Carbohydrate Intake Modulates the Effect of the \textit{ABCA1}-R230C Variant on HDL Cholesterol Concentrations in Premenopausal Women$^{1,2}$

Sandra Romero-Hidalgo,$^3$ Teresa Villarreal-Molina,$^4$ Juan A. González-Barrios,$^6$
Samuel Canizales-Quinteros,$^9,11$ Martha E. Rodríguez-Arellano,$^7$ Lucia B. Yañez-Velazco,$^8$
Demetrio A. Bernal-Alcantara,$^6,12$ Antonio R. Villa,$^{10}$ Barbara Antuna-Puente,$^5$ Víctor Acuña-Alonzo,$^{13}$
José L. Merino-García,$^6$ Hayde N. Moreno-Sandoval,$^6$ and Alessandra Carnevale$^3$*

$^3$Computational Genomics Department, $^4$Laboratory of Cardiovascular Diseases, and $^5$Research Direction, Instituto Nacional de Medicina Genómica, Mexico City, Mexico; $^6$Hospital “1 de Octubre”, $^7$Hospital “Lic. Adolfo López Mateos”, and $^8$Medical Direction, Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, Mexico City, Mexico; $^9$School of Chemistry, and $^{10}$Department of Public Health, School of Medicine, Universidad Nacional Autónoma de México, Mexico City, Mexico; $^{11}$Unit of Molecular Biology and Genomic Medicine, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubrán, Mexico City, Mexico; $^{12}$Department of Research in Biochemistry, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico; and $^{13}$Molecular Genetics Laboratory, Escuela Nacional de Antropología e Historia, Mexico City, Mexico

Abstract

The R230C variant of the ATP-binding cassette transporter A1 (\textit{ABCA1}) gene has been consistently associated with decreased HDL-cholesterol (HDL-C) concentrations in several studies in the Mexican mestizo population. However, information on how diet composition modifies the effect of the \textit{ABCA1}-R230C variant on HDL-C concentrations is very scarce. The aim of the present study was to analyze whether the effect of \textit{ABCA1}-R230C on HDL-C concentrations is modulated by dietary factors in a nationwide population sample of 3591 adults from the National Health and Nutrition Survey conducted by the State’s Employees’ Social Security and Social Services Institute. All participants answered a validated questionnaire to assess health status and weekly food consumption. Fasting blood samples were drawn for biochemical analysis and DNA extraction, and the \textit{ABCA1}-R230C variant was genotyped using TaqMan assays. Statistical analyses consisted of simple linear and multiple regression modeling adjusting for age, BMI, smoking, and alcohol consumption. The overall C risk allele frequency was 9.3% and the variant was significantly associated with low HDL-C concentrations in both sexes. A significant negative correlation between carbohydrate consumption and HDL-C concentrations was observed in women bearing the R230C variant ($P = 0.021$) and a significant gene-diet interaction was found only in premenopausal women ($P = 0.037$). In conclusion, the effect of the \textit{ABCA1}-R230C gene variant on HDL-C concentrations is modulated by carbohydrate intake in premenopausal women. This finding may help design optimized dietary interventions according to sex and \textit{ABCA1}-R230C genotype. J. Nutr. 142: 278–283, 2012.

Introduction

Hypoalphalipoproteinemia (HDL-C$^{14}$ concentrations <40 mg/dl) is an independent risk factor for cardiovascular disease (1) and is the most common dyslipidemia in Mexican adults (2–6). Plasma HDL-C concentrations result from the interaction of both genetic and environmental factors such as diet, smoking, alcohol consumption, and physical activity (7–14). Heritability estimates for HDL-C levels mostly range between 40 and 60% (14) and various genetic polymorphisms of the \textit{ABCA1} gene have been associated with HDL-C concentrations in several populations (15–18). The R230C-ABCA1 gene variant was recently found to be private to the Americas and strongly associated with low HDL-C in Mexican mestizos and Native American populations (19–23). This allele is of particular interest, because it is frequent in the Mexican mestizo population (~10%) and the sole presence of the C risk allele explains almost 4% of plasma HDL-C concentration variation, which is higher than all HDL-C variation associated with a single nucleotide polymorphism identified through genome-wide association studies in European and Asian populations (24).

Recent studies have reported that dietary factors such as protein, carbohydrate, and fat consumption may modify the effect of several gene variants (KCTD10, MMAB, and MVK) on...
HDL-C concentrations, reporting significant gene-nutrient interac-
tions (25–27). However, information on how diet compos-
tion modifies the effect of the ABCA1-R230C variant on HDL-
C concentrations is very scarce. To date, there is only one report
where hyperlipidemic individuals carrying the R230C risk allele
showed a better HDL-C concentration response to a specific
dietary intervention (low-saturated fat diet plus 2.5 g soy protein
and 15 g soluble fiber) (28). The purpose of the present study
was to analyze whether specific diet components modulate the
effect of the ABCA1-R230C variant on HDL-C plasma concen-
trations to provide the bases for optimized dietary inter-
ventions.

Materials and Methods

Participants. The study included 3591 adults (1160 men and 2431
women) recruited from the ENSADER conducted in 2007 by the State’s
Employees’ Social Security and Social Services Institute in Mexico. The
ENSADER is a nationwide, representative, cross-sectional study based on
a 2-stage, stratified, cluster sampling procedure, including government
employees. The purpose of ENSADER was to assess the prevalence,
distribution, and potential impact of risk factors associated with major
nontransmissible diseases as well as the nutritional status of this pop-
ulation. All participants answered a structured, validated, precoded
questionnaire requesting information on age, sex, educational level, and
health. A validated questionnaire assessing weekly food consumption was
also applied. Height, weight, waist/hip circumference, and blood pressure
were measured and BMI was estimated as [weight [kg]/height [m^2]].
Individuals were diagnosed as obese at BMI ≥30 kg/m^2 and overweight
when BMI was ≥25 and <30 kg/m^2. Current alcohol consumption was
analyzed as an ordinal variable where 0 = no consumption, 1 = occasional
consumption, 2 = monthly consumption, 3 = weekly consumption, and 4 =
daily consumption. Fasting blood samples were drawn for DNA extraction
and biochemical measurements. All participants provided informed
consent and the ENSADER protocol was approved by the Research and
Ethics Committee of the National Institute of Public Health.

Biochemical measurements. Lipid concentrations were measured at
the laboratory of the Department of Endocrinology and Metabolism at the
National Institute of Medical Sciences and Nutrition in Mexico. This
laboratory is certified by the External Comparative Evaluation of
Laboratories Program of the College of American Pathologists. All
measurements were performed with commercially available, standard-
ized methods in blood samples obtained after a 9- to 12-h fast. Serum
glucose was measured using the glucose oxidase method. Total choles-
terol was determined using enzymatic hydrolysis and oxidation. TG
concentration was measured after lipase hydrolysis in an automatic
analyzer with a tungsten lamp (Premier 24i, Tokyo Boeki Medical System).
HDL-C was measured by an enzymatic colorimetric direct method
(Synchron CX analyzer, Beckman Systems). LDL-C concentra-
tions were estimated using Friedewald’s formula (29).

Genetic analyses. Genomic DNA was extracted and purified from
peripheral white blood cells using the salting-out procedure (30). The
R230C variant (rs9282541) was genotyped using TaqMan assays (ABI
Prism 7900HT Sequence Detection System, Applied Biosystems). Geno-
typing call exceeded 95% and no discordant genotypes were observed in
180 duplicate samples. Samples previously genotyped by direct sequencing
were used as positive controls. Deviation from the Hardy-Weinberg
equilibrium was not observed.

Statistical analyses. Because of the reduced number of CC homozy-
gous individuals, CR and CC genotypes were analyzed as a single group
using a dominant model. Student’s t and chi-square tests were applied to
test differences between anthropometric and metabolic variables. Lipid
traits were log-transformed due to skewed distribution and are expressed
as geometric means and 95% CI. ANCOVA was used to determine
associations between the R230C variant and metabolic variables,
adjusting for age, BMI, smoking, and alcohol consumption. Simple

\[
\begin{align*}
\text{Table 1} & \quad \text{Anthropometric and serum biochemical measurements, lifestyle characteristics, and genotype distribution of the study participants stratified by sex}^1 \\
\hline
\text{Men} & \quad & \text{Women}^2 \\
\text{Age, y} & \quad & 48.3 \pm 14.2 & 45.9 \pm 12.5^4 \\
\text{Anthropometric variables} & \quad & \\
\text{BMI, kg/m}^2 & \quad & 28.5 \pm 4.3 & 28.3 \pm 5.1 \\
\text{Weight, kg} & \quad & 80.1 \pm 15.0 & 68.7 \pm 14.0^4 \\
\text{Height, cm} & \quad & 167.5 \pm 7.6 & 155.8 \pm 6.9^4 \\
\text{Waist, cm} & \quad & 96.6 \pm 11.5 & 91.2 \pm 13.2^4 \\
\text{Metabolic variables} & \quad & \\
\text{Total-C, mmol/L} & \quad & 5.5 \pm 1.3 & 5.4 \pm 1.1 \\
\text{HDL-C, mmol/L} & \quad & 1.0 \pm 0.2 & 1.2 \pm 0.4^4 \\
\text{LDL-C, mmol/L} & \quad & 3.4 \pm 0.9 & 3.4 \pm 0.9 \\
\text{TG, mmol/L} & \quad & 2.8 \pm 3.0 & 1.9 \pm 1.3^4 \\
\text{Glucose, mmol/L} & \quad & 61.2 \pm 28.3 & 56.3 \pm 20.9^4 \\
\text{Systolic blood pressure, mm Hg} & \quad & 130.3 \pm 16.1 & 122.2 \pm 16.3^4 \\
\text{Diastolic blood pressure, mm Hg} & \quad & 81.9 \pm 10.3 & 77.3 \pm 10.6^4 \\
\text{Dietary intake} & \quad & \\
\text{Total energy, kcal/d} & \quad & 2668 \pm 913 & 2220 \pm 804^4 \\
\text{Proteins, % energy} & \quad & 13.5 \pm 3.2 & 14.2 \pm 3.3^3 \\
\text{Proteins, g/d} & \quad & 87.3 \pm 31.1 & 77.3 \pm 28.8^4 \\
\text{Carbohydrates, % energy} & \quad & 55.3 \pm 9.3 & 57.6 \pm 8.3^4 \\
\text{Carbohydrates, g/d} & \quad & 363 \pm 126 & 318 \pm 122^4 \\
\text{Fats, % energy} & \quad & 28.4 \pm 6.3 & 29.4 \pm 6.3^4 \\
\text{Fats, g/d} & \quad & 84.2 \pm 34.7 & 73.2 \pm 33.3^4 \\
\text{Other characteristics} & \quad & \\
\text{Alcohol consumers, n (%)} & \quad & 986 (85.2) & 1294 (53.5)^5 \\
\text{Daily, n (%)} & \quad & 23 (2.3) & 10 (0.8) \\
\text{Weekly, n (%)} & \quad & 270 (27.4) & 102 (7.9) \\
\text{Monthly, n (%)} & \quad & 122 (12.4) & 82 (6.3) \\
\text{Occasionally, n (%)} & \quad & 571 (57.9) & 1100 (85.0) \\
\text{Physical activity, h/wk} & \quad & 60 \pm 10.7 & 56 \pm 9.8 \\
\text{Daily smokers, n (%)} & \quad & 276 (23.8) & 330 (13.6)^4 \\
\text{Overweight, n (%)} & \quad & 536 (46.7) & 996 (41.6)^4 \\
\text{Obesity, n (%)} & \quad & 362 (31.5) & 731 (30.6) \\
\text{ABCA1-R230C variant, n (%)} & \quad & \\
\text{RR} & \quad & 949 (81.8) & 2010 (82.7) \\
\text{RC} & \quad & 202 (17.4) & 398 (16.4) \\
\text{CC} & \quad & 9 (0.8) & 23 (0.9) \\
\hline
\end{align*}
\]

1 Values are expressed as mean ± SD or n (%). HDL-C, HDL-cholesterol; LDL-C, LDL-
cholesterol; total C, total cholesterol.

2 Values marked with superscripts a and b differ from means for men at P < 0.01 and
P < 0.001, respectively.
overall population and was higher in the southern states, ranging from 4.6% in Guanajuato to 13.9% in the state of Tabasco (Fig. 1). Genotype distribution did not differ from Hardy-Weinberg equilibrium.

**Association of ABCA1-R230C with metabolic traits.** RC/CC genotypes were strongly associated with lower HDL-C concentrations in both sexes. On average, the absolute difference in HDL-C concentrations according to genotype was 0.11 and 0.06 mmol/L for women and men, respectively. The risk allele was also significantly associated with lower total cholesterol concentrations in both sexes and with lower LDL-C concentrations only in men (Table 2).

**Correlation between diet components and HDL-C concentrations according to genotype.** Overall, carbohydrate intake and HDL-C concentrations had a negative correlation in both men and women ($\beta = -0.11\%$; $P = 0.002$ and $\beta = -0.05\%$; $P = 0.041$, respectively). This effect was greater in women carrying the risk allele (RC heterozygous or CC homozygous) ($\beta = -0.16\%$; $P = 0.021$) but not significant in women with RR homozygous genotypes (Fig. 2B). In contrast, men with the RR genotype had a significant negative correlation between carbohydrate intake and HDL-C concentrations ($\beta = -0.13\%$; $P < 0.001$), whereas higher carbohydrate consumption had no effect in males carrying the risk allele (Fig. 2C). Interestingly, the greatest effect of carbohydrate intake on HDL concentrations was observed in premenopausal women ($\beta = -0.30\%$; $P = 0.003$), but it was not significant in postmenopausal women (Fig. 3). The multiple $R^2$ for the model including the main effects of all variables identified as affecting HDL-C concentrations was 15.7% of HDL-C concentration variability. The proportion of energy consumed as protein and fat did not significantly affect serum HDL-C concentrations according to genotype in the entire sample nor in any subgroup (men or premenopausal or postmenopausal women).

Predicted values were calculated from regression models containing the ABCA1-R230C variant, carbohydrate intake, and the interaction term, adjusted for age, BMI, tobacco smoking, and alcohol consumption (Fig. 4). Dietary carbohydrate proportion modulated the effect of the variant on HDL-C plasma concentrations, and the interaction between the polymorphism and carbohydrate intake was significant only in premenopausal women ($P_{interaction} = 0.037$). The gene-diet interaction did not reach significance in men.

**Discussion**

The R230C variant of the ABCA1 gene is of particular interest because of its epidemiological implications; it is frequent and private to Native American and descendant populations, it has been consistently associated with low HDL-C concentrations in various reports (19,21–23) including the present study, and this variant is known to have a functional effect, decreasing cholesterol efflux in vitro by ~30% (22). The overall C risk allele frequency was 9.3%, similar to that reported in previous studies in the Mexican mestizo population (19–23). The highest risk allele frequency was observed in the southern states (11.2%) in accordance with the higher Native American component of these populations and the Amerindian origin of the R230C variant (22,32).

![FIGURE 1](https://academic.oup.com/jn/article-abstract/142/2/278/4630810/280.png) Nationwide distribution of the risk allele (C) ($n = 3591$).

| TABLE 2 Association between ABCA1-R230C polymorphism and serum lipid concentrations stratified by sex¹ |
|----------------------------------|----------|----------|----------|----------|
|                                | RR      | RC       | CC       | $P^2$    |
| **Men**                         |         |          |          |          |
| **n**                           | 766     | 158      | 8        |          |
| **Total-C, mmol/L**             | 5.43 (5.31–5.56) | 5.08 (4.90–5.27) | 5.25 (4.54–6.06) | <0.001  |
| **HDL-C, mmol/L**               | 0.97 (0.94–0.99) | 0.91 (0.88–0.95) | 0.85 (0.72–1.01) | 0.002   |
| **LDL-C, mmol/L**               | 3.30 (3.18–3.41) | 3.08 (2.91–3.25) | 4.12 (3.17–5.35) | 0.020   |
| **Women**                       |         |          |          |          |
| **n**                           | 1769    | 348      | 22       |          |
| **Total-C, mmol/L**             | 5.39 (5.32–5.45) | 5.26 (5.15–5.38) | 4.86 (4.49–5.26) | 0.010   |
| **HDL-C, mmol/L**               | 1.20 (1.18–1.22) | 1.09 (1.06–1.12) | 1.08 (0.98–1.20) | <0.001  |
| **LDL-C, mmol/L**               | 3.30 (3.24–3.36) | 3.26 (3.16–3.37) | 3.10 (2.76–3.47) | 0.328   |

¹ Values are geometric means (95% CI). Means were adjusted for age, BMI, tobacco smoking, and alcohol consumption. HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Total C, total cholesterol.

² $P$ values were obtained by comparing RR vs. RC/CC genotypes.
To date, all studies assessing the effect of the \( \text{ABCA1}-\text{R230C} \) variant on HDL-C concentrations in different populations have been consistent. As expected, the R230C variant was significantly associated with lower HDL-C concentrations in the ENSADER sample, although the effect of the variant was greater in women than in men. This finding is not in accordance with that of Aguilar-Salinas et al. (23), who reported a greater effect in males. This discrepancy could be the result of the different sampling designs and/or different data analyses.

The ENSADER State’s Employees’ Social Security and Social Services Institute population sample has several advantages: it is representative of all Mexico, it is less heterogeneous than the general population in terms of age, educational level, and socioeconomic status; and it includes data on smoking, alcohol consumption, and dietary habits known to affect HDL-C concentrations (14). Thus, this sample allowed the assessment of gene-environment interactions, particularly gene-nutrient interactions in a cross-sectional design. The FFQ was designed and validated by the National Institute of Public Health in Mexico to assess long-term exposure to different nutrients in order to study their potential role as risk factors for chronic diseases (33); however, because of the limitations inherent in diet reporting errors, the results should be interpreted with caution and confirmed in prospective cohort studies.

Multiple dietary factors have been considered key determinants of HDL-C concentrations (34). Higher carbohydrate intake has been correlated with lower HDL-C concentrations in various studies (25,35,36). Consistent with previous reports, we observed an overall negative correlation between carbohydrate intake and HDL-C concentrations in both men and women, with no significant effect of protein and fat percentage intake. Although Guevara-Cruz et al. (28) reported that risk allele carriers (RC heterozygous or CC homozygous) were better responders to a specific diet portfolio (low-saturated fat diet complemented with soy protein and soluble fiber), the interaction of this or any other \( \text{ABCA1} \) variant with specific dietary components has not been previously assessed.

A sex-specific gene-diet interaction was observed in the present study, because the inverse correlation between carbohydrate intake and HDL-C concentrations was greater in women bearing the R230C variant, particularly in those with a premenopausal status. In contrast, the R230C variant and carbohydrate intake showed an independent, nonadditive effect in men. Although the cause of sex-related differences is not fully understood, estrogen is known to have an effect in premenopausal women by increasing HDL-C concentrations (37,38). Moreover, there is evidence that estrogen increases \( \text{ABCA1} \) expression in different tissues (39,40) and estrogen therapy increases \( \text{ABCA1} \) expression in leukocytes and improves lipid profile in menopausal women (41), suggesting that the sex-specific interaction here could be mediated by the effect of estrogen on \( \text{ABCA1} \) expression. On the other hand, although testosterone is known to be responsible for lowering HDL-C levels in men (42), this hormone has been found not to affect apoA-I or \( \text{ABCA1} \) expression in hepatocytes or human monocyte-derived macrophages (43), which is consistent with the lack of \( \text{ABCA1} \)-nutrient interaction observed in males.

To our knowledge, this is the first study reporting a sex-specific interaction between any \( \text{ABCA1} \) variant and carbohydrate intake on HDL-C concentrations, representing the first effort to evaluate gene-nutrient interactions for the \( \text{ABCA1}-\text{R230C} \) gene variant. These findings may provide the bases to

**FIGURE 2** Correlation between HDL-C concentrations and dietary carbohydrate percentage according to genotype in the total population (A), women (B), and men (C). Lines represent simple linear regressions. Gray lines represent RR genotypes and black lines represent risk allele carriers (RC/CC). HDL-C, HDL-cholesterol.

**FIGURE 3** Correlation between HDL-C concentrations and dietary carbohydrate percentage according to genotype in premenopausal (A) and postmenopausal (B) women. Lines represent simple linear regressions. Gray lines represent RR genotypes and black lines represent risk allele carriers (RC/CC). HDL-C, HDL-cholesterol.
design specific dietary interventions for individuals bearing this variant in prospective cohort studies.

Acknowledgments
S.R-H., S.C-Q., and A.C. designed the research; L.B.Y-V., D.A.B-A., and A.R.V . designed the ENSADER 2007 and provided essential materials; J.A.G-B., M.E.R-A., J.L.M-G., and H.N.M-S. conducted the research; S.R-H., T.V-M., S.C-Q., and A.C. analyzed the data; S.R-H. and T.V-M. wrote the paper; B.A-P. and V.A-A. performed a major critical review; and A.C. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


29. Guevara-Cruz M, Tovar AR, Larrieta E, Canizales-Quinteros S, Torres N. Increase in HDL-C concentration by dietary portfolio with soy
protein and soluble fiber is associated with the presence of the ABCA1R230C variant in hyperlipidemic Mexican subjects. Mol Genet Metab. 2010;101:268–72.


42. Asscheman H, Gooren LJG, Megens JAJ, Nauta J, Kloosterboer HJ, Eikelboom F. Serum testosterone level is the major determinant of the male-female differences in serum levels of high-density lipoprotein (HDL) cholesterol and HDL2 cholesterol. Metabolism. 1994;43:935–9.