Journal of Chromatography, 579 (1992) 13–24
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6418

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

Correlation with carbohydrate and alcohol intake based on 24-h dietary recall

A. Kuksis, J. J. Myher, K. Geher, W. C. Breckenridge, T. Feather, V. McGuire and J. A. Little

Banting and Best Department of Medical Research and Department of Medicine, University of Toronto, Toronto M5G 1L6 (Canada)

(First received January 22nd, 1992; revised manuscript received April 27th, 1992)

ABSTRACT

Quantitative gas chromatographic estimates of the major lipid classes and molecular species in fasting plasma were correlated with total carbohydrate, starch, fibre, sucrose and alcohol intake based on 24-h dietary recall. Spearman coefficients (r_s) and tests of significance (P) were obtained for groups of 775 males and 471 females aged 20–59 years from a Toronto-McMaster Lipid Research Clinics Population Study. The most significant correlations varying from r_s 0.1 to 0.2 and P 0.001 to 0.0005 (n = 400–773) were between increased intake of alcohol and increased ratios of C_{50}/C_{54} triacylglycerols, C_{34}/C_{36} phosphatidylcholines and phosphatidylcholine/free cholesterol (PC/FC) of plasma. Increase in total dietary carbohydrate, starch and fibre correlated with decreasing C_{50}/C_{54} triacylglycerol, C_{34}/C_{36} phosphatidylcholine and PC/FC ratios (r_s = −0.1–0.2; P < 0.002–0.04; n = 400–773). In contrast, consumption of high levels of alcohol was associated with increasing C_{50}/C_{54} triacylglycerol, C_{34}/C_{36} phosphatidylcholine and PC/FC ratios. A high intake of alcohol (50–150 ml per day) distinguished itself from other simple carbohydrate-induced lipid profiles by its marked effect on increased C_{50}/C_{52} triacylglycerol and PC/FC ratio.

INTRODUCTION

We have previously reported highly significant correlations between alterations in molecular species of glycerolipids, as determined gas chromatography (GC), and the ingestion of various fats based on 24-h dietary recall [1]. The relationships between dietary lipids and plasma total cholesterol and total triacylglycerols were closely similar to those obtained by conventional methods of lipid analyses [2–4]. However, plasma lipids are known to be affected also by dietary carbohydrates and alcohol, but this relationship has so far been examined only at the level of plasma total cholesterol and total triacylglycerols [2–5]. The availability of the 24-h dietary recall data on the volunteers participating in the Coronary Primary Prevention Trial (CPPT) conducted by the Lipid Research Clinics Program [6] and the results of detailed GC analyses provided an opportunity to correlate the intake of different carbohydrates and alcohol also with alterations in the molecular species of plasma lipids. This report shows that increased consumption of simple car-

Correspondence to: Dr. A. Kuksis, BBDMR, University of Toronto, 112 College Street, Toronto, M5G 1L6, Canada.

0378-4347/92/$05.00

bohydrates leads to increased proportion of lower-molecular-mass species in plasma glycerolipids and cholesteryl esters despite otherwise variable dietary background (fat intake). The most consistent and dramatic effects were associated with increased alcohol consumption. The data confirm the essential accuracy of the 24-h dietary recall as conducted by the CPPT.

EXPERIMENTAL

Subjects

The subjects were 1160 Visit 2 volunteers from the Toronto-McMaster Lipid Research Clinic, aged 20 59 years [1]. The data were restricted to men and women who were fasting (12–16 h), who reported their carbohydrate intake for the previous 24 h and 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 [7]. 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 [7]. The diet composition used for statistical correlations was taken from the last data of interview preceding the blood sampling and represented the dietary intake over several weeks. The dietary carbohydrates specifically tested were: total carbohydrates, sucrose, starch, other carbohydrates and alcohol, as well as fibre. These variables were reported in grams per day, except for alcohol, which was originally reported in ounces per week and recalculated in milliliters per day [2]. The methods used in obtaining the dietary recall data and the potential sources of error have been discussed in great detail by Beaton and coworkers [8–10]. Each dietary variable was correlated with each parameter of the plasma lipid profile, as described below.

Determination of total lipid profiles

Blood specimens were prepared and total cholesterol and triacylglycerols were analyzed according to procedures specified in the Laboratory Manual of the Lipid Research Clinics Program [11]. The GC profiles of plasma total lipids were determined as described [1,12].

Calculations

Total phosphatidylcholine (PC) was calculated as previously described [1,12–14] from the areas of carbon numbers C_{36} and C_{38}, which corresponded to diacylglycerols with a total acyl carbon number of 34 and 36, respectively. The ratio of C_{50}/C_{54} represents the mass ratio of triacylglycerols with acyl carbon numbers of 50 and 54, respectively. Sphingomyelin (SPH) was calculated from the area of peak 34 corresponding to palmitoylsphingosine, which makes up an approximately constant proportion of plasma total sphingomyelin [13,14]. A molar phosphatidylcholine/free cholesterol (PC/FC) ratio was obtained by multiplying the mass ratios by the reverse of the molecular mass ratio of PC (average MW = 740) and FC (MW = 386).

Statistical analyses

Spearman correlation coefficients (r_S) and tests of significance were performed by conventional methods [15].

RESULTS

Increased consumption of total carbohydrate, sucrose and starch gave a highly significant negative correlation with the absolute amounts of plasma 18:1 and 18:0 free fatty acids and their phospholipid and cholesteryl esters for Visit 1 subjects ($r_S = -0.1$–0.15; $P < 0.0001$–0.0004; $n = 1246$), while increased alcohol consumption gave a highly significant positive correlation ($r_S = 0.1$–0.23; $P < 0.0001$–0.0005; $n = 1246$) with the absolute amounts of these lipids. This indicated that plasma total lipids decreased or increased, respectively, with the two types of diets, but that these parameters were not adequate for differentiation between carbohydrate and alcohol

effects. More specific responses were sought and found in the ratios of plasma lipid classes and molecular species of Visit 2 subjects.

Effect of starch

Fig. 1 shows the plasma total lipid profiles recorded for a normolipemic (a) and a Type IV hyperlipoproteinemia (B) subject, who reported high consumption of starch along with large or small amounts of dietary fat, respectively. Neither subject had consumed any alcohol. The peaks represent carbon numbers of the major molecular species of SPH (peak 34), PC (peaks 36–40), cholesteryl esters (peaks 43–47) and triacylglycerols (peaks 48–56). Subject A was a 33-year-old male with a total plasma cholesterol of 182 mg% and total plasma triacylglycerol of 64 mg%. He had consumed 502 g of total carbohydrate per day of which 277 g per day were from starch. In addition, the subject ingested 148 g of saturated fat, 114 g of monounsaturated fat and 40 g of polyunsaturated fat per day along with 1505 mg of cholesterol per day. The plasma total lipid level was estimated at 486 mg%. The lipid profile is characteristic of a normolipemic subject on a mixed diet as reported previously [1,12–14]. The plasma PC contains a high proportion of polyunsaturated species as indicated by the prominent shoulders on the ascending limbs of the C_{38} and C_{40} diacylglycerol species, while the C_{36}/C_{38} peak-area ratio approaches unity. The triacylglycerol profile is characterized only by slight elevation in the proportion of the C_{50} species in relation to C_{54}, with C_{52} being the major species. Subject B was a 48–year–old male with a plasma total cholesterol of 177 mg% and plasma total triacylglycerol of 402 mg%. His plasma total lipid level was 629 mg%. He had consumed 201 g of starch per day along with 37 g of saturated fat, 38 g of monounsaturated fat and 12 g of polyunsaturated fat per day, as well as 377 mg of cholesterol per day. The marked reduction in dietary fat in relation to startch had little effect on the acylglycerol profile, when compared to that recorded for subject A.

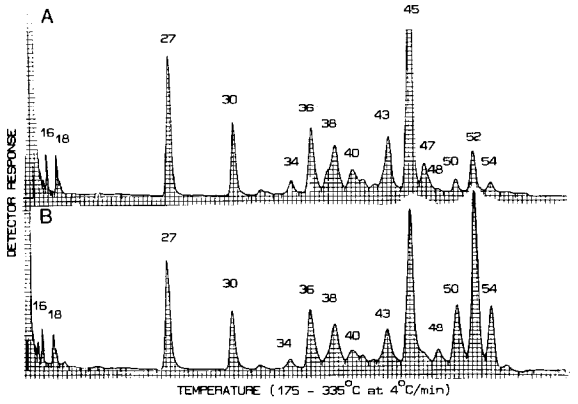

Fig. 1. GC profiles of plasma total lipids for subjects consuming diets high in starch. (A) Normolipemic subject; (B) Type IV hyperlipemia subject. Peaks: 16 and 18 = free fatty acids with 16 and 18 carbon atoms; 27 = free cholesterol; 30 = tridecanoylglycerol, internal standard; 34 = palmitoylsphingosine; 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 conditions of sample preparation as given in text. Retention time of peak 54 was 40 min.

Fig. 2 gives the regression line obtained for the ratios of C_{50}/C_{54} triacylglycerols *versus* starch intake for the male population. With increasing starch consumption, a decrease is seen in the ratio. This negative correlation was also seen for the females not taking hormones and those on the pill. Furthermore the females achieved a comparable degree of change in the C_{50}/C_{54} ratio with about one half the male intake of starch. The correlations in females, however, were not statistically significant even after correction for differences in caloric intake. The decrease in the C_{50}/C_{54} triacylglycerol ratio correlates with the decrease in the relative proportions of the C_{36}/C_{38} peaks, which represent the C_{34} and C_{36} diacylglycerol moieties of the PC.

Fig. 3 gives the regression line for the ratios of C_{34}/C_{36} diacylglycerols *versus* intake of starch for the females not taking hormones. A negative response is seen over the range of the peak-area ratios 0.935–0.975. These changes are similar to those seen for the males over the ratio range of

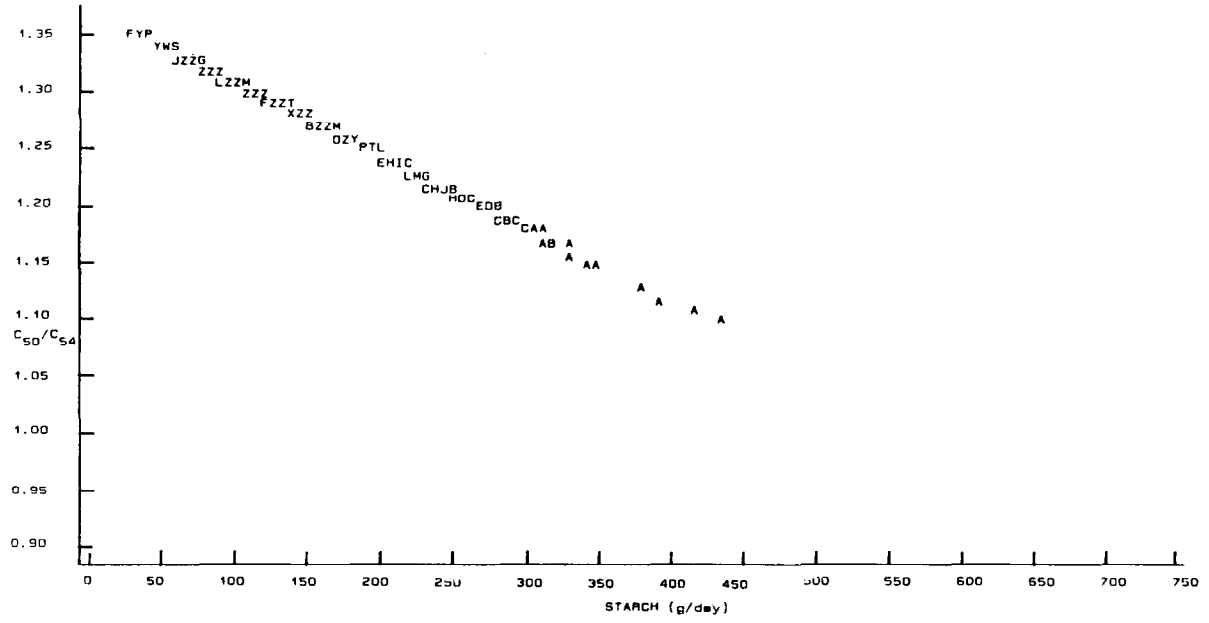

Fig. 2. Regression line for appropriately weighted points of the ratio C_{50}/C_{54} of plasma lipids *versus* starch intake in 769 male subjects. A = one, B = two, C = three observations, etc.

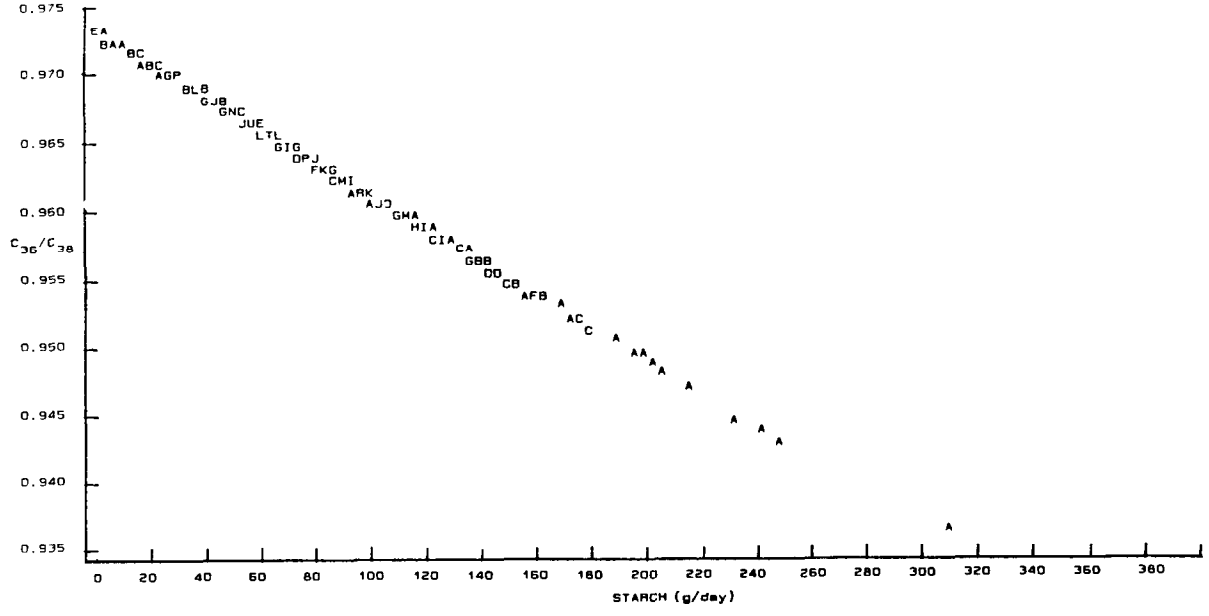

Fig. 3. Regression line for appropriately weighted points of the ratio C_{36}/C_{38} of plasma lipids *versus* starch intake in 250 females not taking hormones. A = one, B = two, C = three observations, etc.

0.90–0.99 (data plots not shown). In contrast, females on the pill showed a positive regression line over a comparable range of ratios, 1.02–1.11 (data not shown). However, the females taking hormones numbered only 80 subjects and the response became negative and statistically non-significant following correction for caloric intake. A negative response was also obtained when all the females were combined (data not shown).

Fig. 4 gives the regression lines for the SPH/PC

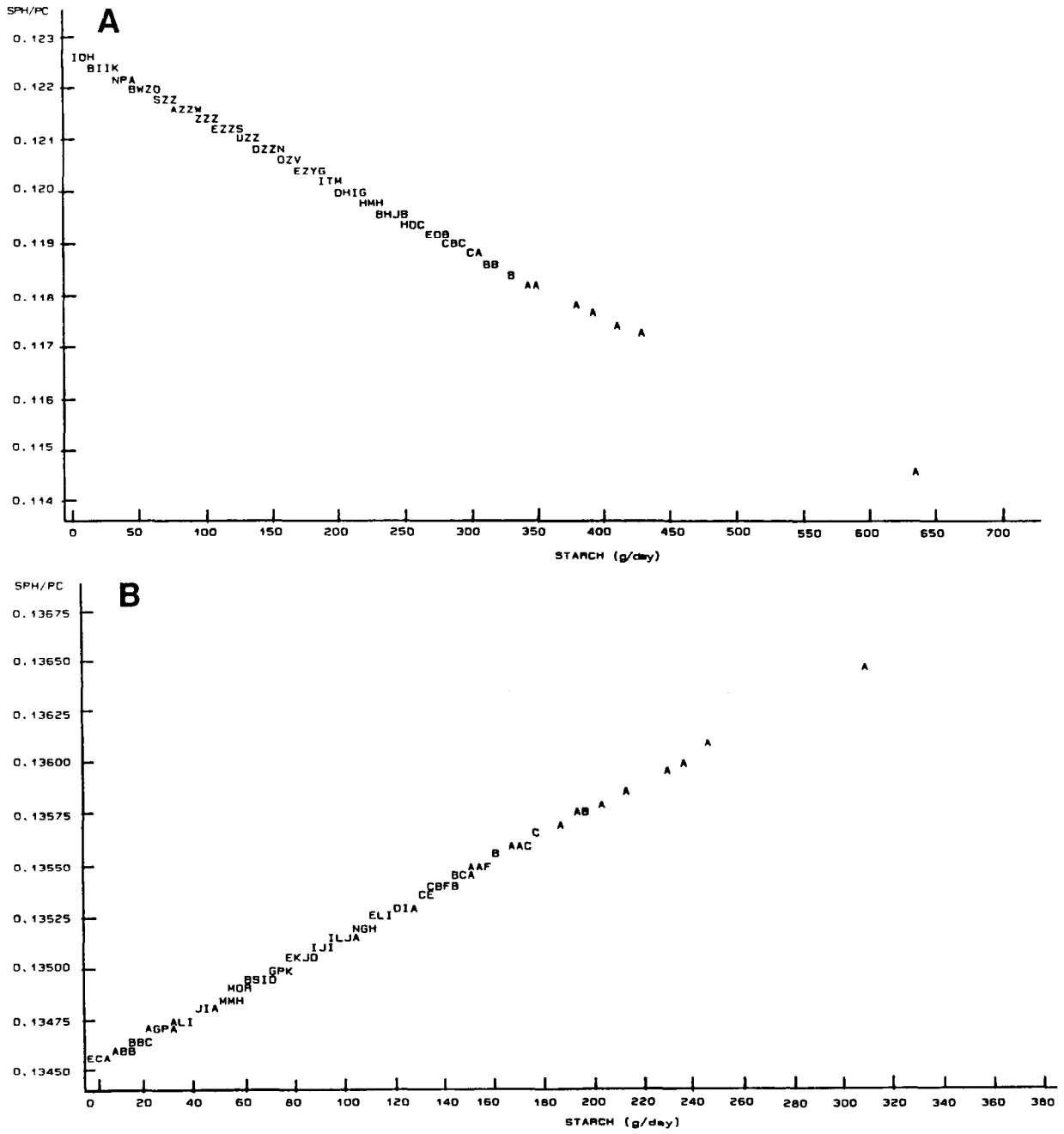

Fig. 4. Regression lines for appropriately weighted points of the ratio SPH/PC of plasma lipids *versus* starch intake for 769 males (A) and 250 females not taking pill (B). A = one, B = two, C = three observations, etc.

ratio *versus* increased starch intake for the male population (upper panel) and the female population not on pill (lower panel). With increasing starch consumption, the males show a negative correlation, which becomes positive after equalization of caloric intake. The females not on pill show a positive correlation, which turns negative following equalization of caloric intake. A positive correlation was obtained also for females on the pill before and after correction for differences in caloric intake (data plots not shown). However, consistent and significant negative correlations were obtained when either the female populations were combined ($r_s = -0.12$; $P < 0.01$; $n = 400$) or when the male and female populations of Visit 1 were combined ($r_s = -0.11$; $P < 0.0001$; $n = 1243$). Both males and females gave significant negative correlations ($r_s = -0.11$–0.12; $P < 0.002$–0.016; $n = 400$–773) for the PC/FC ratios with increasing starch intake (data plots not shown).

Effect of alcohol

Fig. 5 shows the total lipid profiles of plasma from two Type IV hyperlipoproteinemia subjects, who reported high intakes of alcohol during the week prior to blood letting. Subject A was a 33-year-old male with a plasma total cholesterol level of 285 mg% and plasma total triacylglycerol level of 352 mg%. The plasma total lipid level was 908 mg%. This subject had reported an alcohol intake of 162 ml per day along with 36 g of saturated fat, 42 g of monounsaturated fat and 18 g of polyunsaturated fat per day, as well as a daily intake of 1400 mg of cholesterol. The total carbohydrate intake had been 193 g per day with 111 g per day from starch. As a result of the high intake of alcohol in presence of a modest intake of saturated and unsaturated fat, there has occurred a marked distortion of the plasma lipid profile. Thus, there has been a significant increase in the ratio of the C_{36}/C_{38} and especially of C_{36}/C_{40} diacylglycerols. Similarly, there has been a shift towards the shorter chain length among the molecular species of triacylglycerols, as indicated by the increased C_{50}/C_{52} and C_{50}/C_{54} triacylglycerol ratios. There has also occurred a relative increase in the proportion of the cholesteryl palmitate in comparison to the combined cholesteryl oleate and linoleate peaks. The large increase in plasma total lipids is largely due to the elevation of the triacylglycerols. Subject B was a 34-year-old male with a total plasma cholesterol of 198 mg% and total plasma triacylglycerol of 390 mg%. The plasma total lipid content was estimated to be 767 mg%. The subject had consumed 189 ml of alcohol per day along with 90 g of saturated fat, 69 g of monounsaturated fat and 24 g of polyunsaturated fat per day, as well as 790 mg of cholesterol per day. The total carbohydrate intake was reported to be 409 g per day with 199 g per day due to starch. Despite the much higher intake by subject B of the saturated dietary fat (three-fold over that of subject A), the characteristic shift towards the shorter chain length of the glycerolipid species persists during comparable alcohol intake. Likewise, the proportion of cholesteryl palmitate has increased relative to the combined contribution of cholesteryl oleate and linoleate. Similar total lipid profiles were recorded for other hyperlipemic and normolipemic subjects reporting high intakes of alcohol in presence of high or low intakes of dietary fats. With minimal intakes of alcohol the plasma total lipid profiles tended to reflect the patterns seen on mixed diets [1,12–14].

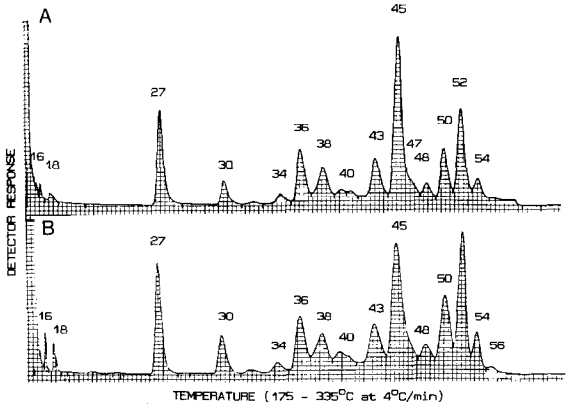

Fig. 5. GC profiles of plasma total lipids for Type IV lipoproteinemia subjects consuming alcohol along with (A) high and (B) low amounts of saturated dietary fat. Peak identification and GC conditions as given in Fig. 1. Retention time of peak 54 was 40 min.

Fig. 6 gives the regression lines for the C_{50}/C_{54} ratios of triacylglycerols *versus* the intake of alcohol for the males. The correlation is positive as the alcohol consumption increases from 0 to 360 ml per day. The C_{50}/C_{54} ratio ranges from 1.2 to 2.4. A similar positive correlation was obtained for the females not on pill (ratio range 1.26–2.4) as the alcohol consumption increased from 0 to 130 ml per day. The females on pill did not give a statistically significant correlation between increased alcohol intake and the C_{50}/C_{54} ratio.

Fig. 7 gives the regression line for the C_{36}/C_{38} ratio of the diacylglycerol moieties of PC *versus* increasing alcohol intake for the male population. Similar results were obtained for females not on pill (data not shown). In both instances a statistically significant positive correlation was realized over the ratio ranges 0.950–1.250 and 0.950–1.175, respectively. The females on pill did not give a statistically significant correlation between increased alcohol intake and the C_{36}/C_{38} ratio.

The regression line for the SPH/PC ratio *versus* increasing alcohol intake for the male population showed a positive correlation over the SPH/PC ratio range 0.120–0.129 ($r_S = 0.017$; $P < 0.6424$; $n = 773$), while the females not on pill gave a negative correlation over the range 0.1340–0.1352 ($r_S = -0.0265$; $P < 0.6763$; $n = 250$). A negative correlation was also obtained for the SPH/PC ratio for the females on pill ($r_S = -0.1311$; $P < 0.2464$; $n = 80$) over a somewhat wider ratio range (0.0900–0.1125) (data plots not shown). These values were not statistically significant and remained unaffected by adjustment for differences in caloric intake.

Effect of other carbohydrates

Table I summarizes the Spearman correlation coefficients and probabilities for all the dietary carbohydrate components and those peak-area ratios of the total lipid profile, which showed high statistical significance. The highest correlations ($r_S = 0.15$–0.21; $P < 0.0001$–0.0022; $n = 396$–773) were found between increased alcohol intake and increasing C_{50}/C_{54} and C_{36}/C_{38} ratios in both male and female subject groups.

Increasing consumption of starch correlated

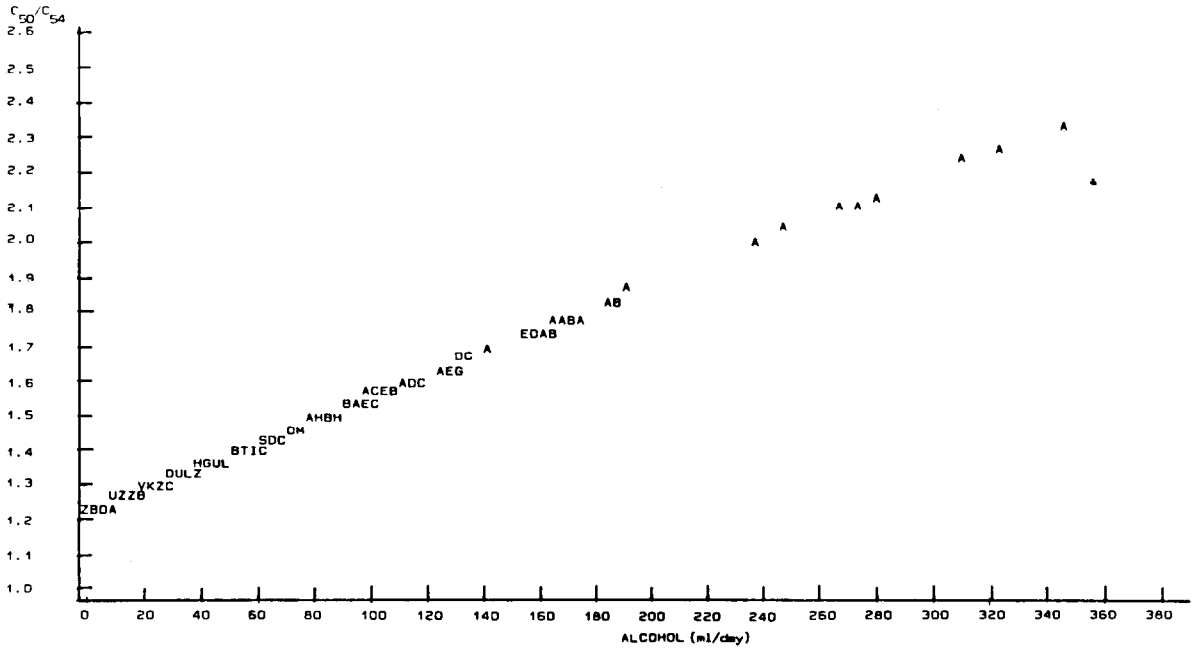

Fig. 6. Regression line for appropriately weighted points of the ratio C_{50}/C_{54} plasma lipids *versus* alcohol intake in 769 male subjects. A = one, B = two, C = three observations, etc.

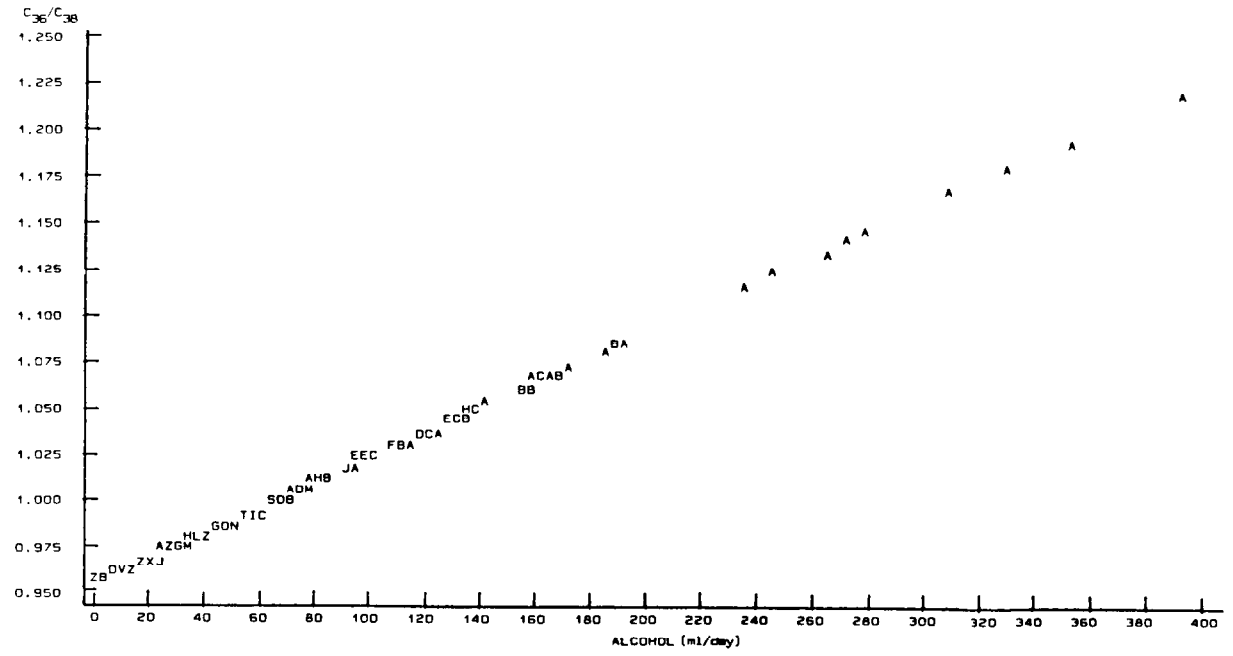

Fig. 7. Regression line for appropriately weighted points of the ratio C_{36}/C_{38} of plasma lipids *versus* alcohol intake in 769 male subjects. A = one, B = two, C = three observations, etc.

significantly ($r_S = -0.07$–0.12; $P < 0.0001$–0.04; $n = 773$) with decreased PC/FC, C_{50}/C_{54} and C_{36}/C_{38} ratios in the male, but not in the female population groups. Likewise, increased consumption of fiber correlated significantly ($r_S = -0.15$; $P < 0.0001$; $n = 769$) with decreased C_{50}/C_{54} ratio in males but not in females. No significant correlations were observed between increased sucrose intake and any of the peak-area ratios of the total lipid profile (data not shown). Table I also includes a positive correlation between increasing water consumption and the C_{50}/C_{54} ratio, which is highly significant for the male ($r_S = 0.1$; $P < 0.007$; $n = 769$) but only marginally significant for the female ($r_S = 0.1$; $P < 0.06$; $n = 396$) population.

Table II gives the correlation coefficients and probabilities for the more significant correlations between the dietary carbohydrate intake and specific parameters of the plasma total lipid profiles following normalization for total caloric intake. There are significant changes in the strengths and direction of the correlations, when compared to the original data given in Table I. Thus, it is now apparent that a relative increase in the consumption of total carbohydrate is highly significantly ($r_S = -0.11$; $P < 0.0013$; $n = 769$) correlated with decreased C_{50}/C_{54} ratio in males but not in females. In addition, a relative increase in the total carbohydrate consumption is accompanied by a highly significant ($r_S = -0.11$–0.13; $P < 0.0015$–0.0065; $n = 400$–773) decrease in the PC/FC ratio in both males and females. The effect of a relative increase in starch consumption, when expressed on basis of equal caloric intake remained about the same for all parameters of the profile as that seen for an increase in the absolute consumption, except that the correlation was now much stronger ($r_S = -0.12$–0.08; $P < 0.01$–0.04; $n = 396$) in the female population group. After normalization for total caloric intake, the relative increase in alcohol consumption remained highly significantly correlated with all parameters of the lipid profile increased on an absolute basis in both males and females. The relative increase in the consumption of fibre is now significantly ($r_S = -0.1$; $P < 0.0024$; $n = 773$) correlated with a decreased PC/FC ratio in males, but

TABLE I

CORRELATION OF DIETARY INTAKE OF SPECIFIC CARBOHYDRATE NUTRIENTS AND SELECTED PARAMETERS OF PLASMA TOTAL LIPID PROFILE

r_S = Spearman coefficient; P = probability; n = number of subjects; other abbreviations as given in text.

Dietary variable	Statistical test	Values of total lipid profile					
		PC/FC		C_{50}/C_{54}		C_{36}/C_{38}	
		Males	Females	Males	Females	Males	Females
Total CHO	r_S			−0.0661	−0.0078		
	P			0.0666	0.8767		
	n			769	396		
Starch	r_S	−0.1113	−0.0101	−0.1207	−0.0666	−0.0724	−0.0211
	P	0.0019	0.8391	0.0008	0.1859	0.0441	0.6739
	n	773	400	769	396	773	400
Alcohol	r_S	0.1880	0.1528	0.1976	0.1858	0.1778	0.2143
	P	0.0001	0.0022	0.0001	0.0002	0.0001	0.0001
	n	773	400	769	396	773	400
Fiber	r_S			−0.1517	−0.0243	−0.0589	−0.1095
	P			0.0001	0.6290	0.1013	0.0285
	n			769	396	773	400
Water	r_S			0.0967	0.0946		
	P			0.0073	0.0599		
	n			769	396		

TABLE II

CORRELATION OF DIETARY INTAKE OF SPECIFIC CARBOHYDRATE NUTRIENTS AND SELECTED PARAMETERS OF PLASMA TOTAL LIPID PROFILE FOLLOWING NORMALIZATION FOR DIFFERENCES IN TOTAL CALORIC INTAKE

r_S = Spearman coefficient; P = probability; n = number of subjects; other abbreviations as given in text.

Dietary variable	Statistical test	Values of total lipid profile					
		PC/FC		C_{50}/C_{54}		C_{36}/C_{38}	
		Males	Females	Males	Females	Males	Females
Total CHO	r_S	−0.1143	−0.1358	−0.1156	−0.0462		
	P	0.0015	0.0065	0.0013	0.3583		
	n	773	400	769	396		
Starch	r_S	−0.1113	−0.1205	−0.1465	−0.1026	−0.0878	−0.0835
	P	0.0019	0.0159	0.0001	0.0412	0.0146	0.0954
	n	773	400	769	396	773	400
Alcohol	r_S	0.1946	0.1553	0.2109	0.1864	0.1820	0.2116
	P	0.0001	0.0018	0.0001	0.0002	0.0001	0.0001
	n	773	400	769	396	773	400
Fiber	r_S	−0.1091	−0.0355	−0.1646	−0.0432	−0.0442	−0.1590
	P	0.0024	0.5035	0.0001	0.3913	0.2193	0.0014
	n	773	400	769	396	773	400
Water	r_S			0.1183	0.0818	0.1270	−0.0339
	P			0.0010	0.1040	0.0004	0.4986
	n			769	396	773	400

not in females. The correlation between increased relative fibre intake and a decreased C_{50}/C_{54} ratio remained highly significant in the males, while in the females the correlation with decreased C_{36}/C_{38} ratio became significant ($r_S = -0.16$; $P < 0.0014$; $n = 400$). Likewise, the positive correlation between increase in water intake and increase in C_{50}/C_{54} triacylglycerol ratio in males remained significant and a new significant ($r_S = 0.13$; $P < 0.0004$; $n = 773$) positive correlation between C_{36}/C_{38} ratio and increased water intake appeared.

DISCUSSION

Dietary carbohydrate and plasma lipid profile

According to the study of Gordon and coworkers [2,4], increased intake of starch and sucrose were positively and significantly related to plasma triacylglycerols in women but not in men. In the present study increased total carbohydrate consumption was positively correlated with plasma triacylglycerol levels in the combined population before adjustment for caloric intake and negatively correlated after adjustment for differences in caloric intake. The magnitude of this effect may depend in part on the type of subjects investigated. Thus, Antar *et al.* [16] found that sucrose was more hyperlipidemic in patients with triglyceridemia (Type III, IV and V) than in patients with hypercholesterolemia (Type II). These studies have been confirmed by detailed analysis of the plasma lipids by high temperature GC [17]. However, normolipemic subjects and diabetics also respond to increases in dietary fructose with increased plasma triacylglycerols [18], although the fatty acid composition has not been studied in detail.

Increased consumption of complex carbohydrate led to a decreased PC/FC ratio in both males and females, when considered as total carbohydrate, starch or fibre, and when corrected for differences in total caloric intake. The reason for this effect is not known. It is possible that the decreased PC/FC ratio resulted from a decreased plasma high-density lipoprotein (HDL), which is normally responsible for a high PC/FC ratio in plasma. Ernst *et al.* [5] also found inverse although weaker relationships between HDL (as estimated from HDL-C) and intakes of total carbohydrate, sucrose and starch (percent calories and grams). Strong negative correlations between carbohydrate and sucrose intake and LDL (as estimated from LDL-C) were found by Schwarz *et al.* [3], but relative decreases in LDL would be expected to increase plasma PC/FC ratio. The decreased SPH/PC ratio with increased starch consumption also could have resulted from decreased LDL, which is rich in SPH, as well as from increases in HDL, which is rich in PC [1,13,14]. There was a strong correlation between increased dietary fibre intake and decreased PC/FC ratio in the male population. A possible explanation for the decreased PC/FC ratio could be reduced uptake of dietary and biliary PC during increased fibre consumption [19].

Increased carbohydrate consumption was anticipated to result in increased C_{50}/C_{54} and C_{36}/C_{38} ratios in both males and females. This effect could be rationalized on the basis of increased biosynthesis of fatty acids, which would represent largely the C_{16} chain length in both plasma triacylglycerols and PC, and for which preliminary evidence had been obtained previously [18]. In the present study, however, consumption of starch and fibre both led to decreased C_{50}/C_{54} and C_{36}/C_{38} ratios, while increased consumption of sucrose had no significant effect on these ratios. There was a highly significant correlation between increased consumption of fibre and decreased C_{50}/C_{54} ratio in males and a decreased C_{36}/C_{38} ratio in females after caloric normalization. It is possible that increased consumption of fibre was associated with decreased consumption and/or biosynthesis of C_{16} saturated fatty acids. The lack of correlation between sucrose consumption and any of the parameters of the plasma total lipid profile is possibly due to the reported opposite effects of the glucose and fructose components of sucrose upon plasma lipids [18]. It was also possible that the variable effect of the carbohydrate upon the plasma lipid profile was due to the uncontrolled nature of the parallel consumption of saturated and unsaturated fats.

There were no means for correcting this problem since the subjects consumed essentially free-choice meals. Nevertheless, certain highly significant correlations were seen.

Dietary alcohol and plasma lipid profile

Cross-sectional data on alcohol consumption in men have shown [20] a positive association with plasma triacylglycerol levels. In the present study increased alcohol intake was not associated with a significant increase in plasma triacylglycerols. According to Gordon and co-workers [2,4], alcohol consumption in men showed a positive correlation with triacylglycerol levels, while for women there was an inverse relation between alcohol consumption and triacylglycerol level (when alcohol level was expressed as percentage of total calories). In the present study, alcohol elicited the most uniform effect on the composition of the molecular species of the plasma glycerolipids. Increased alcohol consumption resulted in highly significant increases in the C_{50}/C_{54} and C_{36}/C_{38} ratios in both males and females. These effects persisted after correction for differences in the caloric intake. The highest statistical significance was observed for the correlation between increased alcohol intake and increased PC/FC ratio in both males and females. The finding can be rationalized on the basis of increased formation of HDL, which possesses a high PC/FC ratio or reduced LDL, which has a low PC/FC ratio [1,13,14]. These observations are consistent with the findings of Ernst *et al.* [5], who observed a strong positive gradient of HDL (estimated as HDL-C) levels with increasing alcohol intake in both men and women. According to Schwarz *et al.* [3], LDL (estimated as LDL-C) levels were negatively related to alcohol consumption in women, but showed weak positive correlation to alcohol intake in men. The significant negative correlation of the SPH/PC ratio with increasing alcohol consumption in the females can be attributed to increased HDL, which is rich in PC, and lowered LDL, which is rich in SPH [1,13,14]. The positive correlation in men between SPH/PC ratio and alcohol consumption must be attributed to the weak positive correlation with LDL levels shown by Schwarz *et al.* [3].

Effect of hormones

The present study allowed also an assessment of the effect of gonadal hormones upon the plasma total lipid profiles, although the number of hormone takers involved was relatively small. In general, the correlations obtained for the non-taker group corresponded fully with the results described for the much larger male group, while the hormone-treated group occasionally showed the opposite trend or failed to give statistically significant correlations. In the study of Schwarz *et al.* [3] no clinical chemistry measurements emerged as correlates of LDL-C in females using hormones. In the present study, increased consumption of alcohol was positively associated with increased C_{50}/C_{54}, C_{36}/C_{38} and PC/FC ratios in the non-takers, while the takers did not give statistically significant correlations. Increased consumption of fibre was negatively correlated with C_{36}/C_{38} ratio in the non-takers.

It is possible that the subdivision of the female population into users and non-users of gonadal hormones resulted in insufficiently large numbers of subjects for obtaining significant correlations in the hormone group in view of the high variance. As a result, many of the apparently significant statistical correlations seen in the total female population were lost. However, the correlations that remained strong were of the same direction and magnitude as those seen for the total female and male population. The lipid class ratio most significantly affected by diet in the two subgroups of the female population was the PC/FC ratio. Both hormone and control groups showed in general the same response as the male population, which indicated that the consumption of gonadal hormones had no significant effect upon the plasma lipid profile. This is in agreement with a previous study [21], which failed to show significant differences in plasma lipid profiles between groups of females taking gonadal hormones and controls. Variable and inconsistent correlations between HDL-C and clinical chemistry data in women taking gonadal hormones and controls have been reported by Pattern *et al.* [22].

Significance of study

The present study was carried out on a pop-

ulation subsisting on free-choice diets, the amounts and composition of which were derived from a 24-h dietary recall. Nevertheless, the observations made appear to attest to the general truthfulness of the 24-h dietary recall. Among the most significant parameters were the PC/FC and C_{50}/C_{54} triacylglycerol ratios, which were associated with the starch and alcohol consumption in quantitatively and qualitatively opposite directions. The present confirmation and extension of previous results is valuable in view of the controversial nature of the effects of high carbohydrate and alcohol consumption upon plasma lipids and lipoproteins. These data and the proposed explanations provide the consistency, responsiveness and mechanisms needed to support the notion of cause [23] in confirming the essential accuracy of the 24-h dietary recall as conducted by the CPPT. Finally, these studies serve as an encouragement to seek more meaningful qualitative and quantitative correlations between diet and plasma lipids by means of high-temperature capillary GC on polarizable liquid phases [24], which allow resolution of glycerolipids and cholesteryl esters based on both carbon number and degree of unsaturation.

ACKNOWLEDGEMENTS

This study was supported by funds from the Heart and Stroke Foundation of Ontario (Toronto, Canada), the Medical Research Council of Canada (Ottawa, Canada) and the United States Heart, Lung and Blood Institute, NIH-NHLI-72-917 (Bethesda, MD, USA).

REFERENCES

1 A. Kuksis, J. J. Myher, K. Geher, W. C. Breckenridge, T. Feather, V. McGuire and J. A. Little, *J. Chromatogr.*, 564 (1991) 11–26.
2 D. J. Gordon, M. Fisher, N. Ernst and B. M. Rifkind, *Arteriosclerosis*, 2 (1982) 502–512.
3 W. Schwarz, D. C. Trost, S. L. Reiland, B. M. Rifkind and G. Heiss, *Arteriosclerosis*, 2 (1982) 513–522.
4 D. J. Gordon, K. M. Salz, K. J. Roggenkamp and F. A. Franklin, *Arteriosclerosis*, 2 (1982) 537–548.
5 N. Ernst, M. Fisher, W. Smith, T. Gordon, B. M. Rifkind, J. A. Little, M. A. Mishkel and O. D. Williams, *Circulation*, 62 (Suppl. IV) (1980) IV-41–IV-52.
6 The Lipid Research Clinics Program, *J. Chron. Dis.*, 32 (1979) 609–631.
7 B. Dennis, N. Ernst, M. Hjortland, J. Tillotson and V. Grambsch, *J. Am. Diet. Assoc.*, 77 (1980) 641–647.
8 G. H. Beaton, J. Milner, P. Corey, V. McGuire, M. Cousins, E. Stewart, M. de Ramos, D. Hewitt, V. Grambsch, N. Kassim and J. A. Little, *Am. J. Clin. Nutr.*, 37 (1979) 2546–2559.
9 G. H. Beaton, J. Milner, V. McGuire, T. E. Feather and J. A. Little. *Am. J. Clin. Nutr.*, 37 (1983) 986–995.
10 G. H. Beaton, *Arteriosclerosis*, 2 (1982) 500–501.
11 Lipid Research Clinics Program, *Manual of Laboratory Operations, Vol. 1, Lipid and Lipoprotein Analysis, DHEW Publication No. (NIH) 75–628*, NIH, Washington, DC, 1974.
12 A. Kuksis and J. J. Myher, *J. Chromatogr.*, 379 (1986) 57–90.
13 A. Kuksis, J. J. Myher, K. Geher, N. A. Shaikh, W. C. Breckenridge, G. J. L. Jones and J. A. Little, *J. Chromatogr.*, 182 (1980) 1–26.
14 A. Kuksis, J. J. Myher, K. Geher, G. J. L. Jones, W. C. Breckenridge, T. Feather, D. Hewitt and J. A. Little, *Arteriosclerosis*, 2 (1982) 296–302.
15 J. H. Zar, *Biostatistical Analysis*, Prentice-Hall, Englewood Cliffs, NJ, 1974, pp. 243–244.
16 M. A. Antar, J. A. Little, C. Lucas, G. C. Buckley and A. Csima, *Arteriosclerosis*, 11 (1970) 191–201.
17 A. Kuksis, O. Stachnyk, G. C. Buckley and J. A. Little, 1972, unpublished results.
18 J. Hallfrisch, *FASEB J.*, 4 (1990) 2652–2660.
19 J. W. Anderson, B. A. Deakins, T. L. Floore, B. M. Smith, and S. E. Whitis, *Crit. R. Food Sci. Nutr.*, 29 (1990) 95–147.
20 W. P. Castelli, T. Gordon, M. E. Hjortland, A. Kagan, J. T. Doyle, C. G. Hames, S. B. Hulley and W. J. Zukel, *Lancet*, ii (1977) 153–158.
21 A. Hedlin, A. Kuksis and K. Geher, *Obstet. Gynecol.*, 52 (1978) 430–435.
22 R. L. Patten, D. Hewitt, G. T. Waldman, G. Jones and J. A. Little, *Circulation*, 62 (Suppl. IV) (1980): IV-31–IV-41.
23 F. Mosteller and J. W. Tukey, *Data Analysis and Regression*, Addison-Wesley, Reading, MA, 1977, p. 210.
24 A. Kuksis, J. J. Myher and P. Sandra, *J. Chromatogr.*, 500 (1990) 427–441.