Dietary indices of atherogenicity and thrombogenicity and ischaemic heart disease risk: the Caerphilly Prospective Study

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The aim of the study was to investigate whether proposed dietary scores of atherogenicity and thrombogenicity predict ischaemic heart disease (IHD) risk in a community sample of men aged 45–59 years. Dietary scores were calculated from consumption of various fatty acids, estimated from 7 d weighed intake data obtained from 665 men. Investigation of associations with blood lipids, lipoproteins and haemostatic factors revealed positive associations with low-density-lipoprotein cholesterol ($P < 0{\cdot}05$) and white cell count ($P < 0{\cdot}05$), and a negative association with antithrombin III ($P = 0{\cdot}05$), after taking into account the effects of age, body mass index and smoking. During a 5-year follow-up period, there were twenty-one new IHD events among the 512 men in whom there was no evidence of IHD at baseline. Men with higher atherogenicity or thrombogenicity scores at baseline tended to have a higher risk of subsequent IHD. The trend was consistent but not statistically significant. A similar trend was observed for total saturates, and an inverse trend for total polyunsaturates, expressed as a percentage of total fatty acids. It is, therefore, concluded that proposed dietary indices of atherogenicity and thrombogenicity may be weak predictors of IHD risk, but that these scores are unlikely to be substantially better predictors than more simple approaches such as intakes of total saturates. To enhance the predictive ability, more complex formulas which take into account other dietary factors as well as fatty acid intakes would probably be required.

Fatty acids: Diet: Ischaemic heart disease

Two processes contribute to the development of ischaemic heart disease (IHD): atherosclerosis and thrombosis. The type of dietary fat consumed may contribute to both of these processes, some fatty acids having a greater role in atherogenesis while others have a greater role in thrombogenesis. Of the saturated fatty acids (SFA), only those with a chain length of 12, 14 or 16 C atoms have a cholesterol-raising effect and are thus atherogenic (Keys *et al.* 1965; Bonanome & Grundy, 1988). SFA with a chain length of 14, 16 or 18 C atoms have been suggested to be thrombogenic (Hornstra & Lussenberg, 1975). Both monounsaturated fatty acids (MUFA) and *n*-6 polyunsaturated fatty acids (*n*-6 PUFA) have been shown to reduce plasma total cholesterol and low-density-lipoprotein cholesterol (LDL-C) concentrations (for review, see Gurr *et al.* 1989). Long chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) have minimal effect on plasma cholesterol level but reduce plasma triacylglycerols thromboxane B_2 and platelet activity and prolong bleeding time and heparin–thrombin clotting time (HTCT) (for review, see Burr, 1989).

In an attempt to take into account the different effects of the various fatty acids, Ulbricht & Southgate (1991) proposed two indices which might better characterize the atherogenic

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and thrombogenic potential of the diet than simple approaches such as total saturates or polyunsaturated: saturated fatty acid (P:S) ratio:

$$\text{atherogenicity} = \frac{12{:}0 + (4 \times 14{:}0) + 16{:}0}{n\text{-}6\ \text{PUFA} + n\text{-}3\ \text{PUFA} + \text{MUFA}},$$

$$\text{thrombogenicity} = \frac{14{:}0 + 16{:}0 + 18{:}0}{(0{\cdot}5\ \text{MUFA}) + (0{\cdot}5\ n\text{-}6\ \text{PUFA}) + (3\,n\text{-}3\ \text{PUFA}) + (n\text{-}3\ \text{PUFA}/n\text{-}6\ \text{PUFA})}.$$

These formulas were best estimates based on data currently available. Whether these dietary scores are predictors of IHD risk is unknown.

We have calculated both of these indices from 7 d weighed food intake data for a community sample of middle-aged men and found the indices to be strongly correlated (r 0·87, Fehily *et al.* 1992*a*). Thus they cannot be interpreted as being indicators of separate processes. Nevertheless, this does not preclude the possibility that they may be predictors of IHD risk. The aims of our study were to investigate whether these indices are determinants of known risk factors for IHD and, more importantly, whether these indices predict risk of subsequent IHD, using data from a longitudinal study of a community sample of middle-aged men in Caerphilly, South Wales.

METHODS

The Caerphilly IHD Study is a community study of 2512 men aged 45–59 years when first seen during 1979–83. Details of the study aims, methods and response rates have been published (Caerphilly and Speedwell Collaborative Group, 1984). A representative subset (665 men) completed 7 d weighed food intake records. The method of weighing and calculation of nutrient intakes have been described in detail previously (Fehily *et al.* 1984). Fatty acid intakes were calculated using data from food composition tables (Paul *et al.* 1980) as well as data from manufacturers on the composition of spreading and cooking fats and oils and calculations from recipes.

A blood sample was taken from each subject after an overnight fast (minimum 8 h, average 12 h). Cholesterol and triacylglycerols were determined by enzymic procedures (Boehringer-Mannheim). High-density lipoprotein (HDL) was separated by precipitation with sodium phosphotungstate and $MgCl_2$ (Lopez-Virella *et al.* 1977) and HDL-C then measured. Very-low-density-lipoprotein (VLDL) lipids were determined directly from a separate aliquot of plasma after ultracentrifugation in a Beckman Airfuge. LDL was calculated by difference.

Plasma fibrinogen concentration was measured nephelometrically after heat precipitation in buffered saline (Thorp *et al.* 1967). Plasma viscosity was determined using a Coulter viscometer (Harkness type). The heparin-neutralizing activity of platelet-poor plasma was measured using the HTCT (O'Brien *et al.* 1975). Antithrombin III was measured by an immunological method (Hedner & Nilsson, 1973). Leucocyte count was measured using a Coulter counter.

Evidence of IHD at baseline (prevalent IHD) was defined as a history of severe chest pain (myocardial infarction (MI)) or angina, according to criteria provided by the London School of Hygiene and Tropical Medicine Chest Pain Questionnaire (Rose & Blackburn, 1968), or ECG ischaemia (12-lead ECG, Minnesota codes 1-1 to 1-3, 4-1 to 4-4, 5-1 to 5-3 and 7-1; Rose & Blackburn, 1968).

New IHD events occurring during 5 years of follow-up (incident IHD) were defined as (1) IHD death; deaths coded to ICD 410–414.

(2) MI; repeat Chest Pain Questionnaire plus Hospital Activity Analysis (HAA) were used as the basis for a detailed search of hospital notes for events which satisfied the World Health Organisation (WHO) criteria for acute MI.
(3) ECG evidence of MI; no Q/QS wave (Minnesota codes 1-1 to 1-3) on baseline ECG with major or moderate Q/QS waves (codes 1-1-1 to 1-2-5 plus 1-2-7) on the follow-up ECG.

Relationships between the atherogenicity and thrombogenicity indices and blood lipids, lipoproteins and haemostatic factors were investigated by linear regression analyses, separate regression analyses being conducted for each of the two scores. Regression coefficients were adjusted for potential confounders: age, body mass index (BMI; kg/m^2) and smoking habit.

Relationships between the indices and risk of subsequent IHD were investigated among men in whom there was no evidence of IHD at baseline. Those who already had IHD were excluded as they may have altered their diet before recruitment. For each dietary score the distribution was divided into thirds and the risk of subsequent IHD examined in each third. Results were expressed as relative odds: the relative odds for the lowest third of men were set to 1·0 and the odds of an incident IHD event in the middle and top thirds calculated relative to this. The 95% confidence intervals for the relative odds in the middle and top thirds were calculated as described by Armitage (1977).

RESULTS

For the 665 men for whom weighed food intake data are available, the mean atherogenicity index was 0·92 (SD 0·20) and thrombogenicity index was 1·27 (SD 0·23).

Relationships between the indices and blood lipids, lipoproteins and haemostatic factors are presented in Table 1. The regression coefficients are standardized and, therefore, indicate the effect on each risk factor of a 1 SD increase in the dietary index. For example, an increase of 0·2 in the atherogenicity index was associated with an increase in plasma cholesterol of 0·05 mmol/l. Regression coefficients were similar for both indices. All the associations were weak and few were statistically significant. LDL-C and leucocyte count were positively associated and antithrombin III was negatively associated with the indices. The proportion of variance in these risk factors which could be explained by the dietary scores was very small. For example, after taking into account the effects of age, BMI and smoking, the atherogenicity index explained only 0·70% of the variance in LDL-C and 0·52% of the variance in leucocyte count. This compares with 0·56 and 0·53% respectively for the thrombogenicity index. Entering both indices together into the multiple regression analysis had little effect on the proportion of variance explained, atherogenicity and thrombogenicity scores together explaining only 0·70% of the variance in LDL-C and 0·56% of the variance in leucocyte count. Plasma triacylglycerol concentration was the only variable examined where entering both indices together into the multiple regression analysis resulted in an increase in the proportion of variance explained: atherogenicity index explained only 0·04% of the variance in plasma triacylglycerol concentration, thrombogenicity index explained 0·13% of the variance, but both indices together explained 1·18% of the variance, the latter being statistically significant ($P < 0·05$). This is presumably because the association between atherogenicity index and triacylglycerols was weakly negative whereas that for thrombogenicity index was weakly positive.

Of the 665 men, 512 had no evidence of IHD at baseline. Among these, there were twenty-one major IHD events during 5 years of follow-up. Mean indices of those who had an IHD event during the follow-up period are compared with those of men who did not in Table 2. Both indices were slightly higher in those who went on to have an IHD event than in those who did not. The difference in the atherogenicity index represented 30% of

Table 1. *Dietary atherogenicity and thrombogenicity indices and ischaemic heart disease (IHD) risk factors among 665 men, ages 45–59 years*†

(Values are means and standard deviations and means with their standard errors)

			Standardized regression coefficient‡			
			Atherogenicity index		Thrombogenicity index	
	Mean	SD	Mean	SE	Mean	SE
Plasma lipids (mmol/l)						
Total cholesterol	5·67	1·18	0·051	0·005	0·057	0·047
HDL-C	1·10	0·32	−0·017	0·012	−0·015	0·012
LDL-C	3·76	1·07	0·089*	0·042	0·080	0·043
Triacylglycerols§	1·66		−0·011	0·022	0·020	0·022
Haemostatic factors						
Fibrinogen (g/l)	3·78	0·82	−0·002	0·031	−0·003	0·031
Viscosity (cP)	1·70	0·09	0·001	0·004	0·004	0·004
Heparin thrombin clotting time (s)	30·9	12·4	0·020	0·502	−0·286	0·504
Antithrombin III (% of standard)	104·2	19·0	−1·131	0·774	−1·521**	0·775
Leucocyte count (cells × 10^9/l)	7·03	1·96	0·142*	0·071	0·144*	0·072

HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol.
* $P < 0·05$, **$P = 0·05$.
† For details of subjects and procedures, see pp. 250–251.
‡ Adjusted for age, BMI and smoking habit.
§ After log transformation.

Table 2. *Mean atherogenicity and thrombogenicity indices and fatty acid intakes in men who had an incident ischaemic heart disease (IHD) event during 5 years of follow-up and those who did not*†

(Mean values and standard deviations)

n...	No incident IHD 491		Incident IHD 21		Difference (% population SD)
	Mean	SD	Mean	SD	
Atherogenicity index	0·93	0·19	0·99	0·22	+30
Thrombogenicity index	1·28	0·23	1·38	0·25	+43*
Saturates (% energy)	17·3	3·3	18·1	2·9	+24
Monounsaturates (% energy)	14·5	2·4	14·9	2·2	+17
Polyunsaturates (% energy)	4·9	1·9	4·4	1·8	−26
Saturates (g/100 g total fatty acids)	46·4	4·7	48·0	4·8	+34
Monounsaturates (g/100 g total fatty acids)	38·9	2·5	39·5	2·6	+24
Polyunsaturates (g/100 g total fatty acids)	13·2	4·9	11·3	3·7	−39

* $P < 0·05$.
† For details of subjects and procedures, see pp. 250–251.

the population SD and the difference in the thrombogenicity index represented 43% of the population SD, the latter being statistically significant ($P < 0·05$).

Table 2 also displays the intakes of SFA, MUFA and PUFA, expressed as a percentage of total energy and as g/100 g total fatty acids. Saturates and monounsaturates were

Table 3. *Atherogenicity, thrombogenicity and fatty acid intakes: relative odds of an ischaemic heart disease (IHD) event during 5 years of follow-up**

(Relative odds values and 95% confidence interval)

Dietary variable	Lowest third†‡	Middle third		Highest third	
	Relative odds	Relative odds	95% CI	Relative odds	95% CI
Atherogenicity index	1·0	1·24	0·39–3·99	1·61	0·53–4·90
Thrombogenicity index	1·0	1·57	0·45–5·47	2·29	0·70–7·46
Saturates (% energy)	1·0	0·84	0·25–2·81	1·57	0·56–4·42
Monounsaturates (% energy)	1·0	0·68	0·21–2·19	1·25	0·45–3·43
Polyunsaturates (% energy)	1·0	0·44	0·15–1·29	0·48	0·16–1·41
Saturates (g/100 g total fatty acids)	1·0	1·47	0·47–4·59	1·49	0·48–4·65
Monounsaturates (g/100 g total fatty acids)	1·0	0·54	0·18–1·64	0·75	0·27–2·06
Polyunsaturates (g/100 g total fatty acids)	1·0	0·69	0·26–1·86	0·43	0·13–1·40

* For details of subjects and procedures, see pp. 250–251.
† No. of men in each third, 156–181.
‡ No. of incident IHD events in each third, 4–11.

slightly higher and polyunsaturates slightly lower among those who went on to have an incident IHD event. None of the differences were statistically significant, but those for saturates and polyunsaturates, expressed as a proportion of total fatty acids, were similar in magnitude to those for the indices.

The relative odds of an incident IHD event in each third of the dietary indices are presented in Table 3. There was a trend of increasing risk of IHD with increasing atherogenicity and thrombogenicity scores. However, the trends were not statistically significant and the 95% confidence intervals for the relative odds were wide. For comparison, the relative odds of an incident IHD event in each third of the fatty acid intake distributions are also presented. When fatty acids were expressed as a percentage of total energy, there was no evidence of an association with incident IHD. There were, however, trends of increasing risk with increasing SFA, and with decreasing PUFA when these were expressed as a percentage of total fatty acids, but these were not statistically significant.

DISCUSSION

We have demonstrated that there are weak associations between dietary indices of atherogenicity or thrombogenicity and certain IHD risk factors. We have also found some evidence to support the hypothesis that high atherogenicity or thrombogenicity scores are associated with increased risk of subsequent IHD.

The association with leucocyte count is particularly interesting, since leucocyte count has been shown to be a strong independent predictor of IHD risk in the Caerphilly and Speedwell Collaborative Heart Disease Studies (Yarnell *et al.* 1991) as well as in a number of other studies (Friedman *et al.* 1974; Grimm *et al.* 1985; Friedman *et al.* 1990). Smoking habit is a well-known determinant of leucocyte count (Zalokar *et al.* 1981; Hansen *et al.* 1990; Schwartz & Weiss, 1991; Yarnell *et al.* 1991) and BMI may also be a determinant (Friedman *et al.* 1990; Schwartz & Weiss, 1991). Our observation of a significant positive association between leucocyte count and fatty acid intake does not appear to have been reported previously. The association remains significant even after taking the effects of age, BMI and smoking habit into account. This finding is of potential importance since

leucocytes, or more specifically neutrophils, produce substances that promote oxidation and tissue damage (Weiss, 1989) and, hence, may play a role in atherogenesis. In addition, leucocytes contain the enzyme lipoxygenase, which transforms arachidonic acid into 5-hydroxyperoxy-eicosatrienoic acid (HPETE) from which leucotrienes are formed, and these possess powerful chemotactic properties (Verstraete & Vermylen, 1984).

LDL-C, but not total cholesterol, is positively associated with atherogenicity and thrombogenicity scores. We have previously reported significant associations between both LDL-C and total cholesterol and the percentage of energy obtained from SFA (Fehily *et al.* 1988). A 1 SD increase in the percentage of energy from SFA is associated with an increase in total cholesterol of 0·1 mmol/l and an increase in LDL-C of 0·2 mmol/l. A 1 SD increase in atherogenicity or thrombogenicity scores is associated with an increase of only 0·05 mmol/l in total cholesterol and an increase of 0·09 mmol/l in LDL-C. There are two possible reasons for the weaker associations between total and LDL-C and the atherogenicity and thrombogenicity scores. The first is that total and LDL-C may in fact be more strongly related to the percentage of energy from SFA than to the atherogenicity and thrombogenicity scores. The second possibility is that since there is presumably a greater degree of error in the estimation of intakes of individual fatty acids than in the estimation of total saturates, there is likely to be a greater degree of underestimation of associations between blood lipids and the dietary scores. Nevertheless, there is a strong positive association between both dietary scores and the percentage of energy from SFA (r 0·6, Fehily *et al.* 1992*a*).

The only haemostatic factor showing a significant association with the thrombogenicity score is antithrombin III (inverse association). An inverse association between antithrombin III and consumption of polyunsaturates has previously been reported in Caerphilly (Rogers *et al.* 1988) as well as in other studies (O'Brien *et al.* 1976*a*, *b*). Deficiency of antithrombin III has been reported to be associated with thrombosis, heterozygote patients suffering from congenital deficiency of antithrombin III having a high risk (Verstraete & Vermylen, 1984). However, an independent and inverse relationship between antithrombin III and IHD risk within the general population has not been established (Yarnell *et al.* 1985; Meade *et al.* 1991).

There is no association between the dietary scores and either plasma fibrinogen or viscosity, despite the fact that the latter are strong and independent predictors of IHD risk in this population (Yarnell *et al.* 1991). Nevertheless, few dietary determinants of fibrinogen or viscosity have been identified. Within the Caerphilly study there is no association between these variables and the amount or type of dietary fat (Rogers *et al.* 1988). Furthermore, in a large, randomized, controlled trial among 2033 post-MI men (Burr *et al.* 1989), advice to reduce the intakes of total fat and saturates or to increase the intake of n-6 PUFA or n-3 PUFA had no effect on plasma fibrinogen or viscosity, even after 2 years.

With regard to incident IHD, our data are consistent with the hypothesis that high dietary atherogenicity or thrombogenicity scores are associated with increased risk of subsequent IHD. The magnitude of the differences is similar to those for saturates or polyunsaturates, expressed as a percentage of total fatty acids; slightly greater than those for saturates and polyunsaturates, expressed as a percentage of total energy; and greater than that for animal fat, expressed as a percentage of total energy (9% of the population SD; Fehily *et al.* 1992*b*).

Several other within-population cohort studies have reported the proportions of energy derived from SFA, MUFA and PUFA. However, there are considerable inconsistencies between these studies. For saturates, intakes were found to be slightly lower in those who subsequently developed IHD than in those who did not in some cohorts (Garcia-Palmieri *et al.* 1980; Gordon *et al.* 1981; Kromhout & Coulander, 1984), but higher in others

(Garcia-Palmieri *et al.* 1980; McGee *et al.* 1984; Kushi *et al.* 1985; Posner *et al.* 1991). Differences in mean intakes ranged from −3 to +39% of the population SD. For monounsaturates there is no evidence of a protective effect, most studies reporting slightly higher intakes among those who went on to have an IHD event. For polyunsaturates, again there is considerable inconsistency, differences between those who subsequently developed IHD and those who did not ranging from −14 to +28% of the population SD. In the Helsinki Study (Miettinen *et al.* 1982) serum phospholipids of those who subsequently developed IHD were found to contain more 16:0 and 18:0 (approximately 60% of the SD) and less 18:2 (38% of the SD) than controls. In two reports, data on IHD risk were presented by tertiles of fatty acid intakes: in the Western Electric Study (Shekelle *et al.* 1981) the relative odds of an IHD event in the top third of the saturates distribution was 1·10 and in the top third of the polyunsaturates distribution the relative odds was 0·72; in the study of London Busmen and Bankers (Morris *et al.* 1977) the relative odds of an IHD event in the top third of the animal fat distribution was 0·75 and in the top third of the P:S ratio distribution the relative odds was 0·31. Two groups of researchers calculated Keys and Hegsted scores from their dietary data and both showed an increased risk of IHD with a high score (Shekelle *et al.* 1981; Kushi *et al.* 1985). These scores take into account the percentage of energy from SFA, the percentage of energy from PUFA and cholesterol intake in mg/4184 kJ (1000 kcal). The difference between men who went on to have an IHD event and those who did not was 19 and 24% of the SD for the Keys and Hegsted score respectively (Kushi *et al.* 1985), and the relative odds of an IHD event in the top third of the score was 1·51 and 1·45 respectively (Shekelle *et al.* 1981). These are slightly lower than we have found for the atherogenicity and thrombogenicity scores.

The relative odds of an incident IHD event is 1·61 among the top third of men for atherogenicity score and 2·29 for thrombogenicity score. However, the confidence limits of the relative odds estimates are wide and the trends not statistically significant, possibly because there is only a small number of IHD events. Nevertheless, these data are extremely valuable: the Caerphilly Study is one of only two prospective studies to have used the weighed inventory method to obtain food intake data (the other being a study of 337 subjects; Morris *et al.* 1977), and there are few population cohort studies in which the predictive ability of these scores can be assessed.

It is concluded that the proposed dietary scores of atherogenicity and thrombogenicity may be weak predictors of IHD but that they are unlikely to be substantially better predictors than more simple approaches such as total saturates or P:S ratios. To enhance their predictive ability, more complex formulas which take into account the effects of other dietary factors as well as fatty acids would probably be needed.

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