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ORIGINAL ARTICLE

Plasma free fatty acid patterns and their relationship with CVD risk in a male middle-aged population

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Background/Objectives: The role of individual fatty acids in the development of cardiovascular disease (CVD) is well established, but the effects of an overall pattern of fatty acids in CVD risk has yet to be elucidated. Circulating fatty acid levels are related to metabolic disturbances associated with the metabolic syndrome and CVD, due to disturbances in the activity of enzymes that catalyse fatty acid desaturation (Δ-desaturases). Therefore, we determined patterns of fatty acids and estimated desaturase activity in plasma and analysed how these patterns were related to a 10-year CVD risk estimates in a middle-aged male population in Northern Ireland.
Subjects/Methods: Principal components analysis (PCA) was performed for defining fatty acid patterns in 379 men aged 30–49 years. Logistic regression analyses were then carried out for analysing the relationship between these fatty acid patterns and the 10-year CVD risk estimates.
Results: The PCA generated three high fatty acid patterns: high saturated fatty acid (SFA), high omega 3 fatty acid (omega 3) and high monosaturated fatty acid (MNFA). Results from logistic regression analyses show that a 1 s.d. increase in the SFA pattern score was significantly and positively associated with an increase in the 10-year CVD risk category (odds ratio 1.71, 95% confidence interval 1.33–2.21, $P<0.0001$) even after adjustment for lifestyle factors. There were no significant relationships between the other two pattern scores and the 10-year CVD risk.
Conclusions: An unhealthy fatty acid pattern representing both dietary intake and *in vivo* fatty acid metabolism is related to the 10-year CVD risk estimates and provide evidence that, as with dietary patterns, the synergistic effect of multiple fatty acids may be more important in relation to the development of CVD risk.
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Keywords: fatty acids; CVD risk; diet

Introduction

Accumulated evidence shows a strong link between intake of saturated fatty acids, hypercholesterolaemia and both cardiovascular disease (CVD) incidence and mortality (Keys, 1980; Hu *et al*., 2001; Kris-Etherton *et al*., 2001) and also on individual risk factors for the metabolic syndrome and CVD, such as high blood pressure and C-reactive protein (Madsen *et al*., 2001; Sacks and Katan, 2002; Pischon *et al*., 2003; Kris-Etherton *et al*., 2005). Altering fat intake has been shown to result in changes in the fatty acid content of plasma phospholipid, cholesteryl ester and triacylgycerol fatty acids that reflect fatty acid intake over the short term (weeks to months). Altering fat intake also alters proportions of free fatty acids (Raatz *et al*., 2001), which are a surrogate marker of adipose tissue fatty acid concentration (Hodson *et al*.,

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2008), and reflect longer-term fatty acid intake. Plasma phospholipid, cholesteryl ester (Lopes *et al*., 1991; Ma *et al*., 1995a, b; Hodson *et al*., 2008) and free fatty acids (Lopes *et al*., 1991; Hodson *et al*., 2008) have been found to be reasonably accurate markers of fatty acid intake.

As well as providing an indicator of dietary intake, circulating levels of fatty acids have also been shown to be related to obesity, insulin resistance and metabolic disturbances associated with the metabolic syndrome and CVD disease (Vessby, 2000; Riccardi *et al*., 2004). In addition, it has been shown that the activity of Δ-desaturases, the enzymes that catalyse the desaturation of fatty acids, is altered in people with such metabolic disturbances (Vessby, 2000), affecting *in vivo* production of some fatty acids.

Individual plasma fatty acids are generally expressed as a percentage of total fatty acids. Levels of each fatty acid are not independent and a change in one fatty acid may affect levels of several other fatty acids. In addition, interconversion of fatty acids occurs *in vivo*; the essential fatty acids, linoleic and α-linolenic, are converted to form γ-linoleic, dihomogammo-linoleic, arachadonic, docosatetranoic, docosapentaenoic, moroctic, eicosapentaenoic and docosahexaenoic acids (Hankey and Jamrozik, 1996), and other fatty acids undergo desaturation and other reactions *in vivo* (Emken, 1994; Rhee *et al*., 1999). The *in-vivo* desaturation of palmitic, oleic, γ-linoleic and arachidonic acids is catalysed by the Δ-desaturases (Δ^5, Δ^6 and Δ^9). These multiple fatty acid conversions can obscure the effects of the individual ingested fatty acids.

Therefore, considering the overall patterns of fatty acids and estimated desaturase activity may be a more useful measure of dietary quality than looking at individual fatty acids. There has been one previous study (Warensjo *et al*., 2006) that has analysed dietary fat patterns and found that they were predictors of the metabolic syndrome. However, no study has to date examined the association between patterns of dietary fat consumption and CVD risk.

Therefore, the aims of this study were to determine patterns of fatty acids and estimated desaturase activity in plasma using principal components analysis (PCA) and to analyse how these patterns are related to the 10-year CVD risk estimates in a middle-aged male population in Northern Ireland.

Subjects and methods

Subjects and study design

This was a cross-sectional study involving male volunteers aged 30–49 years who were recruited from a Belfast-based workforce. These volunteers included all grades of staff from manual to clerical, administrative and executive grades. The participants attended a clinic starting at 0730 h each morning in the Occupational Health Unit at the subjects' workplace, after an overnight fast. A brief medical history was taken and height and weight measured using calibrated instruments. Each subject was asked to complete a self-administered, semi-quantitative, food frequency questionnaire before attending the clinic, in which advice in its completion was available. The food frequency questionnaire used was previously validated in a similar UK population (Yarnell *et al*., 1983) and adapted to collect information on food items whose availability had changed over time. For example, additional questions on the type of milk consumed and cooking oils/spreads were added as more varieties had become widely available. Subjects reported their job status as either manual or non-manual. As this categorization of employment provides a distinction in income and workplace physical activity in this population, it was used as a variable reflecting lifestyle. Blood pressure was measured using a Spengler automated sphygmomanometer (Cochan, France) on the right arm and a fasting venous blood sample was then taken from the left arm. A sample, anti-coagulated with EDTA, provided plasma for fatty acid analysis, whereas a clotted sample was used for lipid analysis. Subjects who were diabetic, who had had a general anaesthetic within the past 3 months or who were using any form of dietary supplementation were excluded from the study. The study was approved by the research ethics committee of Queen's University, Belfast. Further details of the study procedures are described elsewhere (Woodside *et al*., 1999). The 10-year CVD risk estimates were calculated according to the Joint British Societies (JBS) criteria (Joint British Societies, 2005), which uses age, sex, smoking habit, systolic blood pressure and the ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol for calculating risk.

Laboratory methods

Serum lipids were measured on a Cobas Fara automated analyser (Roche, Welwyn Garden City, UK). Cholesterol was measured with an enzymatic CHOD-PAP kit, and triglyceride with the Peridochrom GPO-PAP kit (both from Boehringer Mannheim, Mannheim, Germany). Precipitation for HDL cholesterol was performed using phosphotungstic Mg^{2+} reagents according to the method of Lopes-Virella *et al*. (1977). Free fatty acid methyl esters were measured using gas–liquid chromatography according to the methods of Morrison and Smith (1964) and Folch *et al*. (1957). Results were expressed for each fatty acid as a percentage of total fatty acids present.

Statistical methods

All statistical analyses were performed using SPSS for Windows (version 14, SPSS Inc., Chicago, IL, USA). To determine patterns of free fatty acids in blood, we performed PCA in which *Z*-scores of the following fatty acids plus estimates of estimated desaturase activity were entered into the analysis—palmitic (16:0), stearic (18:0), myristic (14:0), oleic (18:1), palmitoleic (16:1n-7), linoleic (18:2n-6), arachidonic (20:4n-6), DHγ-linolenic (20:3n-6), docosahexanoic (22:6n-3), α-linolenic (18:3n-3), eicosapentanoic (20:5n-3),

docosapentanoic (22:5n-3), moroctic 18:4n-3), γ-linolenic (18:3n-6), docosatetranoic (22:4n-6) and docosapentanoic (22:5n-6). Estimated desaturase activity was estimated by calculating the ratios of desaturase products to their respective precursors:

Δ^9 Stearoyl CoA desaturase activity (SCD): 16:1*n*-7:16:0 (SCD-A) and 18:1:18:0 (SCD-B).
Δ^6 activity: 18:3*n*-6:18:2*n*-6 (D6D).
Δ^5 activity: 20:4*n*-6: 20:3*n*-6 (D5D).

The resulting patterns were interpreted using factor loadings, and factor loadings of >0.4 were considered to be of importance. Only components that were interpretable in terms of plausible fatty acid patterns were retained. Box–Cox power transformations were then applied to these scores to obtain variables with zero skewness and *Z*-scores of these were created.

The 10-year CVD risk estimates were calculated using the JBS Guidelines (Joint British Societies, 2005) using age, sex, smoking status, and the ratio of total to HDL cholesterol levels. The relationship between individual plasma fatty acids and the 10-year CVD risk was assessed using ANOVA and the *post hoc* Student–Newman–Keuls test. Multivariate analyses were carried out using logistic regression (those with a 10-year risk of $<10\%$ compared with those with $\geqslant 10\%$ risk) to analyse the relationship between fatty acid scores and CVD risk. These logistic regressions used five models: (1) unadjusted; (2) adjusted for age; (3) adjusted for age and body mass index; (4) adjusted for age and lifestyle; and (5) adjusted for age, body mass index, lifestyle, carbohydrate and total energy intake. Variables whose distributions were not normal (palmitic, palmitoleic and moroctic plasma fatty acids) were transformed to logarithms.

Results

Plasma fatty acids levels were available for 379 men of mean age 39 years: 22.4% were current smokers and the distributions of lipid and non-lipid CVD risk factors all lay within the usual European range (Table 1). In this study the subjects' diet was typical of a Western diet with fatty acids comprising 38% of total energy, carbohydrates 41% and protein 16%; saturated fatty acids (SFAs) made up 40% of all plasma fatty acids, polyunsaturated fatty acids 37% and monosaturated fatty acids 24% (not shown). The five major individual fatty acids present in plasma were the SFAs, palmitic and stearic, the monosaturated fatty acid, oleic and the polyunsaturated fatty acids, arachidonic and linoleic. These five fatty acids made up 90% of plasma fatty acids. According to JBS guidelines (Joint British Societies, 2005), 174 subjects (46%) had a $<10\%$ risk of developing CVD in the next 10 years, 151 (40%) had a risk of between 10 and 20% and 54 (14%) had a risk of $>20\%$. Higher proportions of palmitic, myristic, oleic, palmitoleic and moroctic acids and lower proportions of stearic, linoleic, arachidonic, docosahexanoic and docosapentanoic acids, and higher ratios of SCD-A and SCD-B, were associated with a higher 10-year risk of CVD (Table 2).

The scree plot of components produced from the PCA (not shown) elbows after the fourth component. As components one to three were interpretable in terms of plausible fatty acid patterns, these were retained. These three patterns had an eigenvalue of $\geqslant 2.0$. Table 3 shows the factor loadings for each of these three components—the first component

Table 1 Characteristics of the study population

	Mean ± s.d.
Age (years)	39.4 ± 5.9
BMI (kg/m^2)	26.4 ± 3.1
Systolic blood pressure (mm Hg)	128 ± 14.7
Total cholesterol (mmol/l)	5.87 ± 1.10
HDL cholesterol (mmol/l)	1.09 ± 0.30
	N (%)
Smokers	85 (22.4)
Manual workers	168 (44.7)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein.

Table 2 Average proportions of free fatty acids in plasma (%) and 10-year risk of CVD[a]

	10-year risk of CVD		
	<10%	*10–20%*	*>20%*
	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
Saturated			
Palmitic (16:0)*	27.8 ± 2.6	28.7 ± 2.8	29.1 ± 3.4
Stearic (18:0)*	9.96 ± 1.4	9.90 ± 1.6	9.33 ± 1.5
Myristic (14:0)***	1.23 ± 0.5	1.59 ± 0.7	1.91 ± 0.8
Monounsaturated			
Oleic (18:1)**	20.6 ± 2.4	21.3 ± 2.5	21.5 ± 3.0
Palmitoleic (16:1n-7)***	2.81 ± 0.9	3.28 ± 1.0	3.72 ± 0.9
Polyunsaturated			
Linoleic (18:2n-6)***	26.7 ± 4.2	24.9 ± 3.8	24.5 ± 4.0
Arachidonic (20:4n-6)***	5.47 ± 1.1	5.08 ± 1.1	4.80 ± 1.3
DHγ-linolenic (20:3n-6)	1.66 ± 0.4	1.71 ± 0.4	1.64 ± 0.4
Docosahexaenoic (22:6n-3)**	1.24 ± 0.4	1.14 ± 0.4	1.08 ± 0.4
α-Linolenic (18:3n-3)	0.60 ± 0.2	0.65 ± 0.3	0.61 ± 0.2
Eicosapentaenoic (20:5n-3)	0.60 ± 0.3	0.56 ± 0.2	0.51 ± 0.2
Docosapentaenoic (22:5n-3)***	0.51 ± 0.1	0.45 ± 0.1	0.41 ± 0.1
Moroctic (18:4n-3)***	0.37 ± 0.1	0.39 ± 0.1	0.42 ± 0.1
γ-Linolenic (18:3n-6)	0.40 ± 2.0	0.327 ± 0.1	0.31 ± 0.1
Docosatetraenoic (22:4n-6)	0.21 ± 0.1	0.21 ± 0.1	0.22 ± 0.1
Docosapentaenoic (22:5n-6)	0.18 ± 0.1	0.18 ± 0.1	0.19 ± 0.1
SCD-A 16:1n-7:16:0***	0.10 ± 0.0	0.11 ± 0.0	0.13 ± 0.0
SCD-B 18:1:18:0**	2.12 ± 0.5	2.20 ± 0.5	2.37 ± 0.5
D6D 18:3n-6:18:2n-6	0.08 ± 1.0	0.01 ± 0.0	0.01 ± 0.0
D5D 20:4n-6: 20:3n-6	3.45 ± 0.9	3.07 ± 0.8	3.39 ± 3.6

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; D6D, Δ6 desaturase; HDL, high-density lipoprotein; MUFA, monosaturated fatty acid; SCD, stearoyl CoA desaturase; SFA, saturated fatty acid.
[a]ANOVA with SNK test. *$P<0.05$, **$P<0.001$, ***$P<0.0001$.

(SFA) loaded positively on palmitic, myristic and palmitoleic acids and on the SCD-A and SCD-B ratios, the second component (omega 3) loaded positively on eicosapentanoic, docosapentanoic and negatively on linoleic acids and the third component (monosaturated fatty acid) loaded positively on oleic acid and negatively on docosatetranoic and docosapentanoic acids.

Table 3 Factor loadings for first four principal components

Fatty Acid	*Component 1: high SFA*	*Component 2: high omega 3*	*Component 3: high MUFA*
Saturated			
Palmitic (16:0)	0.48	0.09	−0.48
Stearic (18:0)	−0.66	0.22	0.06
Myristic (14:0)	0.65	0.16	−0.36
Monounsaturated			
Oleic (18:1)	0.47	−0.17	0.64
Palmitoleic (16:1n-7)	0.86	0.22	0.03
Polyunsaturated			
Linoleic (18:2n-6)	−0.53	−0.52	−0.20
Arachidonic (20:4n-6)	−0.52	0.52	−0.04
DHγ-linolenic (20:3n-6)	−0.40	0.24	0.30
Docosahexaenoic (22:6n-3)	−0.39	0.58	0.19
α-Linolenic (18:3n-3)	0.23	0.05	0.11
Eicosapentaenoic (20:5n-3)	0.08	0.68	0.07
Docosapentaenoic (22:5n-3)	−0.38	0.65	0.33
Moroctic (18:4n-3)	0.43	0.40	0.31
γ-linolenic (18:3n-6)	0.11	0.30	0.25
Docosatetraenoic (22:4n-6)	0.15	0.50	−0.57
Docosapentaenoic (22:5n-6)	0.22	0.39	−0.64
SCD-A 16:1n-7:16:0	0.79	0.22	0.20
SCD-B 18:1:18:0	0.73	−0.24	0.29
D6D 18:3n-6:18:2n-6	0.09	0.28	0.28
D5D 20:4n-6: 20:3n-6	−0.03	0.24	−0.25
Eigenvalue	4.52	2.87	2.21
Total variance (%)	22.6	14.3	11.1

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; MUFA, monosaturated fatty acid; ; SCD, stearoyl CoA desaturase; SFA, saturated fatty acid.

Table 4 shows the relationship between the three PCA scores and CVD risk (split into $<10\%$ risk and $\geqslant 10\%$ risk) using multivariate logistic regression. The SFA score was significantly and positively associated with the 10-year CVD risk in all models. In age-adjusted analyses, a 1 s.d. increase in the SFA pattern score was associated with an odds ratio of 2.01 of having $\geqslant 10\%$ risk of CVD (95% confidence interval1.58–2.56) compared with those with less than $<10\%$ risk. Adjustment for body mass index reduced the relationship (odds ratio 1.71, 95% confidence interval 1.33–2.20) but adjustment for lifestyle, carbohydrate and total energy intake had little effect. There were no significant relationships between the other two pattern scores and the 10-year CVD risk.

Discussion

In this study we found that a fatty acid pattern representing high saturated fat intake, which was also characterized by high SCD-A and SCD-B ratios, both of which are accepted markers of metabolic and CVD function, was significantly associated with increased 10-year CVD risk. In agreement with previous studies we found that individual plasma fatty acid levels were related to CVD risk, with more adverse fatty acid levels in cholesteryl esters, and phospholipids were associated with increased CVD risk (Wang *et al*., 2003). Although there are some differences in the fatty acid composition of cholesteryl esters and phospholipids and free fatty acids, there are strong positive correlations between the fatty composition of cholesteryl esters and phospholipids and free fatty acid levels (Hodson *et al*, 2008).

Our results are comparable with the only other study to analyse data from fatty acid patterns derived from PCA (Warensjo *et al*., 2006), which also found an SFA pattern and an omega 3 pattern. As the negative relationship between individual saturated fats and metabolic and CVD risk, and the positive relationship with individual omega 3 fats (Keys, 1980; Hu *et al*., 2001; Kris-Etherton *et al*., 2001) is well known, it is important to elucidate whether overall patterns of free fatty acid status, characterized by either high

Table 4 Odds ratios (and 95% CI) of having a $\geqslant 10\%$ 10-year risk of CVD for a 1 s.d. increase in plasma fatty acid scores

	SFA score[a]	*Omega 3 score*[a]	*MUFA score*[a]
Unadjusted	2.04 (1.61–2,58)	0.91 (0.74–1.12)	0.96 (0.78–1.18)
Adjusted for			
Age	2.01 (1.58–2.56)	0.88 (0.72–1.09)	0.96 (0.78–1.18)
Age and BMI	1.71 (1.33–2.20)	0.84 (0.67–1.06)	0.93 (0.74–1.16)
Age and lifestyle	2.02 (1.59–2.48)	0.86 (0.69–1.06)	1.00 (0.81–1.23)
Age, BMI, lifestyle, carbohydrate and total energy intake	1.71 (1.33–2.21)	0.80 (0.63–1.01)	0.97 (0.77–1.22)

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; MUFA, monosaturated fatty acid; SFA, saturated fatty acid.
[a]Standardized fatty acid score.

saturated fat or high omega 3 content, would result in similar relationships to CVD risk.

We found that men in the highest category of CVD risk had more adverse levels of individual fatty acids. These are expressed as percentages of total fatty acids, and hence any change in an individual fatty acid has the potential to affect the levels of several other fatty acids. Therefore, it might be suggested that an overall pattern of fatty acid status may be a more appropriate measure for analysing the link with CVD risk. Results from the logistic regression analyses indicate that the overall pattern of plasma fatty acids, which includes known markers of metabolic function, are strongly related to CVD risk. This suggests that although the absolute amount of fat ingested undoubtedly has a role, it may be the synergistic effect of these fatty acids that may ultimately be more important in relation to the development of CVD risk.

However, the use of cross-sectional data does not allow us to determine whether these potentially harmful patterns of estimated desaturase activity lead to changes in CVD risk factors such as total or HDL cholesterol or vice versa. Previous research in this area (Warensjo *et al.,* 2005) indicates that these disturbances in fatty acid conversion are more often observed in those with metabolic syndrome when compared with those without it and that, in a longitudinal study, these disturbances predate the development of metabolic syndrome (Vessby, 2000; Warensjo *et al*., 2006). Another limitation is that it is not known whether factors including lifestyle and genetics are involved in the development of alterations in fatty acid desaturation *in vivo*. In this study, results of the regression analysis remained significant for the high SFA pattern, even after adjusting for lifestyle factors, although we cannot rule out the possibility that other unmeasured confounders may have an important role in this relationship.

We used the recently published JBS score for calculating CVD risk. The JBS guidelines (Joint British Societies, 2005) allow the calculation of CVD risk, taking into account fatal and nonfatal myocardial infarction and new angina plus fatal and non-fatal stroke and cerebral haemorrhage and transient cerebral ischaemia. The JBS coronary risk prediction charts now estimate total risk of developing CVD over 10 years based on five risk factors: age, sex, smoking habit, SBP and the ratio of total cholesterol to HDL-C. They are therefore more likely to closely predict overall risk than the consideration of individual risk factors and provide the most suitable risk scores for this population.

In conclusion, we found that in a population at high risk of CVD, a fatty acid pattern representing high saturated fat intake and disturbance in fatty acid desaturation *in vivo* was significantly associated with an increased 10-year CVD risk. These results suggest that the synergistic effect of combinations of fatty acids may ultimately be more important than individual fatty acid levels in relation to the development of CVD risk. These findings support recommendations for lower saturated fat intake.

Conflict of interest

The authors declare no conflict of interest.

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References

British Cardiac Society; British Hypertension Society; Diabetes UK; HEART UK; Primary Care Cardiovascular Society; Stroke Association (2005). JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* **91**, v1–v52.

Emken EA (1994). Metabolism of dietary stearic acid relative to other fatty acids in human subjects. *Am J Clin Nutr* **60**, 1023s–11028s.

Folch J, Lees M, Sloane-Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**, 497–509.

Hankey GJ, Jamrozik K (1996). Risk factors for stroke: lifestyle factors. *Cardiovasc Risk Fact* **6**, 5–17.

Hodson L, Skeaff CM, Fielding BA (2008). Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* **47**, 348–380.

Hu FB, Manson JE, Willett WC (2001). Types of dietary fat and risk of coronary heart disease: a critical review. *J Am Coll Nutr* **20**, 5–19.

Keys A (1980). *Seven Countries*. Harvard University Press: Cambridge, MA.

Kris-Etherton P, Daniels SR, Eckel RH, Engler M, Howard BV, Krauss RM *et al.* (2001). AHA scientific statement: summary of the Scientific Conference on Dietary Fatty Acids and Cardiovascular Health. Conference summary from the Nutrition Committee of the American Heart Association. *J Nutr* **131**, 1322–1326.

Kris-Etherton PM, Griel AE, Psota TL, Gebauer SK, Zhang J, Etherton TD (2005). Dietary stearic acid and risk of cardiovascular disease: intake, sources, digestion, and absorption. *Lipids* **40**, 1193–1200.

Lopes SM, Trimbo SL, Mascoli EA, Blackburn GL (1991). Human plasma fatty acid variations and how they are related to dietary intake. *Am J Clin Nutr* **53**, 628–637.

Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem* **23**, 882–884.

Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE (1995a). Short and long term repeatability of fatty acid composition of plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Investigators. *Am J Clin Nutr* **62**, 572–578.

Ma J, Folsom AR, Shahar E, Eckfeldt JH (1995b). Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle aged adults. The Atherosclerosis Risk in Communities (ARIC) Investigators. *Am J Clin Nutr* **62**, 564–571.

Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E *et al.* (2001). C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. *Am J Cardiol* **88**, 1139–1142.

Morrison WR, Smith LM (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron trifluoride methanol. *J Lipid Res* **5**, 600–608.

Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB (2003). Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation* **108**, 155–160.

Raatz SK, Bibus D, Thomas W, Kris-Etherton P (2001). Total fat intake modifies plasma fatty acid composition in humans. *J Nutr* **131**, 231–234.

Rhee SK, Kayani AJ, Ciszek A, Brenna JT (1999). Desaturation and interconversion of dietary stearic and palmitic acids in human plasma and lipoproteins. *Am J Clin Nutr* **65**, 451–458.

Riccardi G, Giacco R, Rivellese AA (2004). Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* **23**, 447–456.

Sacks FM, Katan M (2002). Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. *Am J Med* **113**, 13S–24S.

Vessby B (2000). Dietary fat and insulin action in humans. *Br J Nutr* **83**, S91–S96.

Wang L, Folsom AR, Eckfeldt JH (2003). Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Nutr Metab Cardiovasc Dis* **13**, 256–266.

Warensjo E, Risérus U, Vessby B (2005). Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia* **48**, 1999–2005.

Warensjo E, Sundstrom J, Lind L, Vessby B (2006). Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. *Am J Clin Nutr* **84**, 442–448.

Woodside JV, Young IS, Yarnell JW, Roxborough HE, McMaster D, McCrum EE *et al*. (1999). Antioxidants, but not B-group vitamins increase the resistance of low-density lipoprotein to oxidation: a randomized, factorial design, placebo-controlled trial. *Atherosclerosis* **144**, 419–427.

Yarnell JW, Fehily AM, Milbank JE, Sweetnam PM, Walker CL (1983). A short dietary questionnaire for use in an epidemiological survey: comparison with weighed dietary records. *Hum Nutr Appl Nutr* **37**, 103–112.