The Human Cholesteryl Ester Transfer Protein I405V Polymorphism Is Associated with Plasma Cholesterol Concentration and Its Reduction by Dietary Phytosterol Esters[1]

Ana M. Lottenberg,[2,3] Valéria S. Nunes,[3] Edna R. Nakandakare, Mônica Neves, Márcia Bernik, Laurent Lagrost,* José E. dos Santos† and Eder Quintão

*Lipid Laboratory (LIM10), University of São Paulo Medical School, São Paulo Brazil; *INSERM U498, Laboratoire de Biochimie Médicale, Hôpital du Bocage, Dijon, France; and †University of São Paulo Medical School, Ribeirão Preto, Brazil*

ABSTRACT We examined the relationships of I405V cholesteryl ester transfer protein (CETP), Taq1B CETP and apolipoprotein (apo)E polymorphisms with the pattern of response to dietary plant sterol ester (PSE) by plasma lipids and CETP concentrations as well as lecithin-cholesterol acyltransferase (LCAT) activity. Subjects with moderate primary hypercholesterolemia (20–60 y old; 50 women; 10 men) consumed margarine (20 g/d) without (placebo) or with PSE (2.8 g/d = 1.68 g/d phytosterols) for 4 wk each period, in a crossover, double-blind study. Plasma CETP concentration was measured by ELISA; endogenous LCAT activity was expressed as the percentage of esterification (30 min incubation) of the subjects' ^{14}C-unesterified cholesterol HDL. PSE reduced concentrations of plasma total cholesterol (TC) (10%) and LDL cholesterol (LDL-C) (12%). In relation to the I405V CETP polymorphism, the percentage reductions in TC with consumption of PSE for the II, IV and VV phenotypes were 7.2, 4.2 and not significant, respectively, whereas LDL-C significant reductions occurred only for II (9.5%). However, the CETP concentration diminished only in the II phenotype. J. Nutr. 133: 1800–1805, 2003.

KEY WORDS: • *plasma lipoproteins* • *cholesteryl ester transfer protein polymorphism* • *plant sterol ester* • *apoE polymorphism.*

Dietary modifications such as the addition of naturally occurring plant sterols (PSE)[4] are known to lower plasma cholesterol concentration in humans and experimental animals (1–8). In humans, intakes of ~2 g/d of plant sterols achieve this effect without modification of the concentrations of HDL cholesterol (HDL-C) and triacylglycerols (TG) (2–4). These results led to the enrichment of commercially available margarines with esters of plant sterols or stanols (5–9). In addition, the safety and tolerability of esterified phytosterols have been clearly demonstrated (10).

Some studies have dealt with the interaction of genetics with the response of plasma lipid concentrations to changes in dietary fat or cholesterol (11,12). Individuals who carry the apolipoprotein (apo)E4/4 phenotype seem to have the highest response of plasma cholesterol to dietary cholesterol, whereas those with the apoE2/2 phenotype have the lowest (13–15). On the other hand, dietary sitostanol ester increases cholesterol synthesis rates and lowers LDL cholesterol (LDL-C) predominantly in individuals with the apoE4 genotype (16,17).

The mechanisms of control of cholesteryl ester transfer protein (CETP) require investigation because of the association of plasma CETP concentrations with the risk for premature atherosclerosis [for a review, see (18)]. CETP concentration in plasma is dependent on several factors, including environmental components, such as alcohol (19) and dietary cholesterol (20), and on genetic influences, such as the polymorphisms of CETP (21–25) and apoE (20). Furthermore, several studies have demonstrated that plasma CETP and cholesterol concentrations are interrelated; however, the mechanisms involved are not fully understood (26–30). In humans, plasma LDL-C and CETP concentrations decreased simultaneously with consumption of unsaturated fatty acid diets in one study (31), but not in another (32). In addition, the simultaneous variation of LDL-C concentration and of CETP by hypolipidemic drugs has been reported in some (26–30,33) but not all studies (29,34). On the other hand, cholesterol consumption by humans and rabbits simultaneously raises plasma concentrations of CETP and LDL-C, and CETP mRNA in rabbit liver and human adipose tissue (20,35), indicating that the CETP gene may be regulated by diet-induced changes in cholesterol. In one study on cholesterol consumption, the variation of plasma CETP, but not of LDL-C, was associated with the apoE gene polymorphism and

[1] Supported by Gessy Lever Incorporated (Unilever Division), SP, Brazil.
[2] To whom correspondence should be addressed. E-mail: lipideq@usp.br.
[3] A.M. Lottenberg and V. S. Nunes contributed equally to this work.
[4] Abbreviations used: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; HDL-C, HDL cholesterol; LCAT, lecithin-cholesterol acyltransferase; LDL-C, LDL cholesterol; PSE, plant sterol ester; TC, total cholesterol; TG, triacylglycerols.

Manuscript received 2 December 2002. Initial review completed 2 January 2003. Revision accepted 7 March 2003.

was lowest in the apoE4/3 phenotype compared with the E3/2 and E3/3 phenotypes (20).

CETP polymorphisms have been related to variations in plasma HDL-C (22–25,36–38); some polymorphisms, i.e., Taq1B, I405V and R451Q, are associated with simultaneous variations in the concentrations of HDL and CETP (22–25,36,37). However, it is not known whether CETP gene polymorphisms are associated with variations in plasma lipoproteins other than HDL, although the Taq1B CETP polymorphism is related to the LDL size (24). Furthermore, in spite of reports of a close relationship between CETP plasma concentration and the intake of cholesterol, as well as fatty foods, in both humans and monkeys (20,27,31,32,35,39–41), no studies have reported an association of the CETP polymorphisms with the responses of the plasma CETP concentration to the intestinal absorption of cholesterol in food.

Our goals were to gain insight into the effect of the absorption of intestinal cholesterol on the concentrations of plasma lipids and CETP and to determine whether variations in these concentrations relate to common polymorphisms of CETP (I405V and Taq1B), as well as of apoE. This was accomplished by the dietary use of a PSE-enriched margarine, which is known to inhibit the intestinal absorption of cholesterol.

SUBJECTS AND METHODS

Subjects. Sixty patients (50 women and 10 men) between 20 and 60 y of age, with body mass index < 30 kg/m^2, were recruited from the outpatient clinics at the Clinical Hospitals of the University of São Paulo Medical School, São Paulo and Ribeirão Preto campuses. These were individuals with moderate primary hypercholesterolemia (mmol/L, mean ± SD: TC = 7.0 ± 0.8 and TG = 2.0 ± 0.8); only three participants had plasma TG $>$ 3.4 mmol/L, i.e., 3.8, 3.9 and 4.8 mmol/L. The participants had not previously taken hypocholesterolemic drugs, and all secondary forms of hyperlipidemia were excluded. None of the women were using hormonal contraceptives. Subjects gave their informed written consent and the Ethics Committee of both hospitals involved approved the protocol.

Design. The patients ($n = 60$) were enrolled in a double-blind, crossover study with random distribution into two groups of 30 patients each. Before joining the investigative protocol, they had all been consuming a prudent diet (baseline period) in which the energy distribution was protein (15%), carbohydrate (56%) and fat (28%), with a polyunsaturated/saturated fatty acid ratio close to 1.5 and a cholesterol intake of ~170 mg/d. Thereafter, 30 participants were given margarine (20 g/d) without PSE (placebo) for 4 wk; they were next switched to margarine (20 g/d) with PSE (2.8 g/d = 1.68 g/d phytosterols) for the ensuing 4 wk. The other group ($n = 30$) began with consumption PSE and then switched to placebo. The phytosterols added contained ~45% β-sitosterol and 53% as a mixture of stigmasterol, campesterol and brassicasterol, esterified mainly with linoleic acid. Margarine was delivered in cups containing 20 g with the total daily amount eaten evenly distributed among the three major meals as an addition to slices of bread and equaling 8 g fat/d. A nutritionist carefully recorded the total amount eaten after weighing the returned cups once a week. Patients' body weight did not change throughout the study. Three blood samples were drawn at the baseline period, that is, immediately before entering the experimental periods (placebo and PSE), and at wk 3 and 4 of each experimental period (placebo or PSE). Data represent the mean of three determinations for the baseline period and two determinations for each experimental period. The Van den Berg division of Gessy Lever, São Paulo, Brazil, supplied the margarines.

Plasma lipoprotein analysis. After an overnight fast, venous blood samples were drawn into tubes containing 0.1% EDTA. The plasma was obtained by low speed centrifugation (1000 × *g* for 15 min) and the following preservatives were added per mL of plasma: aprotinin (0.1 trypsin inhibitor kU/L), 2 mmol/L benzamidine, 0.5% gentamicin plus 0.25% chloramphenicol, 0.5 mmol/L phenylmethylsulfonyl fluoride in dimethyl sulfoxide. All samples were stored at −70°C until further analysis and all determinations were conducted on the same batch.

ApoB-containing lipoproteins were precipitated with dextran sulfate/magnesium chloride (42) for the separation of HDL-C. Plasma cholesterol, TG and HDL-C were then measured with commercially available kits using the Cobas Mira (Roche) autoanalyzer system. Lipoprotein cholesterol fractions were estimated by the Friedewald formula (43). For the analyses of the responses of plasma lipids according to all polymorphisms, participants with plasma TG concentration $>$ 2.8 mmol/L were excluded because type IIb hyperlipidemics might not be as responsive as type IIa subjects to plasma cholesterol–lowering drugs.

CETP concentration and lecithin-cholesterol acyltransferase (LCAT) activity. CETP concentrations were measured using the ELISA procedure in the laboratory of Laurent Lagrost (INSERM U498, Dijon, France) (44). Plasma endogenous LCAT activity was determined according to the method of Dobiasova (45).

Determination of CETP and apoE polymorphisms. DNA was extracted by the salting-out method from whole blood white cells as described by Miller et al. (46) and was analyzed for apoE (47) and for the I405V (23) and Taq1B CETP polymorphisms (19).

Statistical analysis. Differences in plasma cholesterol, triacylglycerols, LDL-C, HDL-C, CETP concentrations and LCAT activity between placebo and PSE periods, as well as between the apoE phenotypes, were analyzed by the paired Student's *t* test, whereas ANOVA was utilized to compare all values among the CETP genotypes. LDL-C reductions elicited by PSE according to the apoE3/3 and apoE3/4 polymorphisms were compared among three concentration ranges of LDL-C as measured during the baseline phase utilizing one-way ANOVA with Bonferroni's multiple comparison post-test. The Spearman test was utilized to correlate the differences in CETP concentrations with those in total plasma cholesterol or LDL-C, represented by the PSE value minus the placebo value. Differences were considered significant at $P < 0.05$.

RESULTS

Lipids and cholesterol. Fasting plasma lipids, lipoproteins, CETP and LCAT values of all participants are presented during the baseline, placebo (PCB) and PSE-enriched margarine treatment periods (**Table 1**). Total plasma cholesterol and LDL-C concentrations diminished significantly after 4 wk of PSE consumption compared with the PCB and baseline periods. With PSE consumption, cholesterol and LDL-C concentrations were 10 and 12% lower, respectively, compared with the baseline period, and 6 and 8% lower, respectively, compared with the PCB period. TG and HDL-C concentrations did not differ. When all participants were divided into two

TABLE 1

Lipids and lipoprotein plasma concentrations, CETP mass, LCAT activity, during the baseline (BASAL), placebo (PCB) and added plant sterol ester (PSE) periods in male and female subjects with moderate primary hypercholesterolemia[1,2]

	BASAL	PCB	PSE
TC, *mmol/L*	7.0 ± 0.8	6.7 ± 0.8	6.3 ± 0.8*
LDL-C, *mmol/L*	5.0 ± 0.7	4.7 ± 0.7	4.4 ± 0.6*
HDL-C, *mmol/L*	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
TG, *mmol/L*	2.0 ± 0.8	1.8 ± 0.7	1.8 ± 0.7
CETP, *mg/L*	—	2.98 ± 0.78	2.83 ± 0.63†
LCAT, *%*	—	4.03 ± 1.55	3.99 ± 1.72

[1] Values are means ± SD, $n = 60$. * Different from PCB, $P < 0.001$; † Different from PCB, $P < 0.05$; paired Student's *t* test.

[2] Abbreviations: TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triacylglycerols; CETP, cholesteryl ester transfer protein; LCAT, lecithin-cholesterol acyltransferase.

TABLE 2

ApoE3/3 and apoE4/4 polymorphisms: lipids and lipoprotein plasma concentrations, CETP mass, during the placebo (PCB) and added plant sterol ester (PSE) periods in male and female subjects with moderate primary hypercholesterolemia[1,2]

	n	PCB	PSE	% Reduction (PSE − PCB)
TC, *mmol/L*				
3/3	35	6.6 ± 0.7	6.2 ± 0.8*	−6.9 (−28.3; 8.9)
3/4	16	6.6 ± 0.9	6.3 ± 0.9	−4.9 (−14.8; 11.2)
LDL-C, *mmol/L*				
3/3	32	4.7 ± 0.6	4.4 ± 0.6*	−6.3 (−28.9; 11.5)
3/4	16	4.7 ± 0.8	4.5 ± 0.8	−6.8 (−19.2; 14.4)
HDL-C, *mmol/L*				
3/3	32	1.1 ± 0.4	1.1 ± 0.3	0 (−26.2; 29)
3/4	16	1.1 ± 0.3	1.1 ± 0.3	−1.2 (−17.1; 19.5)
TG, *mmol/L*				
3/3	35	1.7 ± 0.6	1.6 ± 0.6	−5 (−50.2; 65.7)
3/4	16	1.7 ± 0.5	1.6 ± 0.6	0 (−42.5; 48.2)
CETP, *mg/L*				
3/3	35	2.92 ± 0.74	2.75 ± 0.60†	−5.60 (−30.90; 61.20)
3/4	14	3.21 ± 0.96	2.96 ± 0.69	−5.10 (−31.30; 5.30)

[1] Values are means ± SD and medians (range), $n = 60$. * Different from PCB, $P < 0.001$; † different from PCB, $P < 0.05$; paired Student's *t* test.
[2] See Table 1 for abbreviations.

groups according to their baseline plasma triacylglycerol levels, i.e., >2.3 mmol/L (68th percentile) and <1.9 mmol/L (51st percentile), they did not differ in total cholesterol (TC) and LDL-C responses to PSE treatment. Interestingly, compared with PCB, CETP concentrations decreased significantly from 2.98 ± 0.78 to 2.83 ± 0.63 mg/L after 4 wk of PSE consumption; however, LCAT plasma activity did not change.

Elevated plasma TG concentration cases (TG > 2.28 mmol/L, $n = 9$) were excluded from further analyses to investigate whether the polymorphisms of apoE and CETP relate to the regulation of plasma cholesterol and CETP concentrations to PSE treatment.

Polymorphisms, lipids and cholesterol. Significant reductions of TC, LDL-C and CETP concentrations were observed in the E3/3 but not in the E3/4 phenotype. However, the percentage reduction did not differ between the phenotypes (**Table 2**). During the placebo phase, TC and LDL-C concentrations were lower in subjects with the VV phenotype than in those with the IV phenotype; however, with PSE consumption, these concentrations no longer differed among the genotypes (**Table 3**). In addition, the significant percentage reductions in TC and LDL-C depended on the presence of the I405V polymorphism because of their occurrence in the II and in the IV, but not in the VV genotype. Although the CETP

TABLE 3

I405V CETP polymorphism: lipids and lipoprotein plasma concentrations and CETP mass, during the placebo (PCB) and the added plant sterol ester (PSE) periods in male and female subjects with moderate primary hypercholesterolemia[1,2]

	n	PCB	PSE	% Reduction (PSE − PCB)
TC, *mmol/L*				
II	15	6.5 ± 0.6	6.0 ± 0.8*	−7.2 (−28.3; 2.4)#
IV	27	6.9 ± 0.7#	6.6 ± 0.8*	−4.2 (−18.2; 8.9)#
VV	9	6.0 ± 1.0	6.1 ± 1.1	−0.4 (−10.0; 11.2)
LDL-C, *mmol/L*				
II	14	4.7 ± 0.5	4.2 ± 0.6†	−9.5 (−28.9; 9.2)#
IV	26	4.7 ± 0.7#	4.6 ± 0.7*	−6.3 (−19.2; 11.5)
VV	9	4.2 ± 0.6	4.2 ± 0.7	4.8 (−13.0; 13.3)
HDL-C, *mmol/L*				
II	15	1.1 ± 0.3	1.1 ± 0.3	−1.4 (−26.2; 18.0)
IV	26	1.1 ± 0.3	0.1 ± 0.3	0 (−17.1; 29.0)
VV	9	1.1 ± 0.3	1.1 ± 0.4	−2.3 (−17.6; 9.1)
TG, *mmol/L*				
II	15	1.6 ± 0.6	1.5 ± 0.5	−4.7 (−28.8; 43.3)
IV	27	1.7 ± 0.6	1.5 ± 0.5	−6.6 (−50.2; 65.7)
VV	9	1.7 ± 0.8	1.8 ± 0.9	5.4 (−42.5; 18.4)
CETP, *mg/L*				
II	14	3.02 ± 0.88	2.82 ± 0.79†	−5.8 (−30.9; 1.4)
IV	27	3.02 ± 0.89	2.86 ± 0.65	−4.9 (−31.3; 61.2)
VV	9	2.94 ± 0.42	2.69 ± 0.28	−6.1 (−27.2; 7.6)

[1] Values are means ± SD and medians (range) for % reduction. * Different from PCB, $P < 0.001$; † different from PCB, $P < 0.05$; # different from VV, $P < 0.05$; paired Student's *t* test.
[2] See Table 1 for abbreviations.

concentration was lowered in the II genotype, plasma CETP was not influenced by the I405V polymorphisms because the percentage reduction did not differ among the three I405V genotypes.

For the Taq1B CETP polymorphism, we observed that PSE treatment diminished plasma concentrations of TC and LDL-C in the B1B1 and B1B2 genotypes, in contrast to the CETP concentration, which was lowered in the B1B2 and B2B2 genotypes. However, the percentage reductions in plasma concentrations elicited by PSE occurred independently of the Taq1B CETP polymorphism (**Table 4**).

Significant correlations were demonstrated between CETP and TC ($r = 0.295$, $P = 0.041$, $n = 48$), or LDL-C ($r = 0.322$, $P = 0.031$ $n = 45$) concentrations during the placebo phase. These correlations were reported (20,26–30), but were no longer present with PSE consumption in our study. However, variations of the plasma concentrations of CETP and of TC brought about by PSE correlated with each other ($r = 0.295$, $P = 0.0491$, $n = 45$), although correlations did not occur between CETP and LDL-C. Finally, we also observed significant inverse correlations between LCAT activity and HDL-C concentration during the placebo ($r = -0.496$, $P = 0.012$) and PSE ($r = -0.525$, $P = 0.007$) periods.

DISCUSSION

The mean percentage reductions in LDL-C elicited by PSE according to the LDL-C baseline range concentrations (in parentheses) were −8.25% (3.4 to 4.6 mmol/L, $n = 18$), −8.60% (4.6 to 5.2 mmol/L, $n = 20$) and −16% (>5.2 mmol/L, $n = 20$). These percentage reductions in LDL-C among the three LDL-C ranges did not differ ($P = 0.06$). Therefore, in the highest LDL-C concentration ranges, e.g., >5.2 mmol/L, there was only a trend for benefiting the most from PSE margarine.

Negative correlations between plasma HDL-C concentration and LCAT activity were compatible with the physiologic roles played simultaneously by LCAT and CETP because LCAT makes HDL cholesteryl ester readily available for transfer to lighter density lipoproteins in exchange for TG, a process mediated by CETP. These findings also agreed with several studies that showed an inverse correlation between plasma CETP and HDL-C concentrations which, nonetheless, was not observed in the polymorphisms investigated here (20,23,25,39,48,49).

Correlations between plasma cholesterol and CETP concentrations indicated that mechanisms of regulation of plasma cholesterol and of CETP were dependent on common but as yet unknown mechanisms. We investigated whether and how variations of plasma cholesterol related to common apoE and CETP polymorphisms.

Previous studies utilizing different experimental approaches have shown that plant sterols (phytosterols) compete with cholesterol for intestinal absorption, thus reducing the cholesterol plasma concentration (1,4,50); this was attributed to an increased expression of the cell LDL receptor (51) or to a lower liver VLDL cholesterol synthesis rate (52). According to Vanhanen et al. (17) and Miettinen et al. (16), dietary sitostanol ester significantly lowered LDL-C in apoE4/4 subjects, but not in subjects with other apoE isoforms, a result attributed to greater absorption of dietary cholesterol in apoE4/4 subjects consuming their regular diets. These ideas were opposed by the recent work of Geelen et al. (53) showing that the LDL-C response to plant sterols was not related to the apoE polymorphism. Furthermore, the role of apoE in the regulation of dietary cholesterol absorption seems contradictory because of a lack of correlation between any apoE isoform and a rise in the LDL-C concentration after cholesterol consumption (20). This was observed in spite of the fact that the apoE phenotype distribution frequency in that work, as well as in our series, was close to that found in the literature (54). On the other hand, Martin et al. (20) indicated that the CETP concentration response to cholesterol feeding differed among the three apoE

TABLE 4

Taq1B CETP polymorphism: lipids and lipoprotein plasma concentrations, CETP mass, during the placebo (PCB) and the added plant sterol ester (PSE) periods in male and female subjects with moderate primary hypercholesterolemia[1,2]

	n	PCB	PSE	% Reduction (PSE − PCB)
TC, *mmol/L*				
B1B1	15	6.5 ± 1.0	6.0 ± 1.0†	−3.9 (−28.3; 7.1)
B1B2	29	6.8 ± 0.7	6.4 ± 0.7*	−6.5 (−18.2; 11.2)
B2B2	7	6.3 ± 0.6	6.1 ± 0.6	−2.6 (−10.1; 5.7)
LDL-C, *mmol/L*				
B1B1	14	4.7 ± 0.8	4.4 ± 0.8†	−7.6 (−28.9; 8.0)
B1B2	29	4.8 ± 0.7	4.5 ± 0.6†	−8.7 (−20.3; 13.3)
B2B2	6	4.3 ± 0.9	4.0 ± 0.8	−3.7 (−13.9; 6.7)
HDL-C, *mmol/L*				
B1B1	15	1.0 ± 0.2	0.9 ± 0.2	−3.4 (−26.2; 19.5)
B1B2	29	1.2 ± 0.3	1.1 ± 0.3	0 (−24.2; 29.0)
B2B2	6	1.3 ± 0.5	1.3 ± 0.5	2.1 (−17.1; 17.4)
TG, *mmol/L*				
B1B1	15	1.6 ± 0.6	1.5 ± 0.6	−1 (−50.2; 30.6)
B1B2	29	1.7 ± 0.6	1.6 ± 0.6	−3.5 (−41.9; 48.2)
B2B2	7	1.6 ± 0.6	1.5 ± 0.5	−13.7 (−31.0; 65.7)
CETP, *mg/L*				
B1B1	14	3.02 ± 0.74	2.93 ± 0.52	−4.5 (−30.9; 61.2)
B1B2	28	3.07 ± 0.87	2.84 ± 0.66†	−6.3 (−31.3; 11.4)
B2B2	7	2.67 ± 0.68	2.52 ± 0.72†	−4.3 (−14.2; 0.4)

[1] Values are means ± SD and medians (range) for % reduction. * Different from PCB, $P < 0.001$; † different from PCB, $P < 0.05$; paired Student's *t* test.

[2] See Table 1 for abbreviations.

genotypes with a greater response in apoE3/2 than in E3/3 and apoE4/3 (E3/2: +37%, E3/3: + 18%, E4/3: + 9%). Contrary to others (16,17), and in agreement with Geelen et al. (53), we showed here that PSE lowered plasma TC and LDL-C concentrations, as well as CETP, independently of the apoE polymorphism. Whether and how a mutual influence occurs between the apoE and the CETP polymorphisms regarding efficiency in absorbing intestinal cholesterol is an open question that requires investigation in large population studies, a suggestion made by others (18).

The present work disclosed that during placebo consumption, plasma cholesterol and LDL-C were lower in the VV genotype, a novel finding not reported by others (18,48). Furthermore, dietary PSE was capable of lowering the TC and LDL-C concentrations in subjects with the II and IV, but not the VV genotype. On the other hand, CETP concentration was lowered by PSE in the II, but not in the IV and VV genotypes. Interestingly, compared with the VV genotype, subjects with the II and the IV genotypes had higher plasma cholesterol and LDL-C concentrations with placebo consumption and, coincidently, both were most responsive to PSE. Seemingly, the VV genotype, which had the lowest plasma cholesterol concentration, was the least responsive to PSE.

Contrary to the present report, other studies found a lower CETP concentration in VV compared with the II and IV genotypes (22,36). However, our data agreed with those from another study in that CETP concentrations were shown to be similar among the phenotypes of the I405V polymorphism (37). On the other hand, although others had reported higher CETP activity in the II (as well as in the B1B1) genotype, plasma LDL-C concentration was not reported (23). Therefore, our work disclosed for the first time that plasma TC and LDL-C concentrations were dependent on the I405V CETP genotypes, but that the concentrations of CETP were independent of both the I405V and the Taq1B CETP polymorphisms during either placebo or PSE consumption in spite of a general effect of PSE of lowering plasma CETP concentrations.

Interestingly, with PSE consumption, correlations between plasma CETP and total cholesterol observed during placebo were maintained, whereas those between CETP and LDL-C disappeared. In addition, TC and LDL-C concentrations with placebo consumption were lower in subjects with the VV phenotype than in those with the II and IV phenotypes; however, these differences were no longer present with PSE consumption. This likely occurred because the effect of the I405V CETP polymorphism on the regulation of plasma cholesterol was blunted once a peak plasma cholesterol was attained. The biochemical mechanisms behind this process require investigation and may be critical for the regulation of plasma cholesterol in populations consuming diets that raise the plasma cholesterol concentration.

In summary, this study demonstrated that PSE brought about a reduction in plasma cholesterol and LDL-C concentrations presumably via an impairment of intestinal cholesterol absorption (5,50). This pattern of response of plasma lipids is related to the I405V CETP polymorphism in which the percentage reductions in TC with consumption of PSE for the II, IV and VV phenotypes were 7.2, 4.2 and not significant, respectively, whereas LDL-C significant reductions occurred only for II (9.5%). However, the CETP concentration diminished only in the II phenotype.

ACKNOWLEDGMENTS

The authors thank the continuous support of João Nóbrega (CardioCenter) and the Clinical Hospitals of the University of São Paulo Medical School, campuses São Paulo (Instituto dos LIM) and Ribeirão Preto, and the technical assistance of Patrícia M. Cazita and Vivian Buonacorso.

LITERATURE CITED

1. Miettinen, T. A. & Gylling, H. (1999) Regulation of cholesterol metabolism by dietary plant sterols. Curr. Opin. Lipidol. 10: 9–14.

2. Less, A. M., Mok, H.Y.I., Lees, R. S., McCluskey, M. A. & Grundy, S. M. (1977) Plant sterols as cholesterol-lowering agents clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28: 325–338.

3. Hallikainem, M. A. & Uusitupa, M. J. (1999) Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am. J. Clin. Nutr. 69: 403–410.

4. Ostlund, R. E., Spilburg, C. A. & Stenson, W. F. (1999) Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans. Am. J. Clin. Nutr. 70: 826–831.

5. Law, M. (2000) Plant sterol and stanol margarines and health. Br. Med. J. 320: 861–864.

6. Andersson, A., Karlström, B., Mohsen, R. & Vessby, B. (1999) Cholesterol lowering effects of a stanol ester-containing low-fat margarine used in conjunction with a strict lipid-lowering diet. Eur. Heart J. (suppl.): S80–S90.

7. Weststrate, J. A. & Meijer, G. W. (1998) Plant sterol-enriched margarines and reduction of plasma total and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur. J. Clin. Nutr. 52: 334–343.

8. Miettinen, T. A., Puska, P., Gylling, H., Vanhanen, H. & Erkki, V. (1995) Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N. Engl. J. Med. 333: 1308–1312.

9. Hallikainen, M. A, Sarkkinen, E. S., Gylling, H., Erkkila, A. T. & Uusitupa, M. I. (2000) Comparison of the effects of plant sterols ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur. J. Clin. Nutr. 54: 715–725.

10. Davidson, M. H., Maki, K. C., Umporowicz, D. M., Igram, K. A., Dicklin, M. R., Schaefer, E., Lane, R. W., McNamara, J. R., Ribaya-Mercado, J. D., Perrone, G., Robins, S. J. & Franke, W. C. (2001) Safety and tolerability of esterified phytosterols administered reduced-fat spread and salad dressing to healthy adult men and women. J. Am. Coll. Nutr. 20: 307–319.

11. Tall, A., Welch, C., Applebaum-Bowden, D. & Wassef, M. (1997) Interaction of diet and genes in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 17: 3326–3331.

12. Williams, C. M. (1998) Gene-nutrient interactions: an important area for consideration. Br. J. Nutr. 79: 115.

13. Gylling, H., Kontula, K. & Miettinen, T. A. (1995) Cholesterol absorption and metabolism and LDL kinetics in healthy men with different apoprotein E phenotypes and apolipoprotein B Xba I and LDL receptor Pvu II genotypes. Arterioscler. Thromb. Vasc. Biol. 15: 208–213.

14. Kuust, G. H, Vanhanen, H. & Miettinen, T. A. (1989) Apolipoprotein E phenotype and cholesterol metabolism in familial hypercholesterolemia. Atherosclerosis 80: 27–32.

15. Kesaniemi, Y. A., Ehnholm, C. & Miettinen, T. A. (1987) Intestinal cholesterol absorption efficiency in man is related to apolipoprotein E phenotype. J. Clin. Investig. 80: 578–581.

16. Miettinen, T. A. & Vanhanen, H. (1994) Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Atherosclerosis 105: 217–226.

17. Vanhanen, H. T., Blomqvist, S., Ehnholm, C., Hyvönen, M., Jauhianen, M., Torstila, I. & Miettinen, T. A. (1993) Serum cholesterol, cholesterol precursors and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J. Lipid Res. 34: 1535–1543.

18. Yamashita, S., Hirano, K., Sakai, N. & Matsuzawa, Y. (2000) Molecular biology and pathophysiological aspects of plasma cholesteryl ester transfer protein. Biochim. Biophys. Acta 1529: 257–275.

19. Fumeron, F., Betoulle, D., Luc, G., Behague, I., Richard, S., Poirier, O., Jemaa, R., Evans, A., Arveiler, D., Marques-Vidal, P., Bard, J.-M., Fruchart, J.-C., Ducimetiere, P., Apfelbau M., Cambien, F. (1995) Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. J. Clin. Investig. 96: 1664–1671.

20. Martin, L. J., Connelly, P. W., Nancood, D., Wood, N., Zhang, Z. J., Maguire, G., Quinet, E., Tall, A. R., Marcel, Y. L. & McPherson, R. (1993) Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. J. Lipid Res. 34: 437–446.

21. Noone, E., Roche, H. M., Black, I., Tully, A. M. & Gibney, M. J. (2000) Effect of postprandial lipaemia and Taq 1B polymorphism of the cholesteryl ester transfer protein (CETP) gene on CETP mass, activity, associated lipoproteins and plasma lipids. Br. J. Nutr. 84: 203–209.

22. Corbex, M., Poirier, O., Fumeron, F., Betoulle, D., Evans, A., Ruidavets, J. B., Arveiler, D., Luc, G., Tiret, L. & Cambien, F. (2000) Extensive association analysis between the CETP gene and coronary heart disease phenotypes reveals several putative functional polymorphisms and gene-environment interaction. Genet. Epidemiol. 19: 64–80.

23. Gudnason, V., Kakko, S., Nicaud, V., Savolainen, M. J., Kesaniemi, Y. A., Tahvanainen, E. & Humphries, S. (1999) Cholesteryl ester transfer protein gene

effect on CETP activity and plasma high-density lipoprotein in European populations. The EARS Group. Eur. J. Clin. Investig. 29: 116–128.

24. Ordovas, J. M., Cupples, L. A., Corella, D., Otvos, J. D., Osgood, D., Martinez, A., Lahoz, C., Coltell, O., Wilson, P. W. & Schaefer, E. J. (2000) Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler. Thromb. Vasc. Biol. 20: 1323–1329.

25. Kuivenhoven, J. A., de Knijff, P., Boer, J. M., Smalheer, H. A., Botma, G. J., Seidell, J. C., Kastelein, J. J. & Pritchard, P. H. (1997) Heterogeneity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. Arterioscler. Thromb. Vasc. Biol. 17: 560–568.

26. Guerin, M., Lassel, T. S., Le Goff, W., Farnier, M. & Chapman, M. J. (2000) Action of atorvastatin in combined hyperlipidemia: preferential reduction of cholesteryl ester transfer from HDL to VLDL1 particles. Arterioscler. Thromb. Vasc. Biol. 20: 189–197.

27. Carrilho, A.J.F., Medina, W. L., Nakandakare, E. R. & Quintão, E.C.R. (1997) Plasma cholesteryl ester transfer protein is lowered by treatment of hypercholesterolemia with cholestyramine. Clin. Pharmacol. Ther. 62: 82–88.

28. Lagrost, L., Athias, A., Lemort, N., Richard, J. L., Desrumaux, C., Chatenet-Duchene, L., Courtois, M., Farnier, M., Jacotot, B., Braschi, S. & Gambert, P. (1999) Plasma lipoprotein distribution and lipid transfer activities in patients with type IIb hyperlipidemia treated with simvastatin. Atherosclerosis 143: 415–425.

29. McPherson, R. (1999) Comparative effects of simvastatin and cholestyramine on plasma lipoproteins and CETP in humans. Can. J. Clin. Pharmacol. 6: 85–90.

30. Homma, Y., Ozawa, H., Kobayashi, T., Yamaguchi, H., Sakane, H. & Nakamura, H. (1995) Effects of simvastatin on plasma lipoprotein subfractions, cholesterol esterification rate, and cholesteryl ester transfer protein in type II hyperlipoproteinemia. Atherosclerosis 114: 223–234.

31. Jansen, S., Lopez-Miranda, J., Castro, P., Lopez-Segura, F., Marin, C., Ordovas, J. M., Paz, E., Jimenez-Pereperez, J., Fuentes, F. & Perez-Jimenez, F. (2000) Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester transfer protein concentrations in young, healthy, normolipemic men. Am. J. Clin. Nutr. 72: 36–41.

32. Lottenberg, A.M.P., Nunes, V. S., Lottenberg, S. A., Shimabukuro, A. F. M., Carrilho, A.J.F., Malagutti, S., Nakandakare, E. R., McPherson, R. & Quintão, E.C.R. (1996) Plasma cholesteryl ester synthesis, cholesteryl ester transfer protein concentration and activity in hypercholesterolemic women: effects of the degree of saturation of dietary fatty acids in fasting and postprandial states. Atherosclerosis 126: 265–275.

33. Ahnadi, C. E., Berthezène, F. & Ponsin, G. (1993) Simvastatin induced decrease in the transfer of cholesterol esters from high density lipoproteins to very low and low density lipoproteins in normolipidemic subjects. Atherosclerosis 99: 219–228.

34. Cheung, M. C., Austin, M. A., Moulin, P., Wolf, A. C., Cryer, D. & Knopp, R. H. (1993) Effect of pravastatin on apolipoprotein-specific high-density lipoprotein subpopulation and low-density lipoprotein subclass phenotypes in patients with primary hypercholesterolemia. Atherosclerosis 102: 107–119.

35. Quinet, E. M., Agellon, L. B., Kroon, P. A., Marcel, Y. L., Lee, Y. C., Whitlock, M. E. & Tall, A. R. (1990) Atherogenic diet increases cholesteryl ester transfer protein messenger RNA levels in rabbit liver. J. Clin. Investig. 85: 357–363.

36. Bruce, C., Sharp, D. S. & Tall, A. R. (1998) Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia. J. Lipid Res. 39: 1071–1078.

37. Goto, A., Sasai, K., Suzuki, S., Fukutomi, T., Ito, S., Matsushita, T., Okamoto, M., Suzuki, T., Itoh, M., Okumura-Noji, K. & Yokoyama, S. (2001) Cholesteryl ester transfer protein and atherosclerosis in Japanese subjects: a study based on coronary angiography. Atherosclerosis 159: 153–163.

38. Agerholm-Larsen, B., Tybjaerg-Hansen, A., Schnobr, P., Steffensen, R. & Nordestgaard, B. G. (2000) Common cholesteryl ester transfer protein mutations, decreased HDL cholesterol, and possible decreased risk of ischemic heart disease. The Copenhagen City Heart Study. Circulation 102: 2197–2203.

39. Bruce, C., Chouinard, R. A., Jr. &Tall, A. R. (1998) Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport [Review]. Annu. Rev. Nutr. 18: 297–330.

40. Cox, C., Mann, J., Sutherland, W., Chisholm, A. & Skeaff, M. (1995) Effects of coconut oil, butter, and safflower oil on lipids and lipoproteins in persons with moderately elevated cholesterol levels. J. Lipid Res. 36: 1787–1795.

41. Fusegawa, Y., Kelley, K. L., Sawyer, J. K., Shah, R. N. & Rudel, L. L. (2001) Influence of dietary fatty acid composition on the relationship between CETP activity and plasma lipoproteins in monkeys. J. Lipid Res. 42: 1849–1857.

42. Bachorik, P. S. & Albers, J. (1986) Precipitation methods for quantification of lipoprotein. Methods Enzymol. 129: 78–100.

43. Friedewald, W. T., Levy, R. I. & Frederickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18: 499–502.

44. Guyard-Dangremont, V., Lagrost, L., Gambert, P. & Lallemant, C. (1994) Competitive enzyme-linked immunosorbent assay of the human cholesteryl ester transfer protein (CETP). Clin. Chim. Acta 231: 147–160.

45. Dobiasova, M., Stribrna, J., Pritchard, H. & Frolich, J. J. (1992) Cholesterol esterification rate in plasma depleted of very low and low density lipoproteins is controlled by the proportion of HDL2 and HDL3 subclasses: study in hypertensive and normal middle-aged and septuagenarian men. J. Lipid Res. 33: 1411–1418.

46. Miller, S. A., Dykes, D. D. & Polesky, H. F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 16: 1215.

47. Hixson, J. E. & Vernier, D. T. (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha* I. J. Lipid Res. 31: 545–548.

48. Yamashita, S., Hui, D. Y., Wetterau, J. R., Sprecher, D. L., Harmony, J. A., Sakai, N., Matsuzawa, Y. & Tarui, S. (1991) Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: plasma CETP regulates high-density lipoprotein concentration and composition. Metabolism 40: 756–763.

49. Nakanishi, T., Tahara, D., Akazawa, S., Miyake, S. & Nagataki, S. (1990) Plasma lipid transfer activities in hyper-high-density lipoprotein cholesterolemic and healthy control subjects. Metabolism 39: 225–230.

50. Wester, I. (2000) Cholesterol-lowering effect of plant sterols. Eur. J. Lipid Sci. Technol. 37–44.

51. Plat, J. & Mensink, R. P. (2000) Consumption of plant stanol esters increase LDL receptor expression in mononuclear cells from non-hypercholesterolemic subjects. Atherosclerosis 151: 86 (abs.).

52. Volger, O. L., van der Boom, H., de Wit, E. C., van Duyvenvoorde, W., Hornstra, G., Plat, J., Havekes, L. M., Mensink, R. P. & Princen, H. M. (2001) Dietary plant stanol esters reduce VLDL cholesterol secretion and bile saturation in apolipoprotein E*3-Leiden transgenic mice. Arterioscler. Thromb. Vasc. Biol. 21: 1046–1052.

53. Geelen, A., Zock, P. L., de Vries, J. H. & Katan, M. B. (2002) Apolipoprotein E polymorphism and serum lipid response to plant sterols in humans. Eur. J. Clin. Investig. 32: 738–742.

54. Assmann, G., Schmitz, G., Funke, H. (1989) Analytical procedures for the differential diagnosis of the disorders of lipid metabolism. In: Lipid Metabolism Disorders and Coronary Heart Disease (Assmann, G., ed.). MMV Medizin Verlag, Munich, Germany.