The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women

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Abstract The objective of the present study was to examine the impact of the T111I missense mutation in exon 3 of the endothelial lipase (EL) gene on HDL and its potential interaction effect with dietary fat. The study sample included 281 women and 216 men aged between 17 and 76 years from the Québec Family Study. Plasma HDL_3-C levels of I111I homozygote women were higher compared with those of women carrying the wild-type allele ($P = 0.03$). These differences were not attenuated when adjusted for levels of obesity and were not observed among men. Dietary PUFA interacted with the T111I mutation to modulate apolipoprotein A-I (apoA-I) and HDL_3-C levels among women. Specifically, a diet rich in PUFA was associated with increased apoA-I levels among women carriers of the I111 allele and with decreased apoA-I among women homozygotes for the wild-type allele ($P = 0.002$). A similar interaction was observed with plasma HDL_3-C levels ($P = 0.003$). These interactions were not observed among men. In conclusion, the EL T111I mutation appears to have a modest effect on plasma HDL levels. The gene-diet interaction among women, however, suggests that the T111I missense mutation may confer protection against the lowering effect of a high dietary PUFA intake on plasma apoA-I and HDL_3-C levels.—Paradis, M-E., P. Couture, Y. Bossé, J-P. Després, L. Pérusse, C. Bouchard, M-C. Vohl, and B. Lamarche. **The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women.** ***J. Lipid Res.*** **2003.** 44: **1902–1908.**

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Endothelial lipase (EL) has recently been cloned by two laboratories using different approaches (1, 2). This lipase has been identified as a new member of the triglyceride (TG) lipase gene family and has been shown to be highly homologous to hepatic lipase (HL) and lipoprotein lipase (LPL), both playing key roles in lipoprotein metabolism. Despite strong similarities and conserved features among members of the lipase family, EL distinguishes itself from other lipases by being the only one expressed in endothelial cells and by its specific substrate preference (1, 2). There is indeed minimal sequence homology between the lid domain of EL and other intravascular lipases (1, 2), a feature known to be critical in determining substrate specificity (3). Previous studies indicated that EL was primarily a phospholipase (1, 2), with particular affinity for HDL compared with other lipoprotein subfractions (4).

In vivo studies in mice have shown that EL plays a major role in modulating HDL metabolism (1, 5–7). Because HDL-cholesterol (HDL-C) levels remain a key component of the lipid risk factors for coronary heart disease (CHD), the discovery of this new player in HDL metabolism warrants extensive investigation among humans. It has been suggested that more than 50% of the variation in HDL-C levels in humans may be genetically determined (8). To date, only two studies have investigated how variants in the EL gene may contribute to variations in HDL concentrations (7, 9). Even though the allele frequency of several mutations in the EL gene identified by deLemos et al. (9) was higher among individuals with high plasma HDL-C levels compared with a control group, none of the newly identified genetic variants showed significant association with plasma HDL-C levels. Among the 17 genetic variants identified in that study, one common mutation in exon 3 deserved greater scrutiny, as it was responsible for a significant amino acid change (T111I) that could potentially be associated with an altered intravascular EL activity. A recent report on a group of subjects from the Lipoprotein and Coronary Atherosclerosis Study (LCAS) observed higher plasma HDL-C and apolipoprotein C-III (apoC-III)

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levels among men carriers of the mutation (I111) as well as higher HDL-C/LDL-C and apoA-I/apoB ratios (7).

The aim of the present study was to characterize further the effect of this T111I missense mutation in exon 3 of the EL gene on the HDL profile of subjects from the Québec Family Study (QFS). As environmental factors such as dietary fat intake are also known to modify plasma HDL-C levels as well as CHD risk (10), we also sought to examine the potential impact of the interaction with the T111I variant and the diet on the HDL profile of these individuals.

METHODS

Subjects

The QFS is a cohort of white men and women who were recruited to participate in studies designed to investigate factors involved in the etiology of obesity (11). A total of 951 subjects from 223 families are currently enrolled in the QFS. Subjects gave their written consent to participate in this study, which received the approval of the Medical Ethics Committee of Laval University. Only subjects with a complete lipoprotein-lipid profile, with anthropometric measurements and genomic DNA available, and in whom dietary intake was assessed, were eligible to be included in the present analysis. None had diabetes or were treated for cardiovascular disease. The subsample used in the present study included 281 women and 216 men aged between 17 and 76 years. Body mass index (BMI) ranged between 16.8 and 64.9 kg/m^2. The study sample comprised men and women from 172 two-generation families. There were 162 and 335 individuals from the parental and offspring generations, respectively. Participants with plasma TG levels >4.5 mmol/l were excluded to allow the calculation of plasma LDL-C concentration with the Friedewald formula (N = 1 woman and 3 men). Inclusion of these individuals did not alter the results (data not shown). Postmenopausal status was defined as having been without menses for a year, and women were asked about the use of hormonal replacement therapy.

Adiposity phenotypes, plasma lipid, lipoprotein, and apolipoprotein measurement

BMI was derived from body weight divided by height squared (kg/m^2). Waist circumference was measured according to procedures recommended by the Airlie Conference (12). The amount of visceral adipose tissue was obtained by computed axial tomography as described previously (13). Plasma cholesterol and TG levels in the various lipoprotein subfractions were determined enzymatically with commercial kits as described elsewhere (14). Plasma LDL-C levels were calculated using the Friedewald formula (15). Plasma VLDL (d < 1.006 g/ml) was isolated by ultracentrifugation (50,000 rpm) using a Beckman 50.3 Ti rotor (Beckman, Palo Alto, CA) (16). HDL particles were isolated from the bottom fraction (>1.006 g/ml) after precipitation of apoB-containing lipoproteins with heparin and $MnCl_2$ (17). HDL_2 was then precipitated from the d > 1.006 g/ml HDL fraction with a 4% solution of low-molecular-mass dextran sulfate (15–20 kDa) obtained from SOCHIBO (Boulogne, France) (18). The cholesterol concentration in the supernatant (HDL_3) was measured and HDL_2 was obtained by subtraction. The TG and cholesterol contents of the infranatant fraction were measured before and after the precipitation step for measurement of LDL and HDL compositions. The apoB and apoA-I concentrations were measured by the rocket immunoelectrophoretic method of Laurell (19) as previously described (16). LDL and HDL size were determined by gradient gel electrophoresis as described earlier (20, 21).

Dietary assessment

Total caloric intake, including carbohydrate, protein, and lipid intakes, was derived from a 3 day activity record, which was based on two weekdays and one weekend day (22).

DNA analysis

In order to design intronic primers for the amplification of exon 3, genomic sequences were sought for the intronic regions surrounding this EL gene exon. To do this, we compared the mRNA sequence of the EL gene with a contiguous genomic DNA region taken from sequences of an overlapping clone from GenBank (Accession Number AC091170). Intronic primers were then designed using the Primer 3.0 software available on the Whitehead Institute/MIT Center for Genome Research server (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). Exon 3 was amplified from genomic DNA using specific primers derived from the 5′ and 3′ ends of the intronic sequence (5′-ATTGGGAAGAAGGTCATATAGAAG-3′ and 5′-CTTAAGAAGATTGGGTTTGAGATCC-3′).

PCR conditions were as follows: reaction volume was 50 μl, 1 unit AmpliTaq DNA polymerase with GeneAmp (Roche) in the buffer recommended by the manufacturer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate, primers at final concentration of 0.6 mM, and 2.5 ng of template genomic DNA. PCR products were purified with hydrophilic GF/C filter (Whatman). Sequencing reactions were performed using BigDye Terminators v3.0 (PE Applied BioSystems, Foster City, CA), and the products were analyzed on ABI 3700 automated sequencers (PE Applied BioSystems). The data files were collected using the Data Collection v1.1.1 and then processed using the Sequencing Analysis v3.7 software (PE Applied BioSystems). Sequence data were analyzed using the Staden Package software (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK).

Statistical analyses

Differences between genotype groups were assessed by ANOVA. The MIXED procedure in SAS (SAS Institute, v.8, Cary, NC) was used to adjust for nonindependence among family members. The prevalence of menopause among T111T, T111I, and I111I women was compared by the χ^2 test. To explore the combined effect of the T111I mutation and dietary fat intake on the HDL profile, two genotype groups (T111T homozygotes vs. I allele carriers) were further divided according to the median of the distributions for the various dietary fats among women (total fat: 33.5% of daily calories; saturated fat: 10.3% of daily calories; monounsaturated fat: 10.9% of daily calories; and polyunsaturated fat: 4.2% of daily calories). Multivariate adjustment was performed to adjust for the potential confounding effect of other variables as indicated in the tables and the figure. Variables abnormally distributed were log transformed prior to analysis. All statistical analyses were performed using the SAS package.

RESULTS

The frequency of the I111 mutated allele among subjects from the QFS was 32%, and the genotype frequencies distribution showed a Hardy-Weinberg equilibrium (χ^2 = 0.01; P = 0.99). **Table 1** shows anthropometric and dietary data in each of the genotypic groups. The three groups of women were similar with respect to age, BMI, energy intake, and dietary fat intake. Among men, differences between the T111T and the T111I groups were observed for the waist circumference (P = 0.03). Approximately 24% of women were menopausal (23% in T111T, 26% among T111I heterozy-

TABLE 1. Anthropometric characteristics of women and men from the QFS and energy and macronutrient intake according to the T111I missense mutation of EL gene

	T111/T111	T111/I111	I111/I111	*P*
Women	N = 153	N = 100	N = 28	
Age (years)[a]	40.2 ± 14.3	39.6 ± 14.4	35.8 ± 14.3	0.47
BMI (kg/m^2)[a]	29.0 ± 8.7	29.1 ± 8.4	27.7 ± 7.1	0.58
Waist circumference (cm)[a]	86.6 ± 17.9[c]	86.9 ± 18.8	84.0 ± 17.3	0.63
	N = 119	N = 73	N = 18	
Energy intake (MJ/d)[b]	8.32 ± 1.92	8.10 ± 2.04	8.02 ± 2.10	0.93
Total fat (% of energy)[b]	34.7 ± 5.9	33.5 ± 6.6	34.3 ± 5.4	0.84
SFA (% of energy)[b]	10.9 ± 3.2	10.2 ± 2.7	10.8 ± 2.9	0.62
MUFA (% of energy)[b]	11.6 ± 3.0	10.9 ± 2.6	11.0 ± 2.7	0.50
PUFA (% of energy)[b]	4.7 ± 1.7	4.4 ± 1.8	4.6 ± 1.9	0.67
Men	N = 77	N = 117	N = 22	
Age (years)[a]	40.5 ± 15.7	40.7 ± 15.4	43.6 ± 16.4	0.90
BMI (kg/m^2)[a]	29.7 ± 7.3	27.6 ± 6.6	28.6 ± 6.2	0.07
Waist circumference (cm)[a]	99.8 ± 18.8	93.6 ± 16.9	98.5 ± 14.9	0.03[d]
	N = 67	N = 95	N = 14	
Energy intake (MJ/d)[b]	11.46 ± 3.10	11.22 ± 3.20	11.88 ± 3.60	0.39
Total fat (% of energy)[b]	35.0 ± 5.5	34.7 ± 6.3	33.4 ± 5.8	0.75
SFA (% of energy)[b]	10.4 ± 3.2	10.4 ± 3.1	11.1 ± 3.9	0.68
MUFA (% of energy)[b]	11.6 ± 3.3	11.5 ± 3.4	11.7 ± 2.3	1.0
PUFA (% of energy)[b]	4.6 ± 1.8	4.3 ± 1.7	4.4 ± 1.9	0.75

QFS, Québec Family Study; EL, endothelial lipase; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; BMI, body mass index. Data are presented as unadjusted values and are expressed as mean ± SD. BMI was log transformed prior to analysis in both men and women, as well as PUFA among men only. Energy intake is expressed in MJ/day. The conversion factor is 1 MJ = 239.23 kcal.

[a] *P* value after adjustment for the nonindependence among family members and age (except when the dependent variable is age) as well as for menopausal status and estrogen therapy (in women).

[b] Multivariate models further adjusted for BMI, carbohydrates (% of daily energy), and proteins (% of daily energy).

[c] N = 151.

[d] T111/I111 different from T111/T111.

gotes, and 14% among I111I homozygotes, $P = 0.43$). The T111I mutation was not associated with variations in plasma levels of apoB-containing lipoproteins or total plasma cholesterol and TG levels among women (**Table 2**). The HDL profile was also very similar among the three genotypes, with the exception of plasma HDL_3-C levels. These levels, which were significantly higher among women homozygous for the mutated allele (I111I), compared with women heterozygous and homozygous for the wild-type allele (T111T and T111I), were independent of familial relationship and age. Further adjustment for menopausal status, the use of hormonal replacement therapy, and levels of visceral fat, BMI, or waist girth did not attenuate the impact of the T111I mutation on plasma HDL_3-C levels (data not shown). Among men, no difference between the three genotypes for any of the lipid variables was observed (**Table 3**).

Among men, dietary intakes of PUFA showed an inverse relationship with plasma levels of HDL-C and HDL_2-C ($r = -0.18$, $P = 0.01$, and $r = -0.17$, $P = 0.02$, respectively). Dietary intakes of monounsaturated fatty acids (MUFAs) were also negatively correlated with plasma HDL-C ($r = -0.23$, $P = 0.002$), HDL_2-C ($r = -0.17$, $P = 0.02$), HDL_3-C ($r = -0.15$, $P = 0.05$), and HDL size ($r = -0.21$, $P = 0.01$). We did not observe any significant relationship among dietary intakes of saturated fatty acids (SFAs) and total fat and the HDL profile in men. Among women, dietary intakes of total, PUFA, MUFA, and SFA showed no association with any HDL-related phenotypes (data not shown).

In order to examine whether the T111I mutation influenced the relationship between dietary fat intake and the HDL profile, Spearman correlations were computed between T111T homozygotes and women carrying the I111 allele. Total fat intake (% of daily energy) was negatively correlated with plasma HDL_3-C levels among women homozygous for the wild-type allele ($r = -0.21$, $P = 0.02$), but positively correlated among I111 carriers ($r = 0.27$, $P = 0.01$). PUFA intake (% of daily energy) showed the same relationship with plasma apoA-I levels among women (T111T: $r = -0.19$, $P = 0.04$; and I111: $r = 0.22$, $P = 0.04$). We did not observe these diverging associations among men. The interaction between the T111I mutation and the HDL profile was further investigated using the PROC MIXED procedure in SAS in both genders. Dietary fat consumption was analyzed using a dichotomous variable defined as high or low based on gender-specific medians of the distribution of each dietary fat separately. As shown in **Table 4**, total dietary fat intake interacted with the T111I mutation in determining plasma apoA-I ($P = 0.009$) and HDL_3-C levels ($P = 0.03$) among women. A significant interaction was also observed among MUFA intake, the T111I mutation, and plasma apoA-I levels ($P = 0.01$), independent of familial relationships, age, BMI, smoking, menopausal status, estrogen therapy, total energy intake, and energy obtained from SFA and PUFA. PUFA interacted significantly with the T111I mutation in modulating plasma HDL_3-C ($P = 0.003$) and apoA-I levels ($P = 0.002$). **Figure 1** illustrates how total dietary fat and PUFA and the T111I mutation interacted to modulate plasma HDL_3-C and apoA-I levels among women. Among homozygotes for the T allele, plasma HDL_3-C and apoA-I levels declined

TABLE 2. Mean plasma lipids among women from the QFS according to the T111I genotype

	T111/T111 N = 153	T111/I111 N = 100	I111/I111 N = 28	P^a	P^b
			mmol/l		
Cholesterol					
Total	4.91 ± 1.03	5.00 ± 1.19	4.66 ± 0.86	0.81	0.82
VLDL	0.46 ± 0.26	0.50 ± 0.48	0.38 ± 0.20	0.28	0.36
LDL	2.97 ± 0.89	3.06 ± 1.00	2.77 ± 0.77	0.97	0.94
HDL	1.20 ± 0.29	1.19 ± 0.29	1.25 ± 0.25	0.09	0.14
HDL_2	0.49 ± 0.22	0.48 ± 0.20	0.49 ± 0.16	0.51	0.66
HDL_3	0.71 ± 0.15	0.71 ± 0.15	0.77 ± 0.17	0.03	0.03
Triglycerides					
Total	1.44 ± 0.62	1.44 ± 0.68	1.25 ± 0.60	0.41	0.49
VLDL	0.86 ± 0.51	0.87 ± 0.58	0.74 ± 0.49	0.32	0.41
LDL	0.30 ± 0.12	0.30 ± 0.11	0.25 ± 0.09	0.26	0.39
HDL	0.28 ± 0.08	0.27 ± 0.08	0.28 ± 0.08	0.45	0.35
			mg/ml		
ApoB					
Total	0.96 ± 0.23	0.97 ± 0.23	0.90 ± 0.16	0.51	0.63
VLDL[c]	0.10 ± 0.05	0.10 ± 0.06	0.09 ± 0.04	0.43	0.50
LDL	0.86 ± 0.21	0.87 ± 0.21	0.81 ± 0.16	0.66	0.74
ApoA-I	1.30 ± 0.18	1.29 ± 0.19	1.31 ± 0.20	0.29	0.21
LDL size (Å)[d]	264.8 ± 4.6	264.0 ± 4.4	264.1 ± 4.8	0.42	0.33
HDL size (Å)[e]	87.8 ± 3.6	87.3 ± 3.4	87.8 ± 3.1	0.82	0.70

ApoB, apolipoprotein B; VLDL-C, VLDL cholesterol; TG, triglyceride. Data are presented as unadjusted values and are expressed as mean ± SD. Total cholesterol, VLDL-C, LDL-C, total triglycerides, VLDL-TG, LDL-TG, HDL-TG, and VLDL-apoB were log transformed prior to analyses.

[a] *P* value of the ANOVA comparing the three mutation groups after adjustment for the nonindependence among family members and age.

[b] Multivariate models were further adjusted for menopausal status, hormonal therapy, and BMI.

[c] N = 152 in the T111/T111 women.

[d] N = 151 in the T111/T111 women.

[e] N = 105, N = 70, and N = 17 among the T111/T111, T111/I111, and I111/I111 women, respectively.

TABLE 3. Mean plasma lipids among men from the QFS according to the T111I genotype

	T111/T111 N = 76	T111/I111 N = 116	I111/I111 N = 22	P^a	P^b
			mmol/l		
Cholesterol					
Total[c]	5.00 ± 0.95	4.96 ± 1.09	5.07 ± 1.04	0.79	0.82
VLDL	0.62 ± 0.42	0.54 ± 0.30	0.56 ± 0.34	0.57	0.99
LDL	3.40 ± 0.79	3.45 ± 0.95	3.42 ± 0.74	0.89	0.93
HDL	0.97 ± 0.21	1.00 ± 0.22	1.04 ± 0.24	0.82	0.96
HDL_2	0.32 ± 0.16	0.33 ± 0.15	0.34 ± 0.14	0.83	0.84
HDL_3	0.65 ± 0.12	0.67 ± 0.14	0.70 ± 0.16	0.68	0.86
Triglycerides					
Total[c]	1.72 ± 0.95	1.53 ± 0.73	1.75 ± 0.89	0.19	0.31
VLDL	1.19 ± 0.80	1.05 ± 0.66	1.11 ± 0.68	0.50	0.84
LDL	0.29 ± 0.12	0.28 ± 0.09	0.29 ± 0.14	0.30	0.56
HDL	0.23 ± 0.06	0.22 ± 0.05	0.22 ± 0.05	0.33	0.66
			mg/ml		
ApoB					
Total	1.03 ± 0.24	1.03 ± 0.25	1.07 ± 0.25	0.93	0.97
VLDL[d]	0.11 ± 0.06	0.11 ± 0.06	0.12 ± 0.06	0.63	0.87
LDL	0.91 ± 0.22	0.92 ± 0.23	0.95 ± 0.22	0.99	0.99
ApoA-I	1.20 ± 0.15	1.21 ± 0.15	1.22 ± 0.25	0.64	0.66
LDL size (Å)[e]	262.4 ± 5.1	262.0 ± 5.0	261.6 ± 5.2	0.87	0.76
HDL size (Å)[f]	84.4 ± 3.0	84.8 ± 3.3	82.7 ± 2.76	0.07	0.17

Data are presented as unadjusted values and are expressed as mean ± SD. VLDL-C, HDL_2-C, total triglycerides, VLDL-TG, and LDL-TG were log transformed prior to analyses.

[a] *P* value of the ANOVA comparing the three mutation groups after adjustment for the nonindependence among family members and age.

[b] Multivariate models were further adjusted for BMI.

[c] N = 77 in the T111/T111 and N = 117 among the T111/I111 men.

[d] N = 115 among the T111/I111 men.

[e] N = 72, N = 114, and N = 21 among the T111/T111, T111/I111, and I111/I111 men, respectively.

[f] N = 51, N = 87, and N = 15 among the T111/T111, T111/I111, and I111/I111 men, respectively.

with increasing amounts of total and dietary PUFA intake (below vs. lower 4.2%). Among women carrying the I allele, however, plasma HDL_3-C, and apoA-I levels increased with a growing intake of dietary PUFA. These interactions between dietary PUFA and the T111I missense mutation in exon 3 of the EL gene remained significant when the three genotypes were considered in the analysis, or when dietary fats were considered as continuous variables in the models (data not shown). The same analysis confirmed that there was no interaction among any type of dietary fat, the T111I mutation, and the HDL profile among men (data not shown).

DISCUSSION

The present study reports the effect of a missense mutation in exon 3 of the EL gene (T111I) on several aspects of the lipoprotein-lipid profile among healthy men and women. In this subsample from the QFS, 230 subjects were T111/T111 homozygotes, 217 were T111/I111 heterozygotes, and 50 were I111/I111 homozygotes. Thus, the 32% allele frequency among this sample of individuals almost exclusively of French Canadian descent is comparable to that observed in another group of Caucasian individuals (31%) (9) and in a group of subjects from the LCAS (26%) (7). Men and women in each of the three genotype groups in the present study had comparable lipid profiles, including plasma HDL-C levels. This observation is consistent with the recent study by deLemos et al. (9), which has also failed to note an increased prevalence of the I allele in subjects with higher plasma HDL-C levels. Our observations, however, conflict with those of a recent study among men and women that noted significantly higher plasma HDL-C levels in I111 carriers (7).

Discrepancies between the results of our study and those of the LCAS may be attributable to the fact that the latter study included patients with angiographic evidence of CHD, who had plasma LDL-C between 115 and 190 mg/dl and were on stable dietary therapy, while subjects from the QFS were healthier. Also, the LCAS study included 27% African Americans, while our study sample was more homogeneous, comprised mostly of Caucasian individuals (7). The lack of effect of the T111I mutation on plasma HDL-C levels in two out of three studies was rather unexpected, considering that this mutation yields a significant amino acid change, and that in vivo studies in animals have demonstrated clearly the importance of EL in modulating HDL metabolism (1, 5–7) through the phospholipase activity of EL and its particular affinity for

TABLE 4. *P* values for the interaction terms between dietary fat intake and the T111I genotype at the EL gene on the HDL profile of women: multivariate regression analyses by the type of fat consumed

Interaction Terms in the Regression Models[a]	Dependent Variable				
	HDL-C	HDL_2-C	HDL_3-C	ApoA-I	HDL Size
	mmol/l	*mmol/l*	*mmol/l*	*mg/ml*	*Å*
Total fat (% of energy) × EL[b]					
Multivariate[c]	0.46	0.41	0.03	0.009	0.43
Further adjustment for carbohydrates[d]	0.44	0.43	0.03	0.009	0.41
SFA (% of energy) × EL					
Multivariate[c]	0.39	0.06	0.23	0.37	0.35
Further adjustment for carbohydrates, MUFA, and PUFA[d]	0.36	0.06	0.29	0.48	0.38
MUFA (% of energy) × EL					
Multivariate[c]	0.86	0.26	0.06	0.01	0.95
Further adjustment for carbohydrates, SFA, and PUFA[d]	0.88	0.26	0.06	0.02	0.91
PUFA (% of energy) × EL					
Multivariate[c]	0.21	0.52	0.003	0.002	0.41
Further adjustment for carbohydrates, SFA, and MUFA[d]	0.24	0.46	0.003	0.002	0.45

Total dietary fat and type of dietary fat were divided into two groups based on their respective median value. (Total fat intake: 33.5%; SFA: 10.3%; MUFA: 10.9%; PUFA: 4.2%.)

[a] Regression models with interaction terms were fitted individually for each type of fat and for each dependent variable.

[b] The T111I variant in the EL gene was examined using two categories, i.e., the T111T homozygotes versus heterozygotes + homozygotes for the I111 allele. Similar results were obtained when the three different genotype groups were analyzed separately or when dietary fats were analyzed as continuous variables.

[c] The multivariate models included T111I genotype, familial relationships, age, BMI, smoking, energy intake, menopausal status, estrogen therapy, and the specific fat intake.

[d] Multivariate models were additionally adjusted for carbohydrates (% of energy) and for SFA, MUFA, or PUFA in order to mutually adjust intakes of specific types of fatty acids to each other.

HDL (4). A greater impact of the T111I mutation on HDL was expected, because the phospholipid content of HDL particles is an important feature in determining apoA-I conformation (23) and HDL function (24).

The T111I mutation results in an amino acid change (from a polar to a nonpolar amino acid) that occurs in a relatively poorly conserved area of the EL sequence, far from important sites encoding for the catalytic activity or defining the tertiary structure of the enzyme. The high prevalence of the I allele also suggests that it may not have a large effect on plasma HDL-C levels in the general population. Our data on HDL subfractions and the interaction with dietary fat among women, however, speak to the contrary. Interestingly, plasma HDL_3-C levels of women homozygous for the mutated allele (I111) were significantly higher compared with those carrying the wild-type allele, independent of BMI, waist circumference, or visceral fat accumulation. Considering that the major lipid component of HDL_3 particles is phospholipid, the specific impact of the T111I variant on HDL_3 is consistent with the substrate preference of EL. Because homozygotes for the mutated allele seem to accumulate HDL_3 particles, we speculate that the amino acid change introduced in the protein may be responsible for a physiologically important reduction in the hydrolytic activity toward phospholipids. This hypothesis will have to be tested and validated by assessing the intravascular EL phospholipase activity and the phospholipid content of lipoprotein subfractions among the various T111I genotypes, as well as by examining the impact of the T111I variant on EL activity by directed mutagenesis. Surprisingly, the three genotypic groups in men were similar for all the lipoprotein-lipid variables. A number of factors, including hormonal differences and differences in plasma lipid levels between males and females, may have attenuated or blunted any potential association between the T111I mutation in the EL gene and the HDL profile in men. The study by Ma et al. (7) did not indicate whether the impact of the T111I mutation on plasma HDL-C levels was gender dependent.

Our second objective was to investigate whether dietary fat may exert an effect on the T111I missense mutation in modulating the HDL profile. Plasma HDL-C and HDL_2-C were negatively correlated with dietary intake of PUFA and MUFA in men but not in women. This contradictory observation is not entirely inconsistent with previous observational studies that have either found or failed to report significant associations between estimated dietary fat and plasma HDL-C levels (25–28). We noted a negative relationship, however, between dietary total fat and PUFA with plasma HDL_3-C and apoA-I levels in T111T, and a positive association among I111 carriers among women only. It may also be argued that the association between estimated dietary fat and HDL levels may have been completely attenuated by diverging associations among the various T111I genotypes.

Recently, the Framingham Offspring Study reported an interaction between fat intake and the C-514T polymorphism in the HL gene promoter and HDL-C levels (29). Among 1,110 women and 1,020 men, they observed that the mutated -514T allele was associated with significantly higher HDL-C levels in subjects consuming <30% of energy from fat and significantly lower among TT homozygotes with a fat intake of ⩾30% (29). Investigating the gene-diet interaction effects on the HDL profile leads us to several interesting observations. Among women, an interaction of the T111I mutation was noted with total dietary

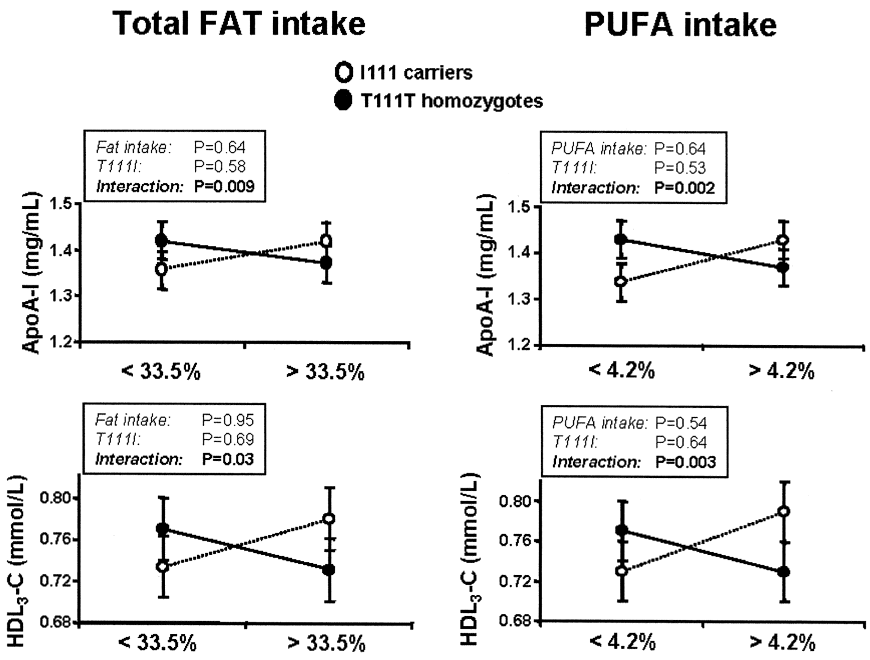

Fig. 1. Interaction among estimated dietary total fat and PUFA intake, the T111I missense mutation in exon 3 of the endothelial lipase gene, and plasma HDL_3-C and apolipoprotein A-I levels. Solid circles represent women homozygous for the T111 allele, while open circles identify women heterozygous and homozygous for the I111 allele. Total dietary fat and PUFA intake were subdivided into high and low, using the median of the distribution for each variable. Values are presented as mean ± SE and are adjusted for the nonindependence among family members, age, body mass index, smoking, total energy intake, menopausal status, and estrogen therapy. *P* values obtained from the PROC MIXED procedure are presented for the dietary fat effect, the genotype effect (T111I), and the dietary fat by genotype interaction.

fat as well as dietary MUFA and PUFA and plasma apoA-I levels. Total dietary fat and PUFA also interacted with the T111I variant to modulate plasma HDL_3-C levels. Increasing amounts of dietary fat were associated with lower apoA-I and HDL_3-C levels in T111/T111 but with more favorable levels among carriers of the mutated allele. This interaction with dietary fat appeared to be mostly attributable to unsaturated fat, and perhaps even more to PUFA specifically, because no gene-diet interactions were noted with dietary SFA intake. Substitution of dietary PUFA and MUFA intake for SFA has been associated with reduced HDL-C levels (10), possibly due to factors such as increased HL activity (30). In light of these observations, we speculate that among T111/T111 homozygotes, a higher dietary PUFA or MUFA intake may have contributed to an increased intravascular EL activity, as previously observed for HL (30), thus contributing to the lowering of plasma HDL_3-C and apoA-I levels. When individuals have an isoleucine rather than a threonine at position 111, however, the resulting EL protein may have reduced affinity for its HDL substrates, thus contributing further to the elevation of plasma HDL_3-C and apoA-I when dietary fat intake increases. Because interactions observed between the various types of fats (saturated vs. unsaturated) and the T111I variant were not of the same magnitude, we speculate that the amino acid change introduced in the protein may have modified its affinity for the various types of fatty acids composing phospholipids, thus altering its impact on HDL_3 subfraction and apoA-I levels. This hypothesis will have to be tested thoroughly in the future. These interactions were not replicated in men of the QFS. Additional studies are therefore needed to elucidate these gender specificities.

From a cardiovascular risk point of view, our data would suggest that women with a mutated EL due to a missense mutation might benefit more than T111T homozygotes if they increase their intake of dietary PUFA. The present observations are based on a modest number of subjects. It is possible that the limited statistical power may have prevented us from detecting an effect of the T111I variant or significant interactions with dietary fat and other variables of the lipoprotein-lipid profile, including HDL-C levels. Further investigations, including larger population-based and intervention studies, will be needed to confirm the observations of the present study. Nevertheless, these preliminary findings may direct research toward targeted dietary recommendations aimed at improving the lipid profile, thereby reducing more efficiently the risk of CHD.

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