The Fatty Acid and Sterol Content of Food Composites of Middle-Aged Men in Seven Countries

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Specific fatty acids and sterols in food composites from seven countries were analyzed. In the 1960s, groups of 8 to 49 men from 16 cohorts, ages 40–59 years and living in the United States, Finland, the Netherlands, Italy, Greece, the former Yugoslavia, or Japan recorded their food intake. In 1987, we collected food composites representing the average food intake per cohort sample in the 1960s. The foods were transported to the Netherlands, pooled, and centrally analyzed for energy, total fat, 42 fatty acids, cholesterol, and four plant sterols. The fat content ranged from 12% of total daily energy in the Tanushimaru, Japan, cohort to 50% in the U.S. cohort sample, and the polyunsaturated to saturated fat ratio ranged from 0.17 in the east Finland cohort to 1.2 in Tanushimaru. The amount of *trans* fatty acids with 16 or 18 carbon atoms varied between 0.2 g/day in Corfu, Greece, and 8.6 g/day in Zutphen, Netherlands, and that of α -linolenic acid between 0.8 g/day in Rome and 2.5 g/day in east Finland. The sum of eicosapentaenoic and docosahexanoic acid ranged from 0.1 (U.S. railroad) to 2.0 g/day (Ushibuka, Japan), and phytosterols from 170 (U.S. railroad) to 358 mg/day (Corfu, Greece). Thus the intake of various fatty acids and sterols with potential relevance for coronary heart disease occurrence varied 10-fold or more between cohorts. Our data can be used to generate new hypotheses about the causes of differences in incidence of diseases between countries. © 1997 Academic Press

INTRODUCTION

Fatty acids and sterols in the diet play a major role in the etiology of coronary heart disease (Willett and Sacks, 1991). Therefore, it is important to know the amounts and types of fatty acids and sterols that are present in the diets of populations, and to know which foods are the main contributors. However, food tables lack reliable data on the content of specific fatty acids, such as *trans* fatty acids (Litin and Sacks, 1993), long-chain polyunsaturated (*n*-3) fatty acids, and plant sterols. As a consequence, the content of these nutrients in diets cannot be calculated from food balance sheets or dietary surveys, using nutrient data bases.

At the end of the 1950s, the Seven Countries Study was designed to study the

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relationships between diet and the occurrence of chronic diseases (Keys, 1980). The food intake was recorded at that time by random samples of men, ages 40–59 years, who were part of a total of 16 cohorts in the United States, Finland, the Netherlands, Greece, Italy, the former Yugoslavia, and Japan. Duplicate portions were collected in 1960, and protein, total fat, and overall fatty acid composition were analyzed. However, a large number of nutrients and other components in the diet which could be important with regard to the occurrence of diseases were not determined. In 1987 we therefore decided to sample equivalent food composites, representing the average reported food intake of the men in the 1960s (Den Hartog *et al.*, 1968; Keys, 1970), and to analyze these chemically for a large number of nutrients and nonnutrients.

One of our aims was to obtain detailed information on the fatty acid and sterol composition of the diets, and we therefore determined the mean content of 42 fatty acids and four sterols in the collected food composites in a central laboratory. We present here the results of these chemical analyses, which can be used to investigate relationships between the composition of diets in the 1960s and the disease outcome in later years.

METHODS

Subjects

Sixteen groups each containing 502 to 2571 men, ages 40–59 years, were selected in the seven countries. Random samples of 8 to 49 men in each cohort (Table 1) recorded their food intake, mostly during 7 days at the baseline survey in the 1960s (Den Hartog *et al.*, 1968; Keys, 1970; Kromhout *et al.*, 1989).

The U.S. participants were railroad employees. One Finnish cohort lived in east Finland, and the other in west Finland. In the Netherlands men living in the commercial town of Zutphen, in the eastern part of the country, participated. Italy contributed three cohorts: Montegiorgio and Crevalcore, both rural villages, and railroad personnel in Rome. Greece contributed two: one on Crete and one on the island of Corfu. Five cohorts were from the former Yugoslavia: two from Croatia included inhabitants of six towns on the Dalmatian Coast and of Dalj, a small town in Slavonia. The other three cohorts consisted of farmers from the village of Velika-Krsna, workers in a large cooperation in Zrenjanin, and university professors in Belgrade in Serbia. Japan had two cohorts: farmers from the village of Tanushimaru and fishermen from the village of Ushibuka.

Food Records

Between 1959 and 1964 samples of participants from 13 cohorts recorded and weighed all they ate and drank for 7 days and a sample of U.S. railroad employees did the same for 1 day. The participants from Rome recorded their food intake in 1969 for 7 days and those from Ushibuka in 1971 for 4 days. Thirteen cohorts reported the foods as weight of edible parts of raw products, and 3 cohorts as prepared foods. The 2 Greek cohorts recorded their intakes in different seasons. As the original 7-day records of the Greek cohorts were no longer available, we reconstructed the diets using a publication of Keys (1970), and information provided by the Greek dietician involved in the original study. We derived missing information on some foods, espe-

TABLE 1

CHARACTERISTICS OF FOOD RECORDS REPORTED BY MEN AGES 40 TO 59 YEARS
IN 16 COHORTS IN SEVEN COUNTRIES IN THE 1960s

Cohort	Country	Year	Number of subjects	Number of recording days
US railroad	United States	1960-62	30	1
East-Finland ^a	Finland	1959	30	7
West-Finland ^a	Finland	1959	30	7
Zutphen	Netherlands	1960	45	7
Crevalcore ^a	Italy	1960	29	7
Montegiorgio ^a	Italy	1960	35	7
Rome railroad	Italy	1969	49	7
Corfu ^{ab}	Greece	1961-63	37	7
Crete ^{ab}	Greece	1960-65	31	7
Dalmatia	Croatia	1960	24	7
Slavonia ^a	Croatia	1960	24	7
Belgrade ^a	Serbia	1964	41	7
Velika-Krsna ^a	Serbia	1963	21	7
Zrenjanin ^a	Serbia	1963	40	7
Tanushimaru ^a	Japan	1964	24	7
Ushibuka ^a	Japan	1971	8	4

Note. The full cohorts contained 502 to 2571 men, of whom a random sample or 8–49 recorded their food intake.

^a Food recorded as amounts of raw products.

^b Original records no longer available.

cially fruits and vegetables, from food balance sheets of Greece from the period 1961–1965 (FAO, 1984).

Food Composites

In 1986, the food intake data were recoded in a standardized way by one dietician. We converted amounts of prepared into amounts of raw foods, taking into account

TABLE 2

TYPES OF MEAT INCLUDING SUBSTITUTIONS MADE FOR THE TYPES OF MEAT CONSUMED IN SMALL AMOUNTS OR NOT AVAILABLE LOCALLY,
SAMPLED FOR FOOD COMPOSITES OF 16 COHORTS IN SEVEN COUNTRIES

Types of meat per cohort	Amount g/d	Including	Types of meat per cohort	Amount g/d	Including	Types of meat	Amount g/d	Including
US cohort			Zutphen			Rome		
pork ^a	43	minced pork	beef liver	2	kidney, pork liver	beef liver	2	
fat pork	15	fat bacon x 2.5	minced beef	21	fat beef	mutton	6	
fat beef	9		pork ^a	30		hambone	5	
lean beef	17		fat bacon	13	lean bacon	beef ^a	147	fat veal
chicken	46	turkey, pheasant	lean beef	35		sausage	14	salami
beef ^a	122		uncooked ham	5	frankfurter, sliced meat	chicken	44	
frankfurter	5	luncheonmeat	smoke-dried beef	2		rabbit	21	horse mat
ham	15	bacon	luncheon-meat	4	liver sausage, bacon, pâté	tripe	3	other intestines
salami	2		saveloy	4	smoked sausage	lamb	8	
			bacon	1		veal	21	cooked veal
East-Finland								
Beef liver	4		Crevalcore			lean beef	5	
lean beef	19	veal ^a	veal liver	2	chicken liver	duck	4	
fat mutton	14	fat pork	pork	9	sausage, drippings	gammon	11	
lean mutton	10		beef	29		mortadella	5	bacon
beef ^a	11		rabbit	8		Corfu		
fat beef	7	canned beef	duck	2		beef	17	
pork ^a	10		chicken	36	pigeon	chicken	18	
fat pork	11		veal	30		Crete		
lean pork	6		horse meat	12	lean pork	mutton	14	
teemakkara	20		salami	9		goat	7	
canned pork	3	bacon (x1.8)	mortadella	8		chicken	14	
smoked ham	2		bacon	6		Balgrade		
			gammon	5		pork liver	4	
West-Finland						drippings	6	
beef liver	1		Montegiorgio			sausage	22	smoked meat
lean beef ^a	17		veal liver	1	pork ^a	pork ^a	40	turkey, goose
pork ^a	10		sausage	15	duck	chicken	14	lamb ^a
fat pork	13	canned pork	goose	5	mutton	veal ^a	7	
lean pork	5	fat beef	beef ^a	4	pigeon	beef ^a	46	pork intestines
teemakkara	24	berlini-makkara	chicken	12	lean veal	pork brains	3	
suomimakkara	8		veal ^a	17		bacon	10	uncooked ham, liver
ryynimakkara	6		rabbit	6	other intestines	smoked ham	4	frankfurter
salted pork	6	saveloy	tripe	6	bacon, canned meat, ham	salami	11	gammon
smoked ham	1		salami	9		canned ham	7	
			mortadella	3				

TABLE 2—Continued

Types of meat per cohort	Amount g/d	Including	Types of meat per cohort	Amount g/d	Including	Types of meat	Amount g/d	Including
Dalmatia			Velika-Krsna			Tanushinari		
lamb liver	2		roasted piglet	22	pork*, smoked meat	beef liver	2	
smoked pork	20	sausage, hot-dog	roasted mutton	5	smoked meat	fat pork	3	fat beef, 25% ham
smoked mutton	5		chicken	2	pigeon	chicken	3	75% ham
lamb ^a	27		lamb ^a	3	roasted lamb	Ushibuka		
beef ^a	28	smoked bacon	smoked bacon	38		fat pork	3	
fileti	6		Zrenjanin			chicken	5	
bacon	9	chicken	pork liver	2	duck and chicken liver			
veal	20	salami, gammon	pork ^a	47	fat pork, 50% mutton			
			duck	14	50% mutton			
Slavonia			sausage	25	sausage, drippings (x2)			
pork liver	3	beef, veal liver	chicken	38	pigeon			
pork ^a	59	sausage	veal ^a	8				
drippings	7		intestines	3	pork brains			
chicken	24	pigeon	beef ^a	10	rabbit, smoked beef			
veal ^a	4		smoked bacon	36				
lamb ^a	10		smoked ham	5	gammon			
beef ^a	16		salami	5				
salami	3		bacon	15	frankfurter			
bacon	58	blood pudding						
gammon	4							

^a Average fat content.

loss of water and loss and gain of fat during cooking. For each cohort sample, we averaged the daily consumption of each food across persons and collected this amount.

Two Dutch dietitians assisted by local researchers and co-workers from the Seven Countries Study bought all foods between April and October of 1987 in the same regions in which the men were living during the baseline survey in the 1960s. In 10 of the 16 locations they bought the duplicates in the same season (± 2 months) during which the food intakes had been recorded. For the United States, Belgrade, Zrenjanin, and Tanushimaru the purchasing of the foods took place in a different season.

The fat compositions of foods have changed between the 1960s and 1987 (FAO, 1994), and therefore we had to select the food items carefully to approximate the composition of the foods as consumed by the men in the 1960s as near as possible. For this purpose, we used food composition tables and data from the literature (Den Hartog *et al.*, 1968). The major food groups contributing to the fat composition of the diets were meat, dairy products, edible fats, and pastries. For meat, we often chose the fatter variants (Table 2). We reduced the quantity of products to be bought by combining foods. We substituted foods consumed in amounts of less than 10 g per day by foods consumed in higher amounts, with a similar nutrient composition. In Table 2, we show the substitutions made for meat. If a food was no longer available locally, we also used a substitute with a comparable composition. Detailed information on the food sampling has been described in a Dutch report (Jansen *et al.*, 1989). The maximum number of different foods bought per cohort was 80. The dietitians vacuum-packed all foods in plastic bags and sent them to the Netherlands in cooled boxes by air express.

The average period between acquiring the foods and their first workup in the laboratory was 2 days (range 1–4 days). Inedible parts were removed so as to produce the edible part as recorded and consumed in the 1960s. For example, we only peeled apples if that is how they were consumed originally. Thereafter, the foods were weighed out and homogenized at the Department of Human Nutrition, Agricultural University Wageningen. Foods were divided into subsamples before homogenizing. Homogeneous foods present in relatively big amounts were blended together into a main sample after addition of t-BHQ (500 mg/kg). Nonhomogeneous foods or products present in small amounts were blended into a subsample first. The sizes of these subsamples were scaled up 5–50 times from the original amount. After that a proportional amount of the subsample was added separately to a part of the main sample. Foods containing vitamin C were also blended in separate subsamples containing 2% oxalic acid. If a sample was too dry to homogenize, a known amount of demineralized water was added. After homogenization, the final combined samples were stored at -20°C .

Chemical Analyses

We determined the amount of total fat according to Folch *et al.* (1957). The fatty acids were assayed after saponification of the Folch extract and derivatization to methyl esters (Metcalf *et al.*, 1966). Fatty acids were analyzed by three different methods, on a Chrompack 438AS gas chromatograph equipped with a flame ionization detector. For determination of C8, C10, C12, C14, C14:1, C15, C16, C16:1, C17, C17:1, C18, C18:1, C18:2, C18:3(*n*-6), C18:3(*n*-3), C20, C20:1, and C20:2, the column was a Durabond-225 fused silica, length $\times$ internal diameter 15 m $\times$ 0.54 mm. The

flow rate of the carrier gas hydrogen was 7.5 ml/min. The oven temperature was programmed from 100 to 180°C at a rate of 10°C/min, then to 225°C at 4°C/min, which was maintained for a further 2.75 min before cooling. The injector temperature was 220°C, and 0.5 μ l was injected directly into a modified liner. The detector temperature was 250°C, the hydrogen flow for the detector was reduced to 17.5 ml/min, and the flow rate of the make-up gas nitrogen was 25 ml/min.

Trans fatty acids were determined by gas chromatography in our laboratory, and these results were checked by gas chromatography and Fourier transform infrared spectrometry in the research laboratory of the Raisio group at Raisio, Finland. The *trans* fatty acid content of the food composite of Zutphen could not be analyzed in Finland due to solvent in the sample, and therefore it was reanalyzed by Fourier transform infrared spectrometry in our own laboratory together with the food composites of east and west Finland and Velika-Krsna.

The geometric and positional isomers of C16:1, C18:1, and C18:2 fatty acid methyl esters were measured using a CP-Sil88 fused silica 50-m $\times$ 0.25-mm column (Chrompack, the Netherlands). The oven temperature was programmed from 160 to 220°C at a rate of 2°C/min plus 5 min at 220°C. The carrier gas was hydrogen, and the inlet pressure was 130 kPa. The injector was a splitter with a split ratio of 1:60, and a volume of 0.5 μ l was injected at a temperature of 250°C. Fourier transform infrared spectrometry in Wageningen was done using a Bio-Rad Digilab Division, Type FTS-7 infrared spectrophotometer. The preparation of fatty acid methyl esters for gas chromatography in Finland was done according to Bannon *et al.* (1982), and for the infrared spectrometry method according to IUPAC 2.301. Duplicate esters of the samples were analyzed as neat solutions according to Sleeter and Matlock (1989). For gas chromatography in Finland a Hewlett–Packard 5890 II was used, and the IR spectrophotometer was a Perkin–Elmer 16PC, Type FTIR.

The fatty acids C18:4(*n*-3), C22, C22:1(*n*-9), C20:3(*n*-3), C20:4(*n*-6), C24, C20:5(*n*-3), C24:1, C22:3(*n*-3), C22:4(*n*-6), C22:5(*n*-3), and C22:6(*n*-3) were assessed separately in a third analysis, again using a CP-Sil88 fused silica 50-m $\times$ 0.25-mm column. The initial oven temperature was 160°C for 4 min, followed by a rise of 25°C/min to 215°C, which was kept for a further 12 min before cooling down. The injector was a Splitter with a split ratio of 1:15, and the injected volume was 1.5 μ l. Injector and detector temperature were 250°C.

We extracted the unsaponifiable part of the lipid (Association of Official Analytical Chemists, 1984) and determined cholesterol, campesterol, stigmasterol, and β -sitosterol by gas chromatography using a Chrompack–Packard Model 439 column CP-Sil119/cb (25 m $\times$ 0.25 mm). The oven temperature was programmed from 240 to 265°C at a rate of 1.25°C/min, then to 285°C by 5°C/min, which was maintained for a further 8 min before cooling down. The carrier gas hydrogen had an inlet pressure of 105 kPa. The injector was a splitter, and the injected volume 1 μ l with a split ratio of 1:30. The temperature of injection was 300°C and of detection 305°C. The flow rate of the make-up gas nitrogen was 25 ml/min. We determined response factors by injection of pure compounds with 5 α -cholestane as internal standard. For campesterol we used the factor for cholesterol. Chromatograms for fatty acids and for sterols were recorded by a personal computer equipped with PCI-GC software Version 4.0 (Chrompack).

We tested the recovery of *n*-3 fatty acids by adding herring of known EPA and

DHA content to 1766 g of the food composite of Zutphen. Recovery was $106 \pm 5\%$ for C20:5 and $98 \pm 6\%$ for C22:6 after addition of 11 g, and $93 \pm 5\%$ for C20:5 and $88 \pm 4\%$ for C22:6 after addition of 25 g of herring.

The conversion factor used for fat to fatty acids was 0.956. We determined protein (Association of Official Analytical Chemists, 1984) and alcohol (Anonymous, 1987) and calculated carbohydrates as the sum of analyzed values of galactose, glucose, saccharose, lactose, maltose, and starch. Atwater factors for the calculation of energy were 17 kJ (4 kcal) for protein, 37 kJ (9 kcal) for fat, 16 kJ (3.75 kcal) for carbohydrates, and 29 kJ (7 kcal) for alcohol.

Calculation by Use of Food Composition Tables

We compared the analyzed values for the food composites from the United States, Finland, the Netherlands, and Italy with values calculated using food composition tables. We used the Dutch computerized nutrient data bank (Kommissie UCV, 1985) for foods with a nutrient composition similar to that of Dutch foods and for foods containing no fat or sterols, and the food composition table of the specific countries (Rastas *et al.*, 1989; Carnovale and Miuccio, 1989; U.S. Department of Agriculture, 1992) for the remainder.

RESULTS

Energy and Total Fat

The energy content varied from 9.6 MJ/day in the diets of the U.S. railroad employees to 15.8 MJ/day in the Croatian cohort Dalmatia, and the fat content in the food composites from 12% of total energy in the Japanese farmers in Tanushimaru to 50% in the United States (Table 3). The ratio of polyunsaturated to saturated fatty acids varied from 0.2 for east and west Finland to 0.9 and 1.2 for Ushibuka and Tanushimaru in Japan, respectively (Table 3).

Saturated Fatty Acids

The saturated fatty acid content was low ($<10\%$ of energy) in the food composites of Greece and Japan, the Italian cohorts Montegiorgio and Rome, and the Croatian cohort Dalmatia. We found high levels of saturated fatty acids ($>15\%$ of energy) for east and west Finland, the Netherlands, the United States, the Serbian cohorts Belgrade and Zrenjanin, and the Croatian cohort Slavonia.

The levels of the medium-chain fatty acids caprylic (C8:0) and capric (C10:0) acid were high in the Finnish samples (Table 4). The contribution of lauric (12:0), myristic (14:0), and palmitic (16:0) acid varied from 65% of saturated fatty acids for Zrenjanin in Serbia to 79% for Corfu and Crete (Table 4). Stearic acid content ranged from 20 (Tanushimaru, Japan) to 32% (Zrenjanin, Serbia) of saturated fatty acids. The highest amount of saturated fatty acids of more than 20 carbon atoms was 2.5 g/day in Zutphen (Table 4).

Monounsaturated Fatty Acids

The range of monounsaturated fatty acids varied from 9.8 g/day in Tanushimaru, Japan, to 84 g/day on the Greek isle of Crete. The levels of monounsaturated fat in the food composites of Greece exceeded 20% of energy.

TABLE 3

TOTAL ENERGY AND FAT CONTENT AND MAJOR CLASSES OF FATTY ACIDS, DETERMINED BY CHEMICAL ANALYSIS OF FOOD COMPOSITES REFLECTING THE AVERAGE RECORDED FOOD INTAKE OF SAMPLES OF MIDDLE-AGED MEN LIVING IN 16 COHORTS IN SEVEN COUNTRIES IN THE 1960s^a

Cohort	Energy MJ/d	Total fat		SFA	MUFA	PUFA	P/S
		g/d	% of energy				
US railroad, USA	9.6	131	50	55	49	21	0.4
East-Finland	14.4	166	42	89	55	15	0.2
West-Finland	14.7	142	36	73	49	14	0.2
Zuiphen, Netherlands	11.3	137	45	61	50	20	0.3
Crevalcore, Italy	15.2	155	38	54	73	21	0.4
Montegiorgio, Italy	12.1	101	31	31	50	15	0.5
Rome railroad, Italy	11.0	96	32	28	54	10	0.4
Corfu, Greece	11.3	105	34	22	63	15	0.7
Crete, Greece	11.8	131	41	28	84	13	0.5
Dalmatia, Croatian	15.8	148	35	40	78	24	0.6
Slavonia, Croatian	15.5	179	43	69	81	22	0.3
Belgrade, Serbia	11.4	143	46	57	54	26	0.4
Velika-Krsna, Serbia	14.3	115	30	52	42	16	0.3
Zrenjanin, Serbia	13.4	149	41	55	65	23	0.4
Tanushimaru, Japan	10.0	33	12	10	10	12	1.2
Ushibuka, Japan	10.2	46	17	14	17	13	0.9

^a SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P/S ratio is the ratio of polyunsaturated to saturated fatty acids.

TABLE 4

SATURATED FATTY ACIDS IN FOOD COMPOSITES FROM 16 COHORTS OF MIDDLE-AGED MEN IN SEVEN COUNTRIES,
REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987

Cohort	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
	<i>grams per day</i>										
US railroad, USA	0.5	1.3	1.8	5.8	0.7	30	0.7	14	0.3	0.3	0.1
East-Finland	1.3	3.3	4.3	14	1.6	41	0.9	22	0.4	0.3	0.1
West-Finland	0.4	2.1	3.4	11	1.1	35	0.7	18	0.3	0.4	0.2
Zutphen, Netherlands	0.6	1.3	2.2	8.0	0.8	30	0.6	15	1.0	1.1	0.5
Crevalcore, Italy	0.5	0.8	1.1	4.2	0.5	31	0.5	15	0.5	0.4	0.2
Montegiorgio, Italy	0.1	0.3	0.3	1.3	0.1	19	0.3	9.4	0.3	0.2	0.1
Rome railroad, Italy	0.2	0.4	0.7	2.4	0.3	17	0.3	6.2	0.3	0.2	0.1
Corfu, Greece	0.2	0.4	0.3	1.1	0.2	16	0.1	3.1	0.4	0.2	0.1
Crete, Greece	0.3	0.7	0.5	1.8	0.3	18	0.2	5.6	0.6	0.3	0.1
Dalmatia, Croatian	0.3	0.5	0.5	2.5	0.3	24	0.4	10	0.5	0.3	0.2
Slavonia, Croatian	0.2	0.5	0.6	3.7	0.5	42	0.5	20	0.2	0.2	0.1
Belgrade, Serbia	0.7	1.2	1.3	5.6	0.8	31	0.7	15	0.4	0.4	0.2
Velika-Krsna, Serbia	0.9	1.3	1.5	5.8	0.8	29	0.5	12	0.1	0.2	0.1
Zrenjanin, Serbia	0.1	0.3	0.4	2.6	0.2	33	0.5	17	0.4	0.2	0.1
Tanushimaru, Japan	0.1	0.0	0.1	0.8	0.1	6.5	0.1	2.0	0.1	0.1	0.1
Ushibuka, Japan	0.0	0.0	0.2	1.0	0.1	9.2	0.2	3.0	0.1	0.1	0.1

TABLE 5

cis-MONOUNSATURATED FATTY ACIDS IN FOOD COMPOSITES FROM 16 COHORTS OF MIDDLE-AGED MEN IN SEVEN COUNTRIES, REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987

Cohort	C14:1	C16:1	(n-7)cis		C17:1	C18:1 ^a		C20:1	C22:1	C24:1	
			total	(n-7)cis		total	(n-9)cis				
			grams per day								
US railroad, USA	0.8	3.0	3.0	3.0	0.4	44	37	2.6	0.4	0.1	0.0
East-Finland	1.3	3.4	3.0	3.0	0.0	49	42	2.4	0.6	0.2	0.1
West-Finland	1.1	2.8	2.5	0.3	0.3	44	38	2.4	0.7	0.3	0.0
Zurphen, Netherlands	0.6	4.3	1.7	0.3	0.3	37	29	2.2	4.3	2.9	0.2
Crevacore, Italy	0.4	2.7	2.3	0.0	0.0	68	62	4.6	1.0	0.3	0.0
Montegiorgio, Italy	0.2	1.7	1.4	0.0	0.0	47	42	3.3	0.9	0.4	0.0
Rome railroad, Italy	0.4	1.6	1.4	0.2	0.2	51	47	2.8	0.8	0.3	0.0
Corfu, Greece	0.1	1.4	1.2	0.1	0.1	61	56	4.9	0.3	0.1	0.0
Crete, Greece	0.2	1.3	1.1	0.2	0.2	82	77	4.7	0.4	0.1	0.0
Dalmatia, Croatian	0.3	2.8	1.9	0.2	0.2	73	67	4.6	1.3	0.2	0.1
Slavonia, Croatian	0.4	5.3	4.1	0.0	0.0	73	64	7.0	2.0	0.3	0.1
Belgrade, Serbia	0.6	3.0	2.8	0.4	0.4	49	43	4.0	1.1	0.1	0.0
Velika-Krsna, Serbia	0.6	2.6	2.2	0.2	0.2	38	33	2.8	0.7	0.3	0.0
Zrenjanin, Serbia	0.3	3.8	3.0	0.4	0.4	59	52	5.5	1.4	0.3	0.0
Tanushimaru, Japan	0.1	0.8	0.6	0.0	0.0	8.0	6.9	0.9	0.0	0.8	0.2
Ushibuka, Japan	0.2	1.6	1.3	0.0	0.0	15	13	1.8	0.0	0.3	0.1

^a No C18:1(*n*-12)*cis*.

Sixty to ninety percent of monounsaturated fat (Table 5) in the food composites occurred as oleic acid [*cis* C18:1(*n*-9)]. The absolute amount varied from 8.0 g/day in Tanushimaru, Japan, cohort to 82 g/day in Crete. The second major monounsaturated fatty acid was palmitoleic acid [*cis* C16:1(*n*-7)] with a highest consumption of about 4 g/day in Slavonia, Croatia. The maximum intake of both erucic [C22:1(*n*-9)] and cetoleic acid [C22:1(*n*-11)] was 2.9 g/day in Zutphen (Table 5).

Total intake of very long chain fatty acids (saturated and unsaturated fatty acids with more than 20 carbon atoms) was about 14 g/day in Zutphen and 2–5 g/day in the other cohorts (Tables 4, 5, and 6).

Trans Fatty Acids

The amount of *trans* fatty acids with 16 and 18 carbon atoms as determined by gas chromatography in Wageningen varied from 0.2 g or 0.1% of energy in Corfu, Greece, to 8.6 g or 2.8% of energy in Zutphen, the Netherlands (Table 6). The highest levels, absolute as well as relative to energy intake, were found in the food composites of the Dutch, U.S., and Finnish cohorts, and the lowest amounts in those of Greece, Italy, and Japan. The main contribution to *trans* fatty acids was formed by *trans* isomers of C18:1 (Fig. 1). The food composite of Zutphen contained the highest amount of *trans* isomers of palmitoleic acid (16:1), while *trans* 16:1 was not present in the food composites of Italy, Greece, Croatia, and Zrenjanin, Serbia. The values measured in Finland by gas chromatography were on average 0.4 ± 0.9 g/day lower than in Wageningen. The value for Zutphen, Netherlands, was 3.5 g/day higher while differences for other cohorts were less than 1 g/day. The total amount of *trans* fatty acids as assessed by infrared spectrometry was in general higher than the amounts of 16:1, 18:1, and 18:2 *trans* fatty acids assessed by gas chromatography (Fig. 2). We found a large difference between the methods for the Zutphen cohort.

Polyunsaturated Fatty Acids

The polyunsaturated fatty acid fraction (Table 7) consisted of 54 to 85% of linoleic acid [18:2(*n*-6)]. The intake of linoleic acid was high in the Serbian cohorts of Zrenjanin and Belgrade and in the United States, and low in Finland. As for the other (*n*-6) polyunsaturates the amount of γ -linolenic acid [18:3(*n*-6)] was at the most 0.29 g, and arachidonic acid [C20:4(*n*-6)] 0.5 g per day. The content of the major *n*-3 (ω -3) polyunsaturated fatty acid, α -linolenic acid, varied from 0.79 g in the diet of Rome railroad workers to 2.5 g per day in east Finland. The food composites of Japan, Dalmatia, east Finland, and Corfu contained the highest and those of the United States, Rome, and Velika-Krsna the lowest levels of eicosapentaenoic [EPA; C20:5(*n*-3)] and docosahexanoic acid [DHA; C22:6(*n*-3); see Fig. 3]. The ratio of linoleic to α -linolenic acid ranged from 3.2 in east Finland to 15 in Velika-Krsna (Table 7).

The (*n*-3) fatty acids have critical roles in membrane structure and are precursors of eicosanoids in the body. A number of studies have shown that the consumption of foods containing EPA and DHA is associated with decreased risk of coronary heart disease (Katan, 1995). α -Linolenic acid is elongated in the body to EPA and DHA, and the efficiency of this conversion is estimated to be about 20% (Emken

TABLE 6

C16 AND C18 *trans* FATTY ACIDS, AS DETERMINED BY GAS CHROMATOGRAPHY, AND TOTAL *trans* FATTY ACIDS AS DETERMINED BY INFRARED SPECTROMETRY IN FOOD COMPOSITES FROM 16 COHORTS OF MIDDLE-AGED MEN IN SEVEN COUNTRIES, REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987

Cohort	C16:1 (<i>n-7</i>) <i>trans</i>	C18:1(<i>n-9</i>) to C18:1(<i>n-12</i>) <i>trans</i>	C18:1 (<i>n-7</i>) <i>trans</i>	C18:2 <i>trans,trans</i>	C18:2 <i>trans,cis</i>	C18:2 <i>cis,trans</i>	Sum of <i>trans</i> - C16:1,C18:1, and C18:2	Total <i>trans</i> fatty acids ^a (infrared)
				<i>gram/day</i>				
US railroad, USA	0.3	3.9	0.0	0.3	0.2	0.4	5.0	6.0
East-Finland	0.8	4.9	0.0	0.6	0.4	0.4	7.1	9.0 ^b
West-Finland	0.6	3.6	0.0	0.2	0.0	0.1	4.5	7.6 ^b
Zutphen, Netherlands	2.6	2.4	3.1	0.0	0.3	0.3	8.6	26
Crevallcore, Italy	0.0	1.1	0.0	0.0	0.0	0.0	1.1	1.8
Montegiorgio, Italy	0.0	0.1	0.3	0.0	0.0	0.1	0.6	1.1
Rome railroad, Italy	0.0	0.7	0.0	0.0	0.0	0.0	0.7	1.6
Corfu, Greece	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.3
Crete, Greece	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.6
Dalmatia, Croatian	0.0	1.1	0.0	0.0	0.0	0.0	1.1	2.3
Slavonia, Croatian	0.0	1.2	0.0	0.0	0.0	0.0	1.2	2.1
Belgrade, Serbia	0.3	0.0	2.0	0.0	0.0	0.0	2.3	3.0
Velika-Krsna, Serbia	0.1	2.2	0.0	0.0	0.0	0.0	2.3	3.8 ^b
Zrenjanin, Serbia	0.0	1.3	0.0	0.0	0.0	0.0	1.3	1.4
Tanushimaru, Japan	0.1	0.0	0.1	0.0	0.0	0.0	0.2	0.4
Ushibuka, Japan	0.0	0.0	0.3	0.0	0.0	0.0	0.3	0.4

^a Determined in the research laboratory of the RAISIO group, Raisio, Finland; value for Zutphen determined in the Wageningen laboratory.

^b Average value of determinations in Raisio, Finland, and in Wageningen.

FIG. 1. The amount of C16:1, C18:1, and C18:2 *trans* fatty acids according to gas chromatography in food composites from 16 cohorts of middle-aged men in seven countries, representing the average food intake in the 1960s, sampled in 1987.

et al., 1992). Therefore, 1 g of dietary α -linolenic acid is not equivalent physiologically to 1 g of EPA or DHA but to much less. In order to allow comparison of the total intake of (*n*-3) fatty acids across cohorts independent of source we multiplied α -linolenic acid intake by 0.2 and combined these values with intakes of EPA and

FIG. 2. Sum of C16:1, C18:1, and C18:2 *trans* fatty acids according to gas chromatography in Wageningen and Raisio, Finland, and total *trans* fatty acids including C20 and C22 isomers according to infrared spectrometry in Raisio, Finland, in food composites from 16 cohorts and according to infrared spectrometry in Wageningen from 4 cohorts of middle-aged men in seven countries, representing the average food intake in the 1960s, sampled in 1987.

TABLE 7

POLYUNSATURATED FATTY ACIDS IN FOOD COMPOSITES FROM 16 COHORTS OF MIDDLE-AGED MEN IN SEVEN COUNTRIES,
REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987

Cohort	C18:3 (n-3)	C18:4 (n-3)	C20:3 (n-3)	C20:5 (n-3)	C22:3 (n-3)	C22:5 (n-3)	C22:6 (n-3)	C18:2 (n-6)	C18:3 (n-6)	C20:4 (n-6)	C22:4 (n-6)	C20:2	C22:2	C18:2(n-6)/ C18:3(n-3)
	<i>grams per day</i>													<i>ratio</i>
US railroad, USA	2.1	0.3	0.0	0.1	0.0	0.1	0.0	17	0.2	0.4	0.1	0.0	0.0	8.3
East-Finland	2.5	0.6	0.1	0.5	0.0	0.2	0.4	8.0	0.3	0.4	0.1	0.0	0.0	3.2
West-Finland	1.7	0.6	0.1	0.2	0.0	0.1	0.2	8.7	0.1	0.4	0.0	0.0	0.0	5.0
Zutphen, Netherlands	2.2	0.2	0.0	0.3	0.1	0.1	0.1	12	0.1	0.5	0.1	1.5	1.2	5.8
Crevalcore, Italy	1.2	0.6	0.1	0.1	0.0	0.1	0.2	17	0.0	0.4	0.1	0.4	0.0	13
Montegiorgio, Italy	1.0	0.0	0.1	0.1	0.2	0.1	0.2	12	0.0	0.2	0.1	0.6	0.0	12
Rome railroad, Italy	0.8	0.0	0.0	0.1	0.1	0.1	0.1	8.1	0.0	0.3	0.0	0.2	0.0	10
Corfu, Greece	1.1	0.1	0.0	0.2	0.0	0.0	0.6	13	0.0	0.1	0.0	0.0	0.0	12
Crete, Greece	1.2	0.0	0.0	0.1	0.0	0.0	0.2	11	0.0	0.2	0.0	0.0	0.0	9.2
Dalmatia, Croatian	1.6	0.9	0.1	0.6	0.1	0.2	1.1	17	0.0	0.3	0.1	0.0	0.0	10
Slavonia, Croatian	1.4	1.5	0.2	0.2	0.2	0.2	0.3	16	0.0	0.5	0.1	0.0	0.0	12
Belgrade, Serbia	2.0	0.6	0.1	0.1	0.1	0.1	0.2	22	0.1	0.3	0.1	0.0	0.0	11
Velika-Krsna, Serbia	1.1	0.7	0.1	0.1	0.0	0.1	0.1	13	0.0	0.3	0.1	0.0	0.0	11
Zrenjanin, Serbia	1.3	0.7	0.1	0.1	0.0	0.2	0.2	19	0.0	0.4	0.1	0.0	0.0	15
Tanushimaru, Japan	1.0	1.0	0.3	0.5	0.2	0.1	0.7	8.0	0.0	0.1	0.0	0.0	0.0	8.4
Ushibuka, Japan	1.3	0.8	0.3	0.8	0.1	0.3	1.1	8.2	0.1	0.1	0.1	0.0	0.0	6.3

FIG. 3. The amount of the major (n -3) fatty acids in food composites from 16 cohorts of middle-aged men in seven countries, representing the average food intake in the 1960s, sampled in 1987, including 20% of the intake of C18:3(n -3), as a measure for C18:3(n -3) converted into longer (n -3) fatty acids in the body. Values for C18:3 were divided by 5 because only about one-fifth of dietary C18:3 is elongated to EPA or DHA (Emken *et al.*, 1992).

DHA (Fig. 3). The total intake of (n -3) fatty acids calculated in this way was highest in Ushibuka, Dalmatia, Tanushimaru, and east Finland (Fig. 3).

Sterols

The amount of cholesterol varied from 170 mg per day in Tanushimaru to 612 mg in Slavonia (Table 8). Intakes of plant sterols were lowest in the United States and highest in Greece. The amounts of campesterol varied from 41 (Slavonia) to 63 mg per day (east Finland), of stigmasterol from 9 (Finland and Rome) to 30 mg per day (Tanushimaru), and of sitosterol from 113 (U.S. railroad) to 281 mg per day (Corfu).

Sources of Visible Fats

The visible fats as reported in the food records (Table 9) contributed from 9.7 to 72% of the total amount of fat. The main source of visible fats in the U.S. and Finnish samples in the 1960s was butter. In the Dutch sample (Zutphen), margarine was more important than butter. Lard consumption was highest in the villages from Italy and the former Yugoslavia. The only visible fat eaten in the Greek cohorts was olive oil which was also an important fat source in Montegiorgio, Rome, and Dalmatia. In Belgrade the major visible fat was sunflower oil. The Japanese used small amounts of rapeseed (Tanushimaru) or peanut oil (Ushibuka).

Comparison with Food Composition Tables

The largest difference between the calculated and the chemically determined amounts of total fat was 9% of energy or 40 g per day for Crevalcore (Table 10). For

TABLE 8

CHOLESTEROL AND PLANT STEROLS IN FOOD COMPOSITES FROM 16 COHORTS OF MIDDLE-AGED MEN IN SEVEN COUNTRIES, REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987

Cohort	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Sum of phytosterols	Sum of unknown compounds ^a
<i>mg per day</i>						
US railroad, USA	582	39	19	113	170	33
East-Finland	537	63	9	159	232	122
West-Finland	492	60	9	149	218	96
Zutphen, Netherlands	476	60	18	146	224	80
Crevalcore, Italy	585	36	12	172	220	93
Montegiorgio, Italy	356	36	16	166	218	108
Rome railroad, Italy	409	33	9	136	179	91
Corfu, Greece	141	60	17	281	358	279
Crete, Greece	211	57	16	252	325	261
Dalmatia, Croatian	388	44	12	215	272	131
Slavonia, Croatian	612	41	24	151	215	89
Belgrade, Serbia	396	59	14	181	254	68
Velika-Krsna, Serbia	359	62	12	181	255	73
Zrenjanin, Serbia	311	54	15	172	242	85
Tanushimaru, Japan	170	44	30	134	208	71
Ushibuka, Japan	403	60	24	137	221	80

^a Refers to all peaks in the nonsaponifiable lipid extract that eluted after the cholesterol peak on gas chromatography.

TABLE 9

TYPE AND AMOUNT OF VISIBLE FATS REPORTED IN FOOD RECORDS IN THE 1960s
BY MIDDLE-AGED MEN LIVING IN 16 COHORTS IN SEVEN COUNTRIES

Cohort	Butter	Margarine	Lard	Olive oil	Sunflower oil	Other
<i>grams per day</i>						
US railroad, USA	26	3.9				3.1 ^a
East-Finland	89	7.7				
West-Finland	66	6.8				
Zutphen, Netherlands	21	56				1.9 ^b
Crevalcore, Italy	13	6.5	32	4.3	2.9	
Montegiorgio, Italy			40	40		
Rome railroad, Italy	3.6		1.8	43		
Corfu, Greece				75		
Crete, Greece				95		
Dalmatia, Croatia			16	72		
Slavonia, Croatia			62	7.8		
Belgrade, Serbia	18		9.0		28	
Velika-Krsna, Serbia			24		5.2	
Zrenjanin, Serbia			44		12	
Tanushimaru, Japan						3.2 ^c
Ushibuka, Japan						7.3 ^b

^a Soy oil.

^b Peanut oil.

^c Rapeseed oil.

the other cohorts the difference varied from 0.1 to 3% of energy. The largest difference for saturated fat was 3.5%, for monounsaturated fatty acids 4%, and for polyunsaturated fat 1.6% of energy. The difference between calculated and chemically determined cholesterol was at the most 6 mg/MJ or 21 mg/day (Table 10).

DISCUSSION

We found large differences in the amounts of total fat, fatty acids, and sterols between the seven countries and between cohorts in the same country. The difference

TABLE 10

COMPARISON OF THE ENERGY AND NUTRIENT CONTENT OF FOOD COMPOSITES REPRESENTING THE AVERAGE FOOD INTAKE IN THE 1960s, SAMPLED IN 1987 OF THE U.S., FINNISH, DUTCH, AND ITALIAN SAMPLES, ACCORDING TO CALCULATION FROM FOOD TABLES (CALC.) AND TO CHEMICAL ANALYSIS (ANAL.)

Cohort	Energy		Fat		Saturated fatty acids		Monounsaturated fatty acids		Polyunsaturated fatty acids		Cholesterol	
	Calc.	Anal.	Calc.	Anal.	Calc.	Anal.	Calc.	Anal.	Calc.	Anal.	Calc.	Anal.
	<i>MJ</i>		<i>% of energy</i>									
	<i>Mg/MJ</i>											
US railroad	10.2	9.6	49	50	20	21	18	19	6.5	8.1	55	61
Finland East	14.6	14.7	39	42	20	22	11	14	2.1	3.8	34	36
Finland West	14.3	14.4	36	36	17	19	10	13	2.3	3.5	34	34
Zurphen	12.0	11.3	46	45	18	20	17	16	5.1	6.6	36	42
Crevalcore	14.6	15.2	29	38	9.7	13	14	18	3.6	5.0	33	38
Montegiorgio	12.6	12.1	28	31	8.6	9.6	13	15	5.0	4.5	25	29
Rome railroad	12.9	11.0	32	32	10	11	17	21	3.6	3.9	36	37

between the highest and the lowest value for *trans* fatty acids in food composites was 8 g per day (2.8% of energy) for those *trans* fatty acids detectable by gas chromatography, and 25 g per day (8.3% of energy) for the total amount of *trans* fatty acids as detected by infrared spectrometry. The sum of the long-chain (*n*-3) fatty acids, EPA and DHA, between the highest and the lowest value differed by almost 2 g per day. Amounts of fish providing these or smaller amounts of EPA and DHA are associated with marked differences in coronary heart disease incidence. Most differences can be explained by the differences in food intake, e.g., higher intakes of hydrogenated fats and milk resulted in higher *trans* fatty acids levels, and higher fish consumption resulted in a higher EPA plus DHA content of the food composites.

Two important questions are: (i) whether chemical analysis of food composites based on food records and composed 30 years after food recording provided valid data and (ii) to what extent the results of samples may be extrapolated to the full cohorts and to their respective countries.

Food Records

We selected the amounts and types of foods for composing the food composites on the basis of food records from the 1960s. Weighed food records give an average underestimation of energy intake of some 10% (de Vries *et al.*, 1994), but they still remain the most accurate method of dietary assessment (Bingham *et al.*, 1995). Body mass index, age, and gender appear to be the major factors associated with underreporting (Black *et al.*, 1993; Jørgensen, 1992; Schoeller *et al.*, 1990), although some studies did not find an effect of obesity (Bandini *et al.*, 1990; Lissner *et al.*, 1989). However, obese men in the Zutphen cohort reported a lower energy and fat intake than their lean counterparts (Kromhout, 1983). Therefore, the energy and fat content of the food composites of Rome, Belgrade, Crevalcore, and U.S. railroad, where the men with the highest relative body weights lived (body mass index >25), might have been underestimated more than in the other cohorts. Nevertheless, the proportional contribution of fat, fatty acids, and sterols might be still reasonably correct, so that the data of these cohorts are still valuable. Gender and age range did not differ between the cohorts.

The reconstructed data of Greece appear to be useful, as they result in a total fat composition for Crete and Corfu that agrees with that reported earlier (Sasaki *et al.*, 1993). Although we derived the food composite of the United States from only 1-day records, it can still be considered representative for the food intake of the cohort, because food records of 30 men were used. It is uncertain if the 8 Ushibuka men were representative of the men in their cohort, but as the total fat level of the food composite agrees with other data of Japanese dietary surveys (Sasaki *et al.*, 1993; FAO, 1994), they probably were.

We do not expect that seasonal variation had a large effect on the results of our study. Seven-day surveys covered seasons of the year, and the intake of energy-yielding nutrients was relatively constant throughout the year (Keys, 1970).

Food Sampling

Most foods to be collected for the food composites were available. We acquired them within 5 months and sampled the foods for all cohorts in the same way. All

food composites were analyzed in one laboratory. Although the relative positions of the cohorts in the distribution of different foods between 1961–1965 and 1975–1977 were maintained (Kromhout *et al.*, 1989), foods probably have changed in their total fat, fatty acid, and sterol composition between the 1960s and the 1980s (FAO, 1994). Meat and dairy products with the same total fat content as recorded by the men were largely still available. We only selected margarines of the stick form made from the same hydrogenated oils as in the 1960s. The main edible fat was margarine only in Zutphen, and as we periodically monitor the fat composition of Dutch margarines (Hulshof *et al.*, 1991), it was possible to choose a margarine with a highly similar fatty acid composition in 1987.

In the 1960s, chemical analysis for determination of a limited number of nutrients of equivalent composites provided the amount of saturated, monounsaturated, and polyunsaturated fatty acids for 13 of the 16 cohorts (Keys, 1970; Den Hartog *et al.*, 1968). The correlation coefficients between these determinations and those found in our study for the same cohorts were 0.92 ($P < 0.001$) for saturated fatty acids, 0.95 ($P < 0.0001$) for monounsaturated fatty acids, and 0.65 ($P < 0.02$) for polyunsaturated fatty acids. Therefore, we apparently succeeded in selecting the right foods and in composing representative food composites.

Chemical Analysis

We were not able to assess butyric (C4:0) and caproic (C6:0) acids, due to workup of the samples and the method used for gas chromatography. Butyric and caproic acid contribute about 5% of total fatty acids in dairy products (Gurr, 1992). We calculated that as a result we could have missed at most about 3 g of fat per day in the cohorts with a high consumption of dairy products.

We considered the methods used well validated for food composites with more than 5% of fatty acid methyl esters. We used internal quality control samples, and within and between variations of determinations of these samples were respectively 1.4 and 1.7% for total fat (mean 18 g, $n = 20$), 1.4 and 2.0% for C16:0 (mean 34 g, $n = 64$), 2.9 and 3.6% for C18:0 (mean 5.2 g, $n = 64$), and 1.8 and 2.4% for C18:2 (mean 15 g, $n = 64$).

Comparisons between *trans* fatty acid levels between assessment by gas chromatography and Fourier transform infrared spectrometry performed in the two laboratories showed good agreement, except for the Zutphen cohort which contains C20 and C22 polyunsaturated fatty acids in the *trans* configuration.

The recovery test for detection of ($n-3$) fatty acids showed that very small amounts of C20:5($n-3$) and C22:6($n-3$) could be determined accurately in the food composites. From all this it appears that our data are valid estimates of the fatty acid and sterol composition of the diets.

Calculation by Use of Food Composition Tables

The results of calculation by use of food composition tables agreed well with those of chemical analyses, except for the high fat content of the Crevalcore composite. This difference may be caused by a decrease of fat content of foods from the sixties to the eighties, although we did not find differences between calculated and analyzed fat content of this magnitude for Montegiorgio and Rome, for which we used the

same food composition tables as for Crevalcore (Kommissie UCV, 1985; Carnovale and Miuccio, 1989). The total fat content assessed in the 1960s corresponds with the lower calculated level. This could point to selections of food with a fat content too low for this cohort. The calculations indicate that the difference in fat content was distributed evenly over the different fatty acids.

Fatty Acid Composition

Medium-chain fatty acids and stearic acid do not raise plasma low-density lipoprotein (LDL) (Keys *et al.*, 1965; Hegsted *et al.*, 1965; Bonanome and Grundy, 1988; Grundy, 1990; Yu *et al.*, 1995). We found small differences in medium-chain fatty acid intake between cohorts, but stearic acid was 10 times higher in Finland and Slavonia than in Japan (Table 4). Palmitic (C16:0), myristic (C14:0), and lauric (C12:0) acid clearly raise LDL cholesterol, and therefore are considered atherogenic (Willett and Sacks, 1991; Grundy, 1990; Zock *et al.*, 1994). Finland had the highest intakes of all three of these fatty acids, due to a high intake of butter and milk, and Tanushimaru, Japan, had the lowest.

Oleic acid in the diet shows beneficial effects on the lipoprotein risk profile for coronary heart disease (Mensink and Katan, 1989; Yu *et al.*, 1995). Cohorts in Greece, Italy, and the former Yugoslavia, with a high consumption of olive oil, showed the highest intake of oleic acid. Long-chain monoenic fatty acids with 20 and 22 carbon atoms occur in margarines containing partially hydrogenated marine oils. That was confirmed for the Zutphen sample, in which these fatty acids contributed 1% of energy. Erucic acid (C22:1) may cause cardiac lipidoses in rats (Gurr, 1992). The effects in humans are not clear, but toxicity is likely to be much less in a mixed diet (Gurr, 1992). A regulation in the European Community limits the erucic acid in foods to 5% of fat content. The highest amount of this fatty acid in our study, 0.9% of energy or 2% of fat content in the sample of Zutphen, was below this level. The erucic acid in Tanushimaru (Table 5) probably came from rapeseed oil.

Trans unsaturated isomers of 18:1 and 18:2 fatty acids are formed during partial hydrogenation of oils rich in linoleic and oleic acid. There is increasing evidence that these *trans* fatty acids raise LDL and reduce high-density lipoprotein cholesterol levels (Katan *et al.*, 1995; Mensink and Katan, 1990; Nestel *et al.*, 1992). Sources for *trans* fatty acids are dairy products, meat, and margarines, cooking fats, and shortenings made with partially hydrogenated oils (British Nutrition Foundation Task Force, 1995; Gurr, 1992; Litin and Sacks, 1993). The data for C16 and C18 *trans* fatty acids assessed by gas chromatography agreed well between the Finnish and the Wageningen laboratory (cf. Methods). We found higher values by using infrared spectrometry. The gas chromatography values could be an underestimation due to a partial overlap of C18:1 *cis* and C18:1 *trans* positional isomers, and because they do not include *trans* fatty acids of 20 carbon atoms and more. The high values for total *trans* fatty acids for Zutphen (Fig. 2) can be explained by a high consumption of margarines with hydrogenated fish oils. These oils contain large amounts of *trans* isomers with 20 or 22 C atoms which can be detected by infrared spectrometry but not by gas chromatography. The high amount of fatty acids with more than 20 carbon atoms in the Zutphen sample (14 g/day) confirmed the presence of hardened fish oils. Also, the high C16:1(*n*-7) *trans* value for Zutphen probably originated from hydrogenated fish oils in margarines.

Aitchison *et al.*, (1977) determined the mean daily intake of *trans* fatty acids by duplicate portion analysis for 11 and van den Reek *et al.* (1986) for 8 American women. Total *trans* fatty acids relative to energy agreed well with our value for the U.S. cohort: Aitchison found 1.8 g/1000 kcal, van Reek found 2.1 g/1000 kcal, and we found 2.2 g/1000 kcal. The average daily intake of *trans* fatty acid for the United States was estimated as 8.1 g/day (about 3.9 g/1000 kcal) by Hunter and Applewhite (1991) and as 13 g/day (about 6.3 g/1000 kcal) by Enig *et al.* (1990), as opposed to 5.0 g/day (2.2/1000 kcal) for our sample. The estimates of Hunter and Applewhite (1991) and Enig *et al.* (1990) were based on disappearance and market share data, respectively, which may overestimate actual intake. Also, *trans* fatty acid intake could have risen between the 1960s and the 1980s as margarines replaced butter in the diet; margarines free from *trans* were available at that time in Europe (Katan, 1994), but not in the United States.

The *trans* fatty acid level determined in a representative Finnish diet in 1987 (Heinonen *et al.*, 1992) was 1.7 g/day or 0.7 g/1000 kcal, while the Finnish value in our composites was 5.8 g/day or 1.7 g/1000 kcal. The difference could be partly explained by a decreased fat intake since the 1960s. Probably, it is even smaller in Finland nowadays, because the intake of fat has declined further, and all margarines and most vegetable fat blends have been reformulated and are very low in *trans* fatty acid content (Dr. Ingmar Wester, Raisio, personal communication).

The amounts of *trans* fatty acids in the duplicate samples of a Dutch market basket study (Van Dokkum *et al.*, 1989, 1990) was estimated to be 10.0 g/day or 3.2/1000 kcal, which agreed well with our value for the Dutch cohort Zutphen (8.6 g/day or 3.1 g/1000 kcal). Although both values may not be considered representative for the Dutch population, they indicate that the intake of *trans* fatty acids in the Netherlands remained the same between the 1960s and the 1980s.

The (*n*-6) and (*n*-3) fatty acids are essential for the formation of membranes and are precursors of eicosanoids which have a variety of functions (Leaf, 1992; Garcia-Closas *et al.*, 1993). The presence of (*n*-3) polyunsaturated fatty acids in the diet may reduce the risk of coronary heart disease (Leaf and Weber, 1988). Fish oils are a rich source of (*n*-3) fatty acids and have a high content of fatty acids with 20 or more carbon atoms. This was reflected by high contents of these fatty acids in the food composites of east Finland, the isle of Corfu in Greece, Dalmatia on the Yugoslavian coast, and Japan, which had a high fish consumption. We found differences in the intake of the (*n*-3) polyunsaturated fatty acids, EPA and DHA, of almost 2 g per day. Some studies suggest that the ratio between (*n*-6) and (*n*-3) polyunsaturated fatty acids is important in the prevention of coronary heart disease (Kinsella, 1988; FAO, 1994). Experts recommend a ratio of linoleic to α -linolenic acid in the diet between 5:1 and 10:1 (FAO, 1994). Only 6 of the 16 cohorts met this advice (Table 5); in the other 10 cohorts the ratio was lower for 1 and higher for 9. A problem with the use of this ratio is that α -linolenic acid is not equivalent to longer chain (*n*-3) fatty acids because only some 10–20% is elongated. Therefore indexes or recommendations for (*n*-3) fatty acid should probably take into account the lower “biological value” of α -linolenic acid relative to EPA and DHA; we have attempted to correct intake figures in this way in Fig. 3.

The values for (*n*-3) and (*n*-6) polyunsaturated fatty acids in our study agreed rather well with those from earlier Finnish and Dutch studies (Heinonen *et al.*, 1992; Van

Dokkum *et al.*, 1990) and corresponded with fish consumption levels of the cohorts (Kromhout *et al.*, 1989).

Sterols

Dietary cholesterol is present in animal foods and is seen by some as an independent risk factor for coronary heart disease (Stamler and Shekelle, 1988). A cholesterol intake of less than 300 mg per day is recommended (FAO, 1994). We found high cholesterol intakes (>475 mg/day) in the U.S. railroad workers, east Finland, west Finland, Zutphen, Crevalcore, and Slavonia; these were generally due to a high intake of milk fat, eggs, and meat. Sterols in plants, phytosterols, are poorly absorbed and interfere with the absorption of cholesterol. Large intakes may lower LDL cholesterol levels (FAO, 1994). A high consumption of vegetable oils and a relative high intake of bread contributed to the high content of plant sterols in the Greek duplicates (Weihrach and Gardner, 1978). Our results confirm previous values for plant sterol intakes of 160–360 mg per day, amounts which probably have little effect on plasma cholesterol (Vanhanen *et al.*, 1993).

Visible Fats

Visible fats were the most important fat source in the diets. Margarine and butter were major contributors to the high saturated and *trans* fatty acid content of the Finnish, Dutch, and American diets, and olive oil to the monounsaturated fatty acid content of the Greek, Italian, and former Yugoslavian diet. A high consumption of lard (e.g., Slavonia) led to high intakes of total and saturated fat, but not of *trans* fatty acids. For the Japanese cohorts visible fats were not an important contribution to fat intake. The amounts and types of visible fats correspond with the fatty acid and sterol composition of the diets, which confirms the accuracy of our data.

CONCLUSION

We conclude that the assessment of fatty acids and sterols in the diets by chemical analysis of equivalent food composite portions is an adequate tool for estimating the average intake of subsamples of populations. However, the results of one or two cohorts do not necessarily reflect the average intake of a country.

We found large differences in the amount of total fat, fatty acids, and sterols between cohorts, which reflected the variations in food consumption patterns. Chemical analysis of equivalent food composite portions allows assessment of more fatty acids and sterols than when using food tables, so that information on the intake of less well known fatty acids and sterols can become available.

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