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RESEARCH PAPER

APOB-516 T allele homozygous subjects are unresponsive to dietary changes in a three-month primary intervention study targeted to reduce fat intake

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Abstract Dietary guidelines aim to control fat intake and reduce cardiovascular risk but an important interindividual variability occurs among subjects. The objective was to investigate whether the response of lipid and glucose homeostasis parameters after a three-month diet aimed at reducing cardiovascular risk could be modulated by the −516C/T polymorphism in the apolipoprotein B gene (APOB). Middle-aged men ($n = 69$) and women ($n = 100$) with moderate cardiovascular disease risk were advised to reduce total energy and fat intakes and replace saturated dietary fat by monounsaturated and polyunsaturated fat. Subjects were genotyped for APOB-516C/T polymorphism. At the entry and at the end of the three-month period, fasting and postprandial plasma lipid analyses were performed. At entry, subjects homozygous for the APOB-516 T allele exhibited significantly lower fasting plasma concentrations of apolipoprotein B 48, triglycerides and triglyceride-rich lipoproteins-triglycerides compared to C carrier subjects. After the diet period, while C carrier subjects presented a clear improvement of most biological parameters, paradoxically T/T subjects did not modify them. In addition, the apoB 48 postprandial response after a standardized mixed test meal was not improved in T/T subjects after the three-month diet, contrary to C allele carriers. Even though their phenotype at entry does not show any significant increase of risk factors when compared to other groups, subjects homozygous for the APOB-516 T allele are unresponsive to a healthy diet that improves cardiovascular risk status in the whole population.

Keywords APOB gene polymorphism · Dietary fat · Cardiovascular risk · Diet–gene interaction

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Introduction

The sanitary, social and economical burden represented by the increasing occurrence of metabolic diseases gave rise during the last decades to a widespread and intensive research in order to identify specific risk factors. Beside risks related to physical inactivity, tobacco consumption or stress, numerous epidemiological studies have evidenced key elements increasing cardiovascular risk, such as dyslipidemia or visceral obesity [4, 29]. This opened up a way for dietary guidelines or recommended diets that emerged over the last decades. They generally agreed on a limited energy intake, a decrease in total fat intake with a shift from saturated (SFA) to monounsaturated or polyunsaturated dietary fatty acids [26, 34]. It is noteworthy that either low-fat prudent diets or Mediterranean-type diets have

been associated with reduced cardiovascular mortality and morbidity [8, 20, 35, 45].

However, as intervention studies multiplied in various populations, it soon appeared that there was an important interindividual and interethnic variability in the metabolic response to recommended diets [28, 36]. For instance, while diets targeted to alter fat intake led to a clear decrease in cardiovascular risk in most subjects [18, 23], part of individuals turned out to behave as poor responders to dietary changes. This led to the emerging concept that such variable metabolic responses might derive from interactions between the individual's genetic background and dietary factors [27, 46]. In that line, the variability of response would thereby result, at least in part, from the presence of allelic variants in the genes coding for proteins involved in the intermediary metabolism such as apolipoproteins, lipid-processing enzymes, lipid transfer proteins and receptors binding lipoproteins [24, 41].

The apolipoprotein B (apoB) plays a central role in lipid metabolism and its disturbances [30]. In humans, apoB is the main apolipoprotein of low-density lipoproteins (LDL) and a major component of triglyceride-rich lipoproteins (TRL). ApoB is also known to be essential for the assembly and secretion of chylomicrons and/or very low-density lipoproteins (VLDL) in the small intestine and the liver. In addition, apoB is the main ligand for LDL receptor-mediated internalization of LDL particles in tissues. In that context, several polymorphic sites in apoB gene have been studied for their potential links with plasma lipid abnormalities [5, 7, 19, 33] and plasma glucose level [6, 13].

In addition, plasma apoB concentration is a well-recognized risk factor for cardiovascular disease [37, 38] and the mechanisms regulating the overproduction of atherogenic apoB-containing lipoproteins have been the focus of much investigation in recent years [3, 11, 14]. In a former work, van't Hooft et al. [40] searched for new polymorphisms in the proximal promoter of the human APOB gene and identified a C to T substitution at position −516. They showed that healthy middle-aged men homozygous for the −516T allele presented higher plasma LDL cholesterol levels than healthy −516C homozygous subjects. However, while this observation has not been confirmed by further works, an interaction between this polymorphism and diet was observed on glucose homeostasis [31, 32].

In the present study, we tested whether the improvement of plasma lipid and glucose homeostasis parameters after a classical diet devoted to reduce cardiovascular risk was modulated by the −516C/T polymorphism in the APOB gene. To this aim, 169 middle-aged men and women at moderate cardiovascular risk were engaged to reduce energy and total fat intakes as well as to replace saturated fat by mono/polyunsaturated fat for 3 months [15, 43]. Their responses to this prudent, Mediterranean-type diet were then correlated with the APOB-516C/T gene polymorphism.

Subjects and methods

Subjects

The design and methods of the intervention study have been previously reported [42]. Briefly, 169 adult volunteers (100 women and 69 men) with moderate untreated cardiovascular risks were recruited: the Framingham score of the whole population was 5.93 ± 3.17. They were provided with nutritional recommendations to consume a low-fat diet for 3 months. Dieticians followed the compliance with dietary recommendations: three-days food records (at entry and after 3 months) and 24 h unscheduled dietary recalls (once a month) were done. To check dietary intakes, the GENI program (Micro6, Nancy, France) based on the French REGAL food database was used [43]. At the entry and at the end of the three-month period, BMI and biochemical analyses (at fasting and/or after a mixed test meal containing 40 g fat) were performed by routine kits in plasma: apoB concentrations, total, HDL, LDL cholesterol, triglycerides, TRL triglycerides, glucose and insulin. Specific concentration of apoB 48 was determined as previously described [42]. Informed consent was obtained for each subject, and the study was approved by the institution's ethics committee (ethics committee no. 98/25).

Polymorphism detection

Genomic DNA was prepared from white blood cells by a standard proteinase K-phenol method. The APOB-516C/T polymorphism (rs 934197, chromosome 2, contig NT 022184.14 position 83395) was genotyped by a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay after Ear I digestion [40].

Primers used for PCR were forward 5′-GGAAACCT AGAAGCTGGTGC-3′, reverse 5′-TCTTCAGATGACCC ACCATG-3′.

Statistical analyses

Statistical analyses were performed with the SAS statistical software (SAS Institute, Inc). A chi square-test was used to determine whether genotypes were in Hardy–Weinberg equilibrium. Logarithmic transformation was performed on individual values of plasma apoB 48, triglycerides, TRL triglycerides, insulin, HOMA and Framingham scores to improve the normality of their distribution.

The effects of the genotypes at baseline were tested with general linear model. Interactions of genotype by gender

were systematically tested. The effect of the genotypes on the response to the diet was tested in repeated measures general linear models. Interactions of genotype by time and by gender were tested. Results are given for men and women separately when interactions were significant.

However, before testing the effect of genotypes on the dependent variables, interfering covariables (adjustment factors) were identified. To this aim, each dependent variable measured at baseline was tested in univariate general linear models with independent qualitative covariables, and by linear correlations concerning quantitative covariables. Covariables were retained as adjustment factors when significant at the 0.05 level. Age was systematically included as adjustment factor at baseline and 3 month. When required, professional activity (activity/retirement/inactivity), BMI (in kg/m^2), alcohol consumption (in kJ/d), antihypertensive treatment (yes/no), smoking status (smoker/former smoker/never smoker), menopausal status in women (yes/no or treated) and plasma apoB concentrations were then entered as adjustment factors into the models. In addition, intragroup comparison between baseline and three-month data was performed with Students *t* paired test or nonparametric Wilcoxon test, as indicated.

Results

The frequency of APOB-516 genotypes was 0.53 for C/C, 0.39 for C/T and 0.08 for T/T. The distribution of genotypes was not significantly different from that expected under the Hardy–Weinberg equilibrium ($P = 0.75$).

The energy intakes expressed as kJ/kg body weight were not different at the entry as well as at the end of the three-month diet period between genders (Table 1) or between genotypes (Table 2). The evaluation of specific nutrient intake patterns showed that, although total protein, total fat

Table 1 Dietary parameters: comparison between genders

Subjects	Men ($n = 69$)		Women ($n = 100$)		P value[e]
	Mean ± SD	% tot energy	Mean ± SD	% tot energy	
Total energy (kJ/kg bw)					
Baseline	112.8 ± 40.7		103.6 ± 30.0		0.090
3 months	84.5 ± 31.7*		77.4 ± 21.5*		0.601
CHO (g/kg bw)[a]					
Baseline	2.79 ± 1.0	39.8 ± 7.34	2.6 ± 0.91	41.8 ± 6.79	0.162
3 months	2.30 ± 0.83*	43.2 ± 6.70	2.1 ± 0.67*	45.2 ± 5.97	0.993
Total protein (g/kg bw)					
Baseline	1.23 ± 0.30	17.7 ± 3.1	1.12 ± 0.31	18.5 ± 3.60	**0.029**
3 months	1.02 ± 0.28*	19.67 ± 3.8	0.91 ± 0.24*	19.9 ± 2.97	0.985
Total fat (g/kg bw)					
Baseline	1.18 ± 0.43	37.2 ± 6.0	1.05 ± 0.39	37.7 ± 6.4	**0.038**
3 months	0.78 ± 0.24*	32.2 ± 5.6	0.69 ± 0.23*	33.5 ± 5.6	0.476
SFA (g/kg bw)[b]					
Baseline	0.44 ± 0.20	13.8 ± 3.5	0.38 ± 0.17	13.7 ± 3.7	**0.042**
3 months	0.23 ± 0.08*	9.7 ± 2.8	0.21 ± 0.09*	10.1 ± 2.8	0.129
MUFA (g/kg bw)[c]					
Baseline	0.43 ± 0.15	13.7 ± 2.6	0.39 ± 0.15	13.9 ± 3.5	0.063
3 months	0.33 ± 0.11*	14.2 ± 3.4	0.29 ± 0.11*	14.3 ± 3.9	0.874
PUFA (g/kg bw)[d]					
Baseline	0.15 ± 0.07	5.04 ± 1.95	0.15 ± 0.08	5.30 ± 2.10	0.609
3 months	0.14 ± 0.06	5.84 ± 2.02	0.12 ± 0.07*	5.85 ± 2.88	0.361

Values in bold denote significant value for P

* $P < 0.05$ for comparison between baseline and after the diet period

[a] CHO, carbohydrates (g/kg body weight)

[b] SFA, saturated fatty acids (g/kg body weight)

[c] MUFA, monounsaturated fatty acids (g/kg body weight)

[d] PUFA, polyunsaturated fatty acids (g/kg body weight)

[e] Comparison between men and women at baseline (tested with general linear models) and in their three-month response to diet (tested with repeated measures general linear models) calculated from raw data (g/kg body weight)

Table 2 Dietary parameters: comparison between genotypes

	C/C ($n = 90$)	C/T ($n = 66$)	T/T ($n = 13$)	P value[a]
Total energy (kJ/kg bw)				
Baseline	106.9 ± 34.7	110.6 ± 36.0	93.6 ± 30.4	0.274
3 months	78.0 ± 25.7*	84.3 ± 26.6*	75.4 ± 27.4*	0.379

Values are mean ± SD

* $P < 0.05$ for comparison between baseline and after the diet period

[a] Comparison between polymorphisms at baseline (tested with general linear models) and in their response to diet (tested with repeated measures general linear models)

and SFA differed at entry between genders, men and women showed a similar reduction in nutrient intakes during the three-month diet period and a similar high compliance with the diet. One outstanding observation was a restriction particularly pronounced in SFA for both genders (Table 1), which is highlighted when fat intakes are expressed as percentage of total fat (−29.5 and −26.3% in men and women, respectively).

Biochemical and anthropometric parameters measured during this study are depicted in Table 3. We did not observe any significant difference at the inclusion for all parameters according to APOB-516C/T polymorphism. In addition, no significant interaction between genotype and gender was observed at entry.

However, after the three-month diet, two noticeable results were observed (Table 3). First, the whole population presented a clear improvement of risk factors, particularly evidenced by decreases in apoB, LDL cholesterol, glucose and insulin plasma concentrations together with a marked reduction of BMI. Second, subjects differed according to their genotype in their response to diet, evidenced for plasma cholesterol and glucose concentrations. Indeed, while C carrier subjects showed a clear decrease of plasma cholesterol concentration after the three-month diet, subjects homozygous for the T allele did not. Plasma glucose concentration also exhibited a markedly different pattern of response according to genotypes since C/C subjects decreased this parameter in response to diet, whereas T/T subjects did not. All together, these results led us to consider T/T subjects as "poor responders" when considering most parameters. We thus compared this genotype to the C carriers (Table 4). As a matter of fact, we confirmed that T/T subjects compared to C carriers markedly differed in their response to diet for fasting glucose and cholesterol. In addition, an interaction between genotype, gender and time pointed out that only men displayed a clear opposite pattern in the plasma glucose concentration at the end of the three-month diet period.

Interestingly, we also observed that compared to C carrier subjects, T/T subjects presented at entry lower fasting plasma concentrations for apoB 48, triglycerides and TRL triglycerides (Tables 4, 5), three biological variables known to be linked to postprandial responses [9, 22]. That led us to analyze the apoB subtype present in intestinally derived TRL (apoB 48) and TRL triglycerides response during the postprandial follow-up (Table 5). Emphasizing the observed differences at fasting, T/T subjects displayed a significant lower apoB 48 response to the test meal at baseline. In addition, T/T subjects did not alter their response to the test meal after the three-month diet while C carrier subjects obviously improved both apoB 48 and TRL triglycerides response at the end of the three-month diet period. These marked discrepancies between genotypes at baseline and in the response to diet were clearly observed at the early 2.5 h postprandial measures.

Discussion

The −516C/T polymorphism in APOB gene has been already pointed out as possibly interfering with lipid parameters at baseline. Indeed, in a previous study, subjects homozygous for the T allele were shown to present moderately enhanced fasting plasma LDL cholesterol levels and conversely C/C subjects presented lower fasting apoB, LDL cholesterol and total cholesterol plasma concentrations compared to T carriers [39]. In contrast, a recent study did not report any modification in lipid parameters associated with this gene polymorphism [31]. Our data actually agree with this latter work since at baseline, neither apoB, nor LDL cholesterol or total cholesterol plasma concentrations were modified by the genotype.

The most relevant result of this study is that the −516C/T polymorphism in APOB gene is associated with an impaired metabolic status, since T/T subjects display a very specific phenotype mainly characterized by a lack of beneficial response to the diet. Indeed, in these subjects, fasting apoB, LDL cholesterol, triglycerides, TRL triglycerides, total cholesterol, insulin and glucose were unchanged at the end of the three-month diet, although these subjects reduced their total energy intake as much as did the other subjects.

Interestingly, C/C subjects showed a beneficial effect of the diet on both cholesterol and glucose levels, while in C/T heterozygous subjects, only cholesterol level was improved by the diet. This positive effect on cholesterol might be linked to the C allele since in T/T subjects no improvement in cholesterol level was observed. Conversely, the absence of effect of the diet on glucose level seemed to be linked to the T allele. This association of the −516 T/T genotype with an impaired glucose homeostasis is in line with the recent observation by Perez-Martinez et al. These authors designed a comparable intervention study in healthy subjects with either C/T or C/C genotype,

Table 3 Fasting biochemical parameters according to APOB-516C/T polymorphism

	Total population ($n = 169$)	C/C ($n = 90$)	C/T ($n = 66$)	T/T ($n = 13$)	P value[a]
ApoB (g/L)					
Baseline	1.24 ± 0.24	1.22 ± 0.22	1.27 ± 0.28	1.22 ± 0.18	0.629
3 months	1.18 ± 0.24	1.17 ± 0.24	1.19 ± 0.25	1.24 ± 0.19	0.228
P value[b]	**<0.001**	**0.008**	**0.001**	0.695	
Total cholesterol (mmol/L)[c, d]					
Baseline	6.52 ± 0.96	6.50 ± 0.85	6.56 ± 1.13	6.49 ± 0.79	0.791
3 months	6.13 ± 0.95	6.11 ± 0.89	6.06 ± 1.07	6.61 ± 0.51	**0.036**
P value[b]	**<0.001**	**<0.001**	**<0.001**	0.529	
HDL cholesterol (mmol/L)[c, e]					
Baseline	1.53 ± 0.45	1.58 ± 0.46	1.48 ± 0.44	1.57 ± 0.36	0.904
3 months	1.52 ± 0.45	1.55 ± 0.45	1.46 ± 0.47	1.60 ± 0.37	0.862
P value[b]	0.443	0.480	0.632	0.441	
LDL cholesterol (mmol/L)[c, f]					
Baseline	4.24 ± 0.92	4.18 ± 0.81	4.33 ± 1.06	4.24 ± 0.83	0.616
3 months	3.90 ± 0.77	3.86 ± 0.71	3.91 ± 0.88	4.24 ± 0.47	0.273
P value[b]	**<0.001**	**<0.001**	**<0.001**	0.807	
Triglycerides (mmol/L)[c, e]					
Baseline	1.53 ± 0.78	1.51 ± 0.69	1.62 ± 0.92	1.25 ± 0.73	0.114
3 months	1.36 ± 0.78	1.34 ± 0.69	1.44 ± 0.96	1.24 ± 0.66	0.344
P value[b]	0.073	0.204	0.223	0.721	
TRL triglycerides (mmol/L)[c, e]					
Baseline	1.09 ± 0.86	1.08 ± 0.92	1.14 ± 0.81	0.80 ± 0.67	0.118
3 months	0.94 ± 0.74	0.96 ± 0.77	1.02 ± 0.80	0.81 ± 0.65	0.690
P value[b]	**0.044**	0.120	0.116	0.929	
Glucose (mmol/L)[d, e, g, h, i]					
Baseline	5.23 ± 0.64	5.31 ± 0.63	5.14 ± 0.65	5.15 ± 0.60	0.197
3 months	5.06 ± 0.59	5.03 ± 0.56	5.03 ± 0.62	5.38 ± 0.59	**0.015**
P value[b]	**<0.001**	**<0.001**	0.087	0.203	
Insulin (μU/mL)[e]					
Baseline	10.58 ± 6.87	10.59 ± 6.92	10.11 ± 6.31	12.21 ± 7.98	0.412
3 months	8.41 ± 4.58	8.21 ± 5.44	8.76 ± 4.68	9.97 ± 5.81	0.154
P value[b]	**<0.001**	**<0.001**	**0.034**	0.279	
HOMA score[e]					
Baseline	2.51 ± 1.83	2.56 ± 1.97	2.36 ± 1.60	2.82 ± 1.99	0.360
3 months	1.97 ± 1.26	1.87 ± 1.29	2.00 ± 1.19	2.42 ± 1.42	0.076
P value[b]	**<0.001**	**<0.001**	**0.027**	0.345	
Framingham score[e, f, i]					
Baseline	5.93 ± 3.17	5.8 ± 3.04	6.12 ± 3.38	5.92 ± 3.12	0.248
3 months	5.02 ± 3.26	4.82 ± 3.15	5.19 ± 3.44	5.69 ± 3.15	0.989
P value[b]	**<0.001**	**0.001**	**0.006**	0.444	
BMI (kg/m$^{2)}$					
Baseline	28.70 ± 5.02	29.0 ± 5.25	28.3 ± 4.60	28.8 ± 5.47	0.732
3 months	27.35 ± 4.65	27.5 ± 4.97	27.1 ± 4.09	27.8 ± 5.07	0.359
P value[b]	**<0.001**	**<0.001**	**<0.001**	**0.003**	

Values are mean ± SD

Values in bold denote significant value for P

[a] Comparison between polymorphisms at baseline (tested with general linear models) and comparison between polymorphisms in their response to diet (tested with repeated measures general models)

[b] P for comparison between baseline and 3 months (calculated by Student t paired test for C/C and C/T and by Wilcoxon test for T/T)

[c] Adjusted for apoB

[d] Adjusted for menopausal status in women

[e] Adjusted for BMI

[f] Adjusted for smoking status

[g] Adjusted for antihypertensive treatment

[h] Adjusted for alcohol consumption

[i] Adjusted for professional activity

although they did not include T/T homozygous subjects [32]. They compared peripheral insulin sensitivity among 59 young healthy men and women at the end of three diets lasting 4 weeks each (SFA rich, monounsaturated fatty acids rich and carbohydrates rich). They demonstrated that this response was gender-specific since only heterozygous

Table 4 Fasting biochemical parameters according to APOB-516C/T polymorphism

	C/C + C/T ($n = 156$)	T/T ($n = 13$)	P value[a]
ApoB (g/L)			
Baseline	1.24 ± 0.24	1.22 ± 0.18	0.733
3 months	1.18 ± 0.24	1.24 ± 0.19	0.115
P value[b]	**<0.001**	0.695	
Total cholesterol (mmol/L)[c, d]			
Baseline	6.52 ± 0.97	6.49 ± 0.79	0.514
3 months	6.09 ± 0.96	6.61 ± 0.51	0.021
P value[b]	**<0.001**	0.529	
HDL cholesterol (mmol/L)[c, e]			
Baseline	1.53 ± 0.45	1.57 ± 0.36	0.679
3 months	1.52 ± 0.46	1.60 ± 0.37	0.673
P value[b]	0.394	0.441	
LDL cholesterol (mmol/L)[c, f]			
Baseline	4.24 ± 0.92	4.24 ± 0.83	0.738
3 months	3.88 ± 0.78	4.24 ± 0.47	0.154
P value[b]	**<0.001**	0.807	
Triglycerides (mmol/L)[c, e]			
Baseline	1.55 ± 0.79	1.25 ± 0.73	0.037
3 months	1.38 ± 0.81	1.24 ± 0.66	0.144
P value[b]	0.077	0.721	
Glucose (mmol/L)[d, e, g, h, i]			
Baseline	5.24 ± 0.65	5.15 ± 0.60	0.782
3 months[j]	5.03 ± 0.59	5.38 ± 0.59	0.023
P value[b]	**0.001**	0.203	
Insulin (μU/mL)[e]			
Baseline	10.39 ± 6.65	12.21 ± 7.98	0.277
3 months	8.45 ± 5.12	9.97 ± 5.81	0.994
P value[b]	**<0.001**	0.279	
HOMA score[e]			
Baseline	2.47 ± 1.82	2.82 ± 1.99	0.397
3 months	1.92 ± 1.25	2.42 ± 1.42	0.550
P value[b]	**<0.001**	0.345	
Framingham score[e, f, i]			
Baseline	5.93 ± 3.18	5.92 ± 3.12	0.851
3 months	4.97 ± 3.27	5.69 ± 3.15	0.886
P value[b]	**<0.001**	0.444	
BMI (kg/m^2)			
Baseline	28.7 ± 4.98	28.8 ± 5.47	0.946
3 months	27.3 ± 4.61	27.8 ± 5.07	0.341
P value[b]	**<0.001**	**0.003**	
Glucose (mmol/L)[d, e, g, h, i]			
Men	($n = 63$)	($n = 6$)	
Baseline	5.38 ± 0.66	5.25 ± 0.65	0.553
3 months[j]	5.15 ± 0.60	5.59 ± 0.54	**0.002**
P value[b]	**0.003**	0.068	
Women	($n = 93$)	($n = 7$)	
Baseline	5.14 ± 0.62	5.07 ± 0.59	0.237
3 months[j]	4.95 ± 0.56	5.19 ± 0.60	0.421
P value[b]	**0.001**	0.917	

Values are mean ± SD

Values in bold denote significant value for P

[a] Comparison between polymorphisms at baseline (tested with general linear models) and comparison between polymorphisms in their response to diet (tested with repeated measures general models)

[b] P for comparison between baseline and 3 months (calculated by Student t paired test for C/C + C/T and by Wilcoxon test for T/T)

[c] Adjusted for apoB

[d] Adjusted for menopausal status in women

[e] Adjusted for BMI

[f] Adjusted for smoking status

[g] Adjusted for antihypertensive treatment

[h] Adjusted for alcohol consumption

[i] Adjusted for professional activity

[j] Significant interaction of genotype × gender × time, $P = 0.018$

men had a significantly greater increase in insulin resistance than C/C subjects for the three diets. In the present study, T/T homozygous men facing a diet challenge that had to improve their insulin sensitivity displayed a nonadapted response (see in Table 4, glucose and HOMA score). It is noteworthy that homozygous individuals for the T allele only represented 8% of the entire population. Although our work would have gained more power by increasing the number of subjects, we had a sufficient number of subjects to define a group of individuals homozygous for the T allele in both genders, which allowed us to clearly attribute this unadaptive phenotype to the T allele. Although the protocols somewhat differed, (i.e., our prudent diet was aimed at reducing both total and saturated fat intake, our subjects were older and presented moderate cardiovascular risks) the convergence of our results and those by Perez-Martinez et al. is particularly striking and clearly points out that the −516 C/T polymorphism in the APOB gene is associated with the glucose homeostasis.

Because we studied a middle-aged population, all subjects were likely to present moderate insulin resistance, which was illustrated by a slightly elevated HOMA score. Insulin resistant states have been extensively shown to be associated with increased hepatic and intestinal TRL secretion resulting in hypertriglyceridemia, concomitant increase in apoB and reduction in plasma levels of HDL cholesterol [2, 10]. They are generally associated with high amplitude of postprandial lipemia [1, 17]. Paradoxically here, parameters such as baseline fasting apoB 48, triglycerides and TRL triglycerides, which are usually linked

Table 5 Postprandial apoB 48 and TRL triglycerides levels according to APOB-516C/T polymorphism

	C/C + C/T (n = 156)	T/T (n = 13)	P value[a]
ApoB 48 T0 (mg/L)[b]			
Baseline	0.25 ± 0.20	0.16 ± 0.14	**0.035**
3 months	0.27 ± 0.26	0.25 ± 0.23	0.052
P value[c]	0.518	0.173	
ApoB 48 T2.5 (mg/L)[d]			
Baseline	0.51 ± 0.39	0.33 ± 0.26	**0.011**
3 months	0.42 ± 0.42	0.36 ± 0.23	**0.009**
P value[c]	**<0.001**	0.382	
ApoB 48 T5 (mg/L)[e]			
Baseline	0.61 ± 0.57	0.35 ± 0.22	0.060
3 months	0.31 ± 0.30	0.33 ± 0.29	**0.048**
P value[c]	**<0.001**	0.422	
TRL triglycerides T0 (mmol/L)[b, f]			
Baseline	1.11 ± 0.87	0.80 ± 0.67	**0.040**
3 months	0.98 ± 0.78	0.81 ± 0.65	0.407
P value[c]	**0.026**	0.929	
TRL triglycerides T2.5 (mmol/L)[d]			
Baseline	1.56 ± 1.04	1.09 ± 0.63	0.156
3 months	1.39 ± 0.92	1.12 ± 0.80	0.857
P value[c]	0.081	0.534	
TRL triglycerides T5 (mmol/L)[e]			
Baseline	1.84 ± 1.12	1.48 ± 0.81	0.312
3 months	1.63 ± 0.96	1.33 ± 0.95	0.809
P value[c]	**0.011**	0.583	

The test meal provided 2,876 kJ, including 53% of energy as fat, 7% of energy as protein and 40% of energy as carbohydrates, and was eaten in 20 min

Values are mean ± SD

All parameters were adjusted for BMI

Values in bold denote significant value for P

[a] Comparison between polymorphisms at baseline (tested with general linear models) and in their response to diet (tested with repeated measures general linear models)

[b] Measured at fasted state

[c] P for comparison between baseline and 3 months (calculated by Student paired test for C/C + C/T and by Wilcoxon test for T/T)

[d] Measured 2.5 h after the test meal

[e] Measured 5 h after the test meal

[f] Adjusted for apoB

to chylomicron remnant metabolism [22, 44], were lower in T/T subjects than in C carriers. In addition, T/T subjects presented a lower postprandial response for apoB 48 levels at entry compared to C allele carriers, a response that was not improved after the three-month diet. These subjects were unable to modify baseline plasma concentrations of other lipid parameters after the three-month diet. Thus, T/T subjects exhibit a very specific and paradoxical phenotype: they can be considered as resistant to dietary changes mainly with regard to fasting glucose concentrations, but do not show any classical features of insulin resistance at intestinal site, such as increased apoB 48 levels of secretion [10, 25]. Previous results had shown that, when directing the transcription of a reporter gene in human hepatic HepG2 cells, the promoter carrying the T allele increased the transcription rate [40], suggesting that it might be more efficient than the C allele promoter. Our apparently contradictory data can be explained if we assume that, although maybe more efficient in the liver, the T allele promoter might be less active in the intestine, thus resulting in lower apoB 48, TRL triglycerides and triglycerides concentrations, as observed at fasting. As a result, the transcription of APOB gene would be less dependent on exogenous regulation, such as during the postprandial period when an increase in insulin concentration, plasma fatty acids or an excess of dietary fat has been shown to modify the apoB 48 secretion in animals [14, 16, 21] as well as in humans [12, 47]. The blunted postprandial apoB 48 and triglycerides peaks that we observed at the entry as well as after the three-month diet in T/T subjects would result from such a poor regulation. The genomic region surrounding the −516 nucleotide of the APOB gene contains several consensus recognition sequences for transcription factors such as USF, SREBP and ADR1, all of which have been shown to regulate genes involved in carbohydrate and lipid metabolism. We also found multiple consensus binding sites for HSF, whose activity is modulated by oxidative stress. More specifically, the C/T polymorphism at this locus alters a consensus site for HSF. This could explain that in each organ (liver or intestine), the regulation of the promoter might differ according to the binding of specific factors on each allele. To what extent such an impaired regulation at the intestinal level might be responsible for the impairment of the glucose homeostasis regulation would deserve further investigation. To address this issue, glucose and fatty acid uptake together with the kinetic of production of apoB 48 and apoB 100 should be studied in relation with polymorphisms at the promoter region of the APOB locus.

In conclusion, our work clearly evidences that subjects homozygous for the T allele at the APOB-516 locus, i.e., about 8% of the subjects, elicited a lack of response for almost all metabolic parameters measured, when they were challenged by a diet that improved cardiovascular risk markers in the other subjects. This unresponsive phenotype probably results from alterations at the intestinal level of apoB 48 synthesis, since the polymorphism lies in the promoter region. The existence of such no-responder phenotypes to dietary change as directed by gene polymorphisms would justify much more important efforts to improve knowledge on diet–gene polymorphism interactions as recently suggested [46] and raises new questions

about how to further improve dietary recommendations to prevent disease occurrence.

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