Fatty acid proportions in cholesterol esters and risk of premature death from cancer in middle aged French men

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Abstract

Objective—To assess the association of proportions of fatty acids in cholesterol esters with the risk of premature death from cancer in middle aged men.

Design—Prospective cohort study.

Setting—Paris, France.

Subjects—3277 working men aged 36-52 in 1981-5.

Main outcome measures—Cancer mortality during an average of 9.3 years of follow up.

Results—59 men died of cancer during follow up. The age adjusted relative risks for men in the highest thirds of the distribution of the proportions of linoleic, palmitoleic, and oleic acid in cholesterol esters as compared with those in the corresponding lowest thirds were 0.16 (95% confidence interval 0.05 to 0.51), 3.39 (1.63 to 7.05), and 4.22 (1.95 to 9.12), respectively. Adjustment for and stratification by smoking, alcohol consumption, serum cholesterol concentration, and body mass index did not alter the results. At the time of examination subjects with cancer had a lower intake of polyunsaturated fats, assessed by 24 hour recall, than those without cancer (13.2 v 17.4 g/day, $P < 0.01$).

Conclusions—Monounsaturated and polyunsaturated fatty acids of cholesterol esters are strong biological markers that predict premature death from cancer in French men. Consistently, intake of polyunsaturated fats did not seem to increase the risk of death from cancer. The association of biological markers of dietary fat intake with incidence of and mortality from cancer should be investigated prospectively in other populations.

Introduction

It has recently been hypothesised¹ that an increased intake of polyunsaturated fat or decreased intake of cholesterol and saturated fat, or both, might explain the inverse association between low concentrations of serum cholesterol and an increased risk of cancer,²⁻⁵ although this association remains controversial.^{6,7} This hypothesis, if supported, would be of great concern because the current policy for prevention of coronary heart disease aims to increase the consumption of polyunsaturated fat and to reduce that of saturated fat.⁸ Kritchevsky reported that diet, assessed by 24 hour recall, cannot explain the association between low serum cholesterol concentration and cancer as men who later developed cancer consumed more dietary cholesterol and saturated fat, which both tend to increase rather than decrease the serum cholesterol concentration.¹ The analysis of this cohort study was based on a method of dietary assessment with low validity at the individual level.⁹ Cohort studies that use more objective biological markers of fatty acid composition of the diet are needed. Recently, fatty acids of cholesterol esters have been proposed,¹⁰⁻¹² even though they might represent only indirect biological markers of dietary fat intake because of their metabolism. The

highest correlations between intake and concentrations of fatty acids were observed for polyunsaturated fatty acids. Particularly, linoleic acid of cholesterol esters could reflect the intake of polyunsaturated fats or the polyunsaturated:saturated ratio of the preceding weeks or months.¹³ Because both saturated and monounsaturated fatty acids can be endogenously produced their intake is weakly reflected in the concentration of corresponding fatty acids esterifying serum cholesterol.

We assessed the independent effects of fatty acids of cholesterol esters on premature death from cancer in middle aged men.

Subjects and Methods

Details of the cohort examined in the Paris prospective study II have been previously reported.^{14,15} Briefly, between 1981-5, 4013 men aged 30-52 years employed in the Parisian civil service and the French Railroad Company participated in the initial screening phase of the study. Participants underwent physical examination, completed a questionnaire on medical history and lifestyle factors, and provided blood samples. Data on alcohol consumption were obtained by a weighted sum of the different types of alcoholic beverages consumed by a subject during a typical week and were expressed in millilitres of alcohol a day. Men were asked about their present and past smoking habits. Daily alcohol consumption was moderately associated with γ -glutamyltransferase activity (correlation coefficient 0.34; $P < 0.0001$) and daily cigarette consumption was strongly correlated with carboxyhaemoglobin concentration (0.72; $P < 0.0001$). Weight (kg) and height (m) were measured, and body mass index was defined as weight(kg)/height (m)².

Two specially trained technicians thoroughly recorded the types and quantities of food consumed by each subject during the preceding 24 hours. After the data were coded by a single dietician a measure of nutrient intake was obtained from a computer program with a food composition table.¹⁶

Serum cholesterol concentration was measured until July 1983 by an automatic procedure based on the Liebermann Burchard reaction.¹⁷ After that an automatic enzymatic procedure was used (Technicon SMA system, Minilyzer, Technicon, New York).

For the measurement of fatty acids of serum cholesterol esters, total serum lipids were extracted by the method of Floch *et al*¹⁸ and fractionated by thin layer chromatography on 0.2 mm silica gel plates (Merck, Darmstadt, Germany). The cholesterol ester fraction was scraped off and treated according to the procedure of Tuckey and Stevenson.¹⁹ After extraction, methyl esters were separated with a glass capillary column (45 m x 0.25 mm inside diameter) coated with 20M Carbowax in a gas chromatograph (Girdel model 300, Girdel, Puteaux, France). Full details of the technical methods and measurements of fatty acids have been

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previously reported.^{14,15} Each identified fatty acid was expressed as a percentage of the total fatty acids recovered. The presentation of results will be limited to the three major components of fatty acids: palmitoleic (C16:1), oleic (C18:1), and linoleic (C18:2) acids. For each fatty acid the precision of the measure was 0.5%. High correlations were observed among the proportions of the three fatty acids. Linoleic acid was closely correlated to oleic and palmitoleic acids (correlation coefficients -0.78 and -0.68, respectively; $P < 0.0001$ for each). Oleic and palmitoleic acid proportions were positively correlated (0.53; $P < 0.0001$).

Follow up—Men were followed up until January 1994 through their medical services at work and causes of death were obtained from death certificates from the National Institute of Health and Medical Research. The vital status after an average of 9.3 (range 7-12) years of follow up was obtained for 3905 subjects (97.3%). Causes of death were defined according to the *International Classification of Diseases, Ninth Revision* (ICD-9). Cancer mortality was defined as ICD-9 codes 140-209. During the follow up 124 deaths occurred from all causes. Of those, 17 were of cardiovascular origin and 59 from cancer, including 16 from lung cancer, three from larynx cancer, 14 from oesophagus, liver, and pancreas cancers, three from colon cancer, 19 from other cancers, and four from cancers of unspecified sites.

Statistical analysis—The relation of the proportions of fatty acids in cholesterol esters with dietary fat intake was assessed by Pearson correlation coefficients. Those with and without cancer were compared by t test by using log transformation for fatty acid proportions and dietary intake. Relative risks adjusted for age and other variables for thirds of each proportion of fatty acid were estimated by Cox's proportional hazards model,²⁰ using the lowest third as the reference category. Age was used as a continuous variable. Models adjusted for several variables by using indicator variables, including smoking habit (never, former, and 1-15 and > 15 cigarettes a day), alcohol consumption (0-30, 31-70, and > 70 ml/day), serum cholesterol (≤ 4.80 , 4.81-6.20, and ≥ 6.21 mmol/l), and body mass index (≤ 24.0 , 24.1-27.0, and > 27.0). Independent effects of the proportions of the three fatty acids on the risk of premature death from cancer were also assessed by Cox's proportional hazards regression by using the "mixture model,"²¹ which specifically takes into account the fact that the three fatty acids were expressed as percentages. Because no deaths from cancer occurred in the 440 men aged 30-35 years at baseline the present study was restricted to the 3277 men aged 36-52 years at baseline after the exclusion of 188 subjects with incomplete data. The SAS statistical package (version 6) was used for analysis.

Results

In this population the proportion of linoleic acid in cholesterol esters was positively associated with intake of polyunsaturated fat assessed by 24 hour recall (correlation coefficient +0.18; $P < 0.0001$). Palmitoleic and oleic acids of cholesterol esters were negatively related to intake (-0.13 and -0.21, respectively; $P < 0.0001$ for each). No significant association was observed between these fatty acids of cholesterol esters and intake of monounsaturated fat (-0.01 for linoleic acid and 0.01 for both palmitoleic and oleic acids).

Men with cancer had a significantly lower proportion of linoleic acid and higher proportions of palmitoleic and oleic acids than those without cancer (table I). Among daily intake of macronutrients only that of polyunsaturated fat was significantly lower in those with compared with those without cancer. Table

I also shows that smoking habits and body mass index were strongly related to premature death from cancer. Men with cancer also had higher alcohol consumption and lower serum cholesterol concentrations, but these trends were not significant.

The age adjusted relative risks for men in the highest thirds of linoleic, palmitoleic, and oleic acid proportions in cholesterol esters as compared with those in the corresponding lowest thirds were 0.16 (95% confidence interval 0.05 to 0.51), 3.39 (1.63 to 7.05), and 4.22 (1.95 to 9.12), respectively (table II). In addition, a negative linear trend for linoleic acid and positive linear trends for palmitoleic and oleic acids were observed. When we tried to separate the independent effects of the three fatty acid proportions on premature death from cancer by using a "mixture model,"²¹ each fatty acid proportion remained significantly associated with risk of cancer and no individual fatty acid seemed to be more important than the others (data not shown).

TABLE I—Mean (SD) values of fatty acid proportions* in cholesterol esters, daily intake of nutrients, and selected potential risk factors in middle aged men with and without cancer (Paris prospective study II cohort)

Variable	Men with cancer (n=59)	Men without cancer (n=3218)	P value†
Fatty acids:			
Linoleic acid (%)	44.6 (6.2)	48.5 (5.7)	<0.001
Palmitoleic acid (%)	5.7 (2.7)	4.2 (1.8)	<0.001
Oleic acid (%)	21.9 (3.9)	19.6 (3.3)	<0.001
Others (%)	27.8 (3.6)	27.7 (3.1)	0.76
Dietary intake:			
Total energy (kcal/day)‡	2439 (667)	2436 (575)	0.97
Protein (g/day)	102.1 (31.5)	100.4 (28.4)	0.68
Carbohydrate (g/day)	235.4 (88.0)	230.5 (74.9)	0.64
Total fat (g/day)	119.8 (37.8)	122.3 (35.6)	0.61
Saturated fat (g/day)	46.8 (16.6)	46.7 (15.2)	0.96
Monounsaturated fat (g/day)	47.0 (17.0)	45.4 (16.3)	0.46
Polyunsaturated fat (g/day)	13.2 (8.8)	17.4 (11.3)	<0.01
Dietary cholesterol (mg/day)	589 (494)	553 (325)	0.57
Other variables:			
Tobacco consumption (g/day)	16.2 (13.6)	6.1 (9.9)	<0.001
Alcohol consumption (ml/day)	52.3 (35.3)	44.1 (32.1)	0.06
Body mass index (kg/m ²)	24.2 (3.3)	25.4 (2.9)	<0.002
Serum cholesterol (mmol/l)	5.54 (1.05)	5.66 (1.0)	0.36

*Each fatty acid was expressed as percentage of total fatty acids.

†Based on t test for difference between those with and without cancer.

‡Total calories were calculated without energy provided from alcohol.

TABLE II—Relative risks (95% confidence interval) adjusted for age and multiple variables* of premature death from cancer by thirds† of fatty acid proportions in cholesterol esters and intake of polyunsaturated fats in middle aged men (Paris prospective study II cohort)

Measurement	No of cases	Relative risk (95% confidence interval)	
		Age adjusted	Multivariate
Linoleic acid:			
Lowest	32	1	1
Middle	24	0.83 (0.49 to 1.41)	1.00 (0.58 to 1.73)
Highest	3	0.16 (0.05 to 0.51)	0.21 (0.06 to 0.70)
P value for trend‡		<0.001	<0.001
Palmitoleic acid:			
Lowest	9	1	1
Middle	12	1.53 (0.64 to 3.65)	1.49 (0.62 to 3.57)
Highest	38	3.39 (1.63 to 7.05)	2.69 (1.22 to 5.87)
P value for trend‡		<0.001	<0.001
Oleic acid:			
Lowest	8	1	1
Middle	15	1.87 (0.80 to 4.43)	1.56 (0.66 to 3.72)
Highest	36	4.22 (1.95 to 9.12)	2.89 (1.30 to 6.43)
P value for trend‡		<0.001	<0.001
Polyunsaturated fat intake:			
Lowest	25	1	1
Middle	23	0.80 (0.46 to 1.42)	0.90 (0.51 to 1.61)
Highest	11	0.39 (0.19 to 0.80)	0.49 (0.23 to 1.01)
P value for trend‡		<0.006	<0.04

*Adjusted by Cox's proportional hazards model for age, smoking, alcohol consumption, body mass index, and serum cholesterol.

†Tertiles=46.5% and 52.0% for linoleic acid; 3.0% and 4.5% for palmitoleic acid; 18.0% and 20.5% for oleic acid; and 10 and 18 g/day for polyunsaturated fats.

‡Tests for linear trends used fatty acids and polyunsaturated fat intake as continuous variables.

TABLE III—Multivariate relative risks of premature death from cancer associated with 1 SD increase in linoleic acid proportion in cholesterol esters and polyunsaturated fat intake stratified by potential risk factors for cancer and by duration of follow up

Variable	No of men with cancer	No of men without cancer	Multivariate relative risks (95% confidence intervals) for 1 SD increase* in	
			Linoleic acid	Polyunsaturated fat intake
Smoking status:				
Non-smokers or former smokers	15	1937	0.58 (0.38 to 0.86)	0.43 (0.21 to 0.94)
Current smokers	44	1281	0.77 (0.58 to 0.98)	0.79 (0.57 to 1.12)
Alcohol consumption (ml/day):				
≤70	42	2629	0.74 (0.55 to 0.95)	0.77 (0.54 to 1.10)
>70	17	589	0.61 (0.38 to 0.91)	0.61 (0.30 to 1.23)
Cholesterol concentration (mmol/l):				
≤4.80	15	650	0.70 (0.45 to 1.16)	0.87 (0.48 to 1.58)
>4.80	44	2568	0.67 (0.52 to 0.86)	0.67 (0.46 to 0.96)
Body mass index:				
<24.0	29	1031	0.78 (0.55 to 1.05)	1.04 (0.70 to 1.54)
≥24.0	30	2187	0.64 (0.45 to 0.86)	0.52 (0.31 to 0.86)
Duration of follow up (years):				
<7	30	3218	0.78 (0.55 to 1.11)	0.80 (0.53 to 1.20)
≥7	29	3218	0.61 (0.42 to 0.82)	0.63 (0.38 to 1.04)

*Relative risks associated with approximately 1 SD increase in linoleic acid (5%) or polyunsaturated fat intake (10 g/day), calculated by Cox's proportional hazards models adjusted for all potential variables (age, smoking, alcohol consumption, serum cholesterol, and body mass index) except the variable of stratification. For non-smokers or former smokers a variable of never having smoked was added to the model.

When we adjusted for possible confounding factors (smoking, alcohol consumption, serum cholesterol, and body mass index) the relative risks were attenuated but remained highly significant (table II). When γ -glutamyltransferase and carboxyhaemoglobin concentrations were substituted for alcohol consumption and cigarette smoking, respectively, in the multivariate models the results for fatty acids were not affected (data not shown).

For intake of polyunsaturated fat the relative risk adjusted for age for men in the highest third was 0.39 (95% confidence interval 0.19 to 0.80). Although the significance of the multivariate adjusted relative risk for men in the highest third was borderline, there was a negative trend ($P < 0.04$) in the risk of death from cancer with increasing intake of polyunsaturated fat (table II). When the proportion of linoleic acid in cholesterol esters was added to the model the trend with intake of polyunsaturated fat was no longer significant.

When separate analyses for proportion of linoleic acid in cholesterol esters and intake of polyunsaturated fat were performed within categories of smoking habits, alcohol consumption, serum cholesterol, and body mass index, consistent relative risks were observed (table III). Moreover, deaths from cancer were divided into two groups according to the median of time to death after baseline (seven years). The pattern of association was found within the first seven years and continued throughout the later years.

When deaths from cancer were grouped into three categories (lung, digestive, and other cancers), the mean concentrations of linoleic acid and intake of polyunsaturated fat in cases were lower than in men

without cancer for all categories, and the multivariate adjusted relative risks associated with an increase of 1 SD were systematically less than 1 (table IV). Consistent results were also observed for the two other fatty acids (data not shown).

Discussion

Findings suggest that fatty acids esterifying serum cholesterol are strong predictors of premature death from cancer among middle aged French working men. Several hypotheses can be formulated to explain the association between fatty acid proportions in cholesterol esters and such mortality.

The association may reflect, in part, the observed difference in intake of polyunsaturated fat at the time of examination between those with and without cancer. Linoleic acid of cholesterol esters has been suggested as a biological marker of dietary polyunsaturated fat or polyunsaturated:saturated ratio, or both,¹⁰⁻¹³ as a major proportion of polyunsaturated fatty acids in humans is of exogenous origin. As each fatty acid was expressed as a percentage of total fatty acids the association of oleic and palmitoleic acids with premature death from cancer could then be explained by their high negative correlations with linoleic acid. The results of the "mixture model" analysis, however, did not suggest that linoleic acid is specifically involved in risk of cancer.

In any case, our results do not imply a causal link between high intake of polyunsaturated fat and some kind of protection against cancer. The fact that the association does not seem to be site specific supports, in our view, the non-causality hypothesis. So does the consistency of the results according to duration of follow up.

The results of epidemiological studies on the relation between dietary fat and cancer in men are controversial.²²⁻²⁵ A positive association of total fat intake with risk of lung, colon, and prostate cancer has been shown in some studies.²⁴⁻²⁷ No association was found in other studies,²⁸⁻³¹ and an increased overall cancer mortality associated with lower intakes of fat and saturated fat was also observed.³² In animal studies, both saturated and polyunsaturated fats seem to increase the risk of cancer.³³⁻³⁴ Nevertheless, our prospective study using an objective biological marker of polyunsaturated fatty acid profile supports the opinion that fearing an increased risk of cancer due to cholesterol lowering diets does not seem to be justified from epidemiological evidence.³⁵ This finding is of great potential importance for the recommendations to increase the ratio of polyunsaturated to saturated fat to reduce the risk of coronary heart disease.

CONFOUNDING FACTORS

The association of fatty acids of cholesterol esters with premature deaths from cancer could be

TABLE IV—Association of proportion of linoleic acid in cholesterol esters and intake of polyunsaturated fat with risk of premature death from cancer in three specific sites in middle aged men (Paris prospective study II cohort)

Measurement	Men without cancer (n=3218)	Digestive cancer*		Lung cancer*		Other cancers	
		Cases (n=26)	Multivariate relative risk for 1 SD† (95% confidence interval)	Cases (n=16)	Multivariate relative risk for 1 SD† (95% confidence interval)	Cases (n=17)	Multivariate relative risk for 1 SD† (95% confidence interval)
Mean (SD) proportion (%) of linoleic acid	48.5 (5.7)	44.1 (10.7) P < 0.001§	0.67 (0.49 to 0.95)	45.4 (6.6) P < 0.10	0.81 (0.45 to 1.00)	44.4 (6.4) P < 0.02	0.64 (0.43 to 0.95)
Mean (SD) intake (g/day) of polyunsaturated fat	17.4 (11.3)	12.4 (6.8) P < 0.001	0.61 (0.36 to 1.04)	11.9 (5.2) P < 0.001	0.58 (0.29 to 1.13)	15.8 (13.1) P < 0.56	0.92 (0.57 to 1.47)

*Digestive cancer ICD-9 codes include 140-150 and 161; lung cancer: 162.

†Relative risks associated with approximately 1 SD increase in linoleic acid (5%) or polyunsaturated fat intake (10 g/day) calculated by Cox's proportional hazards models adjusted for age, smoking, alcohol consumption, body mass index, and serum cholesterol.

§Based on t test for difference between men with and without cancer.

Key messages

- Increased proportions of linoleic acid and decreased proportions of oleic and palmitoleic acids in cholesterol esters are associated with a reduced risk of premature death from cancer in middle aged French working men
- These results and those obtained from 24 hour recall of dietary intake indicate that intake of polyunsaturated fat, at least, does not increase the risk of death from cancer
- The results might vary with population habits (diet, alcohol intake, etc) and should be studied in other countries

due alternatively to confounding factors, especially smoking habits and alcohol consumption. Previous studies,^{36,37} as well as ours,^{14,15} found several significant associations between proportions of linoleic, palmitoleic, and oleic acid in cholesterol esters and alcohol and tobacco consumption, body mass index, and serum cholesterol concentration. In addition, palmitoleic acid has been proposed as a biological marker of alcohol consumption.¹⁵ It is thus important to take these factors into account and not to interpret the results as exclusively reflecting an association between dietary fat intake and cancer. Despite our consistent results after adjusting for and stratifying by potential risk factors, residual confounding cannot be entirely ruled out. It is also possible that fatty acid values are indirect reflections of other unmeasured aetiological factors. Confounding could arise if eating behaviour is part of an "unhealthy" lifestyle which would include other factors that might promote cancer.³⁸ The association would, for instance, mirror differences in intake of other substances like vitamins.

In this cohort, only 17 deaths (13.7%) were due to cardiovascular diseases. The low mortality from coronary and other cardiovascular diseases in middle aged French people has been a constant feature of national mortality statistics for many years,^{39,40} which does not seem to be explained by coding habits.⁴¹ On the other hand, our population was characterised by a relatively high rate of overall and digestive cancers and a high mean consumption of alcohol. Consequently, the associations observed might also apply to the general French male population.

CONCLUSION

This study suggests that the proportions of fatty acids in cholesterol esters are markers that predict premature death from cancer in middle aged French men and that intake of polyunsaturated fat does not increase the risk of death from cancer. The association of biological markers of dietary fat intake with incidence and mortality from cancer should be investigated prospectively in other populations.

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