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The lipoprotein/lipid profile is modulated by a gene–diet interaction effect between polymorphisms in the liver X receptor-α and dietary cholesterol intake in French-Canadians

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Genetic and nutritional factors interact together and modulate the plasma lipid profile. We identified variations in the gene encoding the liver X receptor α (LXRα) and investigated their effects on the plasma lipoprotein/lipid profile. We also examined whether the association between cholesterol intake and plasma lipid profile was modulated by LXRα variants. The LXRα gene was sequenced in thirty-five French-Canadian men with high plasma total cholesterol (>5·0 mmol/l) and LDL-cholesterol (>3·5 mmol/l) concentrations. Dietary cholesterol was obtained from a food-frequency questionnaire. The LXRα c.-115G > A, c.-840C > A and c.-1830T > C genotypes were determined by direct sequencing in 732 subjects. Molecular screening of the LXRα gene revealed sixteen variants. Genotypes c.-115G > A, c.-840C > A and c.-1830T > C (rare allele frequency of 14·3 %, 14·2 % and 11·0 %, respectively) were analysed further. Plasma total cholesterol concentrations were higher in carriers of the -115A, -840A and -1830C allele, compared with the -115G/G, -840C/C and -1830T/T homozygotes ($P \leq 0{\cdot}05$). In a model including the c.-115G > A polymorphism, cholesterol intake, the interaction term c.-115G > A × cholesterol intake (mg/d) and covariates, LXRα-115G > A explained 1·8 % and 2·1 % of the variance in total cholesterol and LDL-cholesterol concentrations ($P=0{\cdot}02$ and $P=0{\cdot}01$), whereas the interaction term explained 2·9 % ($P=0{\cdot}002$) and 2·8 % ($P=0{\cdot}005$), respectively. When subjects were divided into four groups according to the median of cholesterol (290·8 mg) and -115G > A genotypes, high cholesterol intake was associated with higher cholesterol levels in -115A carriers. Similar results were observed for c.-840C > A and c.-1830T > C. These results suggest that cholesterol intake interacts with LXRα variants to modulate the plasma lipid profile.

Liver X receptor-α: Gene–diet interactions: Plasma lipoprotein/lipid profile: Cardiovascular disease: Dietary cholesterol intake

CVD is the leading cause of death in North America (Novak, 1998; Heart and Stroke Foundation of Canada, 2003), emphasizing the need to understand its aetiology and to improve prevention and treatment strategies. Elevated plasma cholesterol concentrations are a well-known risk factor (Wilson *et al.* 1980; Grundy, 1995). The importance of dietary cholesterol in determining plasma cholesterol concentrations has been well documented (Dayton *et al.* 1968; Turpeinen, 1979; Clarke *et al.* 1997; Hopkins, 1992). There exists, however, a large interindividual variability in the plasma cholesterol response to changes in dietary cholesterol intake (Dreon & Krauss, 1997; Grundy & Denke, 1990). Jacobs *et al.* (1983) have previously demonstrated that 9 % of subjects could be characterized as 'hyperresponders' when referring to the effect of a change in diet on plasma cholesterol levels, whereas 9 % were characterized as 'hyporesponders'. Scientific evidence suggests that genetic factors might explain some of the interindividual variability in the plasma cholesterol response following modifications in cholesterol intake (Ordovas & Schaefer, 1999).

The liver X receptor α (LXRα) is a transcription factor expressed predominantly in the liver but also in the kidney, intestine, macrophages, adipose tissue, spleen and adrenal glands (Kohro *et al.* 2000). LXRα regulates the expression of target genes by binding DNA sequence elements, termed LXR response elements (Willy *et al.* 1995). Several genes encoding proteins involved in cholesterol metabolism, for example SREBP-1, ABCA1, ABCG5 and ABCG8, are regulated by LXRα (Peet *et al.* 1998). In addition, oxysterols, which are important in steroid hormone biosynthesis, bile acid synthesis and the conversion of lanosterol to cholesterol, are also potent activators of LXRα, suggesting that LXRα may be an important sensor of cholesterol metabolites (Janowski *et al.* 1996; Lehmann *et al.* 1997) and might be involved in the

Abbreviations: LXRα, liver X receptor-α.

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interindividual variability observed in the plasma cholesterol response to changes in dietary cholesterol intake.

The first aim of the present study was first to identify genetic variants in the LXRα gene and evaluate their effects on the plasma lipoprotein/lipid profile. Second, we examined whether polymorphisms in the LXRα gene could modulate the association between cholesterol intake and plasma lipoprotein/lipid profile.

Subjects and methods

Subjects

The study sample originates from the Saguenay-Lac-St-Jean region, located in the north-eastern part of the province of Quebec. A total of 732 subjects (614 men, 118 women) were recruited through the Chicoutimi Hospital Lipid Clinic. Subjects were excluded if they were diagnosed with type 2 diabetes, type III dysbetalipoproteinaemia, familial hypercholesterolaemia or familial combined hyperlipidaemia. Type 2 diabetes was diagnosed according to the WHO criteria (Alberti & Zimmet, 1998). Written informed consent was obtained from all participating subjects, and the Medical Ethics Committee of Laval University and Chicoutimi Hospital Lipid Clinic approved the protocol.

Metabolic and anthropometric variables

Blood samples from subjects free of medication were obtained in the morning after a 12 h overnight fast. Blood was taken from an antecubital vein into vacutainer tubes containing EDTA. Blood samples were centrifuged within 1 h, and the plasma was frozen (− 80°C) until analysis. Plasma cholesterol and triacylglycerol were measured using enzymatic assays (Burstein & Samaille, 1960; McNamara & Schaefer, 1987). LDL-cholesterol was calculated using the Friedewald formula (Friedewald *et al.* 1972). The HDL-cholesterol fraction was obtained after precipitation of LDL particles in the infranatant with heparin and $MnCl_2$ (Burstein & Samaille, 1960; Havel *et al.* 1955). Apo B concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell (1966), as previously described (Moorjani *et al.* 1987). Serum standards were prepared and calibrated against reference sera obtained from the Center for Disease Control (Atlanta, GA, USA). Body weight, height and BMI were recorded.

DNA analysis

Genomic DNA was extracted using the Qiagen extraction kit (San Francisco, USA). The exons, the exon-intron splicing boundaries as well as the 5′ and 3′ regions of the human LXRα gene were sequenced to screen for DNA variants in thirty-five men exhibiting high plasma total-cholesterol and LDL-cholesterol concentrations (>5 and >3·5 mmol/l, respectively). Table 1 shows the primers used to amplify the different parts of the gene. Primers were designed using sequences available on GenBank (Accession numbers: AC024045) and Primer 3.0 software available on the Whitehead Institute/MIT Center for Genome Research server (Rozen & Skaletsky, 2000). PCR conditions were as follows: reaction volume 50 μl, 1 U RedTaq DNA polymerase (Sigma; St Louis, MO, USA), 5 μl

Table 1. PCR primers for genomic amplification of liver X receptor-α promoter and exons

Gene region	Primer sequences	Annealing temperature (°C)	Product size (bp)
Promoter fragment 4	F-TAACCCGAGCTCCATTTGAC R-TCTACACCCCCTGTCCACTC	55	477
Promoter fragment 3	F-AAGAGAGGAGGCCAGGAGAG R-CACGCCATTCTTCTGTCTCA	66	585
Promoter fragment 2	F-CACAGGGCAGCTTCTCCAC R-GTTGCCTGTCTTCCTTCTGC	66	562
Promoter fragment 1	F-CAGCACAAACCTGTCTCCAA R-CAGGGAAATGCCACTGTTTT	66	581
Exon 1	F-GCAATCAGGTTTCAGCTTCC R-AGAAGTGAGGGGTGATGGTG	66	508
Exon 2	F-CCGCCTAACTGTGTGACCAT R-GGGAGAGGGATGGAATGAAG	66	695
Exon 3	F-AGGTGGAGAAGGGAGCTGAG R-GGAGGCAAGCACGACTAAGA	65	290
Exon 4	F-CCCTCTAAGGGTGGGGATAA R-TCATTTGCCAAGTGCCATTA	58	578
Exon 5	F-GTGGCTGAGTCAGGGAGAAC R- CTCTTTCCCCTCAGCCTCTT	66	381
Exon 6	F-ACCCAGTGCTGTCTGCTTTT R-TCTCCTGCACAGAGACGATG	66	533
Exon 7	F-TCTGACCGGCTTCGAGTC R-GCTTCCCAGCAGATTTGG	59	400
Exon 8	F-TGAAGGGAGAAGCAGAGTGG R-TCACCGTGTTAGCCAGAATG	66	452
Exon 9	F-GGCTCTTCTCGAACTCCTGA R-ATACCCCAAATTGCAACCAA	61	491
Exon 10	F-GTGGAGGCATTTGCTGTGT R-GTCAGGGAATGGGCCTGT	61	286
Exon 11	F-GTGAGTCTCCCCATGGTGTT R-CCTGTGGGAAAAACAGGAAG	65	652

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10 × PCR buffer recommended by the manufacturer, 1·5 mM-$MgCl_2$, 0·2 mM-dNTP, 8·4 μl of each primer at a final concentration of 7·5 μM, 0·1 μg genomic DNA. Betaine was added for sequencing promoter fragments 4, 3 and 1, and exon 4. The annealing temperature for each fragment is shown in Table 1.

Sequencing reactions were performed using BigDyeTerminator v3.0 cycle sequencing (ABI Prism; Applied Biosystems, Foster City, CA, USA) and the products were analysed on ABI 3100 automated DNA sequencer (PE Applied Biosystems). The gel files were processed using the ABI Prism3100 data collection software Applied Biosystems version 1.1 and ABI Prism DNA sequencing analysis software (PE Applied Biosystems) and then assembled and analysed using STADEN preGap4 and Gap4 software (http://staden.sourceforge.net/staden_home.html). Newly identified single nucleotide polymorphisms were genotyped using the same primers and same methods as for sequencing.

Nutritional assessment

A quantitative food-frequency questionnaire was used to evaluate dietary cholesterol intake and was available for 337 subjects. A trained dietitian administered this forty-eight-item questionnaire to participants. The dietitian asked the subjects to recall average use over the previous year. The frequency of food consumption was based on the number of times items were consumed per day, per week or per month. Nutrition Data System for Research version 4.02 (developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA; Food and Nutrient Database 30, published in November 1999) was used to calculate nutrients. The food-frequency questionnaire has been validated, in twenty-five men and women within the same population, using a 3 d food record (V Provencher *et al*. unpublished results). Furthermore, dietary cholesterol intake from food-frequency questionnaires correlated significantly with cholesterol intake as assessed by 3 d food records ($r = 0{\cdot}75$, $P = 0{\cdot}0001$).

Statistical analyses

Variables not normally distributed (triacylglycerol, fasting insulin, cholesterol and total-cholesterol:HDL-cholesterol ratio) were $\log_{10}$ transformed. To establish possible linkage disequilibrium between two polymorphisms, we used the software programs EH and 2LD (Linkage Utility Programs, Rockefeller University, USA: Mathematics & Statistics on the WWW). The Bayesian statistical method implemented in the program PHASE (version 2.1) was used to estimate haplotype frequencies in this study sample (Stephens *et al.* 2001). The association between polymorphisms in the LXRα gene and the plasma lipoprotein/lipid profile was evaluated by analyses of covariance with confounding variables (age and sex or age, sex and BMI) included in the model. Partial Pearson correlation coefficients were used to quantify the age-adjusted interrelationships between the plasma lipoprotein/lipid profile and dietary cholesterol intake in the entire cohort as well as within each genotype group. Gene–diet interactions were evaluated by analyses of covariance using general linear model procedures. Included in the model were the LXRα polymorphism, cholesterol intake, the interaction term and confounding variables (age, gender, energy intake). The source of variation in metabolic parameters was computed using the type III sum of squares. This sum of squares applies to unbalanced study designs and quantifies the effects of an independent variable after adjusting for all other variables included in the model. All the analyses were performed with SAS version 8.2 (SAS Institute, Cary, NC, USA). A value of $P \leq 0{\cdot}05$ was used to identify a statistically significant result.

Results

Molecular screening of the LXRα gene revealed the presence of sixteen polymorphisms (Fig. 1(A)), located principally in the 5′ and intronic regions. One single nucleotide polymorphism was located in exon 5, but it did not alter the predicted serine for this gene at position 99 (c.297C > T or p.Ser99Ser). The relative allele frequency of these variants is shown in Table 2. Database analysis of sequences containing these polymorphisms revealed potential binding sites for the transcription factors Sp-1, HES-1, USF and GATA-1 on the common -115G, -840C and -1830T alleles (Fig. 1(B); using Transcription Element Search Software; http://www.cbil.upenn.edu.tess). The likely functional polymorphisms -115G > A, -840C > A and -1830T > C were therefore selected for further analysis. The genotype distribution of each genetic variant was in Hardy–Weinberg equilibrium. We also observed that these polymorphisms were in perfect linkage disequilibrium ($D' = 1{\cdot}0$, $P < 0{\cdot}0001$ for each pair of single nucleotide polymorphism). Haplotypes were further inferred using PHASE software. Owing to complete linkage disequilibrium between each pair of single nucleotide polymorphisms, two haplotypes were constructed. Therefore, haplotype analyses were not pursued any further.

In order to explore the association of these polymorphisms in the LXRα gene with the plasma lipoprotein/lipid profile, their characteristics were compared between genotype groups (Table 3). Owing to their low number, homozygotes for the rare alleles (-115A, -840A, -1830C; *n* 19), were combined with heterozygotes (*n* 168) in the analyses. Analyses were performed combining results from men and women as there was no sex × genotype interaction for each variable tested (data not shown). We found that plasma total cholesterol concentrations were higher in carriers of the -115A allele than in -115G/G homozygotes after adjusting for age and sex ($P = 0{\cdot}05$; Table 3. The results remained unchanged after adjusting for age, sex and BMI. When age, sex and BMI were included in the model, we observed a significant increase in the triacylglycerol level in carriers of the -115A allele compared with -115G/G homozygotes. Similar associations were found for the LXRα-840C > A and -1830T > C polymorphisms. We also observed similar trends when homozygotes for the rare allele and heterozygotes were analysed separately (data not shown).

As cholesterol metabolites are potent ligands of LXRα, we examined whether polymorphisms in the LXRα gene could modulate the association between dietary cholesterol intake and plasma lipoprotein/lipid profile. Age-adjusted Pearson correlation coefficients were calculated. In the whole group, cholesterol intake was negatively correlated with plasma HDL-cholesterol concentration ($r = -0{\cdot}16$, $P = 0{\cdot}005$) but was not correlated with the total cholesterol:HDL-cholesterol ratio or plasma triacylglycerol, apo B, total cholesterol or LDL-cholesterol concentrations (range $r = 0{\cdot}00$ to $r = 0{\cdot}10$, $P =$ NS). When these analyses were performed for each

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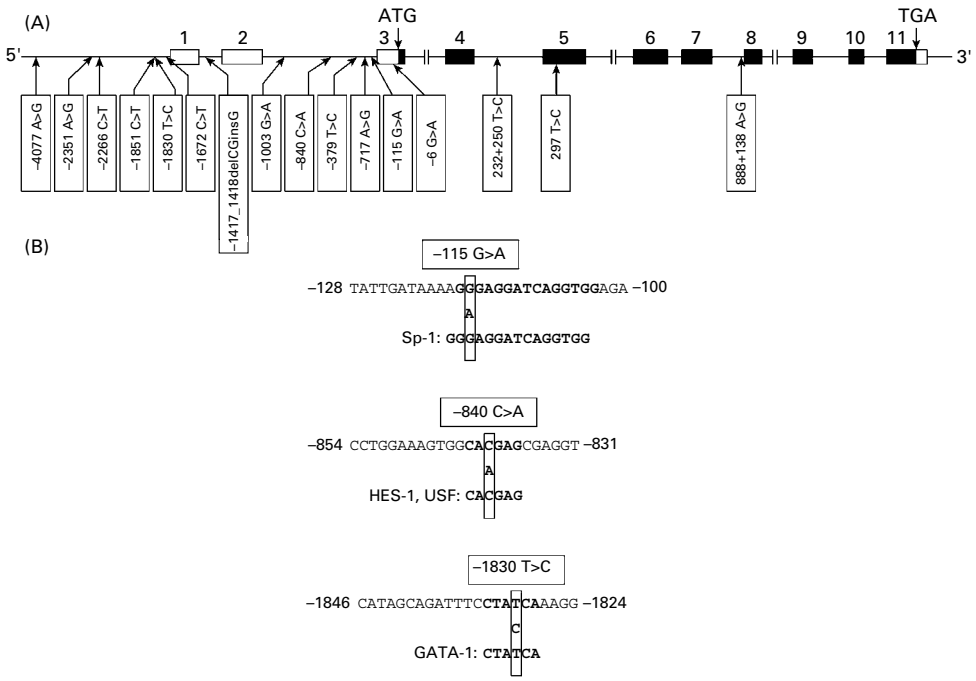

Fig. 1. (A) The schematic structure of the liver X receptor-α (LXRα) gene and polymorphisms identified in the LXRα gene. Black boxes represent coding exons, whereas white boxes represent untranslated exons. (B) The DNA sequences containing each genetic variant identified in the LXRα gene. The consensus sequence for Sp-1, HES-1, USF and GATA-1 binding are shown below these.

genotype group, dietary cholesterol intake was correlated with plasma total cholesterol and LDL-cholesterol concentrations only in carriers of the rare LXRα polymorphisms -115G > A, -840C > A and -1830T > C (range $r = 0{\cdot}28$ to $r = 0{\cdot}31$, $P < 0{\cdot}05$). No correlation with dietary cholesterol was observed in LXRα -115G/G, -840C/C or -1830T/T homozygotes (range $r = -0{\cdot}10$ to $r = -0{\cdot}08$, P=NS).

The interaction between dietary cholesterol and each polymorphism was therefore tested with plasma total cholesterol and LDL-cholesterol concentrations. In a model including the polymorphism, cholesterol intake, the interaction term (polymorphism × cholesterol (mg)), age, sex and energy intake, the LXRα-115G > A polymorphism and the interaction explained 1·82 % and 2·90 % of the variance in plasma total cholesterol concentration (Table 4). Furthermore, 2·12 % and 2·77 % of the variance in plasma LDL-cholesterol concentration was explained by the polymorphism and the interaction between the LXRα-115G > A polymorphism and dietary cholesterol, respectively. Similar results were obtained for the -840C > A and the -1830T > C polymorphism (Table 4). Including saturated fat intake, apo E genotype or BMI in the model did not modify the results.

In order to illustrate these interaction effects, cholesterol intake was separated into two subgroups according to the median value (290·8 mg; Fig. 2). We observed that carriers of the -115A allele were characterized by higher plasma total cholesterol and LDL-cholesterol concentrations when consuming a diet rich in cholesterol (> 290·8 mg). However, in -115G/G homozygotes, plasma total cholesterol and LDL-cholesterol concentrations were similar irrespective of the amount of cholesterol provided from the diet. Similar results were obtained for the effects of the LXRα-840C > A and LXRα-1830T > C polymorphisms on plasma total cholesterol and LDL-cholesterol concentrations (Fig. 2).

Table 2. Relative allele frequency of liver X receptor-α gene variants

Gene variants	Rare allele frequency (%)
c.-4077A > G (rs11039149)	39·39
c.-2351A > G (rs10838683)	26·47
c.-2266C > T (rs11039154)	44·12
c.-1851C > T	25·0
c.-1830T > C (rs3758674)	11·04
c.-1672C > T	6·25
c.-1417_1418delCGinsG	3·28
c.-1003G > A	7·14
c.-840C > A	14·18
c.-379T > C	2·79
c.-171A > G	7·06
c.-115G > A (rs12221497)	14·32
c.-6G > A (rs11039155)	12·72
c.232 + 250T > C (rs2279239)	28·57
c.297 T > C (rs2279238)	11·43
c.888 + 138A > G	4·73

Single nucleotide polymorphism s without 'rs' numbers were newly identified.

Discussion

In the present study, sixteen genetic variants of the LXRα gene were identified. Among them, three polymorphic sites contained potential binding sites for transcription factors on the common allele. The LXRα-115G > A, -840C > A and -1830T > C polymorphisms were associated with moderate elevation of plasma cholesterol and triacylglycerol levels. Moreover, significant interactions between dietary cholesterol

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Table 3. Subjects' characteristics according to each liver X receptor-α genotype
(Mean values and standard deviations)

Variables	− 115G > A						− 840C > A						− 1830T > C					
	G/G			G/A + A/A			C/C			C/A + A/A			T/T			T/C + C/C		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Age (years)	49·5	10·9	545	48·5	10·4	187	49·5	10·8	546	48·5	10·4	186	49·5	10·8	548	48·5	10·4	184
BMI (kg/m^2)	27·1	4·3	539	26·7	4·2	184	27·1	4·3	540	26·7	4·2	183	27·1	4·3	542	26·6	4·2	181
Total cholesterol (mmol/l)	5·92	1·23	545	6·09*†	1·24	187	5·92	1·23	546	6·10*†	1·24	186	5·92	1·23	548	6·10*†	1·25	184
LDL-cholesterol (mmol/l)	3·79	1·01	482	3·89	0·94	168	3·78	1·01	483	3·89	0·94	167	3·78	1·01	485	3·89	0·95	165
HDL-cholesterol (mmol/l)	1·04	0·36	542	1·01	0·33	183	1·04	0·36	543	1·01	0·33	182	1·04	0·36	545	1·01	0·33	180
Triacylglycerol (mmol/l)	2·66	2·02	547	2·91†	2·82	187	2·66	2·02	546	2·91†	2·83	186	2·65	2·02	548	2·92†	2·85	184
Total cholesterol: HDL-cholesterol	6·77	4·62	531	7·10	4·94	178	6·77	4·62	532	7·09	4·95	173	6·78	4·61	534	7·08	4·98	175
Apo B (g/l)	1·16	0·26	394	1·19	0·24	134	1·16	0·26	395	1·19	0·24	133	1·16	0·26	397	1·19	0·24	131
Energy (kcal/d)	2204	640	264	2201	638	73	2204	640	264	2201	638	73	2203	638	266	2204	646	71
Dietary cholesterol (mg)	318·2	141·3	264	328·2	151·2	73	318·2	141·3	264	328·2	151·2	73	319·1	141·1	266	325·1	152·1	71

*Mean values were significantly different from homozygotes after adjusting for age and sex ($P<0·05$).
†Mean values were significantly different from homozygotes after adjusting for age, sex and BMI.

intake and the -115G > A, -840C > A and -1830T > C polymorphisms on plasma total cholesterol and LDL-cholesterol concentrations were observed, suggesting that the association between dietary cholesterol and the plasma lipoprotein/lipid profile was influenced by LXRα gene variants.

To our knowledge, the present study is the first to identify molecular variants in the gene encoding LXRα. Of the newly identified single nucleotide polymorphisms, three were located in regions where transcription factors recognized these sequences to regulate the transcription of LXRα. These sites seem to be abolished when the common allele is substituted for the rare allele at positions -115, -840 and -1830 on the LXRα sequence, suggesting a potential functional role for these genetic variants. In addition, all three polymorphisms are located in well-conserved regions in the mouse LXRα gene. Laffitte *et al.* (2001) have also shown that the promoter region of the human LXRα gene is similar to the mouse LXRα promoter region, suggesting that regions containing these variants are conserved between species and might thus be important in regulating the transcription of LXRα. Functional analyses must, however, be performed to test the functional significance of these three polymorphisms.

Several studies have demonstrated that LXRα is a key regulator of hepatic cholesterol metabolism. Hepatic cholesterol concentrations were increased 15–20-fold in LXRα$^{-/-}$ mice fed a high-cholesterol diet compared with wild-type mice on a similar diet (Peet *et al.* 1998). In addition, mice lacking the LXRα gene did not express the cholesterol 7α-hydroxylase gene, the rate-limiting enzyme in bile acid synthesis normally induced by LXRα (Peet *et al.* 1998), suggesting that LXRα acts as a cholesterol sensor to activate the cholesterol catabolism pathway. In the present study, the plasma lipoprotein/lipid profile was moderately modulated by the presence of molecular variants of the LXRα gene. LXRα also regulates the transcription of genes involved in fatty acid metabolism such as SREBP-1c and FAS, and has been demonstrated to play a role in triacylglycerol metabolism (Peet *et al.* 1998; Jakel *et al.* 2004). According to data obtained in the present study, variants within the LXRα gene promote the accumulation of plasma triacylglycerol, a finding that contrasts with previous results showing that LXRα activation induces lipogenesis. A possible interaction of LXRα with other genes or with dietary fat intake might explain this apparent contradiction as fatty acids induce the expression of LXRα (Tobin *et al.* 2000). Further studies are needed to confirm these results and to understand the effect of LXRα gene variants on plasma triacylglycerol levels.

Interest in studying the interaction between LXRα polymorphisms and dietary cholesterol intake is motivated by the high binding affinity of oxysterols for LXRα (Janowski *et al.* 1996; Lehmann *et al.* 1997). Some of the rate-limiting steps of important pathways in cholesterol metabolism are potent activators of LXRα (Janowski *et al.* 1996). As shown by Peet *et al.* (1998), mice lacking the LXRα gene (LXRα$^{-/-}$) were identical to wild-type mice when fed a standard chow diet. However, the hepatic and plasma cholesterol of LXRα$^{-/-}$ mice fed a diet rich in cholesterol increased dramatically (Peet *et al.* 1998) owing to decreased transcription of the gene encoding the rate-limiting enzyme in bile synthesis, cholesterol 7α-hydroxylase.

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Table 4. Gene–diet interaction effects between liver X receptor-α (LXRα) polymorphism and dietary cholesterol on plasma total cholesterol and LDL-cholesterol concentrations

	Plasma total cholesterol concentrations		Plasma LDL-cholesterol concentrations	
	variance (%)	*P* value	variance (%)	*P* value
LXRα-115G>A polymorphism	1·82	0·02	2·12	0·01
LXRα-115G > A × cholesterol intake interaction	2·90	0·002	2·77	0·005
Model*	4·25		6·93	
LXRα-840C > A polymorphism	1·82	0·01	2·12	0·01
LXRα-840C > A × cholesterol intake interaction	2·90	0·002	2·77	0·005
Model*	4·25		7·23	
LXRα-1830T > C polymorphism	1·87	0·01	2·14	0·01
LXRα-1830T > C cholesterol × intake interaction	3·06	0·001	2·81	0·004
Model*	4·46		7·27	

* Age, sex and energy intake are included in the model.

According to results obtained in the present study and by Peet *et al.* (1998), we hypothesized that the impact of a change in LXRα transcriptional activity (owing to genetic variants) would be relatively small in a 'normal-cholesterol' environment. In the presence of excess of cholesterol from the diet, however, LXRα is not able to handle this surplus, leading to an increase in plasma total cholesterol and LDL-cholesterol concentrations. We could not rely on the absence of induction of cholesterol 7α-hydroxylase, as was seen by Peet *et al.* (1998), to explain our results since cholesterol 7α-hydroxylase is not regulated by LXRα in human subjects (Menke *et al.* 2002; Chiang *et al.* 2001). Considering the large number of genes involved in cholesterol metabolism that are regulated by LXRα, genetic variants affecting the transcriptional regulation of LXRα could still have an impact on the plasma lipid profile in the presence of a diet rich in cholesterol.

The impact of plasma total cholesterol and LDL-cholesterol concentrations on the risk of CVD is well established (Wilson *et al.* 1980; Grundy, 1995). Several strategies have been proposed to manage cholesterol concentration. Among non-pharmacological strategies, a reduction in dietary cholesterol intake appears to be associated with a decreased risk of CVD (Turpeinen, 1979; Dayton *et al.* 1968). A meta-analysis of well-controlled studies confirmed that a diet rich in cholesterol is associated with elevated plasma LDL-cholesterol concentrations (Clarke *et al.* 1997; Hopkins, 1992). However, the plasma cholesterol response to a change in cholesterol intake is highly variable and may be the result of polymorphisms in genes involved in cholesterol metabolism, such as LXRα. An identification of these genetic factors could help us to understand why studies examining the relationship between cholesterol intake and plasma cholesterol concentration are not always consistent. In the present study, we observed that three genetic variants in the LXRα gene modulated the relationship between cholesterol intake and plasma lipoprotein/lipid profile.

Other polymorphisms of genes involved in cholesterol metabolism have been shown to modulate the plasma lipid profile response to cholesterol intake. Among these, it is now well demonstrated that subjects with the apo E4 allele show a greater plasma cholesterol responsiveness to changes in cholesterol intake (Sarkkinen *et al.* 1998). In the present study, an interaction between dietary cholesterol and polymorphisms in the gene encoding LXRα was observed independently of the apo E genotype. In addition to cholesterol intake, physical activity could interact with genes involved in lipid metabolism to modulate total cholesterol and LDL-cholesterol concentrations (Boer *et al.* 1999; Taimela *et al.* 1996). Physical activity was not assessed in this study, but the level of physical activity as reported by Bruce and Katzmarzyk (2002) was relatively low. Indeed, 77 % of women and 74 % of men were physically inactive in Canada (Bruce & Katzmarzyk, 2002).

Nutritional intervention studies will be needed to confirm the role of LXRα as a modulator of the plasma cholesterol response to cholesterol intake. A randomized and cross-over intervention study allows factors such as food intake to be controlled for. In the present study, cholesterol intake was self-reported and might lead to selective underreporting/overreporting. However, the food-frequency questionnaire has been widely used in epidemiological studies and represents a rapid and inexpensive tool for evaluating nutrient intake in large population studies (Willett *et al.* 1987). The presence of other environmental factors such as cigarette smoking might have influenced the observed effects. Statistical adjustment for this covariate did not attenuate the statistical significance of the gene–diet interaction effect (data not shown). Population admixture has been suggested to influence genetic association studies. In the present study, however, subjects were all of French-Canadian descent and population admixture is unlikely to account for the observed effect. Finally, we cannot rule out the possibility that an epistasis effect, not yet identified, might have influenced the effect of the gene–diet interaction.

In conclusion, the results of the present study suggest for the first time an association between human LXRα gene variations and plasma cholesterol concentrations in the presence of a diet rich in cholesterol. The identification of genetic variants of the LXRα gene provides helpful tools for further examining the role of LXRα in terms of the plasma lipoprotein/lipid profile. Although these results need confirmation in other studies, they underline the importance of gene–diet interaction effects in the aetiology of CVD.

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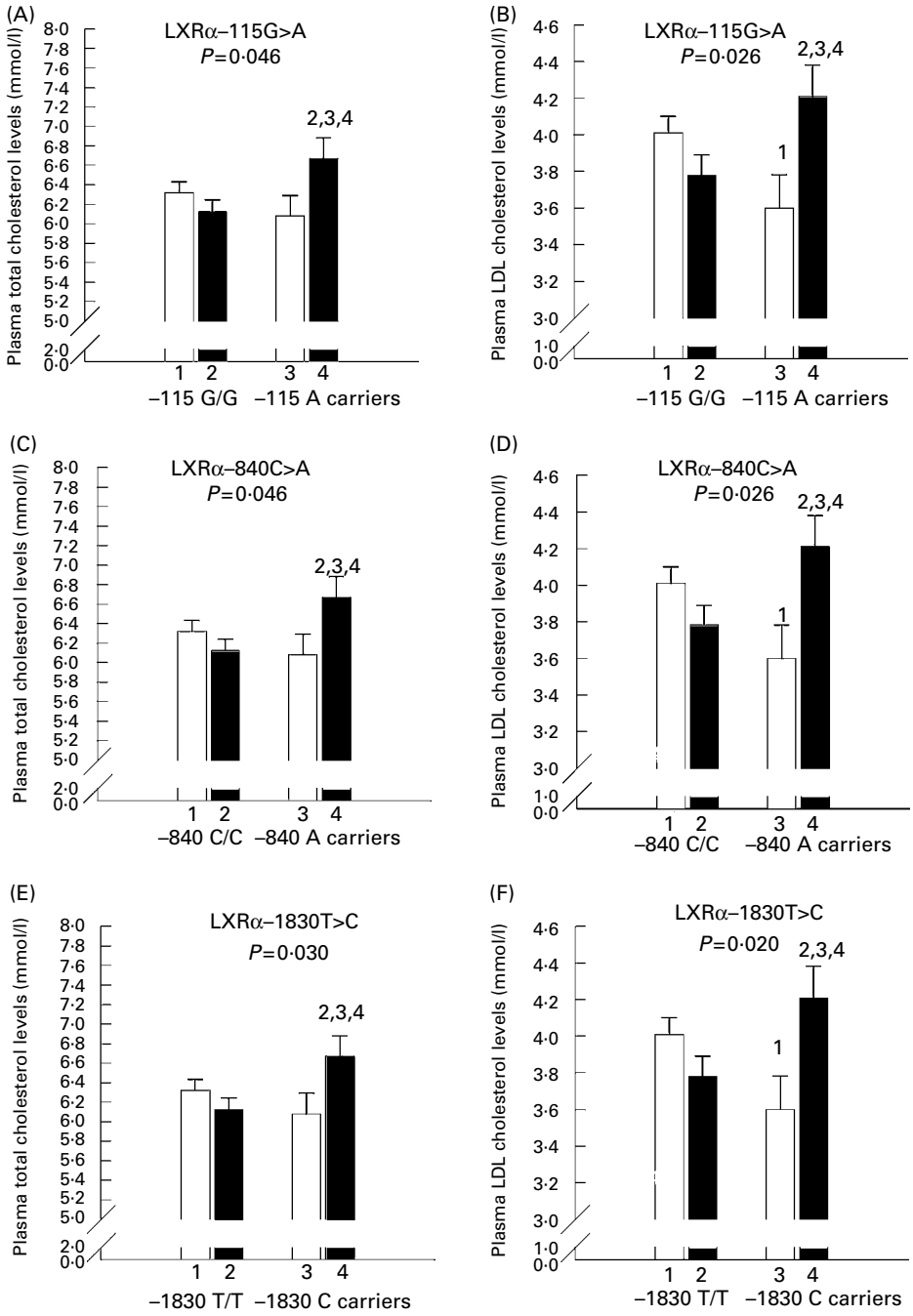

Fig. 2. Effects of the interaction between dietary cholesterol and liver X receptor-α (LXRα) -115G > A ((A), (B)), -840C > A ((C), (D)) and the -1830T > C ((E), (F)) polymorphisms on plasma total cholesterol and LDL-cholesterol concentrations (mean values with their standard errors indicated by vertical bars). Genotype groups are divided on the basis of dietary cholesterol concentrations using the median value (290·8 mg) as a cut-off point. □, Subjects consuming less than 290·8 mg cholesterol; ■, subjects consuming more than 290·8 mg cholesterol. The number at the bottom of each bar identifies each subgroup, whereas the number above the standard error bar indicates the significant difference compared with the corresponding subgroup. *P*, *P* value for the interaction after adjusting for age and sex.

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