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Short Communication

A variant near the melanocortin-4 receptor gene regulates postprandial lipid metabolism in a healthy Caucasian population

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Abstract

The melanocortin-4 receptor (MC4R) is an essential regulator of food intake and energy homeostasis. Previous data suggest an influence of MC4R activity on TAG levels. Thus, the aim of the present study was to determine whether the presence of the rs12970134 polymorphism near the *MC4R* gene could influence postprandial lipoprotein metabolism in healthy subjects. A total of eighty-eight volunteers were selected, fifty-three homozygous for the common genotype (G/G) and thirty-five carriers for the minor A-allele (G/A and A/A). They were given a fat-rich meal containing 1 g fat and 7 mg cholesterol/kg body weight and vitamin A (60 000 IU/m^2 body surface). Fat accounted for 60 % of energy, and protein and carbohydrates accounted for 15 and 25 % of energy, respectively. Blood samples were taken at time 0, every 1 h until 6 h and every 2·5 h until 11 h. Total cholesterol and TAG in plasma, and cholesterol, TAG and retinyl palmitate in TAG-rich lipoproteins (TRL, large and small TRL) were separated by ultracentrifugation. Individuals carrying the G/G genotype displayed a higher postprandial response of plasma TAG ($P = 0{\cdot}033$), total cholesterol ($P = 0{\cdot}019$) and large TRL–TAG ($P = 0{\cdot}023$) than did carriers of the minor A-allele. Furthermore, G/G subjects showed a greater postprandial response of small TRL–apoB48 than did carriers of the A-allele ($P = 0{\cdot}032$). These results suggest that the rs12970134 polymorphism near the *MC4R* gene region may partly explain the inter-individual differences in postprandial lipoprotein response in healthy subjects.

Key words: Polymorphisms: Nutrigenomics: Postprandial lipaemia: TAG-rich lipoproteins

Over the last decade, accumulating evidence has suggested that elevated plasma TAG concentrations, both in the fasting and the postprandial states, may pose a significant independent risk for CVD[1]. Individual variability in postprandial lipaemic response is usually greater than that observed in the fasting state, and, as most other CHD risk factors, that variability appears to be modulated by environmental and genetic factors[2–4]. Regarding the genetic component, the percentage of variability explained by a few loci showing consistent associations is still rather small, supporting the need to identify additional genes implicated in the regulation of fasting and postprandial lipoprotein metabolism and, potentially, CVD risk.

The melanocortin-4 receptor (MC4R) is a G-protein-coupled receptor that is expressed in the central nervous system, and has a role in regulating feeding behaviour, energy homeostasis and blood pressure. *MC4R* is located on chromosome 18q22[5], and variations in this gene have been reported to be associated with common forms of obesity[6,7]. Previously, an independent genome-wide association study identified four variants (rs12970134, rs477181, rs502933 and rs4450508) in the high linkage disequilibrium downstream of *MC4R*

Abbreviations: MC4R, melanocortin-4 receptor; RP, retinyl palmitate; SCD 1, stearoyl-coenzyme A desaturase 1; TRL, TAG-rich lipoprotein.

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associated with obesity-related quantitative traits, of which the most strongly associated variant was the rs12970134 SNP[8]. In addition, previous data from Bronner *et al.*[9] suggested an influence of MC4R activity on TAG levels in cardiovascular patients. Looking for additional physiological pathways underlying MC4R effects on lipoprotein metabolism, we explored whether the presence of the rs12970134 polymorphism near the *MC4R* gene could modulate postprandial lipid metabolism in a healthy population.

Experimental methods

Subjects

A total of eighty-eight healthy men students at the University of Cordoba responded to an advertisement and were recruited for the study. They had a mean age of 23 (SD 4) years. All subjects underwent a comprehensive medical history, physical examination and clinical chemistry analysis before inclusion in the study, and they did not show evidence of any chronic disease (hepatic, renal, thyroid or cardiac dysfunction). Total cholesterol plasma levels were under 6·2 mmol/l, and TAG plasma levels were below 1·7 mmol/l. None of the subjects had a BMI above 30 kg/m^2. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Investigation Review Committee at the Reina Sofia University Hospital. Written informed consent was obtained from all subjects.

After a 12 h fast, volunteers were given a fatty meal enriched with 60 000 IU of vitamin A/m^2 of body surface area. The amount of fat given was 1 g of fat and 7 mg cholesterol/kg body weight. The meal contained 65 % of energy as fat, 15 % as protein and 25 % as carbohydrates, and was consumed in 20 min. The foods provided were bread, whole milk, eggs and butter. After this meal, subjects fasted for 11 h, but they were allowed to drink water. Blood samples were drawn before the meal, every hour until the 6th hour and every 2 h and 30 min until the 11th hour. The detailed design and methods of the study have been described previously[10,11].

Laboratory methods

Blood was collected in tubes containing EDTA to give a final concentration of 0·1 % EDTA. Plasma was separated from erythrocytes by centrifugation at 1500 ***g*** for 15 min at 4°C. The chylomicron fraction of TAG-rich lipoproteins (TRL) (large TRL, Swedberg flotation units (Sf) > 400) and the non-chylomicron fraction (also referred to as small TRL, Sf 20–400) were isolated as described previously [10]. Cholesterol and TAG in plasma and lipoprotein fractions, plasma apoAI and apoB were determined as described previously [10]. HDL-cholesterol was measured by analysing the supernatant obtained following precipitation of a plasma aliquot with dextran sulphate–Mg^{2+}, as described by Warnick *et al.*[12]. LDL-cholesterol was measured as the difference between total cholesterol before ultracentrifugation and that at the bottom part of the tube after ultracentrifugation at a density of 1019 kg/l.

The retinyl palmitate (RP) content and the peak of RP and retinyl acetate were assayed using a method described previously[13,14]. ApoB48 and apoB100 were determined by SDS-PAGE as described by Karpe & Hamsten[15]. Gels were scanned with a video densitometer scanner (TDI, Madrid, Spain) connected to a personal computer for integration of the signals. Background intensity was calculated after scanning an empty lane.

DNA amplification and genotyping

DNA extraction was performed by the standard procedures. Genotyping for the rs12970134 polymorphism at the *MC4R* gene was conducted by Illumina, Inc. (San Diego, CA, USA). Adherence to Hardy–Weinberg equilibrium at each SNP locus was determined using the χ^2 test with 1 df.

Statistical analysis

Several variables were calculated to characterise the postprandial responses of plasma TAG, large TRL and small TRL to the test meal. The area under the curve is defined as the area between the plasma concentration *v.* time curve and a line drawn parallel to the horizontal axis through the 0 h concentration. This area was calculated by a computer program using the trapezoidal rule. Data were tested for statistical significance between genotypes by ANOVA and the Kruskal–Wallis test, and between genotypes and time by ANOVA for repeated measures. In the present analysis, we studied the statistical effects of the genotype alone independent of time in the postprandial study (*P*1), the effect of time alone or change in the variable after ingesting fatty food over the entire lipaemic period (*P*2) and the effect of the interaction of both factors, genotype and time, indicative of the magnitude of the postprandial response in each group of subjects with a different genotype (*P*3). A probability value $<0{\cdot}05$ was considered significant. All data presented are expressed as means and standard deviations. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical comparisons.

Results

The basal characteristics by genotype were analysed according to rs12970134. No differences for any of the variables examined (BMI, total cholesterol, TAG, HDL-cholesterol, LDL-cholesterol, apoB and apoA1) were observed at baseline. Given the low genotype frequencies of individuals homozygous for the minor alleles, and as the analysis did not suggest a recessive mode of action, we analysed the rs12970134 SNP using two genotype categories (G/G subjects (*n* 53) and G/A + A/A subjects (*n* 35)).

The postprandial response of plasma TAG and TAG in different lipoprotein fractions was analysed. A significant effect of time on plasma TAG levels ($P<0{\cdot}001$), HDL-cholesterol ($P<0{\cdot}001$), LDL-cholesterol ($P<0{\cdot}001$), total cholesterol ($P<0{\cdot}001$), small TRL–TAG ($P<0{\cdot}001$), large TRL–TAG ($P<0{\cdot}001$), small TRL-cholesterol ($P<0{\cdot}001$), large TRL-cholesterol ($P<0{\cdot}001$), and in the small TRL–RP ($P<0{\cdot}001$), large

British Journal of Nutrition

TRL–RP ($P=0{\cdot}039$), small TRL–apoB100 ($P<0{\cdot}001$), large TRL–apoB100 ($P<0{\cdot}001$), small TRL–apoB48 ($P<0{\cdot}001$) and large TRL–apoB48 ($P<0{\cdot}001$) were observed, with respect to baseline levels, indicating an increase in these parameters in the different groups of subjects during the postprandial period.

Individuals carrying the G/G genotype displayed a higher postprandial response of large TRL–TAG than did carriers of the minor A-allele (G/A + A/A, $P=0{\cdot}023$; Fig. 1(a)). In addition, the analysis of the interaction between genotype and time showed that carriers of the minor A-allele had an accelerated clearance of large TRL–TAG ($P=0{\cdot}030$; Fig. 1(a)). G/G individuals also presented higher concentrations of total plasma TAG (Fig. 1(b)) and total cholesterol during the postprandial period than did carriers of the A-allele ($P=0{\cdot}033$ and $0{\cdot}019$, respectively). Moreover, G/G subjects showed a greater postprandial response of small TRL–apoB48 than did carriers of the A-allele ($P=0{\cdot}032$; data not shown). The postprandial area under the curve in the study participants according to the rs12970134 polymorphism was analysed; significant differences were observed between genotypes. Consistently, the area under the curve of TAG and large TRL–TAG was greater in G/G individuals than in carriers of the A-allele ($P=0{\cdot}027$ and $0{\cdot}022$, respectively). No other statistically significant genotype-related differences for other parameters were observed.

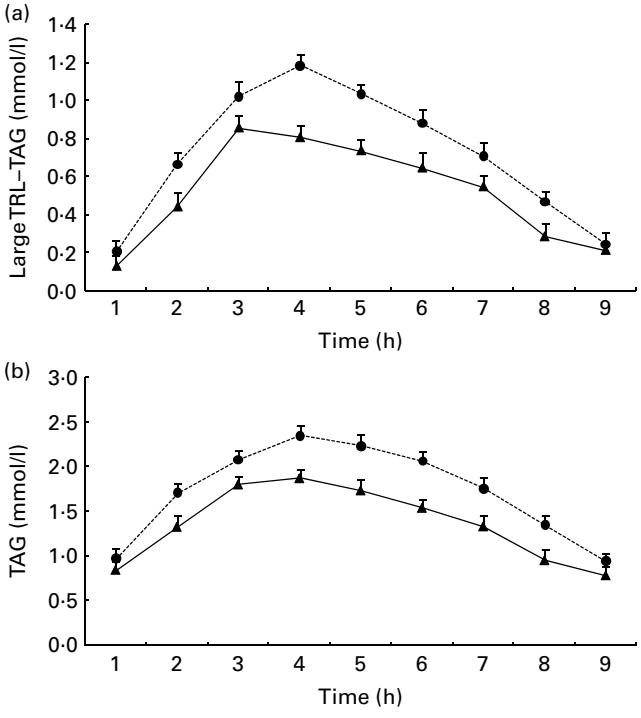

Fig. 1. Evolution of (a) large TAG-rich lipoproteins (TRL)–TAG and (b) TAG depending on the rs12970134 SNP near the *MC4R* gene. Line plots of postprandial large TRL TAG and plasma TAG in G/G subjects (n 53, discontinuous line, (●) and G/A + A/A subjects (n 35, continuous line, (▲). ANOVA for repeated measures. (a) $P1=0{\cdot}023$, genotype effect alone; $P2=0{\cdot}001$, time effect alone; $P3=0{\cdot}030$, genotype–time interaction. (b) $P1=0{\cdot}033$, genotype effect alone; $P2=0{\cdot}001$, time effect alone; $P3=0{\cdot}233$, genotype–time interaction.

Discussion

The present findings show that healthy G/G carriers of the rs12970134 SNP near the *MC4R* gene have a higher postprandial response of TAG and large TRL–TAG compared with carriers of the minor A-allele. These results were derived from a highly controlled, standardised trial of apoE E3/E3 participants who were subjected to a lipaemia test meal. Conducting a study with hourly blood draws is complex and a total duration of 11 h is high; therefore, certainly for large epidemiological cohorts, detailed postprandial assessment is not realistic because of methodological issues. Although other simpler designs for postprandial lipaemia assessment have been reported, our method allows deep evaluation into the postprandial state[16,17].

MC4R gene is expressed in several sites in the brain and has been implicated in mediating most of the effects of melanocortin on food intake and energy expenditure[18]. A recent study conducted in cardiovascular patients has suggested the influence of MC4R activity on TAG levels[9]. However, the influence of the rs12970134 polymorphism, in high linkage disequilibrium with other variants, on the postprandial lipaemic response and its functionality remains unknown. Thus, the present study is, to our knowledge, the first to examine the association between this polymorphism and postprandial lipaemic response in healthy subjects. We have observed that G/G carriers have a higher postprandial response of TAG and large TRL–TAG compared with carriers of the minor A-allele. Moreover, G/G subjects showed a greater postprandial response of small TRL–apoB48 than did carriers of the A-allele, suggesting a delayed postprandial clearance of these particles. In contrast, no other statistically significant genotype-related differences for large TRL–apoB48, large and small TRL–apoB100, and large and small TRL–RP were observed. Several mechanisms might explain the change observed in the catabolism of TRL in subjects with the rs12970134 polymorphism. Central blockage of the melanocortin receptors increased serum TAG levels more than twofold in healthy control rats[19]. Moreover, previous studies explored how MC4R affect liver metabolism. The first clues stem from animal models. In rats, intracerebroventricular infusion of the MC3/4R synthetic agonist melanotan-II reduces the mRNA expression of stearoyl-coenzyme A desaturase 1 (SCD 1) in the liver, whereas the MC3/4R antagonist SHU9119, a synthetic peptide with a β-(2-naphthyl)-D-alanine, has the opposite effect[20]. This suggests that modulation of MC3/4R activity in the brain can affect liver metabolism. SCD1 is required for fatty acid desaturation and, consequently, TAG synthesis. A previous study showed that SCD $1^{-/-}$ mice showed severely reduced VLDL secretion by the liver[21]. Therefore, we could hypothesise that a more active form of the MC4R linked with the minor A-allele entails a reduced SCD 1 activity, resulting in reduced TAG synthesis and a more efficient postprandial response shown by a faster clearance of small TRL–apoB48. In line with this notion, previous studies have also reported the relationship between TAG plasma levels and *MC4R* variations[9,22]. Although the results are still hypothetical, the present study proposes a link between MC4R, SCD1 and plasma TRL. Obviously, we need

to be cautious before extrapolating our conclusions to other populations, and functional analyses of this variant are necessary to support this hypothesis. On the other hand, although rs12970134, rs17782313 and rs17700633 are located relatively far away from the coding region of MC4R (between 188 and 109 kb downstream), we think that the effects of these SNP may be mediated by an alteration in the function/expression of this gene, based on that MC4R known functions which are intimately linked to our phenotypic outcomes, and that close proximity between coding sequences and regulatory elements is not mandatory[23]. Although these results are preliminary, the fact that the present study was carried out in healthy individuals in a controlled interventional trial could be the key to a better understanding of the effect of this polymorphism on postprandial lipoprotein metabolism. In conclusion, allele variability in the *MC4R* gene region could partly explain the inter-individual differences in postprandial lipaemic response in healthy subjects.

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British Journal of Nutrition