Journal of Nutrigenetics and Nutrigenomics

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Received: November 1, 2010
Accepted: March 21, 2011
Published online: June 1, 2011

1661–6499/11/0043–0137$38.00/0
www.karger.com/jnn

Original Paper

Interactions between Dietary Fat Intake and FASN Genetic Variation Influence LDL Peak Particle Diameter

G. Dolley[a, b, d] M.E. Boisclair[a, b] B. Lamarche[a, b] J.P. Després[c, e] C. Bouchard[f] L. Pérusse[a, c] M.C. Vohl[a, b, d]

[a]Institute of Nutraceuticals and Functional Foods (INAF), [b]Departments of Food Science and Nutrition, Laval University, [c]Deparment of Social and Preventive Medicine, Laval University, [d]Endocrinology and Genomics, Laval University Medical Center, and [e]Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Quebec, Quebec, Que., Canada; [f]Pennington Biomedical Research Center, Baton Rouge, La., USA

Key Words
FASN · Fatty acid synthase · Gene-diet interaction · Genome-wide linkage scan · LDL particle size · LDL-peak particle diameter

Abstract
Background: The small, dense LDL phenotype is associated with an increased cardiovascular disease risk. A genome-wide scan performed on the Quebec Family Study (QFS) revealed a quantitative trait locus for LDL peak particle diameter (LDL-PPD) on the 17q21 region. A positional candidate gene – the fatty acid synthase gene *(FASN)* – encodes a key enzyme in the biogenesis of membrane lipids. FASN may play a role in the regulation of feeding and may be a potential therapeutic target for obesity and insulin resistance. ***Methods:*** Analyses were performed on 592 subjects of the QFS. Dietary fat was estimated by a 3-day food record. LDL-PPD was measured by gradient gel electrophoresis on polyacrylamide gradient gels. ***Results:*** Five single nucleotide polymorphisms were genotyped in *FASN* gene. *FASN* rs4246444 was associated with LDL-PPD, but only when fat intake was taken into account ($p = 0.001$). High and low lipid consumers were defined using a cutoff of 35% of dietary fat intake. Carriers of the variant allele showed smaller LDL-PPD only when consuming a high amount of fat. This association remained significant after adjustments for age, sex, body mass index and plasma triglyceride levels. ***Conclusion:*** The results suggest that dietary fat intake may modify the effect of the *FASN* rs4246444 polymorphism on LDL-PPD.

Marie-Claude Vohl
Institute of Nutraceuticals and Functional Foods (INAF)
Pavillon des Services, local 2729K, Université Laval
Quebec, QC G1V OA6 (Canada)
Tel. +1 418 656 2131, ext. 4676, E-Mail Marie-Claude.Vohl@fsaa.ulaval.ca

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Published online: June 1, 2011

Introduction

There is considerable evidence suggesting that LDL particle size varies substantially, even among people with similar plasma cholesterol concentrations [1]. Small, dense LDL particles have been associated with an increased coronary heart disease risk in case-control and prospective studies [1–4]. Data from the Quebec Family Study (QFS) have confirmed the importance of genetic factors influencing LDL particle size [5]. An autosomal genome-wide linkage scan was performed [6], and the strongest evidence of linkage was found on chromosome 17q21 for LDL peak particle diameter (LDL-PPD) after adjustment for age, body mass index (BMI) and triglyceride (TG) levels, with a LOD score of 6.76. In this region, we identified the fatty acid synthase gene *(FASN)* as positional candidate. FASN is a key enzyme in the biosynthesis of fatty acids and could play a role in the pathogenesis of energy metabolism disorders and insulin resistance [7, 8].

Lipoprotein response to dietary fat intake varies widely among individuals [9]. A low-fat diet decreased mean LDL particle size compared with high-saturated-fatty-acid (SFA) diets, with the number of largest and smallest LDL subfractions decreasing, while the intermediate-small fraction increased [10]. Since interindividual variability in LDL particle size is attributable to both genetic [11] and environmental factors, including dietary fat intake [12], numerous studies have investigated dietary fat interaction influencing LDL particle size with polymorphisms located in traditional candidate genes encoding proteins involved in lipoprotein metabolism, notably apolipoprotein (apo) E, apoA4, apoA5 and apoC3 [13–18].

We hypothesized that positional candidate *FASN* gene polymorphisms and dietary fat may modulate LDL particle size. Thus, we examined their potential interaction effects in QFS subjects.

Subjects and Methods

Subjects

Details of recruitment procedures of QFS participants have been published elsewhere [19]. This cohort represents a mixture of random sampling and ascertainment through obese (BMI >32) probands. In the present study, data for both LDL-PPD and fat intake were available for 592 subjects. Characteristics of the study subjects are presented in table 1. The study was approved by the Laval University Medical Ethics Committee, and all subjects provided written informed consent.

Metabolic Measurements

Blood samples were obtained from an antecubital vein in the morning after a 12-hour overnight fast. The plasma was separated immediately after blood collection by centrifugation at 3,000 r.p.m. (850 g) for 10 min for the measurement of plasma lipoprotein/lipid levels. Cholesterol [20] and TG [21] concentrations were determined enzymatically using a Technicon RA-500 automated analyzer (Bayer, Tarrytown, N.Y., USA). The cholesterol content of the infranatant fraction was measured before and after the precipitation step for the calculation of LDL cholesterol. The apoB measurements were performed with the rocket immunoelectrophoretic method [22]. LDL-apoB concentrations were measured in the infranatant ($d > 1.006$ g/ml) obtained after separation of VLDL from the plasma by ultracentrifugation. The measurements were calibrated with reference standards obtained from the Center for Disease Control and Prevention (Atlanta, Ga., USA).

LDL Particle Size Characterization

LDL-PPD was measured by polyacrylamide gradient gel electrophoresis (PAGGE) from plasma obtained after a 12-hour fast. Details of the technique have been previously reported [3]. Briefly, the whole plasma was loaded on nondenaturing 2 to 16% polyacrylamide gradient gel. A 15-min pre-run at 75 V preceded electrophoresis of the plasma samples at 150 V for 3 h. Gels were stained for 1 h with Sudan black and stored until analysis by the Imagemaster 1-D Prime computer software (Amersham Pharmacia Bio-

Table 1. Descriptive statistics of LDL particle size, fat intake and covariates in QFS

Variables	QFS (n = 592)	High fat intake (n = 282)	Low fat intake (n = 310)
Age, years	39.3 ± 16.0	38.7 ± 15.6	39.8 ± 16.5
BMI	27.8 ± 7.7	28.9 ± 8.5	26.9 ± 6.6
TG, mmol/l	1.49 ± 0.8	1.53 ± 0.9	1.46 ± 0.7
LDL-PPD (Å)	263.3 ± 5.1	263.2 ± 5.3	263.3 ± 4.8
$LDL\%_{<255\ Å}$, %	17.0 ± 3.4	17.6 ± 14.7	16.4 ± 11.9
$LDL\text{-}C_{<255\ Å}$, mmol/l	0.52 ± 0.47	0.54 ± 0.5	0.50 ± 0.41
Total fat intake, g	92.7 ± 36.5	111.4 ± 35.8	75.8 ± 27.9
Total fat intake, %	64.5 ± 6.1	39.6 ± 3.7	29.8 ± 3.8
SFA intake, g	27.8 ± 13.2	33.4 ± 14.4	22.7 ± 9.5
PUFA intake, g	12.2 ± 7.1	14.7 ± 7.5	9.90 ± 5.8
Total carbohydrate intake, %	47.6 ± 6.7	43.5 ± 4.9	51.4 ± 5.8
Total protein intake, %	16.0 ± 3.2	15.7 ± 3.2	16.35 ± 3.1

Values are means ± SD.

tech). LDL particle size as defined by the bands was determined on the basis of a calibration curve constructed from plasma standards. Based on PAGGE, a continuous variable was defined as LDL-PPD, reflecting the size of the major LDL subclass in an individual subject. The relative proportion of LDL with a diameter <255 Å (%$LDL_{<255\ Å}$) was ascertained by computing the relative area of the densitometric scan <255 Å [23]. The absolute concentration of cholesterol among particles <255 Å ($LDL\text{-}C_{<255\ Å}$) was estimated by multiplying the total plasma LDL cholesterol (LDL-C) levels by the relative proportion of LDL with a diameter <255 Å.

Dietary Intake

Dietary intake was assessed using a 3-day dietary record, which was completed during 2 weekdays and 1 weekend day. Subjects were asked to record all foods and beverages ingested (except water) with the use of a balance and measuring cups and spoons. All subjects received instructions from a nutritionist on the procedures needed to complete the dietary record and to measure food portions [24]. After completion, the record was verified by a nutritionist. Macronutrient and micronutrient intakes were estimated using a computerized version of the Canadian Nutrient File [25]. We have previously shown that the 3-day dietary record provides reliable estimates of energy and macronutrient intakes [24].

Sequencing and Genotyping

Direct sequencing of coding regions, exon-intron splicing boundaries, and 5′ and 3′ flanking regions was performed to identify new genetic variations in *FASN*. Sequencing of exons and exon-intron splicing boundaries was performed with specific primers derived from the 5′ and 3′ ends of intronic sequences. Primers were designed using the Primer 3.0 software available on the Whitehead Institute/MIT Centre for Genome Research server (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). After PCR amplification, products were purified (Multiscreen, Millipore), and sequencing was performed using BigDye Terminator (version 2.0) and analyzed on ABI 3730XL sequencers (Applied BioSystems, Foster City, Calif., USA). Sequences were then assembled and analyzed using Staden preGAP4 and GAP4 programs [26]. Sequence screening was performed on DNA from 25 unrelated QFS subjects having LDL-PPD at each extreme of the distribution (small: <254 Å; large: >276 Å). Genetic variations were subsequently genotyped on the whole cohort using the TaqMan methodology (Applied BioSystems) [27]. In the case of interspersed repeats or low-complexity DNA sequences, the genetic variations were genotyped by direct sequencing, as described above.

Statistical Analyses

We examined all continuous variables for normality of distribution. TG, fat intake, $\%LDL_{<255\ Å}$ and $LDL\text{-}C_{<255\ Å}$ were log-transformed. The relationship between *FASN* single nucleotide polymorphisms

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Published online: June 1, 2011

Table 2. Allele frequencies of *FASN* SNPs

Gene	SNPs	Allelic frequencies	MAF
FASN	rs2228305	0.02	G:A
	rs2228309	0.40	C:T
	rs2229425	0.10	C:T
	rs4246444	0.38	C:A
	rs17848935	0.17	C:T

MAF = Minor allelic frequency. MAF was calculated in the whole genotyped QFS cohort.

Table 3. Interaction effects between fat consumption and FASN rs4246444 on LDL-PPD

	% fat		SFA (g)		PUFA (g)	
	p^1	p^2	p^1	p^3	p^1	p^3
FASN						
rs4246444	**0.003**	**0.004**	**0.031**	0.115	**0.023**	0.090
Dietary fat	0.357	0.742	0.881	0.949	0.768	0.858
Interaction term	**0.004**	**0.003**	**0.031**	0.059	**0.026**	**0.05**

Interaction terms were determined by analyses of covariance with 3 adjustment models: [1] p values adjusted for the effects of age and sex; [2] p values adjusted for the effects of age, sex, BMI, and TG levels; [3] p values adjusted for the effects of age, sex, energy intake, BMI, and TG levels. Bold values are statistically significant.

(SNPs) and LDL particle features was evaluated by the MIXED procedure in SAS. The sandwich estimator implemented in the MIXED model of the SAS software was used to take into account the non-independence of the data resulting from the familial structure of QFS. This procedure asymptotically yields the same parameter estimates as an ordinary regression method, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. Gene-diet interactions were also evaluated using the MIXED procedure. Statistical analyses for gene-diet interaction with total fat diet were performed following two adjustment models: (1) age and sex, and (2) age, sex, BMI, and TG. SFA and PUFA values were measured in grams. In this regard, further adjustments for energy intake were performed. Therefore, we used (1) age-sex and (2) age-sex-BMI-TG-total energy intake as adjustment models. Since TG values were not normally distributed, the residuals of log-transformed TG values were calculated and used in the model. All statistical analyses were performed with SAS software (version 9.1), and a p value <0.05 was considered as statistically significant. False discovery rate (FDR) implemented in SAS was used to deal with the multiple testing issues.

Results

A total of 18 polymorphisms were identified by direct sequencing of the FASN gene in QFS subjects. Among them, 5 SNPs were selected for two reasons: (1) for their potential impact on the encoded protein structure, and (2) for their coverage of the gene variability. These SNPs were tested for interaction with dietary fat intake on LDL-PPD. Minor allele frequencies for these SNPs are presented in table 2. Genotype frequencies of unrelated QFS subjects did not deviate from Hardy-Weinberg equilibrium expectations.

Journal of Nutrigenetics and Nutrigenomics

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Published online: June 1, 2011

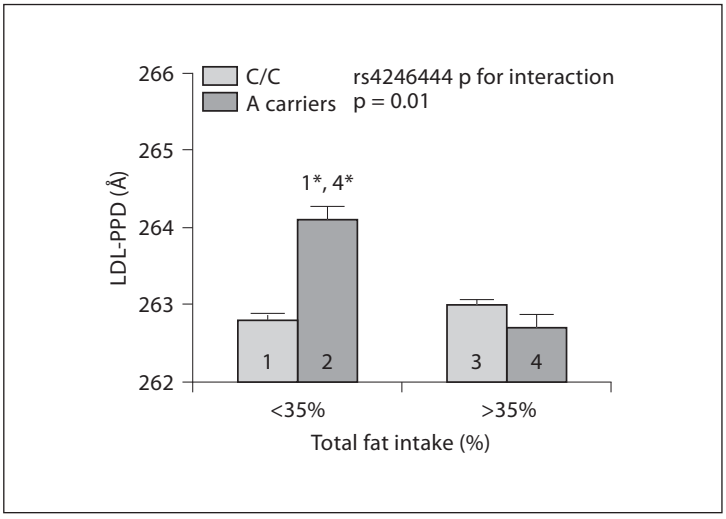

Fig. 1. Mean values of LDL-PPD by *FASN* rs4246444 polymorphism depending on the consumption of fat (low-fat vs. high-fat diet). Percent of energy from fat intake was dichotomized according to the median of the distribution (34.7%, ≈35%). Means + SE (bars). * p = 0.02.

We tested the interaction between *FASN* gene polymorphisms and total fat intake (as continuous variable), expressed as the percentage of the daily caloric consumption, on LDL-PPD. As shown in table 3, the interaction between rs4246444 and fat intake was associated with LDL-PPD (p = 0.004).

To further investigate the interaction between *FASN* rs4246444 polymorphism and dietary fat on LDL-PPD, subjects were divided in two groups on the basis of fat intake using the median value (34.7%, ≈35%) as a cutoff point. A statistically significant interaction (p = 0.01) between *FASN* rs4246444 and total fat intake (>35 or <35%) on LDL-PPD was found (fig. 1). Carriers of the *FASN* rs4246444-A allele had increased LDL-PPD values (p = 0.02) only when consuming ≤35% of fat. LDL-PPD values were similar in CC homozygotes with either a high or a low fat intake. p values for the gene-dietary fat interactions were decreased after adjustment for TG. To verify whether the interaction between *FASN* rs4246444 SNP and fat intake on LDL-PPD was influenced by a potential effect of rs4246444 on TG, we tested the interaction between *FASN* rs4246444 polymorphism and TG in determining LDL-PPD mean values (data not shown). No significant interaction was observed, which suggests that the effect of *FASN* rs4246444 SNP on LDL-PPD was not mediated by plasma TG levels.

Finally, we tested the interaction between *FASN* gene polymorphisms and polyunsaturated fatty acid (PUFA) as well as SFA intake on LDL-PPD. We used absolute consumption of SFA and PUFA in grams, but LDL-PPD mean values were adjusted for daily energy intake. Regarding PUFA consumption, significant interactions were found with *FASN* rs4246444 in determining LDL-PPD (p = 0.026). This result, adjusted for age, sex and total energy intake, remained significant after further adjustments for BMI and TG.

LDL-PPD is only one indicator of LDL particle features. We also tested the interaction between dietary fat and *FASN* rs4246444 on the relative proportion of LDL having a diameter <255 Å (LDL%$_{<255\ Å}$) and on absolute concentrations of cholesterol in this small LDL fraction (LDL-C$_{<255\ Å}$). These variables provide additional information about the pro-atherogenic small LDL fraction. Significant interaction effects were found for LDL%$_{<255\ Å}$ (p = 0.01) and LDL-C$_{<255\ Å}$ (p = 0.008; fig. 2a, b). *FASN* rs4246444-A carriers showed a 3% lower LDL%$_{<255\ Å}$ mean value in the low-fat intake group compared to the high-fat diet group (p = 0.009). In accordance with these results, decreased LDL-C$_{<255\ Å}$ concentrations were found in *FASN* rs4246444-A carriers (p = 0.005) who consumed a low-fat diet (<35% of daily energy intake originating from fat).

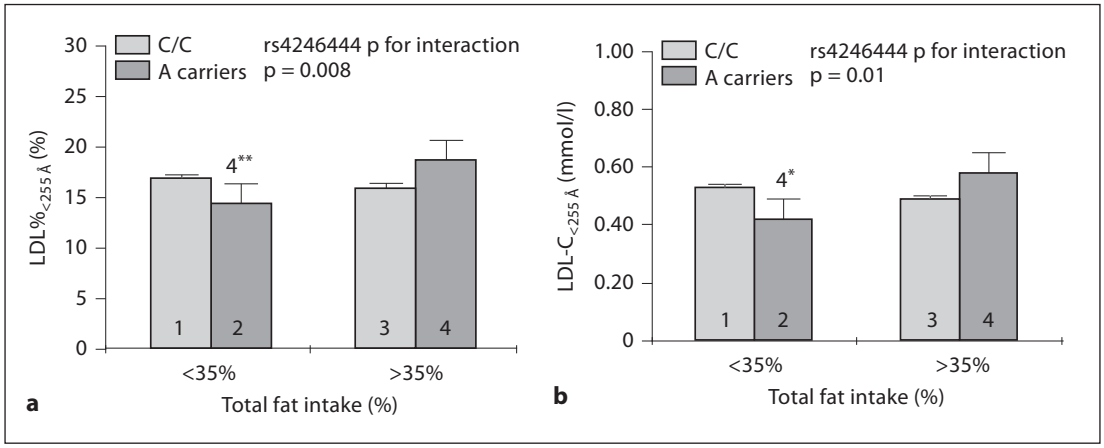

Fig. 2. Mean concentrations of LDL%$_{<255\ Å}$ and LDL-C$_{<255\ Å}$ by *FASN* rs4246444 polymorphism depending on the consumption of fat (low-fat vs. high-fat diet). Percent of energy from fat intake was dichotomized according to the median of the distribution (34.7%, ≈35%). Means + SE (bars). * p = 0.009; ** p = 0.005.

All of these results were presented before corrections for the multiple testing. Five polymorphisms were investigated. Each polymorphism was tested for modifying effects with three dietary fat outcomes in two separate adjustment models. When multiple testing correction was applied using FDR test, only the interaction between rs4246444 SNP and fat intake (expressed as continuous variable) on LDL-PPD remained significant (p = 0.03).

Discussion

Genome-wide linkage scans have become a common strategy for studying the genetics of complex diseases as they are unbiased and without an a priori hypothesis regarding the nature of the genes and sequence variants. A genome-wide linkage scan on LDL-PPD, performed previously on QFS families, revealed a quantitative trait locus on chromosome 17 [6]. In this region, we have recently identified positional candidate genes associated with LDL-PPD [28, 29]. In the present study, we selected *FASN* as a positional candidate gene of the 17q21 region and tested interaction effects between genetic variations in this gene and dietary fat in determining LDL-PPD. *FASN* rs4246444 presented suggestive evidence of interactions throughout the analyses. The analyses of fat intake as a continuous variable revealed significant interaction effects on LDL-PPD. When fat consumption was dichotomized (<35 or >35% of the total energy intake), *FASN* rs4246444-A variant allele was associated with increased LDL-PPD in subjects having a low fat intake, whereas CC homozygotes had comparable LDL-PPD values irrespective of their fat intake. LDL-PPD is a global measurement which provides information on the LDL particle size irrespective of the different LDL subclasses. In these circumstances, we further investigated the interaction effects between FASN rs4246444 and fat intake on the atherogenic small, dense LDL fraction. Thus, lower numbers of small LDL (LDL%$_{<255\ Å}$) as well as lower cholesterol concentrations in these small particles were reported in rs4246444-A allele carriers consuming a low-fat diet, supporting the overall increase in LDL particle size characterized by the higher LDL-PPD observed in these subjects.

FASN is a key enzyme in fatty-acid synthesis. FASN catalyzed the de novo endogenous biosynthesis of fatty acids. An emerging paradigm supports the notion that dysregulation of

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Published online: June 1, 2011

FASN-catalyzed de novo fatty-acid biogenesis could play a role in the pathogenesis of energy metabolism disorders and insulin resistance [7, 8]. Increased *FASN* gene expression in adipose tissue is linked to visceral fat accumulation, impaired insulin sensitivity, and increased circulating fasting insulin and leptin levels, suggesting an important role of lipogenic pathways in the causal relationship between consequences of excess energy intake and the development of obesity and insulin resistance [30]. Several lines of evidence suggest that obesity and insulin resistance influence LDL-PPD [1]. However, no studies have yet reported a relationship between *FASN* and LDL-PPD heterogeneity. Even if there is no proven mechanistic explanation for the impact of FASN on LDL-PPD, one could speculate on the potential mechanism linking FASN and LDL-PPD. The availability of lipids is a major factor in the regulation of apoB assembly and secretion [31]. It plays a central role in apoB targeting, either for intracellular degradation or for assembly as lipoprotein particles. Lipid availability in the liver is modulated by 3 metabolic pathways: lipid synthesis, LDL uptake, and fatty-acid oxidation [32]. Moreover, the hepatic de novo lipogenesis index from TG was associated with the expression of *FASN* gene and is closely reflected by the de novo lipogenesis index of VLDL TG [33]. The strong correlation between plasma TG levels and LDL-PPD has been extensively described [3, 34]. Moreover, increased production of larger, TG-rich VLDL species was associated with a shift from large buoyant LDL to small LDL particles [35]. Our results indicate, however, that the statistical significance of the interaction term between the *FASN* rs4246444 polymorphism and absolute fat intake in determining LDL-PPD decreased with TG adjustment, but remained significant. This observation was confirmed by the lack of interaction between *FASN* rs4246444 polymorphism and TG on LDL-PPD (data not shown). Other evidences support an association between *FASN* polymorphisms and obesity [36]. With regard to the deleterious impact of obesity on the LDL-PPD [23, 37], FASN could potentially affect LDL-PPD in the presence of excess adipose tissue. However, statistical significance was maintained after adjustments for BMI. In this context, further studies are required to identify the potential biological pathway through which FASN may affect LDL-PPD.

Dietary PUFAs suppress the transcription of several hepatic lipogenic genes such as FASN [38]. This suppressive effect of PUFAs occurs through a decrease in in vivo binding of lipogenic transcription factors SREBP-1 (sterol-regulatory-element-binding protein-1) and NF-Y (nuclear factor-Y) at the *FASN* gene promoter. Our results showed that both PUFA and SFA tend to interact with *FASN* rs4246444 polymorphism in determining LDL-PPD values, which suggests a global effect of fat intake, illustrated by the interaction effect observed with total percentage of fat consumption. However, the *FASN* rs4246444 polymorphism is located in an intronic region (intron 37), which minimizes the potential biological significance of this SNP. Nevertheless, it cannot be excluded that *FASN* rs4246444 SNP is in linkage disequilibrium with a functional *FASN* gene variant.

There are, however, some limitations to such genetic association studies. The first one is related to multiple testing. Adjustment for the effect of multiple testing using the FDR test has been performed. After this adjustment, only the interaction between *FASN* rs4246444 and fat intake (expressed as continuous variable) on LDL-PPD remained significant. Another limitation is the lack of replication in independent study samples. Replication studies are essential to confirm an association between gene polymorphism and phenotype(s) in complex trait studies. Before going any further in the investigation of this gene-diet interaction, a replication study should be done.

In conclusion, the interactions between *FASN* gene polymorphisms and fat intake on LDL particle features observed in this study may suggest that higher fat consumption decreases LDL-PPD and increases the number of small, dense LDL particles as well as the cholesterol levels contained in the small LDL fraction in carriers of the *FASN* rs4246444-A allele.

J Nutrigenet Nutrigenomics 2011;4:137–145
DOI: 10.1159/000327778
Published online: June 1, 2011

Acknowledgments

The authors would like to express their gratitude to the subjects for their excellent collaboration and to the staff of the Physical Activity Sciences Laboratory for their contribution to the study. We want also to express our gratitude to A. Houde for his help in collection of genotypic data. J.P.D. is Scientific Director of the International Chair on Cardiometabolic Risk, which is supported by an unrestricted grant awarded to Université Laval by Sanofi Aventis. B.L. is a recipient of the Canada Research Chair in Nutrition and Cardiovascular Health. C.B. is partially funded by John W. Barton Sr. Chair in Genetics and Nutrition. M.C.V. is the recipient of the Canada Research Chair in Genomics Applied to Nutrition and Health.

References

1 Lamarche B, Lemieux I, Despres JP: The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, patho-physiology and therapeutic aspects. Diabetes Metab 1999;25:199–211.
2 St-Pierre AC, Cantin B, Dagenais GR, Mauriege P, Bernard PM, Despres JP, Lamarche B: Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol 2005;25:553–559.
3 St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Bernard PM, Despres JP, Lamarche B: Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. Circulation 2001;104:2295–2299.
4 Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH: A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882–888.
5 Bosse Y, Vohl MC, Despres JP, Lamarche B, Rice T, Rao DC, Bouchard C, Perusse L: Heritability of LDL peak particle diameter in the Quebec Family Study. Genet Epidemiol 2003;25:375–381.
6 Bosse Y, Perusse L, Despres JP, Lamarche B, Chagnon YC, Rice T, Rao DC, Bouchard C, Vohl MC: Evidence for a major quantitative trait locus on chromosome 17q21 affecting low-density lipoprotein peak particle diameter. Circulation 2003;107:2361–2368.
7 Menendez JA, Vazquez-Martin A, Ortega FJ, Fernandez-Real JM: Fatty acid synthase: association with insulin resistance, type 2 diabetes, and cancer. Clin Chem 2009;55:425–438.
8 Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP: Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science 2000;288:2379–2381.
9 Krauss RM, Dreon DM: Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. Am J Clin Nutr 1995;62:478S–487S.
10 Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT: Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. Am J Clin Nutr 2006;83:1025–1031.
11 Bosse Y, Perusse L, Vohl MC: Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations. J Lipid Res 2004;45:1008–1026.
12 Krauss RM: Dietary and genetic effects on LDL heterogeneity. World Rev Nutr Diet 2001;89:12–22.
13 Campos H, D'Agostino M, Ordovas JM: Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake. Genet Epidemiol 2001;20:117–128.
14 Lai CQ, Corella D, Demissie S, Cupples LA, Adiconis X, Zhu Y, Parnell LD, Tucker KL, Ordovas JM: Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. Circulation 2006;113:2062–2070.
15 Herron KL, Lofgren IE, Adiconis X, Ordovas JM, Fernandez ML: Associations between plasma lipid parameters and APOC3 and APOA4 genotypes in a healthy population are independent of dietary cholesterol intake. Atherosclerosis 2006;184:113–120.
16 Gomez P, Perez-Martinez P, Marin C, Camargo A, Yubero-Serrano EM, Garcia-Rios A, Rodriguez F, Delgado-Lista J, Perez-Jimenez F, Lopez-Miranda J: APOA1 and APOA4 gene polymorphisms influence the effects of dietary fat on LDL particle size and oxidation in healthy young adults. J Nutr 2010;140:773–778.
17 Brown S, Ordovas JM, Campos H: Interaction between the APOC3 gene promoter polymorphisms, saturated fat intake and plasma lipoproteins. Atherosclerosis 2003;170:307–313.
18 Moreno JA, Perez-Jimenez F, Marin C, Gomez P, Perez-Martinez P, Moreno R, Bellido C, Fuentes F, Lopez-Miranda J: The effect of dietary fat on LDL size is influenced by apolipoprotein E genotype in healthy subjects. J Nutr 2004;134:2517–2522.
19 Bouchard C: Genetic epidemiology, association, and sib-pair linkage: results from the Quebec Family Study; in Bray GA, Ryan DH (eds): Molecular and Genetic Aspects of Obesity. Baton Rouge, Louisiana University Press, 1996, pp 470–481.
20 Allain CC, Poon LS, Chan CS, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–475.

21 Fossati P, Prencipe L: Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28:2077–2080.
22 Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 1966;15:45–52.
23 Tchernof A, Lamarche B, Prud'Homme D, Nadeau A, Moorjani S, Labrie F, Lupien PJ, Despres JP: The dense LDL phenotype. Association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. Diabetes Care 1996;19:629–637.
24 Tremblay A, Leblanc C, Bouchard C: The reproducibility of a three-day dietary record. Nutr Res 1983;3:819–830.
25 Verdier P, Beare-Rogers JL: The Canadian Nutrient File. J Can Diet Assoc 1984;45:52–55.
26 Bonfield JK, Smith K, Staden R: A new DNA sequence assembly program. Nucleic Acids Res 1995;23:4992–4999.
27 Livak KJ: Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–149.
28 Dolley G, Berthier MT, Lamarche B, Despres JP, Bouchard C, Perusse L, Vohl MC: Influences of the phosphatidylcholine transfer protein gene variants on the LDL peak particle size. Atherosclerosis 2007;195:297–302.
29 Dolley G, Lamarche B, Despres JP, Bouchard C, Perusse L, Vohl MC: Phosphoinositide cycle gene polymorphisms affect the plasma lipid profile in the Quebec Family Study. Mol Genet Metab 2009;97:149–154.
30 Berndt J, Kovacs P, Ruschke K, Kloting N, Fasshauer M, Schon MR, Korner A, Stumvoll M, Bluher M: Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. Diabetologia 2007;50:1472–1480.
31 Davis RA: Cell and molecular biology of the assembly and secretion of apolipoprotein B-containing lipoproteins by the liver. Biochim Biophys Acta 1999;1440:1–31.
32 Maiyoh GK, Kuh JE, Casaschi A, Theriault AG: Cruciferous indole-3-carbinol inhibits apolipoprotein B secretion in HepG2 cells. J Nutr 2007;137:2185–2189.
33 Peter A, Cegan A, Wagner S, Lehmann R, Stefan N, Konigsrainer A, Konigsrainer I, Haring HU, Schleicher E: Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios. Clin Chem 2009;55:2113–2120.
34 McNamara JR, Jenner JL, Li Z, Wilson PW, Schaefer EJ: Change in LDL particle size is associated with change in plasma triglyceride concentration. Arterioscler Thromb 1992;12:1284–1290.
35 Tan CE, Foster L, Caslake MJ, Bedford D, Watson TD, McConnell M, Packard CJ, Shepherd J: Relations between plasma lipids and postheparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women. Arterioscler Thromb Vasc Biol 1995;15:1839–1848.
36 Schleinitz D, Klöting N, Körner A, Berndt J, Reichenbächer M, Tönjes A, Ruschke K, Böttcher Y, Dietrich K, Enigk B, Filz M, Schön MR, Jenkner J, Kiess W, Stumvoll M, Blüher M, Kovacs P: Effect of genetic variation in the human fatty acid synthase gene (FASN) on obesity and fat depot-specific mRNA expression. Obesity (Silver Spring) 2010;18:1218–1225.
37 Goff DC Jr, D'Agostino RB Jr, Haffner SM, Otvos JD: Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. Metabolism 2005;54:264–270.
38 Xu J, Nakamura MT, Cho HP, Clarke SD: Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem 1999;274:23577–23583.