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Dietary predictors of plasma total homocysteine in the Hordaland Homocysteine Study

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Established dietary predictors of plasma total homocysteine (tHcy) include folate, riboflavin, and vitamins B_6 and B_{12}, while information is scarce regarding other dietary components. The aim of this study was to examine the relation between a variety of food groups, food items and nutrients, and plasma tHcy in a large population-based study. The study population included 5812 men and women aged 47–49 and 71–74 years who completed a 169-item FFQ. tHcy was examined across quartiles of dietary components by multiple linear regression analyses adjusting for age, sex, energy intake, various risk factors for elevated tHcy, as well as for dietary and plasma B-vitamins. Among 4578 non-users of vitamin supplements, intake of vegetables, fruits, cereals, eggs, fish and milk, as well as chicken and non-processed meats were inversely associated with tHcy level. The estimated mean difference in tHcy per increasing quartile of intake ranged from −0·11 (95 % CI −0·21, −0·01) μmol/l for milk to −0·32 (95 % CI −0·42, −0·22) μmol/l for vegetables. Positive associations were found for sweets and cakes. Whole-grain bread was significantly inversely related to tHcy only after additional adjustment for dietary and plasma B-vitamins. The nutrients folate, vitamin B_6, B_{12}, and riboflavin were inversely related to tHcy. Complex carbohydrates were inversely, and fat positively associated with tHcy, also after adjustment for dietary and plasma B-vitamins. In conclusion, food items rich in B-vitamins and with a low content of fat and sugar were related to lower tHcy levels. Eggs, chicken, non-processed meat, fish and milk were inversely associated with tHcy.

Homocysteine: Diet: Food groups: Food items: Nutrients

Homocysteine is an intermediate amino acid in the metabolism of the essential amino acid methionine (Castro *et al.* 2006). Elevated plasma total homocysteine (tHcy) concentration has been related to several adverse conditions and diseases, including adverse pregnancy outcomes (Scholl & Johnson, 2000; Daly *et al.* 2005), cognitive dysfunction among elderly (Morris, 2003) and recently osteoporosis (Gjesdal *et al.* 2006). Plasma tHcy also predicts CVD risk in prospective studies (de Bree *et al.* 2002; Wald *et al.* 2002), but intervention trials do not demonstrate risk reduction by tHcy-lowering therapy with high doses of B-vitamins in patients with established CVD (Clarke, 2005; Bonaa *et al.* 2006; Loscalzo, 2006).

Elevated tHcy is observed under a variety of conditions, e.g. deficiency of B-vitamins (Verhoef & de Groot, 2005; Castro *et al.* 2006), smoking (Nygard *et al.* 1998; Ortega *et al.* 2004), high coffee consumption (Ranheim & Halvorsen, 2005; Verhoef & de Groot, 2005) and impaired renal function (Perna *et al.* 2004; Castro *et al.* 2006). The concentration of tHcy in plasma can be lowered by enhanced remethylation of homocysteine into methionine or via degradation through the transsulphuration pathway. Folate or betaine donate a methyl group, while vitamin B_{12}, vitamin B_6 and riboflavin act as cofactors for enzymes involved in homocysteine metabolism (Finkelstein, 1990; Ueland *et al.* 2005; Castro *et al.* 2006).

Several studies report an association between intake of different nutrients and plasma tHcy concentration (Vollset *et al.* 2001; Verhoef & de Groot, 2005; Castro *et al.* 2006). Many studies, particularly the two largest studies – the Framingham Offspring study (*n* 1960) and the Dutch study (*n* 2435) – have found inverse associations between plasma tHcy and intake of folate, vitamin B_{12}, vitamin B_6 and riboflavin (de Bree *et al.* 2001; Jacques *et al.* 2001). Also, mandatory food fortification with folic acid has increased the level of plasma folate and decreased tHcy concentration in the general US population (Jacques *et al.* 1999; Pfeiffer *et al.* 2005; Ganji & Kafai, 2006).

There are contradictory reports on the relation between protein and methionine to plasma tHcy concentration (Verhoef & de Groot, 2005). A few studies that examined the associations

Abbreviations: NNR, Nordic Nutritional Recommendations; tHcy, total homocysteine.

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with total fat, cholesterol, saturated fat, mono- and polyunsaturated fat (Shimakawa *et al.* 1997) and *n*-3 fatty acids (Brude *et al.* 1999) did not show statistically significant associations with tHcy concentration. Although less studied, dietary choline and betaine have been inversely related to plasma tHcy (Verhoef & de Groot, 2005; Cho *et al.* 2006).

The alteration in plasma homocysteine concentration may vary substantially depending on the food source (Vollset *et al.* 2001). Previous studies show that tHcy concentration is inversely related to the intake of food groups such as bread (Vollset *et al.* 2001), cereals (Shimakawa *et al.* 1997; Ganji & Kafai, 2004), fruits (Tucker *et al.* 1996; Vollset *et al.* 2001), vegetables (Tucker *et al.* 1996; Brude *et al.* 1999; Vollset *et al.* 2001), and to individual food items including cruciferous vegetables (Tucker *et al.* 1996; Ganji & Kafai, 2004), peppers (Ganji & Kafai, 2004), citrus fruits and juices (Tucker *et al.* 1996; Vollset *et al.* 2001), cold breakfast cereals (Tucker *et al.* 1996; Shimakawa *et al.* 1997; Ganji & Kafai, 2004), milk (Shimakawa *et al.* 1997; Ganji & Kafai, 2004), yoghurt (Ganji & Kafai, 2004) and liver (Vollset *et al.* 2001). Positive associations have been found with caffeine-containing drinks such as coffee (Jacques *et al.* 2001; Verhoef & de Groot, 2005), Coca Cola (Jacques *et al.* 2001) and tea (Jacques *et al.* 2001; Verhoef & de Groot, 2005), while findings on alcohol are conflicting (Jacques *et al.* 2001; Vollset *et al.* 2001). To understand the associations between plasma tHcy concentration and foods with high and low B-vitamin content, we included major food groups and individual food items in the present study. Moreover, we examined to what extent dietary intake of folate, vitamin B_{12}, vitamin B_6, riboflavin and other nutrients may alter the plasma concentration of tHcy. We also investigated nutrients other than B-vitamins because our comprehensive dietary data allowed us to quantitatively assess the confounding effect of B-vitamins. Thus, the objective of the present paper was to assess the relationship between nutrients and food intake on plasma concentration of tHcy in middle-aged and older men and women in a large population-based study.

Subjects and methods

Subjects

The second round of the Hordaland Homocysteine Study was conducted as part of the Hordaland Health Study (HUSK), from 1997 to 1999 as a collaboration between the National Health Screening Service (now the Norwegian Institute of Public Health), The University of Bergen and local health services. Of the total sample of 9187 men and women born 1925–7 and 1950–1 who were invited to participate in the Hordaland Health Study, 7074 (77 %) agreed to participate. The participants underwent a brief health examination and donated a non-fasting blood sample. Information on diet and lifestyle was collected via self-administered questionnaires. In total 6140 subjects (87 %) completed a FFQ and 6118 of these also had plasma tHcy values measured. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All subjects gave their written consent to participate in the study.

We excluded participants with reported energy intake below the 2·5 percentile (2124 kJ for women 71–74 years and 3899 kJ for women 47–49 years; 3856 kJ for men 71–74 years and 5572 kJ for men 47–49 years) and above the 97·5 percentile (11 098 kJ for women 71–74 years and 12 970 kJ for women 47–49 years; 14 023 kJ for men 71–74 years and 17 590 kJ for men 47–49 years). Thus, 141 men and 187 women were excluded from further analyses, yielding a final number of 5812 participants. Participants with reported seasonal or regular intake of at least one dose of multi- or/and individual vitamin supplement (excluding fish oil and *n*-3 fatty acids) per day were assigned to the vitamin supplement user group (*n* 1234).

FFQ

In the present study we used a 169-item FFQ, a slightly modified version of a FFQ previously described in detail (Andersen *et al.* 1999). This FFQ aimed to capture the habitual diet during the past year. The FFQ includes frequency alternatives (from once a month to several times per day), the number of units eaten and portion sizes (e.g. slices, glasses, cups, pieces, spoons).

The information from the FFQ is presented as individual food items, food groups (consisting of individual food items) and nutrients. Individual food items correspond to the items listed on the questionnaire, whereas 'food groups' include related food items (e.g. the food group 'bread' contains 'bread with low, medium and high quantity of fibre').

In addition to individual food items, the FFQ also includes nine questions about the most commonly used brands of single- and multi-vitamin supplements on the market. Subjects using at least one dose of vitamin supplement per day seasonally or regularly during the past year were classified as vitamin supplement users.

Daily nutrient intakes were computed from a database and software system developed at the Department of Nutrition, University of Oslo (KBS, version 3.2). The nutrient database is mainly based on the official Norwegian food composition table with an update on folate content from 2001 (Rimestad *et al.* 2001). B-vitamin intake in supplements is calculated from information on the contents of vitamin supplements for sale during 1997–9.

Health examination and analytic procedures

A brief examination included measurements of height and weight and drawing of non-fasting blood samples. Tubes with plasma containing EDTA were stored at -80°C. Plasma tHcy was determined by automated HPLC with fluorescence detection. Intra-assay CV was 3 % (Fiskerstrand *et al.* 1993). The concentration of plasma folate was measured by a *Lactobacillus casei* microbiological assay (Molloy & Scott, 1997) and plasma vitamin B_{12} concentration by a *Lactobacillus leichmannii* microbiological assay (Kelleher & Broin, 1991). The concentration of serum creatinine was measured with standard alkaline picrate colorimetric assay.

Statistical analysis

Differences between non-users and users of vitamin supplements were assessed by linear regression with adjustment for age and sex for continuous variables and logistic

regression with adjustment for age and/or sex for categorical variables. Spearman's partial correlation coefficients were used to estimate associations between dietary and plasma B-vitamins, other nutrients and food groups. Associations between predictor variables (food groups and nutrients) and plasma tHcy concentration were analysed separately for non-users and users of vitamin supplements by multiple regression adjusted for age, sex and energy intake. Quartiles of predictor variables were defined separately for vitamin supplement users and non-users. Results were combined for the two age groups and for men and women, because the observed trends were similar in all the four age–sex groups. The potential effect modification (interaction) of vitamin supplement use on associations between diet and plasma tHcy was assessed by linear regression analysis with product terms of supplement use (0 or 1) and each dietary predictor (with both main effects in the model) adjusted for age, sex and energy intake. We found an interaction effect of vitamin user group on the associations with several food groups and nutrients. Thus, due to the present results and the fact that B-vitamin supplementation may substantially reduce plasma tHcy concentration and therefore mask the effects of dietary components, we restricted the study sample to non-users of vitamin supplements in further in-depth analyses.

Multiple linear regression analysis was used to assess the simultaneous relations between various predictors of plasma tHcy concentration. The predictor variables (food groups, food items and nutrients) were categorized in quartiles, excluding refined bread. Due to low consumption of refined bread (29 % of participants), three non-equal categories were used: 'non-user', '0–25 g/d', ' > 25 g/d', separating users in two groups of similar sizes. Thus, the regression coefficients estimated the mean differences in plasma tHcy concentration between quartiles of the predictor variables. Several models were used: Model I was adjusted for age, sex and energy intake (Table 2); Model II included additional adjustment for smoking and coffee consumption, as well as for BMI and serum creatinine (Tables 4 and 5); and Model III was further adjusted for dietary B-vitamin intake (folate, vitamin B_{12}, vitamin B_6, riboflavin) and plasma concentrations of folate and vitamin B_{12} (Tables 4 and 5). Findings with adjustment for dietary but not plasma B-vitamins are presented in the text only. We used additional adjustment for plasma folate and vitamin B_{12} because their associations with tHcy were stronger than between tHcy and dietary B-vitamins. In addition, concentrations of folate and vitamin B_{12} in plasma were relatively weakly correlated with the intake of dietary B-vitamins (Table 3). The continuous predictor variables were categorized in quartiles to use the mean difference of homocysteine per increasing quartile as the main effect measure. All statistical analyses were performed using SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of study population

Descriptive statistics of the study population and its dietary habits are shown in Table 1. There was a higher proportion of women in the group of vitamin supplement users compared to non-users. In age- and sex-adjusted analyses, supplement users had a lower proportion of smokers, lower BMI and coffee consumption, and higher intakes of cereals, fruits, vegetables and fish compared to non-users. The former group also had lower concentrations of tHcy, whereas folate and vitamin B_{12} were higher.

A lower intake than recommended in the Nordic Nutritional Recommendations (NNR) of folate (300 μg/d) was observed among 39 % of vitamin supplement users and among 59 % of non-users; the corresponding proportions were 1 % and 3 % for vitamin B_{12} (NNR: 2 μg/d), respectively. Less than the recommended intake of vitamin B_6 (NNR: 1.2 mg/d for women, 1·6 mg/d for men) was registered among 49 % of women and 57 % of men in the group of non-users of vitamin supplements and 31 % and 38 % in the group of users, respectively. Less than the recommended intake of riboflavin (NNR: 1·3 mg/d for women, 1·7 mg/d for men) was observed among 44 % of the women and 48 % of the men among non-users of vitamin supplements and 22 % of the women and 28 % of the men among supplement users.

Relation between dietary intake and total homocysteine among non-users and users of vitamin supplements

Table 2 shows mean differences in plasma tHcy concentration between quartiles of food group and nutrient intake among non-users and users of vitamin supplements, after adjustment for age, sex and energy intake. While the associations between food groups and nutrients with plasma tHcy were mostly similar between users and non-users of vitamin supplements, there were some marked exceptions. Intake of vegetables was significantly inversely associated with plasma tHcy among non-users only, whereas meat intake was significant only among vitamin supplement users. For these food groups, as well as for eggs, fats, sweets, protein and the nutrient fat, there was a significant effect modification of vitamin supplement use on the associations with tHcy. There was a similar effect for energy intake, which was significantly inversely related to tHcy among non-users only.

Correlations between dietary and plasma B-vitamins, food groups and other macro- and micro-nutrients

Dietary folate intake was significantly positively correlated with the intake of vegetables, fruits and fibre, as well as with most other vitamins, except vitamin D (Table 3). Dietary vitamin B_{12} was most strongly associated with fish intake, and also with meat, milk, protein, cholesterol and vitamin B_6 and riboflavin. Dietary folate, B_6 and riboflavin were positively associated with the intake of complex carbohydrates, and inversely associated with the intake of simple carbohydrates. Fat intake was inversely related to plasma folate and the dietary B-vitamins, except dietary B_{12}.

Plasma concentration of folate was moderately correlated with intake of fruits and vegetables, as well as with dietary folate and vitamin B_6. Plasma vitamin B_{12} was significantly associated with fish and milk intake, as well as with dietary vitamin B_{12} and riboflavin. Plasma tHcy had stronger correlations with plasma folate and vitamin B_{12} than with dietary intake of folate, vitamin B_{12}, vitamin B_6 and riboflavin.

Table 1. Characteristics of the study population and its diet, stratified by vitamin supplement use in the Hordaland Homocysteine Study

	Non-users of vitamin supplements (*n* 4578)		Users of vitamin supplements (*n* 1234)		
Sex					<0·0001
Men (*n*)	2103		447		
Women (*n*)	2475		787		
% (male/female)	46/54		36/64		
Age group (*n*)					0·90
47–49 years	2324		631		
71–74 years	2254		603		
Smoking status (%)					0·004†
Current smokers	24·6		20·8		
Former smokers	38·8		42·4		
Never smokers	34·8		34·9		
	Mean	SD	Mean	SD	*P**
BMI (kg/m^2)	25·8	3·8	25·3	3·8	<0·0001
Coffee consumption (ml/d)	447	292	400	283	<0·0001
Food group (g/d)					
Bread	177	75	171	71	0·81
Cereals	33	30	38	33	<0·0001
Cakes, pies, cookies	26	26	25	23	0·48
Fruit	234	167	267	174	<0·0001
Vegetables	191	143	216	136	<0·0001
Potatoes	121	71	115	67	0·81
Meat	98	59	97	53	0·25
Fish and seafood	83	54	88	51	<0·0001
Eggs	16	11	16	11	0·43
Milk	311	219	310	225	0·29
Cheese	30	26	31	26	0·08
Fats	30	21	30	20	0·27
Sweets, sugar	12	17	13	16	0·09
Nutrient (intake/d)‡					
Energy (kJ)	8120	2573	8299	2366	<0·0001
Protein (g)	77	25	79	23	<0·0001
Fat (g)	69	27	71	26	<0·0001
Complex carbohydrates (g)	208	63	213	61	<0·0001
Simple carbohydrates (sugars) (g)	34	26	33	23	0·95
Fibre (g)	23	8·2	25	7·9	<0·0001
Cholesterol (mg)	263	104	274	95	<0·0001
Folate (μg)	293	112	362	151	<0·0001
Vitamin B_{12} (μg)	6·6	3·8	7·0	3·8	<0·0001
Vitamin B_6 (mg)	1·4	0·5	1·9	1·0	<0·0001
Riboflavin (mg)	1·6	0·6	2·2	1·1	<0·0001
Thiamine (mg)	1·4	0·5	2·0	0·9	<0·0001
Retinol equivalents (μg)	1884	1093	2513	1313	<0·0001
β-Carotene (μg)	5219	3780	5909	3702	<0·0001
Vitamin C (mg)	149	92	185	95	<0·0001
α-Tocopherol (mg)	10·1	5·2	15·7	8·4	<0·0001
Vitamin D (μg)	8·5	7·0	13·4	9·8	<0·0001
Plasma/serum concentration					
Total homocysteine (μmol/l)	11·2	4·0	10·1	3·7	<0·0001
Folate (nmol/l)	7·7	5·4	10·6	8·3	<0·0001
Vitamin B_{12} (pmol/l)	381·0	235·1	400·5	242·3	0·02
Creatinine (μmol/l)	90·8	14·3	88·5	12·8	0·03

* Two-sided *P* value for the difference between non-users and users of vitamin supplements. Logistic regression for the variables age, sex and smoking adjusted for age and/or sex, linear regression analyses for all other variables (categorized in quartiles) adjusted for age and sex.
† Current smokers versus former and never smokers combined.
‡ Includes intake from food and supplements.

Plasma total homocysteine according to food groups and food items intake

Table 4 shows mean differences in plasma tHcy concentration between quartiles of food group and food item intake with two different adjustment models. In comparison with the data in Table 2 adjusted only for age, sex and energy intake, further adjustment for smoking and coffee consumption made the association between tHcy and cakes stronger and statistically significant, whereas the association with fats became weaker and non-significant. For individual variables, significantly lower plasma tHcy was observed for higher intakes of citrus fruits, orange juice, cruciferous vegetables, spinach/green cabbage, non-processed meat and chicken, while a higher intake of processed meat was associated with higher tHcy concentrations. Further adjustments for BMI and serum creatinine only marginally altered the results, and are therefore not presented.

Table 2. Mean difference in plasma total homocysteine concentration per increasing quartile of food group, nutrient intake, smoking and coffee consumption among non-users and users of vitamin supplements in the Hordaland Homocysteine Study

	Non-users of vitamin supplements			Users of vitamin supplements			
	Mean*	95 % CI	*P*†	Mean*	95 % CI	*P*†	*P*‡
Food group							
Bread	−0·09	−0·21, 0·02	0·12	−0·14	−0·34, 0·06	0·18	0·31
Cereals	−0·18	−0·29, −0·06	0·004	−0·23	−0·43, −0·04	0·02	0·21
Cakes, pies, cookies	0·09	−0·02, 0·20	0·10	0·02	−0·17, 0·21	0·59	0·91
Fruits	−0·31	−0·41, −0·20	<0·0001	−0·29	−0·48, −0·11	0·002	0·71
Vegetables	−0·32	−0·42, −0·22	<0·0001	−0·14	−0·33, 0·04	0·14	0·03
Potatoes	−0·03	−0·14, 0·08	0·61	−0·06	−0·26, 0·13	0·52	0·68
Meat	−0·007	−0·13, 0·12	0·91	0·26	0·04, 0·47	0·02	0·002
Fish and sea food	−0·22	−0·33, −0·11	<0·0001	−0·16	−0·35, 0·03	0·11	0·75
Eggs	−0·22	−0·32, −0·12	<0·0001	−0·03	−0·21, 0·15	0·71	0·04
Milk and dairy food	−0·12	−0·23, −0·02	0·02	−0·01	−0·20, 0·17	0·88	0·21
Cheese	−0·04	−0·15, 0·07	0·46	−0·17	−0·36, 0·02	0·09	0·60
Fats	0·15	0·03, 0·27	0·01	0·42	0·21, 0·63	<0·0001	0·003
Sweets, sugar	0·21	0·11, 0·32	<0·0001	0·38	0·20, 0·57	<0·0001	0·02
Nutrient							
Energy	−0·13§	−0·25, −0·02	0·02	0·08§	−0·11, 0·29	0·37	0·02§
Protein	−0·46	−0·64, −0·28	<0·0001	−0·36	−0·66, −0·05	0·02	0·02
Fat	0·49	0·31, 0·67	<0·0001	0·61	0·29, 0·94	0·0002	0·009
Complex carbohydrates	−0·41	−0·59, −0·24	<0·0001	−0·65	−0·94, −0·35	<0·0001	0·31
Simple carbohydrates (sugars)	0·23	0·11, 0·34	0·0001	0·21	0·007, 0·41	0·04	0·33
Fibres	−0·38	−0·51, −0·25	<0·0001	−0·45	−0·67, −0·23	<0·0001	0·41
Cholesterol	−0·10	−0·23, 0·03	0·14	−0·007	−0·23, 0·22	0·95	0·13
Folate	−0·57	−0·71, −0·44	<0·0001	−0·57	−0·77, −0·37	<0·0001	0·87
Vitamin B_{12}	−0·27	−0·39, −0·15	<0·0001	−0·17	−0·38, 0·03	0·10	0·38
Vitamin B_6	−0·54	−0·68, −0·39	<0·0001	−0·54	−0·74, −0·35	<0·0001	0·48
Riboflavin	−0·46	−0·60, −0·33	<0·0001	−0·45	−0·64, −0·26	<0·0001	0·16
Thiamine	−0·64	−0·80, −0·47	<0·0001	−0·47	−0·67, −0·28	<0·0001	0·26
Retinol equivalents	−0·33	−0·44, −0·22	<0·0001	−0·22	−0·41, −0·04	0·02	0·35
β-Carotene (μg)	−0·29	−0·40, −0·19	<0·0001	−0·27	−0·45, −0·09	0·004	0·76
Vitamin C (mg)	−0·42	−0·53, −0·31	<0·0001	−0·40	−0·59, −0·22	<0·0001	0·49
α-Tocopherol (mg)	−0·27	−0·39, −0·15	<0·0001	−0·29	−0·48, −0·11	0·002	0·89
Vitamin D	−0·14	−0·25, −0·03	0·01	−0·18	−0·36, 0·01	0·06	0·90
Lifestyle factors							
Smoking	0·61§	0·47, 0·76	<0·0001	0·62§	0·35, 0·88	<0·0001	0·70§
Coffee consumption	0·33§	0·22, 0·44	<0·0001	0·30§	0·11, 0·49	<0·0001	0·73§

* Model I adjusted for age, sex and energy intake.
† Two-sided *P* value for the effect of food group, nutrient, smoking and coffee consumption.
‡ Two-sided *P* value for interaction of vitamin user group on the association between predictor variables and total homocysteine concentration, adjusted for age, sex and energy intake.
§ Adjusted for age and sex.

Further adjustments for dietary B-vitamins generally resulted in weaker associations for all food variables, and the groups of vegetables, non-processed meat, processed meat, chicken, fish and milk were no longer significantly associated with tHcy (data not shown). The groups of fruits and orange juice were still inversely associated, while cakes and sweets were positively associated with tHcy concentration. After additional adjustment for plasma folate and vitamin B_{12} the associations with fruits, orange juice and cakes became statistically non-significant, while sweets still related positively to tHcy. The inverse association with whole-grain bread and non-processed meat became stronger.

Plasma total homocysteine according to nutrient intake

Differences in plasma concentration of tHcy per increasing quartiles of nutrient intakes are shown in Table 5. After adjustment for smoking and coffee consumption most of the associations became weaker, while for protein, simple carbohydrates, cholesterol, vitamin B_{12} and vitamin D became stronger. The inverse association between cholesterol intake and plasma tHcy concentration became statistically significant. We observed inverse associations between dietary intake of thiamine, vitamins A (retinol equivalent and β-carotene), E (α-tocopherol), D and C, and the plasma tHcy concentration. Adjustment for serum creatinine and BMI only marginally altered the results, and these data are therefore not presented.

After additional adjustment for dietary B-vitamins and plasma concentration of folate and vitamin B_{12}, only complex carbohydrates (inversely), thiamine (inversely) and fat (positively) remained associated with plasma tHcy. Dietary folate and riboflavin (not adjusted for plasma concentrations) remained strongly associated with tHcy even after adjustment for the other dietary B-vitamins. This was not seen for vitamins B_{12} and B_6 after similar adjustment. Vitamin C, retinol equivalent, β-carotene and simple carbohydrates remained significantly associated with tHcy after adjustment for dietary B-vitamins, but not after additional adjustment for plasma folate and vitamin B_{12}.

Table 3. Correlation between dietary intake and plasma B-vitamin concentration, other nutrients and food groups among non-users of vitamin supplements in the Hordaland Homocysteine Study*

	Diet				Plasma	
	Folate	B_{12}	B_6	Riboflavin	Folate	B_{12}
Nutrient						
Folate					0·19	0·04
B_{12}	0·16				0·04†	0·14
B_6	0·58	0·42			0·18	0·08
Riboflavin	0·37	0·40	0·42		0·06	0·15
Thiamine	0·63	0·20	0·57	0·56	0·08	0·10
Vitamin C	0·76	0·08	0·59	0·24	0·20	0·02†
Retinol equivalents	0·39	0·28	0·27	0·22	0·10	0·04†
β-Carotene	0·51	0·10	0·41	0·14	0·13	0·03†
Vitamin D	0·07	0·38	0·22	0·09	0·04†	0·05
α-Tocopherol	0·32	0·14	0·34	0·16	0·10	0·03†
Protein	0·24	0·63	0·48	0·48	0·04	0·15
Fat	−0·33	0·14	−0·26	−0·10	−0·08	−0·02†
Complex carbohydrates	0·46	−0·17	0·24	0·13	0·04†	0·02†
Simple carbohydrates (sugars)	−0·21	−0·19	−0·25	−0·21	−0·08	−0·05
Fibre	0·66	−0·06	0·47	0·10	0·12	0·005†
Cholesterol	0·02†	0·51	0·19	0·23	0·05	0·05
Food group						
Bread	0·06	−0·17	−0·30	−0·14	−0·08	0·02†
Cereals	0·08	−0·01†	0·14	0·08	0·04	−0·006†
Cakes, pies, cookies	−0·03†	−0·06	−0·08	−0·06	−0·05	−0·06
Fruits	0·38	−0·03†	0·44	0·03†	0·16	−0·001†
Vegetables	0·66	0·15	0·50	0·20	0·17	0·02†
Potatoes	0·12	0·02†	0·19	−0·07	−0·02†	0·02†
Meat	−0·08	0·21	0·17	−0·02†	−0·003†	−0·02†
Fish	0·17	0·66	0·49	0·11	0·06	0·09
Eggs	0·07	0·18	0·07	0·13	0·05	0·02†
Milk	−0·04†	0·21	0·06	0·60	−0·08	0·14
Cheese	−0·06	−0·04	−0·15	0·16	0·007†	0·03†
Fats	−0·20	−0·02†	−0·24	−0·22	−0·07	−0·02†
Sweets, sugar	−0·19	−0·14	−0·18	−0·19	−0·03†	−0·05
Plasma total homocysteine	−0·15	−0·07	−0·14	−0·12	−0·37	−0·22

* Spearman's partial correlation coefficients adjusted for age, sex and energy intake. All coefficients are significant, except those marked † ($P > 0·01$).

Discussion

In a large population-based sample of non-users of vitamin supplements, we observed that plasma tHcy is associated with several nutrients and foods. While the intake of complex carbohydrates and protein as well as several B-vitamins were associated with lower tHcy concentrations, the intake of fat and sugar were associated with higher plasma tHcy. Consumption of vegetables, fruits, bread, cereals, fish, non-processed meat, chicken, eggs and milk was associated with lower tHcy concentrations, while intake of sweets, cakes and processed meat was related to higher tHcy concentrations. The present results confirm the importance of lifestyle factors as a major determinant of tHcy.

Limitations and strengths

The current study was conducted in a large population-based sample, using a validated 169-item quantitative FFQ (Andersen *et al.* 1995, 1999). This allowed the investigation of dietary predictors of tHcy in multivariate models. A limitation relates to the collection of dietary data using a FFQ, which has inherent potential problems related to inaccuracy and potential misclassification in the estimation of nutrient intake (Willett & Lenart, 1998; Flood *et al.* 2004): reported associations between dietary non-B-vitamin nutrients and tHcy concentration could be due to confounding effects of dietary B-vitamin intake (Shimakawa *et al.* 1997; Brude *et al.* 1999). We therefore repeated the analyses using a model that included adjustment for dietary and plasma folate and vitamin B_{12} when examining these associations.

Users and non-users of vitamin supplements

In accordance with previous reports (Tucker *et al.* 1996; Nygard *et al.* 1998; Malinow *et al.* 1999; Koehler *et al.* 2001), we observed that users and non-users of vitamin supplements differed with respect to lifestyle, dietary habits and plasma vitamin concentrations. Users were more likely to be non-smokers, consume less coffee and generally have a more healthy diet compared to non-users, and to have lower concentrations of plasma tHcy, but higher concentration of folate and vitamin B_{12} as compared to non-users. There was an effect modification of vitamin supplement use on the association between various nutrients, food groups and plasma tHcy. We are not aware that this has previously been reported.

B-vitamins

The present findings confirm previous studies reporting inverse associations between dietary intake of folate, vitamin

Table 4. Mean difference in plasma total homocysteine concentration per increasing quartiles of food intake among non-users of vitamin supplements in the Hordaland Homocysteine Study

Food group/food item	Mean*	95 % CI	*P*†	Mean‡	95 % CI	*P*†
Bread, group	−0·09	−0·20, 0·03	0·15	−0·17	−0·28, −0·06	0·003
Refined (white) bread	0·14	−0·01, 0·30	0·07	0·04	−0·10, 0·19	0·56
Whole-grain bread	−0·10	−0·21, 0·004	0·06	−0·14	−0·24, −0·04	0·007
Cereals, group	−0·13	−0·25, −0·01	0·03	−0·08	−0·19, 0·03	0·14
Cakes, pies, cookies, group	0·14	0·03, 0·24	0·01	0·03	−0·07, 0·13	0·56
Fruits, group	−0·24	−0·34, −0·14	<0·0001	−0·007	−0·11, 0·10	0·90
Citrus fruits	−0·25	−0·34, −0·16	<0·0001	−0·09	−0·17, −0·0005	0·05
Apples, pears	−0·15	−0·25, −0·05	0·003	−0·04	−0·13, 0·05	0·42
Orange juice	−0·20	−0·29, −0·10	<0·0001	−0·02	−0·11, 0·07	0·69
Vegetables, group	−0·32	−0·42, −0·22	<0·0001	0·002	−0·12, 0·12	0·97
Carrot	−0·29	−0·40, −0·19	<0·0001	−0·16	−0·26, −0·06	0·001
Kohlrabi, turnips	−0·15	−0·25, −0·06	0·002	−0·02	−0·11, 0·07	0·66
Cabbage	−0·09	−0·19, −0·002	0·05	−0·04	−0·13, 0·05	0·36
Cauliflower, broccoli, Brussels sprouts	−0·17	−0·27, −0·07	0·0009	0·08	−0·02, 0·18	0·13
Onions	−0·22	−0·32, −0·13	<0·0001	−0·09	−0·18, −0·004	0·04
Lettuces	−0·16	−0·26, −0·05	0·003	−0·002	−0·10, 0·10	0·96
Cucumber	−0·14	−0·24, −0·04	0·006	0·005	−0·09, 0·10	0·92
Tomato	−0·20	−0·30, −0·10	0·0002	0·02	−0·08, 0·12	0·74
Pepper	−0·21	−0·31, −0·10	0·0001	−0·02	−0·12, 0·08	0·71
Green cabbage, spinach	−0·21	−0·31, −0·11	<0·0001	−0·03	−0·13, 0·07	0·56
Mushrooms	−0·14	−0·24, −0·04	0·007	0·05	−0·07, 0·11	0·69
Potatoes, group	−0·05	−0·16, 0·06	0·41	−0·05	−0·15, 0·05	0·33
Meat, group	−0·06	−0·19, 0·06	0·33	−0·08	−0·20, 0·04	0·17
Non-processed meat	−0·13	−0·23, −0·02	0·02	−0·11	−0·21, −0·004	0·04
Processed meat	0·12	0·002, 0·23	0·05	0·02	−0·09, 0·12	0·75
Chicken	−0·15	−0·25, −0·05	0·003	−0·08	−0·17, 0·02	0·10
Fish and seafood, group	−0·22	−0·33, −0·11	<0·0001	−0·07	−0·20, 0·06	0·28
Egg, group	−0·26	−0·36, −0·17	<0·0001	−0·18	−0·27, −0·09	<0·0001
Milk and dairy food, group	−0·11	−0·21, −0·01	0·03	−0·03	−0·14, 0·09	0·63
Cheese, group	−0·01	−0·12, 0·09	0·83	0·03	−0·07, 0·13	0·56
Fats, group	0·08	−0·04, 0·20	0·18	−0·02	−0·13, 0·09	0·68
Sweets, sugar, group	0·21	0·11, 0·32	<0·0001	0·11	0·01, 0·21	0·02

* Model II adjusted for age, sex, energy intake, smoking and coffee consumption.
† Two-sided *P* value for the effect of food group and food item.
‡ Model III adjusted for age, sex, smoking, coffee consumption, intake of dietary energy, folate, vitamin B_{12}, vitamin B_6 and riboflavin, and plasma folate and vitamin B_{12}.

Table 5. Mean difference in plasma total homocysteine concentration per increasing quartiles of nutrient intake among non-users of vitamin supplements in the Hordaland Homocysteine Study

Nutrient	Mean*	95 % CI	*P*†	Mean‡	95 % CI	*P*†
Energy	−0·15§	−0·26, −0·03	0·01	0·16§	0·001, 0·32	0·08
Protein	−0·47	−0·65, −0·30	<0·0001	−0·18	−0·39, 0·03	0·09
Fat	0·38	0·20, 0·56	<0·0001	0·27	0·10, 0·44	0·002
Complex carbohydrates	−0·35	−0·52, −0·17	0·0001	−0·20	−0·38, −0·02	0·03
Simple carbohydrates (sugars)	0·25	0·13, 0·36	<0·0001	0·06	−0·04, 0·17	0·25
Fibres	−0·31	−0·44, −0·18	<0·0001	−0·06	−0·21, 0·09	0·45
Cholesterol	−0·16	−0·29, −0·03	0·01	−0·03	−0·16, 0·10	0·66
Folate	−0·53	−0·67, −0·39	<0·0001	−0·36‖	−0·53, −0·20	<0·0001
Vitamin B_{12}	−0·29	−0·41, −0·18	<0·0001	−0·13‖	−0·26, 0·007	0·06
Vitamin B_6	−0·49	−0·63, −0·34	<0·0001	−0·15‖	−0·33, 0·04	0·11
Riboflavin	−0·42	−0·55, −0·28	<0·0001	−0·17‖	−0·32, −0·006	0·04
Thiamine	−0·59	−0·75, −0·43	<0·0001	−0·30	−0·50, −0·09	0·004
Retinol equivalents	−0·33	−0·44, −0·22	<0·0001	−0·10	−0·21, 0·01	0·07
β-Carotene	−0·29	−0·39, −0·19	<0·0001	−0·06	−0·17, 0·04	0·25
α-Tocopherol	−0·24	−0·36, −0·12	0·0001	−0·01	−0·13, 0·11	0·87
Vitamin D	−0·15	−0·26, −0·04	0·008	−0·02	−0·13, 0·09	0·70
Vitamin C	−0·38	−0·49, −0·27	<0·0001	−0·003	−0·14, 0·14	0·97

* Model II adjusted for age, sex, energy intake, smoking and coffee consumption.
† Two-sided *P* value for the effect of nutrient.
‡ Model III adjusted for age, sex, smoking, coffee consumption, intake of energy, dietary folate, vitamin B_{12}, vitamin B_6 and riboflavin, and plasma folate and vitamin B_{12}.
§ Not adjusted for energy intake.
‖ Not adjusted for plasma folate and vitamin B_{12}.

B_{12}, vitamin B_6 and riboflavin, and plasma concentration of tHcy (de Bree *et al.* 2001, 2002; Jacques *et al.* 2001; Verhoef & de Groot, 2005). However, only folate and riboflavin remained significantly related to tHcy after adjusting for other dietary B-vitamins. This does not negate an effect of vitamins B_{12} and B_6 on plasma tHcy concentration, because intake of these four B-vitamins is to a significant extent strongly intercorrelated (Shimakawa *et al.* 1997) due to similar dietary sources. Second, because these B-vitamins are all involved in homocysteine metabolism, they have a complementary lowering effect on tHcy concentration (Bostom *et al.* 2002; Huerta *et al.* 2004). Adjustment of an individual B-vitamin for other vitamins in a related metabolic cycle could significantly reduce or mask their individual effect. Among the four B-vitamins, our analysis confirmed that the individual effect of folate was stronger than the effects of riboflavin, vitamin B_{12} and vitamin B_6 (de Bree *et al.* 2001; Jacques *et al.* 2001).

Other nutrients

We found significant inverse associations between plasma tHcy and dietary intake of vitamins C, E and D, retinol equivalent and β-carotene, in accordance with previous reports (Brude *et al.* 1999; Vollset *et al.* 2001). The intake of these vitamins is correlated with dietary B-vitamins, indicating overlapping dietary sources. Nevertheless, after adjustment for dietary B-vitamins, retinol equivalents, β-carotene and vitamin C remained significantly inversely related to plasma tHcy concentration. However, after further adjustments for plasma folate and vitamin B_{12}, these associations were no longer significant.

We observed an inverse association between protein intake and plasma tHcy (Stolzenberg-Solomon *et al.* 1999; Jacques *et al.* 2001). However, there are strong positive correlations between dietary intake of protein and riboflavin, vitamin B_{12} and vitamin B_6, and after adjusting for dietary B-vitamins the association between protein and tHcy became non-significant. As for protein, the association between fibre intake and plasma tHcy was no longer significant after adjustment for the intake of B-vitamins.

The intake of complex carbohydrates was inversely related to tHcy concentration, an association that remained significant after adjusting for dietary and plasma B-vitamins. Complex carbohydrates is a macro-nutrient that is not involved in methionine–homocysteine metabolism, thus the inverse association with tHcy could be due to residual confounding from common dietary sources with B-vitamins. Another possible explanation is related to the construction and limitations of the FFQ. For example, choline and betaine are not included in the Norwegian food composition tables, and we could therefore not examine whether the associations could be due to the dietary content of these factors. Some studies (Sakamoto *et al.* 2002; Zeisel *et al.* 2003) as well as the USDA National Nutrient Database for Standard Reference (US Department of Agriculture, 2005) report a high content of these quaternary ammonium compounds in some plant food, and negative associations between intake and plasma concentrations of choline, betaine and plasma tHcy have been reported in previous studies (Olthof *et al.* 2005; Ueland *et al.* 2005; Cho *et al.* 2006).

Food groups and food items

In line with previous studies (Vollset *et al.* 2001; Ganji & Kafai, 2004), we found inverse associations between plasma tHcy and dietary intake of citrus fruit, orange juice, cruciferous vegetables, spinach/green cabbage and peppers. Furthermore, we observed, as have others, that plasma tHcy is inversely associated with milk (Shimakawa *et al.* 1997; Ganji & Kafai, 2004) and fish (Brude *et al.* 1999). We also found inverse relations with apples, carrots, onions, lettuce, cucumbers, tomatoes, mushrooms and eggs.

The overall food group of meat was not significantly related to tHcy. However, further examination revealed that non-processed meat and chicken were inversely, while processed meat was positively associated with tHcy. Non-processed meat is a source of protein, vitamin B_{12}, vitamin B_6, riboflavin, choline and betaine which all are negatively associated with plasma tHcy (Holm *et al.* 2005; Cho *et al.* 2006). Processed meat contains less protein and B-vitamins but more fat than non-processed meat. The nutrient fat is positively related to tHcy, and this may partly explain the positive association with processed meat (Appel *et al.* 2000). Associations between tHcy and different types of fat will be examined in more detail in a forthcoming paper from our group (P Berstad *et al.*, unpublished results).

The intake of sweets and cakes, high in fat and sugar, was associated with high tHcy concentrations, consistent with the present finding that the intake of simple carbohydrates (sugar) was negatively associated with dietary folate, vitamin B_{12}, vitamin B_6 and riboflavin.

After additional adjustment for dietary B-vitamins and for plasma folate and vitamin B_{12}, whole-grain bread, eggs, sweets, citrus fruits, carrots, onions and non-processed meat remained significantly associated with plasma tHcy concentration. This could be due to residual confounding, or that these food groups or items contain choline, betaine or methionine (Jacques *et al.* 2001; Verhoef & de Groot, 2005; Cho *et al.* 2006). A high content of these compounds has been reported in eggs, whole-wheat bread, meat and liver, and a lower content in fruits and vegetables (Zeisel *et al.* 2003; US Department of Agriculture, 2005). The relationship between tHcy concentration and dietary choline, betaine and methionine should be further examined.

Because people do not eat individual nutrients and food items in isolation, it is important to examine the effect of the total diet on plasma tHcy concentration. Although in the present study we did not evaluate the effect of the total diet, the present results are in general agreement with studies that have examined associations between dietary patterns and tHcy (Fung *et al.* 2001; Gao *et al.* 2003; Lasheras *et al.* 2003; Weikert *et al.* 2005), namely inverse associations between tHcy and diets rich in fruits, vegetables, fish, meat, milk, whole-grain bread and mushrooms (Gao *et al.* 2003; Lasheras *et al.* 2003; Weikert *et al.* 2005). Dietary patterns high in refined cereals (Gao *et al.* 2003), fat (Appel *et al.* 2000; Weikert *et al.* 2005) and sugar have been associated with higher tHcy levels (Fung *et al.* 2001).

Implications and conclusion

The present findings suggest that in a Norwegian adult and elderly population not taking vitamin supplements, plasma

tHcy concentration may be lowered with a diet rich in complex carbohydrates, protein and B-vitamins. Such a diet includes vegetables, fruits, whole-grain bread and cereals, as well as fish, non-processed meat, chicken and eggs. A high consumption of fat and sugar-rich foods such as sweets, cakes and processed meat may increase tHcy concentration. In conclusion, a diet high in B-vitamins and low in fat and sugar is associated with low plasma tHcy concentration in a population not taking vitamin supplements.

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