# Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians[1–3]

*Helga Refsum, Chittaranjan S Yajnik, Milind Gadkari, Jörn Schneede, Stein E Vollset, Lars Örning, Anne B Guttormsen, Anjali Joglekar, Mehmood G Sayyad, Arve Ulvik, and Per M Ueland*

**ABSTRACT**

**Background:** In India, most people adhere to a vegetarian diet, which may lead to cobalamin deficiency.

**Objective:** The objective was to examine indicators of cobalamin status in Asian Indians.

**Design:** The study population included 204 men and women aged 27–55 y from Pune, Maharashtra, India, categorized into 4 groups: patients with cardiovascular disease (CVD) and diabetes, patients with CVD but no diabetes, patients with diabetes but no CVD, and healthy subjects. Data on medical history, lifestyle, and diet were obtained by interviews and questionnaires. Blood samples were collected for measurement of serum or plasma total cobalamin, holotranscobalamin (holoTC), methylmalonic acid (MMA), and total homocysteine (tHcy) and hemetologic indexes.

**Results:** MMA, tHcy, total cobalamin, and holoTC did not differ significantly among the 4 groups; therefore, the data were pooled. Total cobalamin showed a strong inverse correlation with tHcy ($r = -0.59$) and MMA ($r = -0.54$). Forty-seven percent of the subjects had cobalamin deficiency (total cobalamin < 150 pmol/L), 73% had low holoTC (<35 pmol/L), 77% had hyperhomocysteinemia (tHcy >15 μmol/L), and 73% had elevated serum MMA (>0.26 μmol/L). These indicators of impaired cobalamin status were observed in both vegetarians and nonvegetarians. Folate deficiency was rare and only 2.5% of the subjects were homozygous for the *MTHFR* 677C→T polymorphism.

**Conclusions:** About 75% of the subjects had metabolic signs of cobalamin deficiency, which was only partly explained by the vegetarian diet. If impaired cobalamin status is confirmed in other parts of India, it may have important health implications. *Am J Clin Nutr* 2001;74:233–41.

**KEY WORDS** Ethnicity, diet, blood, cobalamin deficiency, holotranscobalamin, homocysteine, folate, methylmalonic acid, methylenetetrahydrofolate reductase, *MTHFR* polymorphism, methionine synthase reductase, *MTRR* polymorphism, vegetarian diet, India

## INTRODUCTION

A substantial proportion of the population of India adheres to a vegetarian diet for cultural and religious reasons. Even the food consumed by nonvegetarian Indians usually contains less animal-derived protein than in the typical Western diet (1). A vegetarian diet is considered to promote health and longevity by protecting against conditions such as cardiovascular disease (CVD) and cancer (2). However, a vegetarian diet may be deficient in some nutrients (3, 4). In particular, a strict vegetarian diet has been associated with an increased risk of cobalamin deficiency (4, 5).

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Symptomatic cobalamin deficiency in white vegetarians is believed to be rare (5). Most whites begin consuming a vegetarian diet early in adult life when they have adequate stores of cobalamin and normal gastrointestinal function. Under these conditions, it may take decades to develop an overt deficiency (5). In contrast, most vegetarians in India begin consuming a vegetarian diet as infants and thus have life-long low cobalamin intakes. Malnutrition is common in India (6) and tropical sprue, gastrointestinal infections, and other nutrient deficiencies frequently result in a malabsorptive state (7, 8); therefore, one would expect a high prevalence of cobalamin deficiency in India. Indeed, reports from both India (9, 10) and studies of Indians living in other countries (11, 12) suggest that nutritional cobalamin deficiency is common. Still, the number of reports on cobalamin status in Asian Indians is surprisingly sparse.

Classic cobalamin deficiency is associated with megaloblastic anemia and neurologic symptoms (13). The typical hematologic changes associated with cobalamin deficiency are easy to detect, but they develop at a late stage of the condition (8) and may even be absent (14). Measurement of serum cobalamin is the most commonly used biochemical test for diagnosing cobalamin deficiency, but it lacks sensitivity (15). A newer approach is to determine the

[1] From the Department of Pharmacology and the Locus for Homocysteine and Related Vitamins, University of Bergen, Norway; Axis-Shield ASA, Oslo; and the Diabetes Unit, King Edward Memorial Hospital and Research Centre, Pune, India.

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[3] Address reprint requests to H Refsum, Department of Pharmacology, University of Bergen, Armauer Hansens Hus, 5021 Bergen, Norway. E-mail: helga.refsum@farm.uib.no.

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amount of cobalamin bound to transcobalamin (holotranscobalamin; holoTC), ie, the fraction of serum cobalamin that becomes internalized by the cells (5, 16). Studies in the past 15 y established that markers of cobalamin function, particularly total homocysteine (tHcy) and methylmalonic acid (MMA), become elevated in subtle cobalamin deficiency states not characterized by the typical clinical symptoms (14). Recently, it was reported that plasma tHcy concentrations are higher in vegans than in omnivores (17). A similar finding of elevated tHcy and MMA was observed in infants in a macrobiotic (vegan) community (18). In the current study we report the cobalamin status of 204 subjects from the state of Maharashtra in India, nearly 40% of whom adhered to a vegetarian diet.

## SUBJECTS AND METHODS

### Subjects

During the period from October 1994 to June 1996, a total of 204 subjects aged 27–55 y were recruited for the study. One hundred of these subjects had confirmed CVD and were consecutively selected from patients attending 1 of the 2 Cardiology Clinics of the King Edward Memorial (KEM) Hospital run by one of the authors (MG) once a week. Of the CVD patients, 86 had a history of myocardial infarction, 9 of unstable angina, 2 of stable angina, and 3 of cerebral or peripheral artery disease. Forty-two percent of the CVD patients had diabetes mellitus. In addition, 104 subjects with no evidence of CVD were recruited. This group included 41 diabetic patients attending the Diabetic Unit at the KEM Hospital and 63 presumably healthy subjects who were staff members of the KEM Hospital or their contacts or outpatients attending the KEM Hospital for treatment of minor illnesses.

Blood samples were collected ≥2 wk after all minor illnesses were treated successfully. Exclusion criteria for all subjects were acute or severe illness or treatment with nitrous oxide anesthesia during the previous 3 mo, evidence of large vessel disease other than atherosclerosis, pregnancy, or excessive alcohol consumption. The study was approved by the Ethical Committee of the KEM Hospital and Research Centre. Informed consent was obtained from all subjects.

### Data collection

Questionnaires were completed by a research assistant. Information on the subjects' personal and family histories of CVD, smoking habits, and intakes of vitamin supplements, drugs, and alcohol were collected. In India, most of the animal-derived food protein consumed is derived from dairy products, eggs, chicken and other poultry, mutton, and fish. For each of these food groups, the frequency of intake was reported in terms of 6 categories (never, ≤1 time/y, ≤1 time/mo, ≤1 time/wk, 2–5 times/wk, and almost daily). Height, weight, waist and hip circumferences, pulse rate, and blood pressure were measured. Pulse rate and blood pressure measurements were repeated twice and the means of the 2 measurements were calculated. All subjects underwent a stress test on a treadmill during which an electrocardiogram was produced.

### Blood sampling and biochemical methods

A fasting blood sample was collected from all subjects. Whole blood was collected into tubes without anticoagulant (for preparation of serum) or into tubes containing the anticoagulant EDTA. The serum fraction was obtained by centrifugation (1300 × *g*, 20 min, 4°C) after storing the whole blood at room temperature for ≈1 h. The EDTA-containing tubes were immediately placed on ice and centrifuged (1300 × *g*, 20 min, 4°C) within 30 min; plasma was collected and stored at −20°C.

Plasma tHcy was analyzed by using a fully automated HPLC method with fluorescence detection (19). The between-day CV was <5% (19). MMA in serum was determined by capillary electrophoresis with laser-induced fluorescence detection (20). The concentrations of both tHcy and MMA were later confirmed by gas chromatography–mass spectrometry (GC-MS) based on ethylchloroformate derivatization (21). Serum concentrations of folate and cobalamin were determined by using microbiological assays (22, 23). The folate and cobalamin assays were both adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland). Plasma holoTC was measured directly by using a novel radioimmunoassay (HoloTC RIA kit; Axis-Shield ASA, Oslo). The assay is based on monoclonal antibodies against transcobalamin. Briefly, plasma (0.4 mL) was diluted 2-fold with 0.1 mol phosphate-buffered saline/L, after which the monoclonal antibody attached to magnetic beads was added. After incubation for 1 h on a roller mixer, the holoTC attached to the beads was precipitated by using a magnetic rack and the supernatant fluid was discarded. The beads were washed once with phosphate-buffered saline. Thereafter, a radioisotope dilution assay using intrinsic factor as binder was performed. The CV of the assay was <8% and the lower limit of detection was ≈5 pmol/L.

Creatinine and the hematologic indexes were determined in the routine clinical chemistry laboratory at the KEM Hospital. Genotyping was carried out by using EDTA-containing blood or serum. To determine the presence of a C-to-T substitution at nucleotide 677 of the gene for methylenetetrahydrofolate reductase (*MTHFR* 677C→T polymorphism), polymerase-chain-reaction (PCR) products were analyzed by using a multiple-injection capillary electrophoresis technique as previously described (24). A real-time PCR assay was used to determine the presence of an A-to-G substitution at nucleotide 66 of the gene for 5-methyltetrahydrofolate–homocysteine methyltransferase reductase (*MTRR* 66A→G polymorphism) (25).

### Description of variables

Several continuous variables were categorized: cobalamin deficiency was defined as a serum cobalamin concentration <150 pmol/L, low holoTC was defined as concentration <35 pmol/L (16), folate deficiency was defined as a serum folate concentration <5 nmol/L, elevated serum MMA was defined as a concentration >0.26 μmol/L, and hyperhomocysteinemia was defined as a tHcy concentration >15.0 μmol/L. In the logistic regression analyses, elevated tHcy and MMA concentrations were used as outcome variables; a tHcy concentration of 20.0 μmol/L and an MMA concentration of 0.50 μmol/L were used as thresholds. Thrombocytopenia was defined as a platelet count $< 140 \times 10^9$/L, macrocytosis as a mean corpuscular volume >100 fL, and anemia as a hemoglobin concentration <135 g/L for men and <115 g/L for women. Age was divided into the following categories: ≤40, 41–45, 46–50, and >50 y. A subject was considered a vegetarian ($n$ = 78) only if he or she never ate mutton, poultry, fish, or eggs; all other subjects were categorized as nonvegetarians ($n$ = 126). In the logistic regression analyses, the frequency

**TABLE 1**
Characteristics of the total population and of the 4 subgroups[1]

| Variable | Total population ($n$ = 204) | Healthy ($n$ = 63) | Diabetes ($n$ = 41) | CVD ($n$ = 58) | CVD + diabetes ($n$ = 42) | $P^2$ |
|---|---|---|---|---|---|---|
| Age (y) | 48 (35–54) | 44[3] | 46[3] | 48[3] | 52 | 0.001 |
| Sex (%) | | | | | | |
| Male | 83 | 78 | 88 | 86 | 81 | 0.50 |
| Female | 17 | 22 | 12 | 14 | 19 | — |
| Vegetarian diet (%) | 38 | 27 | 44 | 45 | 40 | 0.17 |
| Ever smokers (%) | 17 | 24 | 10 | 21 | 10 | 0.12 |
| Diabetes (%) | 41 | 0 | 100 | 0 | 100 | — |
| CVD (%) | 49 | 0 | 0 | 100 | 100 | — |
| Weight (kg) | 66 (53–86) | 66 | 66 | 65 | 67 | 0.99 |
| BMI (kg/m$^2$) | 24.4 (20.2–30.8) | 24.4 | 24.5 | 23.9 | 24.8 | 0.68 |
| Hemoglobin (g/L) | 146 (115–167) | 146 | 153 | 145 | 144 | 0.13 |
| Anemia (%)[4] | 18 | 11 | 13 | 21 | 29 | 0.09 |
| MCV (fL) | 82 (70–94) | 81 | 84 | 81 | 81 | 0.85 |
| Macrocytosis, >100 fL (%) | 1.6 | 1.7 | 2.6 | 1.9 | 0.0 | 0.84 |
| Platelets ($\times$ 10$^9$/L) | 180 (113–363) | 181 | 171 | 176 | 185 | 0.96 |
| Thrombocytopenia, <140 $\times$ 10$^9$/L (%) | 18 | 19 | 19 | 19 | 14 | 0.88 |
| tHcy (μmol/L) | 19.8 (9.8–45.9) | 19.7 | 18.1 | 20.0 | 20.2 | 0.90 |
| tHcy > 15 μmol/L (%) | 77 | 81 | 76 | 74 | 79 | 0.83 |
| Serum cobalamin (pmol/L) | 154 (66–499) | 160 | 130 | 159 | 155 | 0.79 |
| Cobalamin deficiency, <150 pmol/L (%) | 47 | 46 | 54 | 43 | 48 | 0.78 |
| HoloTC (pmol/L) | 21 (8–96) | 24 | 16 | 22 | 18 | 0.62 |
| Low holoTC, <35 pmol/L (%) | 73 | 67 | 76 | 74 | 76 | 0.72 |
| MMA (μmol/L) | 0.49 (0.08–1.67) | 0.40 | 0.47 | 0.50 | 0.55 | 0.33 |
| MMA ≥ 0.26 μmol/L (%) | 73 | 70 | 71 | 79 | 71 | 0.65 |
| Serum folate (nmol/L) | 12.2 (4.9–27.6) | 11.5 | 16.4 | 10.7[5] | 12.7 | 0.002 |
| Folate deficiency, < 5 nmol/L (%) | 5.0 | 7.9 | 2.5 | 5.3 | 2.5 | 0.54 |
| Creatinine (μmol/L) | 72.5 (36.6–104.9) | 71.2 | 73.4 | 72.5 | 79.0 | 0.23 |
| Creatinine > 110 μmol/L (%) | 3.3 | 3.4 | 0 | 5.9 | 2.8 | 0.50 |
| *MTHFR TT* genotype frequency (%) | 2.5 | 0 | 4.9 | 1.7 | 4.8 | 0.88 |

[1] Values are medians or percentages; 5th–95th percentiles in parentheses. CVD, cardiovascular disease; MCV, mean corpuscular volume; tHcy, total homocysteine; holoTC, holotranscobalamin; MMA, methylmalonic acid.

[2] By ANOVA or chi-square test followed by Tukey's post hoc test when the test result was significant.

[3] Significantly different from subjects with CVD + diabetes, $P < 0.05$.

[4] A hemoglobin concentration < 135 g/L for men and < 115 g/L for women.

[5] Signficantly different from subjects with diabetes, $P < 0.05$.

of intake of mutton, poultry, eggs, and fish was divided into 2 groups (≤1 time/mo and >1 time/mo).

### Statistical analyses

Results are presented as medians, percentages, and 5th and 95th percentiles unless otherwise indicated. Student's $t$ test for independent samples, one-way analysis of variance, and the chi-square test were used for comparisons between groups. When significant differences among the means were observed, Tukey's post hoc test was performed to identify significantly different group means. The distributions of the platelet count and of blood concentrations of tHcy, MMA, and vitamins were markedly skewed, and log transformed values were used in the statistical analyses.

Determinants of cobalamin status were identified by Spearman rank-order correlation coefficients, and analysis of variance was used to assess the relation between the various determinants and cobalamin concentrations. Odds ratios (ORs) for tHcy concentrations >20 μmol/L, for MMA concentrations >0.50 μmol/L, or for cobalamin deficiency were obtained by logistic regression analyses. We also performed a logistic regression analysis of the risk of cobalamin deficiency in subjects with infrequent intake (≤1 time/mo) for the various food groups. Subjects who had a frequency of intake >1 time/mo were used as a reference. The independent variables were entered in the model as indicator variables. All relevant associations were tested for linear trend.

A $P$ value <0.05 (two sided) was considered significant. SPSS version 10 for Macintosh (SPSS Inc, Chicago) was used for the statistical analyses. The graph depicting the relation between cobalamin and abnormal tHcy, MMA, and holoTC concentrations was constructed by using the supsmu scatterplot smoother of S-PLUS 2000 (Windows NT version, 1999; MathSoft, Inc, Seattle).

## RESULTS

### Demographic characteristics and hematologic indexes

The demographic characteristics and hematologic indexes of the subjects are listed in **Table 1** by total population and by group. Few significant differences among the groups were observed. The subjects with CVD and diabetes were significantly older than the 3 other groups and serum folate was significantly higher in the diabetic patients than in the nondiabetic patients. Plasma tHcy, the allele frequency of the *MTHFR* 677C→T polymorphism, and serum concentrations of cobalamin,

**TABLE 2**
Spearman rank-order correlation coefficients for biochemical and hematologic indexes in the total study population[1]

| | Cobalamin | HoloTC | Folate | MMA | *MTHFR* 677C→T | Creatinine | Hemoglobin | MCV | Platelets |
|---|---|---|---|---|---|---|---|---|---|
| tHcy | −0.59 (<0.001) | −0.52 (<0.001) | −0.08 (0.25) | 0.48 (<0.001) | 0.14 (0.050) | 0.12 (0.12) | 0.07 (0.36) | −0.08 (0.27) | −0.15 (0.043) |
| Cobalamin | | 0.78 (<0.001) | −0.09 (0.23) | −0.54 (<0.001) | −0.05 (0.49) | 0.05 (0.51) | −0.05 (0.49) | 0.01 (0.88) | 0.08 (0.30) |
| HoloTC | | | −0.10 (0.19) | −0.48 (<0.001) | −0.00 (0.96) | −0.02 (0.82) | −0.04 (0.55) | −0.02 (0.82) | 0.12 (0.11) |
| Folate | | | | 0.05 (0.46) | −0.01 (0.88) | −0.04 (0.56) | 0.08 (0.26) | 0.1 (0.16) | −0.05 (0.51) |
| MMA | | | | | 0.04 (0.59) | 0.14 (0.059) | 0.18 (0.010) | −0.01 (0.85) | −0.15 (0.048) |
| *MTHFR* 677C→T | | | | | | −0.09 (0.24) | 0.01 (0.84) | 0 (0.96) | 0.1 (0.16) |
| Creatinine | | | | | | | 0.01 (0.94) | −0.09 (0.24) | −0.27 (<0.001) |
| Hemoglobin | | | | | | | | 0.18 (0.013) | −0.17 (0.020) |
| MCV | | | | | | | | | 0.15 (0.047) |

[1] $n$ = 204. tHcy, total homocysteine; holoTC, holotranscobalamin; MMA, methylmalonic acid; *MTHFR* 677C→T, C-to-T substitution at nucleotide 677 of the gene for methylenetetrahydrofolate reductase. MCV, mean corpuscular volume. *P* values in parentheses.

holoTC, MMA, and creatinine did not differ significantly among the groups. Thus, to increase the power of the statistical analyses, the data from all 4 groups were pooled.

In this predominantly male population, 38% consumed a vegetarian diet. The median hemoglobin concentration in the total population was within the normal range, but 18% of the subjects had a hemoglobin concentration consistent with anemia. Thrombocytopenia was observed in 18% of the subjects and it was unrelated to anemia (data not shown).

Median total cobalamin and holoTC concentrations were low; the corresponding means ± SDs were 205 ± 225 μmol/L and 35 ± 44 pmol/L, respectively. Nearly 50% of the subjects had cobalamin deficiency and an even higher proportion (73%) had low holoTC concentrations. In contrast, serum folate concentrations were relatively high (median: 12.2 nmol/L) and only 5% of the subjects were folate deficient. Plasma tHcy was markedly elevated in 77% of the total population, with a median of nearly 20 μmol/L ($\bar{x}$ ± SD: 23.2 ± 13.1 μmol/L). About 3 of 4 subjects had a tHcy concentration >15 μmol/L and only 6% had a tHcy concentration <10 μmol/L. Serum MMA was also markedly elevated, with a median of 0.49 μmol/L ($\bar{x}$ ± SD: 0.60 ± 0.60 μmol/L); 73% of the population had a concentration above the normal upper limit of 0.26 μmol/L.

The prevalence of the *MTHFR TT* genotype in the total population was only 2.5%, which corresponds to a *T* allele frequency of 12.5%. This value was markedly lower than the prevalence observed in whites and most other Asian populations (*T* allele frequency of ≈40%) (26, 27). We also determined the *MTRR* 66A→G polymorphism, and found an allele frequency of 45%, which is nonsignificantly lower than that reported in a Canadian study (25). The genotype distributions of both polymorphisms were compatible with Hardy-Weinberg equilibrium.

### Variables associated with cobalamin, holoTC, tHcy, and MMA

Total cobalamin, holoTC, tHcy, and MMA concentrations were not significantly associated with age (data not shown). Concentrations of tHcy, cobalamin, and holoTC did not differ significantly between the sexes (data not shown), whereas MMA was higher in men than in women (0.52 compared with 0.29 μmol/L, $P < 0.01$).

The hemoglobin concentration, mean corpuscular volume, and prevalence of anemia or macrocytosis were not significantly associated with cobalamin concentrations (**Table 2**). Red and white blood cell counts were also not significantly associated with cobalamin concentrations (data not shown). On the other hand, the platelet count was inversely correlated with MMA and tHcy. Thrombocytopenia was observed in 34% of subjects with a total cobalamin concentration <200 pmol/L but in only 8% of subjects with a total cobalamin concentration ≥200 pmol/L ($P < 0.05$; **Table 3**).

Elevated creatinine concentrations showed a weak association with MMA, but not with tHcy, total cobalamin, or holoTC (Table 2). Folate concentrations were not significantly associated with tHcy. In subjects with the *MTHFR* genotype, there was a significant association between the number of *MTHFR T* alleles and tHcy concentrations (**Figure 1**), but no significant differences were observed between the 3 genotypes, probably because few subjects had the *TT* genotype. *MTRR* genotype did not correlate with cobalamin, MMA, or tHcy concentrations (data not shown).

The relation between total cobalamin and holoTC was strong ($r$ = 0.78), and both variables showed strong, significant inverse associations with tHcy and MMA (Table 2). In subjects with a total cobalamin concentration <100 pmol/L, >95% of the subjects had low holoTC and elevated MMA and tHcy concentrations (Table 3, **Figure 2**). As serum cobalamin increased, holoTC increased. At serum cobalamin concentrations of 150–300 pmol/L, the percentage of subjects with low holoTC concentrations declined from nearly 100% to <5% (Figure 2). In contrast, although both MMA and tHcy concentrations declined with increasing cobalamin concentrations, both metabolites were frequently elevated in subjects with normal to relatively high serum cobalamin concentrations. A high creatinine or low folate

**TABLE 3**
Differences in various variables by serum cobalamin concentrations in the total study population[1]

| Variable | Categories of serum cobalamin | | | | $P^2$ |
|---|---|---|---|---|---|
| | <100 pmol/L ($n$ = 50) | 100–150 pmol/L ($n$ = 46) | 150–200 pmol/L ($n$ = 43) | ≥200 pmol/L ($n$ = 65) | |
| Cobalamin (pmol/L) | 77 (47–95) | 115 (101–145) | 163 (151–197) | 274 (205–964) | <0.001 |
| MMA (μmol/L) | 0.68 (0.33–2.64) | 0.63 (0.12–1.75)[3] | 0.47 (0.13–1.76)[3] | 0.26 (0.05–0.84)[3–5] | <0.001 |
| MMA > 0.26 μmol/L (%) | 98 | 78 | 74[3] | 49[3–5] | <0.001 |
| HoloTC (pmol/L) | 11 (6–30) | 18 (9–34)[3] | 22 (10–58)[3] | 55 (18–304)[3–5] | <0.001 |
| Low holoTC (%) | 98 | 98 | 81 | 28[3–5] | <0.001 |
| tHcy (μmol/L) | 29.8 (13.6–80.1) | 22.9 (10.4–42.1)[3] | 19.7 (11.7–37.3)[3] | 15.5 (8.5–27.1)[3–5] | <0.001 |
| tHcy >15 μmol/L (%) | 96 | 83 | 84 | 55[3–5] | <0.001 |
| Serum folate (nmol/L) | 12.2 (6.3–26.0) | 12.6 (5.3–27.2) | 11.6 (2.2–29.3) | 11.3 (3.5–37.9) | 0.26 |
| Folate deficiency (%) | 2 | 2 | 9 | 6 | 0.32 |
| Creatinine (μmol/L) | 72.5 (59.6–94.2) | 70.3 (64.3–96.1) | 72.9 (71.9–93.5) | 71.2 (70.2–93.1) | 0.44 |
| Creatinine >110 μmol/L (%) | 3 | 2 | 0 | 7 | 0.30 |
| Hemoglobin (g/L) | 149 (106–182) | 144 (103–170) | 144 (104–162) | 144 (124–168) | 0.78 |
| Anemia (%) | 19 | 11 | 19 | 22 | 0.55 |
| MCV (fL) | 80.7 (37.1–99.0) | 81.8 (26.5–92.6) | 82.2 (43.6–98.8) | 82.2 (26.8–113.1) | 0.71 |
| Macrocytosis (%) | 0 | 3 | 0 | 3 | 0.45 |
| Platelets ($\times 10^9$/L) | 156 (102–315) | 220 (100–550)[3] | 169 (114–365) | 180 (111–359) | 0.038 |
| Thrombocytopenia (%) | 34 | 12[3] | 23 | 8[3] | 0.004 |
| Vegetarian diet (%) | 42 | 57 | 23[4] | 32[4] | 0.007 |

[1] Values are medians or percentages; 5th–95th percentiles in parentheses. MMA, methylmalonic acid; holoTC, holotranscobalamin; tHcy, total homocysteine; MCV, mean corpuscular volume.
[2] ANOVA or chi-square test followed by Tukey's post hoc test when the test result was significant.
[3] Significantly different from subjects with a cobalamin concentration <100 pmol/L, $P < 0.05$.
[4] Significantly different from subjects with a cobalamin concentration of 100–150 pmol/L, $P < 0.05$.
[5] Significantly different from subjects with a cobalamin concentration of 150–200 pmol/L, $P < 0.05$.

concentration did not explain the elevated tHcy or MMA concentrations observed in these subjects. Even in subjects with concentrations of cobalamin ≥200 pmol/L, of folate ≥12 nmol/L, and of creatinine ≤70 μmol/L ($n$ = 16), MMA and tHcy concentrations remained elevated in 38% and 63% of the subjects, respectively.

The ORs for high tHcy or MMA concentrations increased dose-dependently with declining total cobalamin or holoTC concentrations (**Table 4**). In a multivariate model, low folate and high creatinine concentrations were associated with elevated tHcy concentrations, but the total cobalamin or holoTC concentration was by far the strongest determinant. Hence, only the total cobalamin or holoTC concentration contributed significantly when a high MMA concentration was used as the outcome variable. Thus, in this population, hyperhomocysteinemia and an elevated MMA concentration were predominantly related to impaired cobalamin status.

### Cobalamin status according to diet

All but 3 of the subjects consumed dairy products and 98% of the subjects consumed them daily. Of the 84 subjects who reported that they never ate eggs, 78 were vegetarians. Only one subject adhered to a strict vegan diet, ie, no consumption of mutton, poultry, fish, eggs, or dairy products. Thus, most of the vegetarians ate a lactovegetarian diet.

Of the nonvegetarians, 22 ate animal-derived products rarely, ie, eggs, mutton, poultry, or fish <1 time/mo. Fifty-six subjects reported that they consumed at least one of these food groups between 1 time/wk and 1 time/mo. Forty-eight subjects ate one of these food groups >1 time/mo; of these subjects, 28 ate eggs, mutton, and poultry ≥2 times/wk. There was a strong correlation between the frequency of intake of each of the 4 food groups, from 0.76 (mutton versus fish) to 0.88 (mutton versus poultry).

The vitamin status and hematologic indexes of the vegetarians and nonvegetarians are shown in **Table 5**. Hemoglobin concentrations were somewhat lower and the prevalence of macrocytosis was higher in the vegetarians than in the nonvegetarians; however, the differences, although statistically significant, were not pronounced. Significantly more vegetarians were cobalamin deficient; however, neither the concentrations of cobalamin,

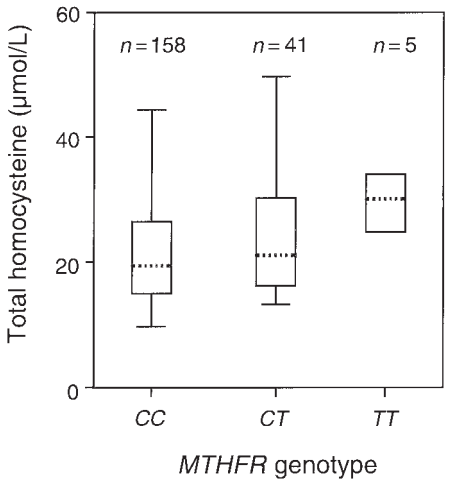


**FIGURE 1.** Relation between *MTHFR* genotype and plasma total homocysteine concentrations shown as medians (dashed line), interquartile ranges (box), and 5th–95th percentiles (bars). $P$ for linear trend = 0.042.

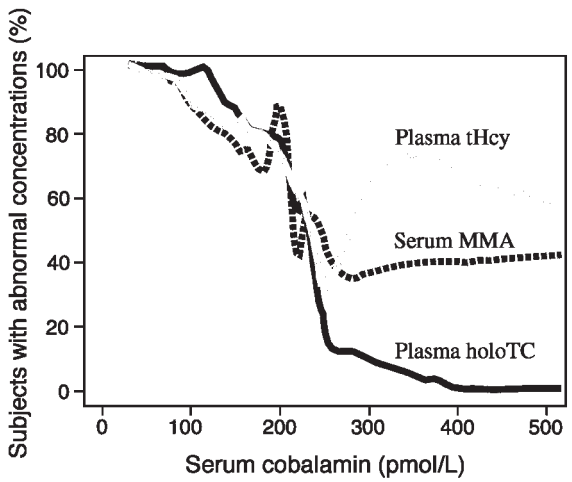


**FIGURE 2.** Relation between serum total cobalamin concentrations and the percentage of subjects with elevated serum methylmalonic acid (MMA; >0.26 μmol/L), elevated total homocysteine (tHcy; >15 μmol/L), or low holotranscobalamin (holoTC; <35 pmol/L) concentrations.

tHcy, and MMA nor the prevalence of hyperhomocysteinemia and elevated MMA concentrations differed significantly between the 2 groups. Of the subjects who reported consuming eggs, mutton, and poultry >2 times/wk ($n$ = 28), 36% had cobalamin deficiency, 61% had elevated MMA concentrations, and 79% had hyperhomocysteinemia.

Overall, the frequency of intake of animal-derived food items was weakly correlated with cobalamin status. The strongest relation was with serum cobalamin, followed by holoTC, tHcy, and MMA concentrations (data not shown). Significant associations were observed between the frequency of intake of mutton and concentrations of cobalamin ($r$ = 0.19, $P$ < 0.01), holoTC ($r$ = 0.17, $P$ = 0.017), and tHcy ($r$ = −0.16, $P$ = 0.038). Serum cobalamin was also significantly associated with the frequency of intake of poultry ($r$ = 0.15, $P$ = 0.038). The frequency of intake of eggs or fish was not significantly associated with cobalamin, holoTC, tHcy, or MMA concentrations (data not shown).

The risk of cobalamin deficiency, adjusted for age, sex, and disease category, was significantly increased in subjects who rarely consumed mutton (OR: 2.93; 95% CI: 1.58, 5.41), eggs (OR: 1.91; 1.06, 3.45), or poultry (OR: 2.24; 1.22, 4.10). Low fish intake was not significantly associated with cobalamin deficiency (OR: 1.55; 0.86, 2.81). In a model including frequencies of fish, poultry, mutton, and egg intakes, only an infrequent intake of mutton remained significantly associated with cobalamin deficiency (OR: 8.36; 1.64, 42.61). Repeating the analyses in only nonvegetarians did not materially alter the results.

## DISCUSSION

The most notable finding in this Indian population was the very high prevalence of cobalamin deficiency. Of the 204 subjects, only 10% had normal concentrations of cobalamin, tHcy, and MMA; 52% had a low serum cobalamin concentration, 76% had hyperhomocysteinemia, and 73% had an elevated MMA concentration. Only 6% of the subjects had a tHcy concentration <10 μmol/L, a threshold sometimes referred to as the upper desirable level. Few subjects ($n$ = 10, or ≈5%) had low serum folate

**TABLE 4**
Odd ratios (OR) for elevated plasma total homocysteine (tHcy) or plasma methylmalonic acid (MMA) concentrations by creatinine concentration or vitamin status in the total study population[1]

| | OR (95% CI) for tHcy > 20 μmol/L | | OR (95% CI) for MMA > 0.50 μmol/L | |
|---|---|---|---|---|
| Variable | Model 1[1] | Model 2[1] | Model 3[1] | Model 4[1] |
| Creatinine | | | | |
| ≤60 μmol/L ($n$ = 47) | 1.00 | 1.00 | 1.00 | 1.00 |
| 60–70 μmol/L ($n$ = 38) | 1.23 (0.38, 4.04) | 0.88 (0.29, 2.70) | 2.13 (0.63, 7.17) | 1.33 (0.43, 4.18) |
| 70–80 μmol/L ($n$ = 46) | 3.15 (0.97, 10.23) | 2.62 (0.88, 7.84) | 1.68 (0.54, 5.24) | 1.43 (0.48, 4.25) |
| >80 μmol/L ($n$ = 52) | 3.12 (0.99, 9.87) | 1.95 (0.67, 5.69) | 2.94 (0.93, 9.31) | 1.66 (0.55, 5.00) |
| $P$ for trend | 0.027 | 0.099 | 0.10 | 0.37 |
| Cobalamin | | | | |
| ≥200 pmol/L ($n$ = 65) | 1.00 | | 1.00 | |
| 150–200 pmol/L ($n$ = 43) | 7.54 (2.43, 23.40) | | 4.99 (1.78, 14.00) | |
| 100–150 pmol/L ($n$ = 46) | 15.95 (5.11, 49.82) | | 14.18 (3.24, 41.12) | |
| <100 pmol/L ($n$ = 50) | 81.89 (20.56, 326.2) | | 119.50 (26.21, 544.8) | |
| $P$ for trend | <0.001 | | <0.001 | |
| HoloTC | | | | |
| ≥35 pmol/L ($n$ = 56) | | 1.00 | | 1.00 |
| 25–35 pmol/L ($n$ = 54) | | 2.61 (0.81, 8.36) | | 12.32 (3.58, 42.45) |
| 15–25 pmol/L ($n$ = 33) | | 6.54 (2.33, 18.40) | | 16.75 (5.14, 54.59) |
| <15 pmol/L ($n$ = 54) | | 21.44 (7.09, 64.87) | | 53.05 (15.02, 187.43) |
| $P$ for trend | | <0.001 | | <0.001 |
| Folate | | | | |
| ≥16 nmol/L ($n$ = 53) | 1.00 | 1.00 | | |
| 12–16 nmol/L ($n$ = 49) | 3.07 (0.94, 10.01) | 2.53 (0.82, 7.77) | | |
| 8–12 nmol/L ($n$ = 58) | 2.68 (0.88, 8.18) | 2.18 (0.75, 6.32) | | |
| <8 nmol/L ($n$ = 40) | 4.61 (1.31, 16.21) | 3.69 (1.19, 11.48) | | |
| $P$ for trend | 0.027 | 0.039 | | |

[1] Each logistic regression model includes the variables shown and was further adjusted for age, sex, cardiovascular disease, and diabetes.

**TABLE 5**
Vitamin status and hematologic indexes by diet[1]

| Variable | Nonvegetarians ($n$ = 126) | Vegetarians ($n$ = 78) | $P$[2] |
|---|---|---|---|
| Cobalamin (pmol/L) | 161 (62–492) | 124 (66–625) | 0.18 |
| Cobalamin deficiency (%) | 39 | 60 | 0.003 |
| HoloTC (pmol/L) | 22.5 (8.4–94.0) | 19.0 (7.0–96.0) | 0.42 |
| Low holoTC (%) | 71 | 75 | 0.61 |
| MMA (μmol/L) | 0.46 (0.06–1.74) | 0.53 (0.13–1.75) | 0.23 |
| MMA > 0.26 μmol/L (%) | 71 | 76 | 0.51 |
| tHcy (μmol/L) | 19.4 (9.7–45.7) | 22.0 (9.6–48.0) | 0.21 |
| tHcy >15 μmol/L (%) | 76 | 79 | 0.59 |
| Serum folate (nmol/L) | 12.1 (4.5–29.4) | 12.3 (4.5–28.9) | 0.75 |
| Folate deficiency (%) | 6 | 4 | 0.60 |
| Hemoglobin (g/L) | 146 (116–171) | 144 (108–164) | 0.031 |
| Anemia (%) | 15 | 22 | 0.21 |
| MCV (fL) | 81.4 (69.9–93.6) | 82.1 (65.3–97.3) | 0.85 |
| Macrocytosis (%) | 0 | 4 | 0.025 |
| Platelets ($\times 10^9$/L) | 175 (112–358) | 188 (112–407) | 0.22 |
| Thrombocytopenia (%) | 19 | 16 | 0.55 |

[1] Values are medians or percentages; 5th–95th percentiles in parentheses. holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine; MCV, mean corpuscular volume.

[2] By Student's $t$ test for independent samples or chi-square test.

concentrations; of these, 8 had low cobalamin concentrations, high MMA concentrations, or both, which suggests that folate deficiency may be secondary to impaired cobalamin function (8).

The biochemical and metabolic findings of cobalamin deficiency were observed in young and middle-aged adults of both sexes. Repeating the analyses separately in the 4 groups with or without diabetes or CVD did not alter the main finding, ie, that most subjects had cobalamin deficiency on the basis of low serum cobalamin concentrations concomitant with high tHcy and MMA concentrations.

A critical question is whether sampling conditions or analytic errors influenced the results. High concentrations of both tHcy and MMA were found with 2 different techniques—tHcy with HPLC (19) and GC-MS (21) and MMA with capillary electrophoresis (20) and GC-MS (21). In the assay for tHcy, we also measured total cysteine. We found a mean cysteine concentration of 243 μmol/L, which is slightly lower than the mean concentration reported in Norwegians (28). Thus, the high tHcy concentrations were not due to analytic errors or to lyophilization of the samples. Moreover, concentrations of both serum cobalamin and holoTC were very low, whereas serum folate concentrations were normal relative to other populations. Finally, serum cobalamin was strongly correlated with both tHcy and MMA concentrations, which is consistent with findings in other cobalamin-deficient populations (29, 30).

One may ask whether the subjects investigated (residents of Pune, Maharashtra) represent the entire Indian population. Data from the Indian subcontinent suggest that cobalamin deficiency is common in healthy subjects (9, 31) and very common in malnourished children (10). Cobalamin deficiency is frequently observed in subjects with anemia (9) and it causes most of the cases of megaloblastic anemia (10, 32). Indian emigrants often have serum cobalamin concentrations that are lower than those of the population in the country of residence, but they rarely have cobalamin deficiency (33, 34). Only a few studies of tHcy concentrations in Indians have been published. In one study, CVD patients from Cochin, India, had tHcy concentrations of ≈10 μmol/L (35). In a recent study in Hyderabad, India, patients with coronary artery disease had tHcy concentrations similar to the values observed in the current study (36). In Indians living in Singapore (34), mean tHcy concentrations were 16.2 μmol/L in men and 11.5 μmol/L in women. In Indians living in the United Kingdom, the mean tHcy concentration was 10.8 μmol/L; this concentration was only marginally higher than the concentration observed in the white population of the United Kingdom (33). Thus, it seems that cobalamin deficiency and hyperhomocysteinemia are common in Indians, but that cobalamin concentrations are markedly influenced by age, sex, diet, and the site of residence, and probably by socioeconomic class.

Severe cobalamin deficiency can occur without the classic signs of anemia or macrocytosis (14). Unexpectedly, anemia was rare in the current study population, even among those with very low cobalamin concentrations concomitant with markedly elevated tHcy and MMA concentrations. A similar observation was made in other Indian populations (31, 37) and recently in pregnant Nepali women (38). One possible reason may be that an adequate folate intake protects against anemia and thereby masks the effect of cobalamin deficiency (13). The fact that even severe biochemical cobalamin deficiency is usually not accompanied by anemia in some Asian populations may explain why there are relatively few articles on cobalamin deficiency in a nation so dominated by vegetarianism.

Interestingly, we observed in our subjects that a low cobalamin concentration was often accompanied by thrombocytopenia. A low platelet count is frequently observed in severe cobalamin deficiency (14, 32), but it is usually believed to arise after the development of anemia (32). Hence, the reason for the thrombocytopenia in our subjects is unclear. It may have been related to impaired cobalamin function or to a lack of other nutrients. Recently, a study showed that vegans with normal cobalamin, tHcy, and hemoglobin concentrations had significantly lower leukocyte and platelet counts than did nonvegetarians (39), possibly because the vegans had protein or energy intakes that were lower than those of the nonvegetarians.

Although the risk of cobalamin deficiency was related to the frequency of intake of animal-derived foods, the low cobalamin

status in the current study population was only partly explained by the population's vegetarian diet. Cobalamin deficiency was common, even in subjects who reported intakes of eggs, poultry, and mutton >2 times/wk (36%). Marked ethnic differences in cobalamin metabolism have been reported (40); therefore, the possibility that Indians have adapted to a chronic low cobalamin concentrations through genetic mechanisms should be considered. For example, the *MTHFR* 677C→T polymorphism is thought to protect against megaloblastic anemia by retaining cellular folates (41). However, the prevalence of the *TT* genotype in Indians was very low, much lower than in other Asian countries and comparable with that in Africa (26, 27).

Other than a vegetarian diet, the cause of cobalamin deficiency in the subjects in the current study was not clear. Tropical sprue, giardiasis, and other gastrointestinal infections are common in India (7), and these may lead to malabsorptive states and cobalamin deficiency (8). Interestingly, Lindenbaum et al (15) reported elevated tHcy and MMA concentrations in 3 patients with tropical sprue, 1 of whom had a normal cobalamin concentration (15). This finding agrees with our observation that even subjects with relatively high cobalamin concentrations can have high tHcy and MMA concentrations. Notably, in the study by Lindenbaum et al, the high MMA concentration was related to anaerobic gut flora and the high tHcy concentration was explained by a low cobalamin concentration.

Some studies suggest that overgrowth of intestinal bacterial may lead to formation and absorption of inactive cobalamin analogues (42, 43). In the current study, we first measured total cobalamin concentrations. To exclude the possibility that the measured cobalamin concentrations did not reflect the circulating cobalamin concentrations available to the mammalian cell, we measured holoTC concentrations. We observed a strong association between holoTC and total cobalamin concentrations. However, the holoTC concentrations measured did not explain the high MMA or tHcy concentrations in subjects with normal total cobalamin concentrations. These findings suggest an unusual regulation or function of cobalamin metabolism in this Indian population.

Megaloblastic anemia and neurologic symptoms occur late in the development of overt cobalamin deficiency (13). Perhaps more important from a general health perspective are the long-term effects of mild, subclinical cobalamin deficiency. Notably, elevated tHcy concentrations, low cobalamin concentrations, or both are related to CVD (44), cancer (45), cognitive impairment (46), delayed mental development in infancy and childhood (47, 48), and birth defects and pregnancy complications (49). A possible relation between impaired cobalamin status and childhood infections, tuberculosis, HIV infection, and reduced cellular immunity (50–53) has also been implicated.

The population of the Indian subcontinent is >1 billion, most of whom consume a diet low in cobalamin. Isolated reports suggest that cobalamin deficiency in India is common; however, this problem has received little attention. The national strategies for improving micronutrient intake do not include cobalamin (54), and a search of MEDLINE (National Institutes of Health, Bethesda, MD) indicated that vitamin A, iron, zinc, and iodine are considered the micronutrients of interest by the Indian medical community. Our data from the city of Pune show that ≈75% of an adult population have metabolic evidence of cobalamin deficiency. If these findings are confirmed in other parts of India, this may have important health implications.

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## REFERENCES

1. Price SR. Observations on dietary practices in India. Hum Nutr Appl Nutr 1984;38:383–9.
2. Appleby PN, Thorogood M, Mann JI, Key TJ. The Oxford Vegetarian Study: an overview. Am J Clin Nutr 1999;70(suppl): 525S–31S.
3. Laidlaw SA, Shultz TD, Cecchino JT, Kopple JD. Plasma and urine taurine levels in vegans. Am J Clin Nutr 1988;47:660–3.
4. Sanders TA. The nutritional adequacy of plant-based diets. Proc Nutr Soc 1999;58:265–9.
5. Herbert V. Staging vitamin B-12 (cobalamin) status in vegetarians. Am J Clin Nutr 1994;59(suppl):1213S–22S.
6. Balaji LN, Dustagheer A. Nutrition scenario in India—implications for clinical practice. J India Med Assoc 2000;98:536–8, 542.
7. Mathan VI. Tropical sprue in Southern India. Trans R Soc Trop Med Hyg 1988;82:10–4.
8. Herbert V. Biology of disease. Megaloblastic anemias. Lab Invest 1985;52:3–19.
9. Kumar S, Ghosh K, Das KC. Serum vitamin $B_{12}$ levels in an Indian population: an evaluation of three assay methods. Med Lab Sci 1989; 46:120–6.
10. Gomber S, Kumar S, Rusia U, Gupta P, Agarwal KN, Sharma S. Prevalence and etiology of nutritional anaemias in early childhood in an urban slum. Indian J Med Res 1998;107:269–73.
11. Ng SC, Kuperan P, Chan KS, Bosco J, Chan GL. Megaloblastic anaemia—a review from University Hospital, Kuala Lumpur. Ann Acad Med Singapore 1988;17:261–6.
12. Chanarin I, Malkowska V, O'Hea AM, Rinsler MG, Price AB. Megaloblastic anaemia in a vegetarian Hindu community. Lancet 1985;2:1168–72.
13. Scott JM. Folate and vitamin $B_{12}$. Proc Nutr Soc 1999;58:441–8.
14. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720–8.
15. Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency. II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. Am J Hematol 1990;34:99–107.

16. Lindgren A, Kilander A, Bagge E, Nexo E. Holotranscobalamin—a sensitive marker of cobalamin malabsorption. Eur J Clin Invest 1999; 29:321–9.
17. Mann NJ, Li D, Sinclair AJ, et al. The effect of diet on plasma homocysteine concentrations in healthy male subjects. Eur J Clin Nutr 1999;53:895–9.
18. Schneede J, Dagnelie PC, van Staveren WA, Vollset SE, Refsum H, Ueland PM. Methylmalonic acid and homocysteine in plasma as indicators of functional cobalamin deficiency in infants on macrobiotic diets. Pediatr Res 1994;36:194–201.
19. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.
20. Schneede J, Ueland PM. Application of capillary electrophoresis with laser-induced fluorescence detection for routine determination of methylmalonic acid in human serum. Anal Chem 1995;34:812–9.
21. Husek P. Simultaneous profile analysis of plasma amino and organic acids by capillary gas chromatography. J Chromatogr B Biomed Appl 1995;669:352–7.
22. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
23. Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin $B_{12}$ with use of a colistin-sulfate-resistant organism. Clin Chem 1987;33:52–4.
24. Ulvik A, Refsum H, Kluijtmans LAJ, Ueland PM. C677T mutation of methylenetetrahydrofolate reductase gene determined in blood or plasma by multiple injection capillary electrophoresis and laser-induced fluorescence detection. Clin Chem 1997;43:267–72.
25. Wilson A, Platt R, Wu Q, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin $B_{12}$) increases risk for spina bifida. Mol Genet Metab 1999;67:317–23.
26. Hessner MJ, Luhm RA, Pearson SL, Endean DJ, Friedman KD, Montgomery RR. Prevalence of prothrombin G20210A, factor V G1691A (Leiden), and methylenetetrahydrofolate reductase (MTHFR) C677T in seven different populations determined by multiplex allele-specific PCR. Thromb Haemost 1999;81:733–8.
27. Franco RF, Araujo AG, Guerreiro JF, Elion J, Zago MA. Analysis of the 677 C→T mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. Thromb Haemost 1998;79:119–21.
28. El-Khairy L, Ueland PM, Nygård O, Refsum H, Vollset SE. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. Am J Clin Nutr 1999;70:1016–24.
29. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 1994;60:2–11.
30. Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 1999;70:904–10.
31. Jathar VS, Patrawalla SP, Doongaji DR, Rege DV, Satoskar RS. Serum vitamin $B_{12}$ levels in Indian psychiatric patients. Br J Psychiatry 1970;117:699–704.
32. Sarode R, Garewal G, Marwaha N, et al. Pancytopenia in nutritional megaloblastic anaemia. A study from north-west India. Trop Geogr Med 1989;41:331–6.
33. Chambers JC, Obeid OA, Refsum H, et al. Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. Lancet 2000;355:523–7.
34. Hughes K, Ong CN. Homocysteine, folate, vitamin $B_{12}$, and cardiovascular risk in Indians, Malays, and Chinese in Singapore. J Epidemiol Community Health 2000;54:31–4.
35. Chacko KA. Plasma homocysteine levels in patients with coronary heart disease. Indian Heart J 1998;50:295–9.
36. Gheye S, Lakshmi AV, Krishna TP, Krishnaswamy K. Fibrinogen and homocysteine levels in coronary artery disease. Indian Heart J 1999;51:499–502.
37. Inamdar-Deshmurkh AB, Jathar VS, Joseph DA, Satoskar RS. Erythrocyte vitamin $B_{12}$ activity in healthy Indian lactovegetarians. Br J Haematol 1976;32:395–401.
38. Bondevik GT, Eskeland B, Ulvik RJ, et al. Anaemia in pregnancy: possible causes and risk factors in Nepali women. Eur J Clin Nutr 2000;54:3–8.
39. Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. Am J Clin Nutr 1999; 70(suppl):586S–93S.
40. Carmel R. Ethnic and racial factors in cobalamin metabolism and its disorders. Semin Hematol 1999;36:88–100.
41. Kvittingen EA, Spangen S, Lindemans J, Fowler B. Methionine synthase deficiency without megaloblastic anaemia. Eur J Pediatr 1997;156:925–30.
42. Brandt LJ, Bernstein LH, Wagle A. Production of vitamin B 12 analogues in patients with small-bowel bacterial overgrowth. Ann Intern Med 1977;87:546–51.
43. Murphy MF, Sourial NA, Burman JF, Doyle DV, Tabaqchali S, Mollin DL. Megaloblastic anaemia due to vitamin $B_{12}$ deficiency caused by small intestinal bacterial overgrowth: possible role of vitamin $B_{12}$ analogues. Br J Haematol 1986;62:7–12.
44. Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31–62.
45. Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B12, and pyridoxal 5′-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
46. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin $B_{12}$, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
47. Schneede J, Dagnelie PC, Refsum H, Ueland P. Nutritional cobalamin deficiency in infants. In: Bhatt H, James V, Besser G, Bottazzo G, Keen H, eds. Advances in Thomas Addison's diseases. Norwich, United Kingdom: Page Brothers, 1994:259–68.
48. Louwman WJ, van Dusseldorp M, van de Vijver FJR, et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 2000;72:762–9.
49. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. Am J Clin Nutr 2000;71:962–8.
50. Chanarin I, Stephenson E. Vegetarian diet and cobalamin deficiency: their association with tuberculosis. J Clin Pathol 1988;41:759–62.
51. Au WY, Hui CH, Chan LC, Liang RH, Kwong YL. Clinicopathological features of megaloblastic anaemia in Hong Kong: a study of 84 Chinese patients. Clin Lab Haematol 1998;20:217–9.
52. Tamura J, Kubota K, Murakami H, et al. Immunomodulation by vitamin $B_{12}$: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin $B_{12}$-deficient patients by methyl-$B_{12}$ treatment. Clin Exp Immunol 1999;116:28–32.
53. Liang B, Chung S, Araghiniknam M, Lane LC, Watson RR. Vitamins and immunomodulation in AIDS. Nutrition 1996;12:1–7.
54. Vijayaraghavan K. Strategies for control of micronutrient malnutrition. Indian J Med Res 1995;102:216–22.