Signs of impaired cognitive function in adolescents with marginal cobalamin status[1–3]

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ABSTRACT

Background: Lack of cobalamin may lead to neurologic disorders, which have been reported in strict vegetarians.

Objective: The objective of this study was to investigate whether cognitive functioning is affected in adolescents (aged 10–16 y) with marginal cobalamin status as a result of being fed a macrobiotic diet up to an average age of 6 y.

Design: Data on dietary intake, psychological test performance, and biochemical variables of cobalamin status were collected from 48 adolescents who consumed macrobiotic (vegan type) diets up to the age of 6 y, subsequently followed by lactovegetarian or omnivorous diets, and from 24 subjects (aged 10–18 y) who were fed omnivorous diets from birth onward. Thirty-one subjects from the previously macrobiotic group were cobalamin deficient according to their plasma methylmalonic acid concentrations. Seventeen previously macrobiotic subjects and all control subjects had normal cobalamin status.

Results: The control subjects performed better on most psychological tests than did macrobiotic subjects with low or normal cobalamin status. A significant relation between test score and cobalamin deficiency ($P = 0.01$) was observed for a test measuring fluid intelligence (correlation coefficient: –0.28; 95% CI: –0.48, −0.08). This effect became more pronounced ($P = 0.003$) within the subgroup of macrobiotic subjects (correlation coefficient: –0.38; 95% CI: –0.62, − 0.14).

Conclusion: Our data suggest that cobalamin deficiency, in the absence of hematologic signs, may lead to impaired cognitive performance in adolescents. *Am J Clin Nutr* 2000;72:762–9.

KEY WORDS Cobalamin deficiency, vegetarian diet, methylmalonic acid, adolescents, cognitive function, macrobiotic diet, vegan diet

INTRODUCTION

Nutritional cobalamin deficiency can develop easily in strict vegetarians because animal products are the main dietary source of cobalamin. Since 1985 we have followed children from macrobiotic families. A strict macrobiotic diet consists of cereals, pulses, and vegetables, with small additions of seaweed, fermented foods, nuts, seeds, and seasonal fruit. Fish may be consumed occasionally, whereas meat and dairy products are avoided. This diet is similar to a vegan diet and has a very low cobalamin content.

In 1986 we observed in a mixed longitudinal cohort study that the group of macrobiotic infants (mean age: 15 mo) had markedly lower cobalamin concentrations (1) and impaired psychomotor functioning (2) compared with control infants. On the basis of these and other findings of the mixed-longitudinal study, dietary recommendations were given and, subsequently, the macrobiotic families in our study population gradually adopted lactovegetarian, lactoovovegetarian, or even omnivorous diets in the years after 1986. In 1995 we found that the mean age at which the diet was changed to include at least some animal products was ≈6 y.

Lack of cobalamin may lead to severe neurologic disorders, which have been described in strict vegetarians, especially in infants and toddlers (3–8). Most of these studies showed isolated cases. There is some tentative evidence that cobalamin deficiency may be one of the factors responsible for delayed psychomotor development and growth retardation in macrobiotic children (9). Graham et al (5) found that intellectual functioning in childhood was poor in 2 of 4 reviewed subjects who had been treated for cobalamin deficiency in infancy. Yet, the clinical significance of these findings is still to be established. On one hand, children who are fed a macrobiotic diet in early childhood are often 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). On the other hand, when we measured cobalamin status in 1995 in adolescents aged 9–15 y who had followed a macrobiotic diet up to the mean age of 6 y, we found that 21% of the subjects had elevated methylmalonic acid (MMA, a specific serum marker for

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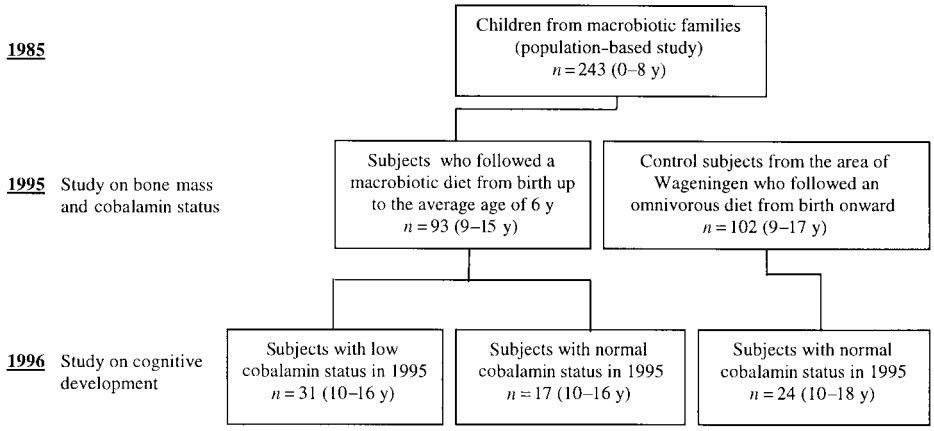

FIGURE 1. Selection of subjects for participation in the 1996 study on the effect of marginal cobalamin status on cognitive development in Dutch adolescents, Wageningen, Netherlands.

early cobalamin deficiency) concentrations and 37% had decreased cobalamin concentrations. This indicated that a strict macrobiotic diet in early childhood resulted in an impaired cobalamin status in many subjects during adolescence, despite their change to a lactoovovegetarian diet after the age of 6 y (11). Recently, it was shown that moderate consumption of animal products is not sufficient for restoring normal cobalamin status in subjects with inadequate cobalamin intakes during the early years of life (11). In the present study we investigated whether there is an association between this prolonged low cobalamin status and cognitive and psychomotor development in these adolescents who consumed a macrobiotic diet in early childhood.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from a sample of 195 Dutch adolescents (92 boys and 103 girls) who participated in a 1995 study on the effect of a macrobiotic diet in early life on bone mass (12) (the subjects' ages at that time ranged from 9 to 15 y) (**Figure 1**). As described by Parsons et al (12), the macrobiotic subjects (43 girls and 50 boys) came from an existing group of macrobiotic families affiliated with the Division of Human Nutrition and Epidemiology, Wageningen Agricultural University. This study covered >80% of the Dutch macrobiotic families when it began in 1985. For practical reasons, we refer to this group as the macrobiotic adolescents, although these adolescents had followed a macrobiotic diet up to an average age of 6 y and subsequently adopted lactovegetarian, lactoovovegetarian, or omnivorous diets. A control group of 42 boys and 60 girls of a similar age range was recruited from local schools. The adolescents in the control group had consumed omnivorous diets since birth.

The subjects in the macrobiotic cohort who were found to have cobalamin deficiency in 1995 were eligible to participate in the present study. Cobalamin deficiency was defined as a cobalamin concentration <229 pmol/L (ie, below the 5th percentile of the 1995 control group) or an MMA concentration >0.29 μmol/L (ie, above the 95th percentile of the control group). Forty-one subjects fulfilled these criteria and 36 agreed to participate. Blood samples were obtained from 31 subjects.

Subjects with normal cobalamin status included 19 subjects from the macrobiotic cohort (blood sample available from 17 subjects); 25 subjects from the 1995 control group, who had always followed an omnivorous diet; and 2 additional control subjects recruited from among healthy adolescents eating omnivorous diets (blood sample available from 24 control subjects). Thus, complete data for 72 adolescents were available for statistical analyses (Figure 1). All participants in the present study were in good health and had no known conditions that might have affected their cognitive or psychomotor functioning.

Protocol

In June and July 1996, psychological test administration and blood collection were carried out on Saturdays between 1000 and 1600 at the Division of Human Nutrition and Epidemiology of Wageningen Agricultural University. Blood samples were taken first in all subjects, after which the subjects had a snack; subsequently, psychological testing took place. The various tests were administered sequentially over a 90-min period by trained psychologists. Dietary intake was also assessed in June and July 1996. Data on socioeconomic status were obtained from the 1995 study by using Attwood scores, ie, a 5-point scale based on occupation and highest level of education attained by both parents. The study was approved by the Ethics Committee of the Division of Human Nutrition and Epidemiology of Wageningen Agricultural University, and all subjects and a parent gave written, informed consent.

Biochemical measurements

Nonfasting serum and plasma samples were collected in evacuated tubes (Venoject II; Terumo Europe NV, Leuven, Belgium). An aliquot of whole blood was taken for hematologic analysis. The remaining blood was placed on ice until centrifugation (1190 × g for 10 min at 4 °C). Serum and plasma were transferred to new vials and stored at –80 °C immediately after centrifugation until analysis. Hemoglobin, mean corpuscular volume, hematocrit, and red blood cell count were measured by using a Coulter counter (model T-860; Coulter Electronics Ltd, Bedfordshire, United Kingdom).

Cobalamin and folate were measured in serum by the IMx B12 assay and the IMx Folate assay, respectively (Abbott Laboratories, North Chicago). The intraassay CVs of the folate assay

TABLE 1
Psychological tests and the functions they measure, administered to 31 subjects with low cobalamin status previously consuming a macrobiotic diet, 17 subjects with normal cobalamin status previously consuming a macrobiotic diet, and 24 control subjects

Test	Functions measured
Raven's progressive matrixes (21)	Fluid intelligence
Block design (22, 23)	Spatial ability
Picture completion (23)	Perceptual closure
Digit symbol (23)	Attention and concentration
Digit span (23)	Short-term memory
Word recall (24)	Memory
Word fluency (25, 26)	Divergent thinking
Pegboard (27)	Psychomotor development
TAART (28)	Information processing time

varied between 3% and 6% and the interassay CVs varied between 6% and 10%, depending on the folate concentration. For cobalamin, the intra- and interassay CVs were <5%. Plasma transcobalamins were determined by using a technique based on specific absorption of saturated transcobalamin II (holoTCII) on heparin-conjugated Sepharose (13) (within-assay CV: 3%). Subsequently, concentrations of saturated transcobalamin I and III could be measured. Total cobalamin minus saturated transcobalamin I and III gave holoTCII.

Serum MMA was assayed with capillary electrophoresis (within-assay CV: 5–10%; between-day CV: 12% at low physiologic concentrations) (14). Plasma total homocysteine, which includes both the free and bound fraction, was determined by using HPLC with fluorescence detection (within-assay CV: 3%; between-day CV: <4%) (15).

Because iron status is a possible confounder (16–19), serum ferritin and transferrin receptor were determined. Ferritin concentrations were measured by using the MAGIC ferritin radioimmunoassay (Ciba Corning Diagnostics Corp, Medfield, MA) (within-assay CV: 3.7%). Transferrin receptor was determined as described earlier (20).

Psychological tests

A broad spectrum of cognitive abilities and some aspects of psychomotor functioning were measured by using standard methods. The name of each test and the functions measured are shown in **Table 1**. A more detailed description of the tests and functions follows.

Raven's progressive matrixes

Raven's progressive matrixes are a computerized version of the standard progressive matrixes, consisting of continuations of series of figures (21). The principle on which the figurative matrix is constructed can be deduced from the design that is presented to the subject. The test measures fluid intelligence, which involves reasoning, the capacity to solve complex problems, the ability to learn, and abstract thinking ability. The score and the time needed to complete the task were recorded.

Block design

For the block design test, the subject is shown a particular pattern and is asked to reproduce this pattern with painted blocks (22, 23). This test measures spatial ability, ie, the ability to break down spatial forms into their constituent elements.

Picture completion

With the picture completion test, each test page has an incomplete drawing of an everyday object and the subject is required to identify which part of each drawing is missing (23). The test measures perceptual closure, which is the ability to combine disconnected, vague visual stimuli into a meaningful whole by matching them with their long-term representations.

Digit symbol

With the digit symbol test, the subject is shown 10 digits, each with a corresponding arbitrary symbol (23). He or she is required to write down the symbol below the number or the number below the symbol, completing as many as possible in 90 s while the code remains in front of the subject as he or she works. This is a test of attention, concentration, speed, and accuracy.

Digit span

For the digit span test, a series of numbers is read aloud to the subject (23). He or she is asked to repeat these numbers in the same order in which they were read (forward) and in reverse order (backward). This is a measure of short-term memory, ie, the ability to retain and immediately reproduce information (digits in this case) after one stimulus presentation.

Word recall

With the word recall test, a list of 15 words is read to the subject, after which he or she is asked to recall as many words as possible immediately after presentation (recall 1), after 30 min (recall 2), and after 60 min (recall 3) (24). This is a measure of memory, ie, the ability to retain and reproduce information (nouns) after a delay after a single presentation of the list.

Word fluency

The word fluency test consists of 5 small tasks: *1*) generating as many animal names as possible in 2 min (D test) and *2–5*) generating in 1 min as many objects as possibly starting with the letters U, N, K, and A, respectively (UNKA test) (25, 26). The test measures divergent thinking, ie, the ability to generate as many items as possible according to some rule, often associated with creativity.

Pegboard test

With the pegboard test, the subject inserts pegs into holes of uniform size that form the outline of a butterfly in a thick transparent plastic board (27). This is a test of psychomotor development and eye and hand coordination. The pegboard test has good test-retest reliability. All the other tests described here have been found repeatedly to have high internal consistencies.

TAART

TAART (28) is a computer-simulated program consisting of one simple reaction task (1) and 2 choice reaction tasks (2, 3). The test measures information-processing time, ie, the ability to react to a simple visual stimulus after a simple visual discrimination and decision task.

Dietary intake

For each subject, two 24-h dietary recalls, conducted 1–2 wk apart, were taken by telephone by 3 trained dietitians. After the subject was interviewed, one of the subject's parents was asked for more details on the subject's diet. Standard portion sizes were used and calculation of nutrients was performed by using

the 1995 release of the Dutch food-composition table (29) and separate reports for folic acid (30) and cobalamin (31).

Statistical analyses

Because of skewed distribution, data for serum folate, cobalamin, MMA, and plasma total homocysteine were log transformed before calculation. Means and SDs or geometric means and 25th and 75th percentiles as estimated from the log-normal distribution were calculated for all blood variables. Differences among groups in characteristics, biochemical variables, and dietary intake were investigated by using analysis of variance with the Bonferroni correction for multiple comparisons. If a significant difference was found, Student's *t* test was used for determining *P* values of differences between the 2 groups.

To facilitate interpretation of different psychological test scores, scores were converted to *z* scores (mean = 0, SD = 1); a negative *z* score indicates a score below the population mean and a positive *z* score indicates a score above the population mean.

To study the association between cobalamin status and psychological test score, multivariate analyses (general linear models) were applied with age and iron status (indicated by ferritin) as covariates. In the event that the addition of the covariate ferritin to the model did not change the β estimate by >10%, ferritin was omitted from the model. We also investigated whether age and iron status alone (ie, without cobalamin status in the model) were associated with the psychological test score. Cobalamin status was judged by serum cobalamin or MMA concentration. We defined normal cobalamin status as serum concentrations above the 5% level of the 1995 control group or MMA concentration below the 95% level of the 1995 control group. Thus, by definition, the subjects with cobalamin deficiency almost exclusively belonged to the macrobiotic cohort, whereas most of the subjects in the control group had normal cobalamin status. As a consequence, a possible association between cobalamin status and psychological test performance could have been due to any difference in characteristics between these 2 groups of subjects. To exclude a possible group effect, we performed multivariate analyses using data of the total study population (n = 72) but also data of adolescents from the macrobiotic cohort alone (n = 48). In these multivariate analyses, the biochemical variable under investigation was also expressed as a *z* score. This was done because it makes the interpretation of the association much easier, because the β estimates can then be interpreted as the partial correlation coefficient between the biochemical variable and the psychological test score. Adjustment for age was performed for the subjects in the subgroup studied, so adjustments are based on 72 subjects for study of the total study population and based on 48 subjects for study of the macrobiotic subgroup.

Iron status as indicated by hemoglobin concentration was also included in the model. Furthermore, we investigated whether cognitive functioning was associated with the number of years of consumption of a strict macrobiotic diet, and homocysteine concentration and holoTCII concentration. In all the analyses, a two-sided significance level of 0.05 was used. Data were analyzed by using SAS (version 6.09; SAS Institute Inc, Cary, NC).

RESULTS

The age distribution and the socioeconomic status of the groups were not significantly different (**Table 2**). The cobalamin-deficient macrobiotic subjects followed a strict macrobiotic diet for a longer time than did the nondeficient subjects, although the difference was not significant. All the macrobiotic subjects followed a strict macrobiotic diet until the age of 2.5 y, and 77% maintained this strict diet until they were 5 y old.

The macrobiotic subjects had lower concentrations of cobalamin and holoTCII and a higher MMA concentration than did the control subjects, whereas hemoglobin, mean corpuscular volume, hematocrit, and folate were not significantly different between the groups (Table 2). We defined cobalamin deficiency as a cobalamin concentration <229 pmol/L or an MMA concentration >0.29 μmol/L, or both (*see* Methods). According to this definition, 24 subjects (77%) in the cobalamin-deficient macrobiotic group had low cobalamin concentrations and 26 (84%) had elevated MMA concentrations. Three macrobiotic subjects (18%) in the group with normal cobalamin status had low cobalamin and 7 (41%) had elevated MMA concentrations.

In the control subjects, serum ferritin concentrations were higher than in the macrobiotic subjects. Five macrobiotic subjects with cobalamin deficiency, 4 macrobiotic subjects with normal cobalamin, and 3 control subjects had serum ferritin concentrations <12 μg/L; only 5 of these subjects also had elevated concentrations of serum transferrin receptor (2 macrobiotic subjects with low cobalamin, 2 macrobiotic subjects with normal cobalamin, and 1 control subject), indicating low iron status.

Mean intakes of fat, carbohydrates, and energy did not differ significantly between groups. Protein intake was lower in the macrobiotic subjects, mainly because of a lower intake of animal protein. Intake of polysaccharides and dietary fiber was higher in the macrobiotic subjects, as was folate intake and iron intake. Intakes of calcium and cobalamin were significantly lower in macrobiotic subjects. The present dietary intake of cobalamin (median) constituted 85%, 99%, and 200% of the recommended daily intakes in deficient macrobiotic subjects, macrobiotic subjects with normal cobalamin concentrations, and control subjects, respectively. Of all the macrobiotic subjects, 15 (31%) had current intakes <50% of the recommended dietary allowance (RDA). A cobalamin intake <25% of the RDA occurred in 16% (n = 5) of cobalamin-deficient macrobiotic subjects and 12% (n = 2) of macrobiotic subjects with normal cobalamin status.

In general, *z* scores on the psychological tests tended to be lower in the macrobiotic subjects (**Table 3**). The scores on the picture completion test were significantly lower in the macrobiotic subjects with normal cobalamin status than in the control subjects. On the test in which both score and time were measured (Raven's progressive matrixes), the cobalamin-deficient macrobiotic subjects performed worse than did the control subjects on both measures, although the effect was more pronounced for score.

Regression analysis showed that, after adjustment for age, there was a significant association between serum MMA concentration and scores on fluid intelligence [correlation coefficient (95% CI): –0.28 (–0.48, –0.08)]. Furthermore, there were marginally significant associations between serum MMA and spatial ability [–0.21 (–0.41, –0.01)] and short-term memory [–0.24 (–0.46, –0.02)]. When the model was applied to the subgroup of macrobiotic subjects, the association between serum MMA and fluid intelligence became more pronounced [–0.38 (–0.62, –0.14)] (**Table 4**). The association with fluid intelligence was also shown in the model with serum cobalamin instead of serum MMA [correlation coefficient: 0.23 (0.01, 0.44) in all subjects

TABLE 2
Characteristics, biochemical variables, and dietary intake of subjects who consumed a macrobiotic diet in early life and had a low or normal cobalamin status at the time of the study and in control subjects with normal cobalamin status

	Macrobiotic subjects		
Characteristic	Cobalamin deficient (n = 31)	Normal cobalamin (n = 17)	Control subjects (n = 24)
Age (y)	13.6 ± 2.0[1]	13.0 ± 1.7	13.5 ± 2.1
Time consuming strict macrobiotic diet (y)	7.3 ± 3.3	6.3 ± 2.6	0
Socioeconomic status[2]	1.95 ± 0.55	1.85 ± 0.72	1.71 ± 0.55
Sex (%)			
M	64	35	50
F	36	65	50
Biochemical variables			
Cobalamin (pmol/L)[3]	177 (136, 227)[4–6]	291 (239, 331)[5]	446 (352, 568)
Methylmalonic acid (μmol/L)[3]	0.41 (0.32, 0.54)[5,6]	0.21 (0.14, 0.36)	0.16 (0.12, 0.22)
Homocysteine (μmol/L)[3]	8.3 (6.8, 9.8)[7]	6.8 (6.2, 6.9)	7.1 (5.4, 9.1)
Folate (nmol/L)[3]	16.9 (15, 20)	16.0 (14, 19)	14.9 (12, 17)
Saturated transcobalamin II (pmol/L)	15 ± 18[5]	17 ± 19[5]	65 ± 43
Hemoglobin (mmol/L)	8.4 ± 0.7	8.3 ± 0.7	8.5 ± 0.6
Ferritin (μg/L)	20.4 ± 8.9[8]	20.8 ± 11	30.3 ± 18
Transferrin receptor (pmol/L)	22.8 ± 4.2	23.7 ± 3.6	21.9 ± 4.3
Mean corpuscular volume (fL)	86 ± 3.9	87 ± 3.4	86 ± 3.5
Hematocrit	0.40 ± 0.03	0.39 ± 0.03	0.40 ± 0.03
Dietary intake			
Energy (kJ/d)	8634 ± 2587	7401 ± 1280	8149 ± 2233
Total protein (% of energy)	12.5 ± 1.9[5]	12.6 ± 2.1[5]	15.4 ± 2.4
Animal protein (% of energy)	3.0 ± 2.3[5]	4.7 ± 2.8[5]	9.2 ± 2.5
Fat (% of energy)	31.9 ± 7.2	34.5 ± 5.7	33.0 ± 7.3
Carbohydrates (% of energy)	52.0 ± 7.0	50.1 ± 6.7	48.8 ± 6.8
Polysaccharides (% of energy)	31.7 ± 7.0[5,9]	27.2 ± 6.2	24.1 ± 4.1
Dietary fiber (g/d)	23.3 ± 7.6[5,7]	17.5 ± 5.5	15.2 ± 5.6
Calcium (mg/d)	709 ± 380[8]	741 ± 286	998 ± 381
Iron (mg/d)	13.9 ± 3.8[8]	11.6 ± 3.5	10.4 ± 3.7
Folate (μg/d)	319 ± 135[8]	281 ± 85.9	226 ± 86.8
Cobalamin (μg/d)	1.5 ± 1.2[5]	1.8 ± 1.3[8]	3.6 ± 1.9

[1] $\bar{x}$ ± SD.
[2] Attwood score (*see* text).
[3] Because of skewed distribution, data were log transformed before calculation.
[4] Geometric mean (25th, 75th percentile).
[5,8] Significantly different from control subjects: [5] $P < 0.0001$, [8] $P < 0.01$.
[6,7,9] Significantly different from non-cobalamin-deficient macrobiotic subjects: [6] $P < 0.0001$, [7] $P < 0.01$, [9] $P < 0.05$.

and 0.38 (0.12, 0.63) in the macrobiotic subjects alone]. Adding ferritin to either model did not change β estimates >10%.

Iron status as indicated by ferritin showed marginally significant associations with results of tests measuring attention and concentration and memory [0.22 (0.03, 0.42) and 0.23 (0.001, 0.46), respectively]. These associations disappeared when the model was applied to the macrobiotic subjects alone. When iron status was indicated by hemoglobin concentration, no significant associations were found with cognitive functioning. Adding hemoglobin to the model instead of ferritin also did not change β estimates significantly.

The number of years of consumption of a macrobiotic diet was not associated with the age-adjusted scores of cognitive function. HoloTCII concentration was not associated with cognitive functioning after correction for age; homocysteine concentration showed an association with fluid intelligence; however, adding this variable to a model that also included cobalamin or MMA did not alter β estimates >10%. The addition of sex to either model also did not change parameter estimates >10% (data not shown).

DISCUSSION

We found a significant association between cobalamin status and performance on tests measuring fluid intelligence, spatial ability, and short-term memory. The association between cobalamin status and fluid intelligence became even stronger within the subgroup of macrobiotic subjects, indicating that the observed relations were not due to concomitant group characteristics. Furthermore, the associations were shown irrespective of whether serum cobalamin or serum MMA was used as the measure of cobalamin status. Fluid intelligence is important because it involves reasoning, the capacity to solve complex problems, abstract thinking ability, and the ability to learn (21). Any defect in this area may have far-reaching consequences for individual functioning.

It is likely that the occurrence of low cobalamin status among the macrobiotic subjects was due to the current intake of moderate amounts of dietary cobalamin in combination with long-term insufficient cobalamin consumption in the past. Macrobiotic diets are known to contain only small amounts of cobalamin (1, 32). Previous studies showed that the cobalamin status of the adolescents studied had been low in infancy (1) and also in

TABLE 3
z Scores on psychological test performance in subjects who consumed a macrobiotic diet in early life and had a low or normal cobalamin status at the time of the study and in control subjects with normal cobalamin status[1]

	Macrobiotic subjects		
	Cobalamin deficient (n = 31)	Normal cobalamin (n = 17)	Control subjects (n = 24)
Raven (score)	−0.15 ± 1.0	0.18 ± 0.7	0.07 ± 1.2
Raven (time)	−0.05 ± 0.9	0.01 ± 1.1	0.06 ± 1.0
Block design	−0.11 ± 1.0	−0.08 ± 1.1	0.20 ± 1.0
Picture completion	0.003 ± 1.0	−0.54 ± 0.9[2]	0.37 ± 0.9
Digit symbol 1	−0.22 ± 1.2	0.14 ± 0.7	0.19 ± 0.9
Digit symbol 2	−0.19 ± 1.0	−0.05 ± 0.8	0.28 ± 1.0
Digit span (forward)	−0.05 ± 1.2	−0.12 ± 0.9	0.15 ± 0.8
Digit span (backward)	−0.03 ± 0.9	−0.23 ± 1.2	0.21 ± 0.9
Word recall 1	−0.05 ± 1.1	−0.33 ± 0.9	0.29 ± 0.8
Word recall 2	−0.09 ± 1.0	−0.18 ± 0.8	0.25 ± 1.1
Word recall 3	−0.10 ± 0.9	−0.23 ± 1.0	0.29 ± 1.1
Word fluency (D)	0.02 ± 1.1	−0.36 ± 0.8	0.23 ± 1.0
Word fluency (U)	0.02 ± 0.9	0.18 ± 1.2	−0.15 ± 1.0
Word fluency (N)	0.06 ± 1.1	−0.12 ± 1.1	0.01 ± 0.8
Word fluency (K)	0.06 ± 1.1	0.24 ± 1.1	−0.25 ± 0.8
Word fluency (A)	0.06 ± 1.0	−0.36 ± 0.9	0.18 ± 1.0
Pegboard	−0.06 ± 0.9	0.02 ± 1.4	0.06 ± 0.9
TAART 1	−0.19 ± 1.1	0.08 ± 1.0	0.19 ± 0.9
TAART 2	−0.02 ± 0.9	−0.02 ± 0.9	0.04 ± 1.2
TAART 3	0.07 ± 0.8	0.07 ± 0.7	0.13 ± 1.4

[1] $\bar{x}$ ± SD.
[2] Significantly different from control subjects, $P < 0.01$.

childhood (11). Since the average age of 6 y, the macrobiotic subjects in our study had changed to lactovegetarian, lactoovovegetarian, or omnivorous diets. However, a moderate intake of animal products was not sufficient to restore and maintain adequate cobalamin status in all subjects by age 9–15 y (11). Although, in the present study, the median intake of cobalamin of the macrobiotic subjects was close to the RDA, 31% still had intakes <50% of the RDA. Because these subjects consumed a diet extremely low in cobalamin from birth up to the age of 6 y, their cobalamin stores may never have reached an optimal level and moderate intakes may not have been sufficient for obtaining normal serum cobalamin status.

We showed previously that infants in the macrobiotic cohort already had markedly impaired cobalamin status and psychomotor delay (2, 33). We attempted to assess mental development in a subgroup of the macrobiotic cohort at the age of 4–5 y (34). However, the test we used could not adjust for the relatively high educational level of the parents. Furthermore, one-third of the macrobiotic children (15 out of 44) did not complete the test, so any conclusions were of limited generalizability.

The mechanisms by which cobalamin deficiency causes cognitive dysfunction are not clear. A wide variety of neurologic symptoms and signs have been described, such as ataxia, loss of cutaneous sensation, diminished or hyperactive reflexes, dementia, loss of memory, psychoses, and disturbances of mood (35, 36). The earliest signs in infants appear to be progressive lethargy and apathy, often accompanied by increasing irritability (37). However, most studies on the effect of cobalamin deficiency showed isolated cases with severe cobalamin deficiency. As far as we know, associations between low cobalamin status in adolescents and cognition have not been studied before. Furthermore, little is known about the consequences of low cobalamin status in childhood for cognitive functioning later in life. Graham et al (5) showed poor intellectual outcome in 2 of 4 subjects who had been treated for cobalamin deficiency in infancy (5).

The fact that only a few single psychological tests yielded significant results should be considered in light of the limited number of subjects available for this study. So far, the effects of cobalamin deficiency during childhood or adolescence on psychological test performance in later life have not been investigated. Therefore, we asked all 41 subjects from the previously macrobiotic group who had marginal cobalamin status in 1995 to participate. However, calculation of sample size on the basis of the present results indicated that 62 subjects in each group would be needed to detect a difference of 0.6 SD in psychological test score with a power of 80% at a 5% significance level. The cobalamin-deficient group had the lowest scores for various psychological tests (Table 1). Both the power analysis and the pattern of differences of Table 1 point to discernible, though small, effects of cobalamin deficiency on intellectual functioning. In some tests, the control subjects

TABLE 4
Results from regression analysis in all subjects and in the macrobiotic subjects only[1]

	All subjects (n = 72)			Macrobiotic subjects (n = 48)		
	β	95% CI	P	β	95% CI	P
Model 1[2]						
Raven (score)	−0.28	(−0.48, −0.08)	0.01	−0.38	(−0.62, −0.14)	0.003
Block design	−0.21	(−0.41, −0.01)	0.04	−0.07	(−0.32, 0.17)	0.57
Digit span (forward)	−0.24	(−0.46, −0.02)	0.03	−0.24	(−0.51, 0.04)	0.10
Model 2[3]						
Raven (score)	0.23	(0.01, 0.44)	0.04	0.38	(0.12, 0.63)	0.01
Word fluency (K)	−0.01	(−0.24, 0.23)	0.96	0.33	(0.04, 0.36)	0.03
Model 3[4]						
Digit symbol 2	0.22	(0.03, 0.42)	0.03	−0.06	(−0.33, 0.21)	0.66
Word recall 3	0.23	(0.001, 0.46)	0.05	−0.05	(−0.35, 0.24)	0.71

[1] Only tests with significant or borderline significant P values in either population are presented. β can be interpreted as the correlation coefficient between the psychological test and methylmalonic acid concentration.
[2] Age + *z* score (methylmalonic acid concentration); adding ferritin to the model did not change β estimates significantly.
[3] Age + *z* score (cobalamin concentration); adding ferritin to the model did not change β estimates significantly.
[4] Age + *z* score (ferritin concentration).

performed worse than did the macrobiotic subjects. These tests (word fluency U and K tests) appeared to have been very difficult for all the subjects and probably lack discriminatory power. In several tests, the macrobiotic subjects with normal cobalamin status performed worse than did the cobalamin-deficient macrobiotic subjects. We defined normal cobalamin status as serum cobalamin concentrations above the 5% level of the 1995 control group or a MMA concentration below the 95% level of the 1995 control group. These cutoffs are, of course, arbitrary because cobalamin and MMA are continuous variables. In the macrobiotic control group, serum concentrations of cobalamin and MMA were still significantly lower and higher, respectively, than in the control subjects (Table 2). The effects of low cobalamin status probably appear before the cobalamin concentration is below the 5% level or the MMA concentration rises above the 95% level. If the definition of abnormal cobalamin status had been less strict (eg, cobalamin below the 90% level and MMA above the 10% level), a better discrimination between the macrobiotic and control groups might have been found. The problem of defining the reasonable cutoffs was avoided in the regression analyses (Table 4) because both cobalamin and MMA concentration were treated as continuous variables in this model.

Because traditional laboratory tests do not completely discriminate cobalamin-deficient patients from persons with normal cobalamin status, we also measured plasma MMA, a metabolite accumulating during cobalamin deficiency (33). In adults, MMA in serum and plasma is a more specific marker for cobalamin status than is total homocysteine, which is also elevated in folate-deficient patients and in some other diseases. MMA also seems to have a somewhat higher sensitivity than does homocysteine. This is probably also true for children and adolescents.

Iron deficiency may affect cognitive performance (16–19); however, we did not consider iron status to be a confounding variable in our study. Hemoglobin concentrations were identical among the groups and iron intake was actually higher in the subjects with cobalamin deficiency, although the iron consumed was nonheme iron, which has reduced bioavailability. Moreover, reliable indicators of iron status, such as serum ferritin and transferrin receptor, did not differ significantly among the groups. Furthermore, adding ferritin or hemoglobin as a covariate did not alter β estimates $> 10\%$.

Marginal associations between iron status and attention, concentration, and memory were observed in the total study population. These associations disappeared when group effects were ruled out (ie, among the macrobiotic subjects). This could have been due to a lack of variation in ferritin status in the macrobiotic subgroup. Most research on the effects of iron status on cognition has focused on infants and very young children. Several studies showed that iron deficiency causes changes in behavior and lowers developmental test scores in infancy (38, 39). Adverse effects on attention and language development in infants and toddlers were observed (40). However, none of the subjects in our study was anemic. In a study on the effects of iron supplementation on cognitive function in adolescent girls with nonanemic iron deficiency, improvement of verbal learning and memory after supplementation was shown (19). In our study, iron status did not seem to be an important determinant of cognitive performance, because the effects were only marginally significant. More importantly, these effects were not found for any other subtest of attention, concentration, and memory.

Our data suggest an association between cobalamin status and cognitive functioning. However, a causal relation cannot be proven from this study. A controlled intervention study would supply evidence for a causal relation. However, there might be a problem in obtaining enough cobalamin-deficient subjects who are comparable with respect to other aspects that may be related to cognitive functioning, such as the cause of their cobalamin deficiency (vegan-type diet, illness, or poverty).

In conclusion, a shortage of cobalamin without the typical hematologic signs of cobalamin deficiency was associated with an impairment of cognitive performance that has consequences for daily life. These findings have implications not only for subjects consuming or previously consuming a macrobiotic diet, but for all subjects who avoid animal products, whether because of medical reasons, beliefs, or poverty.

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