

Fat Intake Influences the Effect of the Hepatic Lipase C-514T Polymorphism on HDL-Cholesterol Levels in Children

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Polymorphisms in the hepatic lipase gene have been associated with variability in plasma HDL-C concentrations, but contradictory results have been reported regarding the effect of diet on this association in adults. In our study, we examined whether dietary fat intake modified the association between lipid levels and the C-514T polymorphism in the hepatic lipase gene (LIPC C-514T) in prepubescent children. The LIPC C-514T polymorphism was determined by PCR and restriction analysis in 1260 healthy school children, aged 6–8. Information on the children's nutrient intake was obtained by means of a validated food frequency questionnaire. We found that regardless of gender, carriers of the minor allele had significantly higher apo A-I levels compared to noncarrier subjects. The effect of the polymorphism, however, was modified by dietary fat intake. In boys, the presence of the LIPC C-514T polymorphism was associated with significantly higher HDL-C among children within the highest tertiles of total, saturated, monounsaturated, or polyunsaturated fat intake. Apo A-I levels were significantly higher in carriers of the LIPC C-514T polymorphism, but only among boys who consumed high total as well as monounsaturated fat and among girls who consumed high total, saturated, monounsaturated, and polyunsaturated fat. Our data show that dietary fat intake modifies the effect of the LIPC C-514T polymorphism on plasma HDL-C and apo A-I levels in prepubescent children, being associated with higher levels of HDL-C and apo A-I only when fat intake is high. This significant gene-nutrient interaction could

help to explain inter-individual variations in the plasma lipid response to fat intake. *Exp Biol Med* 234:744–749, 2009

Key words: hepatic lipase; C-514T polymorphism; HDL-C; dietary fat intake; children

Introduction

Atherosclerosis is a lifelong process (initially asymptomatic) that begins during childhood (1) and whose clinical manifestation is coronary heart disease (CHD). Low plasma HDL cholesterol (HDL-C) and high LDL cholesterol (LDL-C) concentrations are among the main risk factors for the development of atherosclerosis (2). Although diet plays an important role in determining plasma lipid levels (3, 4), individuals differ widely in the response of their plasma cholesterol concentrations to dietary cholesterol and saturated fat (5, 6). This suggests a genetic determination of this variability (7–9). To date, a considerable inter-individual variation in HDL-C levels in response to a similar dietary fat intake has been reported (10, 11).

Hepatic lipase (LIPC) is a lipolytic enzyme synthesized and secreted by the liver. It is involved in HDL-C metabolism and reverse cholesterol transport (RCT), hydrolyzing triglycerides (TG) and phospholipids of all major classes of lipoproteins (12, 13). A common polymorphism located in the LIPC promoter region at position –514 (LIPC C-514T) has been associated with variations in HDL-C concentrations (14–16), although there have been contradictory results regarding this link (17). The interaction of this polymorphism and dietary fat intake affecting plasma lipid concentrations has been analyzed in studies in adults (18–21), but data regarding the nature of this interaction and how much effect dietary fat has are controversial. Some studies have observed an effect of the polymorphism on HDL-C levels when fat intake is low (18), while others

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observed opposite results (20) or investigated populations with different ethnic backgrounds (19–21).

Some studies, such as the Bogalusa Heart Study (22) or the Columbia University Biomarkers Study (23), have described an association between this polymorphism and higher levels of HDL-C in children and young adults, mostly in boys (22). However, to our knowledge, the influence of diet on the effect of the LIPC C-514T polymorphism on plasma lipid levels has not been investigated in children.

Thus, the purpose of our study was to determine whether dietary fat intake modulates the effect of the LIPC C-514T polymorphism on lipid levels in a large population-based sample of Spanish prepubescent children. This age group allows us to avoid confounding factors present in adults, such as high sex hormone levels, alcohol consumption, or smoking.

Materials and Methods

Subjects and Study Design. The population included 1260 healthy schoolchildren (634 males and 626 females), 6–8 years old, who participated in a voluntary survey of cardiovascular risk factors in Spain (24). All were free of any endocrine, metabolic, hepatic, or renal disorder. Sampling was randomized and stratified by pools of school centers in each participating city. The study protocol complied with Helsinki Declaration guidelines and Spanish legal provisions governing clinical research on humans, and was approved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz. Parents were required to sign a written consent form to allow the participation of their children in the study.

Anthropometric Measurements. Measurements were taken with the children lightly dressed and barefoot. Height was measured to the nearest 0.1 cm using a portable stadiometer and weight was recorded to the nearest 0.1 kg using a standardized electronic digital scale. From these measurements, body mass index (BMI, weight (kg) divided by the square of height (m)) was calculated.

Biochemical Data. Fasting (12 hours) venous blood samples were obtained early in the morning by venipuncture into Vacutainer tubes containing EDTA- Na_2 as an anti-coagulant, placed in an ice bath, and centrifuged at low speed. The plasma was separated and used for biochemical determinations, and cells were kept frozen at -70°C for DNA extraction. Cholesterol and triglycerides were measured enzymatically (Menarini Diagnostics, Firenze, Italy) with an RA-1000 Autoanalyzer (Technicon Ltd., Dublin, Ireland). HDL-C levels were measured after precipitation of apo B-containing lipoproteins with phosphotungstic acid and Mg (Roche Diagnostics, Madrid, Spain). Plasma apo A-I concentrations were measured by immunonephelometry (Dade Berhing, Frankfurt, Germany). The interassay coefficients of variation were cholesterol (1.4%), triglyceride (1.7%), and apo A-I (1.55%).

DNA Extraction and Polymorphism Analysis.

Genomic DNA was prepared from leukocytes. For LIPC C-514T genotyping, DNA was amplified by polymerase chain reaction using the primers 5-TCTAGGATCACC-TCTCAATGGGTCCA-3 and 5-GGTGGCTTCCACGT-GGCTGCCTAAG-3, as previously described (16). The 285-base pair amplified fragment was restricted with the enzyme Hha I and the DNA fragments were separated by an 8% polyacrylamide gel electrophoresis.

Nutritional Data. Information on food and nutrition was obtained by means of a food-frequency questionnaire (FFQ), which was initially developed for use in adults and previously validated in Spain for all fats and fat subtypes (25). For the purpose of this study, the questionnaire was adapted to a primary-school population by amending and downscaling the list of foods and portions consumed, eliminating alcoholic beverages, and including some foods frequently found in children's diet (e.g., pizzas, hamburgers, etc.). These amendments were based on a systematic, in-depth review of child population food surveys in Spain (26). The final version of the questionnaire included a total of 77 food items grouped under 11 headings by affinity in nutrient content. For each food, the usual size of the serving eaten was defined (e.g., 1 cup of milk equivalent to 170 cc) and the mean frequency of consumption of such servings was ascertained over the previous year. The blood samples used to measure the plasma lipid levels were obtained concurrently with the FFQ administration. There were 5 consumption frequency scales (never, annually, monthly, weekly, and daily), and the FFQ was filled in by the children's mothers or caretakers, who knew both what food they ate at home and in the school cafeteria. For conversion of foods into nutrients and total caloric intake, standard Spanish food-composition tables were used (27, 28).

Statistical Analysis. Statistical analyses were carried out using the SPSS software package, version 9.0 (SPSS Inc., Chicago, IL). Student's *t*-tests and χ^2 tests were applied to test differences in means and percentages between boys and girls. Due to the skewed distribution, triglycerides were log-transformed before statistical analyses were performed. Because of the small number of children who were homozygous for the less common allele (T), carriers of the CT and TT genotypes were grouped together. A *t*-test was also used to compare lipid and apolipoprotein levels between children with or without the mutation.

To analyze whether the effect of the LIPC C-514T polymorphism on plasma lipid levels is altered by dietary fat intake, the effect of total fat and of the different types of fat (saturated fat, monounsaturated, and polyunsaturated fat intake) were examined. A division of the population according to the World Health Organization recommendations for fat intake was not possible because it was so high that only a very small percentage of the children included in the study were found to comply with the recommendations for total fat and saturated fat intake (29). Therefore, to

Table 1. Anthropometric, Biochemical, Dietary, and Genetic Characteristics of Children According to Gender^a

	Total	Boys (n = 634)	Girls (n = 626)
Age (y)	6.7 (0.7)	6.7 (0.7)	6.7 (0.7)
Weight (kg)	26.7 (5.4)	26.8 (5.3)	26.6 (5.4)
Height (m)	1.25 (0.06)	1.25 (0.06)	1.25 (0.06)
BMI (kg/m ²)	16.9 (2.4)	16.9 (2.4)	16.9 (2.5)
TC (mg/dl)	182.8 (27.4)	181.8 (26.1)	183.3 (28.7)
TG (mg/dl)	72.5 (26.0)	71.2 (25.3)	74.3 (27.2)*
LDL-C (mg/dl)	108.7 (26.1)	107.3 (25.3)	110.0 (26.9)
Apo B (mg/dl)	68.8 (14.0)	68.8 (14.0)	71.3 (14.9)**
HDL-C (mg/dl)	59.5 (13.1)	60.2 (13.0)	58.6 (13.4)*
Apo A-I (mg/dl)	137.0 (18.9)	138.3 (19.0)	135.5 (19.0)**
Total fat intake (% energy)	45.9 (4.4)	46.1 (4.5)	45.7 (4.3)
Saturated fat (% energy)	16.7 (2.7)	16.9 (2.7)	16.5 (2.7)**
Monounsaturated fat (% E)	18.3 (2.5)	18.2 (2.5)	18.3 (2.5)
Polyunsaturated fat (% E)	8.3 (1.8)	8.2 (1.8)	8.4 (2.5)
LIPC C-514T genotype [% (n)]:			
CC	60.6% (766)	60.1% (381)	60.9% (381)
CT	36.1% (456)	37.5% (238)	34.8% (218)
TT	3.3% (42)	2.4% (15)	4.3% (27)

^a Values are given as mean (SD).

*Significantly different from boys ($P < 0.05$).

**Significantly different from boys ($P < 0.01$).

categorize our population as low- and high-consumption children, statistical tertiles were calculated and children in the lowest tertiles of fat intake were considered as low-consumption children and were compared with high-consumption children (those in the middle and high tertiles).

Results

Table 1 shows anthropometric, biochemical, dietary intake, and genetic data from the children according to gender. The mean age was similar in boys and girls (6.7 years). Plasma concentrations of HDL-C and apo A-I were significantly higher in boys compared to girls. Percentage of energy intake from total fat ranged from 31.5% to 64.1%. The ranges of percentage of energy intake from saturated, monounsaturated, and polyunsaturated fat were 9.2–27.4, 11.5–32.6, and 3.3–17.1 respectively. The frequencies of the LIPC C-514T genotypes were 60.6% for CC, 36.1% for CT, and 3.3% for TT. The prevalence of the T allele was 21%. The allelic distribution was consistent with Hardy-Weinberg equilibrium.

Characteristics of children according to the LIPC C-514T polymorphism and gender are given in Table 2. Male carriers of the T allele presented significantly higher plasma concentrations of total cholesterol (TC), TG, and apo A-I compared to noncarrier boys. Female carriers of the CT or TT genotype showed significantly lower BMI and significantly higher apo A-I levels than those with the CC genotype.

In the stratified analyses (Tables 3 and 4), we observed that the relationship between the LIPC C-514T polymorphism and plasma lipid levels was modified by dietary fat intake. We found that boys that were carriers of the

minor allele had significantly higher HDL-C levels than normal boys among those children within the segments of the highest intake (medium and upper tertiles) of total fat and all major types of fat. Boys of both genetic groups in the lower tertiles of fat intake, however, had almost equal HDL-C levels (Table 3). A positive association between fat intake and plasma HDL-C levels was present in T allele carriers, whereas no association was observed in CC homozygotes. Furthermore, male carriers of the LIPC C-514T polymorphism had higher plasma apo A-I levels than normal subjects in the highest tertiles of total fat and monounsaturated fat intake, but not in the lower tertile (Table 3).

We observed significantly higher levels of apo A-I associated with the presence of the LIPC C-514T polymorphism in the upper tertiles of total fat intake and saturated, monounsaturated, or polyunsaturated fat intake in girls (Table 4). Levels of apo A-I between genotypes in girls within the lower tertile of total fat intake or any of the types of fat intake analyzed were nearly identical. Positive associations between the percentage of energy from total fat intake and plasma apo A-I levels, as well as between saturated fat intake and plasma TG levels, were found only in T allele carriers and not in CC homozygotes.

Discussion

While studying the influence of the common polymorphism located at position –514 of the promoter region of the hepatic lipase gene (LIPC C-514T) on plasma lipid levels in a population-based sample of prepubescent children, we found an effect of the LIPC C-514T polymorphism on plasma TC and TG in boys, and apo A-I concentrations in both boys and girls. We also observed that

Table 2. Anthropometric Variables and Plasma Lipid Levels According to the LIPC C-514T Polymorphism^a

	Boys (<i>n</i> = 634)		Girls (<i>n</i> = 626)	
	CC (<i>n</i> = 381)	CT/TT (<i>n</i> = 253)	CC (<i>n</i> = 381)	CT/TT (<i>n</i> = 245)
Weight (kg)	26.8 (5.2)	27.0 (5.5)	26.8 (5.6)	26.3 (5.17)
Height (m)	1.26 (0.06)	1.25 (0.06)	1.24 (0.07)	1.25 (0.06)
BMI (kg/m ²)	16.8 (2.3)	17.0 (2.6)	17.1 (2.6)	16.7 (2.4)*
TC (mg/dl)	180.1 (25.8)	184.7 (26.6)*	182.1 (28.8)	186.0 (28.4)
TG (mg/dl)	68.8 (21.8)	74.6 (29.7)**	73.8 (23.9)	74.3 (30.4)
LDL-C (mg/dl)	106.8 (24.6)	108.1 (26.6)	109.3 (26.3)	111.4 (27.4)
Apo B (mg/dl)	68.5 (13.4)	69.5 (15.1)	70.7 (15.3)	72.5 (14.4)
HDL-C (mg/dl)	59.4 (12.6)	61.5 (13.4)	58.1 (12.9)	59.7 (13.7)
Apo A-I (mg/dl)	137.0 (18.4)	140.3 (19.4)*	134.4 (19.1)	137.7 (18.5)*
Total fat intake (% energy)	46.3 (4.4)	45.6 (4.7)	46.0 (4.3)	45.4 (4.4)
Saturated fat (% energy)	16.9 (2.6)	16.8 (2.8)	16.4 (2.7)	16.5 (2.7)
Monounsaturated fat (% E)	18.3 (2.5)	18.2 (2.6)	18.3 (2.5)	18.1 (2.5)
Polyunsaturated fat (% E)	8.4 (1.9)	8.0 (1.7)*	8.4 (1.8)	8.3 (1.8)

^a Values are given as mean (SD).* Significantly different from CC ($P < 0.05$).** Significantly different from CC ($P < 0.01$).

the association between the LIPC C-514T polymorphism and plasma HDL-C and apo A-I concentrations varied according to total, saturated, and monounsaturated fat intake in our cohort.

As reviewed by Isaacs and colleagues (17), there is a large number of previous studies documenting the association of elevated plasma HDL-C concentration with the presence of the T allele among general populations (14–19, 30–32). While some studies reported a stronger association between the LIPC C-514T polymorphism and HDL-C levels among women (18, 33), other studies either observed this association only among men (14, 34), failed to find the association (35), or reported an association between this polymorphism and apo A-I levels but not with HDL-C levels (36). In our study, we observed a strong association between this polymorphism and apo-AI levels in boys and

girls that was not significantly related to HDL-C concentrations.

Some studies report a strong interaction between this polymorphism and obesity in regard to HDL-C levels (20, 36, 37). In our population of 6- to 8-year-old children, the association between the polymorphism and HDL-C concentrations appears to be independent of BMI (data not shown).

The influence of dietary fat intake on the effect of the LIPC C-514T polymorphism on plasma lipid levels in adults has also been investigated, but data are scarce and controversial (18–21). To our knowledge, no study exists in children. Our study is the first to describe an interaction between the LIPC C-514T polymorphism and fat intake in a prepubescent population. We found that the associations of the LIPC C-514T polymorphism with high levels of HDL-C or apo A-I in boys and with apo A-I levels in girls were

Table 3. Plasma Lipid Levels [mean (SD)] According to the LIPC C-514T Genotype and Fat Intake in Boys

Fat intake	HDL-C (mg/dl)		Apo A-I (mg/dl)		TG (mg/dl)	
	CC	CT/TT	CC	CT/TT	CC	CT/TT
Total:						
Tertile 1 (33–43%)	58.2 (12.6)	58.0 (13.2)	137.1 (19.4)	137.1 (19.2)	69.0 (23.9)	75.3 (23.2)
Tertiles 2+3 (44–64%)	59.2 (12.0)	62.3 (13.5)*	137.3 (18.1)	141.4 (19.9)*	69.6 (20.4)	73.1 (23.1)
Saturated:						
Tertile 1 (11–15%)	59.3 (12.5)	58.5 (11.9)	137.3 (18.2)	139.0 (16.7)	67.7 (21.0)	76.7 (25.3)*
Tertiles 2+3 (16–27%)	58.6 (12.1)	61.9 (14.3)*	137.3 (18.6)	140.3 (21.3)	70.2 (21.7)	72.4 (21.6)
Monounsaturated:						
Tertile 1 (12–17%)	58.7 (11.5)	57.1 (12.1)	138.6 (18.4)	137.8 (20.0)	66.6 (22.0)	71.6 (20.8)
Tertiles 2+3 (18–29%)	59.0 (12.5)	62.5 (13.9)*	136.7 (18.5)	140.9 (19.5)*	70.7 (21.2)	75.1 (24.1)
Polyunsaturated:						
Tertile 1 (3.3–7.3%)	58.2 (12.6)	58.0 (13.2)	137.1 (19.4)	137.1 (19.2)	69.0 (23.9)	75.3 (23.2)
Tertiles 2+3 (7.4–17%)	59.2 (12.0)	62.3 (13.5)*	137.3 (18.1)	141.4 (20.0)	69.6 (20.4)	73.3 (23.1)

* Significantly different from CC ($P < 0.05$).** Significantly different from CC ($P < 0.01$).

Table 4. Plasma Lipid Levels [mean (SD)] According to the LIPC C-514T Genotype and Fat Intake in Girls

Fat intake	HDL-C (mg/dl)		Apo A-I (mg/dl)		TG (mg/dl)	
	CC	CT/TT	CC	CT/TT	CC	CT/TT
Total:						
Tertile 1 (31–44%)	58.6 (13.8)	58.8 (12.5)	136.3 (17.9)	135.0 (17.5)	73.8 (23.1)	77.3 (39.2)
Tertile 2+3 (44–61%)	57.6 (12.6)	60.0 (14.7)	136.6 (20.0)	140.8 (19.7)**	74.9 (23.7)	75.9 (25.3)
Saturated:						
Tertile 1 (11–15%)	59.1 (13.8)	60.7 (13.4)	136.7 (19.6)	138.4 (19.6)	75.6 (26.1)	69.7 (16.2)
Tertile 2+3 (16–27%)	57.3 (12.5)	58.6 (14.2)	133.2 (19.3)	138.6 (18.9)**	74.1 (22.0)	79.3 (35.3)
Monounsaturated:						
Tertile 1 (12–17%)	56.6 (12.0)	60.9 (12.9)	134.3 (20.2)	137.8 (19.3)	77.7 (27.7)	75.7 (26.3)
Tertile 2+3 (18–29%)	58.5 (13.4)	58.3 (14.5)	134.4 (19.2)	139.0 (18.9)*	73.2 (21.2)	76.8 (33.9)
Polyunsaturated:						
Tertile 1 (3.3–7.3%)	59.0 (14.5)	57.4 (11.2)	134.5 (19.6)	134.8 (18.6)	72.3 (20.8)	77.9 (26.3)
Tertile 2+3 (7.4–17%)	57.4 (12.1)	60.2 (15.1)	134.3 (19.4)	140.5 (19.0)**	75.7 (24.7)	75.7 (33.6)

* Significantly different from CC ($P < 0.05$).** Significantly different from CC ($P < 0.01$).

present when consuming a high-fat diet. Furthermore, there was a positive association between fat intake and HDL-C levels in boys and apo A-I in girls, but only among T allele carriers. Our data are consistent with those of other studies which found that higher fat intake enhanced the rising effect of the LIPC C-514T polymorphism on HDL-C concentrations in T allele carriers (20, 21). Conversely, they differ from studies showing that the T allele was associated with higher HDL-C only among subjects that consumed <30% of energy from total fat (18). Comparing our findings with the results of those other four studies is complicated, however, primarily because all those studies were conducted only in adult populations with important ethnic differences. The Framingham study was conducted in Caucasian men and women (18). Zhang and colleagues studied white men with type II diabetes (20), while the study by Tai and colleagues was conducted in a multiethnic Asian population composed of Chinese, Malaysian, and Asian Indian men and women (19). The latter study found that the interaction between TT genotype carriers and dietary fat existed only in the Indian participants. Nettleton and colleagues studied a cohort of Caucasian and African American men and women from the United States and found that the interaction between dietary fat intake and the LIPC genotype was present in African Americans but not Caucasians (21). Moreover, we cannot ignore that the important differences in the amount of dietary fat between our population of prepubescent children and the other investigated populations provide an additional potential confound. Our population is characterized by an excessive intake of fat, particularly saturated fats. None of our children consumed <30% of energy from fat and, in fact, the mean total caloric intake supplied by fats in our cohort was 45.9%, with a mean intake of saturated fat of 16.7% (29). This percentage of energy intake from fats and saturated fats is higher than that reported, for example, in American children (38). Finally, it is likely that other environmental and genetic

factors may also justify the divergent results between studies.

The design of our study did not allow us to investigate the mechanism by which dietary fat intake possibly interacts with the LIPC C-514T polymorphism. This polymorphism seems to be associated with a different HL activity (14, 15), which results in an increase of HDL-C levels. A possible direct effect of fatty acids on HL expression has been suggested. The effect of dietary fat on HL activity could depend on the LIPC C-514T polymorphism.

A limitation of our study is the use of a self-reported 77-item FFQ to assess dietary fat intake that may have yielded results different from other studies using a more comprehensive questionnaire. Unfortunately, the lack of information regarding physical activity, which may affect HDL-C levels, appears as another weakness in our study.

In summary, we conclude that the effect of the LIPC C-514T polymorphism on HDL-C and apo A-I levels is modulated by dietary fat intake in prepubescent children based on the association of the polymorphism with HDL-C and apo A-I levels when fat intake is high. Our data may contribute to elucidate the discrepancies observed when analyzing adult populations. This highly significant gene-nutrient interaction helps to explain the individual differences in the plasma HDL-C response to fat intake.

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