Association between folate intake from different food sources in Norway and homocysteine status in a dietary intervention among young male adults

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The aim of the present investigation was to study the effect of a dietary intervention which combined nutrition information with increased availability of vegetables, fruits and wholegrain bread. The effect of the intervention was determined by changes in the intake of vegetables, fruits, wholegrain bread and estimated nutrients. Furthermore, the study investigated whether changes in relative contribution from different food sources of folate were related to changes in the concentration of plasma total homocysteine (p-tHcy). The 5-month intervention study included 376 male recruits from the Norwegian National Guard, Vaernes (intervention group) and 105 male recruits from the Norwegian National Guard, Heggelia (control group). The study resulted in an increase in the total consumption of vegetables, fruits, berries and juice ($P < 0.001$) and of wholegrain bread ($P < 0.001$). The participants in the intervention group showed a higher increase in the intake of dietary fibre ($P < 0.001$) and folate ($P < 0.001$) compared with the control group. The relative contribution of folate intake from fruits, vegetables and wholegrain bread was higher in the intervention group compared with the control group ($P < 0.001$ for all). The increased intake of folate from wholegrain bread was inversely associated with a reduced concentration of p-tHcy ($P = 0.017$). In summary, the dietary intervention resulted in an increased intake of vegetables, fruits and wholegrain bread and a subsequent increase in folate intake from these food components. Reduction in the concentration of p-tHcy was significantly related to an increased folate intake due to an increased consumption of wholegrain bread.

Dietary intervention studies: Homocysteine: Young male adults

Young male adults are an important but difficult target group to reach when promoting healthy diets. The period when young men are moving away from home for the first time, for example, when they are enrolled in military service, often involves changes in dietary habits[1].

In several European countries, descriptive studies have shown that subjects in all age groups, including children and adolescents, have an increased intake of high-energy foods, lower intakes of vegetables, fruits and wholegrain products and lower level of physical activity[2–6]. Unhealthy dietary habits from childhood and adolescence (11–16 years of age) have been demonstrated to be carried over into adulthood and thereby affect the prevalence of cardiovascular risk factors[7–9]. A follow-up study of young men and women (mean age 25 and 33 years, respectively) showed that their eating behaviour from childhood was a significant predictor for their intake of fruits, vegetables, wholegrain and sugar-rich foods[10]. These studies have confirmed the need to promote and improve healthy eating habits in early age and with special focus on individuals with low intakes of fruits, vegetables and wholegrain products.

A literature review suggested that improved eating habits can be achieved by a variety of approaches in different subgroups in a population[11]. Dietary interventions among adults have often been focused on strategies to increase nutritional knowledge[12,13]. A significant improvement in dietary habits has been shown to require a combination of different approaches when targeting specific population groups[14–16]. Intervention studies in the age group of 6–18 years have proved most effective when dietary information has been combined with increased availability of fruits and vegetables[17,18]. Despite positive effects from these studies mentioned, an intervention programme focusing on both nutritional information and increased availability of healthier foods among pupils in secondary schools showed no significant changes in school-based eating over a 2-year period[19]. Data from various intervention programmes have demonstrated how difficult it is to achieve changes in dietary habits in children and adolescents.

Previously, adults have been the main target group in studies that have evaluated the effects of dietary interventions on changes in cardiovascular risk factors. An 8-month intervention study with a low-fat (< 30 % energy from fat) and high-dietary fibre (> 3 g/MJ) diet showed a reduction in the concentration of total cholesterol among young men and women (mean age 24 years)[20]. Dragsted *et al.*[21] also confirmed a significant decrease in the concentration of total cholesterol and LDL

Abbreviation: p-tHcy, plasma total homocysteine.

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after providing 600 g fruit and vegetables per d to forty-three volunteers for 24 d. Other studies have shown that an increased intake of dietary fibre through vegetables, fruits and wholegrain cereals beneficially affected the lipid profile and the concentration of homocysteine[22,23]. Despite the uncertainty whether plasma total homocysteine (p-tHcy) concentration may be a marker of vascular risk or a causal factor, studies have shown that elevated p-tHcy concentration is associated with an increased risk of CVD[24,25].

The target group of the present intervention study was men between 18 and 26 years of age, since this group seldom has been the focus of nutrition interventions. The aim of the study was to promote an increased intake of vegetables, fruits and wholegrain bread by focusing on both nutrition information and increased availability of these food items. The effect of the intervention was determined by changes in the intake of selected food items and nutrients. Previously it was reported that this dietary intervention had shown a significant increase in the total intake of vegetables, fruit, berries and fruit juice and wholegrain bread ($P < 0.001$ for both) and a reduction in the concentration of p-tHcy (-10%; $P = 0.002$) during the study period[26]. The present study further investigated whether the reduction in p-tHcy was associated with changes in folate intake from specific foods, such as vegetables, fruits, juice and wholegrain bread.

Methods

Study design and participants

In the intervention group, 541 (81·6 %) male recruits (aged 18–26 years) from Værnes military camp agreed to participate in the data collection at baseline and 416 (62·7 %) of the recruits completed the entire study period. In the control group, 209 (88·2 %) newly enrolled male recruits in the Norwegian Army at Heggelia agreed to participate, but only 105 recruits (44·4 %) completed the entire study period. Most of the recruits (89·6 %) who did not complete the entire study had been excluded from military service due to health problems. Supplement use and incomplete datasets were exclusion criteria for the present study. We did not find any sociodemographic differences between participants in the intervention group and the control group that could explain the differences in participation rate.

As Norwegian military service is compulsory for men from the age of 19 years, recruits from different geographical areas and social classes in Norway are randomly distributed to different military camps. The military camps where our data were collected were chosen due to similar levels of physical training during military service. The intervention group was provided with information regarding the benefits of healthy eating habits, as well as easy access to a diet rich in vegetables, fruits and wholegrain bread, and low in fat content. The control group received only practical information regarding the data collection. Blood samples, measurements of body weight and height and information about dietary habits and age were collected during week 1 after the enrolment and after 5 months. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Ministry of Health and Care Services, Regional Committee for Medical Research Ethics and the Data Inspectorate. Written informed consent was obtained from the recruits before participation in the study.

Diet

All recruits were offered free breakfast, lunch, dinner and evening meal in a mess hall or they could choose to buy food in a commercial canteen, both of which were located at the military camps. In the canteen, the recruits had the option to buy fast food, snacks and candies, whereas the availability of fruits and vegetables was very limited. Alternative places to buy food included restaurants, cafeterias and grocery stores located outside Værnes and Heggelia military camps. In the mess hall at Værnes military camp, the commonly used recipes were prepared focusing on vegetables, fruits, wholegrain bread and dietary fat content in line with the Nordic Nutritional Recommendations. The breakfast and evening meals consisted of wholegrain bread and various sandwich spreads. The amount of wholegrain varied between 50 and 100 % in the different bread products. In Norway, folate fortification of bread is not yet allowed. It is also important to notice that refined white flour that is used in Norwegian bread has a high extraction rate (close to 80 %). For lunch, the recruits could choose between a hot dish, wholegrain bread, various sandwich spreads and a salad bar consisting of some selected vegetables. For dinner they were offered one hot dish and they could also choose to have vegetables (cooked or a mixed salad) and wholegrain bread. In the military mess hall, the recruits selected their own food and portion size. The military chefs were trained in how to prepare and present healthy meals. To avoid differences in the composition of meals due to limited seasonal availability of ingredients, the meals were mainly based on ingredients that were available during all seasons. In the control group at Heggelia military camp, there was no change in the food regimen during military service. This food contained therefore more fat, especially saturated fat, and fewer vegetables and fruits were available, compared with the food offered to the intervention group.

Food diary and calculation of nutrient intake

A validated food diary developed at the 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[27]. Frequency and portion sizes of selected food items available in the mess hall were pre-printed choices in the food diary. The food items included vegetables, fruits, wholegrain bread and other cereals, butter, milk products and drinks. When appropriate, the recruits reported their intakes of vegetables, fruits and wholegrain bread in pieces. To assist the recruits and to obtain estimates of the consumption in grams, most of the food items that were printed in the food diary were also pre-weighed. The estimates are the average of ten weighed pieces. Hot dishes offered at lunch and dinners were included in the diary as open-ended questions in which the recruits filled in a number for the menu of the day. To estimate the consumption of different ingredients included in the hot meals, recipes and estimates of the amounts of the ingredients used in every dish were collected. An average content of

ingredients in all dishes and in the small, medium, large and extra-large serving sizes was calculated from these data. For registration of side dishes served at lunch and dinner, the recruits stated the size (small, medium, large or extra-large) and number of servings. Serving sizes for these food items were decided with the help from the kitchen staff at the camp and from other Norwegian surveys[28]. For determination of serving size, the recruits were asked to refer to photographs and/or weighed portion sizes placed in the military mess during the meals in the food diary period. In addition, two research workers were present at every meal to help the recruits to determine their own portion sizes. This support was given to the recruits in both the intervention group and the control group. The food diary also included questions about how often and what they did eat in the military canteen and/or cafeterias and restaurants outside the military camp.

The nutritional values of recipes and intakes of food and nutrients from the food diaries were calculated using the computer program Mat på Data (version 5.0; Matportalen, Oslo, Norway) and the Norwegian food composition table[29]. The average daily consumption for each recruit in the survey period was obtained by adding up their total consumption of selected foods and nutrients and dividing by the number of days during which the subject kept records.

Blood sampling and biochemical analyses

Blood samples were collected after an overnight fast (≥ 12 h). The participants were restricted from drinking and using tobacco 12 h before blood sampling. All blood samples were centrifuged within 4 h at $2000\,g$ for 10 min at 4°C. Serum and plasma samples were immediately frozen in 1 ml blood collection tubes at -20°C before they were sent to Stavanger University Hospital, Norway and stored at -70°C until analysed. Total serum cholesterol, TAG and HDL were measured with enzymic procedures (Roche, Mannheim, Germany) at Stavanger University Hospital. The concentration of LDL was estimated by using the Friedewald formula (total cholesterol $-$ HDL $-$ $0.46 \times$ TAG)[30]. The plasma concentration of tHcy was determined by HPLC[31]. The CV for the measured variables were <0.3 % for total cholesterol, <0.7 % for TAG, <2 % for HDL and 6.4 % for p-tHcy.

Statistical analyses

Changes in dietary intakes of food and nutrients are presented as means and corresponding 95 % CI (Tables 1–3). A multiple regression model calculated the intervention effects as the ratio of means of the intervention group relative to the control group, adjusted for baseline concentrations[32] (Fig. 1 and Tables 1–3). The regression models were checked for normality of residuals, linearity and homogeneity of variance. A multiple regression model was also used to calculate the predictors of changes in p-tHcy concentration (Table 4). Because of skewed distribution, change in the concentration of p-tHcy (dependent variable) was transformed to the natural logarithm before fitting the regression model. All statistical analyses were conducted with SPSS (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of those participants who completed the entire study showed a significantly lower BMI in the intervention group than in the control group (Table 1; $P=0.004$). The concentrations of p-tHcy and serum lipids, however, were not significantly different between the groups.

Changes in the dietary intake of energy from carbohydrates, proteins and different types of fat are listed in Table 2. The results showed a significantly increased energy intake in the intervention group compared with the control group ($P=0.002$). The percentage of energy derived from carbohydrates and PUFA increased in the intervention group compared with the control group ($P<0.001$ for both). On the contrary, a significantly reduced percentage of energy was derived from total fat, SFA, MUFA and proteins in the intervention group compared with the control group ($P<0.001$ for all). The dietary intervention showed no effect on the serum concentration of total cholesterol, LDL and HDL in serum (data not shown). A minor increase in the concentration of TAG was shown in both groups at the end of the study period. The increase in the concentration of TAG, however, was significantly higher in the control group compared with the intervention group ($P=0.045$).

Baseline registration showed a total intake of vegetables, fruits, berries and juice of 434 g/d in the intervention group and 450 g/d in the control group. The total consumption of

Table 1. Baseline characteristics of the study participants who completed the entire study

(Mean values and 95 % confidence intervals)

Variable	Intervention group		Control group		P
	Mean	95 % CI	Mean	95 % CI	
Participants (n)	414		91		
Age (years)	19·6	19·4, 19·7	19·3	19·1, 19·5	0·059
BMI (kg/m²)	23·3	23·0, 23·6	24·5	23·8, 25·3	0·004
tHcy (μmol/l)	11·3*	10·9, 11·7	11·5*	10·8, 12·3	0·830
Total cholesterol (mmol/l)	3·8	3·8, 3·9	3·9	3·7, 4·0	0·770
TAG (mmol/l)	0·7	0·7, 0·7	0·7	0·6, 0·8	0·655
HDL (mmol/l)	1·3	1·3, 1·4	1·3	1·3, 1·4	0·686
LDL (mmol/l)	2·2	2·1, 2·2	2·2	2·1, 2·4	0·575

tHcy, plasma total homocysteine.
* Concentration of tHcy is presented as the geometric mean.

Table 2. Changes in energy intake during the intervention study†

(Mean values and 95 % confidence intervals)

	Baseline		After 5 months			Intervention effect‡	
	Mean	95 % CI	Mean	95 % CI	Change (%)	Mean	95 % CI
Energy (kJ)							
Intervention group	8676	8354, 8997	9349	8998, 9700	+7.8		
Control group	10 905	10 295, 11 514	9234	8650, 9818	−15.3	1006*	377, 1635
Fat (% energy)							
Intervention group	33.1	32.2, 34.0	27.8	26.8, 28.9	−16.0		
Control group	36.3	35.0, 37.7	33.8	32.4, 35.1	−6.9	−3.4**	−5.1, −1.7
SFA (% energy)							
Intervention group	13.0	12.7, 13.3	9.6	9.3, 10.0	−26.1		
Control group	13.9	13.4, 14.4	13.5	13.0, 14.0	−2.9	−2.9**	−3.6, −2.3
MUFA (% energy)							
Intervention group	9.5	9.3, 9.8	6.5	6.3, 6.7	−31.6		
Control group	10.8	10.4, 11.3	10.1	9.7, 10.5	−6.5	−3.0**	−3.4, −2.5
PUFA (% energy)							
Intervention group	8.0	7.6, 8.5	9.1	8.6, 9.6	+13.7		
Control group	8.7	8.0, 9.3	8.0	7.3, 8.7	−8.0	1.5**	0.7, 2.2
Carbohydrates (% energy)							
Intervention group	51.1	50.2, 52.0	58.3	57.4, 59.3	+14.1		
Control group	47.4	46.1, 48.8	50.6	49.2, 52.0	+6.7	5.0**	3.3, 6.7
Protein (% energy)							
Intervention group	15.8	15.5, 16.1	13.8	13.5, 14.1	−12.7		
Control group	16.2	15.6, 16.9	15.6	15.0, 16.2	−3.7	−1.4**	−2.0, −0.9

The effect of the intervention was significant: *P<0.05, **P<0.001.
† Intervention group, *n* 376; control group, *n* 105.
‡ The intervention effect was calculated as the mean of the intervention group relative to the control group, adjusted for baseline values.

wholegrain bread at baseline was 175 and 188 g/d in the intervention and control groups, respectively. After the 5-month dietary intervention, the total intake of vegetables, fruits, berries and juice increased to 538 g/d (+24 %) in the intervention group and 478 g/d (+6 %) in the control group (intervention effect; $P=0.014$). The consumption of wholegrain bread increased to 225 g/d (+29 %) and decreased to 180 g/d (−4.2 %) in the intervention and control groups, respectively (intervention effect; $P<0.001$). The estimated total intake of dietary fibre and folate increased significantly in the intervention group compared with the control group during the study period (Table 3; $P<0.001$ for both). A minor but significant reduction was shown in the dietary intake of vitamin B_2 (riboflavin) in the intervention group compared with the control group ($P=0.018$). A positive correlation, however, was

shown between changes in estimated total intake of dietary fibre and vitamin B_2 (R_p 0.502), dietary fibre and folate (R_p 0.720) and folate and vitamin B_2 (R_p 0.652) during the study period ($P<0.001$ for all).

The total intake of estimated dietary fibre from vegetables, fruits, berries and juice increased from 4.4 g/d to 4.9 g/d in the intervention group and decreased from 4.4 g/d to 3.4 g/d in the control group. At the same time, the total intake of estimated dietary fibre from wholegrain bread increased from 10.2 g/d to 13.2 g/d in the intervention group and from 11.1 g/d to 11.6 g/d in the control group (Fig. 1). Thus, a significant effect of the intervention was shown in the relative contribution of dietary fibre from both vegetables, fruits, berries and juice ($P<0.001$) and wholegrain bread ($P=0.001$). Furthermore, the study showed a significant increase in the estimated folate intake

Table 3. Changes in dietary intake of fibre, folate and vitamin B_2 during the intervention study†

(Mean values and 95 % confidence intervals)

	Baseline		After 5 months			Intervention effect‡	
	Mean	95 % CI	Mean	95 % CI	Change (%)	Mean	95 % CI
Fibre (g/d)							
Intervention group	24.7	23.4, 26.0	31.1	29.6, 32.6	+25.9		
Control group	24.5	22.8, 26.2	24.2	22.3, 26.0	−1.2	5.9**	3.6, 8.3
Folate (μg/d)							
Intervention group	196.3	188.4, 204.2	257.7	248.5, 266.9	+31.3		
Control group	189.9	179.4, 200.5	209.8	196.0, 223.5	+10.5	31.0**	15.3, 46.7
Vitamin B_2 (mg/d)							
Intervention group	1.54	1.45, 1.60	1.49	1.43, 1.56	−2.7		
Control group	1.81	1.69, 1.92	1.76	1.64, 1.92	−2.4	−0.1*	−0.3, 0.0

The effect of the intervention was significant: *P<0.05, **P<0.001.
† Intervention group, *n* 376; control group, *n* 105.
‡ The intervention effect was calculated as the mean of the intervention group relative to the control group, adjusted for baseline values.
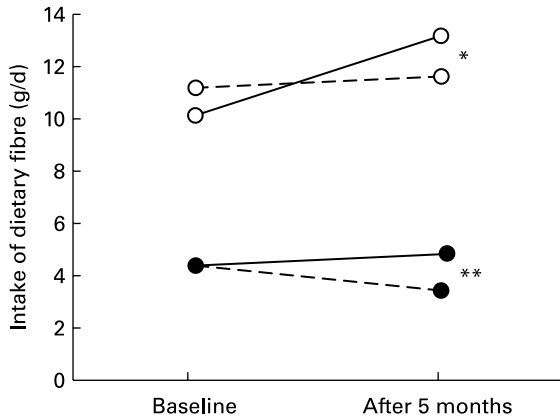
Fig. 1. Changes in intake of dietary fibre from wholegrain bread and vegetables, fruits, berries and juice in the intervention study in the intervention (n 376) and control (n 105) groups. (−○−), Wholegrain bread, intervention group; (--○--), wholegrain bread, control group; (−●−), sum of vegetables, fruits, berries and juice, intervention group; (--●--), sum of vegetables, fruits, berries and juice, control group. The intervention effect was calculated as the mean of the intervention group relative to the control group, adjusted for baseline values. The effect of the intervention was significant: *$P = 0.001$, **$P < 0.001$.

from different food groups, including vegetables, fruits and wholegrain bread (intervention effect, $P < 0.001$ for all; Table 4). The estimated folate intake from different food groups, both at baseline and after the 5-month intervention period, showed that wholegrain bread was the most important single contributor to total folate intake.

Furthermore, we investigated the association between changes in the concentration of p-tHcy and changes in absolute intakes of major food groups (fruits, vegetables, juice, wholegrain bread, milk products and potatoes) during the

intervention study. There was an inverse relationship between the concentration of p-tHcy and the intake of wholegrain bread after adjustment for changes in BMI, physical fitness and the concentration of total cholesterol during the study period (data not shown; $P = 0.034$). A significant association between the estimated increase in dietary intake of folate from wholegrain bread and the decrease in p-tHcy concentration was also shown ($P = 0.010$; Table 5). Changes in folate intake from fruits and vegetables were not significantly related to the changes in p-tHcy concentration. The variables included in the multivariate linear regression analysis explained in combination 16·2 % of the variance in the change of p-tHcy concentration during the study period.

Discussion

The present study was performed to improve dietary habits and reduce cardiovascular risk factors among young men after a 5-month dietary intervention with a focus on vegetables, fruit and wholegrain bread. Despite the random distribution of recruits to different military camps, baseline results for those who completed the entire study showed a significantly lower BMI among recruits in the intervention group compared with the control group. Furthermore, the present study showed a significantly lower energy intake in the intervention group compared with the control group at baseline. However, there were no statistical differences in the concentration of p-tHcy and serum lipids.

A very limited number of studies have focused on reducing modifiable risk factors among young male adults, as this target group has been considered a difficult group to reach when promoting a healthy lifestyle. In the present study, we have demonstrated an inverse relationship between the estimated change in folate intake from wholegrain bread and the

Table 4. Changes in relative contribution of different food groups to total folate intake†

(Mean values and 95 % confidence intervals)

	Baseline (μg/d)		After 5 months (μg/d)			Intervention effect‡	
	Mean	95 % CI	Mean	95 % CI	Change (%)	Mean	95 % CI
Vegetables							
Intervention group	16·4	14·7, 18·0	19·6	17·5, 21·8	+19·5		
Control group	23·6	19·2, 28·1	11·3	8·6, 14·0	−52·1	10·1**	5·9, 14·2
Fruits							
Intervention group	8·2	7·1, 9·3	16·2	14·4, 18·0	+97·6		
Control group	8·4	5·6, 11·2	7·6	5·4, 9·7	−9·5	8·4**	4·7, 12·1
Juice							
Intervention group	13·9	11·6, 16·2	22·9	19·6, 26·2	+64·7		
Control group	15·9	11·7, 20·1	17·8	13·4, 22·2	+11·9	5·2	−1·7, 12·1
Wholegrain bread							
Intervention group	44·2	42·3, 46·1	57·3	55·0, 59·6	+29·6		
Control group	48·7	44·9, 52·5	47·9	43·7, 52·1	−1·6	12·0**	7·4, 16·7
VF							
Intervention group	41·9	38·5, 45·4	61·8	56·9, 66·6	+47·5		
Control group	49·9	42·1, 57·6	40·9	34·3, 47·4	−18·0	22·1**	12·4, 31·8
Total							
Intervention group	82·8	78·5, 87·0	114·3	108·7, 119·8	+38·0		
Control group	97·0	87·3, 106·7	84·8	76·6, 93·1	−12·6	35·7**	24·9, 46·5

VF, sum of vegetables, fruits, berries and juice; Total, sum of vegetables, fruit, juice and wholegrain bread.
** The effect of the intervention was significant ($P < 0.001$).
† Intervention group, n 376; control group, n 105.
‡ The intervention effect was calculated as the mean folate intake of the intervention group relative to the control group, adjusted for baseline values.

Table 5. Unstandardised multiple linear regression coefficients as predictors of changes in plasma total homocysteine (p-tHcy) concentration during the intervention study for 340 subjects*

	Univariate linear regression		Multivariate linear regression†	
Predictor variables	$B_{unstandardised}$	P	$B_{unstandardised}$	P
Changes in				
Folate intake from fruits (μg/d)	0.000	0.258	0.000	0.556
Folate intake from vegetables (μg/d)	0.000	0.651	0.000	0.844
Folate intake from juice (μg/d)	0.000	0.528	0.000	0.211
Folate intake from wholegrain bread (μg/d)	−0.001	0.007	−0.001	0.010
Total folate intake (μg/d)	−0.003	0.031		

* The dependent variable p-tHcy is $\log_e$-transformed before comparison. The dependent variable (p-tHcy) and predictor variables are computed as follow-up concentrations minus baseline concentrations.

† Adjusted for changes in BMI, physical fitness and concentration of total cholesterol during the intervention study.

concentration of p-tHcy. Fat intake, particularly intake of SFA, has been positively associated with serum cholesterol and mortality from CVD in many epidemiological and longitudinal studies[33,34]. The present dietary intervention resulted in an estimated reduction in energy consumption from fat, more in line with the recommendations from the Nordic Council of Ministers[35], but this was not confirmed by changes in the concentration of serum lipids. On the contrary, marginal increases in the concentration of TAG were shown in both the intervention group and the control group, but significantly higher in the latter group. Durstine *et al.*[36] have demonstrated that an increase in physical capacity was associated with a reduced concentration of TAG. Recently published data from the present study showed that the intervention group significantly improved the result from the 3000 m run compared with the control group[26]. Thus, differences in physical exercise among the recruits may also have contributed to differences in the concentration of TAG at the end of the study period.

The baseline intake of vegetables, fruits, berries and juice and wholegrain bread was fairly low among all participants in the present study. The present dietary intervention, based on increased nutrition information and increased availability of vegetables, fruits and wholegrain bread, resulted in an increased consumption of both vegetables, fruits, berries and juice and wholegrain bread. These changes in dietary intake indicate that the main aim of our dietary intervention was achieved.

Another focus of the present study was to investigate baseline intakes of folate, vitamin B_2 and dietary fibre and changes in intake of these nutrients during the study period. Baseline results showed a low daily intake of folate in both groups, whereas the mean intake of vitamin B_2 was low in the intervention group and adequate in the control group compared with the Nordic nutritional recommendations for healthy male adults[35]. Compared with the present results, a higher intake of vitamin B_2 was shown in both Norkost II (2·3 mg/d) and among Irish young male adults (2·2 mg/d) in the Young Hearts Project[37,38]. The latter study also showed a considerable higher dietary intake of folate (369 μg/d) among men aged 20–25 years[37] than what was found in the present study. The intake of dietary fibre, which has been shown to be inversely associated with the risk of CHD in both men and women[39], should range between 25 and 35 g/d according to the Nordic nutritional recommendations[35]. In the present study, the estimated baseline

intake of dietary fibre (24·5 and 24·7 g/d for the control and intervention groups, respectively) corresponded to the results from Norkost II[38] which showed a lower dietary intake of fibre (24 g/d) among men between 20 and 29 years old than recommended[35].

Our intervention study resulted in a significant increase in the estimated total intake of both dietary fibre and folate, which is an outcome of a higher consumption of vegetables, fruits and wholegrain bread. The present study also showed that the increased consumption of wholegrain bread contributed more to the estimated increase in intake of both dietary fibre and folate than the increased consumption of fruits and vegetables. Our findings were in agreement with previous studies, which have demonstrated that wholegrain bread is quantitatively one of the most important contributors to total intake of dietary fibre and folate among both men and women in Norway[38,40]. Despite a minor reduction in the estimated intake of vitamin B_2 on a group level, a positive correlation was shown between the changes in intake of dietary fibre, folate and vitamin B_2 on an individual level. Thus, the recruits who increased their intake of vitamin B_2 seemed to have a greater increase in the intake of dietary fibre and folate than the recruits who decreased their intake of vitamin B_2 during the study period.

Previously reported results from this dietary intervention have demonstrated a significant relationship between an increased concentration of folate and a reduction in the concentration of p-tHcy during the study period[26]. Thus, we expect that the present results may be related to an increased intake of folate from unfortified foods. Few intervention studies have focused on the relationship between changes in folate intake from different food groups and the effect on p-tHcy concentration. Winkels *et al.*[41] demonstrated that wholegrain bread fortified with folic acid significantly increased folate status and reduced p-tHcy concentrations. In Norway, however, fortification of foods with folate is not allowed. The results from the present study showed a weak, but statistically significant, relationship between the estimated increase in folate intake from wholegrain bread, which had not been fortified with folic acid, and the decreased concentration of p-tHcy. On the other hand, changes in the estimated intake of folate from vegetables, fruits or juice did not show any significant relationship with changes in the concentration of p-tHcy, respectively. A possible explanation for the lack of association between p-tHcy concentration and estimated

intake of folate from fruits and juices is the low bioavailability of folate from these food sources[42,43]. Similar to the present results, large cross-sectional studies have not shown any association between the concentration of tHcy in plasma and serum and the folate intake from orange juice or fruits[42,44]. The Framingham Heart Study, however, showed that the intake of green leafy vegetables was related to lower concentrations of p-tHcy[44]. Ganji & Kafai[42] also demonstrated an inverse relationship between the serum concentration of tHcy and intake of cruciferous vegetables, but not with intake of other vegetables or wholegrain bread. The latter study suggested that the lack of association between serum concentration of tHcy and total intake of vegetables was due to the variable B-vitamin content in different vegetables[42].

The lack of association between the p-tHcy concentration and the estimated intake of folate from vegetables in the present study may also be explained by a reduced concentration of folate due to food processing. This is in accordance with the results from a previous study which showed a decreased retention of folate in vegetables caused by different processes used in modern large-scale service systems, including the military service system[45].

Conclusion

Our data showed that a simple, but targeted, nutrition intervention programme based on nutritional information and increased availability of vegetables, fruits and wholegrain bread was effective in modifying food intake of healthy young males. An increased intake of vegetables, fruits and wholegrain bread was associated with beneficial modifications in nutrient intake, with wholegrain bread as the best source of folate. A small, but significant, association was demonstrated between an increased intake of folate from wholegrain bread and a decreased concentration of p-tHcy. There is a debate on the positive and negative aspects of folic acid fortification in many countries. The present study showed evidence of an alternative policy approach based on nutrition education and a food-based alternative to supplementation and/or folic acid fortification.

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