Risk of persistent cobalamin deficiency in adolescents fed a macrobiotic diet in early life[1–3]

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ABSTRACT

Background: Cobalamin deficiency has been described in children consuming macrobiotic diets.

Objective: We investigated whether moderate consumption of animal products is sufficient for achieving normal cobalamin function in 73 adolescents who had received a macrobiotic diet until 6 y of age and had then switched to a lactovegetarian, lactoovovegetarian, or omnivorous diet (macrobiotic adolescents).

Design: Hematologic indexes and serum concentrations of methylmalonic acid (MMA), total homocysteine (tHcy), and folate were measured. Current consumption frequency of animal products and cobalamin intake from dairy products were assessed by questionnaire. Data from 94 age-matched adolescents who received an omnivorous diet from birth were used as a reference.

Results: Serum cobalamin concentrations were significantly lower and concentrations of MMA and folate and mean corpuscular volume (MCV) were significantly higher in macrobiotic adolescents than in control adolescents: of macrobiotic adolescents, 21% had abnormal MMA concentrations (>0.41 μmol/L), 37% had abnormal cobalamin concentrations (<218 pmol/L), 10% had abnormal tHcy concentrations (>12.8 μmol/L), and 15% had abnormal MCV (>89 fL). In macrobiotic adolescents, dairy products (200 g milk or yogurt and 22 g cheese/d) supplied on average 0.95 μg cobalamin/d; additionally, these adolescents consumed fish, meat, or chicken 2–3 times/wk. In girls, meat consumption contributed more to cobalamin status than the consumption of dairy products, whereas in boys these food groups were equally important.

Conclusions: A substantial number of the formerly strict macrobiotic adolescents still had impaired cobalamin function. Thus, moderate consumption of animal products is not sufficient for restoring normal cobalamin status in subjects with inadequate cobalamin intake during the early years of life.   *Am J Clin Nutr* 1999;69:664–71.

KEY WORDS   Vegetarian diet, cobalamin deficiency, adolescents, methylmalonic acid, homocysteine, macrobiotic diet, mean corpuscular volume

INTRODUCTION

The main source of dietary cobalamin (vitamin B-12) for humans is food of animal origin. Therefore, nutritional cobalamin deficiency can develop easily in strict vegetarians (1). Neuro-logic disorders in cobalamin-deficient infants born to vegetarian mothers have attracted attention as a severe health risk (2, 3). The occurrence of megaloblastic anemia has long been an important index in the diagnosis of cobalamin deficiency. However, early cobalamin deficiency may lead to neural damage that results in cognitive dysfunction even before anemia develops. Therefore, new functional tests such as measurements of methylmalonic acid (MMA) and total homocysteine (tHcy) have been developed to discriminate persons with abnormal cobalamin function from those with normal function (4).

A macrobiotic diet is similar to a vegan-like food pattern and consists of whole-grain cereals (mainly unpolished rice), vegetables, and pulses with small additions of seaweeds, fermented foods, nuts, seeds, and seasonal fruit (5). Fish is consumed occasionally whereas meat and dairy products are usually avoided. In 1985 we started a study of the growth and nutritional status of children (n = 243) fed a macrobiotic diet in early childhood (0–8 y) (6). We have revisited these children or subgroups of them several times (in 1987, 1993, and 1995). Furthermore, from 1985 to 1986, a mixed-longitudinal cohort study was performed in 53 infants fed macrobiotic diets and 57 group-matched control infants. Very low cobalamin intakes (7) and low plasma cobalamin concentrations combined with several hematologic abnormalities (8) were observed in the macrobiotic infants. In 41 of these macrobiotic infants, both MMA and tHcy were markedly elevated compared with values in control infants (8-fold and 2-fold, respectively) and both metabolites showed an inverse relation to the plasma cobalamin concentration (9). These findings showed a functional cobalamin deficiency in these subjects. On the basis of these and other

findings of the mixed-longitudinal study, dietary recommendations were given to all macrobiotic families, such as to increase the intake of fat and fatty fish and to add dairy products to the diet. As a consequence, food consumption of the children in the macrobiotic population has changed since 1987. In 1993 a follow-up study in 209 children aged 7–16 y showed an increased intake of animal products and linear catch-up growth (10).

In the present study, data on hematologic indexes, concentrations of cobalamin and metabolites, and intake of animal products are presented; these data were obtained during a study carried out in 1995 of bone density in adolescents from the macrobiotic population (11). These data are compared with data from an age-matched control group. Our objective was to investigate whether a moderate consumption of animal products is sufficient for achieving and maintaining normal cobalamin function in adolescents who had received a macrobiotic diet in early life.

SUBJECTS AND METHODS

Subjects

Between May and July 1995, 93 Dutch adolescents (50 boys and 43 girls) aged 9–15 y who had been fed a macrobiotic diet in early life participated in a study of bone density (11). For practical reasons, we refer to this group as the macrobiotic adolescents, although they consumed a lactovegetarian, lactoovovegetarian, or omnivorous diet at the time of the present study. As described by Parsons et al (11), the macrobiotic adolescents (43 girls and 50 boys) were recruited from an existing group of macrobiotic families affiliated with the Division of Human Nutrition and Epidemiology, Wageningen Agricultural University. Almost all macrobiotic subjects had participated in previous studies of macrobiotic diet and growth in 1985–1987 (6, 12) and in 1993 (10), but not in the mixed-longitudinal study (7–9). A control group of 42 boys and 60 girls of similar ages was recruited from local schools. The control adolescents had received an omnivorous diet since birth.

At the time of the study, 21 adolescents refused to give blood and for another 7 adolescents (1 macrobiotic and 6 control adolescents) the amount of serum collected was insufficient for biochemical analyses of cobalamin status. Thus, complete data for 167 adolescents (73 macrobiotic and 94 control adolescents) were available for statistical analyses. All subjects were of the same ethnic group (white), were in good health, and were not taking drugs that might affect their hematologic status. Socioeconomic status was determined by using Attwood scores, a 5-point scale based on the occupation of and highest level of education attained by both parents. One parent, usually the mother, and the child were also questioned about the duration of the macrobiotic diet. The study was approved by the Ethics Committee of Wageningen Agricultural University and all subjects and a parent gave written, informed consent.

Anthropometry

Subjects were weighed (in underwear) to the nearest 0.1 kg on a digital scale (ED-60T; Berkel, Rotterdam, Netherlands). Standing height was measured to the nearest 0.1 cm with a microtoise.

Dietary assessment

Current consumption of animal products was checked by using a list of 6 food groups (eggs, dairy products, fish, meat,

chicken, and game). Consumption frequency during the past month was indicated by the child, sometimes assisted by a parent (usually the mother), on a 6-point scale: never, seldom, 1–3 times/mo, 1–2 times/wk, 3–5 times/wk, and daily. It was not possible to estimate total cobalamin intake from the diet on the basis of this information. However, from the study on bone density (11) we had detailed information on the consumption of dairy products. All subjects had filled out a validated food-frequency questionnaire for estimation of current calcium intake (13). The questionnaire reference period was the past month and food intake was measured in terms of standardized household portion sizes. (In the questionnaire we did not ask subjects to specify between the use of pasteurized and ultrahigh-temperature-treated milk because ultrahigh-temperature-treated milk is rarely consumed in the Netherlands.) The intake of cobalamin from dairy products was then estimated as follows. Intakes of cheese, pasteurized milk (including chocolate milk), buttermilk, and yogurt (including yogurt drinks, fruit yogurt, custard, and curd cheese in g/d) were calculated. Subsequently, the intake of cobalamin from these 4 product groups was calculated for each macrobiotic and control subject, taking into account the frequency of consumption of a certain product within each product group. The calculated cobalamin contents (μg/g) of these product groups were 0.0035 for milk, 0.02 for cheese, 0.0021 for yogurt, and 0.001 for buttermilk (14). Last, the average intake of cobalamin from these 4 product groups was summed.

Biochemical measurements

Nonfasting blood specimens were obtained [90% within 1 mo (mid-May to mid-June) and the remainder during the subsequent month]. Samples were allowed to clot and were then centrifuged (1190 × g for 10 min at 4°C). Serum was separated from blood after 60 min and serum samples were stored at −80°C until analyzed. Hemoglobin, hematocrit, and red cell indexes were analyzed (Coulter Counter T-860; Coulter Electronics Ltd, Bedfordshire, United Kingdom). Serum concentrations of cobalamin and folate were measured by using a microparticle-based enzyme immunoassay (IMx system; Abbott Laboratories, North Chicago, IL). The intraassay CVs of the folate assay varied between 3% and 6% and the interassay CVs varied between 6% and 10% depending on the folate concentration. For cobalamin, both the intra- and interassay CVs were <5%. MMA was measured by capillary electrophoresis, with a between-day CV of 12% at low physiologic concentrations (15). Concentrations of tHcy were assayed by a method based on HPLC and fluorescence detection, with a between-day CV <4% (16).

Statistical analysis

Because of skewed distributions, data for serum folate, cobalamin, MMA, and tHcy were log transformed before calculations were made. Means and SDs or geometric means (GMs) and GM ± 1.96 SD intervals were calculated from the log-normal distribution for all blood indexes. Group means or GMs were compared by two-sample Student's t tests. Because reference values of MMA, mean corpuscular volume (MCV), folate, and tHcy have not yet been established for adolescents, we used as cutoff points the 95th percentiles for serum MMA, serum tHcy, and MCV and the 5th percentile for serum cobalamin, serum folate, and hemoglobin in the control population. In general, the correlation between indexes was determined by using Pearson correlation coefficients, but Spearman correlation coefficients

TABLE 1
Characteristics of macrobiotic and control adolescents participating in a study of cobalamin status[1]

| | Boys | | Girls | | |
	Macrobiotic (n = 37)	Control (n = 39)	Macrobiotic (n = 36)	Control (n = 55)	P[2]
Age (y)	12.7 ± 1.9	11.7 ± 1.5	11.7 ± 1.6	11.7 ± 1.7	—
Height (m)	1.57 ± 0.1	1.53 ± 0.1	1.50 ± 0.1	1.52 ± 0.1	—
Weight (kg)	42.2 ± 10.5	41.2 ± 9.2	38.4 ± 9.2	41.3 ± 8.6	—
Socioeconomic status[3]	1.8 ± 0.5	2.0 ± 0.8	2.0 ± 0.7	2.0 ± 0.7	—
Consumption frequency per week					
Dairy products	4.4 ± 2.5	6.8 ± 0.4	4.5 ± 2.4	6.7 ± 0.9	<0.0001
Meat	1.3 ± 1.9	5.8 ± 2.0	1.2 ± 1.9	5.7 ± 1.8	<0.0001
Fish	1.0 ± 1.0	0.4 ± 0.3	1.2 ± 1.1	0.4 ± 0.4	<0.001
Chicken	0.3 ± 0.5	0.9 ± 0.5	0.4 ± 0.6	0.8 ± 0.5	<0.001
Egg	1.4 ± 1.2	1.2 ± 0.7	1.3 ± 0.8	1.8 ± 1.5	<0.05
Average cobalamin intake from dairy products (μg/d)[4]					
Pasteurized milk	0.27 ± 0.39	1.31 ± 0.85	0.11 ± 0.21	1.35 ± 0.98	<0.0001
Cheese	0.53 ± 0.61	0.46 ± 0.31	0.43 ± 0.38	0.50 ± 0.30	—
Buttermilk	0.02 ± 0.05	0.04 ± 0.16	0.02 ± 0.06	0.06 ± 0.14	<0.05
Yogurt	0.32 ± 0.32	0.48 ± 0.40	0.19 ± 0.20	0.43 ± 0.34	<0.0001
All dairy products combined	1.13 ± 0.99	2.30 ± 0.90	0.76 ± 0.69	2.34 ± 0.92	<0.0001

[1] $\bar{x}$ ± SD of raw data. Control subjects had followed an omnivorous diet since birth and macrobiotic subjects had followed a macrobiotic diet in early life and had subsequently adopted a lactovegetarian, lactoovovegetarian, or omnivorous diet.

[2] Means of the macrobiotic and control groups (boys and girls combined) were compared by using Student's t test.

[3] Calculated by using Attwood scores (see Methods).

[4] See Methods for a description of the calculation of cobalamin intake from dairy products by using data from a calcium food-frequency questionnaire (13).

were calculated if variables were not normally distributed (eg, for the consumption frequency of products and cobalamin intake from dairy products). Multiple regression analysis was used to investigate the effects of consumption frequencies of animal products on serum concentrations of cobalamin and MMA. Regressions were performed for each sex because of the presence of an interaction between sex and dairy consumption (P = 0.03). The consumption frequency of animal products was expressed as times/wk and was treated as a continuous variable (values: 0–7). Effects were regarded as significant when the addition of the variable to the model significantly increased the multiple R^2 of the model. If a significant effect of diet was found, serum cobalamin and MMA concentrations were compared in subgroups of macrobiotic boys and girls divided by dietary consumption frequency of these products. In all analyses, a two-sided probability level of 0.05 was used. Data were analyzed by using SAS (system release 6.09; SAS Institute Inc, Cary, NC).

RESULTS

Demographic data and food intake

In the macrobiotic group, the adolescents and one of their parents reported that the adolescents had followed a macrobiotic diet from birth until the age of 6.4 ± 2.9 y (n = 73). After that age, the adolescents had switched to a lactovegetarian, lactoovovegetarian, or omnivorous diet. There was no significant group difference in mean age for girls, but macrobiotic boys were on average 1 y older than the control boys. Socioeconomic status was not significantly different between groups for girls, boys, and both sexes combined (**Table 1**).

Game was consumed by only 3% of adolescents in the macrobiotic group and 1% of control adolescents, at frequencies ≤once/mo. Therefore, this food group was omitted from the analyses. The consumption frequencies of chicken and meat are described separately in Table 1, but these frequencies were added and treated as one variable in later analyses. Consumption frequencies of dairy products, meat, and chicken were significantly higher in the control group, whereas macrobiotic adolescents consumed fish more often. None of the subjects consumed chicken >2 times/wk and in comparison with control adolescents, more macrobiotic adolescents totally avoided the consumption of chicken (40% compared with 2%). The consumption frequency of eggs was not significantly different between groups, but control girls had a higher consumption frequency than macrobiotic girls.

The total intake of cobalamin from all dairy products combined was significantly higher in control adolescents than in macrobiotic adolescents. This difference was caused mainly by the lower milk consumption of the macrobiotic group (61 compared with 383 g/d in the macrobiotic and control groups, respectively). Macrobiotic adolescents also consumed less yogurt products (143 compared with 221 g/d) and less buttermilk (18 compared with 51 g/d) than the control group but equal amounts of cheese (22 compared with 25 g/d). Within the macrobiotic group, cobalamin intake from milk and yogurt products was significantly lower in girls than in boys (P < 0.05) but total cobalamin intake from dairy products was not significantly different between the sexes (P = 0.07).

Hematologic values and indexes of cobalamin status

Apparent differences in the cumulative frequency distribution of MCV, cobalamin, and MMA between the macrobiotic and control groups are shown in **Figure 1**. MCV and mean corpuscular hemoglobin mass were significantly higher in macrobiotic adolescents than in control adolescents. Additionally, cobalamin values were significantly lower and MMA concentrations were significantly higher in macrobiotic adolescents than in control
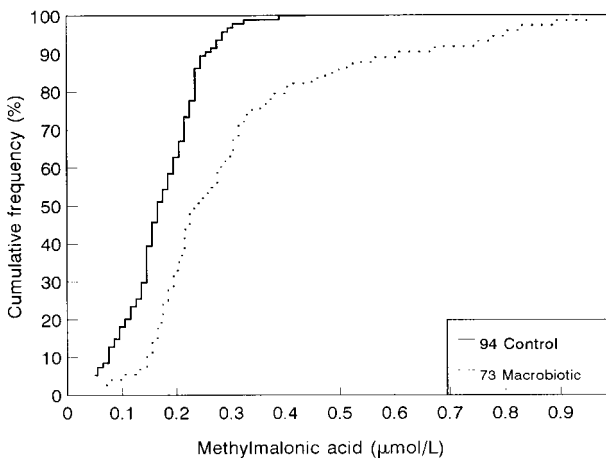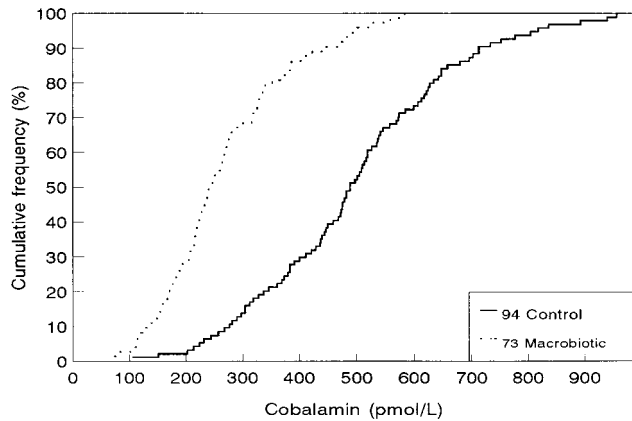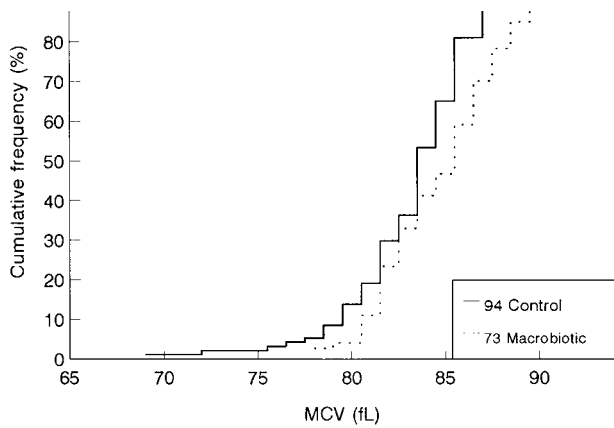
FIGURE 1. Cumulative frequency of mean corpuscular volume (MCV), serum cobalamin, and serum methylmalonic acid in 167 control and macrobiotic adolescents aged 9–15 y. Control subjects had followed an omnivorous diet since birth and macrobiotic subjects had followed a macrobiotic diet in early life and had subsequently adopted a lactovegetarian, lactoovovegetarian, or omnivorous diet.

adolescents (**Table 2**). Twenty-one percent of the macrobiotic adolescents had abnormal MMA concentrations and 37% had abnormal cobalamin concentrations, whereas only 15% had abnormal MCV values (**Table 3**). For almost all subjects, serum folate concentrations were within normal ranges although macrobiotic adolescents had significantly higher concentrations than did control adolescents. Serum tHcy concentrations were only slightly higher in macrobiotic adolescents (Table 2). Differences between groups in blood concentrations of cobalamin, MMA, and tHcy were more pronounced in boys than in girls.

Relation between plasma metabolite concentrations and other indexes

MMA concentrations in serum were inversely correlated with serum cobalamin (r = −0.50, P < 0.0001). Cobalamin had a hyperbolic relation to MMA concentrations, with elevated MMA concentrations (>0.41 μmol/L) occurring only below cobalamin concentrations of ≈280 pmol/L (**Figure 2**). Serum tHcy also showed an inverse relation with cobalamin (r = −0.45, P < 0.0001), but elevated tHcy concentrations occurred at cobalamin concentrations ranging from ≈70 to 570 pmol/L. There was no relation between MCV and serum cobalamin (r = −0.13, P = 0.11).

Serum tHcy was inversely correlated with serum folate (r = −0.34, P < 0.0001) and this correlation was as strong in the macrobiotic group as in the control group (r = −0.45, P < 0.0001 in each group). In the macrobiotic group only, tHcy was positively correlated with MMA (r = 0.35, P < 0.01), whereas in both groups cobalamin concentrations were inversely correlated with serum tHcy concentrations (control group: r = −0.39; macrobiotic group: r = −0.50, P < 0.0001).

Relation with diet

The number of years that subjects in the macrobiotic group reported following a macrobiotic diet from birth onward was, with borderline significance, inversely correlated with serum cobalamin concentrations (r = −0.22, P = 0.06, n = 73). The consumption frequency of meat (r = 0.53), chicken (r = 0.47), and dairy products (r = 0.39) was significantly correlated with serum cobalamin concentrations (P < 0.0001, n = 167). In girls, the correlations between frequency of dairy consumption and cobalamin concentrations and between frequency of meat consumption and cobalamin concentrations were weaker than in boys (0.27 compared with 0.56 for dairy products and 0.45 compared with 0.61 for meat products, respectively). In boys, fish consumption was inversely correlated with serum cobalamin concentrations (r = −0.24, P < 0.05). The cobalamin intake from dairy products was positively correlated with serum cobalamin concentrations (r = 0.47, P < 0.0001) and this correlation was only slightly stronger in boys than in girls (r = 0.52 compared with 0.44).

Shown in **Table 4** is the percentage of adjusted variance explained after adding the consumption frequency of animal products to regression models with serum cobalamin or MMA as the dependent variable. In girls, the consumption of meat and chicken explained 27% of the variance in cobalamin concentrations, whereas consumption of dairy products explained only 7% (although the percentage of variance explained by dairy products was still significant when dairy products were the only animal product added to the model). In boys, the consumption of meat and chicken and the consumption of dairy products both contributed substantially to the variance in cobalamin status, explaining 41% and 35% of the variance, respectively. Further-

TABLE 2
Biochemical and hematologic values in adolescents fed a macrobiotic diet in early life and in control children aged 9–15 y

	Boys		Girls		
	Macrobiotic (n = 37)	Control (n = 39)	Macrobiotic (n = 36)	Control (n = 55)	P[1]
Hemoglobin (mmol/L)	8.5 ± 0.5[2]	8.6 ± 0.6	8.4 ± 0.6	8.4 ± 0.5	—
Hematocrit	0.40 ± 0.02	0.41 ± 0.03	0.40 ± 0.02	0.40 ± 0.02	—
Mean corpuscular volume (fL)	85.2 ± 3.2	83.3 ± 3.7	86.1 ± 3.8	84.4 ± 3.2	<0.01
Red blood cell count ($\times 10^{12}$/L)	4.72 ± 0.28	4.92 ± 0.35	4.63 ± 0.32	4.71 ± 0.29	—
Mean corpuscular hemoglobin mass (fmol)	1.80 ± 0.07	1.76 ± 0.10	1.81 ± 0.09	1.78 ± 0.08	<0.05
Mean corpuscular hemoglobin concentration (mmol/L)	21.1 ± 0.34	21.1 ± 0.35	21.1 ± 0.4	21.1 ± 0.3	—
Folate (nmol/L)	17.9 (10.9–29.4)[3]	14.7 (8.0–27.0)	18.9 (11.4–31.2)	14.5 (7.8–26.8)	<0.0001
Cobalamin (pmol/L)	213 (107–426)	484 (238–985)	288 (112–738)	458 (206–1020)	<0.0001
Methylmalonic acid (μmol/L)	0.29 (0.09–0.93)	0.15 (0.06–0.43)	0.25 (0.09–0.70)	0.17 (0.07–0.40)	<0.0001
Homocysteine (μmol/L)	8.3 (5.2–13.4)	7.0 (4.2–11.7)	7.6 (3.8–15.1)	7.2 (3.8–13.7)	0.07

[1] Means or geometric means of the macrobiotic and control groups (boys and girls combined) were compared by using Student's t test.

[2] $\overline{x}$ ± SD.

[3] Geometric mean; geometric mean ± 1.96 SD in parentheses.

more, in boys the combination of dairy and meat and chicken explained significantly more variance in cobalamin and MMA values than meat or dairy alone, and there was a significant interaction effect of dairy with meat and chicken consumption on MMA values (P = 0.04). Overall, the influence of consumption of animal products on cobalamin status was much higher in boys than in girls. Fish had a significant effect on the percentage of variance explained in boys and increased fish consumption was associated with lower cobalamin and higher MMA concentrations; in girls, the contribution of fish was of only borderline significance (P = 0.05) and was associated with better cobalamin status.

Regression analyses indicated that consumption of meat and chicken and of dairy products contributed most to cobalamin status. We therefore divided subjects from the macrobiotic group (boys and girls combined) into subgroups according to the consumption frequency of meat and chicken and dairy products. As shown in **Figure 3,** those who refrained from the consumption of meat and had a consumption of dairy products of at most 5 times/wk had MMA concentrations above the cutoff of 0.41 μmol/L. In the macrobiotic group, subjects consuming dairy products 0–5 times/wk had a mean intake of cobalamin from dairy products of 0.61 ± 0.61 μg/d (n = 44), whereas those who consumed dairy products daily had a cobalamin intake of 1.5 ± 1.0 μg/d (n = 29). The MMA concentrations (GM, range in parentheses) and percentage of subjects with MMA concentrations >0.41 μmol/L of these 2 subgroups were, respectively, 0.31 (0.07–0.97) μmol/L and 27% and 0.22 (0.07–0.53) μmol/L and 10%.

DISCUSSION

We found significantly lower serum cobalamin and higher MMA concentrations in adolescents who had followed a macrobiotic diet in early life than in an age-matched control group who had followed an omnivorous diet since birth. Because the adolescents in the macrobiotic group were apparently healthy, we assume that their marginal cobalamin status was a result of long-term insufficient cobalamin consumption rather than disease or malabsorption. Macrobiotic diets are known to contain only small amounts of cobalamin (7, 8); in a previous study of macrobiotic infants, cobalamin deficiency was more pro-

nounced and elevations of MMA and tHcy were much more prominent (9). However, since the age of on average 6 y our subjects had switched from a macrobiotic to a lactovegetarian, lactoovovegetarian, or omnivorous diet. The diets of the macrobiotic adolescents at the time of the present study contained on average 200 g milk and yogurt products and 22 g cheese/d; additionally, the macrobiotic adolescents consumed meat, chicken, or fish 2–3 times/wk. Our findings indicate, however, that the consumption of such moderate amounts of animal products over 6–7 y was not sufficient to restore and maintain adequate cobalamin function.

Evidence of cobalamin deficiency

None of our subjects had serum cobalamin values indicative of cobalamin deficiency according to strict, established criteria [<59 pmol/L (17)]. In general, serum cobalamin concentrations in healthy persons range from 148 to 682 pmol/L (18) and 89% of adolescents in the macrobiotic group had cobalamin concentrations in this range. However, MMA concentrations were elevated in 17–24% of the macrobiotic adolescents and tHcy concentrations in 8–11%, compared with (by definition) 5% of the control adolescents, indicating functional cobalamin deficiency.

TABLE 3
Proportions of abnormal serum concentrations of methylmalonic acid (MMA), total homocysteine (tHcy), cobalamin, mean corpuscular volume (MCV), and hemoglobin in adolescents fed a macrobiotic diet in early life[1]

	Percentage abnormal		
Index	Boys (n = 37)	Girls (n = 36)	All (n = 73)
		%	
MMA >0.41 μmol/L	24	17	21
tHcy >12.8 μmol/L	8	11	10
Cobalamin <218 pmol/L	46	28	37
MCV >89 fL	11	19	15
Hemoglobin <7.7 mmol/L	5	3	4

[1] Abnormal values were defined as values above the 95th percentile of healthy control subjects for MMA, tHcy, and MCV and as values below the 5th percentile of healthy control subjects for cobalamin and hemoglobin.
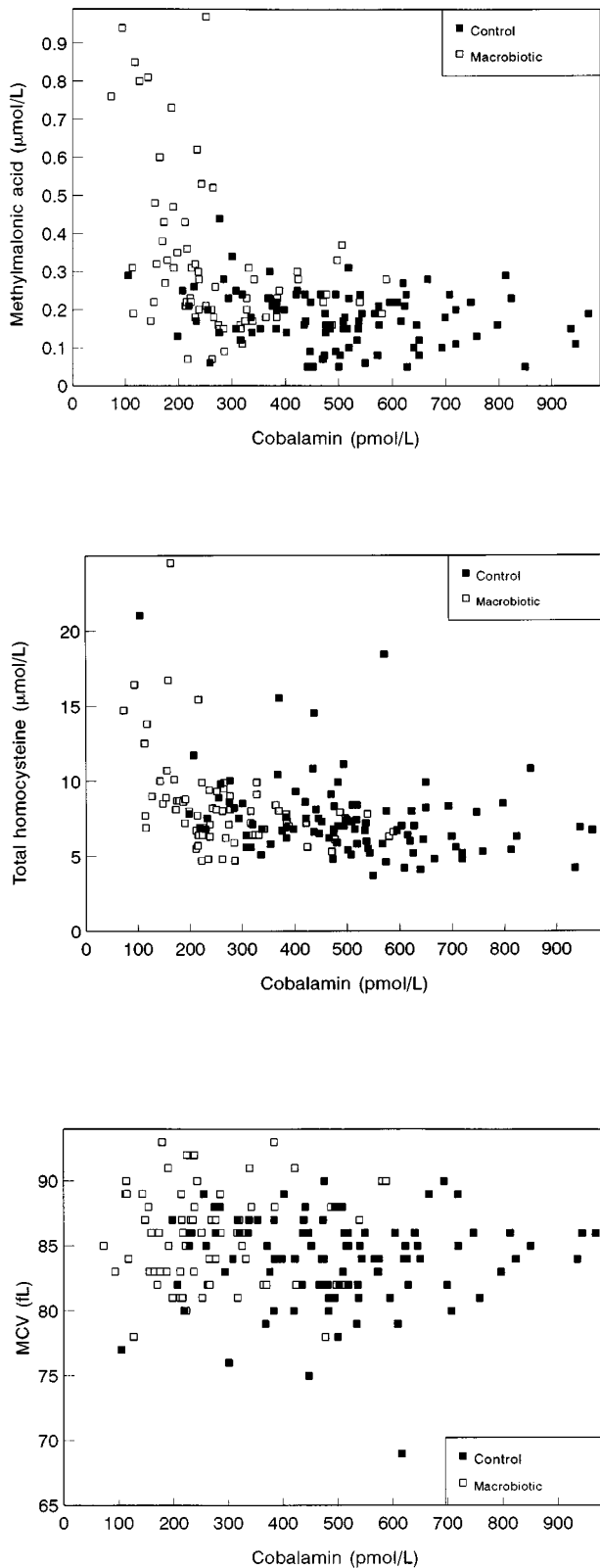
FIGURE 2. Relation between serum cobalamin and serum methylmalonic acid, serum total homocysteine, and mean corpuscular volume (MCV) in 94 control and 73 macrobiotic adolescents aged 9–15 y. Control subjects had followed an omnivorous diet since birth and macrobiotic subjects had followed a macrobiotic diet in early life and had subsequently adopted a lactovegetarian, lactoovovegetarian, or omnivorous diet.

Previous studies showed that MMA is a more specific and sensitive marker of cobalamin function than tHcy (19, 20), with a test sensitivity of 97% and a specificity of 91% in patients with clinically confirmed cobalamin deficiency (21). Even with serum cobalamin and MCV values within normal ranges, elevated MMA concentrations are consistent with impaired intracellular cobalamin status. Furthermore, the distribution of the cumulative frequency curves of the macrobiotic group as a whole was shifted toward higher MCV and MMA and lower cobalamin values than in the control group (Figure 1). This finding supports the view that the macrobiotic group showed signs of functional cobalamin deficiency.

Relation between serum folate and cobalamin

Serum folate concentrations were higher in the macrobiotic adolescents than in the control adolescents. In a study of macrobiotic infants, elevated plasma folate concentrations were hypothesized to be the consequence of cobalamin deficiency (9). In that study, plasma folate correlated positively with tHcy in the macrobiotic infants, whereas an inverse relation existed in the matched control group. In contrast, we found strong inverse correlations between serum folate and tHcy in both the macrobiotic and control groups. We assume that serum folate concentrations were higher as a result of a higher intake of folate-rich products, such as cereals and vegetables, and that this may have counterbalanced the expected increase in tHcy as a result of reduced cobalamin status. The low methionine content of macrobiotic diets may also play a role (22, 23). Thus, tHcy may be less suited for evaluation of cobalamin status in macrobiotic subjects, as can also be concluded from Figure 2.

Relation to diet

As illustrated in Figure 3, serum MMA concentrations decreased with increasing consumption frequency of dairy products and meat. In girls, the consumption of meat and chicken explained much more of the variance in cobalamin status than the consumption of dairy products, whereas in boys no real differences were seen between these 2 animal product groups (Table 4). This finding might be explained by the fact that macrobiotic boys consumed more milk and yogurt products and had lower cobalamin and higher MMA concentrations than girls. Because we did not measure amounts of meat and chicken consumed we do not know whether portion sizes of meat were higher in girls.

In boys, fish consumption was inversely correlated with serum cobalamin, a paradox because fish is regarded as a good source of cobalamin. However, fish is the only animal product accepted in the macrobiotic philosophy, and those boys who still frequently consumed fish were usually those who had adhered more strictly to a macrobiotic lifestyle.

Compared with the control group, the macrobiotic group had higher MMA concentrations although most subjects (96%) consumed dairy products [supplying 1.1 (boys) and 0.8 (girls) μg cobalamin/d] in addition to consuming meat, chicken, or fish 2.6 (boys) to 2.8 (girls) times/wk, which are much richer sources of cobalamin than dairy products (0.01–0.03 μg compared with <0.01 μg/g wet wt) (24). Thus, assuming that 50 g fish, meat, or chicken was consumed each time and that the cobalamin content of these products was 0.02 μg/g (24), the estimated total cobalamin intake from the diet would have been 1.5 μg/d for boys and 1.2 μg/d for girls. On the basis of these assumptions, the current

TABLE 4
Percentage of variance in serum cobalamin or methylmalonic acid (MMA) concentrations of boys and girls aged 9–15 y explained after adding the consumption frequency of animal products to the models[1]

	Boys		Girls	
	Cobalamin	MMA	Cobalamin	MMA
		%		
Model 1[2]	11	3	1	0
Model 1 + dairy	35[3]	24[3]	7[4]	8[4]
Model 1 + meat	41[3]	22[3]	27[3]	12[3]
Model 1 + egg	9	3	2	1
Model 1 + fish	21[4]	11[4]	0	3
Model 1 + dairy, meat	46[3,5,6]	29[3,6,7]	26[3,5]	13[3,7]
Model 1 + dairy, meat, dairy × meat	47	32[8]	27	13
Model 1 + dairy, meat, egg, fish	49[3]	27[3]	29[3]	13[3]

[1] Values represent adjusted R^2 of models in 76 boys or 91 girls.

[2] Covariate was age. Consumption frequencies were treated as a continuous variable.

[3,4] Significant increase in multiple R^2 compared with model 1: [3] $P < 0.0001$, [4] $P < 0.05$.

[5,7] Significant increase in multiple R^2 compared with model 1 + dairy: [5] $P < 0.0001$, [7] $P < 0.05$.

[6] Significant increase in multiple R^2 compared with model 1 + meat, $P < 0.01$.

[8] Significant increase in multiple R^2 compared with model 1 + dairy, meat, $P < 0.05$.

diet of the macrobiotic group supplied, on average, 81% and 67% of the Dutch recommended daily intakes of cobalamin for boys and girls of this age (10–16 y), respectively (25). We realize that this estimate is only a guess and should be interpreted with caution, but it gives us some idea of total cobalamin intake. It is likely that those 29 subjects from the macrobiotic group who consumed dairy products daily (already supplying on average 1.5 µg cobalamin/d) reached recommended cobalamin intakes, but even 10% of these subjects had MMA concentrations >0.41 µmol/L. Thus, subjects previously fed a macrobiotic diet might need cobalamin intakes higher than recommended to obtain normal cobalamin status.

In line with our data, other studies also showed a decreasing number of subjects being cobalamin deficient with increasing consumption of animal products. Dong and Scott (26) reported that of adult subjects not using supplements, 92% of vegans (n = 13), 64% of lactovegetarians (n = 28), 47% of lactoovovegetarians (n = 15), and 30% of semivegetarians (who occasionally consumed meat; n = 10) had serum cobalamin concentrations <148 pmol/L. Likewise, Armstrong et al (27) found that 22% of adult vegetarians (n = 561) who avoided consumption of meat had low cobalamin concentrations (<120 pmol/L), whereas of the vegetarians who consumed meat once per week (n = 84), only 6% had low serum cobalamin concentrations.

Consequences of a macrobiotic diet in early life

Lack of cobalamin may lead to severe neurologic disorders; these have been described in strict vegetarians, especially in infants and toddlers fed vegetarian diets (28–33). Cobalamin deficiency might be one of the factors responsible for delayed psychomotor development and growth retardation in macrobiotic children (34). Yet, there is still skepticism about the clinical significance of laboratory findings indicative of impaired cobalamin status in vegetarians. Children fed a macrobiotic diet in early childhood often are in apparently good health and may show catch-up growth in later life, especially if moderate amounts of dairy products are added to their diets (10). Thus, one may expect that the low content of cobalamin in the macrobiotic diet has no consequences in later life. However, our data show for the first time clear biochemical evidence that a strict macrobiotic diet in early childhood results in impaired cobalamin status in a substantial proportion of subjects during adolescence, even though cobalamin intake in the present population had increased since the age of 6 y. The question of whether consumption of a lactoovovegetarian diet from birth on would be sufficient for obtaining normal cobalamin function needs further investigation.

In conclusion, our data indicate that the adverse effects of cobalamin deficiency in the macrobiotic community may not be
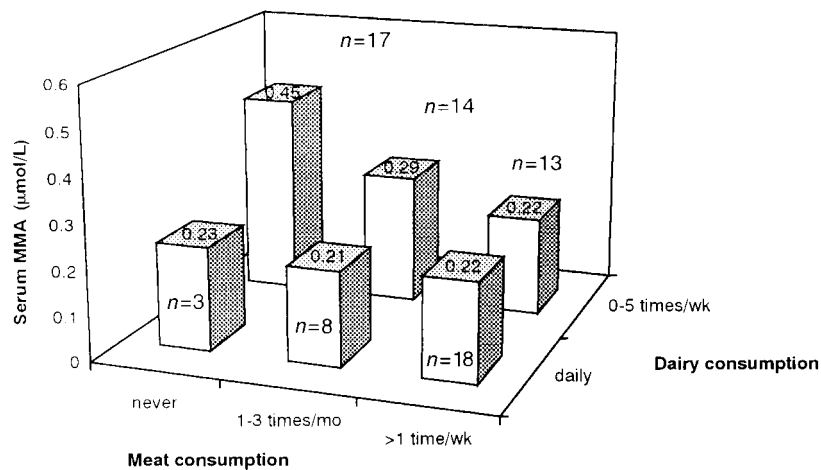

FIGURE 3. Geometric mean concentrations of serum methylmalonic acid (MMA) in 73 subjects aged 9–15 y (boys and girls combined) who had followed a macrobiotic diet in early life and had subsequently adopted a lactovegetarian, lactoovovegetarian, or omnivorous diet, described by current consumption frequency of meat and dairy products.

restricted to just early childhood, but may also cause symptoms related to impaired cobalamin status in later life. Even a change to a lactoovovegetarian or omnivorous diet may not be sufficient for restoring normal cobalamin status if the diet contains only moderate amounts of animal products. Whether the present biochemical findings also have implications for cognitive and psychomotor development in macrobiotic subjects is now under investigation.

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