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Linoleic acid content in adipose tissue and coronary heart disease

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Abstract

The possibility of an inverse relation between essential fatty acids in adipose tissue, in particular linoleic acid, and mortality from coronary heart disease was studied by a cross sectional survey of random population samples of apparently healthy men aged 40-49 from four European regions with differing mortality from coronary heart disease. The proportion of linoleic acid in adipose tissue was lowest in men from north Karelia, Finland, where mortality from coronary heart disease is highest, and highest in men from Italy, where mortality is lowest, with intermediate proportions in men from Scotland and south west Finland. Similar gradients were observed for the desaturation and elongation products dihomog- γ -linolenic and arachidonic acid. The proportion of saturated fatty acids in adipose tissue was

highest in Finland, intermediate in Scotland, and lowest in Italy. Italian men also had the highest proportion of oleate in their adipose tissue and the lowest proportion of myristoleate and palmitoleate. Finnish men were more obese and had a higher blood pressure. Serum cholesterol concentration was higher in north Karelia and south west Finland than in Scotland or Italy. High density lipoprotein (HDL) cholesterol concentrations reflected the regional differences in serum cholesterol, being higher in Finland and lower in Italy. The ratios of HDL cholesterol to total cholesterol, however, did not differ. The regional differences in linoleic acid in adipose tissue remained highly significant when the observed differences in other known risk factors for coronary heart disease among the four areas were taken into account by multivariate analysis.

The gradients in proportions of polyunsaturated fatty acids probably reflect differences in dietary intake of linoleic acid.

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Introduction

The fatty acid composition of adipose tissue provides a stable reflection of long term dietary habits.¹ Population studies suggest that a low proportion of the essential fatty acid linoleic acid (C18:2 n-6) in adipose tissue may be associated with an increased risk of coronary heart disease. The Edinburgh-Stockholm study identified an inverse relation between the proportion of linoleic acid in adipose tissue and risk of coronary heart disease by finding a significantly lower proportion of linoleic acid in adipose tissue in apparently healthy Edinburgh men aged 40 compared with similar men in Stockholm, where the mortality from coronary heart disease was one third of that in Edinburgh.² Similar differences have been observed in apparently healthy men selected at random in Finnish regional studies: men from east Finland, where the mortality from coronary heart disease is almost twice that in west Finland, have lower proportions of linoleic acid in serum lipids than their counterparts in west Finland.¹⁴ A crude inverse correlation can be drawn between plasma cholesterol linoleate and mortality from coronary heart disease in Denmark, Sweden, the United States, Scotland, and Finland.⁵

These findings have been supported by a recent study of a random

healthy population in the Edinburgh region, where it was found that men with hitherto occult coronary heart disease also had a significantly lower proportion of linoleic acid in their adipose tissue than healthy men.⁶ On the other hand, a similar proportion of linoleic acid in adipose tissue to that observed in the healthy Scottish men has been reported from Crete and Greece, where the mortality from coronary heart disease is much lower, but the ratio of polyunsaturated to saturated fatty acids was much higher than that in Scotland.⁷

The hypothesis that there is an inverse relation between linoleic acid in the adipose tissue or the ratio of polyunsaturated to saturated fatty acids and mortality from coronary heart disease has now been studied further by cross sectional surveys of random population samples from four specific areas (three rural and one urban) with differing mortalities from coronary heart disease in 1979—namely, Edinburgh, Scotland (mortality 140/100 000); north Karelia, Finland (212/100 000); south west Finland (146/100 000 (Finnish data 1978-80)), and the area round Sapri, Italy (43/100 000). The results from Finland and Edinburgh, which have some of the highest mortalities from coronary heart disease in the world, are presented in this paper and contrasted with those from the Sapri area, Italy.

Methods

Cross sectional surveys of random samples of apparently healthy men aged 40-49 in four regions with differing mortalities from coronary heart disease were undertaken in 1982 in Finland (February to April) and Edinburgh, Scotland (March to May), and in 1983 in Sapri, Italy (April to June). Regional differences within Finland were also examined by contrasting the results from subjects in north Karelia (high mortality) with those in south west Finland (lower mortality). Ethical approval for the study was obtained in each of the participating centres.

POPULATION SAMPLES

Edinburgh, Scotland—A random sample of 187 men aged 40-49 living and registered with a general practitioner within the city boundary was drawn from the central register of Lothian Health Board. They were invited, with the permission of their general practitioners, to attend a special clinic, and 140 responded, giving an overall response rate of 75%. Of these, two had a reported history of coronary heart disease (one myocardial infarction, one angina pectoris), four gave positive answers to the World Health Organisation (WHO) questionnaire on chest pain,⁸ one was taking a medically prescribed gluten free diet, and two were foreign nationals (from India and

TABLE I—Mean (SEM) anthropometric and serum lipid measurements in Scottish, Finnish, and Italian men

	Country				Significance*					
	Finland		Scotland (n=131)	Italy (n=74)	N Karelia v S W Finland	N Karelia v Scotland	N Karelia v Italy	Scotland v S W Finland	Scotland v Italy	Italy v S W Finland
	N Karelia (n=102)	S W Finland (n=83)								
Height (cm)	173.1 (0.6)	176.5 (0.6)	174.4 (0.6)	166.6 (0.8)	p<0.01	NS	p<0.01	NS	p<0.01	p<0.01
Weight (kg)	84.1 (1.4)	83.8 (1.6)	79.2 (1.0)	72.6 (1.1)	NS	NS	p<0.01	NS	p<0.01	p<0.01
Quetelet's index (kg/cm ²)	2.80 (0.04)	2.68 (0.04)	2.60 (0.03)	2.62 (0.04)	NS	p<0.01	p<0.05	NS	NS	NS
Blood pressure (mm Hg):										
Systolic	143 (1)	146 (2)	135 (1)	128 (2)	NS	p<0.01	p<0.01	p<0.01	p<0.05	p<0.01
Diastolic	88 (1)	90 (1)	83 (1)	84 (1)	NS	p<0.01	NS	p<0.01	NS	p<0.01
% Of smokers	44	35	47	55	NS	NS	NS	NS	NS	NS
Alcohol consumption (g/week)	78 (13)	78 (15)	292 (27)	454 (44)	NS	p<0.01	p<0.01	p<0.01	p<0.05	p<0.01
Serum concentrations†:										
Total cholesterol (mmol/l)	6.65 (0.11)	6.40 (0.15)	5.79 (0.10)	5.37 (0.13)	NS	p<0.01	p<0.01	NS	NS	p<0.01
HDL cholesterol (mmol/l)	1.43 (0.03)	1.36 (0.04)	1.35 (0.03)	1.22 (0.04)	NS	NS	NS	NS	NS	NS
HDL: total cholesterol (%)	22.1 (0.6)	22.1 (0.8)	24.1 (0.4)	23.9 (1.0)	NS	NS	NS	NS	NS	NS
Triglycerides (mmol/l)‡	1.80 (0.15)	1.57 (0.13)	1.70 (0.08)	2.04 (0.22)	NS	NS	NS	NS	NS	NS

Conversion: SI to traditional units—Cholesterol: 1 mmol/l=38.7 mg/100 ml. Triglyceride: 1 mmol/l=88.5 mg/100 ml.

Overall statistical differences between the four regions by Kruskal-Wallis one way analysis of variance not shown: all were highly significant (p<0.001) except for smokers, ratio of HDL to total cholesterol, and triglyceride concentrations.

*Inter-region comparisons were made by pairwise comparisons using the Nemenyi procedure.

†Corrected for variation between laboratories.

‡Non-fasting values.

TABLE II—Fatty acid composition of adipose tissue in Scottish, Finnish, and Italian men (numbers are mean (SEM) percentages)

	Country				Significance*					
	Finland		Scotland (n=131)	Italy (n=74)	N Karelia <i>v</i> S W Finland	N Karelia <i>v</i> Scotland	N Karelia <i>v</i> Italy	Scotland <i>v</i> S W Finland	Scotland <i>v</i> Italy	Italy <i>v</i> S W Finland
	N Karelia (n=102)	S W Finland (n=83)								
Fatty acid†:										
Myristic (14:0)	4.53 (0.13)	4.47 (0.13)	3.62 (0.07)	1.92 (0.05)	NS	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
Myristoleic (14:1n-5)	0.60 (0.03)	0.56 (0.02)	0.39 (0.01)	0.20 (0.01)	NS	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
Palmitic (16:0)	27.00 (0.20)	26.30 (0.20)	26.30 (0.40)	20.40 (0.30)	NS	p<0.01	p<0.01	NS	p<0.01	p<0.01
Palmitoleic (16:1n-7)	7.16 (0.21)	6.78 (0.21)	6.73 (0.16)	4.62 (0.16)	NS	NS	p<0.01	NS	p<0.01	p<0.01
Stearic (18:0)	7.00 (0.48)	7.35 (0.60)	6.36 (0.23)	3.55 (0.10)	NS	NS	p<0.01	NS	p<0.01	p<0.01
Oleic (18:1n-9)	45.00 (0.40)	45.00 (0.50)	46.40 (0.40)	54.30 (0.70)	NS	p<0.05	p<0.01	NS	p<0.01	p<0.01
Linoleic (18:2n-6)	7.36 (0.33)	8.10 (0.27)	8.79 (0.26)	13.45 (0.51)	NS‡	p<0.01	p<0.01	NS	p<0.01	p<0.01
α-Linolenic (18:3n-3)	0.78 (0.02)	0.77 (0.02)	0.82 (0.02)	0.83 (0.03)	NS	NS	NS	NS	NS	NS
Dihomo-γ-linolenic (20:3n-6)	0.10 (0.00)	0.12 (0.00)	0.14 (0.01)	0.19 (0.01)	NS§	p<0.01	p<0.01	p<0.05	p<0.01	p<0.01
Arachidonic (20:4n-6)	0.26 (0.01)	0.27 (0.01)	0.28 (0.01)	0.39 (0.02)	NS	NS	p<0.01	NS	p<0.01	p<0.01
Eicosapentaenoic (20:5n-3)	0.11 (0.00)	0.11 (0.01)	0.07 (0.00)	0.07 (0.00)	NS	p<0.01	p<0.01	p<0.01	NS	p<0.01
Docosahexaenoic (22:6n-3)	0.14 (0.01)	0.14 (0.01)	0.09 (0.00)	0.11 (0.01)	NS	p<0.01	NS	p<0.01	p<0.05	NS
Polyunsaturated: saturated										
fatty acids	0.23 (0.01)	0.26 (0.01)	0.30 (0.01)	0.58 (0.02)	NS	p<0.01	p<0.01	NS	p<0.01	p<0.01
Saturated fatty acids	38.5 (0.5)	38.2 (0.6)	36.3 (0.6)	25.9 (0.4)	NS	p<0.01	p<0.01	p<0.05	p<0.01	p<0.01
Monoenes	52.7 (0.6)	52.3 (0.7)	53.6 (0.5)	59.1 (0.7)	NS	NS	p<0.01	NS	p<0.01	p<0.01
Polyunsaturated fatty acids	8.7 (0.3)	9.5 (0.3)	10.2 (0.3)	15.0 (0.5)	NS	p<0.01	p<0.01	NS	p<0.01	p<0.01

Overall statistical differences between the four regions by Kruskal-Wallis one way analysis of variance not shown: all were highly significant (p<0.001) except for 18:3n-3 (NS).

*Inter-region comparisons were made by follow up pairwise comparisons using the Nemenyi procedure.

†No of carbon atoms: No of unsaturated carbon-carbon bonds and No of carbon atoms in terminal methyl group counting from last double bond.

‡p<0.05 after log transformation.

§p<0.01 after log transformation.

Pakistan): these nine were excluded, leaving 131 subjects (mean (SEM) age 46.1 (0.3) years).

North Karelia and south west Finland—A random sample of 269 men aged 40-49 from semiurban communities (nine from north Karelia and 12 from south west Finland) was identified in connection with the MONICA project (an international project sponsored by the WHO to monitor trends in cholesterol and coronary artery disease). The overall response rate was 81% (80% and 82% respectively). Five subjects were excluded, three because of a history of coronary heart disease (two with myocardial infarction and one with angina pectoris) and two because of medically prescribed diets for diabetes mellitus. In 28 subjects fatty acids in adipose tissue were not analysed, and these were therefore also excluded from this study. The final sample size was 185: 102 men from north Karelia aged 44.3 (0.3) years and 83 men from south west Finland aged 44.1 (0.3) years.

Sapri, Italy—A random sample of 120 men aged 40-49 from rural and semiurban communities in the region of Sapri (south Italy) was drawn for the study. Five had left the country and the remaining 115 were invited to participate, of whom 79 were examined (overall response rate 69%). Of these, five were excluded, four because of a history of coronary heart disease (two with myocardial infarction and two with angina) and one because of a history of intermittent claudication. The final sample comprised 74 men aged 45.6 (0.4) years.

CLINICAL PROCEDURES

Clinical procedures were standardised at each participating centre after an initial common training scheme. The men attended on a Tuesday, Wednesday, or Thursday morning, and in Finland on Monday and Friday mornings also, between 9 am and 1 pm after a light breakfast (tea or coffee and toast). A few men who could not be seen on these days were seen on Saturday in Edinburgh, or on Sunday morning in Italy. A questionnaire was completed to ascertain demographic and social information including main lifetime occupation, medical history, smoking habit, intake of alcohol during the previous week, medically prescribed diet, and consumption of vitamin pills and fruit juice. The WHO chest pain questionnaire was also completed.*

Anthropometric measurements were made with the men stripped to the waist and included height (calibrated measuring stick) and weight (Seca weighing scales, model 76). Duplicate measurements of supine blood pressure to the nearest 2 mm Hg were made by one observer on the right arm using a Hawksley random zero sphygmomanometer with the diastolic pressure measured at phase V.

An intravenous cannula (butterfly with a 21 G needle in Scotland and Italy and a 16 G needle in Finland) was inserted into an antecubital vein without a tourniquet, and 20 ml blood was obtained in two separate aliquots for measurement of serum total cholesterol, triglyceride, and high density lipoprotein (HDL) cholesterol concentrations. Adipose tissue biopsy specimens for the study of fatty acid composition were taken from the anterior abdominal wall under local anaesthesia and stored at -40°C .*

LABORATORY PROCEDURES

Samples of adipose tissue for analysis of fatty acid composition were stored in the coordinating laboratories (Edinburgh, Helsinki, and Naples) and flown frozen on dry ice (-56°C) to the analysing laboratory in Tampere, Finland. The fatty acids extracted from adipose tissue were transesterified with 5% hydrochloric acid-methanol, purified by thin layer chromatography on silica gel G, and analysed on a gas chromatograph (Hewlett-Packard 5880) fitted with a 25 m (0.32 mm inside diameter) fused silica capillary column of OV-351. The peaks were quantified with a flame ionisation detector and Hewlett-Packard 5880A integrator.

High density lipoprotein cholesterol cannot be measured in frozen serum, and serum lipids therefore were analysed in the country of origin. All three laboratories were standardised with the WHO Lipid Reference Centre in Prague. For cholesterol estimations the Boehringer Mannheim monotest kit was used; serum triglyceride concentrations were estimated in Edinburgh by AAI, in Helsinki using a Kone CD parallel analyser, and in Naples by CLA (Carlo Erba) analyser. High density lipoprotein cholesterol was analysed enzymatically after precipitation of very low density lipoprotein and low density lipoproteins using magnesium dextran (identical batch).

Interlaboratory variations in lipid analyses were examined by concurrent analysis of WHO Lipid Reference Centre quality control serum for the duration of the project and by analysis of cholesterol and triglyceride in exchanged frozen samples. The results showed that all three laboratories had excellent agreement virtually free of bias when compared with the WHO Lipid Reference Centre for cholesterol determination (assigned value 5.29 mmol/l (198.8 mg/100 ml): Edinburgh +1.7%, Helsinki -0.4%, Naples

-2.8%). The precipitation technique to determine HDL cholesterol showed a larger bias (assigned value 0.86 mmol/l (33.3 mg/100 ml), using phosphotungstate: Edinburgh -11.6%, Helsinki -8.1%, Naples -2.3%), as did the analysis of triglycerides (assigned value 1.90 mmol/l (168.2 mg/100 ml): Edinburgh -7.4%, Helsinki -5.1%, Naples -10.1%).

The results for cholesterol, triglyceride, and high density lipoprotein cholesterol were adjusted in each of the three participating laboratories using the WHO Lipid Centre quality control data

$$\left(\frac{100\% - \text{bias } \%}{100} \times \text{result} \right).$$

STATISTICAL ANALYSIS

As many of the variables were highly skewed Kruskal-Wallis one way analysis of variance was used for overall comparisons among the four regions, and follow up tests between all pairs of regions were carried out using the Nemenyi procedure.¹⁰ A χ^2 test was used to compare the regions for the proportion of current cigarette smokers. Relations between quantitative variables were tested by Spearman rank correlation. Differences between regions in fatty acid composition after correction for other known risk factors were tested using multiple linear regression on the logarithms of the values for fatty acids. The results are presented as means (SEM) and are considered to be significant if $p < 0.05$.

Results

ANTHROPOMETRIC AND CLINICAL DATA

Table I shows the anthropometric measurements and serum lipid concentrations of the men from the four regions. Men from south west Finland were taller than men from all other regions, and the Italian men were shortest. There was an overall difference in body weight between the countries: Finnish men were heavier than their Scottish and Italian counterparts. These differences were less obvious when allowance was made for the differences in height (Quetelet's index).

Systolic and diastolic blood pressures were significantly different between the regions; the highest pressures were recorded in Finland and the lowest in Italy. There was no significant gradient in blood pressure in Finland. More alcohol was consumed by the Italian men than by their Scottish and Finnish counterparts in that order. The regional difference in coronary heart disease in Finland was not reflected by a gradient in alcohol consumption. There was no difference between regions in the prevalence of smoking.

LIPID DATA

Serum cholesterol concentrations reflected the known gradient in mortality from coronary heart disease: the highest concentrations were recorded in north Karelia, followed by south west Finland, Scotland, and Italy. High density lipoprotein cholesterol concentrations were also highest in Finland and lowest in Italy. "Non-fasting" serum triglyceride concentrations were not significantly different.

FATTY ACID COMPOSITION OF ADIPOSE TISSUE

The proportion of linoleic acid in adipose tissue was lower in north Karelia than in south west Finland and Scotland, and much lower in these centres than in Italy (table II). In comparison with Italy the cumulative frequency distribution curves for the three north European centres (figure) showed a noticeable shift to the left (lower proportions). The biggest shift was observed for north Karelian men, followed by slightly smaller shifts for south west Finnish and Edinburgh men, in that order. The linoleic acid derivative dihomog- γ -linolenic acid (20:3n-6) was highest in adipose tissue in Italy, intermediate in Scotland, and lowest in north Karelia. A simple two sample comparison of the Finnish regions showed a significant difference for both linoleic acid (lower in north Karelia, $p < 0.05$, Student's t test after log transformation) and dihomog- γ -linolenic acid (lower in north Karelia, $p < 0.01$, Student's t test). Arachidonic acid (20:4n-6) in adipose tissue was much higher in Italy than elsewhere. Trace concentrations of eicosapentaenoic acid (20:5n-3) in adipose tissue were higher in Finland than in Edinburgh and Sapri. There was no difference in α -linolenic acid (18:3n-3). Saturated fatty acids were present in higher concentrations in adipose tissue of the men from the areas with high mortality from coronary heart disease, the differences being more pronounced in myristic acid (14:0) and stearic acid (18:0) than in palmitic acid (16:0). As might be expected from the consumption of olive oil in Italy, there was a higher proportion of the

monounsaturated fatty acid oleic acid (18:1n-9) in the adipose tissue of Italian men. Other monounsaturated fatty acids were lower in Italy.

The differences between regions in individual fatty acids in adipose tissue resulted in highly significant gradients in the ratio of polyunsaturated to saturated fatty acids and the sum of saturated, monounsaturated, and polyunsaturated fatty acids. The figure shows the frequency distribution of the ratio of polyunsaturated to saturated fat in adipose tissue.

CORRELATIONS AND REGRESSIONS

Many correlations were made in the four regions between the relative concentrations of linoleic acid, saturated fatty acids, and polyunsaturated fatty acids, the ratio of polyunsaturated to saturated fatty acids in adipose tissue, and the classical risk factors for coronary heart disease: high serum cholesterol concentration, low HDL cholesterol concentration, age, blood

pressure, smoking, and obesity (Quetelet's index). Few were significant (table III). It is noteworthy that the proportion of linoleic acid in adipose tissue (and ratio of polyunsaturated to saturated fatty acids) did not correlate with serum cholesterol concentrations in Italy, south west Finland, and Scotland. The proportion of linoleic acid and the total proportion of polyunsaturated fatty acids in adipose tissue were not related to smoking in Finland and Italy, but in Edinburgh a previously observed negative association was confirmed.

No strong correlation between fatty acid composition of adipose tissue and blood pressure was observed; in north Karelia, however, the proportion of linoleic acid in adipose tissue was inversely related to diastolic but not systolic blood pressure, and in Sapri the total proportion of saturated fatty acids was positively correlated with systolic and diastolic blood pressure. The most consistent negative correlation observed was between the proportion of saturated fatty acids in adipose tissue and obesity in three of the four regions (not in Sapri).

TABLE III—Spearman correlation between fatty acid composition of adipose tissue and classical risk factors for coronary heart disease in Scotland, north Karelia, south west Finland, and Italy

Region	Age	Smoking	Blood pressure		Total cholesterol	HDL cholesterol	Quetelet's index
			Systolic	Diastolic			
<i>Linoleic acid</i>							
Scotland	-0.23*	-0.27**	+0.02	+0.02	+0.02	0.00	+0.01
N Karelia	-0.20	-0.05	-0.11	-0.30**	-0.25*	-0.16	-0.19
S W Finland	-0.10	+0.04	+0.10	+0.15	-0.18	-0.26*	+0.10
Italy	-0.07	-0.02	+0.05	-0.01	+0.13	-0.16	+0.14
<i>Polyunsaturated fatty acids</i>							
Scotland	-0.22*	-0.27**	+0.02	-0.05	+0.02	0.00	+0.01
N Karelia	-0.20	-0.05	-0.09	-0.27*	-0.26*	-0.17	-0.16
S W Finland	-0.08	+0.05	+0.10	+0.17	-0.18	-0.29**	+0.13
Italy	-0.08	-0.01	+0.05	+0.00	+0.13	-0.17	+0.15
<i>Saturated fatty acids</i>							
Scotland	+0.05	-0.06	-0.13	-0.08	-0.03	+0.05	-0.27**
N Karelia	+0.06	+0.01	-0.22*	-0.20	-0.06	+0.14	-0.27**
S W Finland	+0.09	-0.12	-0.17	-0.23*	+0.21	+0.25*	-0.34**
Italy	-0.01	-0.21	+0.31*	+0.32**	+0.09	0.00	+0.16
<i>Polyunsaturated: saturated fatty acids</i>							
Scotland	-0.16	-0.17	+0.07	+0.01	+0.03	-0.01	+0.13
N Karelia	-0.18	-0.07	-0.04	-0.15	-0.18	-0.21*	-0.01
S W Finland	-0.09	-0.05	+0.10	+0.17	-0.28*	-0.40***	+0.26*
Italy	-0.07	+0.06	-0.05	-0.14	+0.09	-0.09	+0.04

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; other correlations not significant.

Cumulative frequency distribution curves of proportion of linoleic acid in adipose tissue and ratio of polyunsaturated to saturated fatty acids in men from three European countries with contrasting mortalities from coronary heart disease.

○—○ = Italy (Sapri). ▲—▲ = South west Finland. ×—× = Scotland (Edinburgh). ■—■ = North Karelia (Finland).

Thus there was generally little relation between the fatty acid composition of adipose tissue and known risk factors for coronary heart disease in the four regions. This suggested that differences among the regions in these factors were unlikely to explain the observed difference in the proportions of fatty acids, and therefore a series of multiple regressions was done to test this. The variables tested were the proportion of linoleic acid in adipose tissue, ratio of polyunsaturated to saturated fatty acids, and total proportions of saturated fatty acids and polyunsaturated fatty acids. In each case logarithms of the values were used to correct for skewness. As the two Finnish regions showed little difference in any of the fatty acid concentrations the data were amalgamated and pairwise comparisons made between the three countries. In addition, the known risk factors for coronary heart disease were included in each regression as "explanatory" variables. The difference in the proportion of linoleic acid in adipose tissue between the three countries remained highly significant when the other variables were taken into account ($p < 0.001$). This was also true for the ratio of polyunsaturated to saturated fatty acids in adipose tissue and the total proportions of saturated fatty acids and polyunsaturated fatty acids.

Discussion

Linoleic acid is an essential fatty acid that cannot be synthesised in man, and these findings probably reflect the dietary habits of these populations. In this study we did not estimate the intake of dietary linoleic acid, but we have previously shown that the relative proportion of linoleic acid in adipose tissue is positively correlated ($r = 0.6$, $p < 0.001$) with the proportion of dietary linoleic acid as determined by seven day prospective weighed diets in 164 Edinburgh men.¹² This observation is true whether dietary intake of linoleic acid is expressed as g/day, proportion of total fatty acids, or proportion of energy. The possibility of selective differences in absorption of fatty acids, transport in plasma, esterification, storage, and mobilisation or oxidation in subjects susceptible to coronary heart disease should not be ignored, and this now requires study.

Within populations the proportion of linoleic acid in plasma cholesterol esters, platelets, red cell membranes, and adipose tissue has been shown to be lower in patients with coronary heart disease. A recent prospective study from Finland showed that a low linoleic acid content in serum phospholipids might be an independent predictor of coronary heart disease.¹³ In a recent Scottish study a low concentration of linoleic acid in adipose tissue was found in men with hitherto occult coronary heart disease, and the saturation-elongation product of linoleic acid, dihomo- γ -linolenic acid (20:3n-6), was even more powerfully and independently related to coronary heart disease.⁶ In this study the proportions of dihomo- γ -linolenic acid were also low in the regions of high mortality.

There were also striking differences between the four regions in proportions of saturated and monounsaturated fatty acids in adipose tissue. Myristic acid is the only fatty acid other than linoleate strongly related to dietary intake ($r = 0.5-0.7$; $p < 0.001$).¹² The higher proportions of myristic acid in adipose tissue in Finnish and Scottish men probably reflect a higher intake of butter fat in these countries, as butter fat is the most important dietary source of this fatty acid. The higher proportion of oleic acid in Italian men was as expected because of the high consumption of olive oil in that country.

Interpretation of differences in proportions of fatty acids is difficult because of the mathematical interdependence of the constituents. Thus the proportion of minor components seems to change as a result of a change in the proportion of a major component. The fact that only two fatty acids, myristic and linoleic acid, strongly correlated with their dietary intake therefore makes these differences even more important. A prospective study on the composition of fatty acids in the diet and adipose tissue in relation to the development of coronary heart disease is indicated.

There is increasing evidence of a relation between the fatty acid composition of adipose tissue and blood pressure.^{11,14} The difference between regions in linoleic acid in adipose tissue could not, however, be explained by the known relation between blood pressure and coronary heart disease. Nor could the difference in linoleic acid content or fatty acid composition of adipose tissue be explained by their relation with other known risk factors for

coronary heart disease—age, Quetelet's index, smoking, and serum and high density lipoprotein cholesterol concentrations. In every case the differences between countries remained highly significant when the other variables were taken into account.

Total serum cholesterol concentrations and high density lipoprotein cholesterol concentrations generally reflected the gradients in mortality from coronary heart disease between the regions, being lowest in Italy, intermediate in Scotland, and highest in north Karelia (19% higher than in Italy). These results agree with observations that populations with high total cholesterol concentrations also have high concentrations of high density lipoprotein cholesterol and that low concentrations of high density lipoprotein cholesterol are related to the development of coronary heart disease only within a certain population,¹⁵ though this has been questioned recently.¹⁶ This regional difference in high density lipoprotein cholesterol concentrations cannot be explained by a high alcohol consumption in Finland or by a higher triglyceride concentration in Italy. The low high density lipoprotein cholesterol concentration in Italy accords with the finding that diets rich in linoleic acid or with a high ratio of polyunsaturated to saturated fatty acids lowered high density lipoprotein cholesterol concentrations.¹⁷ Possibly the amount of high density lipoprotein cholesterol relative to total or low density lipoprotein cholesterol is more important. This view is supported by the finding that the ratio of high density lipoprotein to low density lipoprotein cholesterol appears to be a better predictor for coronary heart disease.¹⁸ Furthermore, not all high density lipoprotein subfractions may be equally effective in preventing coronary heart disease, and neither high density lipoprotein subfractions (HDL₂ and HDL₃) nor apoproteins were measured.

The relation between linoleic acid in adipose tissue, naturally occurring antioxidants (β carotene and vitamins E and C), and coronary heart disease needs investigation. A parallel survey of concentrations of these antioxidants in Finnish, Scottish, and Italian men in relation to the mortality from coronary heart disease in these countries has been conducted and will be reported elsewhere.

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