intake record. Each subject was provided with a food recording kit consisting of standardized dietary scales (Soehlne Digita, Germany), food diary and detailed instructions on how to record their food intake. They were also asked whether they took any dietary supplements. The white subjects were visited twice during the week, but as the Indian subjects required more supervision they were visited about four times. Additionally, the subjects were asked to keep a weighed record of individual recipes of foods cooked at home. Samples of cooking fats and oils used in the individual households were also collected and frozen at −20° until analysed for their fatty acid content. Methyl esters were prepared from the fats by reaction with sodium methoxide and analysed by capillary GLC on a 25 m CpSil88 column (Chromopak, London, UK). Nutrient intakes were subsequently calculated from food composition tables (Holland *et al.* 1991) using a computer program (FOODTABS, T.A.B. Sanders, King's College London, UK) and estimates of fatty acid intakes were made by supplementing the food table data with fatty acid analyses of the spreadable and cooking fats used by the subjects (Reddy & Sanders, 1992). The height, weight and waist circumference of each subject were measured and skinfold thicknesses measured at four sites (biceps, triceps, suprailiac crest and subscapular) using Harpenden calipers. Percentage body fat was calculated according to the method of Durnin & Womersley (1974).

Subjects were asked to make a 24 h faecal collection. A faecal collection kit consisting of a metal frame fitted with a collection box, which could be placed on the toilet, was provided. The contents were kept chilled in an insulated box with cool packs until collected and transported to the laboratory. Detailed instructions on use of the kit and 24 h collection were also included. Faeces were stored in the collection boxes at −20° (for 6–12 months) until analysed for water, faecal pH, bile acids and neutral sterols at Public Health Laboratory Service, Porton Down, Salisbury, Wilts., UK. Frozen faecal samples were homogenized and freeze-dried. A 0·5 g portion of freeze-dried sample was sequentially extracted and fractionated, using lipophilic anion-exchange chromatography, into neutral sterol, free bile acid, and conjugated and sulfated steroid fractions, and steroid concentrations were determined by gas chromatography–mass spectroscopy by the method of Owen *et al.* (1984).

Comparisons between groups were made by ANOVA using the SPSS/PC program (SPSS for Windows, version 6, year 1994; SPSS Inc., Chicago, IL, USA). Where the overall F test approached significance, Dunnet's t test for samples with unequal variance was applied. Simple correlations were estimated using the product moment correlation. Stepwise multiple regression analysis using the forward selection option was undertaken to test for predictive relationships between dietary variables and faecal variables. Ethical approval for the study was given by the Local and Regional Health Authority Ethical Committees.

Results

The Indian women were shorter than the white women (Table 1). They also tended to be slightly fatter as assessed by skinfold thickness measurements but otherwise they were well matched with the omnivorous subjects. Dietary records were obtained in twenty-two white omnivores, twenty-two Asian vegetarians and eighteen white vegetarians. The Indian subjects were life-long vegetarians, the Caucasian vegetarians had followed a vegetarian diet for a period of at least 12 months. Dietary intakes in the omnivorous subjects were similar to those reported in the *Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990). Energy and alcohol intakes were lower in the Indian women than in the other two groups. The proportions of dietary energy derived from saturated fatty acids and protein were lower, and that from polyunsaturated fatty acids was higher, in both groups of vegetarians compared with the omnivores. Cholesterol intakes were lower in both vegetarian groups compared with the omnivores. Dietary fibre intakes were considerably greater in the white vegetarians than in the other two groups. Table 2 shows the sources of dietary fibre in the three groups. The higher intakes in the white vegetarians were mainly derived from cereals. Despite the broad similarities in the intake of proximate nutrients, the Indian vegetarian diets consisted of different types of foods, in particular spiced (curry) and fermented foods and chappatis (unleavened flat bread) were regularly consumed by the Indian subjects, whereas the white vegetarian diets comprised wholemeal bread, salads,

Table 1. Age, height, weight, BMI, percentage body fat and mean daily nutrient intake of the subjects
(Mean values with their standard errors)

	White omnivores (*n* 22)		Indian vegetarians (*n* 22)		White vegetarians (*n* 18)	
	Mean	SE	Mean	SE	Mean	SE
Age (years)	33·8[a]	0·78	34·5[a]	0·86	29·6[b]	0·91
Height (m)	1·64[a]	0·02	1·56[b]	0·01	1·61[a]	0·02
BMI (kg/m²)	24·1	0·70	25·1	0·98	22·5	1·00
Body fat (%)	30·7[a]	0·96	34·1[c]	0·72	26·2[b]	1·32
Energy (MJ)	7·47[a]	0·321	6·07[b]	0·296	7·67[a]	0·373
Energy (kJ/kg body wt)	118	6·16	106	6·83	133	8·66
Energy from protein (%)	15·8[a]	0·58	11·7[b]	0·42	11·9[b]	0·34
Energy from fat (%)	40·3[a]	1·14	37·7[a,b]	1·05	35·1[b]	1·72
Energy from saturated fatty acids (%)	15·7[a]	0·57	11·4[b]	0·60	11·4[b]	1·01
Energy from polyunsaturated fatty acids (%)	6·4[a]	0·66	7·6[b]	0·67	7·8[b]	0·91
Energy from starch (%)	19·7[a]	0·83	23·9[b]	0·86	24·5[b]	1·21
Energy from sugar (%)	16·7	1·41	16·2[b]	1·13	19·6[a]	2·17
Energy from alcohol (%)	3·4[a]	0·78	0·3[b]	0·13	4·9[a]	1·01
Fibre (g)	16·6[a]	0·97	16·2[a]	1·17	29·3[b]	2·57
Cholesterol (mg)	199[a]	22·7	34[c]	12·3	104[b]	20·5

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different; $P < 0.05$.

Table 2. Mean daily dietary fibre contributed by different food groups in premenopausal women according to dietary habit

	White omnivores (*n* 22)		Indian vegetarians (*n* 22)		Caucasian vegetarians (*n* 18)	
Food groups	g/d	% of total dietary fibre intake	g/d	% of total dietary fibre intake	g/d	% of total dietary fibre intake
Cereals	7·9	47·6	7·3	45·1	12·5	42·7
Pulses	2·4	14·5	1·4	8·6	3·2	10·9
Vegetables	3·5	21·1	2·7	16·7	5·9	20·1
Fruits and nuts	1·6	9·6	3·2	19·8	6·1	20·8
Miscellaneous	1·2	7·2	1·6	9·9	1·6	5·5
Total	16·6	100	16·2	100	29·3	100

Table 3. Mean weights of foods consumed (g/d) in premenopausal women according to dietary habit

Food group	White omnivores	Indian vegetarians	White vegetarians
Grains, flours, starches	39	78	66
Bread	88	107	121
Other cereals	73	57	61
Pulses	34	93	111
Vegetables	50	88	102
Potatoes	106	33	59
Other root vegetables	17	6	26
Leafy vegetables	39	27	45
Fruit	80	161	242
Milk	232	166	148
Cheese	15	12	29
Fermented milk	11	35	29
Eggs	17	2	9
Spreading fats	15	6	15
Fish	22	0	0
Meat	148	0	0
Sugars and confectionery	25	13	20
Beverages	1105	443	1052
Miscellaneous	76	214	254
Total weight of food and drink consumed	2182	1541	2389

cheese and vegetable dishes. Table 3 shows the mean intake of foods consumed by the three groups.

Faecal collections were obtained in eighteen white omnivores, twenty-two Asian vegetarians and eighteen white vegetarians. The mean faecal wet weight was significantly greater in the Indian vegetarians compared with the omnivores (Table 4). The faecal water content was greater in the Indian vegetarians compared with both the omnivores and the white vegetarians. Faecal pH was significantly lower in the Indian vegetarians compared with

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Table 4. Faecal weight, water, pH and neutral and acid steroids in premenopausal women with different dietary habits
(Mean values with their 95% confidence intervals)

	White omnivores (*n* 18)		Indian vegetarians (*n* 22)		White vegetarians (*n* 18)		Statistical significance of differences between groups
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Faecal wet wt (g/d)	117[a]	89–146	186[b]	143–228	160[ab]	118–201	$P = 0.036$
Faecal dry wt (g/d)	30·8	27·8–45·0	36·0	29·2–42·7	38·4	31·2–45·6	NS
Faecal water content (%)	72·6[a]	69·9–75·2	78·9[b]	76·3–81·4	74·6[a]	72·4–76·9	$P = 0.001$
Faecal pH	6·65[a]	6·40–6·91	6·18[b]	5·91–6·45	6·55[a]	6·27–6·82	$P = 0.025$
Total bile acids (mg/d)	244	185–303	234	169–301	192	127–257	NS
Total animal derived sterols (mg/d)	848	602–1083	555	393–715	582	396–767	$P = 0.06$

[a,b] Mean values within a row not sharing a common superscript are significantly different, $P < 0.05$.

Table 5. Faecal concentrations of bile acids and neutral sterols (mg/g dry weight) in premenopausal women according to different dietary habits
(Mean values with their 95% confidence intervals)

	White omnivores (*n* 18)		Indian Vegetarians (*n* 22)		White Vegetarians (*n* 18)		Statistical significance of *F*
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Conjugated bile acids	0·75[a]	0·57–0·93	0·61[ab]	0·46–0·77	0·42[b]	0·34–0·49	$P = 0.009$
Free bile acids	7·48[a]	5·68–9·28	5·76[ab]	4·76–6·76	4·36[b]	3·15–5·59	$P = 0.006$
Cholic acid	0·17	0–0·54	0·45	0–0·75	0·02	0–0·14	NS
Lithocholic acid	2·90[a]	2·19–3·60	1·72[b]	1·25–2·21	1·63[b]	1·24–2·01	$P = 0.001$
Chenodeoxycholic acid	0·19	0–0·60	0·4	0–0·75	0·06	0–0·14	NS
Deoxycholic acid	3·21	2·31–4·12	2·54	1·92–3·16	2·07	1·19–2·94	$P = 0.121$
Lithocholic : deoxycholic acid ratio	1·00[a]	0·85–1·15	0·67[b]	0·49–0·86	1·18[a]	0·84–1·52	$P = 0.008$
Plant sterols	15·7	13·7–17·6	14·3	12·0–16·7	13·9	11·6–16·3	NS
Total animal sterols	28·7[a]	22·9–34·6	16·0[b]	13·1–19·0	15·3[b]	11·7–18·9	$P < 0.001$
Coprostanol	20·6[a]	15·7–25·5	7·4[b]	4·3–10·5	9·3[b]	6·3–12·3	$P < 0.001$
Coprostanol : total animal sterols ratio	0·71[a]	0·65–0·77	0·42[b]	0·27–0·5	0·61[ab]	0·49–0·73	$P = 0.003$

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$ (Dunnet's *t* test).

both the omnivores and white vegetarians. Daily faecal animal sterol output tended to be lower in both vegetarian groups compared with the white omnivores but the faecal output of bile acids was not different.

Faecal concentrations of free and conjugated bile acids were significantly lower in the white vegetarian group compared with the omnivores (Table 5). Primary bile acids (cholic and chenodeoxycholic acid) were detected in one omnivore, six Indian vegetarians and three white vegetarians. The secondary bile acids lithocholic acid (LCA) and deoxycholic acid (DCA) were detected in all subjects except for two Indian vegetarians. LCA concentrations were lower in both vegetarian groups than in the omnivores and although DCA concentrations tended to be lower in the white vegetarians this was not statistically significant. The LCA : DCA ratio was significantly lower in the Indian vegetarians than in the other two groups. Faecal pH was positively correlated with the LCA : DCA ratio (r 0·31; $P = 0.009$).

Faecal plant sterol concentrations did not differ significantly between groups. Total animal sterol (cholesterol, coprostanone and coprostanol) and coprostanol concentrations were lower in both vegetarian groups compared with the omnivores. Dietary cholesterol was strongly correlated with total faecal animal sterol concentrations (r 0·46; $P = 0.0001$). However, the intake of dietary cholesterol was considerably less than the daily faecal excretion of animal sterols. Coprostanol concentrations were significantly lower in both vegetarian groups compared with the omnivores with the coprostanol : total animal sterols ratio being significantly lower in the Indian vegetarians than in the omnivores. This ratio was also positively correlated with faecal pH (r 0·397; $P = 0.001$).

Table 6 shows the results of the stepwise multiple regression analysis where intakes of fibre, starch, total carbohydrate, fat, polyunsaturated fatty acids and saturated fatty acids, standardized for energy intake, were entered as predictor variables for faecal pH and secondary bile acid concentrations. Dietary fibre intake was negatively associated with the dry weight faecal concentrations of LCA, DCA and total bile acids. Starch intake was negatively associated with LCA, total free bile acids and faecal pH.

Discussion

The main objective of the present study was to investigate the relationship between dietary intake and faecal sterol and bile acid profiles in different groups of vegetarians. Our study involved a 24 h faecal collection, consequently the estimates for bile acid and neutral sterol excretion rates are only approximately quantitative. It is probably necessary to collect samples for at least 3 d for sterol balance studies. However, a single stool sample has previously been shown

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Table 6. Significant partial correlations of bile acid concentrations and faecal pH, with starch and fibre intakes standardized for energy intake

	Partial correlation			
	Starch	Dietary fibre	r^2	Statistical significance
Lithocholic acid (mg/g dry wt)	−0·27	−0·40	0·31	$P = 0{\cdot}0001$
Deoxycholic acid (mg/g dry wt)	–	−0·44	0·2	$P = 0{\cdot}0007$
Total free bile acids (mg/g dry wt)	−0·23	−0·52	0·38	$P < 0{\cdot}0001$
pH	−0·32	–	0·11	$P = 0{\cdot}013$

to be adequate to provide a profile of faecal sterols and bile acids (McKeigue *et al.* 1989).

Dietary fat and cholesterol are well known to stimulate bile secretion. Total fat intakes were only slightly lower in both vegetarian groups than in the omnivorous group. However, dietary cholesterol intakes were lower in the vegetarians and their excretion of faecal neutral sterols of animal origin also tended to be lower than in the omnivores. Faecal animal sterol concentrations were correlated with dietary cholesterol intakes. The coprostanol : total animal sterols ratio was lower in the vegetarians, especially the Indian subjects. The lower coprostanol : cholesterol ratio implies that there was a reduction in the degradation of cholesterol in Indian vegetarians compared with the white omnivores.

Primary bile acids were present in the faeces of 27 % of the Indian vegetarians, 11.1 % of the white vegetarians and 5.6 % of the white omnivores. Faecal concentrations of secondary bile acids, both free and conjugated were lower in both vegetarian groups but this was more marked in the white vegetarians. Faecal concentrations of bile acids on a dry-weight basis were correlated with the dietary intakes of fibre and starch (both standardized for energy intake). This suggests that the variations in the intake of dietary fibre and starch could explain the differences in faecal bile acid concentrations between vegetarians and omnivores. This conclusion is consistent with a trial of cereal fibre (Alberts *et al.* 1996) which reported decreased faecal secondary bile acid concentrations in subjects randomized to receive 2 or 13·5 g wheat bran daily. The significantly lower concentrations of total bile acids and neutral sterols in the white vegetarians are consistent with their lower risk of gallstones (Pixley *et al.* 1985).

We decided to measure faecal pH because it can influence the microbial degradation of cholesterol and primary bile acids in the large bowel. Ideally, faecal pH should be measured as soon as the stool is passed, but this is not practical in a community-based study. While it remains a possibility that fermentation *ex vivo* led to a reduction of pH, we only observed a lower faecal pH in the Indian vegetarian subjects and not the other subjects. All the samples were collected and stored under the same conditions and it is unlikely that such a difference arose by chance. Moreover, the lower faecal pH is consistent with lower rates of degradation of primary bile acids and cholesterol and higher faecal bulk in the Indian subjects. Furthermore, faecal pH was found to be weakly associated with starch intake. Christl *et al.* (1995) found that starch inhibited the degradation of bile acids more strongly at pH 6 than pH 7 *in vitro*. Resistant starch can be fermented to short-chain fatty acids and, thus, decrease faecal pH. However, we were unable to quantify intakes of resistant starch in the present study owing to a lack of reliable food composition data. It is also possible that the Indian vegetarian diet supplied oligosaccharides that underwent fermentation in the colon. Another explanation could be the lower intake of protein, as undigested protein is fermented to NH_3 which can lead to an increase in gut pH. The Indian vegetarians also regularly consumed fermented foods, particularly live yoghurt and the micro-organisms present in these foods may alter colonic microbial flora.

Owen *et al.* (1986) proposed that the LCA : DCA ratio could be used as an indicator of risk of colorectal cancer. Lower LCA : DCA ratios have been reported in Seventh Day Adventist vegans but not in lacto-vegetarians (Turjiman *et al.* 1984). McKeigue *et al.* (1989) reported faecal steroid concentrations in random stool samples from South Asian (vegetarian and non-vegetarian) subjects with an average age of 39 years compared with white subjects consuming mixed diets (average age 59 years). The LCA : DCA ratio tended to be lower in the South Asian men and women but was not statistically significant. However, the proportion of faecal coprostanol as total sterols in the Asians was significantly lower. In the present study, the same laboratory undertook the analyses but the subjects were matched for age, their dietary habits were carefully assessed and a 24 h faecal collection was made. The LCA : DCA and the coprostanol : animal sterol ratios were significantly lower in the Indian vegetarians compared with both groups of white subjects. The lower LCA : DCA ratio is consistent with the lower risk of colorectal cancer observed in Indian vegetarians.

In conclusion, our observations show that a vegetarian diet is associated with decreased concentrations of faecal animal sterols and secondary bile acids particularly LCA. However, significantly lower faecal LCA : DCA and coprostanol : animal sterols ratios in the Indian vegetarians compared with white vegetarians and omnivores suggest that there was a reduced degradation of bile acids and neutral sterols in this group. The differences in the faecal characteristics of Indian and white vegetarians may be due to the differences in the nature of dietary starch, but in the absence of reliable food composition data this remains a speculation. Further studies are needed to determine the dietary factors that determine faecal pH and microbial degradation of steroids.

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