Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors[1–3]

Annika EM Smedman, Inga-Britt Gustafsson, Lars GT Berglund, and Bengt OH Vessby

ABSTRACT

Background: The fatty acid composition of the diet is known to be partially reflected by the fatty acid composition of serum lipids. **Objective:** We examined whether pentadecanoic acid (15:0) in serum lipids can be used as a marker for intake of milk fat, the major dietary source of 15:0. We also investigated the relations between intake of milk fat and cardiovascular disease risk factors. **Design:** Sixty-two 70-y-old men completed 7-d dietary records. The intake of milk products was studied in relation to the proportions of 15:0 in serum cholesterol esters and phospholipids, as well as to the clinical characteristics of these men, by using Spearman's rank correlation.

Results: The proportions of 15:0 in serum cholesterol esters were positively related to butter intake ($r = 0.36$, $P = 0.004$) and to the total amount of fat from milk products ($r = 0.46$, $P < 0.0001$); 15:0 in phospholipids was related to the amount of fat from milk and cream ($r = 0.34$, $P = 0.008$) and to the total amount of fat from milk products ($r = 0.34$, $P = 0.008$). Inverse associations were found between intake of milk products and body mass index, waist circumference, LDL-HDL ratio, HDL triacylglycerols, and fasting plasma glucose, whereas relations to HDL cholesterol and apolipoprotein A-I tended to be positive.

Conclusions: The results suggest that 15:0 in serum can be used as a marker for intake of milk fat. The explanation for the inverse associations between the intake of milk products and certain cardiovascular risk factors is not known. *Am J Clin Nutr* 1999;69:22–9.

KEY WORDS Pentadecanoic acid, milk fat, biomarker, fatty acid composition, diet, metabolic risk factors, serum, 15:0, elderly men, Sweden

INTRODUCTION

Accurate assessments of dietary intake in free-living subjects are difficult to obtain. Forgetfulness and unwillingness to report actual intakes may result in underestimations of the intakes of, for example, energy and dietary fat, and in relative overestimations of nutrients such as dietary fiber, vitamins, and minerals (1–3). The search for gold standards for estimating the intake of different nutrients and energy has included efforts to identify specific biochemical markers in body tissues and urine.

The fatty acid compositions of serum and adipose tissue are known to partially reflect the relative fatty acid composition of the diet. This knowledge has been used in several ways: for estimations of the average composition of dietary fat (4, 5), as a control for adherence to a given diet (4, 6), as a complement to dietary surveys (7, 8), and in the evaluation and development of dietary assessment methods (9). The fatty acid composition of dietary fat is reflected by the fatty acids in different compartments of the body at different points in time. Thus, dietary fatty acid composition is reflected in the serum triacylglycerols a few hours after a meal; in erythrocyte membranes, serum phospholipids, and cholesterol esters weeks to months later; and in triacylglycerols of adipose tissue 2–3 y later (10–13). When analyzing the fatty acid composition of tissue samples to estimate the fatty acid composition of the diet, strong correlations have been found between essential unsaturated fatty acids and estimated dietary intake (6, 8, 11, 14, 15). Correlations for nonessential unsaturated and saturated fatty acids (SFAs) are weaker.

Human studies have shown that diets rich in milk and butter are hypercholesterolemic, in contrast with diets with a high content of polyunsaturated fatty acids (PUFAs) (16–19). All fatty acids in milk fat are not equal in their effects on serum lipoproteins and apolipoproteins (16, 18). The SFAs lauric (12:0), myristic (14:0), and palmitic (16:0) acids, which constitute ≈40% of the fatty acids in milk fat, are known to be the main cholesterol-raising dietary fatty acids (16, 18). Few studies have addressed the effect of more moderate amounts of milk, butter, and fat from these products on serum cholesterol.

In the rumen of ruminants, the bacterial flora synthesize some fatty acids specific for ruminants, such as pentadecanoic (15:0) and heptadecanoic (17:0) acids (20). Because these fatty acids have an uneven number of carbon atoms, they cannot be synthesized in the human body. A recent study by Wolk et al (21) showed that the proportions of 15:0 and 17:0 in adipose tissue reflected milk fat consumption in women.

The purpose of this project was to study the relation between the estimated intake of fat of dairy origin and the proportion of

[1] From the Department of Geriatrics, Unit for Clinical Nutrition Research, Uppsala University, Uppsala, Sweden.

[2] Supported by The Swedish Dairy Council.

[3] Address reprint requests to AEM Smedman, Department of Geriatrics, Unit for Clinical Nutrition Research, Uppsala University, PO Box 609, S-75125 Uppsala, Sweden. E-mail: annika.smedman@geriatrik.uu.se.

Received December 2, 1997.

Accepted for publication July 2, 1998.

15:0 in serum lipids, with the aim of determining whether this fatty acid could be a biochemical marker for intake of milk products. In addition, the relation between the estimated intake of milk and clinical characteristics considered risk factors for ischemic heart disease was investigated.

SUBJECTS AND METHODS

Subjects

During the years 1970–1973, all 50-y-old men living in the municipality of Uppsala, Sweden, were invited to participate in a prospective survey regarding risk factors for ischemic heart disease. Of the 2841 men invited, 2322 (82%) agreed to take part. At the age of 70 y the subjects were invited to a repeat investigation (22, 23). At this investigation, 1221 of the 1680 (73%) men in the cohort still alive and living in Uppsala participated. Additional physiologic analyses, including fatty acid analysis of serum lipids, were performed on 66 subjects in consecutive order. These subjects were included in the study. Of these 66 men, 62 had completed a 7-d dietary record. The physiologic data at the time of the second survey are shown in **Table 1**. There was no significant difference in the cardiovascular risk profile between the 66 men and the whole cohort at the age of 50 y (data not shown). One of the 66 participants stated that he was restricted to a low-fat diet. The design of the study was approved by the Ethics Committee of the Faculty of Medicine of Uppsala University, and all participants gave their written, informed consent.

Dietary assessment

An optically readable, 7-d, precoded food record was used to assess dietary intake. The dietary recording and the clinical examination were carried out during the winter within 3 wk, except for 3 subjects for whom the time between the dietary survey and the examination was 4–8 mo. A questionnaire identical to the one used in this study, though not optically readable, was used previously by the Swedish National Food Administration and Statistics Sweden in a nationwide dietary survey of 3000 households in Sweden in 1989 (24). This precoded questionnaire has been validated with weighed food records (24). When compared with energy expenditure as measured by the doubly labeled water method, the energy intake reported in the questionnaire was underestimated by 20% (25), which is of the same magnitude as the underestimation observed in earlier validation studies (26). The reported protein intake was validated by urinary nitrogen excretion analysis (27), and was also found to be underestimated by ≈20%. The intake of food and nutrients was calculated by use of the food-composition data of the Swedish National Food Administration and the software program SAS (SAS Institute Inc, Cary, NC).

The amount of energy and the macronutrient and fatty acid compositions of the reported diets are shown in **Table 2**. All data regarding dietary intake used for correlation studies were adjusted for energy intake. The reported intake of milk products and the calculated amount of fat in these products are shown in **Table 3**.

Analysis of the fatty acid composition of serum lipids

The fatty acid composition of the serum lipids was analyzed by gas–liquid chromatography (GLC), as described in detail by Boberg et al (28). The GLC system used for the analyses consisted of a GC 5890, an automatic 7671A sampler, a 3392A integrator (all Hewlett-Packard, Avondale, PA), and a 25-m Nordion fused silica capillary column NS-351 (HNU Systems Inc, Finland), with helium as the carrying gas. The temperature was programmed to 100–210 °C. Standards from NuChek Prep (Elysian, MN) were used for identification of the individual fatty acids and as a control for the GLC system. The amounts of fatty acids are given as the relative percentage of the sum of the fatty acids analyzed. The within-analyses CV of 15:0 was 13.4% in cholesterol esters and 9.1% in phospholipids. The CVs for all other fatty acids analyzed were <1–5.5%, except for 18:0 in cholesterol esters (9.9%) and 17:0 (6.3%) and 18:3n−3 in phospholipids (8.2%).

TABLE 1
Clinical characteristics of the participants[1]

Clinical variable	Value
Age (y)	70.8 ± 0.4[2]
Weight (kg)	77.3 ± 10.5
BMI (kg/m^2)	25.8 ± 2.7
Waist (cm)	92.9 ± 8.9
Hip (cm)	99.0 ± 6.1
Waist-to-hip ratio	0.94 ± 0.05
Smokers (%)	24
Supine SBP (mm Hg)	148 ± 14
Supine DBP (mm Hg)	84 ± 9
Serum triacylglycerols (mmol/L)	1.28 ± 0.59
Serum cholesterol (mmol/L)	5.93 ± 1.01
HDL cholesterol (mmol/L)	1.34 ± 0.26
HDL triacylglycerols (mmol/L)	0.10 ± 0.07
LDL cholesterol (mmol/L)	4.01 ± 0.94
LDL:HDL	3.11 ± 0.96
Apo B (g/L)	1.02 ± 0.25
Apo A-I (g/L)	1.37 ± 0.24
Apo(a) (U/L)	217 ± 262
Nonesterified fatty acids (mmol/L)	0.50 ± 0.17
M (100 mg · kg body wt^{-1} · min^{-1})	5.93 ± 2.08
M:*I* (100 mg · pmol · kg body wt^{-1} · min^{-1} · L^{-1})	1.01 ± 0.46
Plasma glucose, fasting (mmol/L)	5.80 ± 1.77
Oral-glucose-tolerance test (AUC)	189 ± 114

[1] SBP and DBP, systolic and diastolic blood pressure, respectively; Apo, apolipoprotein; *M*, glucose disposal, *M*:*I*, insulin sensitivity index; AUC, area under the curve. n = 62.
[2] $\bar{x}$ ± SD.

TABLE 2
Energy and macronutrients and fatty acid compositions of the reported diets[1]

Nutrient	Value
Energy (kJ)	6900 ± 1600
Fat (% of energy)	33.6 ± 6.7
Total dietary fat (g)	61.3 ± 18.9
Fat from milk products (% of energy)	11.4 ± 8.0
SFAs (% of energy)	14.9 ± 3.6
MUFAs (% of energy)	11.6 ± 2.4
PUFAs (% of energy)	4.9 ± 1.1
Carbohydrate (% of energy)	49.7 ± 7.3
Protein (% of energy)	15.7 ± 2.4
Alcohol (% of energy)	2.4 ± 2.9

[1] $\bar{x}$ ± SD; n = 62. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids. Nutrient analysis by Swedish National Food Administration.

TABLE 3
Reported intake of milk products and the amount of fat provided by each[1]

Milk product	$\bar{x}$ ± SD	Median (range)
	g/d	
Milk	336.1 ± 187.2	328.6 (0–1043)
Cream	1.2 ± 2.4	0 (0–10)
Fat from cream and milk	5.2 ± 4.3	4.1 (0–22)
Cheese	31.8 ± 19.8	28.6 (0–87)
Fat from cheese	7.6 ± 4.9	7.2 (0–23)
Ice cream	10.4 ± 18.8	0 (0–107)
Fat from ice cream	1.2 ± 2.3	0 (0–11)
Butter	7.6 ± 14.3	0 (0–60)
Fat from butter	6.1 ± 11.4	0 (0–48)
Total dairy fat	20.3 ± 14.4	16.7 (4–73)

[1] n = 62.

Lipoprotein concentrations

VLDL and HDL cholesterol were assayed by a combination of preparative ultracentrifugation (29) and precipitation with a sodium phosphotungstate and magnesium chloride solution (30). Triacylglycerol and cholesterol concentrations in serum and in the isolated lipoprotein fractions were measured by enzymatic methods in a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA). The CVs of the analyses of serum cholesterol and serum triacylglycerols were 8.1% and 2.7%, respectively. The concentrations of apolipoprotein A-I and B were measured by immunoturbidimetry with a Monarch apparatus. The CVs of the analyses of apolipoprotein A-I and B were 2.5% and 2.4%, respectively. Apolipoprotein(a) [apo(a)] was determined by an apo(a) radioimmunoassay method [Pharmacia (a) RIA, Pharmacia Diagnostics AB, Uppsala, Sweden], which is based on the direct sandwich technique with 2 monoclonal antibodies specifically directed against apo(a). The CV of the analysis of apo(a) is ≈5% according to the manufacturer.

Glucose metabolic studies

Insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp procedure according to DeFronzo et al (31), as described in detail by Pollare et al (32). The insulin sensitivity index (*M:I*) was calculated by dividing the glucose disposal (*M*; $mg \cdot kg^{-1} \cdot min^{-1}$) by the actual insulin concentration during the test (pmol/L; 1 pmol/L = 0.167 mU/L) multiplied by 100 (*I*). An oral-glucose-tolerance test was performed by giving 75 g glucose in 300 mL water. Plasma glucose was measured before, and 30, 60, 90, and 120 min after the ingestion of glucose. The results of the oral-glucose-tolerance tests were expressed as the area under the glucose curve.

Blood pressure measurements

Supine systolic and diastolic blood pressures were measured with a mercury manometer after subjects rested for 10 min. The value was recorded twice to the nearest even figure. The mean of these 2 figures was used.

Anthropometric measurements

Body weight was determined to the nearest 0.1 kg and height was measured in centimeters. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Waist circumference was measured midway between the lowest rib and the iliac crest and the hip circumference was measured at the widest part of the hips, both in centimeters. The waist-to-hip ratio was calculated from these 2 measures. Physical activity was grouped into 4 categories on the basis of questions included in a medical questionnaire (33).

Statistical analyses

The results of this investigation were analyzed by using SAS and STATA (Stata Corporation, College Station, TX). Pairwise associations were examined by Pearson product-moment correlation analysis when the data were on a continuous scale, and by Spearman's rank correlation analysis when they were skewed (for example, the intake of most of the milk products) or ordinal (for example, the degree of physical activity). For comparison of the correlation coefficients of the associations with and without adjustments for physical activity, Spearman's rank correlation was used in both of the analyses.

To study whether intake of other groups of food items could possibly confound the investigated associations, we studied the relations of intake of other food items to milk products, to 15:0, and to clinical characteristics. From this analysis we concluded that intake of meat, vegetables, root crops, potatoes, and beer could be possible confounders, and we therefore analyzed the associations between milk products and 15:0, respectively, and clinical characteristics both with and without adjustment for intake of these foods. Correlations in the tables with P values < 0.05 are considered significant; all correlations with P values < 0.1 are included to show statistically significant relations as well as not fully significant trends, which may be of interest.

RESULTS

Fatty acid composition of serum lipids

Proportions of 15:0 and 17:0 were analyzed in serum cholesterol esters and serum phospholipids. Although the proportions were low, 15:0 and 17:0 in serum phospholipids and 15:0 in serum cholesterol esters could be quantified in nearly all subjects (**Table 4**).

Relations between dietary intakes and proportions of 15:0 in serum

The proportion of 15:0 in both serum cholesterol esters and phospholipids was positively correlated with intake of fat from milk and cream, with intake of ice cream and fat from ice cream, with intake of butter and fat from butter, and with the total amount of fat provided by milk products, as shown in **Table 5**. No significant associations were found between the proportion of 15:0 and intake of cheese or fat from cheese. Relations between the proportion of 15:0 in serum cholesterol esters and phospholipids, respectively, and the estimated dietary intake of 4:0–10:0 and 14:0 were positive (**Table 6**). Negative associations were found between the proportion of 15:0 in cholesterol esters and the percentage of energy intake as PUFAs. Adjustments for physical activity did not change the associations, except for the one between 15:0 in serum phospholipids and 14:0 in the diet, which was no longer significant.

Relations between the proportions of 15:0 and other fatty acids in serum lipids

The proportion of 15:0 in cholesterol esters was positively related to 15:0 and 17:0 in phospholipids and to 16:0 in choles-

TABLE 4
Proportions of the serum lipid fatty acids analyzed[1]

Fatty acid	Cholesterol esters	Phospholipids
	% of total fatty acids	
Pentadecanoic acid (15:0)	0.22 ± 0.05 (0.15–0.32)	0.25 ± 0.06[2] (0.15–0.32)
Palmitic acid (16:0)	12.12 ± 0.78 (10.71–14.26)	32.29 ± 1.61 (29.17–36.16)
Palmitoleic acid (16:1n−7)	3.88 ± 1.09 (2.03–7.21)	0.83 ± 0.15 (0.44–1.09)
Heptadecanoic acid (17:0)	0.11 ± 0.02[3] (0.08–0.18)	0.49 ± 0.09 (0.33–0.87)
Stearic acid (18:0)	0.97 ± 0.15 (0.70–1.35)	14.03 ± 1.17 (11.41–17.41)
Oleic acid (18:1n−9)	20.64 ± 2.12 (15.58–25.33)	12.48 ± 1.20 (9.25–15.23)
Linoleic acid (18:2n−6)	51.70 ± 4.16 (40.75–59.29)	21.49 ± 2.64 (14.76–26.55)
γ-Linolenic acid (18:3n−6)	0.65 ± 0.26 (0.20–1.47)	ND
α-Linolenic acid (18:3n−3)	0.89 ± 0.19 (0.45–1.36)	0.34 ± 0.09[4] (0.19–0.65)
Eicosatrienoic acid (20:3n−6)	0.66 ± 0.134 (0.35–1.00)	2.71 ± 0.59 (1.43–4.32)
Arachidonic acid (20:4n−6)	5.55 ± 1.12 (3.06–8.34)	7.47 ± 1.20 (4.75–10.17)
Eicosapentaenoic acid (20:5n−3)	1.79 ± 0.97 (0.60–6.91)	1.75 ± 0.96 (0.54–6.91)
Docosahexaenoic acid (22:6n−3)	0.99 ± 0.23[5] (0.41–1.55)	4.89 ± 1.13 (2.29–7.02)

[1] $\bar{x}$ ± SD; range in parentheses. n = 66 unless otherwise indicated. ND, not determined.
[2] n = 65.
[3] n = 37.
[4] n = 63.
[5] n = 58.

terol esters, whereas 15:0 in phospholipids was positively related to 17:0 in phospholipids (**Table 7**). There were also positive relations between 15:0 in cholesterol esters and phospholipids and the long-chain n−3 fatty acids.

Relations between intake of milk products and clinical characteristics

Correlations between the intake of the amount of fat from milk products and the clinical characteristics of the subjects are shown in **Table 8**. After adjustment for physical activity, intake of fat from milk and cream remained significantly related to body weight, waist and hip circumferences, BMI, HDL triacylglycerols, and the results of the oral-glucose-tolerance test (data not shown). The relations between fat from cheese and apo(a), between fat from ice cream and the insulin sensitivity index, and between fat from butter and BMI, HDL cholesterol, LDL-HDL ratio, and apolipoprotein A-I also remained significant after adjustment for physical activity. When adjustments were made for intake of meat, beer, vegetables, and root crops, the intake of fat from milk and cream was still related to body weight, waist circumference, BMI, the waist-to-hip ratio, and the fasting plasma glucose concentration (data not shown). The relations between fat from ice cream and the insulin sensitivity index, and fat from butter and HDL cholesterol, LDL-HDL ratio, and apolipoprotein A-I, respectively, also remained significant. Adjustments for dietary calcium did not alter the relations (data not shown). Although the significance of the relations between intake of milk products and clinical variables were weakened after adjustment for physical activity and intake of other food items, the main pattern of the relations remained.

TABLE 5
Proportions of 15:0 in serum cholesterol esters and phospholipids correlated with energy-adjusted intake of milk products and fat from milk products by using Spearman's rank correlation

	r	P
Cholesterol ester 15:0 (n = 62)		
Fat from milk and cream	0.26	0.039
Ice cream	0.27	0.036
Fat from ice cream	0.27	0.036
Butter	0.36	0.004
Fat from butter	0.36	0.004
Total dairy fat[1]	0.46	<0.0001
Phospholipid 15:0 (n = 61)		
Fat from milk and cream	0.34	0.008
Ice cream	0.25	0.051
Fat from ice cream	0.26	0.041
Butter	0.25	0.051
Fat from butter	0.25	0.051
Total dairy fat	0.34	0.008

Relations between 15:0 and clinical characteristics

The relations between the proportions of 15:0 in serum cholesterol esters and phospholipids and the studied clinical characteristics are shown in **Table 9**. After adjustment for intake of meat, beer, potatoes, root crops, and vegetables the associations were weakened, but significant inverse associations remained between 15:0 in serum cholesterol esters and body weight and BMI (data not shown).

TABLE 6
Correlations between the proportion of 15:0 in serum cholesterol esters and phospholipids and energy-adjusted nutrients and fatty acids[1]

	r[2]	P
Cholesterol ester 15:0		
Percentage of energy as SFAs	0.23	0.073
Percentage of energy as PUFAs	−0.33	0.009
4:0–10:0	0.38	0.002
14:0	0.35	0.006
18:2n−6	−0.24	0.056
Phospholipid 15:0		
4:0–10:0	0.29	0.026
14:0	0.26	0.045

[1] n = 62. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
[2] Pearson correlation coefficient.

TABLE 7
Correlations between the proportion of 15:0 in serum cholesterol esters and phospholipids and other fatty acids analyzed

	r^1	P
Cholesterol ester 15:0		
Phospholipid 15:0 (n = 62)	0.67	<0.0001
Cholesterol ester 16:0 (n = 62)	0.42	0.001
Phospholipid 17:0 (n = 62)	0.52	<0.0001
Cholesterol ester 18:2n−6 (n = 62)	−0.21	0.090
Phospholipid 18:2n−6 (n = 62)	−0.24	0.057
Cholesterol ester 20:5n−3 (n = 62)	0.26	0.035
Phospholipid 20:5n−3 (n = 62)	0.33	0.008
Cholesterol ester 22:6n−3 (n = 58)	0.29	0.025
Phospholipid 15:0		
Phospholipid 17:0 (n = 66)	0.64	<0.0001
Cholesterol ester 20:5n−3 (n = 65)	0.21	0.090
Phospholipid 20:5n−3 (n = 65)	0.26	0.039
Cholesterol ester 22:6n−3 (n = 57)	0.29	0.029

[1] Pearson correlation coefficient.

TABLE 8
Correlations between energy-adjusted intake of fat from milk products and clinical variables[1]

	r	P
Fat from milk and cream		
Weight	−0.35	0.006
Waist circumference	−0.33	0.009
Hip circumference	−0.29	0.022
BMI	−0.33	0.009
WHR	−0.27	0.033
Supine SBP	−0.23	0.070
Serum triacylglycerol	−0.23	0.077
HDL triacylglycerol	−0.28	0.026
Plasma glucose (fasting)	−0.30	0.020
Oral-glucose-tolerance test (AUC)	−0.26	0.043
Fat from cheese		
Supine DBP	0.29	0.024
Apo(a)	−0.37[2]	0.009
Fat from ice cream		
M	0.23	0.072
M:*I*	0.28	0.030
Fat from butter		
BMI	−0.26	0.039
HDL cholesterol	0.31	0.015
LDL:HDL	−0.28	0.027
HDL triacylglycerol	−0.23	0.077
Apo A-I	0.33[3]	0.021
Total dairy fat		
Weight	−0.31	0.016
Waist circumference	−0.25	0.051
Hip circumference	−0.25	0.046
BMI	−0.35	0.006
WHR	−0.21	0.096
Supine SBP	−0.21	0.097
Serum triacylglycerol	−0.22	0.084
HDL triacylglycerol	−0.24	0.064
Apo B	−0.25	0.088
Plasma glucose, fasting	−0.22	0.082

[1] n = 62 unless otherwise indicated. SBP and DBP, systolic and diastolic blood pressure; apo, apolipoprotein; WHR, waist-to-hip ratio; AUC, area under the curve; *M*, glucose disposal; *M*:*I*, insulin sensitivity index.
[2] Spearman rank correlation coefficient.
[3] n = 48.

DISCUSSION

Pentadecanoic acid as a marker of milk fat intake

Our results indicate that 15:0 can be used as a marker of milk fat in the diet (Table 5). The associations between the reported intake of milk products and milk fat, respectively, and the proportion of 15:0 in serum lipids in this study are of the same magnitude as the relations between dietary fatty acids and the proportions of the corresponding fatty acids in serum and plasma shown in several earlier studies (5, 6, 13, 34). For example, Ma et al (5) showed that the Pearson's correlation coefficients between the proportion of 16:0 and 18:2 in plasma and the reported dietary intake of these fatty acids were 0.19 and 0.28 in cholesterol esters and 0.16 and 0.22 in phospholipids, respectively. Ma et al (5) have, as have several other groups, studied the relations between single fatty acids. In contrast, our study was based on intakes of groups of foods rather than on intakes of single fatty acids, which may have weakened the relations.

In contrast with all other milk products, we did not find any associations between the proportion of 15:0 and the intake of cheese or fat from cheese. The reason for this is not clear, but during the study it became evident that some of the participants had difficulty adequately describing the fat content of cheese. There were also difficulties in estimating the size of cheese slices and subsequently estimating the amount of cheese eaten.

As mentioned earlier, 17:0 is also found in milk products, but in low concentrations (35). In this study we also investigated whether 17:0 could be used as a biochemical marker for intake of milk products and how it was related to clinical characteristics. However, the concentrations of 17:0 in serum cholesterol esters were too low to detect in 29 of the 66 subjects. The concentrations of 17:0 in serum phospholipids were detectable, but there were no significant relations to milk fat intake or to clinical characteristics in this study.

Fatty acid composition of the diet and serum lipids

The positive associations found between 15:0 and 16:0 shown in Table 7 could be because they are provided by some of the same dietary sources (35). This is certainly the explanation for the associations between 15:0 and 17:0, which both come mainly from milk products (35). This is also the explanation for the positive relations between the proportion of 15:0 in serum lipids and intake of short-chain fatty acids (4:0–10:0) and of 14:0 (Table 7) because these fatty acids also come mainly from milk products (35). According to this reasoning, 15:0 and 18:2 are probably provided by different dietary sources because they were negatively correlated in this study. This negative correlation may have been due to the choice of cooking fat and bread spread.

The positive correlations between 15:0 in serum and intakes of 20:5 and 22:6, the latter 2 of which are mainly from fatty fish or from endogenous synthesis from 18:3n−3, may have different explanations. One might be that persons who eat a lot of milk products also tend to eat a lot of fatty fish. Another possible explanation could be the enzymatic competition for desaturases and elongases during the endogenous synthesis of long-chain PUFAs from 18:1, 18:2, and 18:3n−3. The same enzymes are used in these reactions, but they have the highest affinity for the formation of 20:5 and 22:6 from 18:3n−3 (36, 37). Thus, a high consumption of 18:3n−3 or a low consumption of 18:2, as sug-

TABLE 9
Correlations between the proportion of 15:0 in cholesterol esters and phospholipids and clinical variables[1]

	r[2]	P
Cholesterol ester 15:0		
Weight	−0.36	0.003
Waist circumference	−0.28	0.022
Hip circumference	−0.32	0.008
BMI	−0.39	0.001
HDL triacylglycerol	−0.23	0.062
M:I[3]	0.21	0.091
Phospholipid 15:0		
Weight	−0.21	0.099
Hip circumference	−0.25	0.042
Apo B[4]	−0.27	0.061

[1] n = 66 unless otherwise indicated. *M:I*, insulin sensitivity index; Apo B, apolipoprotein B.
[2] Pearson correlation coefficient.
[3] n = 65.
[4] n = 50.

gested by the negative correlation between the proportions of 15:0 and 18:2 shown in Table 7, may lead to relatively high proportions of 20:5 and 22:6 in the tissues. This may be true because of more efficient desaturation and elongation of 18:3n−3, even if the intake of these fatty acids has not been very high. The proportions of fatty acids are expressed as relative percentages of the fatty acids analyzed, and not as absolute amounts. Thus, one further possibility is that a result that appears to show an increase in a certain fatty acid is in fact secondary to a pronounced decrease in one or several other fatty acids present in high proportions in the serum.

Milk fat and clinical characteristics

The relations we found between the intake of milk products and several physiologic variables, such as body weight, BMI, waist circumference, and LDL-HDL ratio were negative, whereas the relations to HDL cholesterol and apolipoprotein A-I were positive (Table 8). If these relations reflect real, long-term causal associations, they are hard to reconcile with the opinion that high intakes of milk products, including the main cholesterolemic SFAs 12:0, 14:0, and 16:0 present in milk products, are connected with an increased risk of ischemic heart disease (16, 17, 19, 38). However, the effects of dietary SFAs on serum lipoproteins are complex and probably also depend on several other factors such as genetic disposition, concomitant intake of PUFAs and antioxidants, and other components of food (39). The positive relation between fat from butter and HDL cholesterol seen in this study could be due to the relatively high amounts of SFAs 12:0 and 14:0 in milk fat, fatty acids which have been concluded to increase both total serum and HDL cholesterol (40, 41). Hypocholesterolemic effects of milk products have been observed in some studies (42–45). It is not yet clear what substances in milk products might cause these effects, although several have been discussed, such as hydroxymethylglutaric acid, calcium, orotic acid, lactose, uric acid, or substances in the membrane of the fat particle (42). In several studies the possibly protective role of dietary calcium in the development of atherosclerotic cardiovascular disorders was discussed (46, 47). However, in this study, adjustments for calcium intake did not affect the relations between the intake of dairy fat and metabolic risk factors. This finding indicates that these relations were not explained by the intake of calcium in the dairy products.

It was suggested recently that conjugated linoleic acids, which are found in milk and meat products from ruminants, may have antiatherogenic and serum lipid-lowering effects in rabbits (48). In experimental studies, supplementation with conjugated linoleic acid significantly reduced whole-body fat and increased body protein in rats, mice, and chicks (49). A hypothesis to test could be that the inverse relations in this study between intake of milk fat and proportions of 15:0 in the serum on the one hand and risk factors for ischemic heart disease on the other are partially explained by the concomitant intake of conjugated linoleic acid.

Implication of lifestyle factors

When adjustments were made for physical activity, the relations between the proportion of serum 15:0 and intake of milk fat remained essentially unchanged, indicating that the degree of physical activity does not affect the functioning of 15:0 as a marker of milk fat in the diet. Although the main associations between the intake of milk fat and clinical characteristics also remained after adjustment for physical activity, some of the relations were weakened, indicating that variations in physical activity may confound the associations to some extent. After adjustment for intake of possibly confounding groups of food, such as meat, vegetables, potatoes, root crops, and beer, some of the relations between intake of milk products and serum 15:0 and clinical characteristics lost their significance. The alterations of the associations suggest that the intake of milk products is part of a more complex dietary pattern also characterized by, for example, the intake of meat, vegetables, root crops, and beer. It follows that the relations between the intake of milk products and clinical characteristics shown here should be interpreted with caution.

Study methods

There are some factors in this study that may have confounded the results. First, dietary records seldom show true dietary intake, and should always be interpreted cautiously. However, the imprecision of the dietary data would tend to diminish the strength of the investigated relations. Second, the intake of dairy foods among 70-y-old men may be associated with a health-conscious lifestyle also characterized by good eating habits, lower body weight, and higher physical activity. However, significant associations remained between intake of certain diary products or fat from dairy products and metabolic measures, even after adjustment for other food habits and physical activity. Third, the group of participants was not representative of the total population for age and sex, and other relations may be seen in other populations. A fourth possible confounder was the effect of selection of survivors in a cohort population that has been followed for several years.

Conclusion

From earlier studies it is known that fatty acids, mainly PUFAs, in serum and adipose tissue reflect the fatty acid quality of the habitual diet. The findings in this study of elderly Swedish men suggest that 15:0 in serum can be used as a marker for intake of milk fat at the group level. Although SFAs as a group raise cholesterol concentrations, the effects on the risk of ischemic heart disease of milk fat or dairy products as components of ordinary food need to be studied further.

We are grateful to Siv Tengblad for performing the fatty acid analyses and to Rawya Mohsen for managing the cohort database.

REFERENCES

1. Gibson RS. Sources of error and variability in dietary assessment methods: a review. J Can Diet Assoc 1987;48:150–5.
2. Gibson RS. Measurement errors in dietary assessment. Principles of nutritional assessment. New York: Oxford University Press, 1990.
3. Bingham S. Methodological problems in nutritional epidemiology. Acta Cardiol 1993;48:463 (abstr).
4. Glatz FCG, Soffers AEMF, Katan MB. Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. Am J Clin Nutr 1989;49:269–76.
5. Ma J, Folsom AR, Shahar E, Eckfelt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. Am J Clin Nutr 1995;62:264–71.
6. Sarkkinen ES, Ågren J, Ahola I, Ovaskinen M-L, Uusitupa MIJ. Serum fatty acid composition of cholesterol esters, and erythrocyte and platelet membranes as indicators of long-term adherence to fat-modified diets. Am J Clin Nutr 1994;59:364–70.
7. Katan MB, Deslypere JP, van Birgelen APJM, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res 1997;38:2012–22.
8. London SJ, Sacks FM, Caesar J, Stampfer MJ, Siguel E, Willett WC. Fatty acid composition of subcutaneous adipose tissue and diet in post menopausal US women. Am J Clin Nutr 1991;54:340–5.
9. Willett WC. Future directions in the development of food-frequency questionnaires. Am J Clin Nutr 1994;59(suppl):171S–4S.
10. Hirsch J, Farquhar JW, Ahrens EH, Peterson ML, Stoffel W. Studies of adipose tissue in man. Am J Clin Nutr 1960;8:499–511.
11. Beynen AC. A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. Am J Clin Nutr 1980;33:81–5.
12. Moilanen T, Räsänen L, Vikkari J, Åkerblom HK, Nikkari T. Tracking of serum fatty acid composition. A 6-year follow up study in Finnish youth. Am J Clin Nutr 1992;136:1487–92.
13. Nikkari T, Lukkainen P, Pietinen P, Puska P. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann Med 1995;27:491–8.
14. Ma J, Folsom AR, Eckfelt JH, Lewis L, Chambless LE, the Atherosclerosis Risk in Communities (ARIC) Study Investigators. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. Am J Clin Nutr 1995;62:572–8.
15. van Staveren WA, Deurenberg P, Katan MB, Durema J, de Groot LCPGM, Hoffmans MDAF. Validity of the fatty acid composition of subcutaneous adipose tissue microbiopsies as an estimate of the diet of separate individuals. Am J Epidemiol 1986;123:455–63.
16. Berner LA. Round table discussion on milk fat, dairy foods, and coronary heart disease risk. J Nutr 1993;123:1175–84.
17. Kushi LH, Lenart EB, Willett WC. Health implications of Mediterranean diets in light of temporary knowledge. I. Plant foods and dairy products. Am J Clin Nutr 1995;61(suppl):1407S–15S.
18. Katan MB, Zock PL, Mensink RP. Dietary oils, serum lipoproteins, and coronary heart disease. Am J Clin Nutr 1995;61(suppl): 1368S–73S.
19. Trevisan M, Krogh V, Freudenheim J, et al. Consumption of olive oil, butter, and vegetable oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. [Published erratum appears in JAMA 1990;263:1768]. JAMA 1990;263:688–92.
20. Wu Z, Palmquist L. Synthesis and biohydrogenation of fatty acids by ruminal microorganisms in vitro. J Dairy Sci 1991;74:3035–46.
21. Wolk A, Vessby B, Ljung H, Barrefors P. Evaluation of a biologic marker for dairy fat intake. Am J Clin Nutr 1998;68:291–5.
22. Hedstrand H. A study of middle-aged men with particular reference to risk factors for cardiovascular disease. Ups J Med Sci Suppl 1975;19:1–61.
23. Koupilová I, Leon DA, Lithell HO, Berglund L. Size at birth and hypertension in longitudinally followed 50–70-year-old men. Blood Press 1997;6:223–8.
24. Becker W. Befolkningens kostvanor och näringsintag i Sverige 1989. Metod-och resultatanalys. (Food habits and nutrient intake in Sweden 1989.) Uppsala, Sweden: National Food Administration, 1994 (in Swedish).
25. Gustafsson I-B, Ytterfors A, Vessby B, Bratteby L-E. Validering av energiintaget i en kostundersökning mot vägd kostregistrering och dubbelmärkt vatten. (Validation of the energy intake in a dietary survey compared to food registration and the doubly labelled water method.) The National Convention of the Swedish Medical Society. Hygiea 1995;104:284 (abstr) (in Swedish).
26. Bingham SA. The use of 24-h urine samples and energy expenditure to validate dietary assessments. Am J Clin Nutr 1994;59(suppl): 227S–31S.
27. Isaksson B. Urinary nitrogen output as a validity test in dietary surveys. Am J Clin Nutr 1980;33:4–6.
28. Boberg M, Croon LB, Gustafsson I-B,Vessby B. Platelet fatty acid composition in relation to fatty acid composition in plasma and serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. Clin Sci 1985;68:581–7.
29. Havel RJ, Eder HA, Bragdon JH. The determination and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345–53.
30. Seigler L, Wu WT. Separation of serum high-density lipoprotein for cholesterol determination: ultra centrifugation vs precipitation with sodium phosphotungstate and magnesium chloride. Clin Chem 1981;27:838–41.
31. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:214–33.
32. Pollare T, Lithell H, Selinus I, Berne C. Application of prazocin is associated with an increase of insulin sensitivity in obese patients with hypertension. Diabetologia 1988;29:415–20.
33. Collen MF, Cutler JL, Siegelaub AB, Cella RL. Reliability of a self-administered medical questionnaire. Arch Intern Med 1969; 123:664–81.
34. Nikkari T. Serum fatty acids and coronary heart disease in Finnish populations. Prog Lipid Res 1986;25:437–50.
35. Gunstone FD, Harwood JL, Padley FB. Occurrence and characteristics of oils and fats. In: Padley FB, Gunstone FD, Harwood JL, eds. The lipid handbook. Cambridge, United Kingdom: Cambridge University Press, 1994:47–224.
36. Mayes PA. Metabolism of unsaturated fatty acids and eicosanoids. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. Harper's biochemistry. 22nd ed. East Norwalk, CT: Prentice Hall, 1990.
37. Siguel EN, Maclure M. Relative activity of unsaturated fatty acid metabolic pathways in humans. Metabolism 1987;36:664–9.
38. Artaud-Wild SM, Connor SL, Sexton G, Connor WE. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. Circulation 1993;88:2771–9.
39. Hayes KC. Saturated fats and blood lipids: new slant on an old story. Can J Cardiol 1995;11(suppl):39G–46G.
40. Temme EHM, Mensink RP, Hornstra G. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am J Clin Nutr 1996;63:897–903.
41. Mensink RP, Temme EHM, Hornstra G. Dietary saturated and *trans* fatty acids and lipoproteins metabolism. Ann Med 1994;26:461–4.
42. Eichholzer M, Stahelin H. Is there a hypocholesterolemic factor in milk and milk products? Int J Vitam Nutr Res 1993;63:159–67.
43. Agerbaek M, Gerdes LU, Richelsen B. Hypocholesterolaemic effect

of a new fermented milk product in healthy middle aged men. Eur J Clin Nutr 1995;49:346–52.
44. Andersson H, Boseaus I, Ellegård L, et al. Effects of low-fat milk on cholesterol absorption and excretion in ileostomy subjects. Eur J Clin Nutr 1995;49:274–81.
45. Kiyusawa H, Sugawara C, Sugawara N, Miyake H. Effect of skim milk and yogurt on serum lipids and development of sudanophilic lesions in cholesterol-fed rabbits. Am J Clin Nutr 1984;40:479–84.
46. Cappuccio FP, Elliott P, Allender PS, Pryer J, Follman DA, Cutler JA. Epidemiologic association between dietary calcium intake and blood pressure: a meta-analysis of published data. Am J Epidemiol 1995;142:935–45.
47. McCarron DA, Lipkin M, Rivlin RS, Heaney RP. Dietary calcium and chronic diseases. Med Hypotheses 1990;31:265–73.
48. Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994;108:19–25.
49. Pariza M, Park Y, Cook M, Albright K, Lui W. Conjugated linoleic acid (CLA) reduces body fat. FASEB J 1996;10:3227 (abstr).