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Polymorphisms in Toll-like receptor 4 are associated with factors of the metabolic syndrome and modify the association between dietary saturated fat and fasting high-density lipoprotein cholesterol

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ABSTRACT

Toll-like receptor 4 (TLR4) is a protein of the innate immune system hypothesized to mediate some of the effects of a high-fat diet on inflammation and insulin resistance. As both these factors are associated with the metabolic syndrome (MetS), genetic variation in TLR4 may affect the relationship between dietary lipids and MetS. The objective of the study was to determine whether 2 polymorphisms in TLR4 (rs4986790 Asp299Gly and rs5030728 G>A) modify the relationship between dietary fat and markers of the MetS. Participants were healthy young men and women of various ethnocultural backgrounds. Dietary intake was estimated using a 1-month semiquantitative food frequency questionnaire, and fasting blood samples were taken for genotyping and biomarker measurement. The Asp299Gly polymorphism in TLR4 was associated with increased insulin, homeostasis model assessment of insulin resistance ($P < .05$), and homeostasis model assessment of β-cell function ($P < .05$) and family history of diabetes ($P = .0002$). The intronic polymorphism rs5030728 modified the relationship between dietary saturated fatty acids (SFAs) and high-density lipoprotein (HDL) cholesterol ($P = .003$ for interaction). The SFA intake was inversely associated with HDL cholesterol among individuals homozygous for the G allele ($\beta = -0.015 \pm 0.007$ mmol/L, $P = .04$), whereas a positive relationship was observed for heterozygotes ($\beta = 0.025 \pm 0.01$ mmol/L, $P = .02$). There was no association between dietary SFAs and HDL cholesterol among individuals homozygous for the A allele. These observations suggest that both diet and innate immunity may interact to influence components of the MetS.

1. Introduction

Consumption of a diet high in saturated fatty acids (SFAs) is associated with higher concentrations of proinflammatory markers [1] and has also been shown to increase the expression of proinflammatory genes [2]. Dietary SFA also adversely affects inflammation and factors associated with the metabolic syndrome (MetS) [3,4], and innate immunity has been proposed as a mechanism to explain this relationship.

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Toll-like receptor 4 (TLR4) is a pattern recognition receptor activated by lipopolysaccharide. In addition, TLR4 signaling might also be activated by SFAs [5], although this has been debated [6]. In mouse models, a high-fat diet resulted in the activation of TLR4 and an increase in inflammatory cytokine production and insulin resistance [7]. However, the effect of high-fat diet was absent in TLR4-null mice; and they seem to be resistant to the suppression of insulin signaling and insulin-mediated glucose uptake after lipid infusion [7].

Asp299Gly is the most common exonic polymorphism in TLR4, and studies have indicated that this single nucleotide polymorphism (SNP) may result in attenuated receptor-mediated responses and ligand binding [8]. The Asp299Gly SNP has been associated with lower C-reactive protein (CRP) levels and reduced risk for type 2 diabetes mellitus and coronary artery disease [9]; however, these findings have been equivocal [10]. It is possible that these inconsistencies may be explained by the interaction between this SNP and other risk factors, such as diet. The intronic SNP rs5030728 has not been studied previously; however, it has a relatively high frequency and is near exon 3, which makes it likely to affect messenger RNA processing and stability.

The objective of the present study was to determine whether the Asp299Gly (rs4986790, A1187G) and the intronic rs5030728 SNPs influence biomarkers of the MetS or modify the association between dietary fat and these biomarkers in a population of young adults.

2. Methods

2.1. Study population

The Toronto Nutrigenomics and Health study is an ongoing investigation of the role of genetics and nutrition in health and chronic disease. This population consists of men and women between the ages of 20 and 29 years recruited from the University of Toronto, Canada. For the current analysis, individuals who may have underreported (≤800 kcal/d) or overreported (≥3500 kcal/d if female and ≥4000 kcal/d if male, n = 39) their energy intakes, those with diabetes or other conditions characterized by increased inflammation (n = 14), and individuals with possible acute inflammation (n = 54), as determined by CRP of at least 10 mg/L, were excluded from the analyses. Individuals who were missing data relevant to the MetS were also excluded (n = 51). Finally, all individuals of East Asian descent (n = 440) were excluded from analysis because of low frequencies of the minor alleles (≤1%). The final population consisted of 676 individuals. Written informed consent was obtained from all participants, and the study was approved by the Ethics Review Committee at the University of Toronto.

2.2. Dietary assessment

A 196-item Toronto-modified Willett food frequency questionnaire was used to assess habitual dietary intake over the past month. Each subject was instructed on how to complete the food frequency questionnaire using visual aids of portion sizes to improve the accuracy of self-reported food intake.

2.3. Anthropometrics and energy expenditure

Anthropometric measurements including height, weight, and waist circumference were measured; and body mass index (kilograms per square meter) was calculated. Modifiable activity was measured by questionnaire, and metabolic equivalent (MET) hours per day was calculated. This measurement represents both leisure and occupational activity, not including sedentary hours of sleeping or sitting. One MET is equivalent to 1 kcal expended per kilogram body weight per hour sitting at rest [11].

2.4. Laboratory measurements

Each subject had venous blood drawn after a 12-hour overnight fast to measure biomarkers of glucose and lipid metabolism as well as high-sensitivity CRP (hs-CRP) using standard clinical procedures.

2.5. Genotyping

DNA was isolated from whole blood using the GenomicPrep Blood DNA Isolation kit (Amersham Pharmacia Biotech, Piscataway, NJ). The 2 polymorphisms were detected using TaqMan allelic discrimination assays (Asp299Gly ABI no. C__11722238_20; intron ABI no. C__26954831_10) from Applied Biosystems (Foster City, CA), with real-time polymerase chain reaction on an ABI 7000 Sequence Detection System. The polymerase chain reaction conditions were 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

2.6. Statistical analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC). The GLM procedure in SAS was used to perform a 1-way analysis of variance to test for differences in the characteristics between genotypes. The CONTRAST statement was used for pairwise group comparisons, and the χ^2 test was used to analyze categorical variables. Nonnormally distributed variables were $\log_e$ transformed for analysis, and their antilogs are reported. High-sensitivity CRP was transformed following a gamma distribution using the GENMOD procedure, and the median and interquartile range values for this variable are given.

The 2 TLR4 polymorphisms were not in linkage disequilibrium ($r^2 = 0.001$), which was calculated using the HAPLOVIEW software package [12]. The frequency of the Gly/Gly genotype was rare (n = 5) and was combined with the Asp/Gly heterozygotes, creating a carrier group to facilitate analysis.

The GLM procedure was also used to test whether the effect of dietary total, SFAs, monounsaturated, or polyunsaturated fatty acids (PUFAs) as a continuous or categorical variable on different components of the MetS varied across the 3 genotypes for each polymorphism. A diet-gene interaction was found for dietary SFAs and the TLR4 intronic SNP (rs5030728) on high-density lipoprotein (HDL) cholesterol. Covariates that were associated with the outcome and reduced the variance of the relationship between dietary SFAs and HDL cholesterol were included in the model. Potential covariates that were

associated with HDL cholesterol or differed between genotypes were added individually to the model to test whether the interaction changed. These variables were not included in the final model. No differences or interactions were found between the TLR4 genotypes and any of these potential confounders. Dietary SFA was adjusted for total energy intake by using the nutrient density method (percentage of energy from dietary fatty acids) [13]. Slopes of the 3 lines were estimated using the GLM procedure on a fully adjusted model including the genotype and genotype × SFA interaction term. Departure of genotype distributions from Hardy-Weinberg equilibrium was assessed using a χ^2 test with 1 degree of freedom and confirmed using the HAPLOVIEW software. Significant *P* values are 2-sided and less than .05.

3. Results

Frequencies of the polymorphisms in each ethnocultural group, before subject exclusions, are listed in Table 1. The minor allele frequencies in the population were 6.8% for the Asp299Gly polymorphism and 27.9% for the intronic G>A polymorphism (rs5030728). The SNPs were in Hardy-Weinberg equilibrium (*P* = .51 and *P* = .60 for the Asp299Gly and intronic

Table 1 – Clinical and metabolic characteristics and dietary intake by TLR4 genotype

	Asp299Gly			Intronic (rs5030728)			
	Asp/Asp (n = 585)	Asp/Gly + Gly/Gly (n = 87 + 4)	P [a]	G/G (n = 361)	G/A (n = 260)	A/A (n = 55)	P [a]
Age (y)	23.0 ± 0.1	22.4 ± 0.3	.05	22.8 ± 0.1	22.9 ± 0.2	23.7 ± 0.3	.06
Sex (% female)	67.0%	61.6%	.41	69.5%	63.1%	61.8%	.18
BMI (kg/m^2)	23.3 ± 0.2	23.4 ± 0.4	.96	23.2 ± 0.2	23.6 ± 0.3	23.0 ± 0.5	.35
Smoking status (%)							
Never	80.7	86.8		81.2	82.7	78.2	
Past	10.6	9.9		11.1	9.2	12.7	
Present	8.7	3.3	.19	7.7	8.1%	9.1	.91
Physical activity (MET·h/wk)	8.2 ± 0.1	7.6 ± 0.3	.10	8.0 ± 0.2	8.3 ± 0.2	8.1 ± 0.4	.58
Waist circumference (cm)	75.5 ± 0.5	76.1 ± 0.99	.79	74.5 ± 0.6	76.3 ± 0.7	75.9 ± 1.3	.23
Systolic blood pressure (mm Hg)	115.3 ± 0.6	116.4 ± 1.2	.39	114.5 ± 0.7	116.3 ± 0.8	114.9 ± 1.6	.15
Diastolic blood pressure (mm Hg)	69.5 ± 0.5	70.7 ± 0.9	.19	69.2 ± 0.5	70.0 ± 0.6	70.5 ± 1.1	.28
Total cholesterol (mmol/L)	4.2 ± 0.04	4.4 ± 0.09	.15	4.3 ± 0.05	4.2 ± 0.06	4.1 ± 0.1	.40
LDL cholesterol (mmol/L)	2.3 ± 0.04	2.4 ± 0.07	.22	2.3 ± 0.04	2.2 ± 0.05	2.2 ± 0.09	.24
HDL cholesterol (mmol/L)	1.5 ± 0.02	1.5 ± 0.04	.87	1.5 ± 0.03	1.5 ± 0.03	1.5 ± 0.06	.84
Triglycerides (mmol/L)	0.94 ± 0.03	1.03 ± 0.05	.06	0.94 ± 0.03	0.96 ± 0.03	1.02 ± 0.06	.77
Free fatty acids (μmol/L)	470 ± 13.6	455 ± 26.6	.63	471 ± 15.1	462 ± 17.7	456 ± 34.4	.81
hs-CRP (mg/L) [b]	0.5 ± 1.0	0.5 ± 1.2	.36	0.5 ± 0.9	0.5 ± 1.2	0.5 ± 1.2	.66
Glucose (mmol/L)	4.8 ± 0.02	4.8 ± 0.04	.87	4.8 ± 0.02	4.8 ± 0.03	4.8 ± 0.05	.79
Insulin (pmol/L)	52.0 ± 1.6	60.4 ± 3.1	.03	53.6 ± 1.8	54.7 ± 2.1	50.5 ± 4.0	.81
HOMA-IR	1.6 ± 0.05	1.8 ± 0.1	.03	1.6 ± 0.06	1.7 ± 0.07	1.5 ± 0.1	.84
HOMA-β	117.2 ± 4.2	137.5 ± 8.2	.02	121.0 ± 4.6	123.9 ± 5.4	111.3 ± 10.6	.66
Family history diabetes (%)	10.3	24.2	.0002	13.3%	11.2%	9.1%	.56
Energy intake (kcal)	2019 ± 26.6	1926 ± 67.5	.20	1992 ± 33.9	2008 ± 40.0	2100 ± 87.0	.51
Total fat (% energy)	30.2 ± 0.3	29.1 ± 0.7	.15	29.8 ± 0.4	30.3 ± 0.4	30.8 ± 0.9	.47
SFA (% energy)	9.8 ± 0.1	9.6 ± 0.3	.46	9.7 ± 0.1	9.8 ± 0.2	9.5 ± 0.3	.71
MUFA (% energy)	12.1 ± 0.2	11.5 ± 0.4	.50	11.8 ± 0.2	12.2 ± 0.2	12.4 ± 0.5	.52
PUFA (% energy)	5.6 ± 0.07	5.3 ± 0.2	.20	5.6 ± 0.09	5.6 ± 0.1	6.1 ± 0.2	.14
Cholesterol (mg/d)	242.3 ± 5.5	233.0 ± 13.9	.53	246.8 ± 7.0	235.6 ± 8.2	229.0 ± 17.8	.45
Carbohydrates (% energy)	52.5 ± 0.4	54.5 ± 0.9	.04	52.8 ± 0.5	52.6 ± 0.5	53.3 ± 1.1	.82
Fiber (g/100 g carbohydrates)	9.4 ± 0.1	8.9 ± 0.3	.26	9.3 ± 0.2	9.1 ± 0.2	10.1 ± 0.4	.16
Protein (% energy)	16.9 ± 0.1	17.0 ± 0.4	.86	17.0 ± 0.2	17.0 ± 0.2	15.7 ± 0.5	.03
Alcohol (g/d)	7.6 ± 0.4	4.7 ± 1.1	.048	7.2 ± 0.5	7.0 ± 0.6	8.6 ± 1.4	.53
Genotype frequencies in total population [c]							
White	537 (89%)	66 (11%)		302 (50%)	245 (41%)	56 (9%)	
East Asian	435 (99%)	5 (1%)		434 (98.6%)	5 (1.1%)	1 (0.3%)	
South Asian	100 (74%)	35 (26%)		74 (55%)	56 (41%)	5 (4%)	
Other	84 (88%)	12 (12%)		59 (61%)	30 (31%)	7 (8%)	

Metabolic characteristics are adjusted for subject characteristics and dietary variables that were different between each genotype (ethnicity, age, carbohydrate intake and alcohol or ethnicity and protein intake). Metabolic characteristics include waist circumference; systolic and diastolic blood pressures; total, low-density lipoprotein, and HDL cholesterol; triglycerides; free fatty acids; hs-CRP; glucose; insulin; HOMA-IR; and HOMA-β. BMI indicates body mass index; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid.

[a] *P* values for differences between genotypes were obtained by using a 1-way analysis of variance, and the χ^2 test was used to test for differences between genotypes in categorical variables.

[b] Median ± interquartile range given for hs-CRP.

[c] Genotype frequencies in each ethnic group were calculated before subject exclusions.

SNP, respectively) among the cohort after East Asians were excluded. Individuals who were carriers of the Gly allele were older and consumed more carbohydrates and less alcohol than Asp/Asp homozygotes in this population; and these variables, along with ethnicity, were controlled for when analyzing metabolic characteristics. Carriers of the Gly allele had significantly higher insulin, homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-β) (P = .03, .03, and .02; 1-way analysis of variance) and family history of diabetes (P = .0002; χ^2) compared with Asp/Asp homozygotes. Because of the high frequency of this polymorphism in South Asians, we also analyzed these variables by ethnocultural group; and only family history of diabetes remained significant among whites (P = .004; χ^2). The insulin and HOMA indices followed the same pattern within each ethnocultural group, but did not reach significance, probably because of small sample size. Across the intronic SNP (rs5030728), only a small difference in protein consumption was observed.

The TLR4 genotypes were tested to see if they modified the association between dietary fatty acids and biomarkers of the MetS. A significant interaction was found between TLR4 intronic (rs5030728) genotypes and dietary SFA on serum HDL cholesterol concentrations (P = .003, adjusted for triglycerides, PUFA [percentage energy], ethnocultural group, alcohol intake, energy intake, age, and hs-CRP). Dietary SFA was inversely related to serum HDL cholesterol concentrations in individuals homozygous for the G allele (β = –0.015 ± 0.007, P = .04); however, a positive relationship was found among heterozygotes (β = 0.025 ± 0.01, P = .02). This positive relationship was significantly different from the G/G homozygotes (P = .001), but not A/A homozygotes (P = .15). The relationship between dietary SFA and HDL cholesterol was not significant among A/A homozygotes (β = –0.014 ± 0.02, P = .53), and the slope of this relationship did not differ significantly from the other genotype groups. There were no other interactions with any of the other covariates in the model.

As illustrated in Fig. 1, this interaction was not significant when SFA intake was categorized according to recommended intake levels (<10% of energy) (P = .13); however, this may have been due to the decreased sample size after stratification. Contrasting those who consumed SFAs above the recommendations to those who met recommendations in each genotype, mean HDL cholesterol concentrations were significantly lower in G/G homozygotes consuming diets high in SFA (P = .05). No differences were observed between intake groups in the other genotypes (G/A, P = .35 and A/A, P = .59).

4. Discussion

The objective of this study was to determine whether polymorphisms in TLR4 are associated with components of the MetS and if they modify the effect of diet on these biomarkers. The 299Gly allele has been associated with lipopolysaccharide-related hyporesponsiveness [8], but this association has not been consistent [14,15]. The impact of the Asp299Gly polymorphism on risk of type 2 diabetes mellitus and cardiovascular disease has also been inconsistent. This polymorphism has been associated with reduced odds of clinical diabetes and coronary artery disease [9] and a reduced risk of MetS [16]. Others have found no effect on risk for diabetes [10] or cardiovascular disease–related pathogenesis [17]. In the present study, we found that carriers of the Gly allele have significantly higher serum insulin levels and higher HOMA-IR and HOMA-β indices, suggesting that they have lower insulin sensitivities compared with the Asp homozygotes. This is the first study investigating this SNP in relation to these biomarkers in a young, healthy population. The unique lifestyle and metabolic characteristics of such a young population may be playing a role in influencing the effects of this SNP on glucose metabolism; however, this needs to be further investigated.

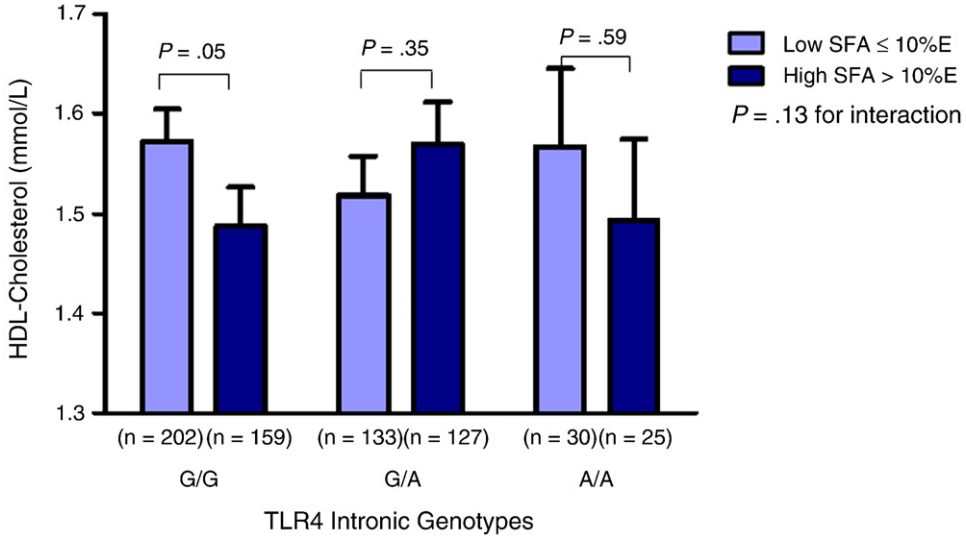

Fig. 1 – Interaction between saturated fat intake and the TLR4 intronic polymorphism (rs5030728) on HDL cholesterol concentrations. Values are means ± SEM adjusted for triglycerides, PUFA (percentage energy), ethnocultural group, alcohol intake, energy intake, age, and hs-CRP.

The second polymorphism, intronic SNP (rs5030728), has not previously been studied for its role in the MetS. Although the polymorphism was not associated with differences in factors associated with the MetS, it did modify the association between dietary SFA and serum HDL cholesterol levels. This interaction was lost after stratifying by category of intake, which may have been because of the reduced sample size, and thus should be investigated in larger populations.

The effects of decreasing SFA intake on HDL cholesterol show a high degree of interindividual variability [18]. In the past, we have also shown polymorphisms in tumor necrosis factor–α and nuclear factor–κB to affect the relationship between dietary PUFA and HDL cholesterol [19,20]. Future studies investigating the effects of combined polymorphisms are needed to clarify potential clinical implications of these genotype association studies in the relationships between dietary fats and HDL cholesterol.

In summary, we found that polymorphisms in TLR4 affect factors related to the MetS in a population of young adults. The Asp299Gly polymorphism was associated with increased insulin, HOMA-IR, HOMA-β, and family history of diabetes, whereas an intronic polymorphism (rs5030728) modified the relationship between dietary SFA and HDL cholesterol. Further studies are needed to confirm these results in other populations as well as to establish mechanisms by which they could be occurring.

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REFERENCES

[1] Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. Arterioscler Thromb Vasc Biol 2006;26: 995–1001.
[2] van Dijk SJ, Feskens EJ, Bos MB, et al. A saturated fatty acid–rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. Am J Clin Nutr 2009;90:1656-64.
[3] Vessby B, Unsitupa M, Hermansen K, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. Diabetologia 2001;44:312-9.
[4] Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. Am J Clin Nutr 2004;79:969-73.
[5] Lee JY, Ye J, Gao Z, et al. Reciprocal modulation of Toll-like receptor–4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem 2003;278:37041-51.
[6] Erridge C, Samani NJ. Saturated fatty acids do not directly stimulate Toll-like receptor signaling. Arterioscler Thromb Vasc Biol 2009;29:1944-9.
[7] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid–induced insulin resistance. J Clin Invest 2006;116:3015-25.
[8] Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000;25:187-91.
[9] Kolek MJ, Carlquist JF, Muhlestein JB, et al. Toll-like receptor 4 gene Asp299Gly polymorphism is associated with reductions in vascular inflammation, angiographic coronary artery disease, and clinical diabetes. Am Heart J 2004;148:1034-40.
[10] Illig T, Bongardt F, Schopfer A, et al. The endotoxin receptor TLR4 polymorphism is not associated with diabetes or components of the metabolic syndrome. Diabetes 2003;52: 2861-4.
[11] Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993;25:71-80.
[12] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
[13] Willet W. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998.
[14] Erridge C, Stewart J, Poxton IR. Monocytes heterozygous for the Asp299Gly and Thr399Ile mutations in the Toll-like receptor 4 gene show no deficit in lipopolysaccharide signalling. J Exp Med 2003;197:1787-91.
[15] van der Graaf C, Kullberg BJ, Joosten L, et al. Functional consequences of the Asp299Gly Toll-like receptor–4 polymorphism. Cytokine 2005;30:264-8.
[16] Steinhardt AP, Aranguren F, Tellechea ML, et al. A functional nonsynonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation. Metabolism 2010;59:711-7.
[17] Norata GD, Garlaschelli K, Ongari M, et al. Effect of the Toll-like receptor 4 (TLR-4) variants on intima-media thickness and monocyte-derived macrophage response to LPS. J Intern Med 2005;258:21-7.
[18] Schaefer EJ, Lamon-Fava S, Ausman LM, et al. Individual variability in lipoprotein cholesterol response to National Cholesterol Education Program Step 2 diets. Am J Clin Nutr 1997;65:823-30.
[19] Fontaine-Bisson B, Wolever TM, Chiasson JL, et al. Genetic polymorphisms of tumor necrosis factor-alpha modify the association between dietary polyunsaturated fatty acids and fasting HDL-cholesterol and apo A-I concentrations. Am J Clin Nutr 2007;86:768-74.
[20] Fontaine-Bisson B, Wolever TM, Connelly PW, Corey PN, El-Sohemy A. NF-kappaB-94Ins/Del ATTG polymorphism modifies the association between dietary polyunsaturated fatty acids and HDL-cholesterol in two distinct populations. Atherosclerosis 2009;204:465-70.