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A single nucleotide polymorphism in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (*HMGCR*) influences the serum triacylglycerol relationship with dietary fat and fibre in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) study

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The objective of the present study was to investigate the influence of the single nucleotide polymorphism (rs17238540) at the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (*HMGCR*) on the relationship between serum lipids and dietary fat and fibre (NSP). FFQ and pyrosequencing were used to assess cross-sectional dietary intake and *HMGCR* genotype in a population study with data for serum lipids available. Genotype frequencies and allele distributions for 23 011 participants were: TT 95·65 %, TG 4·29 % and GG 0·06 %; T 97·8 % and G 2·2 %. In regression analyses, the TG + GG group showed a significant positive relationship between TAG and SFA intake (+0·11 (95 % CI 0·02, 0·20) mmol TAG/l; $P=0·017$; per 3 % SFA energy increase) while the TT individuals showed no change in the TAG levels related to SFA intake (−0·0007 (95 % CI −0·02, 0·02) mmol TAG/l; $P=0·99$). TG + GG individuals showed an inverse relationship between TAG and fibre intake higher (−0·14 (95 % CI −0·22, −0·05) mmol TAG/l than the TT group (−0·04 (95 % CI −0·06, −0·02) mmol TAG/l). In both cases the respective coefficient regressions of TAG were different between the genotype groups ($Z = 2·27$, $P=0·023$ for SFA intake; $Z = 2·19$, $P=0·029$ for fibre intake). Individuals carrying the G allele may show a greater response in lower TAG levels with reduced SFA intake and increased fibre intake compared with those homozygous for the T allele. The effectiveness of different dietary interventions to control serum lipids may vary according to *HMGCR* genotype.

***HMGCR* polymorphism: Serum lipids: Dietary fat: Dietary fibre**

Hypercholesterolaemia and hypertriacylglycerolaemia are well established as risk factors for atherosclerosis and CHD[1,2]. Dietary fats are important determinants of serum cholesterol, lipoproteins and TAG concentrations[3,4]. Links between dietary lipids and changes in serum lipids have been extensively investigated in well-controlled studies. The wide inter-individual variation in the response of the serum lipids to dietary components has been proposed to be partly due to genetic variation[5]. Identification of common variations in genes related to dietary responsiveness may allow individual diet prescription optimising the treatment of dyslipidaemia[6,7]. Candidate genes have been related to the metabolism, synthesis and intestinal absorption of fatty acids, cholesterol and lipoproteins[6–8].

In previous studies, we have examined cross-sectional associations between diet, blood lipids and apoE (*APOE*) single nucleotide polymorphisms (SNP) and, apart from a small proportion (3 %) of individuals who are of the *APOE* e2/e4 genotype, there is little evidence that different *APOE* genotypes respond differently to differences in SFA or total fat intake[9]. Here, we examine gene–nutrient interactions in cross-sectional associations between diet, blood lipids and a SNP in the 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase gene (*HMGCR*).

The HMGCoA reductase enzyme catalyses a limiting step in endogenous cholesterol synthesis converting HMGCoA to mevalonate, a key intermediate in the production of cholesterol and other sterols[10]. Inhibitors of this enzyme, such as simvastatin, pravastatin and lovastatin (statins), lower serum total cholesterol and LDL-cholesterol and are highly effective for cardiovascular risk reduction[1,11]. However, the wide variation in inter-individual response to the therapy

Abbreviations: EPIC-Norfolk, European Prospective Investigation into Cancer and Nutrition in Norfolk; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzyme A; *HMGCR*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene; SNP, single nucleotide polymorphism.

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† Professor S. A. Rodwell (Bingham) read an initial draft of this manuscript, but sadly passed away between submission and acceptance of this article.

suggests that genetic differences may contribute to this variation[12].

Some polymorphisms were identified in the *HMGCR* locus[13–17] and have been studied for associations with lipid levels and CHD. A variable nucleotide tandem repeat at the end of an Alu sequence located 10 kb 3′ of exon 2 consisting of (TTA)n repeats did not show association with cholesterol levels in either children[18] or adults[19], although a trend for hypercholesterolaemia was observed in children carrying alleles more than ten repeats[18]. CHD patients homozygous for the A allele of the 8302AC polymorphism in intron 2 showed higher levels of VLDL and TAG than controls[20]. The association of several SNP in the *HMGCR* gene and the response to statins treatment has been recently studied[17]. Two tightly linked SNP were found to be significantly associated with a difference in the change in the serum lipid response to pravastatin. A significant reduction in the overall efficacy of pravastatin of 22·3 % for the SNP rs17238540 was observed[17].

Our objective was to investigate the influence of the T/G SNP in the *HMGCR* gene (rs17238540) in the relationship between serum lipids and dietary fat in an initially healthy free-living population of the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort study.

Methods

Study protocol

EPIC-Norfolk is a prospective population study of men and women recruited at age 45–75 years from a general practice age–sex register in Norfolk, UK, from 1993 to 1997. Approximately 25 000 individuals participating in the baseline survey, who had filled in a detailed health and lifestyle questionnaire, attended a first health check when blood and urine samples, and data on height, weight and blood pressure were collected by trained nurses[21]. BMI was estimated as weight in kg/(height in m)^2. Medical history was ascertained with the question, 'Has your doctor ever told you that you have any of the following?', which was followed by a list of conditions including 'high blood pressure (hypertension) requiring treatment with drugs' and 'high lipid levels requiring treatment with drugs'. Habitual physical activity was assessed, both in work and during leisure time, during the previous year, and individuals were assigned to one of four ordered categories[22]. Total cholesterol, HDL-cholesterol and TAG were analysed using non fasting blood samples on an RA-1000 (Bayer Diagnostics, Basingstoke, Hants, UK) and LDL-cholesterol was calculated using the Friedewald formula[23].

Genotype determination

DNA for genotyping was extracted from blood samples collected in EDTA or from stored buffy coat samples with a phenol–chloroform procedure after digestion with proteinase K. The *HMGCR* SNP (rs17238540) genotype was assessed using pyrosequencing (Pyrosequencing AB, Uppsala, Sweden). Briefly, forward biotin-labelled (5′-biotinGCAAGC-CTGT TTGCAGGTAT) and reverse (5′-TCAGCCTAAT-CCATTGTGTCC) primers were designed flanking the polymorphic region of the *HMGCR* gene[17]. The PCR reaction tube (12·5 μl) contained 10 ng DNA, 1 × PCR buffer, $MgCl_2$ (2 mol/l), 0·125 mol/l of each dNTP (deoxynucleotide triphosphate), 10 pmol of each primer, and two units of *AmpliTaq* Gold (Applied Biosystems, Inc., Branchburg, NJ, USA). The annealing temperature was set at 56°C at forty-four cycles on the Thermal Cycler (PTC-225; MJ Research, Inc., Watertown, MA, USA). Detailed pyrosequencing sample preparation and procedure have been described elsewhere[9,24–26]. The dispensation order of the nucleotides for the machine was: TAACACGAGTG. Repeated blind genetic analysis for 6 % of the sample was 99·9 % concordant.

Dietary data

A FFQ consisting of 131 items was sent to all participants before the first health check[27]. The questionnaires were coded and analysed for nutrient intake with a custom-made developed program[28]. Participants also completed 7 d food diaries, but the time for processing the diaries is much longer and at the time of the present analysis, data from diaries were available for approximately half of those who had responded to the FFQ, which were available for all genotyped subjects. In this way, to gain enough power for the analysis in the separate genotype groups, the data from FFQ were used in the present study. Dietary fibre intake was estimated as the NSP content of each food, measured by the Englyst method. The response rate for the FFQ in EPIC-Norfolk was 99 % (*n* 25 350). Nutritional analysis of the FFQ was done as previously described[28]. Briefly, individuals with more than ten missing lines were omitted from the dataset (*n* 247, ninety men, 157 women, 0·97 % of data). We also excluded outliers based on the ratio of energy intake:BMR (EI:BMR), BMR estimated using sex-specific equations including age and body weight. Cut points based on the top and bottom 0·5 % of EI:BMI were introduced, identifying another 250 individuals. In addition, nutrient values > 3 SD from the mean of the upper (fifth) quintile for energy, fat, carbohydrate, protein and alcohol were also excluded for each nutrient. For the present analyses additional inclusion and exclusion criteria were used, including availability of results for genotyping.

Statistical analysis

After excluding subjects for whom the genotyping data were not available, the statistical analysis for genetic data was conducted on 23 011 participants while complete data on dietary and serum lipids were available for 21 700 and 20 881 participants, respectively.

Characteristics of individuals in the different categories were compared. Differences in means were tested using ANOVA. Differences in the frequency of the categorical variables as well as the difference between the observed and the expected genotype frequency distributions were examined using the χ^2 test. We compared serum lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG) across the quartiles of dietary fat (as percentage energy of total energy intake) and fibre intake, univariate and then adjusted by sex, age, total energy intake (kJ/d), carbohydrate intake (percentage energy intake) alcohol intake (percentage energy intake), exercise

Table 1. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) SNP29 genotype frequencies in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort study

	All		Men		Women	
Genotype*	*n*	%	*n*	%	*n*	%
TT	22 010	95·7	9512	95·7	12 498	95·6
TG	989	4·3	424	4·3	565	4·3
GG	12	0·06	4	0·04	8	0·06
Total	23 011	100	9940	100	13 071	100·0

* $P=0·77$ (Pearson χ^2 test for differences in the genotype frequencies between men and women).

index, smoking status and use of lipid-lowering drugs for the whole population then stratified by sex and genotype. The Bonferroni correction for multiple tests[29] was used to demarcate significant differences for the further multivariate regression and the *P* value for significance was set as $<0·01$. Regressions between dietary component variables and serum lipids were adjusted as described above and were done for the whole cohort then stratified by sex and genotype. Regression coefficients (β) and standard error were normalised to show the change of serum lipids for every approximate SD change in the dietary lipid intake. The results were expressed as the two-tailed test for significance (*P*) and the 95 % CI. We also compared the regression coefficients of serum lipid on dietary fat intake for the different genotype groups.

The EPIC-Norfolk Study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Norfolk Health District Ethics Committee. Written informed consent was obtained from all subjects.

Results

Genotype frequencies and allele distributions for 23 011 participants were: TT 95·65 %, TG 4·29 % and GG 0·06 %; T 97·8 % and G 2·2 %, respectively (Table 1). The genotype frequencies were in Hardy–Weinberg equilibrium and did not differ between men and women ($P=0·77$).

As there was no difference in the genotype distribution between men and women, the baseline clinical, biochemical and dietary intake variables separated by genotype groups are presented sex combined in Table 2. There were no differences between the genotype groups for these variables. As the GG group had very few individuals (*n* 12), it was pooled with the TG group for the following analyses, forming a G variant allele carriers group (TG + GG).

The comparison between adjusted means of total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG by quartile of lipid (percentage of energy) and fibre intake (g/d) in the two genotype groups is shown in Table 3. Significant differences were observed in the TT genotype group for total and LDL-cholesterol levels which were reduced in the higher quartiles of PUFA and fibre intake and increased in the higher quartiles of SFA intake. In the TT group the HDL-cholesterol increased in the higher quartiles of total fat, SFA and fibre intake and did not change with MUFA and PUFA intake. TAG serum levels in the TT group showed a low inverse association with PUFA and fibre intake ($P=0·04$). Conversely, in the TG + GG group, the only weak relationship ($P=0·048$) observed was an inverse association between LDL-cholesterol and PUFA intake. Similar results were observed in sex-stratified analyses; so, multivariate regression was conducted sex combined, adjusting for sex.

The results presented in Table 4 show the linear relationships between the serum lipids, dietary fat and fibre, stratified by genotype. The results for the whole cohort (not shown)

Table 2. Baseline distribution of variables according to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) genotype in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort study

(Mean values and standard deviations or percentages)

	Genotype									
	TT			TG			GG			
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P*
Age (years)	22 010	58·7	9·3	989	59·3	9·2	12	58·6	6·8	0·08*
BMI (kg/m^2)	21 395	26·3	3·9	965	26·4	4·0	12	26·7	3·8	0·88*
Serum total cholesterol (mmol/l)	20 816	6·19	1·17	938	6·19	1·18	11	6·77	1·28	0·25*
Serum LDL-cholesterol (mmol/l)	20 150	3·97	1·04	903	3·99	1·06	10	4·30	0·85	0·54*
Serum HDL-cholesterol (mmol/l)	20 149	1·43	0·43	903	1·42	0·41	10	1·37	0·43	0·89*
Serum TAG (mmol/l)	20 814	1·81	1·10	938	1·78	1·04	11	2·05	0·93	0·53*
Total energy intake (kJ/d)	20 757	8556	2500		8644	2569		8459	2961	0·57*
Total fat intake (% energy intake)	20 757	32·4	5·8		32·3	5·9		31·0	8·2	0·46*
MUFA intake (% energy intake)	20 757	11·4	2·4		11·3	2·3		10·9	3·4	0·36*
PUFA intake (% energy intake)	20 757	6·2	2·0	931	6·0	2·0	12	6·1	2·1	0·17*
SFA intake (% energy intake)	20 757	12·5	3·3		12·5	3·4		11·7	3·8	0·65*
Fibre (g/d)	20 757	18·5	6·6		18·3	6·6		19·0	6·9	0·59*
Alcohol (g/d)	20 757	8·5	12·8		8·6	13·4		9·3	12·8	0·97*
Current smokers (%)	2514	11·5		125	12·7		1	8·3		0·43†
Lipid-lowering drug users (%)	328	1·5		11	1·1		1	8·3		0·09†
Hypertension drug users (%)	3995	18·2		190	19·2		2	16·7		0·69†

MUFA, monounsaturated fat; PUFA, polyunsaturated fat; SFA, saturated fat.
* *P* value for one-way ANOVA tests between genotype groups.
† *P* value for Pearson χ^2 tests between genotype groups.

Table 3. Adjusted total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG by quartiles of fat intake separated by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) genotype in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort study*

(Mean values with their standard errors)

		Total cholesterol (mmol/l)						LDL-cholesterol (mmol/l)						HDL-cholesterol (mmol/l)						TAG (mmol/l)					
		TT			TG + GG			TT			TG + GG			TT			TG + GG			TT			TG + GG		
Quartiles of nutrient intake		Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†	Mean	SE	*P*†
Total fat (% energy)	1 (<28·7)	6·18	0·02	1·00	6·14	0·10	0·90	3·98	0·02	0·75	3·97	0·09	0·97	1·40	0·01	<0·01	1·46	0·03	0·72	1·84	0·02	0·20	1·59	0·09	0·15
	2 (28·7–32·6)	6·19	0·02		6·23	0·08		3·97	0·02		4·01	0·07		1·42	0·01		1·43	0·03		1·82	0·02		1·74	0·07	
	3 (32·7–36·3)	6·18	0·02		6·20	0·08		3·96	0·02		3·97	0·08		1·44	0·01		1·42	0·03		1·79	0·02		1·85	0·07	
	4 (>36·3)	6·19	0·02		6·18	0·10		3·95	0·02		3·96	0·09		1·45	0·01		1·40	0·03		1·77	0·02		1·90	0·09	
MUFA (% energy)	1 (<10·0)	6·16	0·02	0·06	6·13	0·10	0·23	3·95	0·02	0·055	3·99	0·09	0·24	1·43	0·01	0·84	1·42	0·03	0·83	1·78	0·02	0·37	1·62	0·08	0·27
	2 (10·0–11·4)	6·18	0·02		6·32	0·08		3·98	0·02		4·10	0·07		1·43	0·01		1·41	0·03		1·79	0·02		1·81	0·07	
	3 (11·5–12·9)	6·17	0·02		6·13	0·08		3·94	0·02		3·89	0·07		1·42	0·01		1·42	0·03		1·82	0·02		1·82	0·07	
	4 (>12·9)	6·23	0·02		6·17	0·10		4·00	0·02		3·93	0·09		1·42	0·01		1·45	0·03		1·83	0·02		1·83	0·09	
PUFA (% energy)	1 (<4·7)	6·24	0·02	<0·001	6·18	0·08	1·00	4·01	0·02	<0·001	3·98	0·07	0·96	1·43	0·01	0·11	1·40	0·03	0·048	1·81	0·02	0·04	1·81	0·07	0·29
	2 (4·7–5·8)	6·21	0·02		6·20	0·08		3·98	0·02		3·94	0·07		1·42	0·01		1·46	0·03		1·84	0·02		1·84	0·07	
	3 (5·9–7·3)	6·18	0·02		6·20	0·08		3·96	0·02		3·98	0·07		1·43	0·01		1·46	0·03		1·78	0·02		1·66	0·07	
	4 (>7·3)	6·10	0·02		6·16	0·08		3·90	0·02		4·00	0·07		1·42	0·01		1·38	0·03		1·79	0·02		1·76	0·07	
SFA (% energy)	1 (<10·3)	6·13	0·02	<0·001	6·10	0·09	0·76	3·93	0·02	0·001	3·96	0·09	0·46	1·40	0·01	<0·001	1·41	0·03	0·083	1·82	0·02	0·26	1·66	0·08	0·29
	2 (10·3–12·1)	6·17	0·02		6·23	0·08		3·96	0·02		4·05	0·07		1·41	0·01		1·40	0·03		1·82	0·02		1·71	0·07	
	3 (12·2–14·3)	6·18	0·02		6·19	0·08		3·96	0·02		3·89	0·07		1·43	0·01		1·48	0·03		1·80	0·02		1·81	0·07	
	4 (>14·3)	6·26	0·02		6·22	0·09		4·02	0·02		3·99	0·08		1·46	0·01		1·40	0·03		1·78	0·02		1·88	0·08	
Fibre (g/d)	1 (<14·1)	6·24	0·02	<0·001	6·32	0·08	0·15	4·03	0·02	<0·001	4·08	0·07	0·13	1·40	0·01	<0·001	1·43	0·03	0·84	1·84	0·02	0·04	1·82	0·07	0·24
	2 (14·1–17·7)	6·17	0·02		6·17	0·08		3·96	0·01		3·97	0·07		1·43	0·01		1·41	0·03		1·78	0·02		1·75	0·07	
	3 (17·8–22·0)	6·20	0·02		6·07	0·08		3·96	0·01		3·84	0·07		1·43	0·01		1·43	0·03		1·80	0·02		1·85	0·07	
	4 (>22·0)	6·14	0·02		6·16	0·08		3·91	0·02		4·00	0·08		1·44	0·01		1·43	0·03		1·81	0·02		1·66	0·07	

MUFA, monounsaturated fat; PUFA, polyunsaturated fat; SFA, saturated fat.

* Univariate ANOVA adjusted by sex, BMI, age, total energy intake, carbohydrate intake (% energy), alcohol intake (% energy), exercise index, smoking status and lipid-lowering drug use.

† *P* value for trend of the serum lipid values across the nutrient intake quartiles within each genotype group.

Table 4. Linear regression between total cholesterol, LDL-cholesterol and HDL-cholesterol and dietary fat intake assessed by FFQ multivariate adjusted* and separated according to 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*HMGCR*) genotype in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort study (*n* 20 881)†

(Standardised regression coefficients, standard errors and 95 % confidence intervals)

		TT (*n* 19 969)					TG + GG (*n* 912)				
Serum lipid	Dietary component	β	SE	*P*	95 % CI		β	SE	*P*	95 % CI	
Cholesterol (mmol/l)	Total fat (% energy)	0·066	0·018	<0·001	0·031,	0·100	0·153	0·085	0·073	−0·014,	0·320
	MUFA (% energy)	0·059	0·015	<0·001	0·031,	0·088	0·030	0·072	0·678	−0·112,	0·172
	PUFA (% energy)	−0·049	0·009	<0·001	−0·066,	−0·032	−0·015	0·042	0·723	−0·097,	0·067
	SFA (% energy)	0·076	0·011	<0·001	0·054,	0·098	0·107	0·055	0·053	−0·002,	0·216
	Fibre (g/d)	−0·027	0·010	0·010	−0·047,	−0·006	−0·079	0·053	0·135	−0·182,	0·025
LDL-cholesterol (mmol/l)	Total fat (% energy)	0·059	0·016	<0·001	0·027,	0·090	0·087	0·079	0·275	−0·069,	0·242
	MUFA (% energy)	0·058	0·013	<0·001	0·031,	0·084	−0·037	0·067	0·581	−0·169,	0·095
	PUFA (% energy)	−0·039	0·008	<0·001	−0·054,	−0·024	0·003	0·039	0·938	−0·073,	0·079
	SFA (% energy)	0·062	0·010	<0·001	0·042,	0·082	0·061	0·051	0·234	−0·040,	0·162
	Fibre (g/d)	−0·032	0·010	0·001	−0·051,	−0·014	−0·029	0·049	0·554	−0·125,	0·067
HDL-cholesterol (mmol/l)	Total fat (% energy)	−0·001	0·006	0·843	−0·013,	0·011	−0·025	0·027	0·345	−0·078,	0·027
	MUFA (% energy)	−0·018	0·005	<0·001	−0·028,	−0·009	0·021	0·023	0·356	−0·024,	0·066
	PUFA (% energy)	−0·006	0·003	0·026	−0·012,	−0·001	−0·016	0·013	0·232	−0·041,	0·010
	SFA (% energy)	0·011	0·004	0·004	0·003,	0·019	−0·011	0·018	0·534	−0·045,	0·024
	Fibre (g/d)	0·020	0·004	<0·001	0·013,	0·027	0·021	0·017	0·212	−0·012,	0·054
TAG (mmol/l)	Total fat (% energy)	0·016	0·016	0·315	−0·015,	0·048	0·136	0·072	0·058	−0·004,	0·277
	MUFA (% energy)	0·039	0·013	0·003	0·013,	0·066	0·084	0·061	0·167	−0·035,	0·204
	PUFA (% energy)	−0·002	0·008	0·755	−0·018,	0·013	−0·031	0·035	0·373	−0·100,	0·038
	SFA (% energy)	−0·0007‡	0·010	0·994	−0·020,	0·020	0·109‡	0·047	0·020	0·017,	0·201
	Fibre (g/d)	−0·038§	0·010	<0·001	−0·056,	−0·019	−0·137§	0·044	0·002	−0·224,	−0·051

MUFA, monounsaturated fat; PUFA, polyunsaturated fat; SFA, saturated fat.

* Analysis adjusted by sex, BMI, age, total energy intake, carbohydrate intake (% energy), alcohol intake (% energy), exercise index, smoking status and lipid-lowering drug use.

† The nutrient intake was adjusted to show differences for increase of 1 SD in the intake of total fat (approximately 6 % of energy), MUFA (approximately 2 % energy), PUFA (approximately 2 % energy), SFA (approximately 3 % energy) and fibre (approximately 7 g/d).

‡ $Z = 2{\cdot}27$ ($P = 0{\cdot}023$) for testing differences in the regression coefficients.

§ $Z = 2{\cdot}19$ ($P = 0{\cdot}029$) for testing differences in the regression coefficients.

were similar to the results for the TT group, which presented a significant and positive relationship between total cholesterol and the intake of total fat, MUFA and SFA, with positive regression coefficients of 0·066 (95 % CI 0·031, 0·10), 0·059 (95 % CI 0·031, 0·088) and 0·076 (95 % CI 0·054, 0·098) for increments of 1 SD in the intake of total fat, MUFA and SFA, respectively. The same trend was observed for LDL-cholesterol with coefficients of 0·059 (95 % CI 0·027, 0·09), 0·058 (95 % CI 0·031, 0·084) and 0·062 (95 % CI 0·042, 0·082) for increments of 1 SD in the intake of total fat, MUFA and SFA, respectively. PUFA intake was inversely associated with all blood lipid fractions as was fibre, except for a positive association between fibre intake and HDL. The present results showed no association between total fat and serum HDL or TAG, while SFA was positively associated with HDL but did not show association with TAG. Also, low MUFA intake was associated with high TAG and HDL levels.

The TG + GG group showed overall associations in the same direction as the TT group and mostly were of similar magnitude. However, differences between the regression coefficients for serum TAG and SFA intake and fibre were different amongst the genotype groups; whilst the TT individuals showed no change in the TAG levels related to SFA intake (−0·0007 (95 % CI −0·02, 0·02) mmol TAG/l; P=0·99), the TG + GG group showed a significant positive relationship between TAG and the intake of SFA (+0·109 (95 % CI 0·02, 0·20) mmol TAG/l; P=0·017) for each 3 % SFA energy increase. In the TG + GG group the inverse relationship between TAG and fibre intake was three times higher (−0·137 (95 % CI −0·22, −0·05) mmol TAG/l; P=0·002) than in the TT group (−0·038 (95 % CI −0·06; −0·02) mmol TAG/l; P<0·001). In both cases the respective coefficient regressions of serum TAG were different amongst the genotype groups (Z = 2·27, P=0·023 for SFA intake; Z = 2·19, P=0·029 for fibre intake).

Discussion

In the present paper we report the effect of the SNP rs17238540 at the *HMGCR* gene on serum lipids in response to dietary fat and fibre in a largely Caucasian population from the UK.

While HMG-CoA reductase is the main target of regulatory mechanisms for endogenous cholesterol synthesis, which is being exploited in pharmacotherapy, the SNP was not related to baseline lipid levels. This is in agreement with previous smaller studies[17,30,31].

Serum lipids of TT individuals, according to quartiles of dietary fat and fibre intake, showed significant variation. This is in accordance with previous results of our group for the overall population[9,32]. In contrast, serum lipids, according to quartiles of dietary fat intake, for carriers of the minor allele (G) did not show significant variation. The G allele carriers showed additional relationships that were opposite of those observed for TT individuals, such as the tendency to present lower levels of HDL with higher intake of SFA, suggesting a different serum lipid response to dietary components.

In the general population carbohydrate-rich diets are associated with hypertriacylglycerolaemia more consistently than dietary fat[1,33]. In the Framingham study[34] a positive correlation was found between SFA intake and TAG levels, but the model was not adjusted for carbohydrate and alcohol intakes that are well known to raise TAG levels[1,33]. Adjusting our model for these dietary components (Table 4), we have found that compared with TT individuals, for whom there was no overall relationship between serum TAG and dietary SFA, the G allele carriers showed a higher ($\beta = -0{\cdot}109$, $Z = 2{\cdot}27$) and significantly (P=0·02) positive correlation between TAG and SFA, indicating greater sensitivity of these individuals to SFA intake. This observation may suggest that the G allele is somehow linked to a liver overproduction or lower blood clearance of VLDL-TAG-rich particles elicited by dietary SFA which is not observed in the overall population, which may in turn have implications for CHD risk.

Fibre intake has been previously shown, using both FFQ and 7 d food diaries, to be inversely related to TAG in this population after adjustment for alcohol and carbohydrate intake[9,32]. Cross-sectional inverse associations between fibre intake and serum TAG have also been shown in the Framingham study[34], and we have suggested that serum TAG could be used as a biomarker of fibre intake[32]. The results of the present study showed that individuals carrying the G allele appeared to be more responsive to dietary fibre, presenting lower serum TAG compared with TT individuals. So, adopting healthier dietary patterns such as eating more fibre and less saturated fat would be more beneficial to these individuals.

The mechanisms underlying our observations are speculative. HMGCoA reductase is an enzyme of cholesterol metabolism, and when inhibited by statins has a moderate TAG-lowering efficacy, in the range of 10–35 %, when TAG exceed 150 mg/dl (1500 mg/l)[35,36]. The polymorphism does not apparently alter the basal activity of the enzyme; however, although not confirmed in recently published studies[30,31], it was previously described to lower the response to pravastatin[18]. It is also possible that it is linked to other genetic changes within functional parts of the gene and the observed effect in the present study may reflect this. In fact it is in linkage disequilibrium with another SNP in intron 5 and also with a third SNP in a 3′ untranslated exon of the *HMGCR* gene, which is retained in the mature mRNA[17], but the biological effects of these SNP are also unknown.

The genotype frequencies found are in concordance with the frequencies found in a cohort study of largely Caucasians in the USA (TT 93·23 %; TG 6·70 % and GG 0·07 %)[17]. The frequency found for the minor allele in the present study (0·022) is similar to the frequency (0·019) reported for a study comprising participants from Scotland, Republic of Ireland and The Netherlands[30]. Data about allele frequencies in other ethnicities are scarce. In a recent investigation about the effects of several SNP in the *HMGCR* gene, the frequency of the G allele was found to be 0·09 in African-Americans[37]. This finding might indicate that our observations can have more impact in populations with different genetic backgrounds in which the number of individuals carrying the G allele is higher.

The present study has several limitations. First, it is a cross-sectional study with consequent limitations concerning the effect of dietary components and changes on blood lipids over time. However, the positive association of SFA intake and the negative association of PUFA intake with total serum cholesterol and LDL-cholesterol are consistent with

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the directions predicted by carefully controlled intervention studies[3,4,38]. Second, we measured serum lipids in the non-fasting state. Nevertheless, blood sampling would not be expected to have a major effect on our analysis as indicated by a meta-analysis that showed no differences in IHD risk between non-fasting and fasting participants for TAG levels[2]. Finally, the phenotypic variability of serum lipids is probably related to several common genetic variants in different genes[5,7] which were not considered.

The strength of the present study is the large number of individuals for whom both data on genotype and dietary variables were available along with data concerning important covariates such as physical activity and alcohol intake that allowed us to detect the interactions. Also, a previous study[9] with the same population had found highly significant associations between fatty acids and fibre, assessed by the FFQ, and serum lipid fractions in the same directions predicted from carefully controlled intervention studies on blood lipids[3,4]. Although the FFQ cannot be the best method for assessing these nutrients compared with more detailed methods[39], it might rather attenuate the interactions.

The present results suggest that individuals carrying the G allele for the SNP may show a greater response to dietary fibre intake with lower TAG levels and higher TAG levels with increased SFA intake compared with those homozygous for the major allele. In this way the individuals carrying the minor allele may benefit more from dietary intervention to control serum TAG (for example, substituting SFA for PUFA and increasing fibre intake). However, even though the present study involved a large cohort, only a small number of individuals carried the minor allele. Thus, whether this conclusion would be substantiated by data on a larger population of minor allele carriers remains unknown. Nonetheless, the present study does provide unique information on diet, genotype and blood lipids.

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There are no conflicts of interest to declare.

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