Iron status of predominantly lacto-ovo vegetarian East Indian immigrants to Canada: a model approach[1-3]

Gursh S Bindra, PhD and Rosalind S Gibson, PhD

ABSTRACT   Iron status of East Indian predominantly lacto-ovo vegetarian immigrants (59 males, mean age 37.7 ± 10.5 yr; 55 females, mean age 33.3 ± 7.4 yr) was assessed using dietary and biochemical-iron indices, including a Tri-index (TI) model. Iron deficiency was higher among females than males: 33% vs 5%, respectively, via the TI model (serum ferritin, serum-transferrin saturation, and mean corpuscular-hemoglobin concentration) and 18–42% vs 2–22%, respectively, via individual biochemical-iron indices. Rates of anemia calculated via the TI model in combination with low hemoglobin and mixed-distribution analysis (MDA) were similar and higher for the females (TI + Hb = 16%; MDA = 12%) than for the males (TI + Hb = 5%; MDA = 3%). High prevalence among females was attributed to low available iron intakes, concomitant with high intakes of dietary fiber, phytate, and tannins. We recommend the TI-model approach to estimate relative prevalence of iron deficiency in small surveys.   *Am J Clin Nutr* 1986;44:643–52.

KEY WORDS   Iron status, vegetarians, Tri-index model, iron deficiency, anemia, available iron, dietary fiber, phytate, tannins

Introduction

Iron-deficiency anemia is a major nutritional problem in India, despite dietary iron intakes of up to 30 mg/day in adults (1). Impaired absorption of iron resulting from high intakes of various *anti-nutrients* (such as, phytate (2, 3), dietary fiber (4), and tannins (5)) and low intakes of flesh foods has been proposed as the most likely cause of this inadequate iron status (6). Suboptimal iron nurture has also been documented in East Indian immigrant populations residing in South Africa (7, 8), the United Kingdom (9), and the USA (10) and attributed to their retention of traditional East Indian eating patterns. To date, no information exists on the iron status of East Indian immigrants residing in Canada.

Most previous surveys of iron status have relied on individual biochemical tests for classifying subjects as iron deficient or replete (11–16). This is not the best method of assessing the prevalence of suboptimal iron status on a population basis because of the marked degree of overlap in laboratory results between iron-deficient and iron-sufficient populations (17–19). Instead, a model approach that simultaneously incorporates several biochemical-iron indices has been proposed (20, 21). With this method, a positive diagnosis of iron deficiency is made only when values from at least two or three of the biochemical tests included in the model are abnormal (17, 20, 21).

In this study we have determined the iron status of a sample of predominantly lacto-ovo vegetarian East Indian Punjabi immigrants to Canada, using dietary and biochemical-iron indices, including a Tri-index model. The latter is based on three independent tests of biochemical-iron status, representing the three different stages in the development of iron-

deficiency anemia (22). These tests determined serum ferritin, which identifies depletion of storage iron (23); serum-transferrin saturation, an index of reduced transport-iron supply (24); and mean corpuscular-hemoglobin concentration (MCHC), a red cell index that measures the concentration of hemoglobin in an average red corpuscle (25). Low MCHC values (ie, < 32.0%) are indicative of the final stage of iron deficiency, a classic hypochromic, microcytic anemia (25, 26).

Subjects and methods

Subjects were 59 adult males (mean age, 37.7 ± 10.5 yr) and 55 adult females (mean age, 33.3 ± 7.4 yr) from the East Indian communities of Guelph and Kitchener-Waterloo, Ontario, Canada. Many were from Punjabi families who had emigrated from India between 1970 and 1975, and all were consuming self-selected diets. The majority of subjects surveyed ate meat, poultry, and fish no more than twice a week.

The socioeconomic status (SES) of these Punjabi immigrants was determined according to the scale of Blishen and McRoberts (27), which generates an index value based on the father's occupation. Mean SES index scores for males were 45.8 (range, 26.5–74.2) and 44.0 (range, 26.5–74.2) for females; these mean values correspond to the skilled-labor class. Median parity for the mothers in the study was two. Consent was obtained from the subjects after the nature of the study had been fully explained to them. The study protocol was approved by the Human Ethics Committee of the University of Guelph.

Biochemical results were not included for women known to be pregnant, lactating, or taking oral contraceptive agents (n = 5). It is unlikely that any of the Punjabi females included in the study used intrauterine devices, known to increase menstrual bleeding, because the Sikh religion discourages invasive methods of contraception.

Biochemical assessment

Laboratory analyses. Peripheral venous blood samples were drawn from each subject in the morning between 0700 h and 1000 h after an overnight fast, using trace-element-free vacutainers with siliconized needles (Sarstedt, Montreal, PQ). Serum was separated using acid-washed pipettes and frozen in trace-element-free polyethylene vials at −20°C for subsequent analysis of serum iron, total iron-binding capacity (TIBC), and serum ferritin.

Immediately following blood collection, whole anticoagulated blood was used to determine hemoglobin and hematocrit levels by the cyanomethemoglobin and microhematocrit methods (28), respectively. The mean corpuscular-hemoglobin concentration was calculated by the formula: MCHC (%) = hemoglobin/hematocrit × 100.

We determined serum iron and TIBC by a colorimetric procedure, using ferrozine as the chromogen (29) and iron-UIBC-TIBC kits from Sigma Chemical Company of St Louis, MO. Serial replications of a quality-control sera (Serachem Level 1, Fisher Diagnostics, Orangeburg, NY) and a pooled serum sample were used to check on the

accuracy and reproducibility of the method, respectively. The mean results ± SD from eight measurements of serum iron and TIBC were 166 ± 10 μg/dl and 296 ± 22 μg/dl, respectively. Certified values for serum iron and TIBC using the Sigma method were 158 ± 16 μg/dl and 290 ± 32 μg/dl, respectively. Analysis of eight pooled serum samples yielded a mean result of 118 ± 6 μg/dl for serum iron and 257 ± 18 μmg/dl for TIBC. The percentage saturation of serum transferrin (a derived index) was calculated (25). We assayed serum ferritin via the two-site immunoradiometric procedure described by Miles et al (30), using kits supplied by Bio-Rad Labs (Richmond, CA). Eight serial replications of four levels (anemia, I, II, and III) of immunoassay control sera (LYPHOCHEK, Bio-Rad) gave (mean ± SD) values of 5.5 ± 0.8, 48.0 ± 6.7, 138.5 ± 14.4, and 390.7 ± 73.7 ng/ml, respectively. These compared with certified values of 6.0 ± 1.4, 42.0 ± 5.0, 147.0 ± 18.0, and 422.0 ± 64.0 ng/ml, respectively.

Cut-off values for adult males and females for the Tri-index model indices were selected from the literature and were: serum ferritin (SF) < 12 ng/ml (31, 32), serum transferrin percentage saturation (STS) < 16% (33), and MCHC < 32% (34). Because these criteria are equally applicable to older and younger adults (21), all males 18–64 yr were combined into one group and all females 18–64 yr were combined into another for the Tri-index data analysis.

The criteria used by the Nutrition Canada Survey (34) to represent the upper limits of *moderate risk* of deficiency for hemoglobin concentration (males > 17 yr = 14.0 g/dl, females > 17 yr = 12.0 g/dl) were chosen in our analysis because they closely approximate hemoglobin cutoff points used in several previous surveys (35–38).

Diagnosis of iron-deficiency anemia. In this study, we determined relative prevalence of iron-deficiency anemia using low hemoglobin concentration combined with two or three abnormal values for indices in the Tri-index model and mixed-distribution analysis of hemoglobin values (hemoglobin-percentile shift). Mixed-distribution analysis is based on the premise that the presence of anemia resulting from iron deficiency or inflammatory disease in a population causes a lowering of the population's hemoglobin concentration. Hemoglobin values for the nonanemic individuals are assumed to follow a Gaussian distribution, so the relative prevalence of anemia can be estimated by determining the change, or shift, in the median hemoglobin concentration (50th percentile) after excluding subjects who have one or more laboratory value indicative of iron deficiency (21, 38, 39).

Dietary assessment

Three-day, weighed-food dietary records were completed by the subjects on two consecutive weekdays and one weekend day, using dietary scales (Hanson, Shubuta, Ms; Model No 1440) and bilingual food diaries. Records were checked on completion by a Punjabi nutritionist (GSB). Daily intakes of energy, protein, dietary iron, and dietary fiber were calculated from the coded three-day records using food-composition data from the condensed Canadian Nutrient File (40), food composition tables (41, 42), and the literature (43, 44).

The computer program used for the dietary calculations also provided data on the total content of iron and ascorbic acid per meal or snack for each day of the 3 days as well

as the total weight of meat, poultry, and fish consumed at each meal or snack. We used this information to calculate available iron content of the diets using the original and refined models of Monsen et al (45) and Monsen and Balintfy (46), respectively. The factors used for the calculation of absorption of iron per meal were based on the assumption that the level of iron stores for the men were higher than for the premenopausal women (ie, ~500 mg compared to 250 mg). Mean intakes of heme and nonheme iron for the males and females were also determined. Foodstuffs were divided into nine food groups to determine the major sources of dietary iron.

Statistical analyses

Serum-ferritin concentrations followed a skewed distribution that was normalized by logarithmic transformation. We evaluated other biochemical and dietary data using standard parametric statistical methods (47), with the exception of correlation analyses. For the latter, Spearman rank-correlation coefficients (r_s) were used (48).

Results

Biochemical assessment

The mean hemoglobin, hematocrit, MCHC, serum iron, TIBC, serum-transferrin saturation, and median serum-ferritin values for Punjabi subjects aged 18–39 and 40–64 yr, respectively, are shown in **Table 1.**

The major significant correlations observed between biochemical indices of iron status of Punjabi females were hemoglobin and hematocrit ($r = 0.87$, $p < 0.001$) and hemoglobin and log serum ferritin ($r = 0.5$, $p < 0.001$). The only significant correlations for males were between hemoglobin and hematocrit ($r = 0.79$, $p < 0.001$) and hemoglobin and MCHC ($r = 0.61$, $p < 0.001$). No significant correlations were found between dietary and biochemical indices for either male or female subjects.

Table 2 shows the percentages of individuals classified as iron deficient, based on abnormal values for each index of iron status, individually and in combination, in the Tri-index model.

In general, fewer males and females were classified as iron deficient by the Tri-index model than by the *cutoff* method of using individual biochemical indicators of iron status (Table 2). The exception was for serum ferritin in the male group.

Frequency distributions presented in **Figure 1** illustrate the absence of any distinct separation between normal and deficient values for

TABLE 1
Biochemical indices of iron status of Punjabi adult males and females*

Group	n	Hemoglobin	Hematocrit	MCHC†	Serum iron	TIBC	Serum-transferrin saturation†	Serum ferritin‡
		g/dl	%	%	μg/dl	μg/dl	%	ng/ml
Young-Adult Males (18–39 yr)	37	15.5 ± 1.4	46.2 ± 3.4	33.6 ± 2.0	83.2 ± 47.0	318.7 ± 54.0	25.9 ± 12.5	70.0 (13.1, 258.8)
Older-Adult Males (40–64 yr)	22	15.4 ± 1.8	45.3 ± 3.6	34.0 ± 2.2	83.9 ± 24.8	323.8 ± 29.7	25.9 ± 7.6	91.4 (14.8, 176.6)
Young-Adult Females (18–39 yr)	38	13.1 ± 1.6	39.5 ± 3.3	33.0 ± 2.7	81.8 ± 70.2	375.2 ± 118.6	22.8 ± 17.3	14.8 (2.0, 117.2)
Older-Adult Females (40–64 yr)	12	13.6 ± 1.4	42.6 ± 3.6	31.9 ± 2.4	67.4 ± 37.6	343.2 ± 42.0	19.8 ± 11.6	18.3 (8.6, 140.0)

* Values are reported as Mean ± SD, except those for serum ferritin.
† Derived index.
‡ Values expressed as median, with 90% confidence intervals.

TABLE 2
Percentage of Punjabi males and females classified as iron deficient using a selection of biochemical indices of iron status individually, and combined into the Tri-index model

		Biochemical indices (with criteria used for classifying iron deficiency)*					
Group	n	Serum ferritin (12 < ng/ml)	Serum-transferrin saturation (<16%)	MCHC (<32%)	Hemoglobin (M < 14 g/dl) (F < 12 g/dl)	Tri-index model†	Low hemoglobin + Tri-index model‡
Males (18–64 yr)	59	1.7 (n = 1)	13.6 (n = 8)	22.0 (n = 13)	15.3 (n = 9)	5.1 (n = 3)	5.1 (n = 3)
Females§ (18–64 yr)	51	41.2 (n = 21)	41.2 (n = 21)	33.3 (n = 17)	17.7 (n = 9)	33.3 (n = 17)	15.7 (n = 8)

* Refer to text for source of cutoff points.

† The Tri-index model combines serum ferritin, transferrin saturation, and MCHC. Individuals are classified as iron deficient with two or three abnormal values for these indices.

‡ Individuals classified under this category have low hemoglobin concentrations (age- and sex-specific) and two or three abnormal values for the Tri-index model.

§ Pregnant women are excluded (n = 3).

hemoglobin, MCHC, transferrin saturation, and serum ferritin in the males (Fig 1A) or females (Fig 1B). This finding emphasizes the importance of using more than one biochemical index for assessing iron status.

The prevalence of iron-deficiency anemia, rather than biochemical iron deficiency, was estimated by two methods in this study. In the first approach, the Tri-index model was used in conjunction with low hemoglobin concentration (Table 2). A second approach (hemoglobin-percentile shift) utilized the cumulative frequency distribution of hemoglobin values for the entire male (n = 58) and female (n = 50) subject populations (excluding pregnant women n = 3), plotted on a probability scale (Figure 2). The linear component of both the male and female plots represents the majority of subjects who were nonanemic. In contrast, the curved, nonlinear components of the plots represent those individuals who had low hemoglobin values that fell outside the Gaussian distribution.

The shaded areas that fall between the total (curved) and extrapolated linear segment depict the estimated proportion of anemic subjects, ie, the relative prevalence of anemia. The latter was calculated for both male and female groups by a hemoglobin-percentile-shift analysis, as described earlier. Table 3 presents these results.

Dietary assessment

The calculated mean daily intakes of energy; protein; dietary fiber; dietary iron; heme and nonheme iron; available iron; meat, poultry, and fish (in grams/day); and vitamin C for the diets of 47 adult male and 44 female subjects who returned valid 3-day, weighed-food records are shown in Table 4.

Average dietary-iron intakes (Table 4) (without iron supplements) for the Punjabi men and women were 234% and 103%, respectively, of the Canadian recommended nutrient intake (RNI) for iron (49). All Punjabi males in the dietary sample had mean total dietary-iron intakes greater than both the Canadian RNI (49) and US recommended dietary allowances (RDA) (50). However 46% and 82% of the Punjabi premenopausal females had total dietary-iron intakes lower than the RNI and RDA, respectively (Fig 3), 7% and 30% of these women receiving less than two-thirds of these recommended levels. Only 3% of the adults took iron supplements regularly on a daily basis with a further 5% reporting less frequent use, so that their contribution to dietary-iron intakes was negligible.

The calculated mean daily intake of available iron, assuming 500 mg of iron stores for adult males (46), was 1.27 mg, 134% of the Canadian RNI (49) to cover basal losses (0.95 mg). Premenopausal women have a higher average daily RNI for available iron, 1.12 mg/day, a figure comprising a mean basal loss of 0.67 mg and a median menstrual loss of 0.45 mg (49). Their mean available iron intake calculated assuming iron stores of 250 mg (45) was 1.08 mg, just below their RNI. In this study, 23% of the males and 64% of the pre-
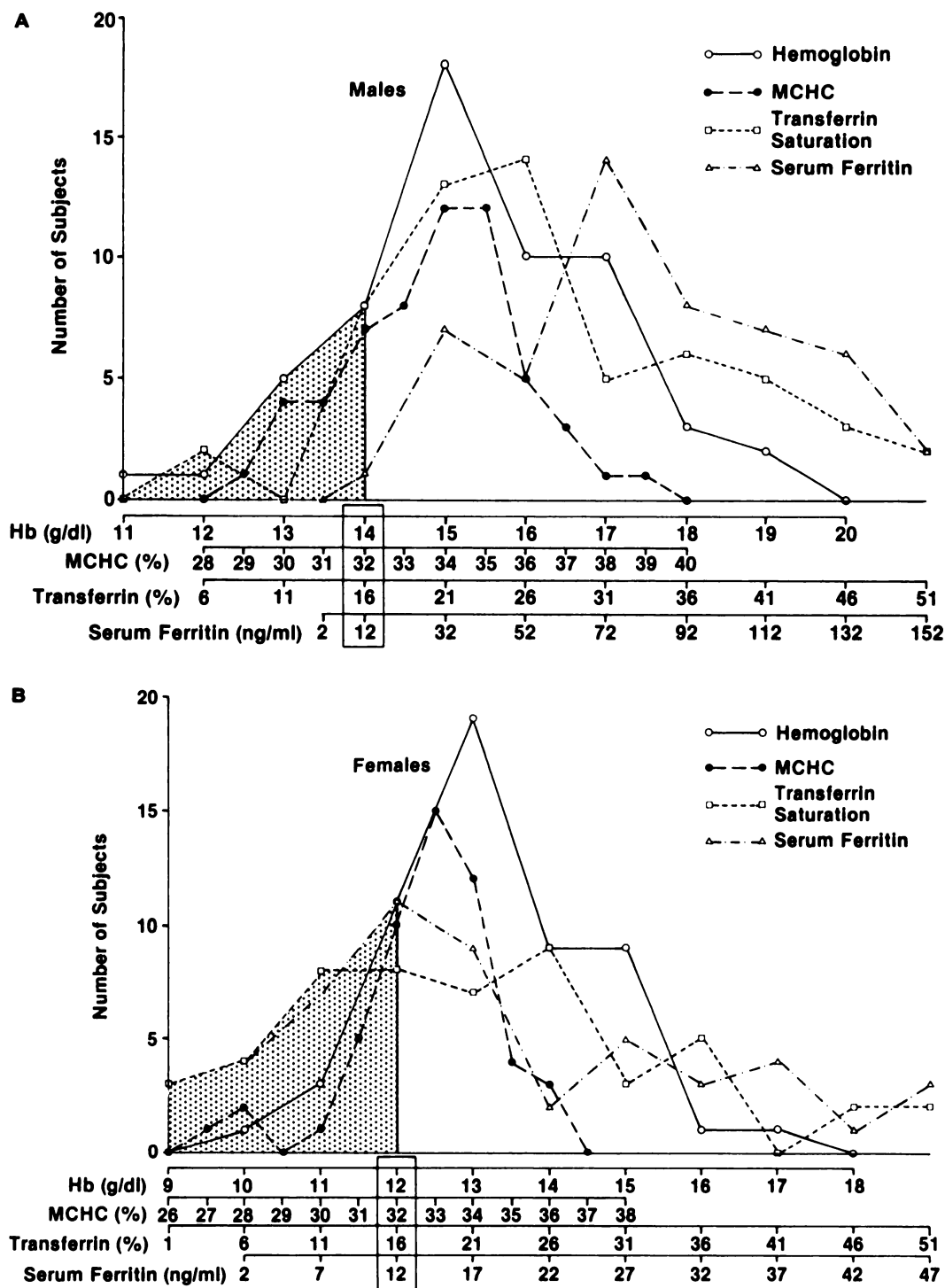
FIG 1. Frequency distribution of indices of iron status for male (*A*) and female (*B*) Punjabis. The shaded areas include values considered to represent iron deficiency.
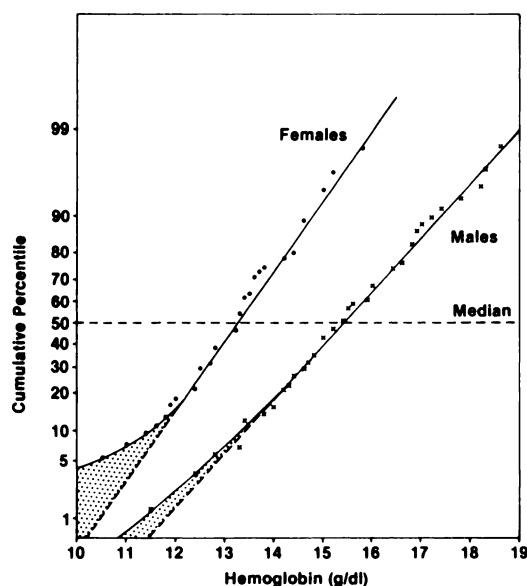
FIG 2. Probability plot of the cumulative frequency distributions of hemoglobin values for Punjabi adult males (×, $n = 58$) and females (●, $n = 50$), 18–64 yr old. Pregnant females are excluded ($n = 3$). Shaded areas represent estimated proportion of anemic individuals.

menopausal women had calculated available iron intakes below their estimated RNI.

Grain products contributed over 50% of the total dietary iron, whereas flesh foods provided only 10%, emphasizing the important contribution of grain products to the dietary-iron intakes of the Punjabis.

Discussion

Biochemical assessment

The results from our Tri-index model analysis (employing serum ferritin, serum-transferrin saturation, and MCHC in combination) confirm the advantages of using a minimum of two independent biochemical measures to identify iron deficiency (17, 51). For instance, we found that one or a combination of two or three abnormal values for any of our Tri-index model indices increased the sensitivity of detecting anemia (as measured by a low hemoglobin value) sequentially from 19%, to 42% and 75%, respectively. In contrast, when these biochemical-iron indices were used individually, the sensitivity was so poor that an excess of false-positive classifications could easily be made (Fig 1A, 1B). Hence, these results emphasize that iron deficiency cannot be correctly identified when only one biochemical test is used.

In general, the application of the Tri-index model, both with and without the inclusion of low hemoglobin, produced lower prevalence rates for anemia (with) and iron deficiency (without) than the more traditional approach utilizing the biochemical-iron indices individually (Table 3). The only exception was the use of serum ferritin alone to identify biochemical-iron deficiency among males. This result was not entirely unexpected because serum-ferritin values for men are rarely found to be below the 12 ng/ml cutoff point, mainly due to their larger reserve of body-iron stores (52).

The relative sensitivity of determining the prevalence of anemia using the Tri-index model in combination with low hemoglobin concentration was compared with that of a mixed-distribution analysis of hemoglobin values. It is noteworthy that the prevalence rates for iron-deficiency anemia in Punjabi

TABLE 3
Determination of the hemoglobin-percentile (Hb-%) shift for male and female Punjabi adults

	50th percentile Hb		Hb-% of original population represented by reference sample's 50th-% value	Hb-% shift (relative prevalence of anemia)†
	Original population	Reference sample*		
	g/dl			
Males (18–64 yr)‡	15.45 (n = 58)	15.5 (n = 39)	53%ile	3%
Females (18–64 yr)‡§	13.30 (n = 50)	13.45 (n = 24)	62%ile	12%

* Created after excluding individuals with either serum-transferrin saturation < 16% or MCHC < 32% from original population.
† The term, *relative prevalence*, indicates that values are merely estimates because $n < 100$ for both males and females. Hence standard errors or ranges are not given.
‡ Hb values were pooled across ages 18–64 because for both sexes they were found to be independent of age.
§ Pregnant females are excluded ($n = 3$).

TABLE 4
Mean daily energy, protein, dietary fiber, dietary iron, heme, nonheme iron, available iron, and iron-density intakes of Punjabi men and women

Dietary intake	Males n = 47 (Mean ± SD)	Females n = 44 (Mean ± SD)
Energy (kcal)	2381 ± 557	1711 ± 451
Protein (g)	96.2 ± 24.3	68.2 ± 20.8
Total dietary fiber (g)	29.3 ± 10.6	23.6 ± 8.1
Dietary iron (mg)	18.7 ± 4.6	14.4 ± 4.2
Heme iron (mg)	0.86 ± 0.71	0.40 ± 0.53
Nonheme iron (mg)	17.84 ± 4.42	14.01 ± 4.20
Ratio heme:nonheme iron	0.05 ± 0.04	0.03 ± 0.04
Iron density (mg/1000 kcal)	8.0 ± 1.6	8.5 ± 1.7
Available iron (mg)*	1.27 ± 0.40	1.08 ± 0.39
Meat, poultry, & fish (g)	114 ± 78	53 ± 53
Vitamin C (mg)	135.0 ± 92.8	120.6 ± 94.4

* Available iron calculated assuming body-iron stores of 500 mg for men and 250 mg for women.

men and women estimated by these two methods were comparable (Fig 3).

In contrast, the prevalence rate for iron deficiency for Punjabi females derived from the Tri-index model alone was higher (Fig 3). These differences are due to the large number of low serum-ferritin values in Punjabi women (n = 21). Serum ferritin is an index of early depletion of body-iron stores, so that slightly elevated prevalence values are to be expected

FIG 3. Prevalence of impaired iron status among Punjabi adults, estimated by the Tri-index. model, Tri-index model and low hemoglobin concentration combination, and the hemoglobin-percentile shift.

if a model includes this index. A similar trend was observed following data analysis of the NHANES II survey (21).

Prevalence rates for iron-deficiency anemia derived from the Tri-index model and low hemoglobin concentration combined and from the mixed-distribution analysis ranged from 12–16% for the Punjabi adult women (most of whom were menstruating) and 3–5% for Punjabi adult men (Fig 3). These levels were all higher than those observed (based on low hemoglobin values) for noninstitutionalized adult populations living in North America (34, 36–38) and Western Europe (53) but remarkably similar to rates observed for other East Indian populations living outside Canada (8).

It is unlikely that the presence of chronic disease and/or inflammatory disorders accounted for the high prevalence of anemia in these Punjabis. Eight of the eleven subjects classified as anemic in this study using the Tri-index model and low-hemoglobin classification had serum-ferritin values below 12 ng/ml. Low serum-ferritin values are not associated with inflammation; in the latter condition, serum-ferritin levels are elevated (23, 54). Also six subjects had TIBC values > 400 μg/dl, values indicative only of iron-deficiency anemia, not chronic disease (20, 55).

In large-scale surveys, the prevalence of anemia in a given population is usually defined in terms of the percentage of individuals with hemoglobin concentrations below the 2.5 percentile value of a 95% reference range (or confidence interval), created by examining all in-
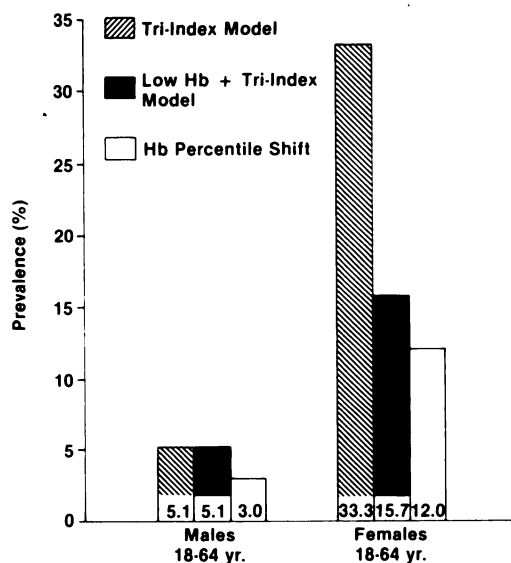
dividuals in the survey (38). Such a reference range cannot be reliably determined, however, in small-survey situations—like this one—in which the sample size is smaller than 200 (56). An alternative procedure is to use non–race-specific cutoff values for individual biochemical indices derived from larger national surveys. But this approach often results in excessive false-positive or false-negative classifications of anemia (39, 57, 58). Results presented in this study confirm that it is preferable to use a Tri-index model approach in combination with a low hemoglobin for assessing iron-deficiency anemia in small-survey studies.

Dietary assessment

Food-consumption patterns and energy and nutrient intakes of the Punjabi subjects were examined in relation to biochemical-iron indices. In this way, possible dietary factors associated with the etiology of iron-deficiency anemia in the study group could be identified.

It is noteworthy that, in this study, available iron intakes represented only 6.8% of the total iron intake for the males and 7.5% for the female Punjabis. These levels are below the upper limits of absorption for dietary iron assumed in mixed Canadian diets (ie, 20% for premenopausal women and 15–16% for men, ref 49) and slightly lower than the estimated average for absorption (10%) used by the US Food and Nutrition Board RDA for iron (50).

The relatively low intakes of available iron by the Punjabi subjects is due to their predominantly lacto-ovo vegetarian diet. For instance, grain products were the major source of dietary iron (as nonheme), a form less readily absorbed compared to the heme iron of meat, poultry, and fish. The latter was the primary source of iron for adults in the Nutrition Canada survey (59).

The major source of cereal grains in the Punjabi diet is unleavened chapatti bread made from whole-wheat flour with or without added bran, which is very high in phytic acid and dietary fiber (manuscript submitted). Both phytate (60–62) and dietary fiber (63–66) are known to depress the absorption of iron. Hence, it is not surprising that absorption of iron from whole-meal flour chapatti can be as low as 2.2 (67) to 4.5% (68).

Polyphenols, especially the nonhydrolyzable tannins (69) may also be potent inhibitors of iron absorption in Punjabi diets because condiments, spices (tamarind, chillies, turmeric, coriander), and tea (frequently used by Punjabis) are high in tannins (5, 69, 70). Consequently, the actual level of absorption of iron in the diets of the Punjabi subjects is probably lower than the proportion of total iron calculated as available in this study (ie, 6–8%) because Monsen's model (45, 46) does not take into account the inhibitory effects of phytate, dietary fiber, and tannins on iron absorption. Absorption of iron is probably nearer the level observed for other Indian diets (1–3%, ref 71).

In view of these dietary findings, it is not surprising that the Punjabi subjects of this study (especially the females) had a higher prevalence of iron deficiency compared to North Americans consuming mixed diets (34, 36–38). Indeed, the very high intakes of dietary fiber, tannins, and phytate and a possible competitive antagonism between phytate and calcium (72, 73) may counteract the effect of any physiological adaptation to a decreased level of iron absorption. Such an adaptive mechanism has been hypothesized to explain the absence of iron deficiency in long-term North American Caucasian vegetarians (74, 75).

In summary, the high prevalence of iron deficiency noted particularly among the female Punjabis of this study was attributed to inadequate intakes of readily available dietary iron from flesh foods concomitant with high intakes of calcium, dietary fiber, phytate, and tannins that inhibit absorption of dietary iron. Application of the Tri-index model approach to estimate the relative prevalence of iron deficiency in small surveys, rather than the more traditional use of individual biochemical indices, is recommended. ☒

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