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RESEARCH PAPER

Cholesterol absorption status and fasting plasma cholesterol are modulated by the microsomal triacylglycerol transfer protein −493 G/T polymorphism and the usual diet in women

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Abstract An important inter-individual variability in cholesterol absorption has been reported. It could result from polymorphisms in genes coding for proteins involved in the absorption process and in interaction with dietary intakes. To assess whether the extent of cholesterol absorption or synthesis is modified in adult women according to the −493 G/T polymorphism in the microsomal triglyceride transfer protein gene (MTP) and/or the habitual diet. Cholestanol and sitosterol, as well as desmosterol and lathosterol, surrogate markers of cholesterol absorption or synthesis, respectively, were analyzed in the fasting plasma of 69 middle-aged women under a Western-type diet (WD) and after 3 months on a low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD). Genotypes for MTP −493G/T polymorphism were determined. Under an usual WD, subjects homozygous for the MTP −493 T allele exhibited higher ($P < 0.05$) fasting serum concentrations of cholestanol (199.0 ± 30.0 vs. 133 ± 7.4 × 10^2 mmol/mol cholesterol) and lathosterol (188.7 ± 21.8 vs. 147.6 ± 9.1 × 10^2 mmol/mol cholesterol), as well as total cholesterol (7.32 ± 0.22 vs. 6.63 ± 0.12 mmol/l) compared to G carrier subjects. After 3 months on a LFLCD, level of absorption markers decreased in TT subjects with no change in synthesis ones, leading to values comparable to those measured in G carriers. The lowering of plasma total and LDL cholesterol due to dietary change was 2.4- and 2.3-fold greater in TT women than in G carriers. The polymorphism −493G/T in MTP modulates the level of cholesterol absorption but not synthesis in women under a WD, an effect abolished under a prudent LFLCD.

Keywords Nutrition · Intervention · MTP gene · Non-cholesterol sterols · Mediterranean-type diet · Cardiovascular risk factor

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Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality in industrialized countries and is thus a major health outcome. Many epidemiological studies have indicated that the elevation in plasma cholesterol is a key and independent risk factor in the etiology of coronary heart disease [26]. The high incidence of CVD is partly ascribed to dietary habits [30], and more specifically, diets rich in saturated fat or cholesterol are known to increase concentrations of fasting plasma total cholesterol and LDL cholesterol [31].

Along with de novo endogenous synthesis, intestinal cholesterol absorption regulates the body cholesterol homeostasis. While the mechanisms controlling cholesterol metabolism and levels have been widely investigated during the last decades [44], they do not yet explain the large inter-individual variability observed (30–80%) in humans for the rate of cholesterol absorption by the small intestine [4, 39].

Cholesterol absorption is a complex multifactorial and polygenic process that is thought to result from an interaction between an individual's genetic background [44] and various environmental factors. Specifically, the efficiency of intestinal cholesterol absorption depends on dietary factors such as the dose of dietary cholesterol [29], the amount of dietary phytosterols [28] and the amount or type of dietary fat [33].

Regarding genetic factors, present knowledge is limited. Upon digestion, biliary and dietary cholesterol are stepwise absorbed by the small intestinal mucosa and re-secreted into chylomicrons reaching the circulation [1]. Several cellular trans-membrane transporters such as SR-B1, NPC1L1 and ABCG5/G8 have already been implicated in the process of cholesterol absorption by the small intestine [12], but their respective roles are still under investigation. Only few studies have documented the influence of allelic variants in genes coding for such transporters on the extent of cholesterol absorption [5, 8, 36].

In fact, many other intracellular proteins play key roles in cholesterol and lipid trafficking, packaging into chylomicrons and baso-lateral secretion [12]. The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein mainly present in hepatocytes and enterocytes. MTP has an essential role in the assembly process and secretion of very low-density lipoproteins (VLDL) and chylomicrons into the plasma [40]. Indeed, it is able to transfer cholesterol esters [13] and triglycerides from the endoplasmic reticulum membranes to nascent apo-B lipoproteins [46]. For instance, the loss of activity resulting from mutations in the coding regions of the MTP gene expresses the rare genetic disorder abetalipoproteinemia, with a suppressed chylomicron secretion and hypocholesterolemia [40, 45]. The MTP gene is polymorphic, with several genetic variants especially in the promoter region [14, 17]. A number of studies were undertaken to demonstrate associations between this single nucleotide polymorphisms (SNP) at position −493 (G/T) in the promoter region of the MTTP and the concentration of circulating cholesterol but provided inconsistent data [6, 7, 14, 19, 38, 41, 49].

The purpose of this study was to test for the first time the hypothesis of an association between the common MTP −493 G/T polymorphism and the extent of cholesterol absorption or synthesis in humans and its interaction with habitual diet. To that aim, we measured in 69 adult women surrogate markers of cholesterol absorption (cholestanol and sitosterol) and synthesis (desmosterol and lathosterol) [16, 23, 24], as well as plasma and lipoprotein cholesterol under two different dietary regimen, namely a Western diet (WD) or a low-saturated fat and low-cholesterol/Mediterranean-type diet (LFLCD).

Subjects and methods

Subjects

The population and the dietary intervention have previously been described for the whole Medi-RIVAGE cohort [42, 43]. In the present study, the subgroup population was composed of 69 Caucasian women with moderate and untreated cardiovascular risk factors (Table 1). Briefly, women aged 28–70 were recruited at the Center for Detection and Prevention of Atherosclerosis at La Timone University Hospital (Marseille, France). They presented at least one of the following eligibility criteria: fasting plasma cholesterol, 6.5–7.7 mmol/l; plasma triacylglycerols, 2.1–4.6 mmol/l; plasma glycemia, 6.1–6.9 mmol/l; systolic and diastolic blood pressure between 140–180 and 90–105 mm Hg, respectively; BMI > 27 kg/m^2; smoking; sedentary lifestyle; family history of CVD. Hypolipidemic treatment was an exclusion criterion [43].

At entry, subjects ($n = 69$) were used to follow a WD as published [43] and were advised to follow, for 3 months, a Mediterranean-type diet ($n = 35$) or a low-fat type diet ($n = 34$), both intended to reduce saturated fatty acids and cholesterol intakes. The compliance to dietary recommendations was checked by dieticians, and 3-day food records were obtained at entry and after 3 months. The dietary records were analyzed by dieticians using the GENI program (Micro6, Nancy, France) based on the French REGAL food database [42].

Analytical methods (laboratory determinations)

At entry and at the end of the 3-month (3-mo) intervention period, body mass index (BMI: kg/m^2) was calculated, and blood samples collected after an overnight fast. The triglyceride-rich lipoprotein (TRL) fraction ($s_f = 20$–400) was isolated by ultracentrifugation, as previously described [10]. Biochemical analyses were performed with commercial kits on fasting plasma samples as reported in details [43]. The sum of cholesterol carried out by apoB-containing lipoproteins (LDL- plus TRL-cholesterol) was calculated. Fasting serum non-cholesterol sterols (cholestanol, sitosterol, desmosterol and lathosterol) were quantified by gas chromatography-mass spectrometry (GC–MS) on a Hewlett-Packard 6890 GC-5973 MS apparatus, using the reference method of Miettinen et al. [23] using a Zebron-1 capillary column (50 m × 0.25 mm i.d., 0.50 μm

Table 1 Characteristics of subjects enrolled at entry and after 3-month dietary intervention

Women $n = 69$		P value[a]
Age years	53.3 ± 1.0	
Menopausal status (0/1)[b]	41/28	
Activity (1/2/3)[c]	41/7/11	
Current smokers (0/1/2)[d]	43/5/9	
Weight (kg)		
Entry	70.8 ± 1.5	**<0.001**
Month 3	67.7 ± 1.4	
Body mass index (kg/m^2)		
Entry	28.1 ± 0.6	**<0.001**
Month 3	26.9 ± 0.5	
Systolic blood pressure (mm Hg)		
Entry	125.8 ± 2.3	ns
Month 3	124.6 ± 1.5	
Diastolic blood pressure (mm Hg)		
Entry	76.1 ± 1.2	0.069
Month 3	77.8 ± 0.9	
Total cholesterol (mmol/l)		
Entry	6.73 ± 0.11	**0.001**
Month 3	6.40 ± 0.11	
LDL cholesterol (mmol/l)		
Entry	4.33 ± 0.11	**0.030**
Month 3	4.12 ± 0.09	
Triglycerides (mmol/l)		
Entry	1.38 ± 0.09	0.087
Month 3	1.24 ± 0.06	
Insulin (μU/ml)		
Entry	10.03 ± 0.78	**<0.001**
Month 3	8.23 ± 0.56	
Glucose (mmol/l)		
Entry	5.16 ± 0.07	**0.002**
Month 3	4.96 ± 0.06	
Framingham score		
Entry	6.3 ± 0.4	**0.001**
Month 3	5.6 ± 0.4	

Values are mean ± SEM

[a] Comparison between subjects at entry (Western diet, WD) and after 3-mo intervention (low-saturated fat, low-cholesterol/Mediterranean-type diet, LFLCD) tested with Student's t paired test

[b] 0: no menopausal or treated women/1: menopausal women

[c] 1: activity/2:retirement/3: inactivity

[d] 0: never smoker/1: smoker/2: former smoker

film thickness). Epicoprostanol (0.2 ml) was added to serum (0.2 ml) as an internal standard. After alkaline hydrolysis, extraction and derivatization, non-cholesterol sterol concentrations were determined from the same GC–MS run. To eliminate the effect of changing lipoprotein level, the non-cholesterol sterol values were standardized and expressed in terms of 10^2 mmol/mol cholesterol, as usual [21, 24].

Polymorphisms detection

Genomic DNA was prepared from white blood cells by a standard proteinase K-phenol method as reported [7]. The MTP (or MTTP) −493G/T polymorphisms (rs 1800591, chromosome 4, contig NT 016354.18 position 25043208) were genotyped by a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay, the restriction cleavage being performed by *Hph*1 enzyme [14]. Primers used for PCR were as follows: forward 5′-AG TTTCACACATAAGGACAATCATCTA-3′ and reverse 5′-GGATTTAAATTTAAACTGTTAATTCATATCAC-3′ (mutated).

Statistical analyses

Statistical analyses were conducted with SPSS software (v17.0, SPSS Inc., Chicago, IL, USA). Hardy–Weinberg equilibrium between genotypes of MTP −493G/T was assessed by a chi square-test. All data are expressed as means ± SEMs.

Prior to analysis, the distribution of each outcome variable was checked for normality, and logarithmic transformations were performed on individual values of plasma and Framingham score.

Statistical significances between Mediterranean-type diet and low-fat-type diet subgroups were tested with univariate general linear models on plasma cholesterol parameters and serum non-cholesterol sterols (data not shown). These two subgroups of women did not differ according to the followed diet, in agreement with observations previously reported for the whole mixed cohort [42]. The two subgroups could thus be merged for the present analysis as done before [7].

Before testing the effect of genotypes on the dependent variables, Pearson correlation coefficients were calculated to check interrelations between absorption and synthesis markers of cholesterol.

Age, BMI, alcohol consumption, smoking status and menopausal status were identified as interfering covariables and used as adjustment factors (tested by univariate general linear models at entry). The effects of the genotypes at entry, 3-mo and on the response to the diet were tested with general linear models. Intragroup comparison between entry and 3-mo data was made with Student's t paired test or non-parametric Wilcoxon test, as indicated. A P value <0.05 was considered statistically significant.

Table 2 Dietary intakes of subjects according to *MTTP*−493G/T polymorphism

69 women	G/G (2) $n = 30$	G/T (1) $n = 30$	T/T (3) $n = 9$	P value[a]
Total energy (kJ)				
Entry	7,237.6 ± 288.3	7,117.0 ± 310.9	7,929.4 ± 825.8	ns
Month 3	5,285.4 ± 226.6	5,279.8 ± 175.9	5,640.8 ± 281.2	ns
P value[b]	**<0.001**	**<0.001**	**0.011**	
Protein (g/d)				
Entry	79.5 ± 2.6	73.9 ± 3.1	83.1 ± 7.4	ns
Month 3	61.1 ± 1.7	63.6 ± 2.7	69.4 ± 4.3	ns
P value[b]	**<0.001**	**0.011**	**0.021**	
Carbohydrate (g/d)				
Entry	173.4 ± 8.3	181.4 ± 9.9	214.3 ± 19.8	ns
Month 3	141.0 ± 6.8	145.5 ± 6.3	155.1 ± 10.6	ns
P value[b]	**<0.001**	**<0.001**	**0.008**	
Total fat (g/d)				
Entry	75.7 ± 4.1	73.5 ± 4.4	77.0 ± 13.6	ns
Month 3	48.4 ± 3.1	45.3 ± 2.1	49.0 ± 3.3	ns
P value[b]	**<0.001**	**<0.001**	**0.038**	
SFA (g/d)				
Entry	27.2 ± 1.7	27.8 ± 2.2	28.6 ± 5.1	ns
Month 3	14.2 ± 0.9	13.8 ± 1.1	13.9 ± 1.6	ns
P value[b]	**<0.001**	**<0.001**	**0.008**	
MUFA (g/d)				
Entry	28.2 ± 1.7	26.9 ± 1.8	29.5 ± 5.4	ns
Month 3	19.9 ± 1.4	18.3 ± 0.9	19.8 ± 1.3	ns
P value[b]	**<0.001**	**<0.001**	0.051	
PUFA (g/d)				
Entry	11.7 ± 0.9	9.4 ± 0.8	8.7 ± 1.7	ns
Month 3	8.9 ± 0.9	7.7 ± 0.6	9.1 ± 1.3	ns
P value[b]	**0.029**	0.074	0.767	
Cholesterol (mg/d)				
Entry	293.4 ± 17.4	276.7 ± 18.3	282.6 ± 33.3	ns
Month 3	145.9 ± 9.7	171.1 ± 15.9	141.7 ± 16.2	ns
P value[b]	**<0.001**	**<0.001**	**0.008**	

Values are mean ± SEM

SFA saturated fatty acids (g/day); *MUFA* monounsaturated fatty acids(g/day); *PUFA* polyunsaturated fatty acids (g/day)

[a] Comparison between genotypes at entry with Western-type diet (WD) and in their response to low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD) tested with general linear models. ns: non-significant ($P \geq 0.05$)

[b] $P < 0.05$ for comparison between entry and 3-mo within groups (calculated by Student's t paired test for G/G and G/T and by Wilcoxon test for T/T)

Results

In the women sample studied ($n = 69$), the frequency of MTP −493G/T genotypes was 0.43 for GG, 0.43 for GT and 0.13 for TT. The distribution of genotypes was not significantly different from that expected under the Hardy–Weinberg equilibrium ($P = 0.729$). The T allele frequency was 0.35 in this population and was 0.15 in a reference population presented in Hapmap for 24 European-caucasian subjects [25].

At entry (Table 1), women fasting plasma cholesterol ranged 4.26–9.54 mmol/l, 58% women having hypercholesterolemia (cholesterol >6.5 mmol/l). Moreover, 62% women had a BMI above 25, 25% had a systolic blood pressure higher than 140 mm Hg, 19% had triglyceridemia >1.7 mmol/l, and 7% had hyperglycemia (glucose >6 mmol/l).

As shown in Table 2, BMI, weight and energy intake (kJ) were not different between genotypes. Moreover, protein, carbohydrate and fat (saturated, monounsaturated

Table 3 Subjects fasting biochemical parameters according to *MTTP*−493 G/T polymorphism at entry on a Western diet (WD)

	G/G ($n = 30$)	G/T ($n = 30$)	T/T ($n = 9$)	P value[a]
Cholestanol[b]	129.6 ± 9.8	136.4 ± 11.3	199.0 ± 30.0	**0.034**
Sitosterol[b]	103.8 ± 12.5	114.1 ± 15.5	261.1 ± 104.7	**0.037**
Desmosterol[b]	114.2 ± 6.9	106.1 ± 5.7	109.2 ± 4.2	ns
Lathosterol[b]	152.8 ± 15.2	142.3 ± 10.3	188.7 ± 21.8	0.081
Cholesterol (mmol/l)	6.62 ± 0.14	6.64 ± 0.19	7.37 ± 0.22	0.106
HDL cholesterol (mmol/l)	1.68 ± 0.09	1.65 ± 0.09	1.65 ± 0.19	ns
LDL cholesterol (mmol/l)	4.26 ± 0.17	4.25 ± 0.19	4.81 ± 0.22	ns
TRL cholesterol (mmol/l)	0.92 ± 0.12	0.98 ± 0.11	1.37 ± 0.31	ns
ApoB-cholesterol (mmol/l)	5.18 ± 0.22	5.15 ± 0.24	6.07 ± 0.47	ns
TRL TG (mmol/l)	0.87 ± 0.11	0.85 ± 0.11	0.86 ± 0.17	ns
TRL PL (mmol/l)	0.29 ± 0.03	0.30 ± 0.03	0.33 ± 0.06	ns
ApoB (g/l)	1.22 ± 0.05	1.33 ± 0.03	1.21 ± 0.09	ns
Insulin (μU/ml)	11.23 ± 1.29	9.21 ± 1.15	8.78 ± 1.66	ns

Values are mean ± SEM. ApoB-cholesterol is the calculated sum of apoB-containing LDL and TRL-cholesterol levels

[a] Comparison between genotypes at entry tested with general linear models and adjusted for age, menopausal status and BMI. *ns* non-significant ($P \geq 0.05$)

[b] 10^2 mmol/mol cholesterol

and polyunsaturated) intakes were comparable at entry and after 3-mo intervention whatever the genotype. The 3-mo dietary intervention with LFLCD clearly resulted in significant changes in nutrient intakes, especially in reduction of saturated fat and cholesterol intakes ($P < 0.05$) independently of the allele present.

At entry under a Western diet, cholestanol and sitosterol plasma levels gradually increased from homozygous GG toward homozygous TT (Table 3). The comparison of homozygous TT with G carriers (grouping GT and GG alleles) showed higher plasma levels of cholestanol and sitosterol, along with a moderately higher level of lathosterol but not desmosterol (Table 4).

The levels of the various cholesterol parameters measured at entry were not different between genotype groups except for a trend to higher plasma cholesterol in subjects having the T allele (Table 3). In contrast, women homozygous for T allele exhibited higher values for plasma cholesterol, TRL-cholesterol and ApoB-containing lipoprotein cholesterol than the G carriers at entry (Table 4). Comparable HDL cholesterol, TRL-Triglycerides and ApoB levels were observed in TT or G carriers.

After the 3-mo dietary intervention with LFLCD, the levels of all synthesis and absorption markers were no longer significantly different between G carriers and TT women (Table 4), although values for cholestanol, sitosterol and lathosterol remained marginally higher. No differences were found between genotypes for other lipid or cholesterol or ApoB levels.

However, the effect of LFLCD appeared different according to genotypes. Indeed, TT women, but not G carrier women, showed a decrease in cholestanol and sitosterol levels ($P = 0.045$ and $P = 0.035$ adjusted to age and menopausal status; $P = 0.057$ and $P = 0.043$ adjusted to age, menopausal status and BMI, respectively; Table 4). This reduction in cholesterol absorption markers was not balanced by a noticeable elevation of desmosterol or lathosterol levels. Finally, only TT women showed a significant reduction in TRL-cholesterol level after 3-mo on the LFLCD.

Discussion

The results of the present study show, for the first time in adult women, an association of the −493G/T polymorphism in MTP gene with cholesterol absorption markers, as well as the metabolic response to a dietary challenge. This association is modulated by the chronic diet of the subjects in such a way that it is observed only under a Western-type diet (WD) and not a low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD).

As in numerous previous studies, we measured herein serum non-cholesterol sterols as surrogate markers of cholesterol metabolism [16, 20, 23, 24]. Cholestanol and sitosterol well reflect the extent of intestinal cholesterol absorption, whereas lathosterol and desmosterol are precursors and markers of endogenous cholesterol synthesis. It is noteworthy that we found in the present study (data not shown) as expected that the two cholesterol absorption markers were positively correlated and they were both negatively correlated with synthesis markers,

Table 4 Fasting biochemical parameters under a Western-type diet (WD) at entry and after a 3-mo low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD) according to *MTTP*−493G/T polymorphism

69 women	G/G + G/T $n = 60$	T/T $n = 9$	P value[a]
Cholestanol[b]			
Entry	133.0 ± 7.4	199.0 ± 30.0	**0.002**
Month 3	143.3 ± 7.8	173.5 ± 20.1	ns
Mean change	9.98 ± 6.14	−25.59 ± 24.21	**0.045**
Sitosterol[b]			
Entry	109.0 ± 9.9	261.1 ± 104.7	**0.002**
Month 3	133.9 ± 11.9	215.7 ± 80.0	0.081
Mean change	23.67 ± 11.08	−45.37 ± 43.37	**0.035**
Desmosterol[b]			
Entry	110.1 ± 4.5	109.2 ± 4.2	ns
Month 3	107.7 ± 4.4	102.5 ± 6.2	ns
Mean change	−3.81 ± 4.00	−6.64 ± 6.86	ns
Lathosterol[c]			
Entry	147.6 ± 9.1	188.7 ± 21.8	0.101
Month 3	144.8 ± 10.7	195.5 ± 22.0	**0.032**
Mean change	−4.42 ± 8.89	6.77 ± 12.80	ns
Cholesterol (mmol/l)			
Entry	6.63 ± 0.12	7.37 ± 0.22	**0.023**
Month 3	6.35 ± 0.12*	6.70 ± 0.21*	ns
Mean change	−0.277 ± 0.096	−0.663 ± 0.267	ns
HDL cholesterol (mmol/l)			
Entry	1.66 ± 0.06	1.65 ± 0.19	ns
Month 3	1.66 ± 0.08	1.63 ± 0.23	ns
Mean change	−0.004 ± 0.046	−0.013 ± 0.093	ns
LDL cholesterol (mmol/l)			
Entry	4.25 ± 0.13	4.81 ± 0.22	0.107
Month 3	4.08 ± 0.10	4.41 ± 0.18*	0.130
Mean change	−0.177 ± 0.104	−0.403 ± 0.164	ns
TRL cholesterol (mmol/l)			
Entry	0.95 ± 0.08	1.37 ± 0.31	0.091
Month 3	0.93 ± 0.08	0.88 ± 0.17	ns
Mean change	−0.017 ± 0.078	−0.493 ± 0.294	**0.050**
ApoB-cholesterol (mmol/l)			
Entry	5.16 ± 0.16	6.07 ± 0.47	0.056
Month 3	5.07 ± 1.08	5.42 ± 0.98	ns
Mean change	−0.14 ± 0.13	−0.90 ± 0.40	**0.049**
Triglycerides (mmol/l)			
Entry	1.592 ± 0.088	1.623 ± 0.321	ns
Month 3	1.453 ± 0.081	1.199 ± 0.173	ns
Mean change	−0.137 ± 0.064	−0.424 ± 0.370	ns
TRL TG (mmol/l)			
Entry	0.86 ± 0.08	0.85 ± 0.17	ns
Month 3	0.81 ± 0.07	0.78 ± 0.18	ns
Mean change	−0.023 ± 0.067	−0.071 ± 0.094	ns
ApoB (mmol/l)			
Entry	1.27 ± 0.03	1.21 ± 0.09	ns
Month 3	1.21 ± 0.03*	1.21 ± 0.10	ns
Mean change	−0.065 ± 0.026	−0.041 ± 0.047	ns
Insulin (μU/ml)			
Entry	10.22 ± 0.87	8.78 ± 1.66	ns
Month 3	8.33 ± 0.56*	7.41 ± 2.02	ns
Mean change	−2.04 ± 0.54	−1.21 ± 1.14	ns

Values are mean ± SEM. Mean changes are calculated as 3-mo value minus entry value. ApoB-cholesterol is the calculated sum of apoB-containing LDL and TRL-cholesterol levels

[a] Comparison between genotypes at entry with Western-type diet (WD) and in their response to low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD) tested with general linear models and adjusted for age, menopausal status and BMI. *ns* non-significant ($P \geq 0.05$)

[b] 10^2 mmol/mol cholesterol

* $P < 0.05$ for comparison between entry and 3-mo (calculated by Student's t paired test for G/G and G/T and by Wilcoxon test for T/T)

reflecting the well-known cholesterol homeostasis regulation [24].

It has been shown using a stable isotope method that the rate of cholesterol absorption is not different in women and men [4]. Nevertheless, some previous studies showed a sex-specific association between MTP polymorphisms and anthropometric, blood and lipid parameters [3, 48, 49]. Moreover, the effects of a dietary challenge can be differently modulated by MTP genotypes according to sex. For instance, previous results from our laboratory showed that insulinemia and Framingham score displayed a genotype by sex interaction for the −493 locus in MTP gene during this 3-mo dietary intervention [7]. This therefore highlights the importance of considering genders separately in such gene–diet interaction studies.

Our first finding was the association of the −493G/T polymorphism in MTP gene with cholesterol absorption markers, but not synthesis ones, in adult women under a Western-type diet (WD). As we did not find differences in the dietary intakes of subjects according to the gene locus at entry under the WD, this observation supports the existence of a clear effect of the polymorphism at −493 MTP gene locus on the cholesterol absorption process in this cholesterol- and saturated fat-rich dietary context. Indeed, the T allele is associated with an increased cholesterol absorption status with TT homozygotes having a significantly higher level of surrogate markers (+49.6 and +139.4% for cholestanol and sitosterol, respectively) compared to G carriers. In contrast, this locus does not seem to be noticeably associated with the extent of cholesterol synthesis. It was very interesting to observe that, under the WD, TT homozygote women showed higher

fasting levels for plasma cholesterol, TRL-cholesterol and ApoB-containing lipoprotein cholesterol, illustrating the clear relationship existing between the augmented extent of intestinal cholesterol absorption and the increased levels of circulating lipoproteins carrying cholesterol derived from the small intestine, and also the liver. This is in line with previous data reporting an association of LDL cholesterol with cholesterol absorption level [20, 22, 32, 39]. It is noteworthy that fasting HDL cholesterol and TG levels were not affected by this gene locus, suggesting that it predominantly interacts with the intestinal processing of cholesterol in a WD context.

Our second finding highlighted the modulatory effect of the −493G/T MTP polymorphism on the cholesterol absorption status and the metabolic response of women to a low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD) 3-mo challenge. As for entry, no differences in the subject's dietary intakes according to the locus were observed after the 3-mo intervention period, but the response to LFLCD differed between homozygous TT and G carriers. The key observation was that the LFLCD resulted in a significant reduction in both cholesterol absorption markers in TT women (−12.9 and −17.4% for cholestanol and sitosterol, respectively), whereas not noticeable change was observed in G carriers. This contrasted response abolished the significant difference in absorption status as observed between TT and G carriers under the WD. This indicates that under a LFLCD, the−493G/T MTP polymorphism does not modulate the cholesterol absorption phenotype as observed under the WD. This points out that while a diet rich in saturated fat and cholesterol promotes a high-absorption status in TT women, a low-saturated fat, low-cholesterol diet does not illustrating a strong gene–diet interaction. In contrast, the endogenous cholesterol synthesis markers were not altered by the 3-mo dietary challenge, while the homozygote TT women showed an unconsistent change with comparable desmosterol and increased lathosterol levels compared with G carriers. This modest rise can likely be explained as the result of a compensatory mechanism somewhat increasing cholesterol synthesis when cholesterol absorption decreased as reported [22].

The decrease observed in cholesterol absorption status in TT women after the LFLCD was accompanied by a 26.3% lowering of TRL-cholesterol levels which are the primary circulating cholesterol carriers derived from both the small intestine (chylomicrons) and the liver (VLDL). This well supports the concept that the T allele at −493 MTP gene locus could favor the packaging of cholesterol into TRL particles.

Overall, the low-saturated fat, low-cholesterol/Mediterranean-type diet (LFLCD) promoted a lower cholesterol absorption status which can explain, at least partly, the observed beneficial reductions in total, TRL-cholesterol and ApoB-containing lipoprotein cholesterol. In contrast, it has recently been reported that a restricted weight-loss diet led to a reduction in cholesterol synthesis without changes in absorption capacity in women [35]. Moreover, variations in intakes of dietary fat or cholesterol were not accompanied by noticeable changes in cholesterol absorption markers in a short-term study in men [27].

The mechanisms behind the observed effects of the −493G/T MTP polymorphism deserve more specific comments. In fact, some other studies have highlighted that polymorphisms in MTP gene can alter various health-related indexes such as circulating cholesterol [2, 14, 17, 19] and insulinemia [7]. Both functional genetic variant in the MTP gene and diet are likely to alter the expression and intracellular concentration of MTP and subsequently, the amount of assembled chylomicrons in the small intestine. The lower level of cholesterol absorption in G carrier versus TT women could likely result from the twofold lower activity of the MTP gene promoter bearing the G allele versus T as found in vitro [14]. It has also been reported that the activity of the MTP gene promoter could also be modulated by the −164T > C polymorphism [34] which was not studied herein. On the other hand, the MTP gene is up-regulated by cholesterol [9] and down regulated by insulin [18] and the transcription factor SREBP1 [9, 37]. We can thus suggest that a higher intake of dietary cholesterol, as occurring under the Western diet (WD) period, could enhance the expression of MTP in human intestinal cells and hypothesize that this could preferentially occur in the presence of the T allele in the −493 locus of the MTP gene promoter. When changing for a low-cholesterol-type diet as herein, homozygous TT women would more markedly than G carriers lower the intracellular MTP gene transcription through lowering of cholesterol intake and decreasing insulinemia resulting in lower MTP expression, and finally lower cholesterol uptake and chylomicron assembly. Further studies should aim to validate this hypothesis.

The present data raise questions in terms of public health nutrition and cardiovascular risk. It has largely been described that upon controlled dietary interventions dedicated to lower plasma or LDL cholesterol, part of subjects showed a marked response, while others did not exhibit any change [5, 11, 15, 50], thus highly suggesting an important gene–diet interaction. According to other authors, the minor variant −493T allele in MTP promoter has been associated with lower serum LDL cholesterol [2, 14, 17, 19] but not in all studies [38, 49]. Finally, a higher cholesterol absorption level in subjects with elevated risk of CHD [32, 20] has recently been reported. Taken together, these data and our present findings support the concept of a strong link between usual diet, gene polymorphisms,

ApoB-containing lipoprotein cholesterol and CHD risk. This is reinforced by the higher frequency of TT homozygotes found as herein in cohorts of subjects at moderate/high cardiovascular risk [1, 14, 38] compared to healthy subjects [14, 17, 38]. Large-scale intervention studies are needed to confirm our findings owing to the low frequency of minor allelic variants and possible sex-specific associations. Nevertheless, the present observation that 13% women at moderate cardiovascular risk are TT homozygote for the −493G/T MTP polymorphism and have a high-absorber cholesterol status under a unbalanced diet raises the need for more targeted dietary recommendations in the perspective of personalized nutrition based on nutrigenetics [47].

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Conflicts of interest statement None.

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