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Gas chromatographic profiles of plasma total lipids as indicators of dietary history

Correlation with fat intake based on 24-h dietary recall

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ABSTRACT

Fasting plasma total lipid profiles were determined by high-temperature gas chromatography on a total of 1246 free living urban subjects, ages 20–59 years, from the Toronto–McMaster Lipid Research Clinic Population Study. Quantitative estimates of the major molecular species, lipid classes and lipid class ratios were correlated with a total of twelve dietary lipid components, including total saturated and unsaturated fats, oleic and linoleic acids, and cholesterol, to give appropriate Spearman coefficients (r_S) and tests of significance (P) for groups of 775 males and 471 females. The intake of the various nutrients was derived from a 24-h dietary recall. The most significant correlations varying from $r_S \pm 0.1$–0.4 and $P < 0.0001$–0.0005 were between the intake of total fat, individual saturated and unsaturated fats, and the ratios of C_{50}/C_{54} triacylglycerols and the C_{34}/C_{36} phosphatidylcholines, which reflected the nature and quantity of the dietary fat consumed. Increases in dietary cholesterol and saturated fat produced small increases in plasma cholesterol and saturated triacylglycerols, while unsaturated dietary fat produced small decreases in saturated and increases in unsaturated plasma triacylglycerols. These changes in the plasma lipid parameters are consistent with those observed previously in much more limited dietary experiments with accurately known composition of ingested fats. It is, therefore, concluded that direct gas chromatographic profiling of plasma total lipids provides a simple and rapid method of verifying the overall correctness of the dietary recall.

INTRODUCTION

Previous studies [1–4] have shown that high-temperature gas chromatography (GC) provides a rapid and accurate assessment of plasma lipid profiles. Furthermore, a limited number of studies has suggested that these profiles exhibit characteristic changes with diet [5–7], ischemic vascular disease [8–10], hyperlipoproteinemia [8,11] and sitosterolemia [12]. The availability of the 24-h dietary recall data on the subjects participating in the Coronary Primary Prevention Trial (CPPT) conducted by the Lipid Research Clinics Program [13] provided an opportunity to use the determination of the plasma total lipid profiles for a ver-

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ification of the dietary recall. On the basis of previous work [5–7] it was anticipated that specific changes would take place in the total levels and relative proportions of different glycerolipids and cholesterol due to the dietary choices made by the participants in the study, provided they were accurately reported.

This report presents the correlations obtained between the intakes of various nutrients calculated from the 24-h dietary recall [14] and the changes in the plasma lipid parameters measured by direct GC of the plasma total lipids at the Toronto–McMaster Lipid Research Clinic. The data show that the predicted characteristic changes were largely realized confirming the essential accuracy of the 24-h dietary recall as conducted during the CPPT. A preliminary report has appeared [15].

EXPERIMENTAL

Subjects

The subjects were 1246 Visit 2 volunteers from the Toronto–McMaster Lipid Research Clinic, aged 20–59 years [8]. The data were restricted to 775 men and 471 women, who were fasting, who reported their alcohol intake for the previous week and for whom lipid and lipoprotein determinations were made. The study included 80 women on gonadal hormones.

Diets

The diets were of free choice, but each subject was questioned about his dietary intake by three trained interviewers certified by the Lipid Research Clinics Program [13]. Food intake records were completed from 24-h dietary recall data, coded and nutrient intakes computed by the Nutrition Coding Center using a food composition data base generated by the Lipid Research Clinic and Multiple Risk Factor Intervention Trial Program [14]. For the nutrients included in the survey, composition values were available for all food items reported in the intake records. The diet composition used for statistical correlations was taken from the last data of interview preceding the blood sampling. The dietary ingredients specifically tested were: total calories, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, oleic acid, linoleic–linolenic acid, total *cis–cis* unsaturated acids, dietary cholesterol, total carbohydrates, sucrose, starch, other carbohydrates, fiber and alcohol. Except for alcohol, which was reported in milliliters, these variables were reported in grams per day. Dietary cholesterol was expressed as milligrams per day. Total calorie intake and energy intake are used interchangeably and both are expressed as kilocalories. Not all subjects provided all data. Hence, the populations actually assessed differ somewhat among the variables tested. The methods used in obtaining the dietary recall data and the potential sources of error have been discussed in great detail by Beaton and co-workers [16–18]. In this report, the dietary lipid variables were correlated with the various plasma lipid parameters, which were determined as explained below.

Determination of total lipid profiles

Blood specimens were prepared according to procedures specified in the Laboratory Manual of the Lipid Research Clinics Program [19]. The GC profiles of plasma total lipids were determined as described earlier [20], except that the column temperature was raised at either 4 or 8°C/min. Comparative studies on the accuracy and precision of the GC technique and the Technicon Autoanalyzer routine have given comparable estimates for total cholesterol and total triacylglycerols [3]. Likewise, comparative studies of the plasma phosphatidylcholine and sphingomyelin by GC and by manual phosphorus analyses have also given good agreement [4].

Calculations

Total phosphatidylcholine was calculated as previously described [4,20] from the areas of peaks 36 and 38, which correspond to diacylglycerols with a total acyl carbon number of 34 and 36, respectively, but sphingomylin from the area of peak 34, which corresponds to palmitoylsphingosine. A molar phosphatidylcholine/free cholesterol (PC/FC) ratio was calculated by multiplying the mass ratios by the reverse of the molecular weight ratio of phosphatidylcholine (average MW = 740) and free cholesterol (MW = 386). The molar ratio of sphingomyelin/phosphatidylcholine (SPH/PC) was calculated similarly. The ratios of C_{50}/C_{54} represent the mass ratios of triacylglycerols with a total acyl carbon number of 50 and 54.

Statistical analyses

Spearman correlation coefficients (r_S) were determined and tests of significance were performed by conventional methods [21]. Some of the data were expressed on basis of equal caloric intake [22].

RESULTS

Effect of total fat, carbohydrate and protein intake

Table I gives the correlation coefficients for total fat, carbohydrate and protein intake, as estimated from a 24-h recall data, and selected parameters of the plasma total lipid profiles from the entire population of subjects. Increasing intake of total calories was significantly correlated with decreasing levels of glycerophospholipids (as indicated by decreased amounts of the C_{34} diacylglycerols) and increasing amounts of plasma triacylglycerols (as indicated by increased levels of C_{50}, C_{52} and C_{54} triacylglycerols). An identical correlation was seen with increasing protein intake, while increased total fat intake was significantly correlated with decreasing levels of glycerophospholipids and increasing levels of triacylglycerols, as indicated by the C_{34} diacylglycerols and the C_{52} triacylglycerols. Increased intake of total carbohydrate gave a significant correlation only with decreased levels of plasma glycerophospholipids (C_{34} and C_{36} diacylglycerols).

TABLE I

CORRELATION OF DIETARY VARIABLES WITH SELECTED PARAMETERS OF PLASMA TOTAL LIPID PROFILE (ALL SUBJECTS)

Dietary parameter	Statistical test[a]	Values of total lipid profile				
		C_{34}	C_{36}	C_{50}	C_{52}	C_{54}
Calories	r_s	−0.09711		0.10036	0.12710	0.08502
	P	0.0006		0.0004	0.0001	0.0027
	n	1246		1246	1246	1246
Total fat	r_s	−0.09954			0.07698	
	P	0.0004			0.0066	
	n	1246			1246	
Total CHO[b]	r_s	−0.11016	−0.09834			
	P	0.0001	0.0005			
	n	1246	1246			
Protein	r_s	−0.11000		0.10352	0.14281	
	P	0.0001		0.0003	0.0001	
	n	1246		1246	1246	
After normalization for differences in caloric intake.						
Total fat	r_s	−0.06309	−0.06948	−0.09410	−0.05785	
	P	0.0259	0.0156	0.0009	0.0412	
	n	1246	1246	1246	1246	
Total CHO[b]	r_s		−0.13700	−0.0970	−0.08412	
	P		0.0001	0.0006	0.0030	
	n		1246	1246	1246	

[a] r_s = Spearman coefficient; P = probability; n = number of subjects; other abbreviations as given in text.
[b] CHO = carbohydrate.

Since the data were greatly affected by the total calories consumed, further comparisons were made following correction for differences in caloric intake [22]. The lower half of Table I shows a negative correlation between total dietary fat intake and the plasma levels of both glycerophospholipids and triacylglycerols. Likewise, the negative correlation between plasma glycerophospholipid and triacylglycerol levels and increasing intake of total carbohydrate was maintained. In addition, significant correlations became apparent between the total fat intake and the C_{34}/C_{36} ($r_s = -0.1$; $P < 0.0009$; $n = 1236$) and C_{50}/C_{54} ($r_s = 0.1$; $P < 0.0005$; $n = 1236$) ratios of the glycerolipids. Fig. 1 gives the original data plot for the ratios of C_{50}/C_{54} triacylglycerols *versus* the total fat intake in the male subjects along with the regression line for appropriately weighted points.

Effect of type of dietary fat and cholesterol

Fig. 2 shows the plasma total lipid profiles recorded for two of the subjects, who most likely had consumed high amounts of dairy fat and saturated animal

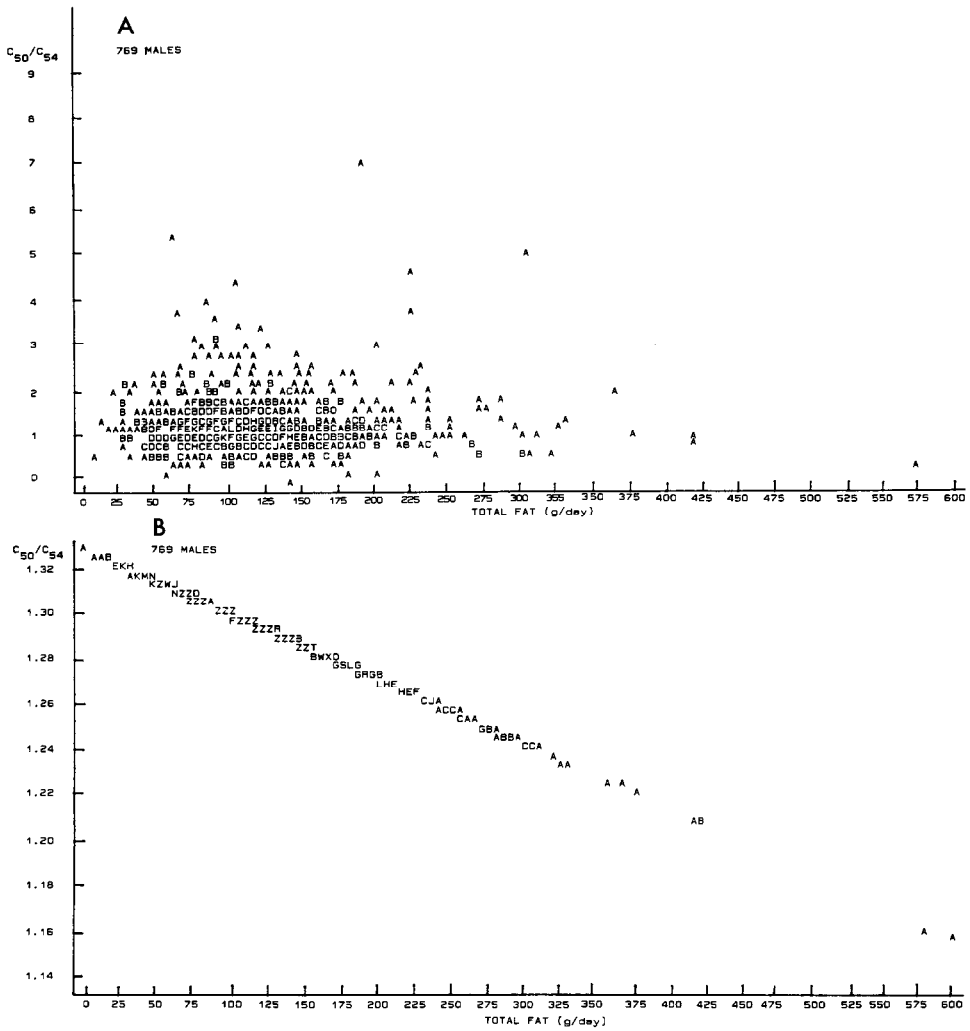

Fig. 1. (A) Original data plot for the ratios C_{50}/C_{54} of plasma triacylglycerols *versus* total fat intake by male subjects. (B) The regression line for appropriately weighted points. A = one, B = two, C = three, etc. observations.

fat, respectively. There is the anticipated [2] increase in the proportion of the C_{34} (peak 36) and C_{36} (peak 38) diacylglycerols and in the C_{48} and C_{50} triacylglycerols (Fig. 2A). Similar plasma lipid profiles would be anticipated for other subjects consuming increased amounts of saturated dairy and animal fats, respectively. Fig. 3 gives the original data plot for the ratios of C_{50}/C_{54} triacylglycerols *versus* the saturated fat intake in the female subjects along with the regression line for appropriately weighted points. The slope of the regression line is positive. A

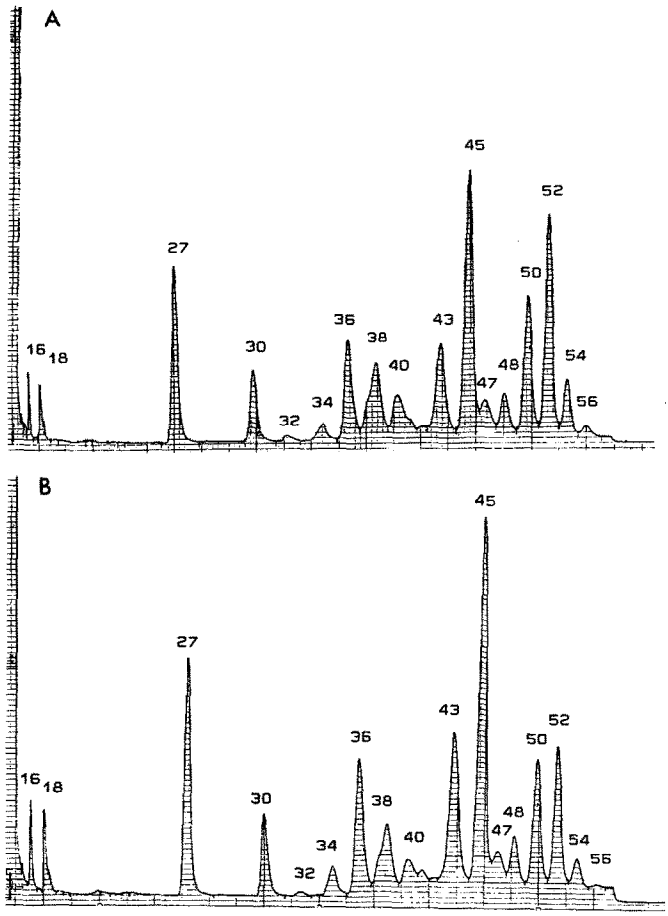

Fig. 2. GC profiles of plasma total lipids for subjects consuming diets high in saturated animal fats: A, butter?; B, lard? Peaks: 16 and 18 = free fatty acids with 16 and 18 carbon atoms; 27 = free cholesterol; 30 = tridecanoylglycerol, internal standard; 34 = palmitoylsphingosine; 36–40 = diacylglycerols with 34–38 acyl carbons; 43–47 = cholesteryl esters with 16–20 acyl carbons in the fatty acids; 48–56 = triacylglycerols with 48–56 acyl carbons. Instrument, Hewlett-Packard Model 5700 gas chromatograph equipped with a column (50 cm × 2 mm I.D.) packed with 3% OV-1 on Gas Chrom Q (100–120 mesh). Carrier gas, nitrogen at 80 ml/min. Detector temperature, 350°C. Column temperature programmed from 175 to 335°C at 4°C/min. Sample, 1 μl of a 1% solution of total lipid in silylation mixture. Other GC conditions as given in text.

similar positive slope was obtained for the C_{50}/C_{54} ratio of the triacylglycerols in the male population. These trends are opposite to those associated with increased total fat consumption, which gave a negative slope.

Fig. 4 shows the plasma total lipid profiles recorded for subjects with a high dietary intake of monounsaturated and polyunsaturated fats. While the subject on the monounsaturated fat (Fig. 4A) shows a plasma lipid profile not unlike that

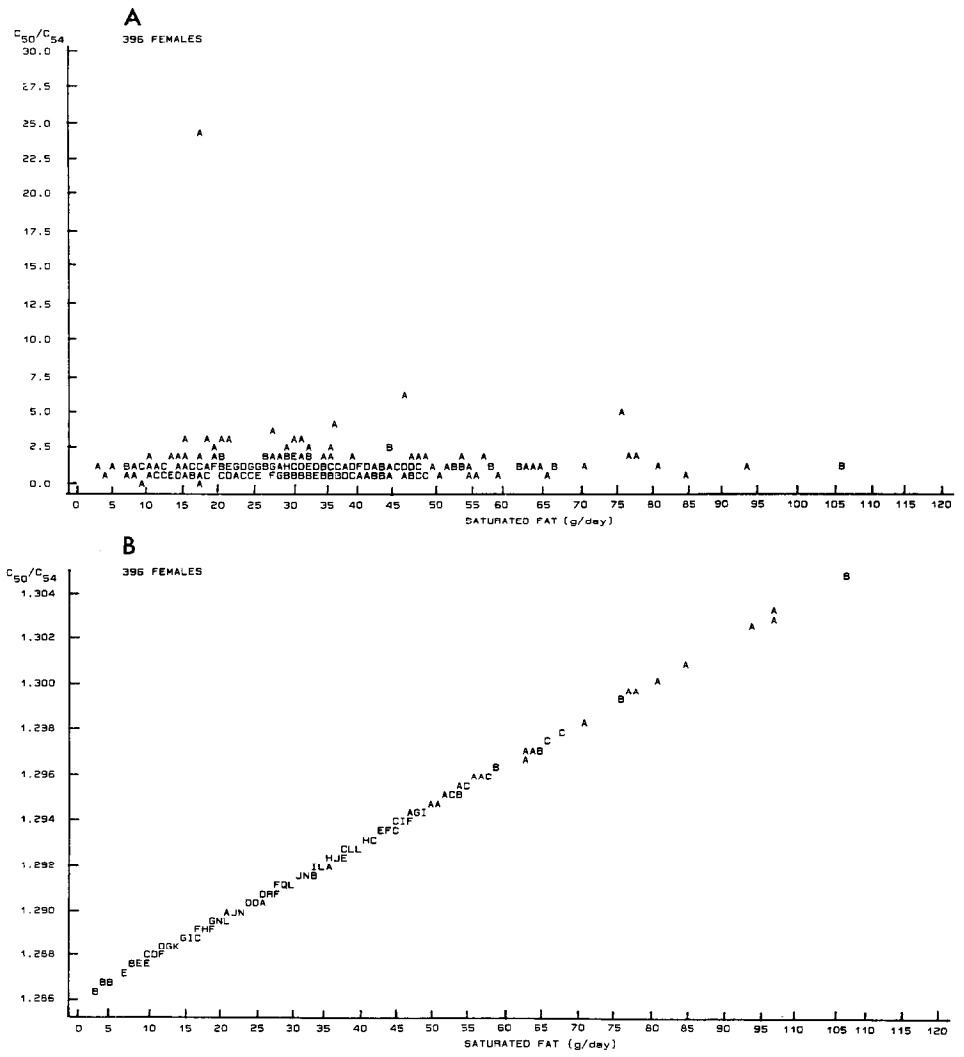

Fig. 3. (A) Original data plot for the ratios C_{50}/C_{54} triacylglycerols of plasma *versus* the saturated fat intake in female subjects. (B) The regression line for appropriately weighted points. A = one, B = two, C = three, etc. observations.

obtained on the high saturated animal fat diet (Fig. 2B), the subject on the polyunsaturated fat diet (Fig. 4B) possesses increased proportions of the C_{36} (peak 38) diacylglycerols and C_{54} triacylglycerols in the total lipid profile. The finding of increased C_{50}/C_{54} triacylglycerol ratio in subjects claiming high saturated fat consumption can be rationalized on the basis of the anticipated increase in 16:0–16:0–18:1 and 14:0–18:1–18:1, and a decrease in the 18:1–18:1–18:2 and 18:1–

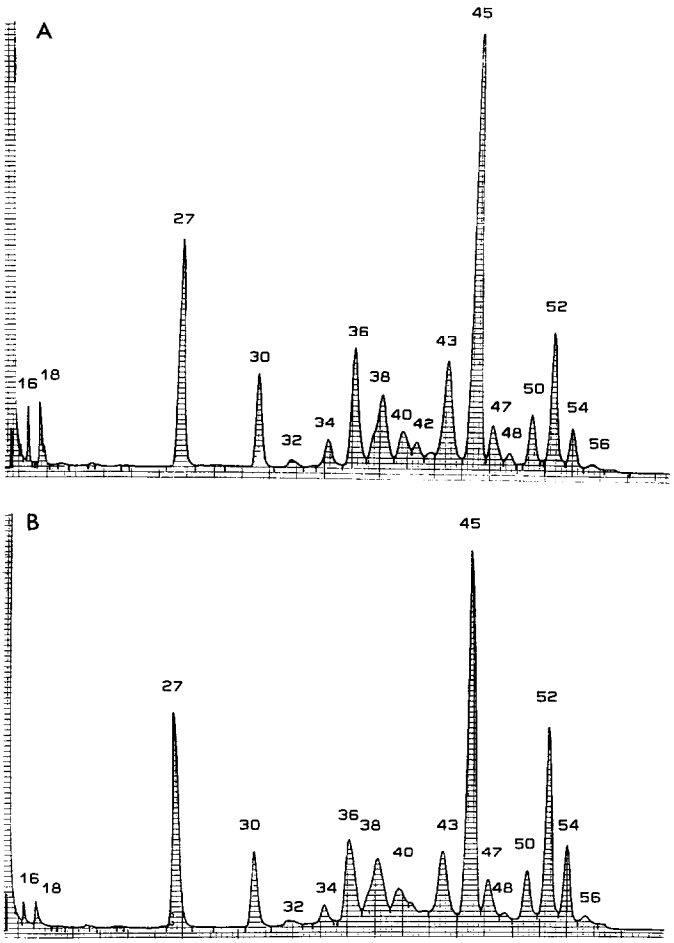

Fig. 4. GC profiles of plasma total lipids for a subject consuming large amounts of polyunsaturated fats (A) and a subject consuming large amounts of polyunsaturated fats (B) in the diet. Peak identification and GC conditions as given in Fig. 2.

18:1–18:1 triacylglycerols, which are the major triacylglycerol species in human plasma corresponding to the C_{50} and C_{54} carbon numbers, respectively.

Table II gives the correlation coefficients for the various plasma lipid parameters and the saturated, monounsaturated and polyunsaturated fat or fatty acids in the diet. Increased consumption of monounsaturated and polyunsaturated fats led to significant decreases in the concentration of the C_{34}, C_{36} and C_{50} glycerolipids. A similar trend was seen when the consumption of total linoleate was monitored. At the same time there was a significant increase in the content of the C_{54} triacylglycerols.

The consumption of increased amounts of polyunsaturated fats was strongly correlated with decreased C_{34} and C_{36} diacylglycerols and decreased C_{50} tria-

TABLE II

CORRELATION OF DIETARY VARIABLES WITH SELECTED PARAMETERS OF PLASMA TOTAL LIPID PROFILE FOLLOWING NORMALIZATION FOR TOTAL CALORIC INTAKE

Dietary variable	Statistical test[a]	Values of total lipid profile				
		C_{34}	C_{36}	C_{50}	C_{52}	C_{54}
Saturated fat	r_s			−0.05307	−0.07646	−0.14084
	P			0.0611	0.0069	0.0001
	n			1246	1246	1246
Monoun-saturated fat	r_s	−0.07121	−0.05206	−0.06670		
	P	0.0119	0.0662	0.0185		
	n	1246	1246	1246		
Polyun-saturated fat	r_s	−0.08183	−0.08917	−0.09943		0.07089
	P	0.0038	0.0016	0.0004		0.0123
	n	1246	1246	1246		1246
Linoleic acid	r_s	−0.06139	−0.07403	−0.08088		0.06224
	P	0.0303	0.0089	0.0043		0.0280
	n	1246	1246	1246		1246

[a] r_s = Spearman coefficient; P = probability; n = number of subjects; other abbreviations as given in text.

cylglycerols, but increased C_{54} triacylglycerols. A similar trend was seen for linoleic and linolenic acid consumption. Fig. 5 gives the original data plot for the ratios of C_{50}/C_{54} triacylglycerols *versus* the linoleic acid intake in the male subjects along with the regression line for appropriately weighted points. The slope of the regression line is negative indicating that increased consumption of the polyunsaturated fats resulted in increased proportion of the longer-chain triacylglycerols. Likewise, a negative slope was observed for the ratio of C_{34}/C_{36} diacylglycerols (peak 36/peak 38).

Sex differences

In general the total plasma lipid profiles of the males and females responded similarly to the type of fat and cholesterol in their diet. The main difference was in the strength of the correlation and not in the direction. An apparent exception was provided by the observation that increased consumption of saturated fat was associated with significantly decreased C_{34}/C_{36} diacylglycerol ratios in males but significantly increased C_{34}/C_{36} diacylglycerol ratios in females. Fig. 6 compares the regression line plots for the ratios of peak 36/peak 38 (C_{34}/C_{36} diacylglycerols) and saturated fat intake in the males and females. Table III shows the more significant correlations recorded for the males and females with respect to the dietary lipids and selected parameters of plasma total lipid profiles. In addition to the changes in the proportions of the major molecular species of the glycerophos-

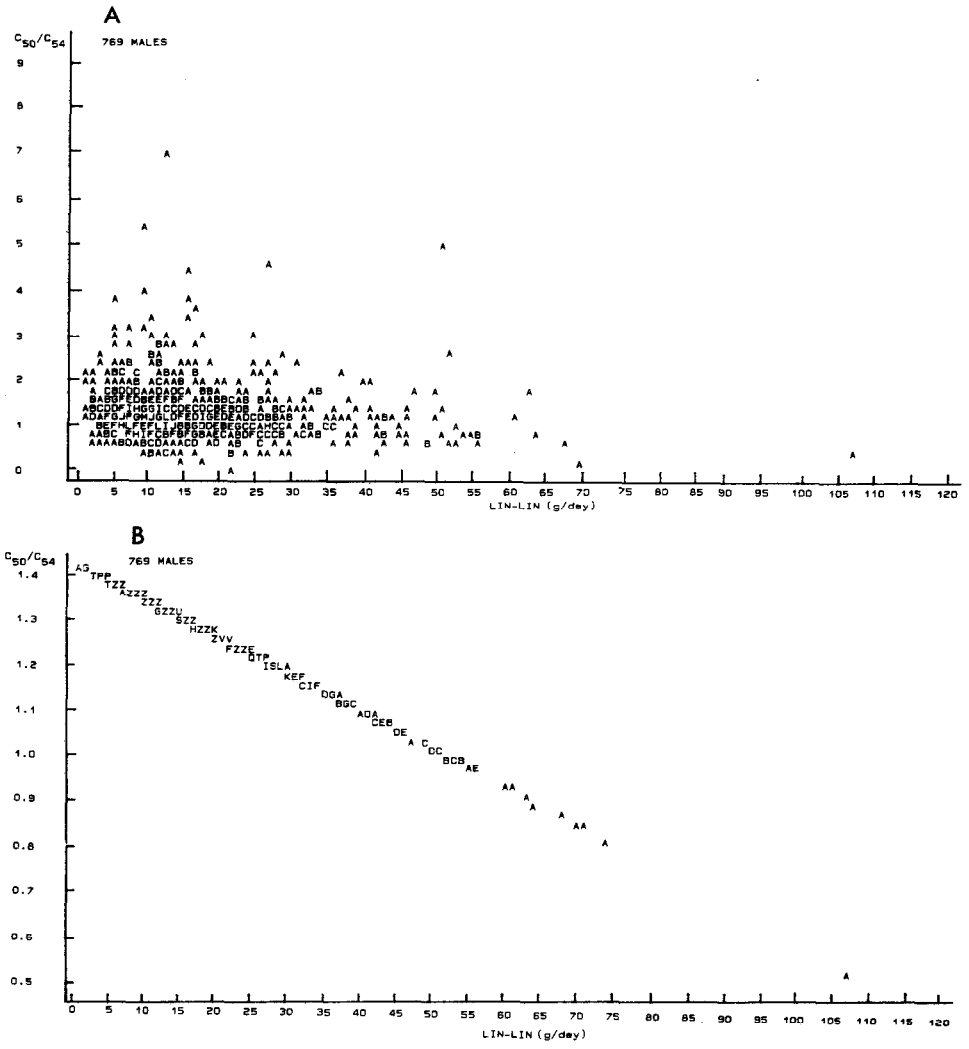

Fig. 5. (A) Original data plot for the ratios C_{50}/C_{54} triacylglycerols *versus* linoleic–linolenic acid intake in the male subjects. (B) The regression line for appropriately weighted points. A = one, B = two, C = three, etc. observations.

pholipids and triacylglycerols, the male population showed a significant correlation with decreases in the PC/FC ratio with increasing saturated fat in the diet, while increased amounts of monounsaturated fat in the diet yielded a significant correlation with decreases in the SPH/PC ratio in the males.

When corrected for differences in caloric intake, the males showed a significant positive correlation between increasing cholesterol intake and decreasing C_{34}/C_{36} diacylglycerol ratio. In addition, the female population showed a highly

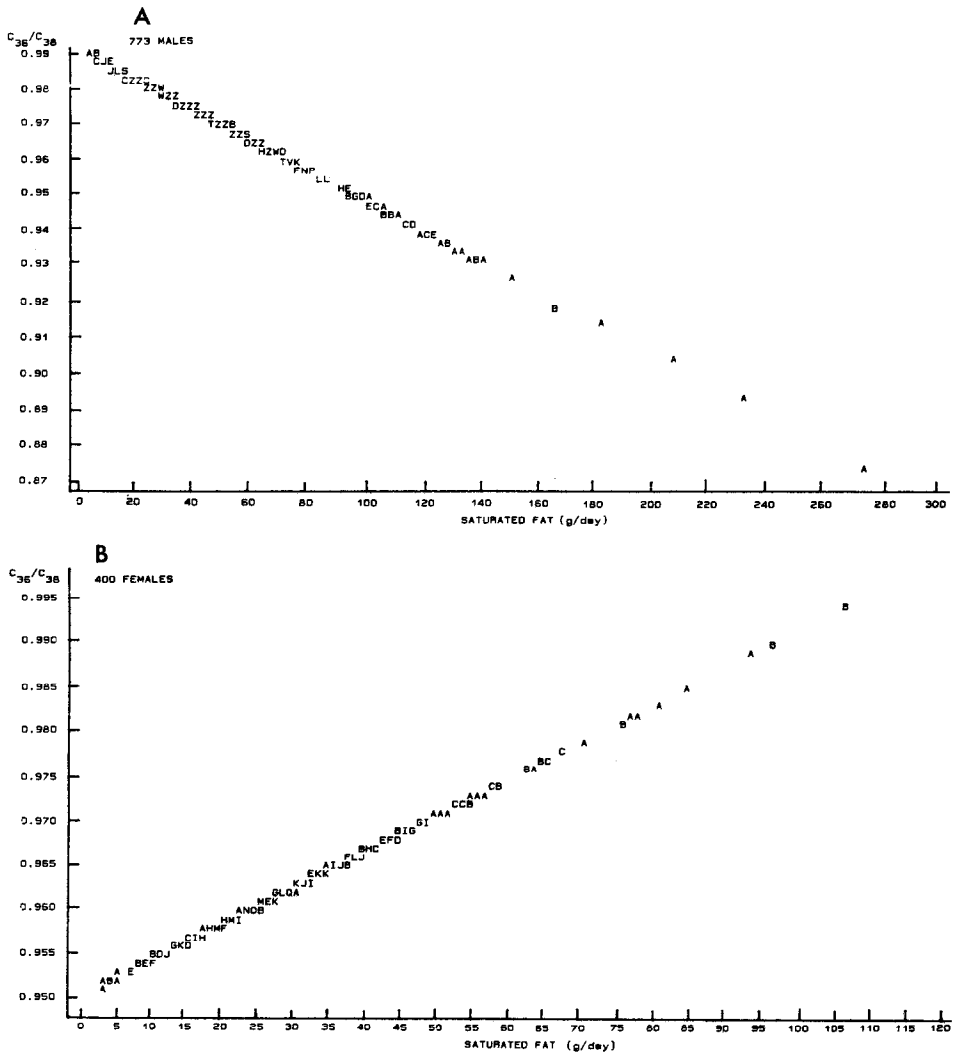

Fig. 6. Regression lines for the ratios C_{34}/C_{36} phosphatidylcholines (peak 36/peak 38) *versus* saturated fat intake in male (A) and female (B) subjects for appropriately weighted points.

significant negative correlation between increased total fat, monounsaturated fat and oleic acid intake and decreasing free cholesterol/(cholesteryl ester plus triacylglycerol) (FC/CETG) ratio (r_s = 0.1–0.3; $P < 0.0001$; n = 250), as well as increasing free cholesterol/triacylglycerol (FC/TG) ratio (r_s = 0.1; $P < 0.0001$; n = 250). This correlation was not seen in the female population on the pill, or in the male population. It should be noted that there was a highly significant correlation (r_s = 0.2–0.3; $P < 0.0001$; n = 250) between increasing age of the female population and increasing C_{34}, C_{36}, C_{43}, C_{45}, C_{50} and C_{52} components of the

TABLE III

CORRELATION OF DIETARY VARIABLES WITH SELECTED PARAMETERS OF PLASMA TOTAL LIPID PROFILES FOLLOWING NORMALIZATION FOR DIFFERENCES IN TOTAL CALORIC INTAKE

Dietary variable	Statistical test[a]	Values of total lipid profile							
		PC/FC		SPH/PC		C_{50}/C_{54}		C_{34}/C_{36}	
		Males	Females	Males	Females	Males	Females	Males	Females
Saturated fat	r_s	−0.1037	0.0759			0.0793	0.2065	−0.0946	0.1021
	P	0.0039	0.1295			0.0277	0.0001	0.0085	0.0412
	n	773	400			769	396	773	400
Mono-unsaturated fat	r_s			−0.0901	−0.0239	−0.1479	−0.0950	−0.1393	
	P			0.0122	0.7061	0.0001	0.0588	0.0001	
	n			773	250	769	396	773	
Oleic acid	r_s							−0.1302	
	P							0.0003	
	n							773	
Poly-unsaturated fat	r_s			−0.0660	−0.0048	−0.3034	−0.3129	−0.0836	
	P			0.0665	0.9397	0.0001	0.0001	0.0200	
	n			773	250	769	396	773	
Linoleic acid	r_s					−0.2513	−0.2540	−0.0800	
	P					0.0001	0.0001	0.0260	
	n					769	396	773	
Cholesterol	r_s							−0.0961	
	P							0.0075	
	n							773	

[a] r_s = Spearman coefficient; P = probability; n = number of subjects; other abbreviations as given in text.

plasma total lipid profile, including increasing amounts of free and esterified cholesterol. The age effect was not seen for males.

DISCUSSION

Effect of dietary fat on plasma lipid classes

Previous publications arising from the Lipid Research Clinics Program [23–25] have described the association between diet fat and high- (HDL-C), low- (LDL-C) or very-low (VLDL-C) density lipoprotein cholesterol levels as well as with VLDL triacylglycerol levels, which are reflected to a greater or lesser extent in the plasma total lipid profiles. Thus, the absence of response of HDL-C levels to dietary saturated, monounsaturated and polyunsaturated fat and total fat [23] can be seen in the absence of a significant change in the plasma total PC/FC ratio in males, which has been shown to be closely related to changes in HDL-C [8]. However, in females a strong positive response was seen for saturated, mono-

unsaturated and total dietary fat, as well as total cholesterol. Ernst *et al.* [23] also found a positive although weak correlation between dietary cholesterol and HDL-C in women not taking gonadal hormones. Gordon *et al.* [25] reported a statistically significant positive association of LDL-C and total cholesterol with the percentage of total calories from fat. This effect could not be confirmed in regards to total plasma cholesterol or to PC/FC ratio in the present study. The present study also failed to confirm the inverse association of plasma triacylglycerol levels with intake of polyunsaturated fat (grams per day), percentage of calories from total fat, saturated fat or polyunsaturated fat, which were significant for both men and women in the Lipid Research Clinics Study [23]. Schwarz *et al.* [24] found that the associations between LDL-C and individual lipid nutrients computed from a 24-h dietary recall were generally of a low order of magnitude. Gordon *et al.* [26] noted that diet-associated changes in VLDL-C were even less well marked, although in the same direction. These dietary changes were also weakly associated with a lowering of HDL-C [26].

The absence of uniform changes in plasma lipids with alterations in dietary fat is consistent with other published reports. Thus, McNamara *et al.* [27] found that 70% of free living subjects could effectively compensate for an increase in dietary cholesterol by down-regulating cholesterol synthesis, while Katan *et al.* [28] have shown that a correlation may exist between sensitivity to changes in dietary saturated fat intake and sensitivity to dietary cholesterol in normocholesterolemic individuals. Clifton *et al.* [29] have recently shown that hypercholesterolemic individuals, who are sensitive to dietary fat reduction, show, as a group, a significantly larger response to dietary cholesterol than do either fat-insensitive hypercholesterolemic subjects or normocholesterolemic individuals.

Effect of dietary fat on molecular species of plasma lipids

Although dietary fatty acids become incorporated into plasma glycerolipids together with endogenous acids, they exert a significant influence on the molecular species or carbon number of the phosphatidylcholines and triacylglycerols. This aspect of plasma lipid response has received very limited attention for lack of adequate methodology. Thus, feeding of butterfat and corn oil with or without cholesterol to normolipemic subjects has been shown [2] to lead to distinctly different qualitative and quantitative changes in the molecular species of plasma glycerolipids. Likewise, the consumption of saturated and unsaturated fats has been shown [5] to effect a marked change in the total lipid level and in the composition of the molecular species of various plasma lipids, as well as in the ratios of lipid classes in the different plasma lipoproteins. In another study [7] supplementation of arthritis patients with a fish oil preparation (MAXEPA) has been associated with the appearance of long-chain molecular species in plasma glycerolipids and cholesteryl esters. In the present study, it was specifically anticipated that increased consumption of polyunsaturated fats and linoleic acid would lead to an appearance in plasma of glycerolipids enriched in C_{18} fatty acids,

which are associated largely with C_{54} triacylglycerols and the C_{36} phosphatidylcholines. A similar effect would be anticipated for the monounsaturated fats, especially oleic acid and the partially hydrogenated vegetable oils found in margarines. This should result in decreased C_{50}/C_{54} and C_{34}/C_{36} phosphatidylcholine ratios. In contrast, increased consumption of animal fats, such as butterfat, and palm and coconut oils would be anticipated to lead to an appearance in plasma of glycerolipids enriched in C_{16} fatty acids, which are associated largely with C_{50} and C_{52} triacylglycerols and C_{34} phosphatidylcholines. This should result in increased C_{50}/C_{54} and C_{34}/C_{36} ratios, which was to a large extent realized. Thus, increased consumption of polyunsaturated fats and linoleic acid led to an appearance of plasma of glycerolipids enriched in C_{18} fatty acids, which was associated largely with the C_{54} triacylglycerols and the C_{36} phosphatidylcholines. Indeed there was a highly significant decrease in C_{50}/C_{54} triacylglycerol ratio resulting from an increased consumption of polyunsaturated fat in both males and females and of the C_{34}/C_{36} phosphatidylcholine ratio in males. The lack of significant correlation for the C_{34}/C_{36} ratio in the females is not immediately obvious, although the female population was numerically smaller (400 females *versus* 773 males). A similar effect was observed for the monounsaturated fats. The saturated fat and oleic acid failed to show any statistically significant correlation with the C_{50}/C_{54} or C_{34}/C_{36} ratios, possibly due to too large variance. It is probable that this effect resulted from a failure to differentiate between saturated fat representing hydrogenated vegetable oils and animal fats. Interestingly, increased consumption of total fat was associated with significantly decreased C_{50}/C_{54} and C_{34}/C_{36} ratios in the males but significantly increased C_{50}/C_{54} and C_{34}/C_{36} ratios in females. There is no obvious explanation for the sex difference, except that the number of subjects was smaller in the female group. There was a marked strengthening of this correlation following normalization of the total caloric intake. In addition, increased intake of saturated fat now resulted in an increased C_{50}/C_{54} triacylglycerol ratio in both males and females, and in an increased C_{34}/C_{36} phosphatidylcholine ratio in females, as expected. However, in males increased intake of saturated fat and oleic acid resulted in a decreased C_{34}/C_{36} ratio, which remains to be explained. Finally, the increased consumption of dietary cholesterol was associated with a highly significant decrease in the C_{34}/C_{36} ratio in the female but not in male population. This was reflected in changes in the PC/FC ratios. There was a tendency for FC/PC and the PC/SPH ratios to decrease in response to increased consumption of polyunsaturated fat.

These changes in the proportions of the molecular species of plasma glycerolipids clearly indicate that the subjects had very likely consumed the type and the quantities of the dietary lipids claimed in the 24-h recall. The limited response of plasma cholesterol to dietary cholesterol and dietary saturated and unsaturated fat, therefore, must be taken as supporting the general consensus in the literature that dietary cholesterol provides only a small increase in plasma cholesterol in most large groups of subjects. The change in plasma cholesterol in response to

increased consumption of saturated or unsaturated fat was also insignificant in our groups of subjects.

Significance of study

It is possible that the present lack of any marked effect of dietary fat or cholesterol upon plasma lipid levels is due to a high background fat intake, and that higher rise in plasma cholesterol and glycerolipids might have been realized if the starting levels of plasma lipids had been lower. Many previous studies have used plasma lipid levels established on fat-free or monounsaturated fat diets as reference points. Furthermore, the present expression of data on an equal caloric intake also constitutes a deviation from current practice [22], where caloric intake is adjusted to maintain body weight. The present study shows that the total caloric intake is closely related to increases in plasma cholesterol and glycerolipids. It remains to be established, whether the calories originating in fat, carbohydrate and protein all contributed equally to the increase in plasma lipid levels. The limited response of plasma lipids to dietary fat and cholesterol, however, should not be considered as a recommendation for disregarding the potential adverse effects of high fat and cholesterol consumption, which may be related to development of atherosclerosis without affecting the plasma lipid values.

The present study shows that high-temperature GC on a non-polar liquid phase provides an effective method for verifying the overall correctness of a 24-h dietary recall. It anticipates the application of high-temperature GC with polarizable liquid phases, which permit separations of plasma lipids based on both carbon number and degree of unsaturation [30].

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