CLINICAL RESEARCH

Fatty-acid composition of serum lipids predicts myocardial infarction

T A MIETTINEN, V NAUKKARINEN, J K HUTTUNEN, S MATTILA, T KUMLIN

Abstract

During a follow-up of five to seven years 33 out of 1222 middle-aged men initially free of coronary heart disease sustained fatal or non-fatal myocardial infarction or died suddenly. The fatty-acid composition of serum triglycerides, phospholipids, and cholesterol esters had been measured at the start of the surveillance in these men and in a control group of 64 men matched for age, serum cholesterol and triglyceride concentrations, blood pressure, obesity, smoking, and one-hour glucose tolerance. Palmitic and stearic acids of phospholipids were significantly higher and linoleic and most polyunsaturated fatty acids, including arachidonic acid and eicosapentaenoic acid, of phospholipids were lower in the subjects who sustained coronary events compared with the controls. Linoleic acid tended to correlate negatively with blood pressure while other polyunsaturated fatty acids, especially eicosapentaenoic acid, exhibited a negative correlation with blood pressure and relative body weight in the controls but not in the subjects who sustained coronary events.

These findings suggest that the fatty-acid pattern of serum phospholipids is an independent risk factor for coronary heart disease.

Introduction

Patients with ischaemic heart disease have a low content of linoleic acid in plasma cholesterol esters.¹⁻³ In patients with peripheral arterial disease the development of myocardial infarction was significantly associated with reduced concentrations of dienoic acids in plasma cholesterol esters at entry to the trial.⁴ No data are available on the predictive value of the fatty acid content of serum lipids in the development of coronary heart disease in populations initially free of clinical signs of ischaemic heart disease. We conducted a study to assess this.

Subjects and methods

In 1974-5 a group of 3400 middle-aged men (40-55 years old), who had previously participated voluntarily in health examinations organised by their companies, were screened for risk factors for ischaemic heart disease. Of these men, 1222 were free of ischaemic heart disease but had one or more of the following risk factors: hypertension (systolic blood pressure >159 mm Hg or diastolic >94 mm Hg); hypercholesterolaemia (>7.0 mmol/l (271 mg/100 ml)); hypertriglyceridaemia (>1.6 mmol/l (142 mg/100 ml)); smoking (over nine cigarettes/day); obesity (>119% of ideal body weight); and abnormal oral glucose tolerance (one-hour value >8.9 mmol/l (160 mg/100 ml)). During a subsequent follow-up period of five to seven years 33 of these 1222 men experienced fatal or non-fatal myocardial infarction (verified by chest pain, raised enzyme concentrations, and electrocardiographic changes, or by the appearance of a Q wave on the electrocardiogram) or sudden death. For these 33 subjects 64 controls were selected from the remaining 1189 men so that the two groups were matched for age, blood pressure, cholesterol and triglyceride concentrations, smoking, obesity, and glucose tolerance at the start of the follow-up. Since fasting serum samples had been obtained at entry in 1974-5 and stored frozen for the follow-up period at -20°C the initial fatty-acid composition of the serum lipids could be analysed in the two groups.

The fatty-acid composition of phospholipids, cholesterol esters, and triglycerides was determined. The lipids were extracted with chloroform-methanol and the lipid fractions separated on plastic silica gel chromatoplates using heptane:ethyl ether:acetic acid (80:20:2, v/v/v) as a solvent. The fatty-acid methyl esters were analysed with a Varian 2100 gas chromatograph equipped with a 35 m glass capillary BDS (butane-1,4-diol succinate) column. The temperature programme was 2°C/min, from 130°C to 200°C. The peaks were identified on the basis of retention times recorded for

Second Department of Medicine, University of Helsinki, 00290 Helsinki 29, Finland

T A MIETTINEN, MD, professor of medicine

Jorvi Hospital, Espoo, Finland

V NAUKKARINEN, MD, senior physician

National Public Health Institute, Helsinki, Finland

J K HUTTUNEN, MD, professor and director

Pension Insurance Company Kalervo, Helsinki, Finland

S MATTILA, MD, senior physician

Finnair Co, Helsinki, Finland

T KUMLIN, MD, senior physician

different standards. The peak areas were measured with an electronic integrator. The sum of fatty acids from 14:0 to 18:3 was taken as 100 so that the longer-chain polyunsaturated fatty acids could be expressed as a percentage of them.

Results

Table I shows that the initial levels of the risk factors were similar in the subjects who developed ischaemic heart disease and in those who did not. Coronary events accumulated in subjects with hyperlipidaemia, those with hypertension, and those who smoked.

TABLE I—Risk factors in different groups at start of follow-up (means $\pm$ SE)

Risk factor	Controls (n = 64)	Patients with infarcts* (n = 33)	Whole group (n = 1222)
Cholesterol concentration (mmol/l)	7.4 $\pm$ 0.1	7.6 $\pm$ 0.2	7.1 $\pm$ 0.03
Triglyceride concentration (mmol/l)	1.9 $\pm$ 0.1	2.0 $\pm$ 0.2	1.8 $\pm$ 0.01
Blood pressure (mm Hg):			
Systolic	156 $\pm$ 4	154 $\pm$ 3	143 $\pm$ 0.5
Diastolic	101 $\pm$ 1	100 $\pm$ 2	92 $\pm$ 0.3
Relative body weight (%)	116 $\pm$ 2	115 $\pm$ 2	116 $\pm$ 0.3
One-hour glucose tolerance (mmol/l)	7.6 $\pm$ 0.3	7.3 $\pm$ 0.4	7.5 $\pm$ 0.1
No (%) of smokers:			
Non-smokers	28 (44)	14 (42)	758 (62)
$\leq$ 10 cigarettes/day	5 (8)	1 (3)	61 (5)
$<$ 10 cigarettes/day	22 (34)	15 (46)	293 (24)
Pipe or cigar	9 (14)	3 (9)	110 (9)

*Includes sudden deaths and myocardial infarcts found during follow-up of five to seven years.

Conversion: SI to traditional units—Cholesterol: 1 mmol/l = 38.7 mg/100 ml. Triglyceride: 1 mmol/l $\approx$ 88.6 mg/100 ml. Glucose: 1 mmol/l $\approx$ 18 mg/100 ml.

The relative amounts of the saturated fatty acids 16:0 and 18:0 were significantly higher in the phospholipids of the subjects destined to have a coronary event than in those of subjects who remained healthy during the follow-up period (table II). The values of 18:2 and 18:3 and the sum of the remaining polyunsaturated fatty acids were correspondingly lower. Of the individual polyunsaturated fatty acids, 20:3 ω 6, arachidonic acid (20:4 ω 6), eicosapentaenoic acid (20:5 ω 3), and 22:6 ω 3 were significantly decreased in the subjects who subsequently had cardiac events (fig 1).

Subgrouping the study population according to the increasing proportion of 18:2 in phospholipids (fig 2) showed a negative correlation between the incidence of new cardiac events and the initial proportion of 18:2.

In triglycerides and cholesterol esters the fatty-acid patterns were less consistent. Thus, in the group who sustained cardiac events compared with the control group, 14:0 was lower and 18:1, 22:5, and 22:6 higher in triglycerides and 18:1 higher in cholesterol esters (table II, fig 1).

Plotting fatty acids against the different risk factors in the control group (table III) showed negative correlation coefficients for 18:2 and positive ones for 16:0 and the polyunsaturated fatty acids. The correlations were significant between blood pressure and 18:2, 16:0, and the polyunsaturated fatty acids and between relative body weight and the polyunsaturated fatty acids of phospholipids and cholesterol esters but not of triglycerides. Also, 20:5 of phospholipids and cholesterol esters was positively correlated with the serum triglyceride concentration. In the group who sustained coronary events the correlations were mostly non-significant. An exception, however,

FIG 1—Pattern of individual polyunsaturated fatty acids (means $\pm$ SE) in serum lipids in controls and subjects with infarcts. (Overall pattern given as total polyunsaturated fatty acids in table II.)

* $p < 0.05$ compared with controls.

was the correlations of relative body weight with 18:2 in all the lipid classes (negative correlations) and with 16:0 and the polyunsaturated fatty acids (positive correlations).

The correlation of 18:2 with 16:0 or the polyunsaturated fatty acids was negative in phospholipids and cholesterol esters, especially in the controls (r ranging from -0.349 to -0.630) but less consistently in the group who sustained coronary events (r ranging from -0.051 to -0.824).

Discussion

Our findings show for the first time that the fatty-acid composition of serum lipids—in particular, a low content of polyunsaturated fatty acid in phospholipids—is predictive of ischaemic heart disease. Furthermore, since the subjects who experienced coronary events had been matched with the controls at the start of follow-up for age, blood pressure, serum lipid concentrations, smoking, obesity, and glucose tolerance it may be postulated that this fatty-acid pattern of serum lipids is an independent risk factor for ischaemic heart disease. The

TABLE II—Fatty-acid patterns of serum lipids at start of follow-up (means $\pm$ SE)

Group	Fatty acids (%)							Total PUFA†
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	
Controls	0.79 $\pm$ 0.03	13.24 $\pm$ 0.13	5.06 $\pm$ 0.25	1.23 $\pm$ 0.03	25.31 $\pm$ 0.49	53.89 $\pm$ 0.77	0.57 $\pm$ 0.02	3.93 $\pm$ 0.18
Infarcts	0.81 $\pm$ 0.05	14.38 $\pm$ 0.28	4.98 $\pm$ 0.30	1.25 $\pm$ 0.05	25.69 $\pm$ 0.66	52.34 $\pm$ 1.16	0.56 $\pm$ 0.02	3.90 $\pm$ 0.27
Controls	2.29 $\pm$ 0.15	29.49 $\pm$ 0.43	4.85 $\pm$ 0.15	6.42 $\pm$ 0.28	41.96 $\pm$ 0.41	14.16 $\pm$ 0.72	0.83 $\pm$ 0.04	2.11 $\pm$ 0.20
Infarcts	1.76 $\pm$ 0.16*	29.95 $\pm$ 0.60	4.84 $\pm$ 0.19	6.07 $\pm$ 0.27	43.63 $\pm$ 0.63*	12.95 $\pm$ 0.73	0.80 $\pm$ 0.08	2.67 $\pm$ 0.34
Controls	0.34 $\pm$ 0.21	35.17 $\pm$ 0.28	1.30 $\pm$ 0.06	19.30 $\pm$ 0.16	17.52 $\pm$ 0.30	26.15 $\pm$ 0.46	0.23 $\pm$ 0.01	17.74 $\pm$ 0.51
Infarcts	0.34 $\pm$ 0.03	36.54 $\pm$ 0.51*	1.18 $\pm$ 0.07	20.04 $\pm$ 0.22*	18.29 $\pm$ 0.27	23.40 $\pm$ 0.59*	0.20 $\pm$ 0.01*	14.63 $\pm$ 0.61*

* $p < 0.05$ or less.

†PUFA (polyunsaturated fatty acids) include 20:3 ω 9, 20:3 ω 6, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, and 22:6 ω 3, expressed as percentage of total fatty acids up to 18:3.

TABLE III—Correlation coefficients between fatty acids of serum lipids and risk factors in control subjects (n=64)

Risk factor	Fatty acids				
	16:0	18:2	20:4	20:5	PUFA
<i>Phospholipids</i>					
Blood pressure:					
Systolic	0.393**	-0.259*	0.196	0.323**	0.257*
Diastolic	0.448***	-0.218	0.246*	0.395**	0.284*
Cholesterol	0.132	-0.088	0.155	0.157	0.203
Triglycerides	0.155	-0.160	0.051	0.249*	0.150
Relative body weight	0.224	-0.193	0.245*	0.279*	0.254*
<i>Cholesterol esters</i>					
Blood pressure:					
Systolic	0.219	-0.279*	0.222	0.280*	0.241
Diastolic	0.177	-0.206	0.282*	0.386**	0.327**
Cholesterol	0.115	-0.045	0.212	0.243	0.233
Triglycerides	0.216	-0.009	0.110	0.277*	0.181
Relative body weight	0.183	-0.132	0.241*	0.204	0.266*

PUFA = Polyunsaturated fatty acids.
*p<0.05, **p<0.01, ***p<0.001.

FIG 2—Distribution of controls (○) and subjects who had coronary events (●) according to linoleic acid content of phospholipids. Figures in parentheses are total numbers of subjects in each linoleic acid category.

similar serum cholesterol concentrations in the two groups suggests that the high incidence of ischaemic heart disease in the subjects with low antecedent concentrations of polyunsaturated fatty acid is not mediated by hypercholesterolaemia induced by intake of saturated fat. In fact, no significant correlation was found between fatty acids of serum lipids and the serum cholesterol concentration.

The importance of the inverse association of 18:2 with blood pressure (table III) remains unknown. Associations of 16:0 and polyunsaturated fatty acids—20:5 in particular, and, somewhat less consistently, 20:4—with blood pressure were correspondingly positive because these fatty acids correlated negatively with 18:2. There is some clinical and experimental evidence that a diet high in polyunsaturated fatty acids reduces blood pressure.⁵⁻⁸ This type of diet is well known to increase polyunsaturated fatty acids in body lipids. The 18:2 content of adipose tissue correlates negatively with blood pressure in the normal population.⁹

Patients with clinically manifest coronary heart disease or atherosclerosis in general have a low linoleic acid content in plasma cholesterol esters^{1-4 10 11} and in adipose tissue provided that no specific dietary changes have occurred.¹² Apart from in Eskimos, an approximate inverse correlation exists between essential fatty acids of cholesterol esters and mortality from coronary heart disease.¹³ The content of 20:3ω₉, however, a

sensitive indicator of deficiency of essential fatty acids,¹⁴ was not increased in the patients who sustained coronary events in our study. Thus these subjects had hardly any such deficiency at the start of the follow-up. Furthermore, the linoleic acid content of cholesterol esters was fairly high at entry, also excluding deficiency of essential fatty acids.

The present study suggests that the incidence of ischaemic heart disease is predicted more consistently by the fatty-acid pattern (high in saturated and low in polyunsaturated fatty acid) of phospholipids than by that of cholesterol esters or triglycerides. Patients with acute myocardial infarction without previous symptoms of ischaemic heart disease also have a low linoleic acid content in phospholipids but, in contrast to our findings, the more unsaturated fatty acids are increased.⁴ Phospholipids of normal muscle from human heart with severe atherosclerosis have a low 18:2 content; in sudden death due to recent myocardial infarction phospholipids of cardiac muscle also contain relatively little polyene fatty acids.^{15 16} In addition, a high concentration of diunsaturated lecithin, found in plasma from patients with myocardial infarction and those with premature atherosclerosis, has been suggested to be a risk factor for atherosclerosis, independent of triglyceride and cholesterol concentrations.¹⁷ Furthermore, a low level of polyunsaturated fatty acids with more than 18 carbons has been found in plasma phospholipids of elderly women with cardiovascular diseases.¹⁸

The actual reason for the different antecedent fatty-acid pattern in the group who sustained coronary events can only be hypothesised. One explanation is that metabolism of linoleic acid to arachidonic acid and of polyunsaturated fatty acids as a whole was enhanced in subjects prone to atherosclerosis or that existing asymptomatic atheromatosis at entry was associated with increased consumption of these fatty acids. More probably, however, dietary intake of unsaturated fatty acids had been low in the subjects who sustained coronary events. Since the major risk factors of ischaemic heart disease were similar in the two groups, possible diet-induced change in the fatty-acid pattern and subsequently in the incidence of ischaemic heart disease may not be mediated via serum lipid concentrations or blood pressure. We speculate that the low polyunsaturated fatty acid content of phospholipids is associated with unfavourably altered prostaglandin metabolism, which was an ultimate reason for the increased incidence of ischaemic heart disease by an unknown mechanism.

This study was supported by the Finnish National Council for Medical Research.

References

- Lewis B. Composition of plasma cholesterol ester in relation to coronary-artery disease and dietary fat. *Lancet* 1958;ii:71-3.
- Schrade W, Biegler R, Böhle E. Fatty-acid distribution in the lipid fractions of healthy persons of different age, patients with atherosclerosis and patients with idiopathic hyperlipidaemia. *Journal of Atherosclerosis Research* 1961;1:47-61.
- Kirkeby K, Ingvaldsen P, Bjerkedal I. Fatty acid composition of serum lipids in men with myocardial infarction. *Acta Med Scand* 1972;192:513-9.
- Kingsbury KJ, Morgan DM, Stovold R, Brett CG, Anderson J. Polyunsaturated fatty acids and myocardial infarction. *Lancet* 1969;iii:1325-9.
- Iacono JM, Marshall MW, Dougherty RM, Wheeler MA, Mackin JF, Canary JJ. Reduction in blood pressure associated with high polyunsaturated fat diets that reduce blood cholesterol in man. *Prev Med* 1975;4:426-43.
- Hoor FT, van de Graaf HM. The influence of a linoleic acid-rich diet and of acetyl salicylic acid on NaCl induced hypertension, N⁺- and H₂O-balance and urinary prostaglandin excretion in rats. *Acta Biol Med Ger* 1978;37:875-7.
- Comberg H-U, Heyden S, Hames CG, Vergroesen AJ, Fleischman AI. Hypotensive effect of dietary prostaglandin precursor in hypertensive man. *Prostaglandins* 1978;15:193-7.
- Stern B, Heyden S, Miller D, Latham G, Klimas A, Pilkington K. Intervention study in high school students with elevated blood pressures. Dietary experiment with polyunsaturated fatty acids. *Nutr Metab* 1980;24:137-47.

- ⁹ Oster P, Arab L, Schellenberg B, Heuck CC, Mordasini R, Schlierf G. Blood pressure and adipose tissue linoleic acid. *Res Exp Med (Berl)* 1979;**175**:287-91.
- ¹⁰ Böttcher CJF, Woodford FP. Lipid and fatty-acid composition of plasma lipoproteins in cases of aortic atherosclerosis. *Journal of Atherosclerosis Research* 1961;**1**:434-43.
- ¹¹ Kingsbury KJ, Brett C, Stovold R, Chapman A, Andersson J, Morgan DM. Abnormal fatty acid composition and human atherosclerosis. *Postgrad Med J* 1974;**50**:425-40.
- ¹² Kirkeby K, Nitter-Hauge S, Bjerkedal I. Fatty acid composition of adipose tissue in male Norwegians with myocardial infarction. *Acta Med Scand* 1972;**191**:321-4.
- ¹³ Oliver MF. Dietary prevention of coronary heart disease: the role of essential fatty acids. In: Gotto AM Jr, Smith LC, Allen B, eds. *Atherosclerosis V*. New York: Springer-Verlag, 1980:235-43.
- ¹⁴ Holman RT. The ratio of trienoic: tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J Nutr* 1960;**70**:405-9.
- ¹⁵ Gudbjarnason S, Öskarsdóttir G, Doell B, Hallgrímsson J. Myocardial membrane lipids in relation to cardiovascular disease. In: Manninen V, Halonen PI, eds. *Advances in Cardiology*. Basle: Karger, 1978:130-44.
- ¹⁶ Gudbjarnason S. Pathophysiology of long-chain polyene fatty acids in heart muscle. *Nutr Metab* 1980;**24**, suppl 1:142-6.
- ¹⁷ Gershfeld NL. Selective phospholipid adsorption and atherosclerosis. *Science* 1979;**204**:506-8.
- ¹⁸ Deby-Dupont G, Ducarne H, Lefevre A, Deby C, Lennes G, Goutier R. Recherches sur les relations entre acides gras polyinsaturés et accidents vasculo-cardiaques. *Acta Cardiol* 1980;**2**:83-91.

(Accepted 13 July 1982)

Arterial hypertension developing 10 years after radiotherapy for Wilms's tumour

OLLI KOSKIMIES

Abstract

Three patients developed arterial hypertension more than 10 years after receiving irradiation for Wilms's tumour. Scattered radiation appeared to have caused changes in the remaining kidney which were not severe enough to inhibit compensatory hypertrophy but which produced a rise in blood pressure at a later date. Since arterial hypertension appears to be a delayed complication of radiotherapy which is easily detected and controlled and can occur at any age long-term surveillance after successful treatment of malignant tumours in childhood is necessary.

Introduction

An increasing number of children with malignant diseases can now be cured or have their lives prolonged substantially by surgery, cytostatic drugs, and ionising radiation. The last two methods of treatment in particular are known to have many side effects, some appearing immediately and others only several years after treatment. Many organs are known to be adversely affected by radiation. The effects on the kidney have been graded by Luxton¹ as follows: acute and chronic radiation nephritis, asymptomatic proteinuria, benign arterial hypertension, and malignant arterial hypertension.

In this paper I describe three patients who first developed arterial hypertension more than 10 years after irradiation treatment for Wilms's tumour. These findings emphasise the necessity of long-term surveillance of patients with successfully treated malignancies in childhood.

Case reports

CASE 1

In 1959 a girl aged 2 years 7 months was admitted with a history of haematuria and a palpable tumour on the right upper part of her abdomen. On admission haematuria and proteinuria were both

present, and her systolic blood pressure was 105 mm Hg. A right nephrectomy was performed and a large tumour removed subradically. The tumour, which originated in the right kidney, had dislocated the liver, extended to the left side and infiltrated the renal vein and vena cava. Histological examination showed an undifferentiated nephroblastoma. During the operation 50 mCi of radioactive gold were instilled into the space of the extirpated right kidney. Postoperatively, irradiation of 4073 rads was given to the right upper abdominal quadrant (table).

When the child was 3 years old a metastasis in the right lower pulmonary lobe was detected and a lobectomy performed. Postoperatively, irradiation of 3650 rads was given to the right lower chest. At the time of the operation her blood pressure was 105/60 mm Hg. She was followed up at the outpatient department until the age of 7 years. During this period her blood pressure was well within normal limits.

In 1974, at the age of 17 years, she was admitted with proteinuria and arterial hypertension, which had been detected by the school physician during a routine examination. She was small in stature (height 152 cm, weight 53 kg) with underdevelopment of the right side of the chest and the right breast. Her blood pressure was 160/120 mm Hg. An overnight urine specimen gave a urinary osmolality of 959 mmol/kg (959 mosmol/kg); protein and cell excretions were within normal limits. Sporadic urine analyses showed intermittent protein up to 5 g/l and at times an increased number of leucocytes. Her haemoglobin concentration was 13 g/dl and serum creatinine 55.7 µmol/l (0.63 mg/dl). Intravenous pyelography showed the left kidney to be compensatorily hypertrophied as it was > 2 SD above the mean in length. There was no evidence of coarctation of the aorta or intracranial processes. Serum electrolyte concentrations, calcium and phosphorus, thyroid function, and urinary catecholamine excretion were found to be normal, and there was no family history of hypertension. As no cause other than radiation injury to the remaining kidney was found treatment with reserpine was started. Blood pressure control was adequate, diastolic pressure being well below 95 mm Hg.

CASE 2

In 1960 an 11-month-old girl presented with a three-month history of tiredness and lack of appetite. She was admitted because there was palpable resistance in the left kidney area and her blood pressure was 220/130 mm Hg. A left nephrectomy was performed and a partially undifferentiated nephroblastoma originating in the left kidney removed. Postoperatively, she received irradiation to the left upper abdominal quadrant and actinomycin D stoss therapy (table). After the operation her blood pressure fell to 110/80 mm Hg and remained at that level during the follow-up period. She was admitted every