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Food patterns, inflammation markers and incidence of cardiovascular disease: the Malmö Diet and Cancer study

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Abstract. Hlebowicz J, Persson M, Gullberg B, Sonestedt E, Wallström P, Drake I, Nilsson J, Hedblad B, Wirfält E (Lund University, Skåne University Hospital, Malmö, Sweden). Food patterns, inflammation markers and incidence of cardiovascular disease: the Malmö Diet and Cancer study. *J Intern Med* 2011; doi: 10.1111/j.1365-2796.2011.02382.x.

Objectives. To examine the associations between food patterns constructed using cluster analysis and markers of systemic and vascular inflammation, and incident cardiovascular disease (CVD) after 13 years of follow-up.

Design. Population-based, prospective cohort study.

Setting and subjects. Cluster analysis identified six food patterns from 43 food group variables among 4999 subjects, aged 45–68 years, who participated in the Malmö Diet and Cancer cardiovascular programme between 1991 and 1994. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), C-reactive protein concentration and white blood cell (WBC) count were measured using blood samples at baseline. Incidence of CVD (coronary events and ischaemic stroke) was monitored over 13 years of follow-up.

Results. The fibre-rich bread pattern was associated with favourable effects on WBC count in women, and the low-fat and high-fibre pattern with favourable effects on Lp-PLA_2 mass in women, and on Lp-PLA_2 activity in men. However, the milk fat and sweets and cakes patterns were both associated with adverse effects; the former on WBC count in women and on Lp-PLA_2 mass in men, and the latter on WBC count and Lp-PLA_2 mass in women. The milk fat and sweets and cakes patterns were associated with increased CVD risk in women.

Conclusions. The results of this study support the present Nordic dietary recommendations indicating that diets rich in high-fibre, low-fat and low-sugar foods are favourably associated with markers of inflammation and, potentially, with CVD risk.

Keywords: CVD, epidemiology, inflammation, lipoproteins, lymphocytes, nutrition.

Introduction

Atherosclerosis is the major cause of acute myocardial infarction (MI), stroke and peripheral artery disease. Markers of systemic inflammation such as C-reactive protein (CRP) [1, 2] and white blood cell (WBC) count [3–6] are associated with increased risk of cardiovascular disease (CVD). Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a novel inflammatory marker associated with metabolic syndrome, low-density lipoprotein (LDL) cholesterol, carotid artery atherosclerosis and prevalent and incident CVD [7, 8]. The enzyme Lp-PLA_2 is produced by inflammatory cells and circulates in the blood mainly bound to LDL cholesterol. Lp-PLA_2 converts oxidized phospholipids on the surface of LDL cholesterol into the highly pro-inflammatory metabolites lysophosphatidylcholine and oxidized-free fatty acids [9]. Epidemiological studies have shown an association between Lp-PLA_2 mass, Lp-PLA_2 activity and incident CVD [8, 10–14].

The relationship between dietary factors and CVD has been studied extensively. There is strong evidence that dietary patterns with high intakes of vegetables, legumes, fruits, nuts, whole-grain products, cheese or yoghurt and monounsaturated rather than saturated fats (i.e. 'prudent' and Mediterranean diets) offer protection against coronary heart disease (CHD) [15]. However, the intake of trans-fatty acids and

foods with a high glycaemic index or load increases the risk of CHD [15]. By grouping individuals with similar dietary characteristics, the effects of the whole diet can be elucidated, rather than the effects of individual foods and nutrients [16]. Dietary patterns determined using cluster analyses have proved useful when assessing diet–disease associations [17]. Diet has consistently been shown to modulate inflammation [18–23]. However, information about the possible interactions between dietary factors and Lp-PLA_2 activity and mass and WBC count in vascular inflammation is still limited. In addition, data regarding the link between food patterns and these inflammation markers (i.e. Lp-PLA_2 activity and mass, and WBC count) are lacking, and the prospective association with CVD needs to be examined further. Thus, the aim of the present study was to examine whether food patterns constructed using cluster analysis are cross-sectionally associated with markers of systemic and vascular inflammation. A secondary aim was to explore the association between food patterns and risk of incident CVD during 13 years of follow-up in the same Swedish urban population-based cohort, to potentially yield supportive evidence.

Subjects and methods

Study population

The Malmö Diet and Cancer (MDC) study is a prospective population-based cohort study designed to explore the effects of diet on cancer risk [24]. All men (aged 46–73 years) and women (aged 45–73 years) living in the Swedish city of Malmö were eligible for inclusion; 11 063 men and 17 035 women took part in the study, with baseline examinations between March 1991 and October 1996. Approximately 40% of the total population participated, either spontaneously or after receiving an invitation by post. Between October 1991 and February 1994, every other participant was invited to take part in a study of the epidemiology of carotid artery disease. This group underwent additional examinations to assess carotid arteriosclerosis by ultrasound (n = 6103) and donated blood after an overnight fast (n = 5533) [7, 8]. Both dietary data and blood samples were obtained from a total of 5135 individuals. Of these, 136 were excluded because they were either using medication for or had previously been diagnosed with diabetes mellitus. At baseline, 126 subjects had a history of CVD (hospitalization for stroke or MI). The remaining 4999 individuals (2040 men and 2959 women; including those with a fasting whole blood sugar level of ≥5.6 mmol L^{-1} (corresponding to plasma-glucose ≥6.1 mmol L^{-1}) but without diabetes mellitus) constitute the present study population. The Ethics Committee of Lund University approved the MDC study. All participants provided informed consent. This study is registered in the US National Library of Medicine as trial number NCT01216228.

Dietary, lifestyle and other variables

Participants in the cardiovascular sub-cohort of the MDC study visited the study centre on three occasions. During the first visit, trained staff provided groups of participants with detailed instructions about the data collection procedure, distributed the study questionnaires and conducted anthropometric measurements. At the second visit, trained interviewers conducted a diet history interview and checked the correctness of questionnaires completed at home. Fasting blood samples were drawn from the participants during the third visit and stored in a biobank. Dietary history data were obtained for the MDC study by combining a 7-day menu book ('current' diet information) and a diet history questionnaire ('usual' diet information) with a 1-h interview. The reference period for the questionnaire was the preceding year. A total of 13 interviewers conducted the diet interviews. Studies of the reproducibility and validity of these methods have been published previously [25–27].

Information on smoking habits, socioeconomic status, marital status and physical activity was retrieved from a self-administered questionnaire. Detailed descriptions of these variables have been published previously [16, 17]. In short, subjects were defined as current smokers, former smokers or never smokers. Socioeconomic status was recorded according to the Swedish population census as blue-collar workers, white-collar workers or employers/self-employed [28]. Marital status was categorized as single, divorced or widowed versus married or cohabiting. Physical activity at work was self-rated as very light, light, medium, heavy or very heavy. Leisure-time physical activity questions were adapted from the Minnesota Leisure Time Physical Activity Questionnaire, and activity was categorized as low, moderate or high [29, 30]. Information on prior medical history was acquired from the questionnaire (regarding diabetes) and by record linkage with local or national registers using the unique 10-digit civil registration number (e.g. regarding diagnoses of cancer, MI or stroke). Weight (kg), height (m) and blood pressure (mmHg) after a 10-min rest in the supine position

were measured by trained nurses at one of the visits to the MDC centre. Body mass index (BMI) was calculated from the weight and height measurements (kg m^{-2}). Body composition was estimated using the single-frequency bio-impedance methodology. Waist circumference was measured midway between the lowest rib margin and the iliac crest; hip circumference was measured horizontally at the level of the greatest lateral extension of the hips. A categorical variable delineating the four seasons (winter, spring, summer and autumn) was defined from the date of the dietary interview. Information on age and gender was obtained through the personal identification number. In addition, a past change in diet was based on the following questionnaire item 'Have you, because of ill health or other reasons, substantially changed your food habits?' with yes or no response categories.

The construction of food patterns, using cluster analysis of the subjects (n = 5375) in the cardiovascular sub-cohort for whom dietary data were complete, has been described previously [16]. Briefly, all available dietary information was converted from grams of the specific foods consumed to the percentage of total energy contributed by these foods and was then aggregated into 43 food group variables. Six clusters seemed to reflect the food patterns in this sample most appropriately. The terms given to these food patterns indicate the major sources of food energy: 'many foods and drinks' (MFD), 'fibre bread', 'low fat and high fibre' (LFHF), 'white bread', 'milk fat' and 'sweets and cakes'. In the sweets and cakes cluster, 18% of the total energy was derived from sugar, sweets and jam; other major energy sources were cakes, biscuits and soft drinks. In the milk fat pattern, 12% of the total energy was derived from Bregott (a spread consisting of butter and rapeseed oil), and other major energy sources included cheese and whole milk and, to a lesser extent, white bread and sweets. In the white bread cluster, 16% of the total energy was derived from white bread, and the other major energy sources were low-fat margarine, both high-fat and low-fat meats and sweets. In the LFHF cluster, 10.5% of the total energy was derived from fruit, and 8% from low-fat milk, both high-fat and low-fat meats and sweets. Individuals in the fibre bread cluster obtained 15% of their energy from fibre-rich bread. Other major energy sources were meats, sweets and fruits; low-fat margarine and boiled potatoes were also comparatively important.

Vegetable consumption was highest in the LFHF and the fibre-rich bread clusters. The LFHF cluster had the lowest mean intake of total energy (8.70 MJ) and the lowest contribution from fat (34.3%), whereas the milk fat cluster had the highest energy (10.90 MJ) and fat intakes (44.5%). The LFHF cluster had the highest mean intake of total energy from protein (17.6%), and the milk fat and sweets and cakes clusters had the lowest protein intakes (15.0% and 15.1%, respectively). The milk fat cluster had the lowest intake of total energy from carbohydrates (40.6%), whereas the fibre bread and the LFHF clusters had the highest carbohydrate intakes (48.1% and 48.2%, respectively). The MFD cluster had the highest median intake of alcohol (10.8 g), whereas the LFHF and sweets and cakes clusters had the lowest median alcohol intakes (5.5 g for both). The milk fat cluster had the lowest mean fibre intake (17.5 g), and the fibre-rich bread and LFHF clusters had the highest mean fibre intakes (26.6 and 25.7 g, respectively). Compared with subjects in all other clusters, those in the fibre bread and LFHF food clusters were more likely, and those in the MFD, milk fat, and sweets and cakes clusters less likely, to report a past change in diet. As reported previously, more women belonged to the LFHF food cluster, whereas fibre bread and white bread clusters both included a higher proportion of men [17].

The following dietary variables were also examined: total energy (kcal), the percentage contribution of protein, fat and carbohydrate intakes to total energy and the relative intake of total fibre (g fibre per 1000 kcal).

Laboratory tests

Blood levels of glucose, insulin, haemoglobin (Hb)A1c, triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured after an overnight fast, according to standard procedures at the Department of Clinical Chemistry, Malmö University Hospital, as described previously [17]. LDL cholesterol level was calculated according to Friedewald's formula [31]. WBC count ($\times 10^9$ cells per L) was analysed using an automatic counter, in accordance with the standard methods at the laboratory of Malmö University Hospital. Twenty-eight participants were excluded from the original cohort of 4999: six had no WBC count, 16 had a WBC count $<2.5 \times 10^9$ cells per L and six had a count $>15.0 \times 10^9$ cells per L. Measurements of Lp-PLA_2 activity and of CRP concentration, using the high-sensitivity CRP (hsCRP) test, have been described in detail previously [7].

Classification of cardiovascular events

Record linkage with the Swedish Hospital Discharge Register, the National Myocardial Infarction Register, the Stroke Register of Malmö (STROMA) and the Cause of Death Register provided information on morbidity and mortality from CHD and stroke in the MDC study, including subjects that moved out of the city during follow-up [32–34]. Information on case retrieval, validity and ascertainment of cases in the MDC study has been described in detail previously [8]. Underlying causes of death were coded in accordance with the 9th and 10th versions of the International Classification of Diseases (ICD-9 and ICD-10, respectively). In brief, all subjects were followed from the baseline examination until first-time CVD event, emigration from Sweden or until 31 December 2006. A CVD event was defined as fatal or nonfatal MI (ICD-9: 410A–410X; ICD-10: I21), death because of ischaemic heart disease (ICD-9: 410–414; ICD-10: I20–I25) or cerebral infarction (ICD-9 code: 434; ICD-10: I63), or nonspecific stroke (ICD-9: 436; ICD-10: I64). Cerebral infarction (i.e. ischaemic stroke) was diagnosed when computed tomography, magnetic resonance imaging or autopsy could verify the infarction and/or exclude haemorrhage and nonvascular disease. If neither imaging nor autopsy was performed, the stroke was classified as nonspecific. By definition, patients with transient ischaemic attacks were excluded. Two cases with nonspecific stroke were categorized as cerebral infarctions.

Statistical methods

All statistical calculations were performed in spss (version 14.0, 2005; SPSS Inc., Chicago, IL, USA) and pasw Statistics (spss Statistics, version 18, 2009; SPSS Inc.) for Windows. The distributions of levels of triglycerides, glucose, insulin, HbA_1c, and hsCRP and of systolic and diastolic blood pressure were skewed and were therefore log-transformed. The general linear model examined the distributions of BMI, waist : hip ratio and percentage body fat by food pattern and gender, controlling for age, total energy and season of data collection. Similarly, the distributions of levels of HbA_1c, insulin, glucose, triglycerides and hsCRP, LDL : HDL ratio, systolic and diastolic blood pressure, WBC count and Lp-PLA_2 activity and mass were examined by food patterns and gender, controlling for age, total energy, season of data collection, percentage body fat and waist : hip ratio. Chi-squared analysis was used to examine the distribution of smoking across the six food patterns. Continuous variables are presented as the mean ± standard error. All analyses were gender specific, and *P*-values <0.05 were considered significant.

Logistic regression analysis was first used to examine the association between WBC count (in quartiles), hsCRP, Lp-PLA_2 activity and mass (in tertiles) and each food pattern (including risk trends across categories of inflammation markers), with all other food patterns as the reference, controlling for age, total energy, season of data collection, body fat, waist : hip ratio and smoking. Models were also formulated that included, systolic blood pressure, LDL : HDL ratio, socioeconomic status and levels of education, leisure-time physical activity, housework and physical activity at work. Lastly, sensitivity analyses were conducted in which only those subjects reporting no change in food habits were analysed. A model was also formulated controlling for alcohol consumption. Cox proportional hazards regression was used to examine the incidence of CVD in relation to each food pattern using the largest cluster (MFD pattern) as the reference and to estimate adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs). The initial model controlled for age, total energy, season of data collection, percentage body fat, waist : hip ratio, smoking and history of CVD. Additional models were formulated controlling for systolic blood pressure, antihypertensive medication, total cholesterol and triglycerides.

Results

Associations between food patterns and markers of inflammation and other cardiovascular risk factors

Body mass index was highest in both men and women in the LFHF cluster (Table 1), whereas percentage body fat was highest in women only in the LFHF cluster. The HbA_1c levels were highest in women in the milk fat cluster, while high LDL : HDL ratios were most common in women in the white bread cluster and in men in the sweets and cakes cluster, and high triglyceride levels were most common in women in the white bread cluster. There were no differences in plasma insulin and glucose levels or in systolic and diastolic blood pressure between the food patterns (Table 2). When the distribution of the four inflammation markers was examined across food patterns, the level of Lp-PLA_2 mass was highest in the milk fat cluster, and lowest in the LFHF cluster, in both women and men (Table 3). In women, the WBC count was highest in the milk fat cluster, and the Lp-PLA_2 activity was highest in the white bread

Table 1 *Obesity indicators according to gender and food patterns, in the Swedish Malmö Diet and Cancer cardiovascular cohort (n = 4999)*

	Mean (SE)[a]							
	1	2	3	4	5	6		
	Many foods and drinks	Fibre bread	Low-fat and high-fibre foods	White bread	Milk fat	Sweets and cakes	*F*-test	
	(*n* = 1399)	(*n* = 460)	(*n* = 755)	(*n* = 713)	(*n* = 638)	(*n* = 1034)	*P*-value	Multiple comparisons*,†
Body mass index (kg m^{-2})								
Women	25.53 (0.14)	24.92 (0.28)	26.15 (0.18)	26.00 (0.24)	24.95 (0.23)	24.79 (0.17)	<0.001	2 < 1, 1 < 3; 2 < 3, 4; 5, 6 < 1, 3, 4
Men	26.44 (0.15)	25.85 (0.22)	26.70 (0.28)	26.13 (0.17)	25.57 (0.20)	25.64 (0.17)	<0.001	2, 5, 6 < 1, 3
Waist : hip ratio								
Women	0.79 (0.002)	0.79 (0.003)	0.79 (0.002)	0.79 (0.003)	0.78 (0.003)	0.79 (0.002)	0.072	5 < 1, 4
Men	0.95 (0.003)	0.94 (0.004)	0.94 (0.005)	0.95 (0.003)	0.94 (0.003)	0.94 (0.003)	0.06	6, 5, 2 < 1
Percentage body fat[b]								
Women	31.39 (0.16)	30.62 (0.32)	31.85 (0.20)	31.71 (0.27)	30.83 (0.26)	30.71 (0.20)	<0.001	2, 5, 6 < 3, 4; 6, 5 < 3, 4
Men	21.52 (0.22)	20.70 (0.32)	21.18 (0.41)	21.47 (0.25)	20.97 (0.28)	20.81 (0.24)	0.087	2, 6 < 1; 6 < 4

[a]Adjusted for age, total energy and season of data collection. [b]Data are missing from seven subjects. *P < 0.05, †Least Significant Difference test.

cluster. No differences in hsCRP levels were observed between the various clusters (Table 3). Women and men in the milk fat cluster were more often current smokers, whereas former smokers were more common in the sweets and cakes cluster among women, and in the fibre bread cluster among men. More women in the fibre bread cluster and men in the LFHF cluster were never smokers (data not shown).

Multivariate analysis

In women, Lp-PLA_2 mass was inversely and significantly associated with the LFHF pattern [tertiles 2 and 3: odds ratio (OR) 0.89, 95% CI 0.71–1.12 and OR 0.69, 95% CI 0.54–0.87, respectively, P = 0.002] and positively associated with the sweets and cakes pattern (tertiles 2 and 3: OR 1.20, 95% CI 0.96–1.50 and OR 1.29, 95% CI 1.02–1.62, respectively, P = 0.030), taking age, total energy, season of data collection, body fat, waist : hip ratio and smoking into account (data not shown). When women who reported a past change in diet were excluded from the analysis, the associations between Lp-PLA_2 mass and both the LFHF and the sweets and cakes patterns were attenuated (P = 0.098 and P = 0.149, respectively).

In men, Lp-PLA_2 activity was inversely associated with the LFHF pattern (tertiles 2 and 3: OR 0.92, 95% CI 0.61–1.38 and OR 0.62, 95% CI 0.40–0.96, respectively, P = 0.036), and Lp-PLA_2 mass was positively associated with the milk fat pattern (tertiles 2 and 3: OR 1.17, 95% CI 0.85–1.62 and OR 1.50, 95% CI 1.10–2.05, respectively, P = 0.011), taking age, total energy, season of data collection, body fat, waist : hip ratio and smoking into account. When men reporting a past change in diet were excluded from the analysis, the inverse association between Lp-PLA_2 activity and the LFHF pattern was no longer significant (P = 0.352), but the association between Lp-PLA_2 mass and the milk fat pattern was stronger (P = 0.009) (data not shown).

When alcohol consumption was also added to the models, the associations between food pattern and Lp-PLA_2 mass and activity did not change.

In women, WBC count was inversely and significantly associated with the fibre bread pattern (quartiles 2–4: OR 0.69, 95% CI 0.48–0.99, OR 0.76, 95% CI 0.52–1.13 and OR 0.50, 95% CI 0.33–0.76, respectively, P = 0.003), and positively associated with the milk fat (quartiles 2–4: OR 1.08, 95% CI 0.77–1.52, OR 1.40, 95% CI 0.98–2.00 and OR 1.39, 95% CI 0.97–1.98,

Table 2 *Cardiovascular risk factors according to gender and food patterns in the Swedish Malmö Diet and Cancer cardiovascular cohort (n = 4999)*

	Mean (SE)[a]							
	1	2	3	4	5	6		
	Many foods and drinks	Fibre-rich bread	Low-fat and high-fibre foods	White bread	Milk fat	Sweets and cakes	*F*-test	
	(*n* = 1399)	(*n* = 460)	(*n* = 755)	(*n* = 713)	(*n* = 638)	(*n* = 1034)	*P*-value	Multiple comparisons*,†
HbA_1c (%)[b]								
Women	4.82 (1.00)	4.81 (1.01)	4.80 (1.00)	4.86 (1.00)	4.88 (1.00)	4.79 (1.00)	0.017	6 < 3; 6, 3 < 4; 3, 6 < 5
Men	4.86 (1.00)	4.81 (1.01)	4.74 (1.01)	4.82 (1.00)	4.83 (1.01)	4.81 (1.00)	0.549	
Plasma insulin (mIU L^{-1})								
Women	65.01 (1.04)	63.39 (1.08)	66.83 (1.05)	68.71 (1.06)	69.50 (1.06)	67.45 (1.05)	0.878	
Men	93.76 (1.05)	86.70 (1.08)	93.33 (1.11)	90.78 (1.06)	98.63 (1.07)	87.70 (1.06)	0.773	
Glucose (mmol L^{-1})								
Women	4.88 (1.00)	4.85 (1.01)	4.86 (1.00)	4.85 (1.01)	4.88 (1.01)	4.85 (1.00)	0.952	
Men	5.19 (1.01)	5.18 (1.01)	5.08 (1.01)	5.13 (1.01)	5.09 (1.01)	5.12 (1.01)	0.285	
LDL : HDL ratio[c]								
Women	2.84 (0.04)	2.92 (0.07)	2.98 (0.04)	3.13 (0.06)	2.93 (0.06)	3.07 (0.04)	<0.001	1 < 3, 4, 6; 2, 3, 5 < 4, 5 < 6
Men	3.52 (0.05)	3.68 (0.08)	3.59 (0.10)	3.64 (0.06)	3.58 (0.07)	3.70 (0.06)	0.004	1 < 6
Triglycerides (mmol L^{-1})								
Women	1.21 (1.03)	1.31 (1.06)	1.24 (1.04)	1.46 (1.05)	1.26 (1.05)	1.38 (1.04)	0.015	1 < 4, 6; 6, 5, 3 < 4; 3 < 6
Men	1.92 (1.05)	1.90 (1.07)	1.94 (1.09)	2.00 (1.05)	1.90 (1.06)	1.88 (1.05)	0.972	
Systolic blood pressure (mmHg)								
Women	137.72 (1.00)	139.32 (1.01)	138.36 (1.00)	139.32 (1.01)	138.04 (1.01)	140.00 (1.00)	0.603	
Men	142.23 (1.00)	142.56 (1.01)	144.21 (1.01)	141.58 (1.01)	142.56 (1.01)	141.91 (1.01)	0.715	
Diastolic blood pressure (mmHg)								
Women	84.72 (1.00)	84.72 (1.01)	84.92 (1.00)	84.53 (1.01)	84.53 (1.00)	85.11 (1.00)	0.916	
Men	88.51 (1.00)	88.10 (1.01)	88.92 (1.01)	87.70 (1.00)	89.33 (1.01)	88.10 (1.00)	0.216	

LDL, low-density lipoprotein; HDL, high-density lipoprotein. [a]Adjusted for age, total energy, season of data collection, percentage body fat and waist : hip ratio. [b]Data are missing from 13 subjects. [c]Data are from 60 subjects. *$P < 0.05$, †Least Significant Difference test.

Table 3 *Selected markers of systemic and vascular inflammation according to gender and food patterns in the Swedish Malmö Diet and Cancer cardiovascular cohort (n = 4999)*

	Mean (SE)[a]							
	1	2	3	4	5	6		
	Many foods and drinks	Fibre-rich bread	Low-fat and high-fibre foods	White bread	Milk fat	Sweets and cakes	*F*-test	
	(*n* = 1399)	(*n* = 460)	(*n* = 755)	(*n* = 713)	(*n* = 638)	(*n* = 1034)	*P*-value	Multiple comparisons*,†
Lp-PLA_2 mass (ng mL^{-1})[b]								
Women	258.72 (2.65)	257.15 (5.17)	250.64 (3.26)	263.62 (4.40)	269.25 (4.23)	265.42 (3.19)	0.004	3 < 1, 5; 3 < 4, 5, 6
Men	287.39 (3.76)	286.51 (5.48)	284.55 (6.97)	291.74 (4.22)	308.03 (4.84)	296.33 (4.17)	0.009	1, 2, 3, 4 < 5
Lp-PLA_2 activity (ng mL^{-1})[c]								
Women	41.59 (0.42)	42.98 (0.82)	42.01 (0.52)	44.06 (0.70)	43.27 (0.67)	43.40 (0.51)	0.007	1 < 4, 5, 6; 3 < 4
Men	49.17 (0.61)	50.70 (0.89)	47.58 (1.13)	49.89 (0.68)	50.09 (0.78)	49.93 (0.67)	0.291	
White blood cell count (10^6 cells)[d]								
Women	5.54 (0.06)	5.31 (0.11)	5.50 (0.07)	5.60 (0.10)	5.88 (0.09)	5.68 (0.70)	0.001	2 < 1, 5, 6; 1, 3, 4 < 5
Men	5.51 (0.10)	5.67 (0.15)	5.42 (0.19)	5.90 (0.12)	5.98 (0.13)	5.69 (0.12)	0.022	1 < 4, 5; 3 < 4, 5
hsCRP (mg L^{-1})[e]								
Women	1.01 (1.08)	0.81 (1.16)	0.97 (1.10)	1.11 (1.14)	0.94 (1.13)	1.04 (1.09)	0.711	
Men	0.93 (1.11)	0.88 (1.17)	1.02 (1.2)	1.21 (1.12)	1.20 (1.15)	1.03 (1.12)	0.439	

hsCRP, high-sensitivity C-reactive protein. [a]Adjusted for age, total energy, season of data collection, percentage body fat and waist : hip ratio. [b]Data are missing from 80 subjects. [c]Data are missing from 76 subjects. [d]Data are from six subjects. [e]Data are missing from 157 subjects. *$P < 0.05$, †Least Significant Difference test.

respectively, $P = 0.030$) and sweets and cakes patterns (quartiles 2–4: OR 1.01, 95% CI 0.79–1.31, OR 1.22, 95% CI 0.93–1.60 and OR 1.25, 95% CI 0.96–1.63, respectively, $P = 0.045$), taking age, total energy, season of data collection, body fat, waist : hip ratio and smoking into account. When women reporting a past change in diet were excluded from the analysis, the positive association between WBC count and the milk fat pattern was stronger ($P = 0.022$) and the inverse association with the fibre bread pattern remained significant ($P = 0.006$), but the association with the sweets and cakes pattern was no longer significant ($P = 0.127$) (data not shown).

In men, WBC count was inversely and significantly associated with the MFD pattern (quartiles 2–4: OR 0.74, 95% CI 0.56–0.97, OR 0.66, 95% CI 0.49–0.89 and OR 0.57, 95% CI 0.42–0.77, respectively, $P < 0.001$), and a positive association was seen with the milk fat pattern (quartiles 2–4: OR 1.05, 95% CI 0.72–1.53, OR 1.35, 95% CI 0.92–1.98 and OR 1.36, 95% CI 0.92–2.00, respectively, $P = 0.056$), taking age, total energy, season of data collection, body fat, waist : hip ratio and smoking into account. When men reporting a past change in diet were excluded from the analysis, the WBC count was still inversely and significantly associated with the MFD pattern ($P = 0.005$), but the association with the milk fat pattern was weaker ($P = 0.110$) (data not shown).

When smoking was excluded as a confounding factor from the models, the association between Lp-PLA_2 mass in women and Lp-PLA_2 activity in men and the LFHF pattern did not change. However, the associations between Lp-PLA_2 mass and Lp-PLA_2 activity and the milk fat pattern in women, as well as between Lp-PLA_2 mass and the milk fat pattern in men, were stronger ($P = 0.025$, $P = 0.002$ and $P = 0.034$, respectively) (data not shown). Similarly, the association between WBC count and the milk fat pattern was stronger in both men and women ($P = 0.003$ and $P = 0.001$, respectively) when smoking was excluded from the multivariate model. However, the associations did not change when other lifestyle factors (e.g. socioeconomic status and levels of education, leisure-time physical activity, household work and physical activity at work) or alcohol consumption was included in the analysis (data not shown).

Food patterns and incidence of cardiovascular events

During the 13-year follow-up period, a total of 449 subjects (180 women and 269 men) had an incident cardiovascular event (273 had an MI and 250 had a stroke; 202 events were ischaemic). An increased CVD risk was seen among women in both the milk fat and sweets and cakes patterns when compared to the MFD pattern (Table 4). Additional adjustment in the analysis for systolic blood pressure, antihypertensive medication and levels of total cholesterol and triglycerides did not substantially affect the associations (data not shown).

Discussion

The results of this study demonstrate cross-sectional associations between food patterns and markers of systemic and vascular inflammation, and longitudinal associations between food patterns and incident CVD in apparently healthy middle-aged subjects. Women and men were analysed separately because of gender differences in food selection and confounders across food patterns in this cohort. The milk fat pattern was found to be associated with a high WBC count in women and with Lp-PLA_2 mass in men. The fibre bread food pattern was associated with a low WBC count in women. Similarly, the LFHF pattern was associated with high Lp-PLA_2 mass in women and high Lp-PLA_2 activity in men. The sweets and cakes pattern was associated with a high WBC count and high Lp-PLA_2 mass in women. The dietary patterns dominated by milk fat or sweets and cakes were associated with increased risk of CVD in women.

To the best of our knowledge, this is the first study to examine associations between dietary factors and not only Lp-PLA_2 activity but also Lp-PLA_2 mass, WBC counts and hsCRP levels in a large cohort of middle-aged men and women. Lp-PLA_2 is an enzyme that is produced by inflammatory cells and contributes to atherosclerosis [35, 36]. The pro-inflammatory action of Lp-PLA_2 has also been supported by a number of epidemiological studies suggesting that Lp-PLA_2 concentration [10, 37–40] and Lp-PLA_2 activity [8, 10–13] are predictors of CVD. Hatoum *et al.* observed in a recent epidemiological study that smoking and alcohol and protein consumption may influence Lp-PLA_2 activity [20]. However, Beulens *et al.* [41] found in an intervention study that moderate alcohol consumption did not affect Lp-PLA_2 activity. In the present study, a high rate of current smoking was particularly common in the milk fat pattern in both women and men. Although residual confounding cannot be excluded, the results of our analysis suggest that both the milk fat pattern itself and smoking are associated with elevated levels of LpPLA_2 mass and activity. In addition, the protective effect of the fibre bread food pattern supports previous

Table 4 *Incidence of cardiovascular events (coronary heart events or ischaemic stroke) in relation to gender, by food patterns, in the Swedish Malmö Diet and Cancer cardiovascular cohort (n = 4999)*

	HR[a] (95% CI)					
	Many foods and drinks	Fibre-rich bread	Low-fat and high-fibre foods	White bread	Milk fat	Sweets and cakes
	(*n* = 1399)	(*n* = 460)	(*n* = 755)	(*n* = 713)	(*n* = 638)	(*n* = 1034)
Women						
Cases (*n* = 180)	38	13	38	21	26	44
Follow-up period (years)[b]	13.51 (0.07)	13.81 (0.14)	13.54 (0.08)	13.29 (0.12)	13.66 (0.11)	13.61 (0.08)
CVD risk	1.00	1.69 (0.70, 4.10)	1.76 (0.91, 3.40)	1.61 (0.72, 3.61)	2.20 (1.09, 4.44)	2.14 (1.17, 3.93)
Men						
Cases (*n* = 269)	57	36	20	58	40	58
Follow-up period (years)[b]	13.00 (0.11)	13.38 (0.16)	13.31 (0.20)	13.27 (0.12)	13.02 (0.14)	13.25 (0.12)
CVD risk	1.00	1.23 (0.74, 2.04)	0.61 (0.29, 1.27)	1.12 (0.65, 1.62)	1.18 (0.72, 1.92)	1.10 (0.72, 1.71)

HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease. [a]Adjusted for age, total energy, season of data collection, body fat percentage, waist : hip ratio, smoking and history of CVD. [b]Mean (SE).

reports of the benefits of whole-grain foods with regard to CHD [15]. Diets with a low-glycaemic index and/or low-glycaemic load have been reported to be associated with a reduced risk of type 2 diabetes and heart disease [42], comparable to the reduction in risk observed with high intakes of dietary fibre and whole-grain products. The fibre bread pattern could indicate a more frequent consumption of low-glycaemic index, high-fibre foods. By contrast, the sweets and cakes pattern indicates a more frequent consumption of high-glycaemic index, low-fibre foods. The results of the present study demonstrate cross-sectional associations between the sweets and cakes pattern and WBC count and Lp-PLA_2 mass, and also a potential longitudinal association with increased incident CVD in women.

In a recent small study, supplementation of the diet with marine n-3 polyunsaturated fatty acids showed no effect on Lp-PLA_2 compared with olive oil capsules [43]. In another trial, it was found that low-calorie diets (no more than 20% of energy intake from the same macronutrient) were associated with 10% weight loss and a reduction in the levels of Lp-PLA_2 in obese women [18]. These observations can be interpreted as supporting our findings, as a food pattern dominated by milk fat is likely to be high in energy, whereas food patterns dominated by high-fibre and low-fat foods are likely to have a low energy level.

Many hypotheses have been proposed to explain the associations between WBC count and CVD, including the induction of endothelial dysfunction by leucocytes [44], giving rise to mechanisms that mediate inflammation [5]. It has also been suggested that an elevated WBC count is simply a marker of a chronic inflammatory state, and that other aspects of inflammation may be the direct cause of vascular disease. There have been few studies to assess the relationship between WBC count and food patterns [19, 20]. Healthy eating patterns were found to be associated with reduced WBC count, but not CRP level, in a small study in overweight and obese postmenopausal women [21]. The Mediterranean diet, which is low in saturated and high in monounsaturated fat (mainly from olive oil), high in complex carbohydrates and fibre (mostly from legumes, other vegetables and fruit) and high in micronutrients (beta-carotene and vitamins B6, B12, C and E), has been associated with a reduction in WBC count and levels of interleukin-6, CRP and fibrinogen [19]. In addition, it has been suggested that vitamin E and omega-3 fatty acids play a role in T-cell differentiation [45, 46]. In the present study, the milk fat pattern was characterized by the

highest proportion of total energy derived from fat, the lowest fibre intake, the lowest intake of micronutrients (e.g. vitamin E) and the lowest ratio of polyunsaturated to saturated fatty acids [16]. By contrast, and probably because of the rapeseed oil in butter-based spreads in this population, the ratio of omega-3 and omega-6 fatty acids was comparatively high. The results of our study demonstrate a cross-sectional adverse association between the milk fat pattern and WBC count, as well as a potential longitudinal association with incident CVD in women. Together with the high prevalence of current smoking in the milk fat cluster (a factor that is closely related to inflammation), the observed nutrient intakes in this cluster may partly explain why it is associated with high WBC counts, in both men and women.

Previous studies on the relation between CRP and dietary patterns have reported inconsistent results. The healthy dietary patterns characterized by high intakes of vegetables, fruit and whole-grain products were shown to be related to lower concentrations of CRP in some [19, 22, 23], but not all [47, 48] studies. However, dietary patterns characterized by high intakes of fat and meat are more consistently associated with higher concentrations of CRP [22, 23, 47, 48].

As reported previously, low energy intake, obesity and past changes in diet were more common in the LFHF cluster [16]. This may reflect dietary change and dieting in overweight individuals, which are important confounders in nutritional epidemiology [49] and could explain why no significant associations were observed in the LFHF cluster when subjects with past dietary changes were excluded from the analysis. Those in the fibre bread cluster were more likely to have a low BMI and less likely to have changed their eating habits in the past [16]. Thus, when excluding individuals with potentially unstable food habits, associations between the milk fat and fibre bread patterns and inflammation markers remained or were strengthened.

A major advantage of this study is the high relative validity of the dietary data. Few epidemiological studies have had access to similar dietary data. The cross-sectional analysis of associations between inflammation markers and food patterns cannot establish causality, but may contribute to the formulation of hypotheses. Therefore, access to an extensive data set with information on many potential confounders (not available in other studies) is an important advantage. For instance, in the cross-sectional analysis, in which both inflammation markers and food patterns are assessed at one moment in time, associations were generally stronger when the analysis was limited to those individuals without self-reported previous change in food habits (i.e. excluding individuals with potentially unstable habits). This sensitivity analysis adds support to the observed associations in our study. Nevertheless, the power to detect significant associations between food patterns and CVD was low. Although a chance finding cannot be excluded, the observed significant association in women still adds some support to the hypothesis (i.e. diet is linked to CVD risk via inflammation processes). However, the association needs to be examined further after a longer follow-up period, and in other cohorts and populations. In addition, this is the first population-based study measuring not only dietary and lifestyle factors, but also hsCRP levels, WBC counts and Lp-PLA_2 mass and activity, and prospectively assessing the incidence of CVD in the same cohort. As hsCRP level, WBC count, and Lp-PLA_2 mass and activity were only measured once, misclassification is one potential cause of bias that might underestimate true associations. Also, as in many other large urban population-based cohorts, the participants were generally healthy and middle-aged, which may limit the possibility of extrapolating the results to the general population. Another potential limitation is that only 40% of the eligible population was included in the study sample, which potentially could result in selection bias. However, previous reports from the MDC cohort indicated that the socio-demographic structure and prevalence of obesity and smoking were similar compared to a postal health survey in the same population (with a participation rate of 74.6%), whereas the proportion reporting good health was higher [50].

In conclusion, the results of this study demonstrate the relations between food patterns and markers of inflammation in apparently healthy middle-aged subjects. The food pattern characterized by a large proportion of energy derived from milk fat (and with low intakes of several micronutrients) was associated with high WBC counts in women and increased Lp-PLA_2 mass in men. Also, in women, the food pattern characterized by a large proportion of energy derived from foods high in sugar was associated with high WBC counts and increased Lp-PLA_2 mass. After 13 years of follow-up, an increased incidence of CVD was observed in women reporting dietary patterns dominated by milk fat or sweets and cakes. An inverse association was found between the fibre bread cluster (with high intakes of several micronutrients) and WBC levels in women, whereas the pattern

dominated by low-fat, high-fibre foods was favourably associated with levels of Lp-PLA_2 mass in women and Lp-PLA_2 activity in men. As WBC counts and Lp-PLA_2 mass and activity are biomarkers associated with increased risk of CVD, it is necessary to identify means of influencing these factors. Thus, our study supports the present Nordic dietary recommendations by indicating that diets characterized by high-fibre, low-fat and low-sugar foods are favourably associated with markers of inflammation and, potentially, CVD risk.

Authors' contributions

The authors' contributions were as follows: JH, MP, BG, PW, JN, BH and EW contributed to the design of the study; JH, BH and EW performed the statistical calculations. JH and EW wrote the first draft of the manuscript and MP, BG, ES, PW, ID, JN, BH and EW made critical revisions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

No conflicts of interest to declare.

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