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Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores

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Full blood counts, serum ferritin, vitamin B_{12} and folate, erythrocyte folate concentrations and nutrient intakes were estimated in twenty-three Indian vegetarian, twenty-two Caucasian omnivores and eighteen Caucasian vegetarian women aged 25–40 years. Energy and copper intakes were lower in the Indian women than in the Caucasians. Intakes of dietary fibre, vitamin C and folate were greater and the proportion of energy derived from fat was lower in the vegetarians than in the omnivores. Vitamin B_{12} and protein intakes were lower in both vegetarian groups than in the omnivores. Fe intake was similar in all the groups but haem Fe provided one-quarter of the Fe intake of the omnivores. Haemoglobin concentrations were generally inside the normal range in all groups, but were lower in the Indians as were mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Higher MCV, MCH and lower erythrocyte (RBC) counts were observed in Caucasian vegetarians compared with the Caucasian omnivores. In both groups of vegetarians, concentrations of serum vitamin B_{12} and ferritin were markedly lower than in the omnivores. RBC folate concentrations were lower in the Indians than in either of the Caucasian groups when subjects taking supplements were excluded. It is concluded that vegetarians need to ensure they have adequate intakes of Fe and vitamin B_{12}.

Vegetarians: Vitamin B_{12}: Folate: Haematology: Indian

Nutritional anaemias are relatively common among women of childbearing age in both developed and developing countries. In developed countries microcytic Fe-deficiency anaemia is the most prevalent. The Indian Hindu population of the UK, the majority of whom are lifelong vegetarians, appear to have a higher incidence of nutritional megaloblastic anaemia than the general population. This appears to be a consequence of combined folate and vitamin B_{12} deficiencies (Britt *et al.* 1971; Matthews & Wood, 1984; Chanarin *et al.* 1985). The popularity of vegetarianism among the Caucasian population in the UK has increased for a variety of reasons: an abhorrence of cruelty to animals, a distaste for meat, the belief that vegetarian diets are more healthy and natural. Haematological studies of Caucasian vegetarians have generally concluded that they are no more prone to nutritional anaemias than the general population (Armstrong *et al.* 1974; Sanders *et al.* 1978; Gear *et al.* 1980). In the present paper we compare the dietary intakes, biochemical indices of Fe, vitamin B_{12} and folate status and haematological status in non-pregnant, premenopausal Indian vegetarian women compared with Caucasian vegetarian and omnivorous women.

METHODS

Subjects

Indian vegetarian women and Caucasian omnivores aged 25–40 years were randomly selected from general practice lists in North London. Caucasian vegetarians were recruited through the London branch of the Vegetarian Society (11 Barley Mow Passage, Chiswick).

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Subjects from all groups were studied simultaneously from July 1988 to April 1989 and the measurements spanned all four seasons equally. They were visited at home and given a food-recording kit, including dietary balance (Soehnle Digita, West Germany) and detailed instructions on how to record their food intake for seven consecutive days. In the event of foods being consumed outside the home, the subjects were asked to keep the wrappers giving the details of weights, ingredients, etc. and in some cases average portion sizes were estimated according to Crawley (1988). They were also asked if they took dietary supplements. Dietary intakes were verified by home visits and checking the records with the individual subjects. Fasting venous blood samples were obtained from subjects following the measurement of food intake and heights and weights were recorded. Blood samples were obtained 12–17 d following the onset of the last menstrual period.

Nutrient intakes

Nutrient intakes were calculated using the FOODTABS program (T. A. B. Sanders, unpublished) based on the McCance and Widdowson's food tables of Paul & Southgate (1978) and supplements (Tan *et al.* 1985; Holland *et al.* 1988). Other sources of food composition data were used for foods not included in that database, such as information provided by the manufacturers and US food tables (United States Department of Agriculture, 1979). Particular attention was paid to obtaining data on foods supplemented with vitamin B_{12}. The intake of Fe provided by meat was assumed to be haem-Fe.

Haematology

Serum vitamin B_{12}, folate and erythrocyte folate were determined by commercial radioimmunoassay kits (Becton Dickinson & Co., New York) in fresh samples. Serum ferritin was determined in serum frozen at $-20°$ by radioimmunoassay. Full blood counts were made on a Coulter counter and examined by the Haematology Department of the Central Middlesex Hospital, London.

Statistical methods

Data were analysed by analysis of variance. Data that were not normally distributed were log-transformed before analysis. Statistical correlations were made using the product-moment correlation. The Chi square test was used to test for differences between groups in the proportion of individuals with certain characteristics outside the normal range.

RESULTS

Blood samples were obtained from twenty-two Caucasian omnivorous women, twenty-three Indian vegetarians and eighteen Caucasian vegetarians, and dietary intakes from twenty-two, twenty-one and eighteen subjects respectively. The Indian and omnivorous subjects were well matched for age and socio-economic status but the Caucasian vegetarians were younger and 94·4% were nulliparous compared with 20·8% omnivores and 16·7% of the Indians (Table 1). Intakes of energy and copper were lower in Indian women compared with the other two groups (Table 2). Vitamin B_{12} and protein intakes were lower in both vegetarian groups. Dietary fibre, vitamin C and folate intakes were greater and the proportion of energy derived from fat was lower in the Caucasian vegetarians compared with the omnivores. Total Fe intakes from diets were similar in all the groups, but haem-Fe supplied approximately 25% of total Fe intakes of the omnivores (mean 2·83, SEM 0·34). Two omnivores, four Indian vegetarians and four Caucasian vegetarians were taking a multi-vitamin and mineral supplement.

Table 1. *Characteristics of the subjects*

(Mean values with their standard errors)

	Caucasian omnivores (n 22)		Indian vegetarians (n 21)		Caucasian vegetarians (n 18)	
	Mean	SEM	Mean	SEM	Mean	SEM
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
Weight (kg)	64·6	2·41	61·4	2·67	59·4	2·60
Body mass index (wt/ht^2)	24·1	0·70	25·1	0·98	22·5	1·00
Nulliparity (%)	20·8	—	16·7	—	94·4	—
Mean interval since recent childbirth (years)	6·3 (n 19)	1·08	8·5 (n 20)	1·15	2·0 (n 1)	—

[a, b] Values with unlike superscript letters were significantly different (analysis of variance; between group comparison made with a t test using the pooled estimate of standard deviation): $P < 0·05$.

Table 2. *Mean dietary intakes of the subjects excluding nutrient supplements*

(Mean values with their standard errors)

	Caucasian omnivores (n 22)		Indian vegetarians (n 22)		Caucasian vegetarians (n 18)	
	Mean	SEM	Mean	SEM	Mean	SEM
Energy (MJ/d)	7·47[a]	0·32	6·11[b]	0·29	7·67[a]	0·37
Percentage energy from:						
Protein	15·8[b]	0·58	11·6[a]	0·40	11·9[a]	0·34
Fat	40·3[a]	1·14	37·7[ab]	1·05	35·1[b]	1·72
Carbohydrate	40·3[b]	1·33	50·3[a]	1·08	48·0[a]	2·19
Fibre (g/d)	16·6[a]	0·97	16·2[a]	1·17	29·3[b]	2·57
Iron (mg/d)	12·1	1·5	12·7	1·3	13·8	1·0
Copper (mg/d)	1·42[a]	0·16	0·75[b]	0·05	1·49[b]	0·12
Vitamin C (mg/d)	68·7[a]	9·03	66·6[a]	5·7	114·1[b]	18·8
Folate (μg/d)	169·6[a]	13·9	141·7[a]	10·2	262·0[b]	34·1
Vitamin B_{12} (μg/d)	5·5[b]	1·25	0·87[c]	0·13	1·52[a]	0·16

[a, b, c] Values with unlike superscript letters were significantly different (analysis of variance; between group comparisons were made with t test using pooled estimate of standard deviation. Vitamin B_{12} comparisons between Indian vegetarians and Caucasian vegetarians were made using the pooled estimate of standard deviation for those two groups): $P < 0·05$.

Haemoglobin concentrations were generally inside the normal ranges, with the exception of two Indian vegetarian subjects (108 and 109 g/l). However, several differences in haemoglobin concentration and blood counts were noted between the three groups (Table 3). Haemoglobin concentration, packed cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly lower and platelet counts were significantly higher in the Indian women compared with Caucasian omnivores. Erythrocyte counts (RBC) and erythrocyte distribution width (RDW) were significantly lower and MCV and MCH significantly greater in Caucasian vegetarians compared with the omnivores and MCH concentration (MCHC) was significantly greater compared with that

Table 3. *Blood counts and haemoglobin concentrations in Caucasian omnivores, Indian vegetarians and Caucasian vegetarians*

(Mean values with their standard errors)

	Caucasian omnivores (n 22)		Indian vegetarians (n 23)		Caucasian vegetarians (n 18)	
	Mean	SEM	Mean	SEM	Mean	SEM
WBC ($\times 10^9$/l)	6·0	0·37	6·2	0·28	5·7	0·28
RBC ($\times 10^{12}$/l)	4·7^a	0·10	4·6^a	0·07	4·4^b	0·07
Hb (g/l)	136^a	2·0	126^b	2·3	136^a	1·7
PCV (l/l)	0·41^a	0·006	0·39^b	0·007	0·40^a	0·005
MCV (fl)	88·8^a	1·52	84·7^b	1·16	92·7^c	0·89
MCH (pg)	29·5^a	0·70	27·7^b	0·47	31·2^c	0·36
MCHC (g/l)	331·0^{ab}	3·3	327·0^b	1·7	337·0^a	1·8
RDW (%)	15·1^a	0·61	16·3^a	0·55	12·3^b	0·15
Platelets ($\times 10^9$/l)	256·0^b	11·2	311·0^a	14·5	279·0^{ab}	18·2
MPV (fl)	8·2	0·36	7·9	0·17	8·3	0·19
Neutrophils ($\times 10^9$/l)	3·5	0·25	3·6	0·19	3·7	0·27
Lymphocytes ($\times 10^9$/l)	1·9^{ab}	0·12	2·0^b	0·14	1·6^a	0·07
Monocytes ($\times 10^9$/l)	0·36	0·032	0·35	0·022	0·34	0·023

Hb, haemoglobin; PCV, packed cell volume; WBC, leucocyte count; RBC, erythrocyte count; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, MCH concentration; RDW, erythrocyte distribution width; MPV, mean platelet volume.

$^{a, b, c}$Values with unlike superscript letters were significantly different (analysis of variance, between group comparisons made with a t test using the pooled estimate for standard deviation): $P < 0·05$.

of the Indians. The intake of supplements did not alter the statistical significance observed for blood counts and haemoglobin concentrations. However, the mean concentrations of haemoglobin were lower when subjects taking supplements were excluded from the analysis.

All the subjects seemed healthy and none complained of any symptoms of anaemia, except one Indian subject who was prescribed Fe supplements by her doctor. One omnivorous subject had low MCV (68·5 fl), MCH (21 pg) and haemoglobin (111 g/l) and raised RDW (23 %) and was diagnosed as anaemic with microcytosis, although levels of serum vitamin B_{12}, folate and ferritin were within normal ranges. This subject was of Italian origin and target cells were also observed in the blood film indicating impaired haemoglobin production. The two Indian subjects with haemoglobin concentrations lower than 110 g/l showed a raised RDW, but only one subject showed reduced MCV and MCH. Eleven Indian women had RDW greater than the upper limit of normality (16 %), as compared with eight omnivores. None of the Caucasian vegetarians had RDW outside the normal range.

The mean serum vitamin B_{12} concentration was lower in both Indian and Caucasian vegetarians than in the omnivores and was associated with low dietary intakes (Table 4). This difference became more marked if the subjects taking supplements of vitamin B_{12} were excluded from the analysis and resulted in similar serum vitamin B_{12} concentrations in both groups of vegetarians. A high proportion of vegetarian subjects had serum vitamin B_{12} levels below the normal range (180 ng/l). Four vegetarians (two Indians and two Caucasians) had serum vitamin B_{12} concentrations below 120 ng/l, the level below which clinical signs of deficiency usually appear (Matthews & Wood 1984).

Serum folate concentrations were greater in the Caucasian vegetarians compared with

Table 4. *Serum ferritin, vitamin B_{12}, folate and erythrocyte folate concentrations of Caucasian omnivores, Indian vegetarians and Caucasian vegetarians*

(Geometric mean (GM) and 95% confidence limits (CL))

		Ferritin (μg/l)		Vitamin B_{12} (ng/l)		Folate (μg/l)		Erythrocyte folate (μg/l)	
	n	GM	CL	GM	CL	GM	CL	GM	CL
Caucasian omnivores									
All subjects	22	20	15·9–25·1	341	265–439	4·43	3·71–5·29	278	253–305
Excluding supplement users	20	18	11·2–29·2	336	276–409	4·27	3·55–5·15	273	224–332
Indian vegetarians									
All subjects	19	7·9	4·8–12·9	199	159–256	5·85	4·9 6·99	211	174–255
Excluding supplement users	17	6·4	3·8–11·8	178	145–219	6·06	4·98–7·38	201	163–248
Caucasian vegetarians									
All subjects	18	11·1	6·7–18·4	197	153–253	6·93	5·73–8·4	284	235–344
Excluding supplement users	14	10·4	5·8–18·4	158	125–198	5·79	4·66–7·2	230	185–286

Statistical method: analysis of variance of log transformed data.

Table 5. *Number of Caucasian omnivores, Indian vegetarians and Caucasian vegetarians outside the normal ranges for haematological values*

(Total number of subjects given in parentheses)

	Caucasian omnivores	Indian vegetarians	Caucasian vegetarians	Statistical significance*
Hb (< 110 g/l)	0(22)	2(23)	0(18)	NS
MCV: < 74 fl	1(22)	1(23)	0(18)	NS
> 99 fl	1(22)	0(23)	1(18)	NS
RDW (> 16%)	8(22)	11(23)	0(18)	$P < 0·01$
Serum ferritin (< 12 μg/l)	6(22)	15(19)	10(18)	$P < 0·01$
Serum vitamin B_{12} (< 180 ng/l)	0(22)	9(21)	9(18)	$P < 0·01$
Serum folate (< 2 μg/l)	0(22)	0(21)	0(18)	NS
(< 3 μg/l)	5(22)	0(21)	0(18)	$P < 0·01$
Erythrocyte folate (< 200 μg/l)	5(19)	8(18)	7(18)	NS

Hb, haemoglobin; MCV, mean corpuscular volume; RDW, erythrocyte distribution width; NS, not significant.
* Statistical method: Chi squared test.

the other groups and reflected intakes of folate. Serum folate concentration is more liable to change with recent dietary intakes, unlike erythrocyte folate which is known to be a reliable indicator of folate status. If subjects taking supplements were excluded from the analysis, the mean erythrocyte folate concentration was significantly lower in the Indian women compared with the other two groups. Five omnivores, eight Indians and seven Caucasian vegetarians had erythrocyte folate concentrations below 200 μg/l (Table 5).

Serum ferritin concentrations were significantly lower in both vegetarian groups compared with the omnivores. In the subjects not taking supplements, mean ferritin concentrations in Caucasian and Indian vegetarians were less than half the mean value of 24·7 μg/l (SE 3·92). Intake of haem-Fe was positively correlated with serum ferritin concentration (r 0·59, $P < 0·01$, n 18) in omnivorous subjects not taking supplements. Ferritin concentrations in Caucasian and Indian vegetarians were on the borderline of the lower level of normality (12 ng/ml). However, when subjects taking supplements were excluded, mean ferritin concentrations in both vegetarian groups fell below the normal range.

DISCUSSION

The Indian vegetarian subjects were well matched with the Caucasian omnivore controls with regard to age and socio-economic status. Of Caucasian omnivores 71 % were married compared with 83% of Indian subjects. The Caucasian vegetarians differed in several respects from the other two groups. They were mainly unmarried (67%) and had a high awareness of health and environmental issues and were eager to demonstrate the benefits of vegetarian diet. However, they may be representative of the Caucasian vegetarian population in the UK.

The energy intakes of all three groups were lower than the UK recommended daily amounts of 9 MJ/d (Department of Health and Social Security, 1979). Although energy intakes were substantially lower in the Indian women, this was not reflected in a lower body mass index. Many of the Indian subjects fasted 1 d/week. The intakes of vitamin B_{12} were low in both vegetarian groups and were less than the US recommended daily amounts of 2 μg (National Academy of Sciences, 1989). The intake of vitamin B_{12} in the omnivores was similar to that described in the UK National Food Survey and was derived mainly from meat (Lewis & Buss, 1988). It is perhaps surprising that the intakes of vitamin B_{12} were so low in the vegetarians, in view of the availability of a large number of processed foods supplemented with the vitamin. This might suggest that these supplemented foods are not popular with vegetarians. Although we may have underestimated vitamin B_{12} intakes because of unknown values in the food tables, the low serum vitamin B_{12} concentrations in the vegetarian subjects suggest that their dietary intake was inadequate. Several (eighteen) vegetarians had serum vitamin B_{12} concentrations below the normal range. Vitamin B_{12} deficiency of dietary origin may take several years to develop owing to liver stores of the vitamin. The risk of vitamin B_{12} deficiency in vegans, who exclude all food of animal origin, has been recognized for some years (Sanders *et al.* 1978). Our results suggest that vitamin B_{12} deficiency is a possible hazard in both Indian and Caucasian vegetarians.

Folate intakes were relatively high in Caucasian vegetarians, but low in the Indian vegetarians. It is well known that high folate intakes inhibit the development of megaloblastic anaemia caused by inadequate intakes of vitamin B_{12}, while permitting the development of the more insidious neurological symptoms of vitamin B_{12} deficiency such as sub-acute combined degeneration of the spinal cord. It is likely, therefore, that Caucasian vegetarians would present neurological rather than haematological symptoms of vitamin B_{12} deficiency. Vitamin B_{12} is involved in the transport of folate to the erythrocyte. In vitamin B_{12} deficiency serum folate concentration can be raised and that of erythrocyte folate depressed. It is notable that although serum folate concentration tended to be slightly greater in the Indian women compared with the omnivores, erythrocyte folate concentrations were significantly lower.

Fe intakes were similar in all three groups and close to the recommended daily amount of 12 mg. However, the availability of Fe from their diets is variable. While haem-Fe derived from meat is well absorbed, Fe from plant sources is generally poorly absorbed and substances present in plant foods, such as phytate and fibre, may render the Fe unavailable. It has also been suggested that a high intake of vitamin C may aid Fe absorption by conversion of ferric-Fe to ferrous-Fe. Ferritin concentrations are generally considered to be good indicators of Fe stores (Cook & Skikne, 1982). Ferritin concentrations in both groups of vegetarians were approximately half the value found in the omnivores. It seems likely that this difference can be attributed to the haem-Fe present in the diets of the omnivores which provided approximately 3 mg/d. A significant correlation (r 0·59, $P < 0·01$) was observed between haem-Fe intake and serum ferritin concentrations in the omnivores. Ferritin concentrations were below the normal range in the majority of vegetarian subjects not taking supplements.

Haemoglobin concentrations were significantly lower in the Indian vegetarians than in either the Caucasian vegetarians or omnivores. MCV and MCH values were also lower. If Fe deficiency leading to microcytosis co-exists with megaloblastosis, macrocytosis may be masked and the MCV may not be increased (McKenzie, 1988). This may explain the absence of macrocytosis in the Indians, although their biochemical measurements of folate and vitamin B_{12} indicated deficiency. RDW is a measure of erythrocyte heterogeneity and a high value is indicative of maturation defect of erythrocytes, regardless of MCV or degree of anaemia. Changes in RDW can be detected before abnormal cells can be identified in the blood smear. Although RDW may not aid in diagnosing the type of nutritional anaemia, it is a useful predictor of an individual's susceptibility to anaemia. RDW was higher in the Indian vegetarians than in the other groups, which implies that they are more susceptible to anaemias caused by a maturation defect. As folate, Fe and vitamin B_{12} status were similar in both vegetarian groups, it may be that some other nutrient is responsible for this. Cu is a possible haemopoetic nutrient and its intake was lower in the Indians. However, it is recognized that Cu intakes may have been underestimated. Further studies should consider measuring indicators of Cu status. Erythrocyte (RBC) and lymphocyte counts were lower in the Caucasian vegetarians and MCV was greater. This observation is similar to that made by Sanders *et al.* (1978) in vegans and may indicate hypoproliferation of blood-forming cells.

Although the subjects of the present study were generally in good health and could not be classified clinically as anaemic, it does not follow that they do not differ with regard to risk of anaemia. Precipitating factors such as pregnancy, illness and excessive blood loss are implicated in the causation of anaemia. Our results suggest that both Indian and Caucasian vegetarians are at increased risk of Fe-deficiency anaemia and that Indian vegetarian women are also at greater risk of developing nutritional megaloblastic anaemia. This is in agreement with studies carried out on Indian populations in other developed countries (Ganapathy & Dhanda *et al.* 1980; MacPhail *et al.* 1981; Bindra & Gibson, 1986). There is clearly a need for nutritional advice targetted at Indian vegetarians.

Meat, especially red meat, plays an important role in supplying Fe and vitamin B_{12} in the average British diet. Individuals who change to a vegetarian diet need to ensure that they have adequate intakes of these nutrients. Wholemeal cereals and dark green leafy vegetables are good sources of Fe, but the Fe from these sources is poorly absorbed. Vitamin B_{12} is generally absent from plant foods unless they are contaminated with micro-organisms that produce the vitamin. Milk products are not particularly good sources of vitamin B_{12}, but eggs are. Non-animal sources of vitamin B_{12} are added to several processed foods such as marmite and cornflakes. However, these may not be consumed by those vegetarians who avoid processed foods. These individuals may well benefit from taking supplements.

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