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ORIGINAL CONTRIBUTION

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Changes in predictors and status of homocysteine in young male adults after a dietary intervention with vegetables, fruits and bread

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Abstract *Background* Elevated plasma total homocysteine (p-tHcy) is associated with increased risk of cardiovascular disease, and an inverse association has been shown between the dietary intake of B-vitamins, B-vitamin profile and the concentration of p-tHcy. *Aim of the study* The main objective of this investigation was to study the effect of a dietary intervention focusing on an increased intake of vegetables, fruits and bread. The effect of the dietary intervention was determined by the changes in plasma concentrations of tHcy, cysteine (cys), riboflavin, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) and serum concentrations of folate and vitamin B_{12}. *Method* An intervention study with duration of 5 months, including 541 male recruits from the Norwegian National Guard, Vaernes and a control group, including 209 male recruits from the Norwegian Army, Heggelia. *Results* The dietary intervention resulted in decreased concentration of p-tHcy (−10%, $P = 0.002$), p-cys (−6%, $P < 0.001$) and FMN (−11%, $P = 0.310$) and increased concentration of riboflavin (+23%, $P < 0.001$) and FAD (+10%, $P = 0.008$) in the intervention group compared with the control group. The change in p-tHcy concentration was positively related to the change in the concentration of p-cys ($P < 0.001$) and FMN ($P = 0.035$) and inversely related to the change in concentration of folate ($P = 0.021$). *Conclusions* A dietary intervention program focusing on an increased intake of vegetables, fruits and bread showed a favourable effect on the concentration of p-tHcy and its metabolites. Our findings suggest that the changes in the concentration of p-cys, folate and FMN seem to be predictors of changes in the p-tHcy concentration.

Key words dietary intervention – homocysteine – B-vitamins – young men

Introduction

Increased concentration of p-tHcy is a widely acknowledged risk factor for cardiovascular disease (CVD) and is considered to be an independent predictor for mortality [7, 12, 19]. Despite structural similarities between p-tHcy and p-cys, an increased concentration of p-cys have not shown to be a predictor for mortality and morbidity [14]. However, it has been suggested that high p-tHcy and p-cys might act synergistically as cardiovascular risk factors [27,

36]. After comparing and evaluating several techniques for the determination of p-tHcy (interassay CV: 3–11%), Ueland et al. [43] suggested a normal range of p-tHcy concentrations between 5 and 15 μmol/l in healthy subjects. On the other hand, Moore et al. [34] have shown that the normal range for p-tHcy may vary in different ethnic groups.

B-vitamins including folate, vitamin B_{12}, vitamin B_6, riboflavin and the flavo-coenzymes, FMN and FAD, are involved in the metabolism of homocysteine (Fig. 1). Observational studies have confirmed an inverse relationship between the concentration of p-tHcy and the concentration of both folate and vitamin B_{12} in serum [15, 40]. Data on the relationship between p-tHcy and riboflavin is limited, however Hustad et al. [21] have demonstrated that riboflavin in plasma may be inversely related to p-tHcy.

The best natural sources of B-vitamins in the Nordic countries are dairy products, whole-grain products, fruits and green vegetables [4, 13, 42]. Studies focusing on an increased intake of natural rich sources of B-vitamins have shown a decrease in p-tHcy concentration [10, 11]. Huerta et al. [20] reported that an adequate dietary intake of folate and vitamin B_{12} may synergistically decrease the risk of hyperhomocysteinemia. Jacques et al. [23] reported an inverse association between the dietary folate, vitamin B_6 and riboflavin and the concentration of p-tHcy. Similarly, Ganji and Kafai [16] confirmed an inverse association between the dietary intake of folate and riboflavin and the concentration of p-tHcy. However, no association between the dietary intake of vitamin B_{12} and vitamin B_6 and the concentration of p-tHcy was shown in the study of Ganji and Kafai [16]. These results may indicate that many components in the total diet may have an effect on the concentration of p-tHcy.

Most intervention studies, however, have focused on the association between supplementation of folic acid, riboflavin, vitamin B_6 and vitamin B_{12} and the concentration of p-tHcy [25, 26, 29, 30]. A newly published population study indicated that a fall in mortality caused by stroke in the United States was related to an average reduction in p-tHcy after fortification of grain products with folic acid [47]. Studies of patients with CVD have also confirmed that high doses of folic acid significantly reduce the concentration of p-tHcy, but not the mortality rate of the patients [6, 26]. Supplementation with a combination of folic acid and vitamin B_{12} has been suggested to be more effective in lowering p-tHcy in patients with CVD than vitamin B_6 [25]. A study with healthy elderly, replete in folate and riboflavin, reported however that vitamin B_6 effectively lowered p-tHcy concentration [29]. Supplementation of riboflavin has in a newly published study shown to have an inverse effect on the concentration of p-tHcy, depending on the methylenetetrahydrofolate reductase (MTHFR) 677C/T genotype [31].

Several studies have focused on elderly subjects [26, 37] showing that aging independently increase p-tHcy concentration. Measurement of p-tHcy concentrations in children have also been reported [3]. According to our knowledge, limited data is available on the concentration of p-tHcy in young men.

The aim of the present study was to investigate the effect of a dietary intervention focusing on an increased consumption of vegetables, fruits and bread, on the concentration of B-vitamins in blood samples. The effect of the dietary intervention was determined by changes in plasma concentrations of tHcy, cysteine, riboflavin, FAD and FMN and serum concentrations of folate and vitamin B_{12}. In addition, the relationship between the concentrations of p-tHcy

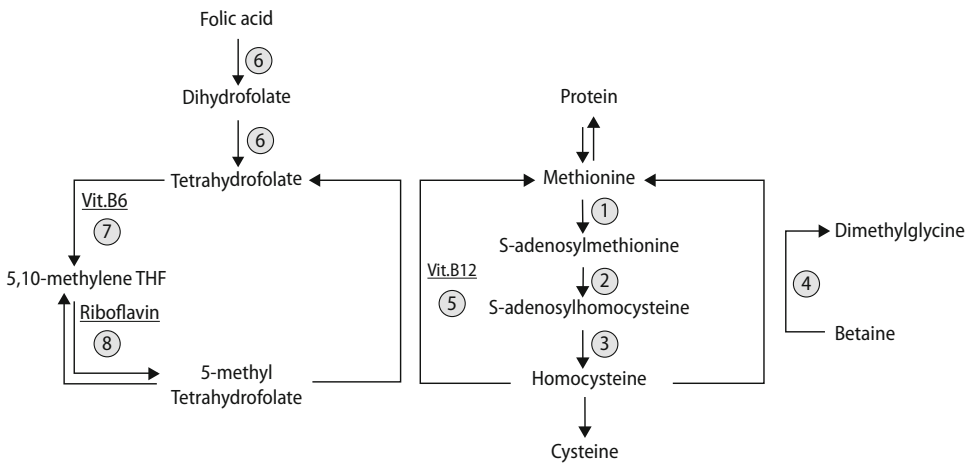

Fig. 1 The role of B-vitamins in homocysteine metabolism. Enzymes: 1 Methionine adenosyltransferase, 2. Methyltransferase, 3. S-adenosylhomocysteine hydrolase, 4. Betaine-homocysteine methyltransferase, 5. Methionine synthase, 6. Dihydrofolate reductase, 7. Serine hydroxy methyltransferase, 8. 5,10-methylene tetrahydrofolate reductase

and B-vitamins, smoking habits, body mass index (BMI) and physical performance were investigated.

Subjects and methods

Study design

A diet rich in vegetables, fruits and bread and low in fat was provided and information on benefits of healthy eating habits was given to the intervention group throughout the whole intervention period. The control group received only practical information regarding the data collection. Blood samples, measurements of body weight and height and information of physical fitness, dietary habits and smoking habits were collected during the first week after the enrolment and after 5 months. The Ministry of Health and Care Services, Regional Committee for Medical Research Ethics and the Data Inspectorate approved the study protocol. Written consents were obtained from the recruits prior to participation in the study.

Participants

Male recruits (18–26 years old) from two consecutive enrolments at Værnes military camp, in January 2004 (n = 231) and July 2004 (n = 432) were asked to participate in the intervention group. In this group, 541 (81.6 %) recruits agreed to complete the baseline registration and 416 (62.7%) recruits completed the entire study. In August 2005, 237 enrolled male recruits in the Norwegian Army at Heggelia were asked to participate in the study as a control group. For baseline registration 209 recruits agreed to participate (88.2%) but only 105 recruits (44.4%) completed the entire study. Most of the recruits who did not participate in the follow up study had been excluded from the military service due to health problems. This was the case both for the intervention and the control group.

Diet

All recruits were offered free breakfast, lunch, dinner and an evening meal in the military mess hall. For the intervention group, the recipes for food used in the military were changed according to the Nordic Nutritional Recommendations with special focus on fruits and green vegetables. The breakfast and evening meals consisted of bread and different sandwich spreads and seasonal fruits. For lunch, the intervention group could choose between bread, sandwich spreads, a hot dish with adjusted fat content and vegetable ingredients and a salad bar. For dinner, they were served a hot dish with adjusted fat content, vegetable ingredients or vegetables (cooked or a mixed salad) as a side dish. In the control group, there was no change in the food regime during military service. This food contained therefore more fat, especially saturated fat and less fruits and green vegetables compared with the food served to the intervention group. The nutritional value of the original and the modified recipes were compared by using a food database developed at the Department of Nutrition, University of Oslo (Mat på Data, version 5.0). In the military mess hall, the recruits chose their own food and also decided the number of servings and the serving sizes by themselves. A commercial military canteen, restaurants/cafeterias and grocery stores were located in or close to the military camps of both the intervention group and the control group. At their own expenses, all of the recruits could also purchase food at these places.

Measurements

A validated food diary developed at Department of Nutrition, University of Oslo (sef.no/assets/11002260/vedlegg1_ungkost.PDF) was modified to appropriately reflect the diet served in the military mess hall [44]. The diary described choices of frequency and portion sizes of some selected food items like vegetables, fruits and bread. To obtain estimates of the consumption in grams, most of the food items that were described in the food diary were also preweighed. The estimates were the average of 10 weighed pieces. The food diary also included questions about how often and what they ate in the military canteen and/or cafeterias/restaurants outside the military camp.

Blood samples were collected from the individuals after an overnight fast (≥12 h). The participants agreed to refrain from smoking and drinking 12 h before blood sampling. The EDTA blood samples were immediately cooled on ice, protected from daylight, centrifuged at 2000g for 10 min at 4°C, and frozen at −20°C before they were sent and stored at −70°C for analyses at Stavanger University Hospital, Norway. The concentrations of p-tHcy and p-cys were determined by HPLC [28]. Plasma concentration of riboflavin, FAD and FMN were determined by a modified HPLC method (Mansoor et al. manuscript in preparation). Serum concentrations of folate and vitamin B_{12} were determined by immunoenzymatic assay (Access, Beckman Instruments, Inc.1998, Chaska, MN, USA). The coefficients of variation for the measured variables were: 6.4% for total homocysteine; 6.8% for cysteine; 8.77% for folate; 5.19% for

vitamin B_{12}; 4.89% for riboflavin; 4.12% for FAD and 7.46% for FMN.

Body weight was measured in kilogram after overnight fasting and height of the recruits were measured to the nearest centimetre. Two physical examinations were done: time, in minutes, for running 3,000 m and score for a test of strength. According to the military requirements, 15 min was the maximum time limit in order to obtain an approved result from the 3,000 m run. Individual strength was measured as number of push-ups, sit-ups and lift-ups, and the total results of the tests were categorised on a 6-point scale. Grade 6 was the highest possible score whereas grade 2 was the lowest score required for approved performance according to the military guidelines. To obtain grade 2, the recruits had to accomplish 16 push-ups, 20 sit-ups and 4 lift-ups. Information on age, smoking habits, use of dietary supplements and medicines was collected using a self-administered questionnaire.

Statistical analyses

To obtain normality, all variables were logarithmically transformed and pearson correlation coefficients were computed to examine the correlations between baseline characteristics. Parameters that correlated significantly with p-tHcy were used in a multiple regression model. Furthermore, a multiple regression model calculated the intervention effects as ratio of geometric means of the intervention groups relative to the control group, adjusted for baseline concentrations [46]. A multiple regression model was also used to calculate the predictors of changes in p-tHcy concentration. All regression models were checked for normality of residuals, linearity, and homogeneity of variance. Because of skewed distribution, p-tHcy concentration (but no other blood values) was transformed to their natural logarithm before fitting regression models. All statistical analyses were conducted with SPSS, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline data collection

Baseline registration showed a total intake of vegetables, fruit, berries and fruit juice (VF) of 434 g/d in the intervention group and 450 g/d in the control group. Total consumption of bread at the baseline was 175 and 188 g/d in the intervention group and the control group, respectively. Biochemical characteristics, BMI, physical fitness and smoking habits of the recruits participating in the baseline data collection are summarized in Table 1. A total of 24.8% of the recruits had p-tHcy concentration >15 μmol/l. Table 2 shows that the concentration of p-tHcy and p-cys was positively correlated ($P < 0.001$). An inverse correlation was shown between the concentration of p-tHcy and the concentration of folate ($P < 0.001$), vitamin B_{12} ($P < 0.001$), riboflavin ($P < 0.001$) and FMN ($P = 0.011$). The concentration of p-tHcy was inversely correlated with the score on the 3,000-m run. The variables, significantly correlated with p-tHcy concentration, explained in combination

Table 1 Baseline characteristics of the study sample[a]

Variable	Intervention group	Control group	*P*-value
No. of participants	541	209	
Smoking (%)			
Current smokers	23.1	17.6	
Former smokers	2.7	3.5	
Nonsmokers	71.9	71.9	
Missing	2.3	7.0	
Age	19.8 (19.6–19.9)	19.2 (19.1–19.4)	<0.001
tHcy (μmol/l)	11.8 (11.3–12.3)	12.5 (12.0–13.1)	0.043
Cys (μmol/l)	233.4 (227.9–239.1)	331.7 (319.3–344.7)	<0.001
Folate (nmol/l)	13.3 (13.0–13.7)	10.1 (9.6–10.6)	<0.001
Vitamin B_{12} (pmol/l)	344.0 (330.1–353.2)	284.8 (267.8–302.8)	<0.001
Riboflavin (nmol/l)	7.7 (7.3–8.1)	6.1 (5.4–7.0)	0.002
FAD (nmol/l)	48.3 (47.7–49.0)	51.9 (49.4–54.5)	0.006
FMN (nmol/l)	13.7 (13.3–14.1)	8.0 (7.5–8.6)	<0.001
BMI (kg/m^2)	23.4 (23.1–23.7)	24.2 (23.6–24.8)	0.014
Physical status; running 3,000 m (min)	14.5 (14.2, 14.7)	14.9 (14.3–15.4)[b]	0.213
Test of strength (grade)	2.2 (2.1, 2.3)	2.0 (1.9–2.2)[c]	0.135

Concentration of tHcy, cys and B-vitamin profile are presented as geometric mean and 95% CI in parentheses
tHcy total homocysteine, *Cys* cysteine, *FAD*, flavin adenine dinucleotide, *FMN* flavin mononucleotide, *BMI* body mass index
[a]The maximum proportion of missing value in different variables is 16.18%
[b]Corresponds to 60.7% approved results according to the military guidelines
[c]Corresponds to 52.2% approved results according to the military guidelines

Table 2 Pearson correlation coefficients for total plasma homocysteine (tHcy) and concentrations of selected vitamins involved in homocysteine metabolism[a]

	tHcy	Cys	Folate	Vitamin B_{12}	Riboflavin	FAD	FMN	BMI	Smoking	No. sig./day	Test1: running	Test2: strength
tHcy	1.000	0.386**	−0.418**	−0.327*	−0.170**	−0.055	−0.120*	0.012	0.056	−0.007	−0.121*	−0.057
Cys		1.000	−0.242**	−0.137**	−0.069	0.020	−0.332**	0.140**	0.029	−0.024	−0.035	−0.021
Folate			1.000	0.337**	0.251**	−0.087	0.321**	0.041	−0.115**	0.040	0.089	0.047
Vitamin B_{12}				1.000	0.221**	0.081	0.165**	−0.077	−0.037	−0.037	0.070	0.133**
Riboflavin					1.000	0.067	0.648**	−0.020	−0.043	−0.051	0.047	0.083
FAD						1.000	0.037	0.051	−0.026	0.012	0.058	0.062
FMN							1.000	−0.137**	−0.053	−0.091*	0.181**	0.160**
BMI								1.000	−0.019	0.041	−0.209**	−0.330**
Smoking									1.000	0.522**	−0.136**	−0.049
No. sig./day										1.000	−0.264**	−0.288**
Test1: running											1.000	0.378**
Test2: strength												1.000

$n = 641$
tHcy total homocysteine, *Cys* cysteine, flavin adenine dinucleotide, *FMN* flavin mononucleotide
[a]All variables in the table have been $\log_e$ transformed before comparison
*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

23.2% of the variance of p-tHcy concentration in a linear regression model. The regression analysis showed a significant positive effect for the concentration of p-cys ($P < 0.001$) and significant negative effects for the concentrations of folate ($P < 0.001$), vitamin B_{12} ($P < 0.001$) and riboflavin ($P = 0.022$) as predictors of p-tHcy. The score on the 3,000-m run and the concentration of FMN, however, were not associated with the concentration of p-tHcy.

The intervention study

The participants in the intervention group that completed the entire study had a significantly higher baseline concentration of folate ($P < 0.001$), riboflavin ($P = 0.046$) and FMN ($P < 0.001$) compared with the control group. On the other hand, the baseline concentration of p-cys ($P < 0.001$), FAD ($P = 0.003$) and level of BMI ($P = 0.004$) were lower in the intervention group than in the control group. Baseline data showed no significant differences between dietary intake of VF and bread, the concentration of p-tHcy, vitamin B_{12} and the result of the 3,000-m run between the intervention group and the control group.

During the study period, total intake of VF and bread was increased in the intervention group (+24 and +29% respectively) compared with the control group (+6 and −4% respectively). The change in consumption of VF and bread was significantly higher in the intervention group than the control group ($P < 0.001$ for both).

A significant decrease in the concentration of p-tHcy ($P < 0.001$) was observed in the intervention group. No significant change in p-tHcy concentration was shown in the control group (intervention effect, $P = 0.002$, Fig. 2 and Table 3). Compared to the control group, a significant decrease in the concentration of p-cys was shown in the intervention group ($P < 0.001$). Both groups showed a significant increase in the concentration of serum folate ($P < 0.001$ for both). The control group, which had a significantly lower baseline concentration of serum folate, showed a greater increase in serum folate during the study period than the intervention group ($P = 0.009$). The dietary intervention did not show a significant effect on the concentration of vitamin B_{12}.

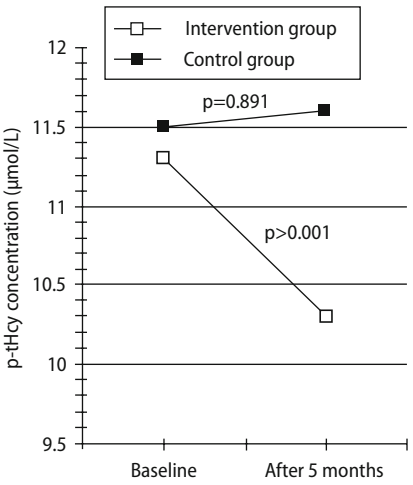

Fig. 2 Changes in the concentration of homocysteine during the intervention

The concentration of riboflavin increased in the intervention group ($P = 0.004$) and decreased in the control group ($P = 0.001$) during the study period, which resulted in a significant intervention effect ($P < 0.001$). A significant effect of the intervention was also shown in the concentration of FAD ($P = 0.008$) but not FMN.

Table 3 Changes in concentration of plasma total homocysteine (p-tHcy), plasma cysteine and B-vitamins[a]

	Baseline	After 5 months	Changes (%)	Intervention effect[b]
tHcy (μmol/l)				
Intervention group	11.3 (10.9–11.7)	10.3 (9.9–10.7)	−8.8	0.90 (0.85–0.96)[c]
Control group	11.5 (10.8–12.3)	11.6 (10.8–12.4)	+0.9	
Cys (μmol/l)				
Intervention group	234.1 (228.5–240.0)	231.0 (223.9–237.4)	−1.3	0.68 (0.62–0.76)[d]
Control group	320.5 (301.6–340.6)	336.4 (323.4–350.0)	+5.0	
Folate (nmol/l)				
Intervention group	12.9 (12.6–13.3)	13.8 (13.4–14.2)	+7.0	0.91 (0.85–0.98)[c]
Control group	10.2 (9.5–10.9)	13.1 (12.4–13.8)	+28.4	
Vit.B12 (pmol/l)				
Intervention group	335.6 (324.3–347.3)	347.8 (336.7–359.2)	+3.6	0.99 (0.94–1.04)
Control group	317.0 (287.2–349.9)	333.5 (310.9–357.7)	+5.2	
Riboflavin (nmol/l)				
Intervention group	7.6 (7.2–8.0)	8.1 (7.6–8.5)	+6.6	1.29 (1.17–1.42)[d]
Control group	6.4 (5.5–7.5)	5.3 (4.6–6.2)	−17.2	
FAD (nmol/l)				
Intervention group	48.3 (47.7–48.9)	49.7 (49.1–50.4)	+2.9	1.05 (1.01–1.09)[c]
Control group	52.6 (49.8–55.5)	48.9 (46.0–52.1)	−7.0	
FMN (nmol/l)				
Intervention group	13.7 (13.3–14.1)	13.2 (12.8–13.6)	−3.6	0.96 (0.90–1.03)
Control group	8.0 (7.3–8.8)	8.6 (7.8–9.4)	+7.5	

Intervention group; $n = 414$, Control group; $n = 91$. Dependent variables in the table are $\log_e$ transformed before comparison
tHcy total homocysteine, *Cys* cysteine,*FAD* flavin adenine dinucleotide, *FMN* flavin mononucleotide
[a]Blood concentrations are presented as geometric mean. 95% CIs in parentheses
[b]Intervention effect calculated as ratio of geometric means of the interventions groups relative to the control groups, adjusted for baseline values
[c]Significant intervention effect, $P < 0.01$
[d]Significant intervention effect, $P < 0.001$

A repeated measure of BMI at the end of the study showed an increase in both the intervention group (+1.46 kg/m^2) and in the control group (+0.76 kg/m^2) compared with the baseline ($P < 0.001$ for both). The results from the physical examinations showed a higher score in running performance for both the intervention group and the control group at the end of the study period. Between the baseline registration and the final registration after 5 months, mean result from the 3,000 m run improved with 1.02 min in the intervention group ($P < 0.001$), whereas an improved result of only 0.42 min ($P = 0.002$) was shown in the control group. In the intervention group, the test of strength also showed a significantly improved mean grade among the recruits at the end of the study ($P < 0.001$).

A change in the concentration of p-tHcy was dependent on the changes in the concentration of p-cys, folate and FMN (Table 4). The variables included in the analysis explain in combination 27.3% of the variance in the change of the p-tHcy concentration during the study period.

Table 4 Standardised multiple linear regression coefficients as predictors of changes in plasma total homocystein (tHcy) concentration in recruits in the Norwegian National Guard

Predictor variable[a]	β-value	P-value
Cys (μmol/l)	0.001	<0.001
Folate (nmol/l)	−0.003	0.021
Vitamin B_{12} (pmol/l)	0.000	0.274
Riboflavin (nmol/l)	−0.002	0.084
FAD (nmol/l)	−0.001	0.125
FMN (nmol/l)	0.004	0.035
$R^2 = 27.3\%$		

The dependent variable, tHcy is $\log_e$ transformed before comparison
Cys cysteine, *FAD* flavin adenine dinucleotide, *FMN* flavin mononucleotide
[a]Computed as follow-up concentrations minus baseline concentrations. All 7 variables were included in a single analysis

Discussion

This intervention study was performed in the Norwegian military, which is one of the best-suited organizations to obtain a representative group of young men in our country. As military service is compulsory, men from different social classes and geographical areas are randomly distributed to different military camps in Norway. The dietary intervention in the present study was designed to create conditions that encourage and lead to healthy food choices according to preferences of the selection offered in the military mess. Therefore, this study does not have a strictly controlled dietary intake.

Relationship between the baseline characteristics

The baseline results showed that the concentration of p-tHcy was quite high and the concentration of B-

vitamins was quite low, in particular in the control group. This association is in line with several cross-sectional studies which have demonstrated an inverse relationship between the concentration of p-tHcy and several B-vitamins [15, 21, 40].

In agreement with previous studies, the present study showed a positive association between the concentrations of p-tHcy and p-cys [14, 27, 36]. This positive association has been suggested to reflect the structural and metabolic relationship between p-tHcy and p-cys [8]. Furthermore, the present study shows an inverse relationship between the concentration of p-tHcy and the concentration of vitamin B_{12} and folate, which is in agreement with other studies [2, 15, 18].

The present study showed that p-tHcy status was related to the plasma concentration of riboflavin. Few previous studies have focused on this relationship. A study by Moat et al. [33], however, demonstrated an inverse correlation between p-tHcy and riboflavin, whereas p-cys was inversely related to folate, vitamin B_{12} and FMN, but not riboflavin. In our study a weaker relationship between p-cys and B-vitamin status compared with the relationship between p-tHcy and B-vitamin status was found, which is in agreement with Bates et al. [3]. Folate, vitamin B_{12} and FMN was positively correlated to riboflavin in the present study. A positive, but weaker association was also shown between riboflavin, folate, vitamin B_{12} and FMN in the study of Moat et al. [33].

In agreement with our results which showed no relationship between the running performance and the p-tHcy concentration in regression analyses, a newly published study found no relationship between physical fitness and p-tHcy concentration in male adolescents [39]. Previously, an inverse association is shown between physical activity and the concentration of p-tHcy in middle aged men and women [17, 35].

The intervention study

A marked reduction in the concentration of p-tHcy was shown among Norwegian recruits in the study after 5 months with increased availability and information about healthy foods. A similar dietary intervention program [38] reported a significant reduction in the concentration of p-tHcy after 2 years of follow-up. In contrast to our investigation, this latter mentioned study included only high-risk individuals.

Results from the present study showed that the control group in our study had a greater increase in the concentration of folate after the 5-months of the intervention period compared with the intervention group. The concentration of folate however, both at baseline and at the end of the study period, was higher in the intervention group compared with the control group. Thus failure to demonstrate any positive intervention effect on folate status in the present study may result from the fact that the subjects in intervention group had achieved a higher concentration of serum folate prior to the study than the control group.

In line with the result from the present study, Venn et al. [45] showed that an increased intake of natural folate after individual dietary counselling improved the concentration of serum folate and decreased the concentration of p-tHcy. This study, however, included only subjects with p-tHcy concentrations ≥10 μmol/l.

In addition to dietary information, the present study included increased availability of healthy foods. In accordance with our findings, several studies have demonstrated an increased concentration of serum folate and a decreased concentration of p-tHcy after an increased intake of fruits and vegetables [5, 10, 11]. Another study, however, showed no impact of fruits and vegetables on the concentration of p-tHcy [1]. Different findings in previous studies may suggest that there is a difference in folate availability from mixed diets depending on the different sources of folate and methods of food processing [11, 41].

In agreement with the present study, no association between the changes in the concentration of vitamin B_{12} in serum and the changes in the concentration of p-tHcy could be detected in the study of Appel et al. [1].

The present dietary intervention with increased focus and availability of fruits, vegetables and bread resulted further in an increased concentration of riboflavin and FAD in plasma. Our study also showed that the concentration of p-tHcy was inversely related to the concentration of serum folate and positively related to the concentration of plasma FMN. The change in the concentration of p-tHcy reflected a decrease in the concentration of p-tHcy during the intervention period. It is expected that during the intervention, a possible increase in the concentration of riboflavins could take place because the participants of this study increased their intake of vegetables, fruits and bread. Previously, genetic studies have also shown that effect of riboflavin is dependent on the MTHFR 677C/T genotype [21, 22, 24].

There are indications that dietary FAD and FMN are hydrolyzed by the enzymes phosphatases or pyrophosphatases of the gut mucosa, which liberate free riboflavin [9]. However, it remains to be established whether during increased dietary intake of FAD and FMN, the liberation of riboflavin from FAD or FMN is identical or similar or has any association with each other. Therefore, we could suggest that more studies might be required to address this question in healthy subjects as well as in patients with CVD.

A decreased concentration of p-tHcy caused by an increased intake of folic acid and other B-vitamins however, has not been shown to alter the risk of cardiovascular events or mortality in some clinical investigations [6, 26]. The concentration of tHcy may on the other hand be a useful marker of vascular risk and not a causal factor.

As studies have shown adverse effects of folic acid supplementation in certain individuals, the optimal dose and supplementation period of folic acid have been discussed by a number of researchers [32, 48]. Therefore, interventions to increase the intake of B-vitamins by increasing the consumption of vegetables, fruits and bread may be the safest way of decreasing the p-tHcy concentration in patients with CVD and healthy subjects.

Conclusions

Our findings show that a dietary intervention with increased focus and availability of vegetables, fruits and bread, significantly reduced the concentration of p-tHcy. The reduction in the concentration of p-tHcy seems to be due to the increase in the concentration of serum folate and reduction in FMN.

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