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Eating patterns are associated with biomarkers in a selected population of university students and employees

Sigrunn Hernes[1], Rona N. Cabo[2], Mohammad Azam Mansoor[2] and Margaretha Haugen[1,3]*

[1]*Department of Public Health, Sport and Nutrition, Faculty of Health and Sports, University of Agder, Kristiansand, Norway*
[2]*Department of Natural Sciences, Faculty of Engineering and Science, University of Agder, Kristiansand, Norway*
[3]*Department of Food Safety and Nutrition, Norwegian Institute of Public Health, Oslo, Norway*

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Abstract

The association between diet and CVD cannot be assigned to a single nutrient, but rather to a set of nutrients and non-nutrients, and eating pattern analyses have become an important tool in investigation of this relationship. Our objective was to investigate eating patterns in relation to nutrient intake and serum concentration of folate, vitamin B_{12} and TAG in ninety-five healthy adult participants. Dietary information was collected by an FFQ, and eating patterns were obtained by principal components analyses of thirty-three food groups. Three eating patterns were extracted, a sweet eating pattern identified by intakes of cakes, snacks, sugar-sweetened drinks and chocolates; a prudent eating pattern identified by vegetables, fruits and olive oil; and a traditional food pattern identified by red meat, lean fish and cheese. Blood samples were collected in the morning after an overnight fast. Linear regression analyses adjusted for age, BMI and smoking showed a negative association between the sweet eating pattern scores and the serum concentration of folate (β = −2·31 (95 % CI −4·14, −0·45)) and a positive association with serum concentration of TAG (β = 0·35 (95 % CI 0·12, 0·57)). The prudent eating pattern scores were positively associated with the serum concentration of folate (β = 1·69 (95 % CI 0·44, 2·92)). In conclusion, a sweet eating pattern was associated with risk factors for CVD, whereas a prudent eating pattern was associated with protective factors.

Key words: Eating patterns: Biomarkers: Serum folate: TAG: CVD

An unbalanced diet contributes to the development of chronic diseases, whereas a healthy diet promotes health and reduces the risk. Findings from the Health Professionals Follow-Up Study and the Nurses' Health Study cohorts suggest that an eating pattern associated with high consumption of fruits, vegetables, whole grains, legumes and poultry (known as the prudent pattern) is associated with a lower risk of CVD[(1)]. An eating pattern, known as the Western pattern, and characterised by high consumption of refined cereals, processed and red meats, desserts and high-fat dairy products, has been associated with an increased risk of CHD and type 2 diabetes[(2–4)]. The eating pattern approach intuitively has an appeal because the human diet does not consist of single nutrients, but of food items composed of many nutrients[(5)]. However, combination studies referring to nutrient intake and eating patterns might add further insight into the impact of nutrition on disease[(6)].

A reduced intake of folate, vitamins B_{12} and B_6 results in an increased concentration of plasma homocysteine, and an increased concentration of plasma homocysteine has been found to be associated with CVD[(7–9)]. Large epidemiological studies have shown a clear association between intake of vitamin B and CVD[(10,11)], and intervention with folic acid fortification of grain products has resulted in reduction in stroke mortality outcomes[(12)]. Meta-analyses of folic acid supplementation studies have also indicated a protective effect[(13,14)], although not convincing[(14)].

The metabolic syndrome is a known risk factor for CVD, and one of the criteria for this syndrome is dyslipidaemia

* **Corresponding author:** Margaretha Haugen, fax +47 21076686, email margaretha.haugen@fhi.no

with elevated serum TAG concentration[15]. High dietary intake of carbohydrates, especially sucrose and fructose, is known to increase TAG concentration, and dietary patterns with a high content of sucrose have also been associated with dyslipidaemia[16,17]. Thus, both nutrient intake and eating patterns are useful to explore diet and disease associations. The aim of this study was to evaluate eating patterns in relation to nutrient intake and serum concentrations of folate, vitamin B_{12} and TAG.

Methods

Design and subjects

Data collection was carried out from March to May 2008 at the University of Agder, Kristiansand, Norway. This project (ref: S-0627a, 2008/1851) was part of an intervention study with folic acid supplementation for 2 weeks, where the participants were given 800 μg/folic acid per d in a double-blind cross-over trial. Healthy students and employees were invited to participate in the study. Exclusion criteria were pregnancy, those on medication and use of vitamin B supplements for the last 3 months. Different approaches were used to recruit the participants. The students were contacted in the student canteen and the employees were recruited from three different faculties and among the technical and library staff. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Committee of Ethics and the Data Inspectorate in Norway. Written informed consent was obtained from all subjects.

Blood sample

Blood samples were collected in the morning between 07.30 and 08.30 hours after an overnight fast. The blood samples were placed on ice and plasma was isolated within 30 min. The serum and plasma samples were stored at −80°C. The concentrations of serum folate, vitamin B_{12} and TAG were measured with a Cobas 6000 at the Laboratory of Medical Biochemistry, Sørlandet Hospital, Arendal. The analytical method for the measurement of serum TAG has a CV of 2·0–2·6 %. The analytical methods for the measurement of serum folate and vitamin B_{12} have a CV of 5 and 10 %, respectively.

Dietary data

A validated semi-quantitative FFQ was used for the dietary information, which included 255 different food items grouped into twenty-six main questions[18]. In the FFQ, habitual dietary intake for the last 12 months was asked. Nutrient calculations were performed with the use of FoodCalc, a software program for nutrient calculation on the Internet[19], and the Norwegian Food Composition table[20]. All participants had a calculated energy intake between 4500 and 20 000 kJ[21]. A total of thirty-three different food groups were included in the principal components factors analysis to identify independent clusters of eating patterns. In all, three different food patterns were recognised with an eigenvalue >2·5 and accounting for 34 % of the total variation. The three patterns were extracted on the basis of the scree plot and the factor loading matrix was evaluated after orthogonal (varimax) rotation. Food groups with loadings >0·4 were used to identify the eating patterns. For each participant, factor scores were produced by multiplying factor loadings with the standardised value for each food and summing across the food items. These factor scores were used for further analyses. A high factor score will indicate a high intake of the foods in the special food pattern, and a negative factor score will indicate a low intake of foods in that pattern.

Statistics

Data are given as mean values and standard deviations if normally distributed and as median and interquartile range if non-normally distributed. Group differences for normally distributed variables were analysed using independent samples *t* tests and skewed distribution group differences were analysed with the Mann–Whitney *U* test. The χ^2 test was used for nominal data. To investigate correlations between factor sores and nutrient intakes, we used the Spearman correlation coefficient. Multiple linear regression models were built with serum folate, vitamin B_{12} and TAG as dependent variables and factor scores of the food patterns were added as continuous variables. The models were checked for normality of the residuals and because of non-normality the model for vitamin B_{12} could not be used. In all regression models, all three eating patterns were included in addition to energy as suggested by Newby *et al.*[22]. In the adjusted models, age, sex, smoking categories (non-smokers and daily smokers) and BMI were adjusted. All analyses were performed using PASW version 18 (SPSS Inc.) and $P < 0·05$ was regarded as statistically significant.

Results

We recruited forty-five university students and fifty-three university employees. Blood samples from ninety-five subjects were available for biochemical analyses (Table 1). There was no significant difference between the groups except for age. Mean age was 52 years among employees and 23 years among the students. Intake of energy and nutrients was similar in the two groups, except intake of added sugar, where the students had a higher intake.

In all, three different eating patterns were extracted with the use of the principal components analysis method: a 'sweet' eating pattern that was identified by sweets and snacks; a prudent eating pattern identified by vegetables, fruits and poultry; and a traditional eating pattern identified by red meat, fish products and boiled potatoes and jam (Table 2). There was a statistically significant difference in food intake between the first and third tertile in the sweet and prudent eating patterns, but not for the traditional eating pattern (data not shown). The sweet eating pattern score correlated positively with the total intake of energy, protein, fat and carbohydrate (Table 3). When energy adjusted the correlations with protein, fibre, folate and vitamin

Table 1. Demographic characteristics of the participants
(Mean values and standard deviations, median values and interquartile ranges (IQR) or numbers of participants and percentages)

	Employees (*n* 50)		Students (*n* 45)	
	Mean	SD	Mean	SD
Sex: females				
n	37		35	
%	74		78	
Age (years)	52·8	6·9	23·0*	2·9
Weight (kg)	73·6	14·2	69·9	11·7
BMI (kg/m²)	24·4	3·5	23·8	3·5
Daily smokers				
n	8		3	
%	16		7	
Serum folate (nmol/l)	17·9	6·8	16·6	5·2
Serum vitamin B_{12} (pmol/l)				
Median	295		277	
IQR	176		129	
Serum TAG (mmol/l)				
Median	1·24		1·00	
IQR	0·60		0·79	
Energy intake (kJ)	9·7	3·0	10·1	2·4
Protein (E%)	16·8	2·5	16·8	2·4
Fat (E%)	32·8	4·8	31·2	5·4
Saturated fat (E%)	12·3	2·7	11·6	2·5
Polyunsaturated fat (E%)	6·0	1·3	6·0	1·5
Carbohydrates (E%)	49·0	5·2	50·8	5·4
Added sugar (E%)	7·6	3·5	8·9*	4·8
Fibre (g/10 MJ)	36·0	10·4	34·9	9·0
Folate (μg/10 MJ)	295	85	299	87
Vitamin B_{12} (μg/10 MJ)	7·3	3·4	7·0	2·5
Vitamin C (mg/10 MJ)	158	78	163	83

E%, Energy percentage of total energy intake.
* Mean value was significantly different from that of the employees (P<0·05).

B_{12} were changed and negatively correlated. The prudent eating pattern correlated positively with the intake of fibre, folate and vitamin C, and these correlations did not change when energy was adjusted. The traditional eating pattern correlated positively with protein, fat, carbohydrates, fibre and fat, but not with the energy-adjusted intakes.

Significantly, more employees than students were categorised in the third tertile of the traditional eating pattern, 55 % compared with 9 % (P < 0·001, χ^2 test), while there was no significant difference between the two groups for adherence to the sweet and prudent eating patterns. Of the males, 12 % were categorised in the third tertile for the prudent diet, while 41 % of the females were categorised into this tertile (P = 0·001).

The concentrations of serum folate, vitamin B_{12} and TAG did not differ between the students and the employees (Table 1). Serum folate concentration was positively associated with energy-adjusted intake of folate (β = 0·03, P = 0·001). In the sweet eating pattern, serum folate concentration decreased with increased adherence, whereas in the prudent eating pattern serum folate concentration increased with increased adherence (Table 4). No association was identified for serum vitamin B_{12} concentration and the tertiles of the eating patterns, but there was a positive correlation between the energy-adjusted calculated intake and the serum concentration of vitamin B_{12} (β = 0·03, P = 0·005). For the sweet eating pattern, there was a positive association with the serum concentration of TAG, whereas there was a negative association with the prudent eating pattern and serum TAG. The serum concentration of TAG was significantly associated with intake

Table 2. Varimax rotated loading coefficients of the three eating factors identified

Pattern	Food	Loading coefficient	Cumulative variance explained (%)
Cakes and sweets	Cakes and sweet rolls	0·72	15
	Snacks	0·69	
	Sugar-sweetened drinks	0·68	
	Chocolate bars	0·58	
	Desserts	0·56	
	Mayonnaise	0·52	
	Waffles and pancakes	0·48	
	Milk	0·45	
	Potatoes, fried	0·43	
	White bread	0·41	
	Tomatoes and ketchup	0·42	
	Pizza and tacos	0·42	
Prudent	Salads and raw vegetables	0·64	25
	Fruit and berries	0·58	
	Chicken and poultry	0·57	
	Vegetables, boiled	0·57	
	Muesli and hot cereals	0·51	
	Olive oil	0·47	
	Fish spreads	0·46	
	Herbal tea	0·41	
	Processed meat	−0·65	
Traditional	Jam and honey	0·60	34
	Fresh red meat	0·54	
	Coffee	0·50	
	Potatoes, boiled	0·45	
	Cheese	0·45	
	Lean fish	0·43	
	Chicken and poultry	−0·42	
	Pasta	−0·58	

Table 3. Spearman correlation coefficients of dietary pattern scores and calculated intake of nutrients from an FFQ asking about food intake in the last 12 months

Nutrients	Sweet eating pattern	Prudent eating pattern	Traditional eating pattern
Energy (kJ)	0·69***	0·18	0·29**
Protein (g)	0·50***	0·29**	0·33**
Protein (E%)	−0·65***	0·15	−0·07
Fat (g)	0·66***	0·06	0·28**
Fat (E%)	0·15	−0·18	0·02
Saturated fat (g)	0·70***	−0·11	0·31**
Saturated fat (E%)	0·30**	−0·36**	0·16
Polyunsaturated fat (g)	0·52**	0·10	0·16
Polyunsaturated fat (E%)	0·02	−0·04	−0·20
Carbohydrates (g)	0·66***	0·20	0·22*
Carbohydrates (E%)	0·15	0·15	−0·05
Added sugar (g)	0·87***	0·08	0·12
Added sugar (E%)	0·62***	−0·02	0·00
Fibre (g)	0·26*	0·49***	0·22*
Fibre (g/10 MJ)	−0·45***	0·55***	0·05
Folate (μg)	0·18	0·66***	0·15
Folate (μg/10 MJ)	−0·51***	0·60***	−0·10
Vitamin B_{12} (μg)	0·12	0·34**	0·29*
Vitamin B_{12} (μg/10 MJ)	−0·45***	0·24*	0·04
Vitamin C (mg)	0·09	0·51***	0·12
Vitamin C (mg/10 MJ)	−0·28*	0·41***	−0·02

E%, energy percentage of total energy intake.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$.

of added sugar (β = 0·03, P = 0·012), but not with total carbohydrate or starch intake.

In the multiple regression models, adherence to the sweet eating pattern was negatively associated with serum folate concentration, whereas adherence to the prudent diet was positively associated with serum folate concentration (Table 5). Adherence to the sweet eating pattern was positively associated with TAG concentration.

Discussion

In the present cross-sectional study, we found three eating patterns explaining 34 % of the variance in food intake among students and employees at a university in southern Norway. The sweet eating pattern was identified by intake of cakes, snacks, sugar-sweetened drinks and chocolates, and the prudent eating pattern was identified by intake of vegetables, fruits and olive oil. The traditional food pattern was identified by intake of red meat and cheese. In the multiple linear regressions, we found that the sweet eating pattern was negatively associated with serum folate concentration and positively with increased serum concentration of TAG, while the prudent eating pattern was associated with an increase in serum folate concentration.

Dietary patterns have been recognised as useful tools in nutritional science to evaluate the multidimensional nature of

Table 4. Serum concentrations of biomarkers according to tertiles of eating pattern
(Mean values and standard deviations or median values and interquartile ranges (IQR))

	Tertile 1		Tertile 2		Tertile 3		
Eating patterns	Median	IQR	Median	IQR	Median	IQR	P for trend
Serum folate concentration (nmol/l)							
Sweet							0·011
Mean	18·4		18·0		15·6		
SD	6·9		7·0		5·1		
Prudent							< 0·001
Mean	14·8		16·8		20·1		
SD	4·7		5·1		7·1		
Traditional							0·345
Mean	16·6		18·1		17·0		
SD	6·4		6·3		5·6		
Serum vitamin B_{12} concentration (pmol/l)							
Sweet	259	125	328	168	302	112	0·829
Prudent	308	117	324	166	311	149	0·824
Traditional	312	127	314	156	317	169	0·935
Serum TAG concentration (μmol/l)							
Sweet	1·19	0·59	1·26	0·88	1·53	0·98	0·004
Prudent	1·59	0·96	1·34	0·68	1·06	0·68	0·004
Traditional	1·55	0·90	1·24	0·84	1·18	0·59	0·063

Table 5. Multiple linear regression models with serum folate and TAG as dependent variables

	Model 1†			Model 2‡		
	R^2	β	95 % CI	R^2	β	95 % CI
Serum folate as dependent variable						
	0·24			0·31		
Sweet		–2·89**	–4·76, –1·02		–2·31*	–4·14, –0·45
Prudent		1·92**	0·75, 3·08		1·69**	0·44, 2·92
Traditional		0·22	–0·95, 1·40		–0·44	–1·92, 1·04
Serum TAG as dependent variable						
	0·32			0·51		
Sweet		0·45**	0·19, 0·70		0·35**	0·12, 0·57
Prudent		–0·16*	–0·30, –0·02		–0·11	–0·24, 0·02
Traditional		–0·14	–0·29, 0·00		–0·32***	–0·48, –0·15

* *P* < 0·05, ** *P* < 0·01, *** *P* < 0·001.
† Model 1 adjusted for energy intake.
‡ Model 2 adjusted for energy, BMI, age, smoking and sex.

diets[22]. In dietary studies with disease outcomes, different methods have been used to construct not only eating patterns, such as the Healthy Eating Index, but also posterior methods such as cluster and factor analyses have been used. However, very few studies have exploited biomarkers in relation to different food patterns[23,24]. In the present study, we investigated eating patterns developed by principal components analysis, which is a data-driven statistical method designed to isolate and identify independent clusters[5].

Our extraction with the sweet eating pattern explaining most of the variance was surprising, because in many studies most variance is explained by the healthy (i.e. a prudent) or the traditional eating pattern[5,25]. Although students had a higher intake of added sugar compared with the employees, as many of the students were ranked in the third tertile of the sweet eating pattern as the employees. This might indicate that students are a less homogeneous group, with both health-conscious and not-so health-conscious individuals, and students are known to have a more irregular life style. The only eating pattern that was preferred by the employees was the traditional eating pattern, indicating a different life style in the two groups[26]. It has been demonstrated that women more often adhere to a prudent diet, i.e. eat more vegetables, and that was also seen with this method, where significantly more women were included in the third tertile of the prudent eating pattern[27].

Calculated nutrient intake correlated significantly with the sweet and the traditional eating pattern scores, similar to results from earlier studies[28]. However, adjusted for energy, the correlations were negative between the nutrients and the sweet eating pattern, which indicates a poor-nutrient-dense pattern (i.e. nutrient per kJ). The energy adjustment of fibre, folate, vitamin B_{12} and vitamin C did not remove the correlations with the prudent eating pattern, confirming a more nutrient-dense eating pattern.

An increase in serum folate concentration with increased adherence to the prudent eating pattern and a reduction with adherence to the sweet eating pattern might indicate an association between these patterns. This was not seen and would have also been in disagreement with the principal components analysis method[22]. In the linear regression model, the inclusion of all three patterns did not weaken the associations, which indicates that the three patterns were not correlated factors.

None of the extracted dietary patterns correlated with serum vitamin B_{12} concentration. Important sources of vitamin B_{12} are animal proteins, and although only meat and fish were identified in the traditional food pattern, all participants had a diet including both meat and fish. An evenly distributed intake of these food items in the three eating patterns might explain why vitamin B_{12} did not correlate with any of the extracted eating patterns in the present study, while there still was a positive correlation between calculated vitamin B_{12} intake and the serum concentration.

A high concentration of serum TAG is an indicator and predictor of CVD, and has been shown to correlate with intake of disaccharides[29]. In the present study, we found a positive association between TAG and the sweet eating pattern and an indication of an inverse association with the traditional eating pattern. The sweet eating pattern was also the only eating pattern that correlated with the intake of added sugar. The positive associations with TAG and the sweet eating pattern add strength to the association between sugar intake and CVD.

The present study is limited by the low power, with only ninety-five participants, and by a selected population consisting of university students and employees. Furthermore, with an FFQ it is impossible to register all food items eaten by an individual, and the nutrient calculations are always dependent on the quality of the nutrient database. However, the study included only healthy individuals who were not on medication and without vitamin B food supplements during the last 3 months. In addition, folate fortification was not present in Norwegian food at the time of the study inclusion. In most epidemiological studies, this factor is impossible to adjust for because medication or food supplements are rarely reported. Furthermore, in Norway there is virtually no fortification with vitamins and minerals, which strengthen the nutrient calculations.

In conclusion, the present findings indicate that adhering to the prudent eating pattern is associated with reduced known risk factors for CVD, whereas adhering to the sweet eating pattern may be associated with increased risk for chronic disease. The eating pattern approach is interesting and can be used in epidemiological studies in combination with the more traditional nutrient approach to shed light on the impact of diet on disease.

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