# Association between dietary fiber intake and the folate status of a group of female adolescents<sup>1–3</sup>

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ABSTRACT The main objective of this study was to assess the association between dietary fiber intake and the folate status of Canadian female adolescents. We also assessed dietary folate intakes and evaluated the prevalence of biochemical folate deficiency in these subjects. Female adolescents aged 14-19 y (n =224) were recruited and fasting blood samples were collected. Dietary intakes (3-d food record) were recorded and participants were classified as lactoovovegetarians, semivegetarians, or omnivores on the basis of food-consumption patterns assessed with food-frequency questionnaires. Fourteen percent, 17%, and 26% of lactoovovegetarians, semivegetarians, and omnivores, respectively, had dietary folate intakes below their predicted requirements; 1%, 4%, and 23%, respectively, had serum folate concentrations indicative of deficiency. Despite low dietary folate intakes and serum folate concentrations, few subjects had homocysteine concentrations indicative of deficiency, suggesting that the degree of folate depletion had not yet produced functional consequences. Most important, results suggest that the consumption of nonstarch polysaccharide is significantly associated with serum folate concentrations (P < 0.001). For each 1-g increase in nonstarch polysaccharide intake, a 1.8% increase in serum folate concentration is expected. In summary, we propose that an increase in nonstarch polysaccharide intake may promote the intestinal biosynthesis of folate, providing a complementary strategy to enhance the folate nutriture of humans. Am J Clin Nutr 1997;66:1414-21.

**KEY WORDS** Folate status, fiber, dietary fiber intake, microbial biosynthesis of folate, Canada, female adolescents

# INTRODUCTION

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Consumption of  $\geq 400 \ \mu g$  folate/d during the periconceptional period has been recommended as a public-policy strategy by some governments to reduce the number of neural tube defect-affected pregnancies (1-4). Unfortunately, usual folate intakes of women typically fall well below this amount (3, 4) and even diets carefully designed according to national food guidelines are unlikely to provide 400  $\mu g$  folate/d unless they are devised meticulously to emphasize folate-rich foods (3-6). Several strategies are proposed to remedy this situation, including the recommendations that a folic acid supplement be consumed before conception and during the early months of pregnancy and that cereal-grain products be fortified with folic acid (7). We propose that dietary manipulations that increase the bacterial biosynthesis of folate in the large intestine may en-

hance the folate status of women. An early study by Denko et al (8) suggests that an average of 300  $\mu$ g folate/d is excreted in the feces of healthy adults. Rodriquez (9), in a comprehensive review of the literature on folate, suggested that the feces of human beings contain 5–15 times more folate than that ingested daily; however, until recently the metabolic fate of this pool of folate was unknown and had not been considered seriously. Data obtained by using a rat model (10) and organ-cultured endoscopic biopsy specimens from the human colon (11) provide evidence to suggest that bacteria-synthesized folate in the large intestine can, in fact, be absorbed across the large intestine and utilized by the host organism.

Fermentable substrates of dietary origin are important determinants of the number and type of bacteria in the human colon. Presumably, one of the most notable fermentable substrates, in quantitative terms, is dietary fiber (12). Hence, the stimulation of bacterial growth by the fermentation of increased quantities of dietary fiber may, in turn, enhance the net microbial biosynthesis of folate. Therefore, in this study we assessed the effect of dietary fiber, expressed as nonstarch polysaccharides (NSPs), on the folate status of young women consuming vegetarian or omnivorous diets. Second, because the soluble sources of dietary fiber are fermented more easily and are therefore more likely to stimulate bacterial growth (13, 14), we also investigated whether soluble fiber intake, specifically, was associated with folate status.

# SUBJECTS AND METHODS

# Subjects

Two hundred twenty-four female adolescents aged 14–19 y were recruited for the study via publicity in local newspapers, schools, and community groups. One-hundred subjects were

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recruited between May 1991 and October 1991. The remaining subjects were recruited from September 1994 to April 1995.

Subject eligibility was determined by a self-administered questionnaire. Because of the confounding effect of specific health circumstances and lifestyle practices on blood folate values, the following young women were ineligible to participate in the study: 1) women taking drugs known to interfere with folate metabolism (eg, phenytoin, sulfasalazine, phenobarbital, primidone, and cimetidine), and 2) pregnant women or women who had a pregnancy in the previous year lasting > 20 wk (15). Other exclusion criteria included cancer, gastrointestinal disease, history of renal insufficiency, diabetes mellitus, and other chronic diseases. Those taking oral contraceptives or folate supplements were not excluded.

The study protocol was approved by the University of Guelph Human Ethics Committee. Informed, written consent was obtained from each subject after the purpose, significance, and protocol of the study were explained fully. Written consent was also secured from a parent or legal guardian for those subjects aged < 16 y.

#### **Data collection**

Subjects attended an early morning clinic during which fasting peripheral blood samples were collected by venipuncture. A 1-mL sample of heparin-containing whole blood was used to measure the hemoglobin concentration and hematocrit. Whole blood not containing an anticoagulant was separated by centrifugation  $(620 \times g \text{ for } 20 \text{ min at 5 °C})$  to obtain serum for folate and homocysteine analyses. Sodium ascorbate (1%) was added to the samples designated for folate determination. Blood samples were then stored at -70 °C. A self-administered questionnaire was used to collect demographic data on each participant's age, source of income, education, medical and menstrual history, and cigarette, alcohol, nutrient, contraceptive, and supplement use.

## **Dietary** assessment

Dietary intakes of energy, protein, total fat, dietary fiber, folate, and select micronutrients were estimated by using weighed food records completed by each subject for 3 consecutive days, including 1 weekend day. Care was taken to ensure proportional distribution of week and weekend days among subjects. Each subject was trained to record accurately the types and quantities of all food and beverages consumed and was instructed to provide brand names and methods of food preparation. Participants were provided with an electronic digital (Soehnle; CMS Weighing Equipment Ltd, London) or pan (model 1440; Hanson Dietetic Scale, Shubuta, MI) scale to weigh edible portions of all food and beverages consumed. Energy and nutrient intakes were tabulated by using the Department of Family Studies Nutrient Intake System (16). The database was derived by using the most recent food-composition values from the condensed Canadian Nutrient File (17), US Department of Agriculture Handbook no. 8 (18), and other food-composition tables (19, 20). NSP values as analyzed by the method of Englyst et al (21) were used to estimate dietary fiber intake. Total soluble and insoluble NSP values were obtained from the fourth and fifth editions (20, 22-26) of McCance and Widdowson's The Composition of Foods and from other publications (27, 28).

Participants were classified into three categories on the basis of their food-consumption patterns as assessed with a food-frequency questionnaire: 1) 88 lactoovovegetarians—defined as individuals who consumed red meat, poultry, and fish less than once per month but included dairy or egg products or both in their diet; 2) 25 semivegetarians—defined as individuals who consumed red meat less than once a month, but included poultry, fish, or both in their diets more than once a month; and 3) 111 omnivores—defined as individuals who consumed a mixed diet.

## **Biochemical assessment**

Serum folate concentrations were measured with a singleincubation, <sup>125</sup>I competitive-binding radioassay (Quantaphase Folate Radioassay; Bio-Rad Laboratories, Mississauga, Canada). The accuracy and reproducibility of the radioassay methods were assessed through serial replication of control sera (Lymphochek; Bio-Rad Laboratories). The CV for this assay was 6.2%.

Total serum homocysteine concentrations were measured by HPLC according to the fluorometric method of Vester and Rasmussen (29). This method results in both the complete reduction of free oxidized homocysteine in the nonprotein fraction of serum (ie, homocystine and cysteine-homocysteine mixed disulfide) and the release of protein-bound homocysteine. Aliquots of serum (150  $\mu$ L) were treated with 20  $\mu$ L 10% (by vol) tri-*n*-butylphosphine in dimethylformamide. Samples were treated with 10% trichloroacetic acid (125  $\mu$ L) and centrifuged (13 000 × g for 10 min at 4 °C). The supernate was removed immediately (100  $\mu$ L) and derivatized with ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate. Samples were stored at 4 °C for  $\leq 2$  d.

Homocysteine was separated and quantified with a Waters Millipore system (Pump UK6, 600E Systems Controller, M470 scanning fluorescence detector; Waters, Milford, MA) equipped with an analytic column (Phenomenex Hypersil 3 C-18 column,  $250 \times 4.6$  mm; Phenomenex, Torrance, CA). Fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm. The detection signal was recorded and peak areas quantified with a Millipore Millennium 2010 Chromatograph Manager Integrator (Waters). Addition of mercaptoproprionylglycine as an internal standard served two purposes: as a control of the derivatization procedure and as a direct standardization of the size of the homocysteine peak. The CV for this assay was 4.6%.

#### Statistical analysis

All data were analyzed by using the general linear model of the Statistical Analysis System (30) and all tests were conducted at the 5% level of significance. Differences in mean energy, fiber, and nutrient intakes among the three dietary groups (lactoovovegetarians, semivegetarians, and omnivores) were assessed by two-way analysis of variance. Factors in each model included dietary group and time of data collection (1991 or 1994–1995). The least-significant-difference procedure was performed with adjusted dietary group means. Chi-square tests were used to ascertain whether differences existed in the proportion of lactoovovegetarians, semivegetarians, and omnivores with nutrient intakes less than two-thirds of the recommended nutrient intakes (RNIs; 31).

Analysis of covariance was used to determine whether there were any statistically significant relations between dietary fiber intake and the dependent variables serum folate and serum homocysteine. Factors included in the analysis of the relation between NSP intake and serum folate were time of data collection (1991 or 1994-1995) and dietary group (diet). Covariates in the model included supplemental folic acid intake and chronologic age. Analysis of covariance did not show any effect of dietary folate intake, cigarette smoking, or alcohol or oral contraceptive use on serum folate concentrations (P >(0.15); therefore, these factors were not included in the final model. Examination of the relation between NSP intake and serum homocysteine included alcohol consumption as a factor and dietary folate intake (including supplemental folic acid) as a covariate in the model. The least-significant-difference procedure was performed by using adjusted means to assess differences in serum folate and serum homocysteine concentrations among the three dietary groups.

Goodness of fit and normality assumptions were checked with residual plots. When departures from normality were detected, the dependent variable was transformed appropriately. All values in the text are reported as medians (first and third quartiles).

## RESULTS

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## **Participant characteristics**

Most subjects in this survey were white (89%), 6% were Asian, and 5% were black. Approximately 95% of subjects attended either high school or a university or college. Parents of the subjects were, on average, well educated and the sample as a whole represented a middle-class income group, although both lower- and upper-income groups were represented. Subjects ranged in age from 14 to 20 y. Ninety-nine percent of the subjects had a gynecologic age of  $\geq 2$  y, suggesting that linear growth had either slowed or ceased for most of the subjects (32). Fifteen percent of subjects smoked cigarettes and 21% were taking oral contraceptives. Self-reported alcohol consumption showed that 60% of subjects consumed alcohol. Of the subjects consuming alcohol, 48% and 32% consumed alcohol one to two times and three to four times per month, respectively. Forty-four percent of subjects consumed a vitamin or mineral supplement or both at some time during the 6 mo before the clinic visit, and 23% were consuming a folic acid-containing supplement at the time of the clinic visit. Average folic acid consumption (from supplements alone) was 231  $\mu$ g/d among supplement users.

## **Dietary intakes**

The reported median (first and third quartiles) dietary intakes of energy, macronutrients, and fiber (per day and per megajoule) for the lactoovovegetarians, semivegetarians, and omnivores are presented in Table 1. Energy and protein intakes (total per day) were similar among all dietary groups; however, the median protein intake expressed per megajoule among lactoovovegetarians was significantly lower than that of the omnivores and semivegetarians (P < 0.05). Although the fat intake (per day) of the omnivores did not differ significantly from that of either vegetarian group, lactoovovegetarians had significantly higher fat intakes (per day) than semivegetarians. When expressed per megajoule, fat intakes of the lactoovovegetarians and omnivores were significantly higher than those of the semivegetarians (P < 0.05). NSP intakes (per day and per megajoule) of lactoovovegetarians and semivegetarians were significantly higher than those of the omnivores (P < 0.05).

The percentage contribution of fat, protein, and carbohydrate to total energy intake differed among lactoovovegetarians, semivegetarians, and omnivores (**Table 2**). The lactoovovegetarians and omnivores consumed a significantly greater percentage of energy in the form of fat than did semivegetarians

## TABLE 1

Median and first and third quartiles of energy, macronutrient, and dietary fiber intakes for a group of female adolescents consuming a lactoovovegetarian, semivegetarian, or omnivorous diet'

	Per day			Per megajoule		
Nutrient	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	Omnivorous (n = 111)	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	$\begin{array}{l} \text{Omnivorous} \\ (n = 111) \end{array}$
Energy (kJ)						
Median	7489	7577	7077	_		
First quartile	6153	6061	6019	_		_
Third quartile	9038	9421	8702	_	_	
Protein (g)						
Median	52.8	66.6	57.3	7.0 <sup>a</sup>	7.5 <sup>b</sup>	7.9 <sup>6</sup>
First quartile	41.7	40.4	47.2	6.2	6.7	6.7
Third quartile	60.3	77.3	71.0	7.9	9.2	9.2
Fat (g)						
Median	60.8 <sup>b</sup>	50.2 <sup>a</sup>	57.7 <sup>a.b</sup>	8.3 <sup>b</sup>	7.0 <sup>a</sup>	8.4 <sup>b</sup>
First quartile	49.0	37.4	43.8	7.6	5.4	6.8
Third quartile	77.4	68.6	77.4	9.7	8.7	9.5
Dietary fiber (g) <sup>2</sup>						
Median	14.6 <sup>b</sup>	16.1 <sup>b</sup>	10.0 <sup>a</sup>	1.8 <sup>b</sup>	2.0 <sup>b</sup>	1.3 <sup>a</sup>
First quartile	9.9	9.0	7.1	1.4	1.6	1.0
Third quartile	18.2	22.3	12.7	2.5	2.4	1.7

<sup>1</sup> Values in the same row with different superscript letters are significantly different, P < 0.05.

<sup>2</sup> Nonstarch polysaccharide values were used to determine dietary fiber intake.

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Macronutrient	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	Omnivorous $(n = 111)$
Fat			
$\bar{x} \pm SD$	$31.8 \pm 6.1^{b}$	$26.8 \pm 8.8^{a}$	$31.0 \pm 6.5^{b}$
Median	31.5	26.5	31.8
Range	9–45	12-42	15-46
Protein			
$\bar{x} \pm SD$	$11.9 \pm 2.4^{a}$	$13.4 \pm 3.2^{b}$	$13.6 \pm 3.2^{b}$
Median	11.8	12.6	13.3
Range	6-23	7-21	7–24
Carbohydrate			
$\bar{x} \pm SD$	$58.5 \pm 7.4^{b}$	$61.7 \pm 9.8^{\circ}$	$55.9 \pm 8.0^{a}$
Median	58.1	63.7	56
Range	44-86	37-82	33–77

<sup>1</sup> Values in the same row with different superscript letters are significantly different, P < 0.05.

(P < 0.05). The semivegetarians and omnivores consumed a significantly higher percentage of energy from protein than did lactoovovegetarians, whereas all three dietary groups differed with respect to the amount of energy consumed in the form of carbohydrate (P < 0.05). Despite differences among dietary groups, intakes of fat and protein as a percentage of total energy were close to the recommended amounts in Canada (30% and 10–15%, respectively) (31).

Median micronutrient intakes (per day) and micronutrient density of the diets (per megajoule) were comparable among the three dietary groups, except for folate, vitamin C, and riboflavin (**Table 3**). Both lactoovovegetarians and semivegetarians had higher intakes of folate (per day and per megajoule) than omnivores (P < 0.05). Although semivegetarians did not differ from lactoovovegetarians and omnivores with respect to vitamin C intakes, lactoovovegetarians had higher intakes of vitamin C (per day and per megajoule) than omnivores (P < 0.05). When expressed as a ratio of riboflavin to energy, lactoovovegetarians had lower dietary intakes than omnivores (P < 0.05); however, no difference in total riboflavin intakes per day was found among the three dietary groups.

The proportion of individuals (as a percentage) with intakes less than two-thirds of the Canadian RNI (31) and the percentage of adolescents with dietary intakes likely less than their individual requirement are summarized in Table 4. Anderson et al's (33) probability approach was used to predict the percentage of subjects with dietary intakes below their own individual requirement. No significant differences were found among dietary groups with respect to the proportion of subjects with nutrient intakes below two-thirds of the RNI. A comparison of each subject's dietary folate intake with the RNI found that 7%, 12%, and 16% of lactoovovegetarians, semivegetarians, and omnivores, respectively, consumed less than twothirds of the RNI for folate. Use of the probability approach showed that 14% of lactoovovegetarians, 17% of semivegetarians, and 26% of omnivores had dietary folate intakes below their own individual requirements.

#### Folate status

The biochemical indexes of folate status for lactoovovegetarians, semivegetarians, and omnivores are summarized in Figure 1. Lactoovovegetarians had higher ( $\approx$ 52%) serum folate concentrations than omnivores; however, the serum folate concentrations of semivegetarians did not differ from those of lactoovovegetarians or omnivores. Serum homocysteine concentrations were similar among lactoovovegetarians, semivegetarians, and omnivores.

One percent, 4%, and 23% of the lactoovovegetarians, semivegetarians, and omnivores, respectively, had serum folate concentrations < 6.8 nmol/L, a concentration indicative of folate deficiency (34). Thirty-nine percent, 48%, and 53% of lactoovovegetarians, semivegetarians, and omnivores had serum folate concentrations indicative of marginal folate status (6.8–13 nmol/L). Omnivores had a higher prevalence of marginal and deficient folate status than lactoovovegetarians and semivegetarians when serum folate concentration was used as an index of folate nutriture (P < 0.001).

## **Regression analysis**

A significant relation was found between dietary fiber intakes of subjects and their serum folate concentrations (P < 0.001, ANCOVA; **Table 5**). Additional significant factors and covariates included in this model were time, diet, supplemental folic acid intake, and chronologic age. Because the fermentability of fiber by intestinal bacteria is somewhat dependent on its solubility, data were analyzed by using soluble NSP as the independent variable. Results of the analyses showed that the influence of soluble NSP on serum folate concentrations was nearly significant (P = 0.0512). No relation was found between NSP intakes and serum homocysteine concentrations.

# DISCUSSION

#### Relation between dietary fiber intake and folate status

Results suggest that NSP intakes were positively associated with serum folate concentrations (P < 0.001). This relation remained significant even after the following covariates were included in the model: time of data collection (1991 compared with 1994–1995), diet (semivegetarian, lactoovovegetarian, or omnivorous), supplemental folic acid intake, and chronologic age. After confounding variables were controlled for, data herein provide evidence to suggest that each 1-g increase in NSP intake is associated with a 1.8% increase in serum folate concentration.

We propose that an increase in NSP intake will promote bacterial synthesis of folate in the large intestine. Unlike their host, certain microorganisms in the large intestine can synthesize folate by condensing 6-hydroxymethyl-7,8-dihydropterin pyrophosphate, first with *p*-aminobenzoic acid (PABA) and then glutamic acid to form dihydropteroate and dihydrofolic acid, respectively (35). As reviewed recently by Gibson and Roberfoid (36), there is bountiful evidence to suggest that certain food components can significantly affect the quantity and type of microorganisms in the large intestine. Indeed, it has been shown that an increase in dietary fiber can lead to an overall increase in the number of bacteria in the large intestine (14, 37). However, it is not clear whether this increase is due to a consistent rise in all types of bacteria or is a result of the selective stimulation of certain bacterial species.

Like total NSP intakes, soluble NSP intakes tended to be positively associated with serum folate concentrations (P =

#### TABLE 3

Median and first and third quartiles of micronutrient intakes for a group of female adolescents consuming a lactoovovegetarian, semivegetarian, or omnivorous diet'

		Per day		Per megajoule		
Nutrient	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	Omnivorous (n = 111)	Lactoovovegetarian (n = 88)	Semivegetarian $(n = 25)$	Omnivorous $(n = 111)$
Folate $(\mu g)^2$			****			
Median	228.3ª	236.4 <sup>a</sup>	163.7 <sup>b</sup>	29.5 <sup>a</sup>	31.5 <sup>a</sup>	22.5 <sup>b</sup>
First quartile	165.2	153.9	130.9	23.7	20.6	18.1
Third quartile	303.7	360.6	217.5	41.2	35.5	29.6
Iron (mg)						
Median	11.5	12.9	11.2	1.5	1.6	1.6
First quartile	8.8	8.3	9.3	1.3	1.2	1.2
Third quartile	14.0	15.8	14.1	1.8	1.9	1.9
Zinc (mg)						
Median	7.0	7.9	7.0	0.9	1.0	1.0
First quartile	5.5	5.5	5.8	0.8	0.8	0.8
Third quartile	8.3	9.6	8.9	1.1	1.2	1.2
Calcium (mg)						
Median	709.1	730.6	687.2	96.2	114.8	96.5
First quartile	488.0	574.7	559.3	75.2	77.7	73.0
Third quartile	922.2	1236.5	853.1	118.1	140.2	123
Vitamin A (RE)						
Median	648.1	763.7	595.9	87.3	86.4	85.6
First quartile	468.8	504.1	416.1	63.4	70.5	61.5
Third quartile	929.0	896.8	831.3	125.2	135.2	111.8
Vitamin C (mg)						
Median	122.3"	134.7 <sup><i>a</i>,<i>b</i></sup>	103.9 <sup>b</sup>	17.2 <sup>a</sup>	19.2 <sup><i>a</i>,<i>b</i></sup>	14.1 <sup>b</sup>
First quartile	70.2	63.0	65.9	9.9	8.5	8.9
Third quartile	211.3	200.6	156.5	26.8	24.9	21.8
Thiamine (mg)						
Median	1.3	1.6	1.3	0.17	0.17	0.17
First quartile	1.0	1.0	1.0	0.14	0.15	0.13
Third quartile	1.6	1.8	1.6	0.21	0.23	0.21
Riboflavin (mg)						
Median	1.3	1.6	1.4	0.17 <sup>a</sup>	0.19 <sup>a,b</sup>	0.2 <sup>b</sup>
First quartile	1.0	1.1	1.1	0.15	0.16	0.16
Third quartile	1.7	2.0	1.7	0.22	0.24	0.23

<sup>1</sup> Values in the same row with different superscript letters are significantly different, P < 0.05.

<sup>2</sup> Dietary intakes exclude the amount consumed from vitamin, mineral, or vitamin-mineral supplements.

0.0512). In contrast, insoluble fiber intakes were unrelated to serum folate concentrations. These observations are consistent with the view that soluble sources of dietary fiber, like fruit and vegetables, and gel-forming polysaccharides, are those that are fermented, and therefore likely to stimulate bacterial growth (13, 14). Insoluble fiber, eg, cellulose, wheat bran, and other cereal grains, is fermented much less by the intestinal microflora and results in little bacterial growth (13). However, insoluble fiber tends to speed up intestinal transit time (38), which influences the profile of bacterial species, overall bacterial growth, and the pH in the colonic lumen. These effects, along with the presence of soluble fiber, would result in the optimum conditions for folate synthesis and absorption and serve to explain the stronger relation with total NSP than with soluble NSP alone.

Although there is a great deal of evidence suggesting that net microbial biosynthesis of folate is related to diet (39–45), less is known about the effect of fiber intake on folate biosynthesis in the large intestine. Using a rat model, Keagy and Oace (46) examined the effect of wheat bran and xylan on the microbial

synthesis of folate in the intestine. Results from the study showed that xylan stimulated bacterial folate synthesis with subsequent utilization by the host as shown by both increased fecal and liver folate concentrations. In contrast, wheat bran did not increase tissue folate concentrations, probably because it is resistant to fermentation. Denko et al (8) compared urinary and fecal excretions of folate with the daily dietary folate intakes of healthy adults. Results revealed an approximately threefold increase in fecal folate compared with dietary intake. Note that this determination of fecal folate is most likely an underestimate.

Since the time of these analyses, new methods have been developed to extract folate from biological samples, and most laboratories now add ascorbate to samples to protect labile folate during storage. In addition, there have been improved procedures introduced to analyze more appropriately all available folate in the sample (47). Thus, it is apparent that healthy human adults excrete far more folate in their feces than is ingested in their diet, due presumably to the intestinal microbial synthesis of the vitamin.

Nutrient	<2/3 of RNI <sup>7</sup>			Probability analysis		
	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	Omnivorous (n = 111)	Lactoovovegetarian $(n = 88)$	Semivegetarian $(n = 25)$	Omnivorous $(n = 111)$
Energy	23	24	23			
Protein	6	8	5	11	12	8
Folate	7	12	16	14	17	26
Iron	22	28	17	32	34	33
Zinc	31	36	29	49	40	46
Calcium	20	16	20	31	25	27
Vitamin A <sup>2</sup>	34	32	41	_		
Vitamin C	2	12	5	3	12	7
Thiamine	0	0	0	5	8	7
Riboflavin	0	8	0	16	10	8

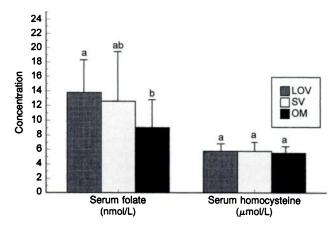
Percentage of lactoovovegetarian, semivegetarian, and omnivorous female adolescents with inadequate intakes of energy, folate, and selected nutrients determined as less than two-thirds of Canadian recommended nutrient intakes (RNIs) and by probability analysis

<sup>1</sup> Chi-square analyses were used to ascertain whether differences existed in the proportion of lactoovovegetarian, semivegetarian, and omnivorous subjects with nutrient intakes from dietary sources alone was < 2/3 of the RNI. No significant differences were found.

<sup>2</sup> Probability analyses were not applied to vitamin A intakes because of the absence of reliable estimates of mean requirements and variability and uncertainty concerning food-composition values for provitamin A carotenoids (33).

# Folate status

Our dietary results suggest that a significant proportion of adolescent girls in our sample had folate intakes below current recommendations. When the probability approach was used, 14%, 17%, and 26% of lactoovovegetarians, semivegetarians, and omnivores, respectively, had folate intakes below their own individual requirement on the basis of the recommendation of 3.1  $\mu$ g/kg body wt (31). This recommendation predates current advice that consumption of  $\geq 400 \mu$ g folate/d (approximately twice the amount of folate) during the periconceptional period will reduce the risk of a neural tube defect-affected pregnancy (2-4). These results came as a surprise to us given the generally health-conscious attitude of the girls recruited, their relatively high socioeconomic status, and specifically the high education level of their parents. However, the low folate intake data herein are consistent with other recent intake data



**FIGURE 1.** Biochemical and functional indexes of folate status for lactoovovegetarian (LOV), semivegetarian (SV), and omnivorous (OM) female adolescents. Each bar represents the median concentration and the top of the vertical line indicates the third quartile for that group of subjects. Values with different superscript letters are significantly different, P < 0.05.

on young Canadian women collected by using the 24-h recall method (5, 48).

Note that the folate values available in even the most carefully devised food-composition tables are not perfect. As a result, it is likely that the dietary folate intakes described herein, as in other published reports, are an underestimate of true folate intake. Nonetheless, the estimates of folate intake in this study are a reasonable indicator of "relative" folate intake. In examining the relation between dietary fiber intakes and serum folate concentrations, it is critical that the composition values for foods typically consumed by subjects consuming high-fiber diets are not over- or underestimating true folate intake in a fashion systematically disproportionate from that of subjects consuming low-fiber diets. To assure ourselves that this was not the case, we examined the relation between folate intakes and serum folate concentrations within each diet group and the Pearson correlation coefficients were similar for both omnivores (r = 0.53, P < 0.0001) and vegetarians (r = 0.46, P < 0.0001). Likewise, the correlation between folate intakes and serum homocysteine concentrations were similar for both omnivores (r = 0.18, P = 0.07) and vegetarians (r = 0.28, P =

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Analysis of covariance for the dependent variable serum folate'

Source	Parameter estimate	SE	Percentage change
			%
Intercept	1.539	0.392	
Nonstarch polysaccharide	0.018	0.005	1.8
Age	0.038	0.021	3.9
Folate supplement	0.001	0.0002	0.13
Type of diet			
Omnivorous	-0.133	0.097	-14.2
Lactoovovegetarian	0.095	0.092	9.96
Semivegetarian	0	0	0
Time			
1991	-0.096	0.065	-10.04
1994-1995	0	0	0

 $r^{2} = 0.38.$ 

0.007). Therefore, if the folate intakes presented herein are an underestimate of true intake, it seems likely that both the omnivorous and vegetarian groups were affected equally.

The median (first and third quartiles) serum folate concentrations of lactoovovegetarians, semivegetarians, and omnivores were 13.8, 12.6, and 9.1 nmol/L, respectively. Further, 1%, 4%, and 23% of lactoovovegetarians, semivegetarians, and omnivores, respectively, had serum folate concentrations indicative of deficiency, indicating a greater probability of inadequate folate intakes in omnivores than in lactoovovegetarians and semivegetarians. In contrast with serum folate concentrations, few subjects had homocysteine concentrations indicative of folate depletion and no significant differences were found in mean homocysteine concentrations among lactoovovegetarians, semivegetarians, and omnivores. Thus, despite low dietary folate intakes and serum folate concentrations, our data suggest that the degree of folate depletion has not yet produced functional consequences, as determined by serum homocysteine concentrations.

## Summary

Results from this study suggest that an increase in dietary fiber intake is associated with an increase in serum folate concentration. We propose that this is due, at least in part, to changes in the intestinal environment that promote a net increase in microbial biosynthesis of folate. To clearly establish that an increase in dietary fiber causes an increase in blood folate concentrations (effect), a randomized controlled trial with various fiber intakes is necessary. Second, many adolescent subjects, regardless of whether or not they were vegetarian, had inadequate intakes of dietary folate. It is possible that there was a general underreporting of folate intake by the adolescents and that the quality of current food-composition tables further yielded low folate intakes; however, the serum folate concentrations reported herein tend to corroborate our findings about dietary folate. Given the relatively high socioeconomic status and education level of these adolescents and their families, we suggest that inadequate intake of folate in adolescents is widespread. ÷

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