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RAPID COMMUNICATION

The A-204C Polymorphism in the Cholesterol 7α-hydroxylase (CYP7A1) Gene Determines the Cholesterolemia Responsiveness to a High-Fat Diet

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Summary

The aim of the study was to ascertain whether the A-204C polymorphism in the cholesterol 7α-hydroxylase (CYP7A1) gene plays any role in determining LDL-cholesterol (LDL-C) concentration responsiveness to a high-fat diet. The concentrations of total cholesterol and LDL-cholesterol were measured in eleven healthy men (age: 30.9±3.2 years; BMI: 24.9±2.7 kg/m^2) who were homozygous for either the -204A or -204C allele, after 3 weeks on a low-fat (LF) diet and 3 weeks on a high-fat (HF) diet. During both dietary regimens, the isocaloric amount of food was provided to volunteers; LF diet contained 22 % of energy as a fat and 2.2 mg of cholesterol/kg of body weight a day, HF diet 40 % of fat and 9.7 mg of cholesterol/kg of body weight a day. In six subjects homozygous for the -204C allele, the concentrations of cholesterol and LDL-cholesterol were significantly higher on HF than on LF diet (cholesterol: 4.62 vs. 4.00 mmol/l, $p<0.05$; LDL-C: 2.15 vs. 1.63 mmol/l, $p<0.01$, respectively); no significant change was observed in five subjects homozygous for the -204A allele. There were no other differences in lipid and lipoprotein-lipid concentrations. Therefore, the polymorphism in the cholesterol 7α-hydroxylase promotor region seems to be involved in the determination of cholesterol and LDL-C responsiveness to a dietary fat challenge.

Key words

LDL-cholesterol • Cholesterol 7α-hydroxylase • Diet responsiveness • Genetics

A high concentration of cholesterol, especially low-density lipoprotein (LDL) cholesterol in plasma, is a prominent risk factor for coronary heart disease and the strategy of both primary and secondary prevention of ischemic heart disease is aimed at lowering cholesterolemia. However, it has been repeatedly shown that dietary manipulation is not fully efficient in all subjects and that there is a strong inter-individual variability in the response of cholesterolemia to low-fat dietary regimens (Beynen *et al.* 1987). Presumably, the responsiveness of cholesterolemia to a dietary change is determined genetically, and several genes seem to be involved (Ordovas and Galuzzi 1999).

Cholesterol 7α-hydroxylase is likely to be one of

the critical genes, which can play a role in responsiveness determination (Cohen 1999). We have recently documented in a cohort of 131 men from the Czech MONICA study that A-204C polymorphism in the cholesterol 7α-hydroxylase gene is strongly associated with the magnitude of changes in cholesterolemia (Hubáček *et al.* 2003). During 8-year follow-up, there was a pronounced change in the diet of the Czech population associated with a decrease in cholesterolemia. In our study, the serum cholesterol of individuals homozygous for the -204A allele dropped by 0.4 mmol/l, whereas the cholesterol of those homozygous for -204C decreased by 1.3 mmol/l.

In order to confirm such an observation in a well-controlled setting of a clinical study, we measured the response of cholesterolemia to a high-fat diet challenge in healthy men homozygous for the -204A or -204C allele in a preliminary study with a cross-over design.

Eleven men (age: 30.9±3.2 years; BMI: 24.9± 2.7 kg/m^2; cholesterol: 4.74±0.73 mmol/l; triglycerides: 1.03±0.57 mmol/l; HDL-cholesterol: 1.40±0.25 mmol/l; LDL-cholesterol [calculated from Friedewald's formula]: 2.87±0.61 mmol/l), six homozygous for -204C and five homozygous for the -204A allele, were included into the study. Carriers of both alleles did not differ in age, BMI or lipid concentrations on entering the study. They were matched with respect to apolipoprotein E gene polymorphisms. All volunteers consumed a low-fat (LF) diet for 3 weeks and a high-fat (HF) diet for another 3 weeks. The order of these dietary periods was randomized. During both dietary periods, the subjects received food of isocaloric content. The composition of diets differed in the content of fat, cholesterol, and carbohydrates (LF diet: 22.2 % of energy as fat, 62.2 % of energy as carbohydrates, 2.2 mg of cholesterol/kg of body weight a day; HF diet: 39.5 % of fat, 45.3 % of carbohydrates, 9.7 mg of cholesterol/kg of body weight a day). Animal fat represented 31 % and 86 % of total fat in LF and HF diets, respectively. The body weight of the subjects was 1.5 % higher at the end of the HF dietary period than at the end of LF dietary period ($p<0.01$), with no difference observed between both compared groups. At the end of each dietary period, blood was drawn after 12-h fasting to determine cholesterol, triglycerides and serum lipoprotein concentrations.

The A-204C polymorphism in the cholesterol 7α-hydroxylase (CYP7A1) gene was determined during the screening phase of the study as described earlier (Hubáček *et al.* 2003).

Very low-density, intermediate-density, low-density and high-density lipoproteins (VLDL, IDL, LDL, and HDL, respectively) were isolated by sequential ultracentrifugation (Havel *et al.* 1955), cholesterol and triglyceride (TG) in serum and lipoprotein fractions were determined with Roche COBAS MIRA autoanalyzer (Hoffmann-La Roche, Switzerland) using enzymatic kits from the same manufacturer. The recovery of cholesterol and TG in ultracentrifugation-isolated lipoproteins was 82 and 95 %, respectively. The differences in lipid and lipoprotein-lipid concentrations between -204A and -204C subjects on both LF and HF diets were evaluated using Student's t test, the differences in lipid and lipoprotein-lipid concentrations on LF and HF diets in both -204A and -204C subjects were evaluated using the paired t test.

Table 1. Cholesterol in serum and ultracentrifugally isolated lipoproteins in subjects homozygous for either the -204C or -204A allele on low-fat and high-fat diets.

	-204C		-204A	
	Low-fat diet	**High-fat diet**	**Low-fat diet**	**High-fat diet**
Cholesterol (mmol/l)	4.00 ± 0.34	4.62 ± 0.35*	4.92 ± 1.04	4.79 ± 1.00
VLDL-C (mmol/l)	0.23 ± 0.11	0.24 ± 0.07	0.24 ± 0.19	0.21 ± 0.18
IDL-C (mmol/l)	0.08 ± 0.05	0.10 ± 0.03	0.11 ± 0.04	0.10 ± 0.03
LDL-C (mmol/l)	1.63 ± 0.34	2.15 ± 0.28**	2.26 ± 0.70	1.88 ± 0.56
HDL-C (mmol/l)	1.32 ± 0.29	1.44 ± 0.23	1.56 ± 0.34	1.49 ± 0.30

Data are means ± S.D., *, ** $p<0.05$, $p<0.01$ (low-fat vs high-fat diet using paired t-test). There was no significant effect of diet change on triglyceride concentration in serum and isolated lipoproteins (data not shown). No significant difference in cholesterol and TG concentrations in serum and ultracentrifugally isolated lipoproteins between -204C and -204A subjects on both LF and HF diets were observed.

In -204A allele carriers, feeding of HF diet had no effect either on cholesterolemia and triglyceridemia, or on any lipoprotein concentration. On the other hand, in -204C allele carriers, cholesterol and LDL-C were 16 % and 32 % higher on a HF diet (Table 1, Fig. 1). No other parameter of lipoprotein metabolism was affected by the change in diet.

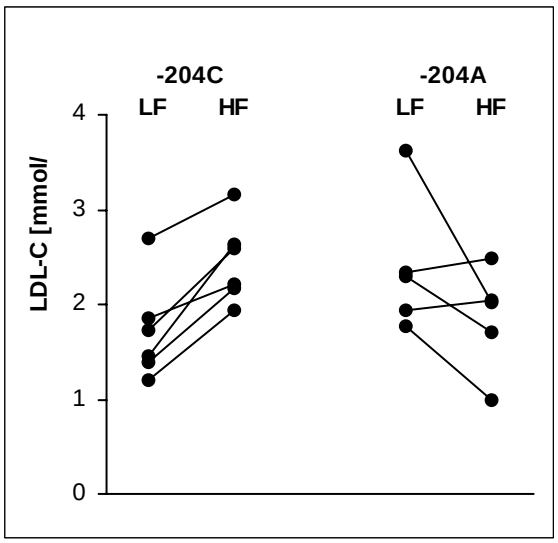

Fig. 1. LDL-cholesterol change between low-fat (LF) or high-fat (HF) diets in subjects homozygous either for the -204C or -204A allele.

The finding that subjects homozygous for the -204C allele respond to a HF diet by an increase in LDL- and total cholesterol (whilst the carriers of -204A allele not) is in support of our previous finding that a polymorphism in the promotor region of CYP7A1 affects the responsiveness to a HF diet.

It has been repeatedly shown in population studies that A-204C polymorphism in the CYP7A1 gene is partially responsible for the variation in cholesterolemia (Wang *et al.* 1998, Couture *et al.* 1999). In these studies, the -204C allele was associated with an increased cholesterol concentration and, judging by our results, it could be assumed that such findings could be explained by a hyperresponsiveness of the carriers of this allele to a HF diet.

It can be speculated that subjects carrying the -204C allele are unable to respond to a high-fat and/or high-cholesterol load by upregulation of cholesterol 7α-hydroxylase activity. Indeed, cholesterol and dietary fats have been shown to modulate cholesterol 7α-hydroxylase activity and gene expression (Cheema *et al.* 1997). However, the exact mechanism by which different fatty acids can modulate gene expression and the activity of CYP7A1 remains to be elucidated.

In conclusion, we have shown, in this group of healthy young men, that the promotor variant of the CYP7A1 gene affects the responsiveness of cholesterolemia to extensive dietary changes. Although our study was not large enough to allow for definite conclusions with respect to the role played by CYP7A1, our data has furnished evidence (for the first time) strongly supporting its involvement in direct regulation of cholesterol and LDL-cholesterol concentration responsiveness to a dietary change.

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Reprint requests

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