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Nutrition

Total Homocysteine, Vitamin B_{12}, and Total Antioxidant Status in Vegetarians

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Background: **Decreasing or eliminating animal products from the diet decreases the intake of some essential nutrients, such as vitamin B_{12}, which may lead to hyperhomocysteinemia. We investigated vitamin B_{12}-dependent metabolism and oxidative stress in groups with various or no intake of meat or animal products.**

Methods: **We investigated 44 high meat eaters, 19 low meat eaters, 34 lacto-ovo/lacto vegetarians, and 7 vegan vegetarians. Homocysteine (HCY) was assayed by HPLC, methylmalonic acid (MMA) by capillary gas chromatography–mass spectrometry, serum folate and vitamin B_{12} with a chemiluminescence immunoassay, and total antioxidant status (TAS) by a Randox method.**

Results: **The mean serum HCY concentration of vegetarians was significantly increased, and in vegans the median concentration exceeded 15 μmol/L. Vegetarians had a higher serum concentration of MMA but a lower TAS. Vitamin B_{12} and folate did not differ significantly between vegetarian and omnivorous subjects. Overall, HCY and MMA were significantly correlated. Vitamin B_{12} correlated negatively with MMA, HCY, and folate, whereas the correlation with TAS was positive. Backward regression analysis revealed an independent influence of MMA on HCY, of HCY and vitamin B_{12} on MMA, and of vitamin B_{12} on TAS. The increased MMA concentration suggested a 25% frequency of functional vitamin B_{12} deficiency in all vegetarians. Serum vitamin B_{12} was below the lower reference limit in only five subjects.**

Conclusions: **Functional vitamin B_{12} deficiency in vegetarians may contribute to hyperhomocysteinemia and decreased TAS, which may partly counteract the beneficial lifestyle of vegetarians. However, increased serum HCY is most likely not responsible for the lower TAS values in vegetarians. We recommend assaying of MMA and HCY to investigate functional vitamin B_{12} status.**
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Hyperhomocysteinemia has been recognized as an important independent cardiovascular risk factor *(1)*. It is hypothesized that homocysteine (HCY)[3] alters endothelial and smooth muscle cell function by generating reactive oxygen species *(2–4)*. The resulting increase in oxidative stress diminishes antioxidative capacity, which increases the risk for atherosclerotic vessel diseases in these subjects *(5, 6)*. Vitamin deficiencies (B_2, B_6, B_{12}, and folate), enzyme mutations with only limited loss of enzymatic activity (cystathionine-β-synthase, methionine synthase, and thermolabile methylenetetrahydrofolate reductase polymorphisms), and renal insufficiency may produce moderate hyperhomocysteinemia (15–30 μmol/L) *(7–9)*. Dietary folate deficiency causes insufficient formation of 5-methyltetrahydrofolate, which is needed as a methyl-group donor in the remethylation of HCY to methionine (Fig. 1). However, most relevant to vegetarians is vitamin B_{12} deficiency (dietary or inadequate absorption), which leads to impaired methyl transfer from 5-methyltetrahydrofolate to HCY during remethylation and, subsequently, increased serum HCY concentrations.

The principal difference among various vegetarian diets is the extent to which animal products are avoided. Some vegetarian diets provide less fat, less saturated fat, and fewer calories than typical omnivorous diets and have a higher content of fruits, vegetables, and whole-grain products. By total elimination of food of animal origin, vegetarians decrease their intake of some essential nutrients, including vitamin B_{12}. Vitamin B_{12} typically is

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[3] Nonstandard abbreviations: HCY, total homocysteine; MMA, methylmalonic acid; HME, high meat eater; LME, low meat eater; LOV, lacto-ovo vegetarian; LV, lacto vegetarian; TAS, total antioxidant status; and ABTS, 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate).

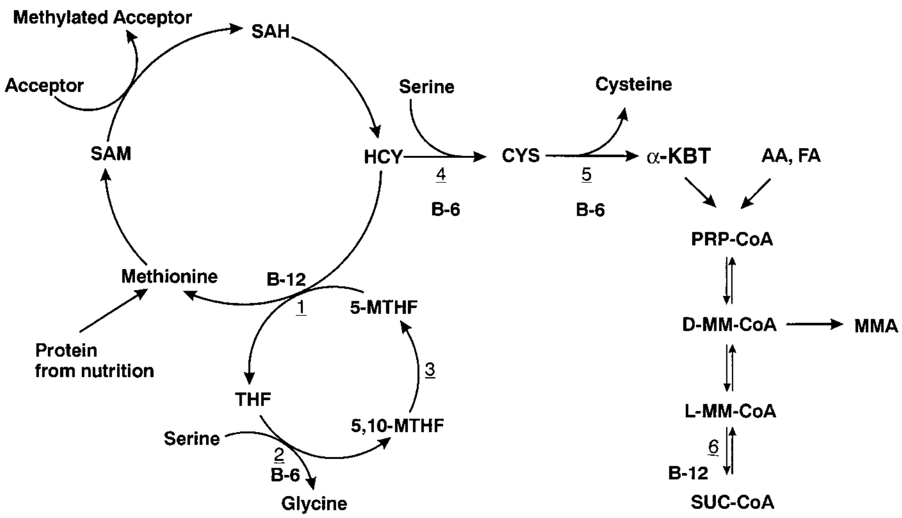

Fig. 1. Metabolism of HCY.

SAM, *S*-adenosylmethionine; *SAH*, *S*-adenosyl homocysteine; *CYS*, cystathionine; *α-KBT*, α-ketobutyrate; *PRP-CoA*, propionyl-CoA; *D-MM-CoA*, D-methylmalonyl-CoA; *L-MM-CoA*, L-methylmalonyl-CoA; *SUC-CoA*, succinyl-CoA; *THF*, tetrahydrofolate; *5-MTHF*, 5-methyltetrahydrofolate; *5,10-MTHF*, 5,10-methylenetetrahydrofolate; *1*, methionine synthase; *2*, serine hydroxymethyltransferase; *3*, N^5,N^{10}-methylenetetrahydrofolate reductase; *4*, cystathionine-β-synthase; *5*, cystathionase; *6*, L-methylmalonyl-CoA mutase; *AA*, amino acids; *FA*, fatty acids.

found only in foods of animal origin. Thus, the avoidance of animal products in association with a strict vegetarian diet may lead to a deficiency of vitamin B_{12} *(10, 11)*. The ultimate source of all vitamin B_{12} is microbial synthesis. Lacto-ovo and lacto vegetarians ingest adequate amounts of vitamin B_{12} from egg and dairy products *(12–14)*. Omnivorous subjects typically ingest ~26 μg of vitamin B_{12} per day and excrete ~5–10 μg of vitamin B_{12} from their livers via bile into their intestines. When no intestinal reabsorption problems are present, the bodies of omnivorous subjects reabsorb ~3–5 μg of vitamin B_{12} per day. High liver stores combined with effective enterohepatic recirculation prevent healthy adult vegan vegetarians from developing vitamin B_{12} deficiency *(15)*. However, people with low body storage of vitamin B_{12}, impaired absorption or metabolism of vitamin B_{12}, and physiological conditions with increased demands (e.g., pregnancy and breast feeding) may develop deficiency symptoms much faster. Prolonged vitamin B_{12} deficiency as a clinical disease usually manifests in neurologic and gastrointestinal disorders as well as anemia *(16, 17)*.

Vitamin B_{12} (cobalamin) functions as an essential cofactor for only two enzymes in mammalian cells: L-methylmalonyl-CoA mutase requires adenosyl-cobalamin, and methionine synthase requires methylcobalamin *(18)*. In vitamin B_{12} deficiency, increased concentrations of methylmalonyl-CoA are hydrolyzed and lead to increased amounts of methylmalonic acid (MMA). Increased serum HCY is an indicator of functional intracellular deficiency of vitamin B_{12} and folate, whereas increased MMA is a more specific indicator of functional vitamin B_{12} deficiency and is not dependent on folate status *(19–22)*.

In the present study, we investigated omnivorous subjects and vegetarians with different dietary habits to determine the influence of vegetarian lifestyles on HCY and vitamin B_{12} status. The vegetarians in this study differed from omnivorous subjects not only in their dietary habits but also in their lifestyle, e.g., they consumed less alcohol, smoked less, and exercised more (Table 1). Although the actual B_{12} content of the different diets was not calculated, we have reason to believe that the vitamin B_{12} content of the food in these dietary groups was different. We tried to clarify whether MMA and HCY concentrations reflect dietary habits better than total vitamin B_{12} in serum. This could indicate that these metabolites are better early markers of a disturbed vitamin B_{12} status.

Table 1. Patient characteristics.

	Group				
	HME	All vegetarians[a]	LME	LOV/LV	Vegans
Smoke >5 cigarettes/day	20%	5%	0%	9%	0%
Alcohol consumption[b]	30%	8%	16%	6%	0%
Exercise >7 h/week	26%	61%	57%	62%	66%
BMI,[c] kg/m^2	22.5 ± 3.1	21.6 ± 4.1[d]	21.9 ± 2.3	21.4 ± 3.2[d]	21.4 ± 5.2
Sex, F/M	30/14	36/14	17/2	24/10	5/2

[a] Includes LME group.
[b] Less than the equivalent of 1 alcoholic drink/day.
[c] BMI, body mass index.
[d] Significant at 5% level compared with the HME group (Mann–Whitney test).

Subjects and Methods

SUBJECTS

A total of 104 apparently healthy subjects (randomly selected) living in the same region were investigated and classified into four groups based on their habitual dietary intake. All vegetarians were volunteers recruited at a conference of the German Federation of Vegetarians. High meat eaters and low meat eaters were selected from students and staff members. All participants were interviewed, and the average of three 24-h dietary recalls from the previous 3 days was used to calculate qualitative daily consumption of different nutrients. The participants had to meet the following criteria: constant dietary pattern for at least 1 year, values for basic hematologic variables within the appropriate reference intervals, no renal disease, no vitamin supplementation, no lipid-lowering drugs, no weight-loss diets, no medications or metabolic diseases that influence nutritional status, and no pregnancy. The use of oral contraceptives was not an exclusion criterion. The Human Ethics Committee at the Faculty of Medicine, University Leipzig, Germany approved the investigation. For additional subject characteristics, see Table 1. The principal difference among various vegetarian diets was the extent of which animal products were avoided; the subjects were therefore divided into the following groups:

- High meat eaters (HME; n = 44), who were the controls and consumed a typical omnivorous diet.
- Low meat eaters (LME; n = 19) usually excluded red meat and ate white meat or fish once or twice per week.
- Lacto-ovo vegetarians did not consume meat, poultry, or fish, but had no restrictions as to egg or dairy product consumption. Lacto vegetarians also excluded eggs. These subjects were combined into one group (LOV/LV; n = 34).
- Vegans (n = 7) excluded all foods of animal origin.

LABORATORY TESTS

All tests, with the exception of total antioxidant status (TAS), were performed on serum, which was collected after an overnight fast. The blood was allowed to clot on ice, and serum was obtained by centrifugation (4 °C) within 45 min after venipuncture and stored at −70 °C.

HCY was measured by HPLC with fluorescence detection according to the method of Araki and Sako *(23)* (between-day CV, 4.5%). We found no significant difference between the HCY concentrations in plasma and serum. The HCY results for serum were ~5% higher than results for optimally prepared plasma. MMA was assayed by a modified capillary gas chromatography–mass spectrometry method according to the method described by Allen et al. *(24)* (capillary gas chromatograph Model 6890 with a Model 5973 mass-selective detector; Hewlett-Packard). We used a serum pool prepared in-house for quality control (within-day CV, 2.9%; between-day CV, 6.3%). Serum folate and vitamin B_{12} were measured with a chemiluminescence immunoassay (Bayer) on an ACS Centaur (Bayer). Control sera were obtained from the same company (between-day CVs, 9% for serum folate and 2.7% for vitamin B_{12}).

Plasma TAS was measured on a Hitachi Analyzer with a Randox reagent set (Randox). Control samples were obtained from the same company (between-day CV, <5%). The latter determination is based on the reaction of 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS®) with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS$\cdot^{+}$:

$$\text{HX-Fe}^{3+} + H_2O_2 \rightarrow \text{X-[Fe}^{4+}\text{=O]} + H_2O$$

$$\text{ABTS} + \text{X-[Fe}^{4+}\text{=O]} \rightarrow \text{ABTS}\cdot^{+} + \text{HX-Fe}^{3+}$$

where HX-Fe^{4+} is metmyoglobin. The radical cation has a relatively stable blue-green color, which is measured at

Table 2. Medians (5th/95th percentiles) of metabolites, vitamins, and TAS in vegetarians.

	Group				
	HME (n = 44)	All vegetarians[a] (n = 60)	LME (n = 19)	LOV/LV (n = 34)	Vegans (n = 7)
Age, years	23 (21/53)	22 (19/56)	24 (17/61)	22 (19.5/49.3)[b]	22 (19/30)
HCY, µmol/L Pathologic range >15 µmol/L	9.8 (5.9/16.7)	11.6 (6.3/19.3)[c]	11.8 (6.1/17.0)[b]	11.0 (5.7/20.8)	15.2 (9.3/18.5)[c]
TAS, mmol/L Reference interval, 0.98–1.64 mmol/L	1.21 (1.05/1.43)	1.17 (0.98/1.34)[b]	1.10 (0.95/1.24)[c]	1.21 (0.97/1.40)	1.14 (1.02/1.21)
MMA, nmol/L Reference interval, 73–271 nmol/L	169 (111/280)	209 (111/522)[b]	210 (95/257)	205 (110/572)	246 (153/292)[b]
Vitamin B_{12}, pmol/L Reference interval, 156–674 pmol/L	276 (172/406)	243 (148/386)	240 (118/331)[b]	253 (153/376)	217 (153/438)
Folate, nmol/L Pathologic range <7 nmol/L	17.3 (7/36.5)	17.7 (9.1/33.8)	19.1 (10/26.1)	17.3 (7.9/44)	15.4 (10.4/24.5)

[a] Includes LME group.
[b,c] Significant compared with the HME group (Mann–Whitney test): [b] significant within 1% level; [c] significant within 5% level.

Table 3. Correlation analysis by Spearman ρ.

	Spearman ρ		
	HME (n = 44)	All vegetarians (n = 60)	All (n = 104)
MMA-HCY	0.340[a]	0.351[b]	0.390[b]
B_{12}-MMA		−0.285[a]	−0.253[a]
B_{12}-HCY		−0.299[a]	−0.202[a]
B_{12}-Folate		0.329[a]	0.285[b]
B_{12}-TAS			0.256[b]

[a,b] Significant within the [a] 5% or [b] 1% level.

Table 4. Influence of different variables on HCY, MMA, and TAS concentrations calculated by backward multiple regression analysis.

Independent variable	Variables in the order of their removal	Variables with significant influence: Variable	*P*
HCY	Vitamin B_{12}, TAS, folate	MMA	0.033
		Sex	0.001
		Age	0.000
MMA	Sex, folate, TAS	Age	0.006
		Vitamin B_{12}	0.031
		HCY	0.005
TAS	HCY, MMA, folate, age	Vitamin B_{12}	0.008
		Sex	0.000

600 nm. Antioxidants contained in the serum sample suppress the formation of this color.

STATISTICAL ANALYSIS

Median values and 5th and 95th percentiles were calculated, and the Mann–Whitney test, correlation analysis by the Spearman ρ, and backward regression analyses were performed with the software package SPSS (Ver. 9.0 for Windows; SPSS).

Results

All results were median values because the test results showed skewed distribution, with the exception of TAS and vitamin B_{12}. Compared with age- and sex-matched members of the HME group, the serum HCY concentrations of vegetarians were significantly increased (Table 2). Vegetarians also had higher serum MMA but slightly lower TAS. The serum concentrations of vitamin B_{12} and folate did not differ significantly between both groups. Only vegans showed a median HCY >15 μmol/L. Compared with the HME group, the HCY median value was increased in the other subgroups, but did not reach the pathologic range of >15 μmol/L. Similarly, only the serum MMA concentration of the vegans was significantly increased compared with the HME group, whereas in the other subgroups, the MMA concentration was only slightly increased. Compared with the HME group, all subgroups of vegetarians showed lower vitamin B_{12} serum concentrations, but the median serum vitamin B_{12} was significantly lower only in the LME group. For folate, we found rather uniform concentrations in all subgroups. The TAS of all vegetarian subgroups was lower than that of the HME group, reaching statistical significance only in the LME group. Additionally, vegetarians differed from the omnivorous HME group not only in their dietary habits but also in their lifestyle, e.g., they consumed less alcohol, smoked less, and exercised more (Table 1).

Correlation analysis revealed a highly significant correlation of MMA with HCY (Table 3). Vitamin B_{12} correlated with the other investigated variables (MMA, HCY, folate, TAS) at a 5% significance level. From backward regression analysis, it followed that the HCY concentration was significantly and independently influenced by MMA, age, and sex (Table 4). MMA was independently modulated by age, vitamin B_{12}, and HCY. The TAS was influenced by vitamin B_{12} and sex only.

We found a high frequency of subjects with pathologically increased metabolite concentrations (HCY and/or MMA), whereas serum vitamin B_{12} and folate were pathologically decreased in only five cases (Table 5).

The scatter plots of MMA vs vitamin B_{12}, HCY vs vitamin B_{12}, and MMA vs HCY are depicted in Fig. 2. From Fig. 2A it follows that increased MMA was found only in subjects with serum vitamin B_{12} concentrations up to 360 pmol/L. This concentration is approximately twice as high as the upper reference limit for vitamin B_{12}.

Table 5. Frequency of increased metabolites and decreased vitamins in vegetarians.

	HME (n = 44)	All vegetarians (n = 60)	LME (n = 19)	LOV/LV (n = 34)	Vegans (n = 7)
Increased metabolites, %					
HCY >15 μmol/L	7	20	21	12	57
MMA >271 nmol/L	5	25	5	32	43
HCY and/or MMA[a]	9	37	21	38	71
Decreased vitamins, %					
Folate <7 nmol/L	5	2	0	3	0
B_{12} <156 pmol/L	0	8	11	6	14
Folate and/or B_{12}[b]	5	10	11	9	14

[a] At least one of the metabolites was increased.
[b] At least one of the vitamins was decreased.

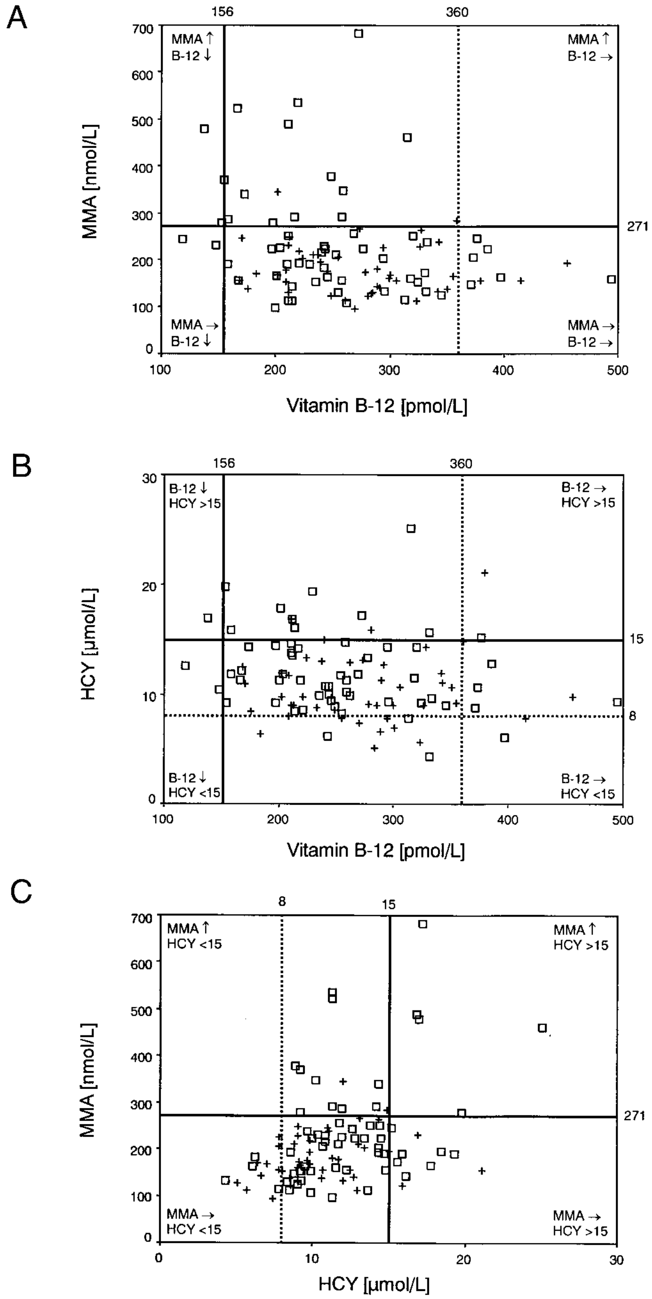

Fig. 2. Scatter plots illustrating the relationship between MMA and vitamin B_{12} (*A*), HCY and vitamin B_{12} (*B*), and MMA and HCY (*C*). □, all vegetarians; +, HME group; ↑, increased; ↓, decreased; →, within the reference interval. The *lines* in each panel indicate the cutoff values.

Decreased serum vitamin B_{12} was linked with increased as well as normal MMA concentrations at almost the same frequency. Fig. 2B shows that, in our subjects, decreased serum vitamin B_{12} concentrations were detected only at HCY serum concentrations >8 μmol/L. Additionally, we found that increased MMA already occurred at HCY serum concentrations >8 μmol/L (Fig. 2C). The plots in Fig. 2 clearly indicate that MMA showed the highest discriminative power between the dietary groups in our study. Therefore, MMA represents the most sensitive test for early vitamin B_{12} deficiency. The odds ratio for all vegetarians, compared with the HME group, to have an increased MMA was 7 (95% confidence interval, 1.51–32.46) and to have an increased HCY was 3.42 (95% confidence interval, 0.90–12.95). An odds ratio for decreased vitamin B_{12} was not calculable.

Discussion

Our investigation clearly demonstrates that vegetarians have higher serum concentrations of HCY than omnivorous controls. The median value for all vegetarians was 11.6 μmol/L, compared with 9.8 μmol/L in omnivorous controls. The HCY concentration increased as the vegetarian diet became more restrictive and peaked in the group of vegans. Twenty percent of all vegetarians (inclusive the LME group) had moderate hyperhomocysteinemia (>15 μmol/L). To date, there has been no unanimous definition of hyperhomocysteinemia. In an European Concerted Action Program, hyperhomocysteinemia had been defined as HCY >12 μmol/L *(25)*. Stampfer et al. *(26)* reported 90th and 95th percentiles for HCY of 14.1 and 15.8 μmol/L, respectively, for men free of a diagnosed vascular disease. They also found a 3.4-fold increased risk for myocardial infarction among men with a HCY concentration >15.8 μmol/L. In the Framingham Heart Study *(27)*, it was shown that the risk for vascular disease was increased at concentrations ≥11.4 μmol/L. It has been suggested that HCY concentrations should be lowered to 9–10 μmol/L and that HCY values <10 μmol/L may be considered desirable *(28, 29)*. HCY values <12 μmol/L are considered as optimal, the range 12–15 μmol/L as borderline, and values >15 to 30 μmol/L are defined as moderate hyperhomocysteinemia *(3)*. The median age of our vegetarian group was 22 years and differed in this respect from the studies mentioned above. However, an age-related risk definition for HCY values is not suggested. Therefore, it can be assumed that a greater proportion of our vegetarians have HCY values in an unfavorable range.

The correlation analysis indicated a significant correlation between MMA and HCY and inversely between vitamin B_{12} and HCY, which is in agreement with results obtained by other investigators *(30, 31)*. Compared with the occurrence of decreased vitamin B_{12} in serum, the vegetarians showed a higher frequency of increased MMA, which has also been reported in elderly subjects *(30, 31)*. Additionally, from backward regression analysis it follows that the HCY is significantly and independently modulated by the MMA concentration but not by serum vitamin B_{12}. This analysis also indicated that MMA is significantly influenced by HCY and serum vitamin B_{12}. It should be mentioned that smoking, alcohol consumption, and physical exercise were not included in the multiple regression model. The results from backward regression analysis were confirmed by a study on elderly subjects who also have a high frequency of vitamin B_{12} deficiency

(31). The relationship between HCY and MMA is most likely caused by impaired functional vitamin B_{12} status because only two enzymes exist that are vitamin B_{12} dependent, L-methylmalonyl-CoA mutase and methionine synthase.

From our study it can be concluded that MMA is a sensitive and specific predictor of dietary group. We may assume that the "dietary groups" represent different degrees of likelihood of subtle cobalamin deficiency. Therefore, it may be expected that MMA is an early, sensitive, and specific marker of impaired cobalamin status. The scatter plot shown in Fig. 2A, presenting the relationship between MMA and vitamin B_{12}, demonstrates that at up to 360 pmol/L vitamin B_{12} in serum, several subjects had increased serum MMA concentrations. Thus, serum vitamin B_{12} concentrations within the reference interval do not exclude a functional vitamin B_{12} deficiency, and conversely, low serum vitamin B_{12} does not confirm functional cobalamin deficiency (only three of five individuals with "low" vitamin B_{12} in serum had increased HCY or MMA). At serum vitamin B_{12} concentrations >360 pmol/L, a functional vitamin deficiency did not occur.

At conventional cutoff values, serum vitamin B_{12} had the highest diagnostic specificity, but at the expense of sensitivity. Furthermore, the vitamin B_{12} measurement in serum detected only four vegetarians, but no members of the HME group, as vitamin B_{12} deficient. At an arbitrary cutoff value of 360 pmol/L, the vitamin B_{12} test would gain diagnostic sensitivity, but would lose all discriminative power. A possible explanation for the low diagnostic efficiency of serum vitamin B_{12} could be that ~80% of total serum vitamin B_{12} is bound to haptocorrin, a late indicator for vitamin B_{12} deficiency, and only ~20% typically is bound to the early indicator, serum transcobalamin II, which is responsible for cellular vitamin B_{12} supply (half-life of only 6 min) *(32)*. The serum vitamin B_{12} concentration does not differentiate between those vitamin B_{12} fractions. Subjects with serum vitamin B_{12} concentrations between 156 and 360 pmol/L and increased MMA have a functional vitamin B_{12} deficiency, which could possibly be attributable to a lowered fraction of holotranscobalamin II. Using the cutoff values for vitamin B_{12} and MMA, we found increased MMA in 25% of the vegetarians, whereas only 8% had serum vitamin B_{12} below the lower reference limit. Thus, our study confirms the findings of other investigators who postulated that the serum MMA concentration is a sensitive indicator of a functional intracellular vitamin B_{12} shortage *(18–20, 31, 33)*. Additionally, the scatter plot presenting the relationship between MMA and HCY (Fig. 2C) shows that only subjects with HCY concentrations >8 μmol/L had increased serum MMA. Similarly, the scatter plot of vitamin B_{12} vs HCY (Fig. 2B) demonstrates that serum vitamin B_{12} concentrations below the lower reference limit were found only in subjects with HCY concentrations >8 μmol/L. Furthermore, the number of cases with increased serum MMA was twice as high as the number of subjects with decreased vitamin B_{12}. Nevertheless, because there is no "gold standard" for vitamin B_{12} deficiency, the role of MMA as a sensitive indicator for vitamin B_{12} deficiency has to be confirmed by further studies. The use of transcobalamin II as a vitamin B_{12} marker together with MMA possibly provides deeper insights *(34)*. Concerning the treatment of hyperhomocysteinemia, our findings support the suggestion that HCY should be lowered to 9–10 μmol/L and that HCY values <10 μmol/L may be considered desirable *(28, 29)* because only subjects with HCY concentrations this low had no imbalances in vitamin B_{12} markers.

The increased HCY concentration in a greater portion of vegetarians may possibly contribute to an increased atherosclerotic risk in these subjects *(13, 35)*. In general, antioxidants play a significant role in the pathogenesis of atherosclerotic and age-related diseases *(6)*. Epidemiologic data strongly support the hypothesis that high consumption of fruits and vegetables that are rich in monounsaturated and polyunsaturated fatty acids, minerals, fiber, complex carbohydrates, antioxidant vitamins, flavonoids, and nutrients together with a otherwise healthy lifestyle protects against degenerative diseases *(36–40)*. A recent publication *(41)* reports that 1 week after a change to a vegan diet-based lifestyle, HCY was significantly reduced (~13%). The authors concluded that because of the short duration of this lifestyle change, factors other than B vitamins are involved in lowering HCY. However, it can be supposed that the generally healthier lifestyle of vegetarians could be partly reversed by increases in HCY as a consequence of vitamin B_{12} deficiency. Mezzano et al. *(35)* reported that increased platelet function and HCY may counteract the known cardiovascular health benefits of a vegetarian diet.

We were able to show that vegetarians, especially the LME and vegan groups, had a reduced TAS, whereas the TAS of LOV/LV was not different from that of the HME group. The TAS decreased with increasing avoidance of vitamin B_{12}-containing animal products, whereas HCY increased in the same order. The total antioxidant concentration correlated highly significantly with the vitamin B_{12} concentration in serum but not with HCY. In addition, the correlation as well as backward regression analysis demonstrated that serum vitamin B_{12}, but not MMA, as a marker for a functional B_{12} status is the variable that influences the TAS. Therefore, from our study we cannot totally exclude that components other than vitamin B_{12} in cobalamin-rich food could possibly improve TAS. The missing significant correlation between HCY and TAS might be attributable to the fact that of total HCY, 98% is oxidized HCY and only ~2% is reduced (free) HCY, which can be oxidized and in this way modulate TAS. Rauma and Mykkanen *(6)* reported that measurements of antioxidant status in vegetarians showed that a vegetarian diet maintains a high antioxidant vitamin status (vitamins C and E, β-carotene) but a variable antioxidant trace element status compared with omnivorous diet. They therefore recommended evaluation of the total antioxidant capacity rather than the status of a single antioxidant nutrient. Our results underscore this statement and add that in subjects on restrictive vegetarian diets, insufficient vitamin B_{12} intake is a very important factor that influences the TAS. Therefore, sufficient vitamin B_{12} supplementation for persons on restrictive vegetarian diets is of great importance.

Additional studies confirming our results are needed. These studies should focus on determining the diagnostic value of vitamin B_{12} markers, such as transcobalamin II and MMA, compared with vitamin B_{12}. Studies on well-characterized vegetarian groups having quantitative dietary protocols could investigate the influence of different vegetarian diets on HCY metabolism, taking special consideration of the content of vitamin B_{12} and other vitamins. A possible influence of confounders, such as renal function, alcohol and coffee consumption, smoking habits, intake of supplements, sex hormones, physical exercise, duration of dietary habit, and other factors should be taken into account and, if possible, excluded or minimized. Last but not least, the importance of supplements, especially vitamin B_{12}, to compensate for the adverse effects of certain vegetarian diets should be considered.

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